IMMUNOSUPPRESSION INDUCED BY ULTRAVIOLET IRRADIATION AND THE ROLE OF UROCANIC ACID

Thesis submitted for the Degree of Doctor of Philosophy

by

Jill W Gilmour

University of Edinburgh

December 1992

I dedicate this Thesis to my parents,

whose constant support and encouragement

have made this possible

DECLARATION

I declare that studies presented in this Thesis are the result of my own independent investigation, with the exception of the fluorescent flow cytometric analysis and HPLC analysis which were carried out with the assistance of W Neill and J Crosby. Some routine tests presented in Chapter 6 were carried out by the Hospital laboratory service at the Royal Infirmary of Edinburgh. Dr. J P Vestey recruited, monitored and managed all the patients and obtained the clinical material for the studies outlined in Chapter 6 and 7. Dr. S George assisted in recruiting additional subjects for the study outlined in Chapter 7.

This work has not been, and is not being currently submitted for candidature for any other degree.

Jill W Gilmour

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to thank the Medical Research Council for the award of a research studentship and also the Edinburgh Dermatological Research Fund for their sponsorship over the past three months.

My deepest gratitude is extended to my supervisor Mary Norval whose constant enthusiasm, guidance and support (beyond the call of duty) have made the past three years not only an invaluable training but also a real pleasure. My sincere thanks are also extended to Dr James Vestey for collecting all the clinical material and also for his patience, encouragement and friendship over the past three years.

Thanks also to Bill Neill for all his assistance with the fluorescent flow cytometry and along with the other members of the VRL team, Dave Yirrell, Fiona Charleson and Mel Jackson for their invaluable advice (concerning matters both inside and outside the lab) and for providing some of the more amusing moments through out my studies.

I wish to acknowledge the volunteers who kindly donated the clinical material used in this study. I would also like to thank John Verth and the staff in the animal house for their help through out the project.

My sincere thanks to my friends in Edinburgh, Jane Matthews, Fiona Thomson, Lisa Kendall, Dave MacEwan for providing a welcome distraction from my studies especially when the going got tough.

Finally, I would like to thank my mum and dad for their love and constant moral support.

ABBREVIATIONS USED IN TEXT

AC	adherent cell
AIDS	acquired immune deficiency syndrome
APC	antigen presenting cell
BCC	basal cell carcinoma
BSA	bovine serum albumin
$C_{3(4)}$	complement component 3 (4)
cAMP	adenosine 3', 5'-cyclic monophosphate
CH	contact hypersensitivity
Con A	concanavalin A
cpm	counts per minute
DC	dendritic cell(s)
DD	dermal dendrocyte(s)
DETC	dendritic epidermal T cells
DH	delayed hypersensitivity
DLN	draining lymph node
DMSO	dimethylsulphoxide
DNFB	dinitrofluorobenzene
EC	epidermal cell(s)
E cell	effector cell(s)
ELAM	endothelial lymphocyte adhesion molecule
ELISA	enzyme-linked immunosorbent assay
E:T	effector to target cell ratio
FACS	fluorescence activated cell sorter
FCS	foetal calf serum
FGF	fibroblast growth factor
FITC	fluorescein isothiocyanate
fTEC	frozen T enriched cells
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte/macrophage-colony stimulating factor
h	hour(s)
H1 (2)	histamine type 1 (2) receptor
HIV	human immunodeficiency virus
HPLC	high pressure liquid chromatography
HS	horse serum
HSV-1 (2)	herpes simplex virus type-1 (2)
5HT	5 hydroxy tryptamine (serotonin)

ICAM-I	intracellular adhesion molecule
IFN-α (β γ)	interferon-α (β γ)
IL-1(-2)	interleukin-1 (-2)
Ig	immunoglobulin
KC	keratinocyte(s)
KLH	keyhole limpet haemocyanin
LC	Langerhans cell(s)
LFA-1(3)	leukocyte function associated antigen-1(3)
LN	lymph node
LPR	lymphoproliferative response
LPS	lipopolysaccharide
MAIDS	murine acquired immunodeficiency virus
M-CSF	macrophage-colony stimulating factor
MED	minimal erythemal dose
MHC class I or II	major histocompatability complex class I or II
MLR	mixed lymphocyte reactions
MM	malignant melanoma
NBCS	new born calf serum
NK cell	natural killer cell
NMSC	non-melanoma skin cancers
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PDGF	platelet-derived growth factor
pfu	plaque forming units
PG	prostaglandin
PHA	phytohaemagglutinin
PUVA	psoralen with ultraviolet A therapy
SALT	skin associated lymphoid tissue
sem	standard error of mean
SI	stimulation index
SIS	skin immune system
TCM	tissue culture medium
TEC	T enriched cells
TGF-α (β)	transforming growth factor-α (β)
Th	T helper cell
TNF-α(β)	tumour necrosis factor
UCA	urocanic acid
UV	ultraviolet

UVA (B)	ultraviolet A (B)
UVR	ultraviolet radiation
WC	white cells

ABSTRACT

Ultraviolet radiation (UVR) results in a transient suppression of selected immune responses to antigens encountered following exposure. Urocanic acid (UCA), found in the stratum corneum of the epidermis as the *trans*-isomer, absorbs UV light whereupon it changes to the *cis*-isomer. *Cis*-UCA mimics many of the suppressive effects of UVR on immune responses.

Previously *cis*-UCA has been shown to produce a dose dependent, antigen specific suppression of the delayed hypersensitivity (DH) response to herpes simplex virus (HSV) in a murine model of infection, in an identical manner to UVB-irradiation. A time course of DH revealed an initial response 1 hour after antigen challenge, followed by a second response at 24 hours, both of which were suppressed if the mouse had been UV irradiated or treated with *cis*-UCA prior to infection. Skin painting murine ears with *cis*-UCA resulted in a reduction in the number of ATPase positive cells (Langerhans cells) in epidermal sheets prepared 24 hours later, while *trans*-UCA had no effect. In an attempt to elucidate the mechanism of action of *cis*-UCA two histamine receptor antagonists were employed; cimetidine (H2) and terfenadine (H1). When *cis*-UCA was applied together with either of the antagonists no significant reduction in the number of ATPase positive cells was observed. Similarly if *cis*-UCA was applied together with either antagonist the suppression of the DH response to HSV was blocked. Thus *cis*-UCA may act through a histamine-like receptor. A number of structural analogues of UCA were tested for their ability to suppress the DH response to HSV to further elucidate the structures required to induce immunosuppression. Tumour necrosis factor-α (TNF-α) has been implicated to be of major importance in the induction of UV-induced immunosuppression. Mice pretreated with neutralizing antibodies to TNF-α were found to be resistant to the immunosuppressive effects of UV, but there was little effect on *cis*-UCA induced

suppression of the DH response to HSV. These results extend the mechanism by which *cis*-UCA modulates suppression of immune responses.

UVB and UVA plus psoralens (PUVA) are successfully employed in the management of psoriasis but their use is largely empirical and little is known of their effects on immunity. A number of parameters of immunity were measured in patients with psoriasis undergoing UVB or PUVA therapy. These were compared with patients receiving coal tar treatment and with normal subjects undergoing UVB irradiation. Serum immunoglobulin (Ig) isotypes, complement components and percentages of subsets of peripheral blood mononuclear cells (PBMC) were within normal ranges in most of the psoriasis patients. These, and lymphoproliferative response to HSV and to the mitogen, concanavalin A (Con A), remained unchanged throughout therapy. Epidermal cells and blood adherent cells were used to present HSV to PBMC enriched T cells, in a lymphoproliferative assay. The functional antigen presenting ability of adherent cells remained unchanged throughout therapy while that of epidermal cells was suppressed and recovered, in most instances, after UVB therapy had been completed. The epidermis of patients with psoriasis contained about three times the quantity of UCA of normal subjects but no difference was observed in suction blister fluid. During UVB irradiation, the percentage of *cis*-UCA rose in both the epidermis and suction blister fluid of all subjects and it remained elevated in the blister fluid after therapy had finished. TNF-α was measured in suction blister fluid and its concentration did not alter consistently as a result of therapy. Natural killer (NK) cell activity was measured in subjects receiving broad band UVB, PUVA or TLO1 narrow band (311 - 313 nm) therapy. Four weeks of PUVA and TLO1 therapy resulted in a significant suppression in NK cell activity which took greater than four weeks after the last treatment to recover to normal. *Cis*- but not *trans*-UCA resulted in a dose dependent suppression in NK cell activity *in vitro*. Whether any immune modulation measured in psoriasis patients contributes to the efficacy of phototherapy remains unknown.

CHAPTER 1

GENERAL INTRODUCTION

1.1 THE SKIN IMMUNE SYSTEM

1.1.1 The Evolution Of The Concept Of The Skin Immune System

The skin is the largest organ of the body and it carries out a number of physiological functions including the maintenance of body temperature, production of endocrine mediators in addition to containing peripheral neural receptors and nerve endings. It prevents undesirable water loss and acts as a barrier to the penetration of exogenous compounds. In addition it acts as a physical barrier to mechanical trauma, potentially pathogenic microorganisms and the photons of sunlight.

The skin is more than a passive protective covering as it is an active component of the immune system. This fact was potentially recognized as far back as 460 BC by Hippocrates; Plato and Galen also believed that the skin was not merely a physical barrier but acted like a fisherman's net, linking elements of the body which were otherwise isolated. There is a strong historical link between early immunology and skin physiopathology, exemplified by the experiments of Jenner, in which observations of immune induction after vaccination with cowpox through the skin were made. Classical subtypes of DH have been characterised in the skin, and skin tests to evaluate DH remain one of the most reliable indicators of cell mediated immunity.

The concept of the skin-associated lymphoid tissue (SALT) was introduced by Streilein in 1978. This included the major antigen presenting cell of the epidermis known as the Langerhans cell (LC) , epidermotropic recirculating subpopulations of T cells, the cytokine producing keratinocytes (KC) and also the skin-draining peripheral lymph nodes. A number of other immunologically active cells and mediators have since been included with SALT, for example mast cells, tissue macrophages, neutrophilic granulocytes, indeterminate cells, veiled cells, vascular

endothelial cells and also the afferent lymphatic endothelium (starting in the dermis). Taken in combination with the SALT, this system was nominated the skin immune system (SIS) by Bos and Kapsenberg (1986).

The following section is a concise summary of the immunological functions of a number of populations of skin cells, and briefly discusses the pathways by which they may operate in generating immune responses to antigens which are encountered in the skin. An excellent account and full literature citation is given in "The Skin Immune System" by Bos (1990).

1.1.2 Langerhans Cells

LC were first described in 1868 by Paul Langerhans and were originally thought to be of neural origin and function. They are now known to be the primary antigen presenting cells (APC) of the skin, and are pivotal in generating immune responses to antigens encountered in the skin including contact sensitizers, haptens and protein antigens (reviewed in "Epidermal Langerhans Cells" Ed Schuler (1991); Kapsenberg *et al.*, 1990). The LC is the main DC of the epidermis, located below the upper stratum granulosum, but rarely in the basal layer, and it constitutes approximately 3-4 % of the total number of epidermal cells. The dendritic processes almost connect to form a complete network covering the epidermis as illustrated in Chapter 3 (Fig 3.2 (a) and (b)). Similar to other DC, LC are bone marrow derived (Katz *et al.*, 1979), have a low buoyant density (Teunissen, *et al.*, 1988) and low phagocytic capacity. LC are capable of inducing allogeneic T cell responses, antigen specific T cell responses and cytotoxic T cell activation (see below).

LC exhibit unique trilaminar cytoplasmic organelles known as Birbeck granules, which allows their identification by electron microscopy. Resident, as well as freshly isolated LC share a number of phenotypic characteristics in common with mononuclear phagocytic cells. A range of cell markers identified on LC are outlined

in Table 1.1 (adapted from Romani *et al.*, 1991). Histochemically they exhibit nonspecific esterase and ATPase activity. Immunophenotypically they bear Fc-IgG II and complement receptors as well as MHC class II antigens. The phenotype of LC is not stable. In culture LC undergo profound morphological changes. They acquire a pronounced DC morphology and the expression of some markers continuously decreases (eg Fc-IgG II receptors, ATPase activity, Birbeck granules) whereas others greatly increase (eg MHC II, RFD 1, CD25, CD40, CD54, CD58).

Table 1.1

LC markers

Marker	**Resident LC**	**Freshly Isolated LC**	**Cultured LC**
CD1	+	+	—
CD4	±/-	?	?
FcR IgG	+	+	—
C3 R	+	+	—
HLA-DR	+	+	++
HLA-DP	+	+	++
HLA-DQ	+	+	++
RFD-1	—	+	++
ICAM-1	?	+	+
LFA-3	?	±	+/++
LFA-1	—	—	—
ATPase	+	+	+/-
Non-specific Esterase	+	—	—
Birbeck Granules	+	+	—

Human LC express CD4 in low quantities but murine LC do not (Schmidt *et al.*, 1984). Murine LC express IL-2 receptor (Steiner *et al.*, 1986).

These phenotypic changes are thought to be induced by granulocyte/macrophage colony stimulation factor (GM-CSF) and interleukin (IL) -1 (Heufler *et al.*, 1988). Tumour necrosis factor alpha (TNF-α) does not appear to affect the morphology of LC but does maintain their viability (Koch *et al.*, 1990). TNF-α may also be involved in LC migration from the epidermis to the draining lymph node (DLN) (Cumberbatch and Kimber , 1992). As discussed below LC may migrate from the skin to the DLN, and are thought to undergo maturation during this process. Essentially cultured LC are indistinguishable from certain DC of the lymphoid tissue, and have been proposed to represent the difference between LC in the epidermis and DLN.

In vivo, LC are thought to initiate the induction of immune responses against antigens encountered in the skin by providing the primary sensitizing signal to T cells. A substantial amount of experimental evidence indicates that LC pick up antigen in the epidermis, mature functionally and phenotypically as they move from the epidermis through the afferent lymphatics (possibly as veiled cells) into the DLN where they reside as mature DC or interdigitating cells and present antigen to T cells, resulting in proliferation of antigen responsive T cells (Barker and Billingham, 1968; Bergstresser *et al.*, 1980; Botham *et al.*, 1987; Kinnaird *et al.*, 1989; Knight *et al.*, 1985a; Knight *et al.*, 1985b; Macatonia *et al.*, 1986; Siberberg-Sinakin *et al.*, 1976). Following sensitization T cells may recirculate and preferentially home to the skin. The most compelling evidence in support of this hypothesis derives from a report by Kripke *et al* (1990). In this study skin from C3H mice was grafted onto BALB/c recipient mice. Sensitization of the mice through the grafted tissue with FITC (fluorescein isothiocyanate) resulted in the accumulation of antigen bearing DC in the DLN which were found to be of graft donor origin. A number of the DC in the draining LN were found to contain Birbeck granules.

Epidermal cell preparations containing LC induce primary and secondary T cell responses to alloantigens, haptens and also soluble protein antigens (reviewed by Stingl and Shevach, 1991) including herpes simplex virus (HSV) proteins (Williams *et al.*, 1991). An area of controversy is the effect of culturing LC on their antigen presenting function. A number of groups have reported that cultured LC have a greatly enhanced antigen presenting capacity (Shimada *et al.*, 1987; Teunissen *et al.*, 1990). This finding has been disputed by the work of Cohen and Katz (1992) and Romani *et al* (1989) who found no enhancement after culturing. The contradiction may arise from differences in the antigens employed, differences in the activation state of the responder cells or may result from differences in the extent or method of LC enrichment.

KC are a rich source of cytokines (see section 1.1.4.) but little is known of the secretory capacities of LC. Sauder *et al* (1982) demonstrated that epidermal cell suspensions which were greatly enriched for LC (84-97%) constitutively produced significant IL-1 activity. This finding must still be treated with a degree of caution as it is possible that contamination by KC may affect this finding. More recently P D Cruz has demonstrated the production of IL-1 and IL-6 by LC (personal communication).

1.1.3 Dermal Dendrocytes

A population of DC has been identified which reside in the dermis (Cerio *et al.*, 1989; Sontheimer *et al.*, 1989). Dermal dendrocytes (DD) have a folded nucleus and a very ruffled irregular surface. The cytoplasm contains organelles which are required for active metabolism, but does not contain Birbeck granules. Phenotypically DD express ATPase and non-specific esterase activity as well as CD45, CD11b, CD11c and MHC class II antigens. They do not express CD1, CD14 or CD15 and are therefore distinct from endothelial cells, but share certain

features with LC. Phenotypically they share features with other APC. Evidence in the mouse indicates that they may in fact be capable of initiating cell-mediated contact hypersensitivity (CH) *in vivo* (Tse and Cooper, 1990).

1.1.4 Keratinocytes

For many years keratinocytes (KC) were thought to function merely as building blocks in the epidermis but it has become apparent that they are very efficient producers of a number of cytokines (outlined in Table 1.2), which have profound effects in the skin. The first of these factors to be identified, and the most well characterized is ETAF (epidermal cell thymocyte activating factor) (Luger *et al.*, 1981; Sauder *et al.*, 1982) which is now known to be identical to monocyte derived IL-1 (Kupper *et al.*, 1986; Luger *et al.*, 1983 (a); Luger *et al.*, 1983 (b)). It is produced constitutively whereas the other cytokines tend to be produced following stimulation by trauma, chemicals, UVR (Nickoloff *et al.*, 1990) or by other cytokines which may be KC derived themselves (Ansel *et al.*, 1990).

Table 1.2

Keratinocyte Produced Cytokines

Interleukin (IL)	**Colony-Stimulating Factors (CSF)**	**Others**
IL-1 α	GM-CSF	IFN–α (Interferon-α)
IL-1 β	G-CSF (Granulocyte-CSF)	IFN-β (Interferon-β)
IL-3	M-CSF (Macrophage-CSF)	TNF-α
IL-6		PDGF (Platelet-derived growth factor)
IL-8		TGF-α (Transforming growth factor-α)
		TGF-β
		FGF (Fibroblast growth factor)
		PGE-2 (Prostaglandin E-2)

KC are capable of phagocytosis and endocytosis (Luger *et al.*, 1983 (b)). *In vitro* the T cell cytokine IFN-γ (but not α or β) induces HLA-DR expression on human and murine KC (Basham *et al.*, 1985; Czernielewski and Bagot, 1986). MHC class II expression on KC in human graft versus host disease has also been reported (Lampert *et al.*, 1981). Furthermore, examination of a number of other dermatoses revealed HLA-DR expression on KC only when there was a lymphoid infiltration into the skin eg mycosis fungoides, eczema, discoid lupus erythematosus and lichen planus (Lampert 1984), which may indicate that IFN-γ is also of importance *in vivo*. IFN-γ induced the expression of OKM5 on KC (Simon and Hunyadi, 1987). OKM5 is found on a number of cell types including endothelial cells, monocytes, platelets and C32 melanoma cell line. ICAM-1 may be absent from KC or expressed at very low levels, but its synthesis may be enhanced by treatment with IFN-γ or TNF-α (Dustin *et al.*, 1988). The expression of adhesion molecules on KC by IFN-γ may play an important role in the trafficking of T cells through the epidermis, particularly in inflammatory dermatoses.

Despite the fact that KC constitutively produce IL-1 and may be induced to express MHC Class II antigens and ICAM-1, both necessary for antigen presentation, it has not been possible to demonstrate antigen presentation by these cells (Breathnach *et al.*, 1986; Chu *et al.*, 1990). This may be due to inadequate antigen processing, despite their being capable of phagocytosis and endocytosis, or perhaps the low expression of Class II.

1.1.5 Lymphocyte Populations of the Skin

The overwhelming majority of lymphocytes in the skin are T cells. Early attempts to locate lymphocytes in normal human skin gave very inconsistent results. However reinvestigation more recently by Bos *et al* (1987) revealed that 90% of T cells in the skin were located in the dermis. Here they were clustered and localised around post-capillary venules and adjacent to cutaneous appendages. In contrast to circulating T

lymphocytes the majority of dermal T cells were activated, ie expressed HLA-DR and IL-2 receptors. In addition there was an approximately even distribution of $CD4^+CDw29^+(4B4^+)$ (helper / memory) and CD8 (suppressor / cytotoxic) T cell subsets; $CD4^+CD45^+(2H4^+)$ naive T cells were found to be relatively rare in the skin (less than five percent), despite forming 50% of peripheral blood $CD4^+$ T cells (Bos *et al.*, 1987; Bos *et al.*, 1989). It is unclear whether these memory cells have immigrated into the skin in an activated state or whether they have been locally activated, or reactivated. It has been demonstrated that $CD4^+CDw29^+$ T cell subsets show enhanced endothelial cell binding, compared with that of $CD4^+CD45^+$subsets which may indicate that $CD4^+CDw29^+$ cells preferentially immigrate into the skin (Pitzalis *et al.*, 1988). Intraepidermal T cells were randomly distributed and were found to be predominantly $CD8^+$, but they account for only 10% of the total skin lymphocytes.

Two types of T cell receptors (TCR) have been identified. The most common is a disulfide-linked heterodimer comprising an α and a β transmembranous polypeptide, with variable regions at the periphery. The TCR is noncovalently associated with the CD3 complex, forming the CD3-TCR complex, in which the CD3 complex is thought to transduce the activation signal upon recognition of the appropriate antigen by the TCR. The second TCR is present on a small percentage of thymocytes (0.1-1 %) and peripheral T cells (1-9%). It consists of a heterodimer with transmembranous polypeptide designated the γ and δ subunit. This receptor is also linked to the CD3 complex, but the antigen specificity remains unknown.

T cells with the γ/δ-TCR are extremely rare in the human skin (Bos *et al.*, 1988; Foster *et al.*, 1988). In contrast, murine skin contains a population of DC which are $CD45^+$ $Thy1^+$ $CD5^-$ $CD4^-$ $CD8^-$ MHC II^- and were originally known as $Thy\text{-}1^+$ dendritic epidermal cells (Bergstresser *et al.*, 1983; Tschachler *et al.*, 1983). These cells are now known to be T cells which uniformly express the γ/δ-TCR on their

surface (Koning *et al.*, 1987; Kuziel *et al.*, 1987; Stingl *et al.*, 1987) and have more recently been renamed dendritic epidermal T cells (DETC) (Steiner *et al.*, 1988). The functional role of DETC remains unknown, and despite attempts by several groups, no human equivalent has been identified.

Murine $CD4^+$ cells can be further subdivided into T helper 1 (Th1) and T helper 2 (Th2) cells on the basis of their cytokine production profile. Th1 cells produce predominantly IL-2, IFN-γ and TNF-β whereas Th2 produce IL-4 but not IL-2. Th1 appear to be more important in mediating DH responses, whereas Th2 cells appear to preferentially support antibody production by B cells. The situation is probably more complicated in man.

One other population of Thy-1^+ cells found in the skin is necessary for the elicitation of contact hypersensitivity (CH) responses (reviewed by Askenase *et al.*, 1991). CH responses in mice are biphasic, with an early response at around two hours followed by the classical reponse 24-48 h following antigen challenge (Van Loveren *et al.*, 1983; Lavaud *et al.*, 1991). Elicitation of the response is due to the sequential action of two different antigen specific Thy-1^+ cells. The early acting, or initiating cell, was revealed to be an unusual phenotype, for an antigen specific cell; Thy-1^+, $CD5^+$, $CD4^-$, $CD8^-$, $CD3^-$, sIg^-, $CD45RA^+$ (not found on mature T cells), $Mac1^+$, IL-$2R^-$ and Il-$3R^+$ (present on pre-B and pre-T cells) (Herzog *et al.*, 1989). This cell type produces antigen-specific non-IgE factors which sensitize mast cells for the release of the vasoactive amine serotonin (5-hydroxytryptamine; 5HT) at the local site of antigen challenge. 5HT initiates CH by acting on endothelial cells which allows the recruitment of effector cells. The effector cells in contrast to the initiator population were $Thy1^+$, $CD5^+$, $CD4^+$, $CD8^-$ and $CD3^+$ (Herzog *et al.*, 1989). The 24 h swelling is therefore dependent on the initial response at two hours.

It has also been found that monoclonal IgE is capable of initiating CH (Ptak *et al.*, 1991). When a low dose (1-10 μg per mouse) of IgE was injected a one hour peak

and subsequent CH responses were observed, but when a higher dose was given (10-100 μg per mouse) there was a large immediate hypersensitivity reaction but no CH response. It was thought that the higher dose of IgE may have been sufficient to induce histamine release from mast cells. Histamine has previously been shown to suppress DH reactions (Norval *et al.*, 1989b). Treatment of the mice with cimetidine (histamine class 2 receptor (H2) antagonist) permitted the higher dose of IgE to initiate the DH response (Ptak *et al.*, 1991). Histologically there is evidence that an early initiating event occurs in human DH responses. One hour after antigen challenge mast cell degranulation occurs, followed by the induction of ELAM-1 on post capillary venules at two hours, peaking at 24 h (Waldorf *et al.*, 1991).

Cellular infiltrates in inflammatory dermatosis consist predominantly of T cells. This has been demonstrated in a number of diseases varying from very common disorders such as eczema and psoriasis, to uncommon malignancies such as cutaneous T cell lymphomas.

1.1.6 Mast Cells

Mast cells constitute a heterogeneous population, as demonstrated histochemically, morphologically, biochemically and functionally. They occur in large numbers in the skin (7000 mm^{-3} mast cells in human skin) and are located predominantly in the perivascular region of the papillary dermis, less frequently in the deeper dermis and very rarely in normal epidermis. Activation by immunological or non immunological stimuli results in the release of mediators which are stored in cytoplasmic granules, or are synthesised very rapidly. Mast cell mediators are very diverse and have complex functions. They include vasoactive substances (such as serotonin and histamine), chemotactic factors, active enzymes and structural proteoglycans. Serotonin is a necessary requirement for the elicitation of DH responses (Askenase *et al.*, 1991). Mast cells are a principal source of histamine. Histamine is capable of inducing a variety of cellular functions through its

heterogenous receptors (Hill 1990; Mitsuhashi and Payan, 1992). Histamine also has specific effects on immune responses (reviewed by Falus and Merétey 1992; Melmon and Kahn 1987).

Mast cells express and release a number of cytokines including IL-1, 3, 4, 5, 6, 8, TNF-α, GM-CSF and IFN-γ. In addition they are capable of phagocytosis and on stimulation with IFN-γ they express MHC-II antigens (reviewed by Möller *et al.*, 1991). Therefore mast cells may play an impotant role in regulating immune responses, in addition to being of major importance in initiating immune responses.

1.1.7 Endothelial Cells

The vascular endothelium consists of a monolayer of cells lining the circulatory system, and forms a mechanical barrier between the blood and extravascular tissues. The dermal endothelium therefore plays a vital role in emigration of cells from the blood into the skin. The majority of lymphocytes found in the skin are memory T cells (Bos *et al.*, 1989) indicating that the selection of circulating cells is not a random process.

Large families of adhesion molecules have been identified on the surface of cells and multiple ligand receptor interactions are thought to play an important role in trafficking of cells (Albelda and Buck 1990; Shimizu *et al.*, 1992). A number of adhesion molecules have been identified on endothelial cells, and their corresponding ligands on T cells (Nickoloff, 1991b). The expression of several adhesion molecules is greatly enhanced during inflammatory reactions as a result of the release of cytokines such as IL-1 IFN-γ and TNF eg IL-1 and TNF-α both induce the expression of ICAM-1 and ELAM.

MEL-14 in the mouse (Gallatin *et al.*, 1983) and Hermes 1 in humans (Jalkanen *et al.*, 1986) are found on the surface of lymphocytes and are thought to be involved in lymphocyte homing to peripheral lymph nodes (LN). *In vitro*, antibodies to these

molecules block the binding of these cells to high endothelial venules, and *in vivo* they also block the homing of lymphocytes to the peripheral LN. To date the molecules involved in "cell homing into the skin" remain to be identified.

1.2 HERPES SIMPLEX VIRUS

1.2.1 Herpes Simplex Virus Infection

There are six members of the family Herpesviridae which are known to infect man, herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella zoster virus, cytomegalovirus, Epstein Barr virus and human herpes virus 6. HSV-1 is an enveloped virus possessing an icosahedral capsid, approximately 100 nm in diameter, which encases linear double stranded DNA of approximately 152 000 base pairs with the potential to code for approximately 70 polypeptides.

HSV-1 has a predilection for the oral mucosa where it is commonly responsible for mucocutaneous infections, more commonly known as "cold sores" (Fig 1.1). Typically lesions in most individuals progress from erythema through stages of papules, vesicles, erosions or ulcers before healing. Following a primary infection with HSV-1 the virus is thought to travel up the sensory nerves serving the site of infection and become latent in the sensory neurons. In certain individuals reactivation of the virus may occur, resulting in a recrudescent perioral lesion. UVR is a common triggering factor. In a previous study of healthy subjects suffering from recrudescent HSV infection who were recruited in Edinburgh, at least 30% noted UV to be a triggering factor for their recrudescent HSV (Vestey *et al.*, 1989).

1.2.2 Immune Responses to HSV

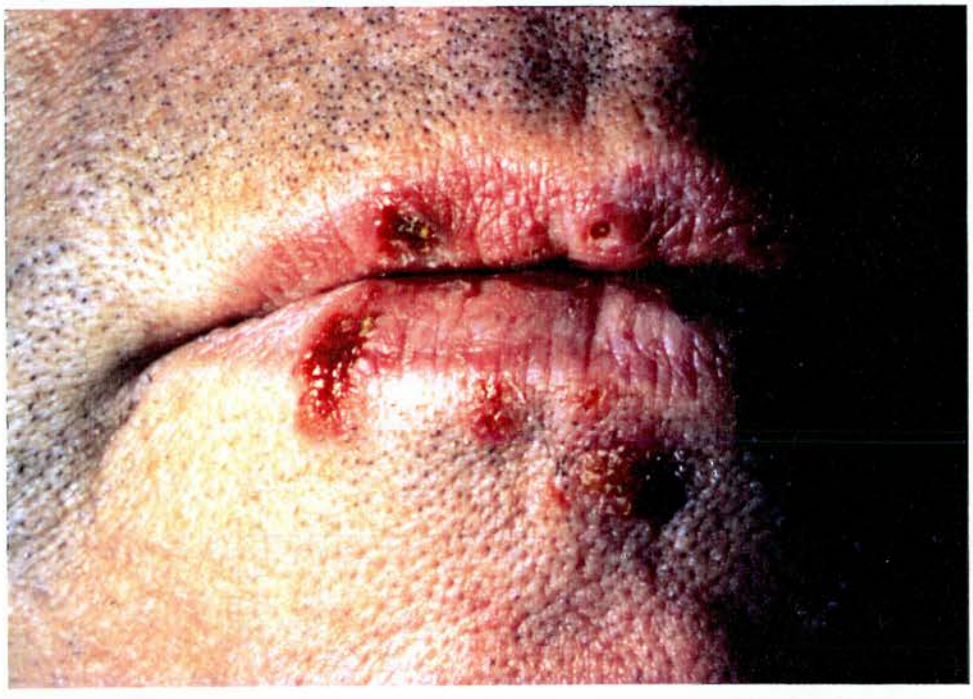
Most information pertaining to immune responses to HSV have been gained from animal models (Wildy and Gell, 1985). The immune response to HSV is complex and involves most arms of the immune response. Cell mediated immune responses appear crucial in the clearance and recovery from HSV infection, while antibody responses are relatively unimportant.

During HSV infection the virus is virtually exclusively confined to the epidermis.

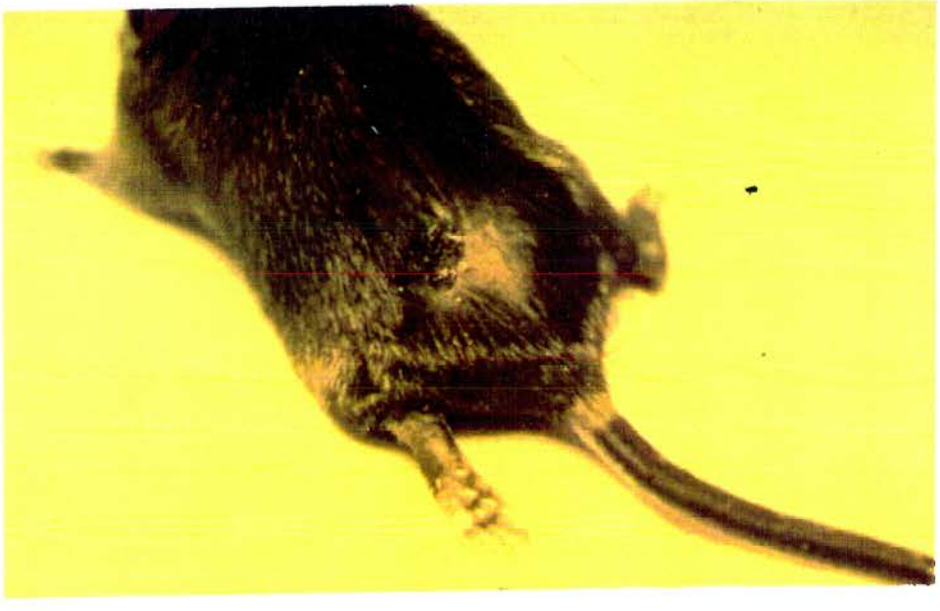
Figure 1.1

HSV Infection. (a) A typical human recrudescent orofacial lesion. (b) Epidermal infection with HSV in the murine model of infection used in this study

(a)



(b)



Sprecher and Becker reported that the pathogenicity of HSV-1 in mice was dependent on epidermal LC density (Sprecher and Becker, 1987; Sprecher and Becker, 1989). LC are thought to take up the viral antigens and travel to the DLN where presentation to T cells takes place. Following sensitization activated T cells recirculate to the skin. The infiltrating T cells secrete IFN-γ which results in the expression of MHC class II antigens and ICAM-1 on KC, and the release of IL-1 from LC and KC. Expression of adhesion molecules on KC may enhance T cell/KC interactions and migration of $CD8^+$ lymphocytes into the epidermis as is found late in HSV lesions. IL-1 release may attract cells to the site and amplify immune responses. Both cytotoxic and DH resposes are required for the efficient clearance of HSV from the skin (Martin and Rouse 1987; Wildy and Gell 1985). Macrophages, which are resistant to HSV replication, are important in the the control of infection (Morahan *et al.*, 1985). NK/K cells are found adjacent to KC which exhibit the greatest cytopathic effect (Heng *et al.*, 1989).

Most subjects develop high antibody titres to the virus during a primary infection which remain elevated throughout the life of the subject, and vary little even with recrudescences (Vestey *et al.*, 1989). High antibody titres do not appear to prevent recrudescences but there is some evidence that antibody dependent cell-mediated cytotoxicity may play a role early in HSV infections (Kohl, 1991). Cell-mediated immune responses to HSV in man have been monitored in peripheral blood predominantly. Patients develop T_h responses (T helper cell), as determined by *in vitro* lymphoproliferative responses to HSV antigens, during primary infections. Lymphoproliferative responses are usually similar for HSV-1 and -2 antigens, reflecting the multiplicity of type-common antigens (Vestey *et al.*, 1989). The degree of lymphoproliferation varies between individuals but is not related to the severity or the likli hood of subsequent recrudesences. In addition to T_h HSV-specific cytotoxic T cell reponses are found and DH responses to intraepidermal HSV

challenge is also detectable. Both of these persist indicating immunological memory. Analysis of NK cell activity, HSV-specific lymphoproliferation and IFN production suggests that a temporary depression in immune responses may occur just before or during a recrudescence (Vestey *et al.*, 1989). Thus recrudescent lesions may occur if there is a delay in, or suppression of, the normal cell mediated immunity to HSV. It is possible that trigger ing factors for recrudescent HSV infection, such as exposure to UVR for example, might play a role in this.

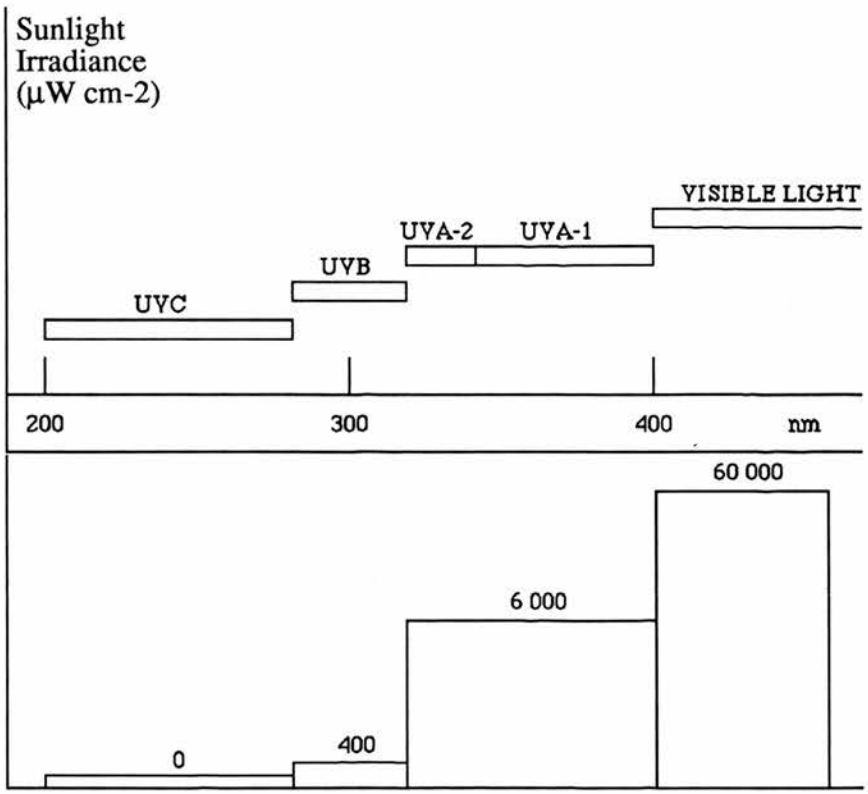
1.3 ULTRAVIOLET RADIATION

1.3.1 Sources

Ultraviolet radiation (UVR) consists of electromagnetic radiation of wavelengths ranging from 200-400 nm. This has been broken into defined wavebands as shown in Fig 1.2. Unfiltered sunlight contains the complete UV spectrum, but the amount reaching the earth's surface is directly dependent on its wavelength. Nitrous oxide in the thin upper atmosphere blocks the germicidal short UVC wavelengths. At lower altitudes of the stratosphere (15-30 km) molecular oxygen and ozone absorb the lower UV wavelengths. The relative irradiance from the sun is shown in Fig 1.2, but the UV spectrum of incident radiation from the sun on the earth's surface at a given time is dependent upon the latitude, time of day and year, weather, air pollution and reflection from the earth's surface.

Fig 1.2

The UV-Spectrum



In 1985 it was reported that a "hole" in the ozone layer had developed over Antarctica each Spring since 1979 (Farman *et al.*, 1985). Between 1969 and 1986 the average concentration of ozone in the stratosphere had decreased by approximately two percent per year, much faster than models had predicted (NASA, 1988). Ozone depletion leads to a greater amount of UV penetrating the atmosphere. Increased environmental exposure to UV may have important effects on crop yields, plankton and fish populations, which may have important implications to the food chain (Cullen *et al.*, 1992) as well as human health.

As world travel becomes more accessible, many people now expose themselves to intense sunlight for a few weeks in a year. Some also expose themselves to artificial UV sources for cosmetic purposes in solaria or as phototherapy in the treatment of a number of common dermatoses such as psoriasis and eczema. Other possible sources of exposure include halogen desk lamps, fluorescent light bulbs and welders lamps.

1.3.2 The Biological Effects Of UV

The biological effects of UV are determined by the interactions at the body surface particularly in skin and eyes. One important role of UVR is in the synthesis of Vitamin D in the skin which is necessary for bone formation. Many people find that exposure to UVR is linked to a general feeling of well being. Cosmetically, some Caucasians believe tanning to be beneficial. The formation of melanin, the pigment which produces the tan, does protect other cells from UVR damage. UV exposure is highly effective in the treatment of a large number of skin diseases. However these positive effects are possibly outweighed by some negative effects of UV exposure.

UVR, particularly in the UVB range induces erythema, more commonly known as "sunburn". This is a local effect caused by the dilation of blood vessels and increased blood flow to the skin. If the UV dose has been particularly high this may

be followed by blistering and peeling of the skin. Susceptibility to erythema corresponds to the skin type of the subject.

Frequent and prolonged exposure to UVR over a substantial period results in permanent damage to the skin shown as dryness and wrinkling (Kligman, 1989). This process is known as photoageing and can be distinguished histologically from the natural ageing process by elastosis and the formation of mature collagen (Kligman, 1979).

Non-melanoma skin cancers (NMSC) are the most common of all tumours in the Caucasian population, with a low mortality rate of one percent or less (Pollock Shea, 1988). Two main forms exist; basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), both of epidermal origin. NMSC are directly related to UVR. They usually occur on areas of the body which have been most exposed to UV and have a higher prevalence close to the equator. Mice chronically exposed to UVR develop NMSC (Blum 1959). The action spectrum for skin tumour production is similar to that for erythema, that is UVB wavelengths are more efficient than UVA (De Gruijl and van der Leun, 1991). Malignant melanoma (MM) is much rarer than NMSC, but is increasing at an alarming rate, particularly in Northern countries (MacKie *et al.*, 1992). The mortality rate from MM is much greater than NMSC. The correlation between UV exposure and MM is not as clear cut as that of NMSC, although it does occur more frequently in fair-skinned individuals with a history of blistering sunburn in their childhood. It is not restricted to UV-exposed areas of the body, and is not found in people such as outdoor workers with a very high UV exposure.

UVR also affects the eye. Absorption of UVR by the eye can give rise to inflammation of the cornea (keratitis) and conjunctiva (conjunctivitis; "snow blindness"). UV light may be an important factor in senile cataract formation (Pitts *et al.*, 1986; Taylor *et al.*, 1988).

1.3.3 The Effect of UVR on Immune Responses

Cutaneous irradiation with UVB results in a transient systemic suppression of selected immune responses to antigens encountered thereafter (reviewed by Baadsgaard *et al.*, 1991; Gallo *et al.*, 1990; Morisson *et al.*, 1989a). This was first shown in 1977 for the transfer of UV-induced tumours, which are highly immunogenic, to sygeneic receipient mice. The tumours were rejected unless the mouse had previously been UV-irradiated (Fisher and Kripke, 1977). This suppression was transferrable by lymphocytes (Daynes and Spellman, 1977). In these experiments high doses of UV were administered, but subsequent experiments revealed suppressed induction of CH responses and reduction of $ATPase^+$ cells in mice following much lower, sub-erythemal doses of UVB (100-300 J m^{-2}) (Toews *et al.*, 1980). Studies of the effects of UV on immune responses have focussed on the induction of CH responses to a range of allergens in mice (reviewed by Gallo *et al.*, 1990) and recently similar experiments have been performed in man (Cooper *et al.*, 1992; Yoshikawa *et al.*, 1990).

Some reports have indicated differences between high dose and low dose models of UV-induced immunosuppression, showing that two different mechanisms of suppression may occur depending on dose, and wavelength of UV (reviewed by Cruz and Bergstresser, 1992; Kripke, 1991), as outlined in table 1.3 (taken from Cruz and Bergstresser, 1992).

However Noonan and De Fabo (1990) reported that the dose-response curves for local and systemic immunosuppression were identical, although the time course of generation of local and systemic suppression were different. Thus the effects of high dose and low dose may be very similar with the local effects of low dose irradiation requiring more time to become apparent systemically.

To confuse the issue further, experiments have also been conducted in mice to

determine whether low-dose UVB treatment has a similar suppressive effect on the expression of immunity. When the skin of hapten immune mice were exposed to a low dose of UVB and then challenged with the hapten the expression of the CH response was actually enhanced, compared with non-irradiated controls (Polla *et al.*, 1986; Yoshikawa *et al.*, 1992).

Table 1.3

Comparison of Low- and High-dose Models of UV-induced Immunosuppression.

	LOW-DOSE UVB	**HIGH-DOSE UVB**
Irradiance	$< 5 \times 10^3$ J m^{-2}	$> 10^5$ J m^{-2}
Location of unresponsiveness to hapten	Only in irradiated skin	Both irradiated skin and distant unirradiated sites
Action spectrum for peak unresponsiveness	< 300 nm	320 nm
Generation of hapten-specific T_S cells	Present	Present
Demonstrated impairment in antigen-presenting cells	Epidermal LC	Splenic macrophage
Implicated sources or mediators of immunosuppression	Irradiated LC Thy-1$^+$ EC I-J, Ia+ EC T6$^-$DR$^+$ cells	*cis*-UCA Prostaglandins IL-1 KC derived mediator(s)

T_S: suppressor T lymphocytes

Few reports have examined the effect of UV exposure on the outcome of microbial infection, but what information is available indicates that suppressed immunity is induced to a range of organisms including protozoa, yeast, bacteria and viruses as outlined below.

Leishmania is a parasitic protozon. Ulcerating nodular skin lesions may occur and in mucocutaneous leishmaniasis there maybe severe and mutilating. In humans these

lesions appear more commonly on UV-exposed regions of the body. Using a murine model, Giannini found that UV irradiation resulted in impaired immunity to the protozoa (Giannini, 1986). The severity of the lesion induced by *Leishmania* was significantly reduced in mice exposed to UVB, but there was no effect on dissemination of the organism to other organs of the body. There was a significant suppression in the DH response to the organism in irradiated mice, and no protection was induced against a second inoculation at a distant site.

Candida albicans is a commensal yeast of the oral cavity, gut, skin and vagina which can cause opportunistic infections at these sites. In a murine model of infection UV irradiation markedly suppressed the DH response (Denkins *et al.*, 1989).

Mycobacteria cause diseases such as leprosy and tuberculosis. BALB/c and C3H mice exposed to UVB either before or during infection with *Mycobacterium bovis* had significantly reduced DH responses to antigen challenge (with BCG) when the mice had been infected at a site distant from the UV exposure compared with unirradiated controls (Jeevan and Kripke 1989). The total number of bacteria isolated from the spleen and LN was significantly higher in the UV-irradiated animals, which demonstrated systemic suppression. In addition supernatants from KC which had been UV irradiated *in vitro* suppressed the DH response to BCG when injected intravenously into mice prior to infection (Jeevan *et al.*, 1992b).

Further studies by this group examined the effect of low dose UVB prior to infection at the site of irradiation with *Candida albicans*, BCG or *Schistosoma mansoni* in mice. The induction of DH responses to *C. albicans* and BCG were unaffected by UVR, but the number of viable organisms recovered from the lymphoid organs of UVR mice infected with BCG was significantly enhanced relative to unirradiated controls. UVR had no effect in mice infected with *S. mansoni* (Jeevan *et al.*, 1992a).

It was suggested that the involvement of the epidermal LC as the primary APC in the induction of immunity may be critical in determining whether the immune response will be affected by local UVR. Preliminary experiments by Chung *et al.*, (1988) showed that UV-irradiated mice injected intravenously with *Staphylococcus aureus, Escherichia coli* and *Candida albicans* had a suppressed ability to clear the organisms in comparison with unirradiated controls.

UVR also has important implications for the outcome of viral infections. Thus it has been proposed that UVB may enhance immunosuppression in individuals with human immunodeficiency virus (HIV). It has also been shown that UVB can activate HIV viral replication (Zamudzka and Beer, 1990), and also that HIV infects epidermal LC (Zambruno *et al.*, 1991). Therefore HIV infected patients may be at considerable risk from the deleterious effects of UVR (Flegg, 1990). This theory has recently been substantiated in a murine model of infection with murine leukemia virus, which induces an immunodeficiency syndrome (MAIDS) which resembles the early stages of human AIDS. *In vivo* irradiation of mice infected with the virus resulted in more pronounced changes in immune responses due to MAIDS infection, including IgM levels, mitogenic responses to PHA (phytohaemagglutinin,) Con A and LPS (lypopolysacharide), mixed lymphocyte reactions (MLR), antigenic responses to sheep red blood cells and histopathologic changes in the spleen (Brozek *et al.*, 1992).

UV exposure is a common triggering factor for the reactivation of latent HSV-1, resulting clinically in a "cold sore" (Perna *et al.*, 1987; Spruance 1985; Vestey *et al.*, 1989). Previously in this laboratory a murine model of HSV infection had been employed to study the effects of UVB on primary immune responses to the virus. Mice were shaved dorsally and irradiated with a single low dose (96 mJ cm^{-2}) of UVB prior to infection with the virus, either epidermally (see Fig 1.1) or subcutaneously. Eight to ten days later the mice were challenged with inactivated

HSV intradermally and the DH responses measured. The UVB irradiated group displayed a 60-90% suppression of DH which was antigen specific (Howie *et al.*, 1986 (a)) if the mice were infected between two and fourteen days after irradiation. Spleen cells from UV-irradiated mice which had been infected with HSV shortly after irradiation, were transferred to syngeneic mice which were already infected with HSV. This resulted in an antigen specific suppression of the DH response when the recipients were challenged with HSV antigen. The cells transferring the suppression were T lymphocytes of two phenotypes, $Ly1^+2^-$ ($CD4^+$) and $Ly1^-2^+$ ($CD8^+$) (Howie *et al.*, 1986a). The suppression could also be transferred by UV-irradiated epidermal cells, but these had to be injected at the same time and location as the virus, suggesting that a local interaction between the two had to take place for suppression to be induced. In this instance T-cells were generated which mediated the suppression, but these were of one phenotype only; $Ly1^+2^-$ $L3T4^+$ $Thy1^+$ (Howie *et al.*, 1987). Epidermal cells derived from mice which had been irradiated three days previously had an impaired capacity to present HSV antigen (Howie *et al.*, 1986b).

Although UV-induced immunosuppression of CH and DH responses appear to be very similar the dose responses are different (Kim *et al.*, 1990). Much higher doses of UV are required to suppress CH responses, which also has a different wavelength dependency from DH (discussed in section below) suggesting that the mechanism may be different.

1.3.4. The Mechanism(s) of UV-induced Immunosuppression

The Effect of UVR on LC and Antigen Presentation

Low doses of UVB were shown to reduce the number and alter the morphology of epidermal LC (Elmets *et al.*, 1983; Towes *et al.*, 1980). This may be due to an altered expression of MHC II or enzyme ATPase activity, destruction of the LC or

migration of LC from the epidermis. This is a controversial issue as some authors have reported a reduction in the expression of MHC II (Aberer *et al.*, 1981) while others have reported no alteration, or possibly enhanced expression (Czernielewski *et al.*, 1984; Norlund *et al.*, 1981). A recent report (Spencer *et al.*, in press) examined the number of LC and the expression of MHC class II in ultra-thin sections through MHC II^+ epidermal cells by electron microscopy before and after a standard course of UVB phototherapy. This revealed a reduction in the number of MHC II^+ cells but those which remained exhibited a significantly increased expression of MHC class II. No MHC class II negative LC were found following UVR. This observation ties in well with the findings of Ashworth *et al.* (1989) who reported that PUVA therapy depleted the number of LC in the epidermis. The residual class II positive cells retained normal HLA-DR and CD1a expression, and on a per cell basis *in vitro* alloantigen expression was unaltered. Following UVB irradiation of mice an increased number of DC are found in the DLN, suggesting that UVB induced DC migration from the epidermis (Moodycliffe *et al.*, 1992).

In 1980 Toews demonstrated that C57BL mice exposed to low doses of UVB (< 200 J m^{-2} a day for four days) failed to mount a CH response to DNFB (dinitrofluorobezene) when it was applied to the site of irradiation. This was accompanied by a reduction of epidermal $ATPase^+$ cells and loss of dendritic morphology in those which remained. Further experiments revealed that antigen specific suppressor inducer cells were present in the spleen of the irradiated mice (Elmets *et al.*, 1983), suggesting that altered APC function was responsible for activating specific suppressor pathways. Following application of FITC (fluorescien isothiocyanate) to murine skin, FITC-bearing epidermal LC leave the epidermis and collect in the DLN, where they interact with T cells to initiate the CH response (Kripke *et al.*, 1990). Normally, the DLN cells induce CH responses when injected into the footpad of a syngeneic mouse (Kripke *et al.*, 1990) but following UV-irradiation they failed to induce CH, despite FITC-bearing cells being present.

Instead, suppresssor T lymphocytes were induced in the recipients (Okamoto and Kripke, 1987). FACS (fluorescence activated cell sorter) purified, hapten derivatised LC irradiated *in vitro* with a single dose of UVB (200 J m^{-2}) failed to induce contact allergic responses when injected intravenously into syngeneic mice. In addition these mice failed to mount a CH response when sensitized at a later time with the same hapten (Cruz *et al.*, 1989). This indicated that UVB did not prevent antigen presentation but induced tolerance. Further experiments (reviewed by Simon *et al.*, 1992) demonstrated that UVB irradiation of purified hapten derivatised LC reversed the normal capacity of these cells to preferentially activate Th1 rather than Th2 cell clones *in vitro*. The Th1 cells in these experiments were tolerant to subsequent stimulation with unirradiated hapten derivatised LC. They retained the capacity to proliferate in response to exogenous IL-2, indicating that they were not physically deleted, but were functionally anergic.

Simon *et al.*, (1991) had prevously reported that mouse Th1, but not Th2, clones required an ICAM-1-mediated activation signal from LC for optimal proliferation. UVB depresses the expression of ICAM-1 on human (Krutmann *et al.*, 1990) and murine (Tang and Udey, 1991) LC, and therefore it is possible that ICAM-1 serves as a UVB-sensitive accessory factor for Th1 cells alone.

On *in vitro* UV irradiation human or murine epidermal cell populations containing LC, lose their ability to act as APC to T cells in a lymphoproliferation assay using a range of antigens (Austad and Braathan, 1985; Czernielewski *et al.*, 1984; Stingl *et al.*, 1981). Irradiated epidermal cells exhibit suppressed alloactivation properties (Cooper *et al.*, 1985). There is also evidence in humans that following irradiation the epidermis is re-populated with $T6^{-}$ Dr^{+} ($OKM5^{+}$) cells which preferentially activate suppressor inducer T cells (Baadsgaard *et al.*, 1990b).

Altered APC capacity may not account for all the effects of UVR on the immune system, particularly in the case of systemic suppression when UV irradiation and

antigen sensitization are undertaken at different sites.

The Effect of UVR on Keratinocytes

KC are the major cytokine producing cells of the epidermis, and therefore UVB induced changes of these cells may play an important role in alteration of cutaneous immune responses. UVB irradiation of human KC both *in vitro* and *in vivo* increases the synthesis of IL-1 (Kupper *et al.*, 1987; Murphy *et al.*, 1989; respectively) which can suppress CH responses in mice (Robertson *et al.*, 1987). Murine KC irradiated *in vitro* also produce a factor which blocks the activity of IL-1 (contra-IL-1; Schwarz *et al.*, 1987) and this factor is also found in the serum of mice following *in vivo* irradiation (Schwarz *et al.*, 1988). Other soluble factors are released by KC following UVR which are capable of suppressing DH responses (Kim *et al.*, 1990; Schwarz, 1986b). UVA irradiation of KC released factors which were capable of suppressing CH responses but not DH responses, but UVB induced factors were capable of suppressing DH but not CH responses (Kim *et al.*, 1990), indicating that CH and DH responses may be different. UV-irradiation of epidermal cells resulted in increased TNF-α production *in vitro* (Köck *et al.*, 1990) and *in vivo* (Oxenholm *et al.*, 1987). Following UVB, but not UVA irradiation, local production of IL-6 is increased (Kirnbauer *et al.*, 1991), and correspondingly increased IL-6 has been detected in serum from UVB irradiated individuals (Urbanski *et al.*, 1990), which may play an important role in the systemic sunburn reaction.

The Effect of UVR on Circulation of Lymphocytes

In mice, UV irradiation on six consecutive days induced an alteration in lymphocyte distribution in the regional LN (Spangrude *et al.*, 1983). The number of radio-labelled lymphocytes that migrate to the peripheral LN in UV exposed mice was increased compared with unirradiated controls, and this effect persisted for two months. The prolonged retention of lymphocytes in the LN is possibly due to PG,

which are released on UV irradiation, as PG can induce efferent lymphatic blockage (Chung *et al.*, 1986). Another possibilty for the prolonged retention of lymphocytes could be that UVB alters the morphology of high endothelial venule structures, which play a pivotal role in lymphocyte homing. UVB irradiation may also alter the expression of adhesion molecules such as ICAM-1, which may upset the balance of cell recirculation (Norris *et al.*, 1990; Tang and Udey, 1991).

The effect of UVR on circulating cells of the blood in man is a controversial issue and it is possible that any detected changes are transient. Thus, some authors reported a temporary decrease in circulating T cells after UVB irradiation or PUVA therapy (Cormane *et al.*, 1977; Haftek et al., 1979; Morison *et al.*, 1979a; Rivers *et al.*, 1989), but others found no modulation following a variety of UV regimens (Harper *et al.*, 1979; Hersey *et al.*, 1988; Morison *et al.*, 1979b; Mutzas *et al.*, 1991). Differences in the source, wavelengths, duration or dose of UV, as well as the time of sampling may explain these apparently conflicting data.

The Effect of UVR on Mast Cells

Low dose UVB has been shown to result in suppression of mast cell degranulation (Danno *et al.*, 1986). In contrast, high dose UVB irradiation induces mast cell degranulation (Danno *et al.*, 1986). Mast cells are located in the dermis of normal skin and UVB is thought to penetrate the skin poorly. It is therefore likely that the effects of UVB are mediated through the release of soluble factors in the epidermis, such as IL-1, PG and IL-3. Recently the effect of UV-irradiation on LC in mast cell deficient mice was studied (Ikai and Kanauchi, 1991). Mast cell deficient mice had a reduced number of LC compared with normal mice but on UV-irradiation they exhibited the same reduction in LC ($ATPase^+$ cells) as normal controls, although no measurement of immunity was made.

The Role of Photo-receptors in UV-Induced Immunosuppression

UVB penetrates the skin poorly and therefore it mediates at least part of its action on the immune system through specific photo-receptors in the skin. A number of constituents of the epidermis absorb UV-light and have been proposed as candidates including DNA, membrane associated compounds such as arachidonic acid (Kaleta *et al.*, 1988), tryptophan and UCA (urocanic acid). TNF-α is also thought to play an important role in determining UV-induced immunosuppression.

UV irradiation results in dimer formation between adjacent pyrimidine base pairs which may play an important role in erythema. The role of of dimer formation in UV-induced immunosuppression was tested by using enzymes which repair pyrimidine dimer damage to DNA, such as T4 endonuclease, which were incorporated into liposomes. They were found to increase the repair of UV-induced pyrimidine dimers in murine skin (Kripke *et al.*, 1991). Application of T4 endonuclease liposomes immediately following UV exposure restored a 70% suppression of the DH response to almost normal values by this group.

A further possibility is a membrane asociated molecule which may be activated following UV to release cytokines or other immunomodulatory compounds. An example may be the release of arachidonic acid from membrane phospholipids (Kaleta *et al.*, 1991) which is a precursor for prostaglandins, which are important inflammatory mediators.

Inbred strains of mice display varying genetic susceptibility to the immunomodulatory effects UV-irradiation (Streilein and Bergstresser, 1988), and it is possible that genetically determined UV-susceptibility may occur in humans also (Yoshikawa *et al.*, 1990). In mice the trait appears to be restricted to polmorphisms at the LPS and TNF-α loci (Yoshikawa and Streilein, 1990). Thus TNF-α is increased on UV-irradiation in UV-sensitive strains. Intradermal injection of TNF-α

at the site of hapten application mimics the effects of UVB irradiation by impairing the induction of CH responses and altering the numbers and morphology of MHC II positive cells (Vreemer and Streilein, 1990; Yoshikawa and Streilein, 1990). As UVB irradiation enhances DC migration to the DLN (Modycliffe *et al.*, 1992), and TNF-α increases the migration of epidermal LC to the DLN (Cumberbatch and Kimber, 1992) it is possible that increased TNF-α on UV-irradiation induces DC migration to the DLN, which may result in altered antigen presentation.

A particularly interesting candidate as an important photo-receptor of UV-induced immunosuppression is UCA, and this is considered in detail in the following section.

1.4 UROCANIC ACID

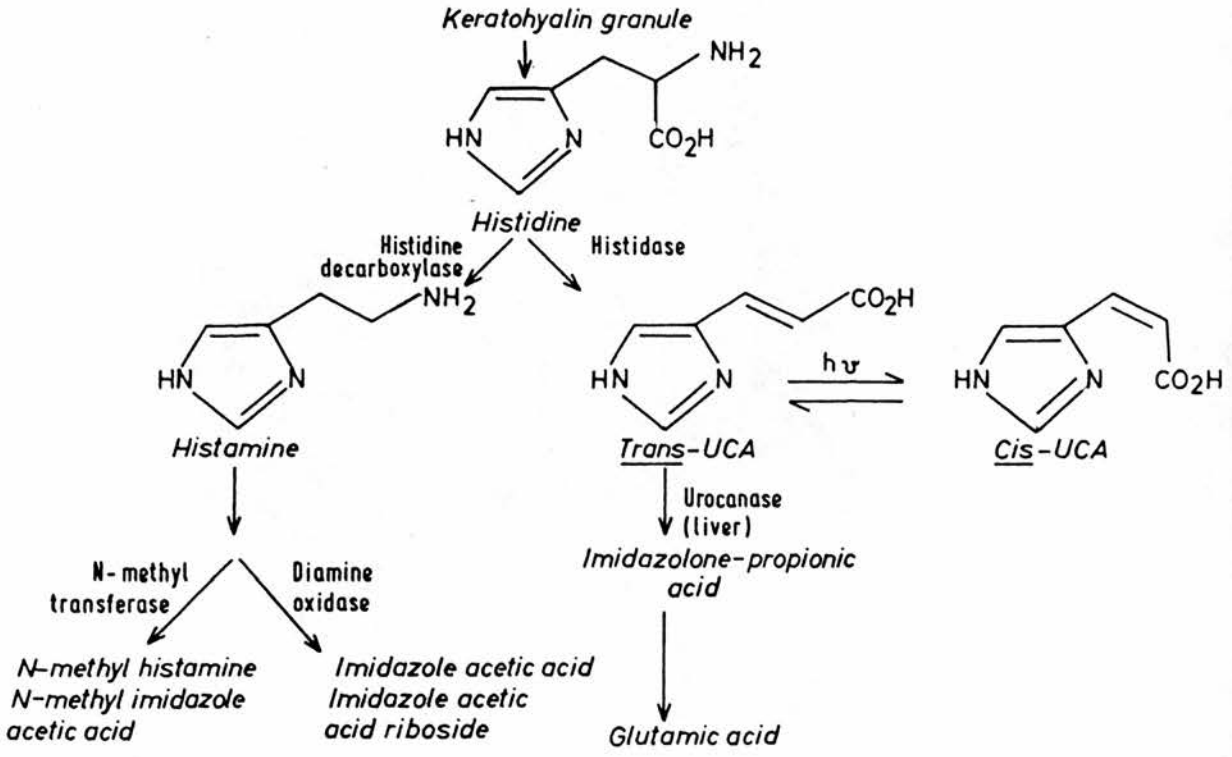
1.4.1 Location and Biochemistry

UCA (deaminated histidine, 4-imidazoleacrylic acid) was discovered in 1874 in the urine of dogs (Jaffe). It is a major component of the epidermis, consisting of approximately 0.6% of the total dry weight (Tabachnick, 1957). It is formed from histidine in a one step deamination by the enzyme histidase (histidine-ammonia lyase) during the process of keratinization, when histidine rich proteins are released from the keratohyalin granule (Baden and Pathak, 1967). Histidase is synthesised in the stratum spinosum and granulosum but is only active in the stratum corneum, but the mechanism of enzyme activation is unknown (Scott, 1981). The plasma membrane of keratinized cells is highly impermeable, and urocanase is not found in the epidermis, therefore UCA accumulates in the superficial layers of the stratum corneum (Baden and Pathak, 1967; Zannoni and La Du, 1963). Six to twelve times as much histidine is synthesized into UCA in the epidermis compared with protein (Baden and Pathak, 1967; Reavan and Cox, 1965). Histidase activity is also found in the liver but in that organ UCA is further metabolised by the enzyme urocanase to imadazoalone proprionic acid and finally to glutamic acid which is excreted in the urine. It is of interest that urocanase only metabolises the trans-isomer but not the *cis*-isomer. The metabolism of histidine is outline in Fig 1.3 (taken from Norval *et al.*, 1989c).

Serial tape stripping of normal human epidermis down to the stratum granulosum, followed by alkali extraction and analysis for UCA by HPLC (high pressure liquid chromatography) revealed that the greatest amounts of UCA were situated between the third and seventh strip, out of a total of 14 consecutive strips, the median value being 4 $\mu g\ cm^{-2}$ (Schwartz *et al.*, 1986a). Similar results were obtained by Norval *et al.*, 1989a) UCA is soluble and it is possible that the UCA of the extreme superficial layers is lost in sweat.

Figure 1.3

The metabolic Pathway of Histidine, Histamine and Urocanic Acid



1.4.2 The Photobiology of UCA

UCA is a major absorber of UV light in the epidermis. On UVR the naturally occurring *trans*-isomer undergoes a UV-dependent isomerization to form the *cis*-isomer (illustrated in fig1.3). While the absorption maximum is 264 nm *in vitro* the quantum efficiency of isomerization is maximal at 313 nm (Morrison *et al.*, 1984). UVA irradiation *in vivo* results in isomerization of UCA (Schwarz *et al* ., 1987; Rücker *et al.*, 1991), but Shwartz *et al* also reported that the same dose of UVA *in vitro* did not. They proposed that a unknown photosensitizing agents produced *in vivo* may have been responsible for the isomerization, a theory also recently proposed by De Fabo *et al.* (1991). However it has been demonstrated that *in vitro* PUVA treatment of a solution of *trans*-UCA resulted in isomerization (Gruner *et al.*, 1992), although the possibility exists that the psoralen acts as a photosensitizing agent. Others have found that *in vitro* UVA irradiation of *trans*-UCA in the presence of psoralens had little or a slightly protective effect on the isomerization (H. Morrison personal communication; our unpublished observation). More recently, using monochromatic light it has been demonstrated that isomerization of UCA *in vitro* takes place up to 320 nm (Gibbs *et al.*, submitted for publication). This was also true when mice were irradiated *in vivo* (Gibbs *et al.*, submitted for publication).

UVB irradiation of human forearm followed immediately by sequential tape stripping revealed that the highest percentage of *cis*-UCA was found in strips 1-8 (Norval *et al.*, 1989a). The relative percentage of *cis*-UCA produced was dependent on the dose of UVB employed in the study eg 6 mJ cm^{-2}, 12 mJ cm^{-2} or 24 mJ cm^{-2} resulted in production of 20-39 %, 35-50 % and 35-60 % *cis*-UCA respectively. A number of reports indicate that *cis*-UCA may not be confined to the epidermis. Radiolabelled *trans*-UCA was applied to mouse epidermis. Two hours after irradiation ^{14}C was detected in the serum and spleen, but at six days it was no longer detectable in the serum with reduced quantities remaining in the spleen (Reeve *et al.*,

1991). A monoclonal antibody which recognises *cis*-UCA but not *trans*-UCA was employed in a competitive inhibition ELISA to detect *cis*-UCA in the serum of mice following UVB (A. Moodycliffe, personal comunication). In addition *cis*-UCA has been detected in human urine following whole body UVB (Prof. J D Bos, personal communication) and also in suction blister fluid, which is thought to be mainly derived from the dermis (Pasanen *et al.*, 1990).

UCA was proposed to be a natural sun screen because of it's UV-absorbing properties (reviewed by Morrison, 1985). Topical application of UCA in three subjects did afford modest protection against sunburn (Baden and Pathak, 1967). A number of commercially available sunscreens containing UCA were recently shown to form *cis*-UCA on UVR *in vitro* (Reeve and Mitchell, 1991). When the irradiated lotion or the lotion plus a normally sub-immunosuppressive dose of UVB were given to hairless mice a 68% and 56% suppression in the CH response (respectively) was measured. This demonstrates the potentially hazardous nature of UCA as a constituent of a sun screen lotion. The absorption spectrum of UCA overlaps the photomutagenesis action spectrum considerably which suggests that it may be a more efficient photoprotecting agent against the UV damage to DNA than that of erythema (Morrison 1985).

1.4.3 UCA and Immunosuppression

In 1983 De Fabo and Noonan published an action spectrum of UV-induced immunosuppression of CH response in BALB/c mice using 10 different wavelengths of narrow band UV-light of 5-15 nm intervals. This spectrum resembled the UV-absorption spectrum of *trans*-UCA very closely, which led to the proposal that *trans*-UCA was the photoreceptor involved in UV-induced immunosuppression (De Fabo and Noonan, 1983a). Following this report experiments in histidenaemic mice, which had a mutant histidase gene, and formed less than 10% of the normal quantity of UCA, revealed that these mice were resistant to UV-induced immunosuppression (De Fabo *et al.*, 1983b). Conversely mice fed on a histidine rich diet, and therefore with an enhanced epidermal UCA content were more sensitive to the suppressive affects of UV on CH responses (Reilly and De Fabo, 1991). There is now a large body of experimental evidence which indicates that *cis*-UCA alone is capable of mimicking many of the effects of UV-induced immunosuppression (reviewed Noonan and De Fabo, 1992; Norval *et al.*, 1989a).

Previously the immunosuppressive effects of low dose UV on the DH response in a murine model of HSV infection had been characterized (Howie *et al.*, 1986 a; Howie *et al.*, 1986b; Howie *et al.*, 1987; discussed in section 1.3.3). Subsequently the effects of applying UV-irradiated UCA were found to mimic the effects of UVR exactly. Application of a mixture of UCA isomers to the epidermis or injected subcutaneously, followed by infection with HSV five hours or three days later resulted in a dose dependent suppression of DH response when the mice were challenged with inactivated HSV antigen eight to ten days later (Ross *et al.*, 1986). Using purified *cis*-UCA a significant suppression of the DH response to the virus resulted at doses as low as 1 μg of *cis*-UCA per mouse. The suppression was mediated by the induction of antigen specific T cells of two phenotypes, $L3T4^+$ $Ly2^-$ and $L3T4^-$ $Ly2^+$ (Ross *et al.*, 1987). Mice were treated epidermally

with a mixture of UCA isomers and 24 h later epidermal cells from the treated site were transferred to naive syngeneic mice at the same time and place as infection with HSV. A suppressed DH response resulted on challenge with the virus, which was again found to be antigen specific but was due to the induction of T cells of only one phenotype, $L3T4^+$ $Ly2^-$ (Ross *et al.*, 1987/88). This suggested that two signals are possibly generated by *cis*-UCA, one locally inducing the $L3T4^+$ $Ly2^-$ phenotype and one systemically inducing the $L3T4^-$ $Ly2^+$ alone or inducing the $L3T4^+$ $Ly2^-$ also, (as found with UVR; section 1.3.3). A series of UCA analogues were synthesised and their potential to suppress DH carried out (Norval *et al.*, 1989b).

A number of other groups have reported that *cis*-UCA suppresses CH responses in mice (Harriott-Smith and Halliday, 1988; Reeve *et al.*, 1989). In a similar manner to UVR, *cis*-UCA has been reported to produce soluble suppressive factors in the serum of mice (Harriot-Smith and Halliday., 1988). Treatment of chronically UV-irradiated mice with UCA enhanced UV-induced tumour yield per mouse, and a greater number were aggressive squamous tumours (Reeve *et al.*, 1989). UCA forms photoadducts with DNA *in vitro* (reviewed Morrison, 1985) which may play a role in this process, but it is more likely that UCA suppresses immune responses to tumour antigens generated in the skin following UVR. Further experiments by Reeve *et al* revealed that mice treated with cimetidine, a H2 receptor antagonist, had a reduced number of UV-induced tumours suggesting that a histamine receptor may be involved in the process (Matheson and Reeve, 1991).

It has previously been shown that UVB or PUVA treatment of a recipient prolonged the graft survival (Pamphilon *et al.*, 1991). *Cis*-UCA prolonged the survival of allogeneic MHC disparate skin grafts in mice in a dose dependent manner (Gruner *et al.*, 1992). In 30 % of cases treatment with *cis*-UCA, but not *trans*-, prevented the onset of acute lethal graft-versus-host disease and delayed it in others (Gruner *et al.*, 1992). Likewise permanent acceptance of heart allografts in rats (Oesterwitz *et al.*,

1990), enhanced survival of rabbit corneal transplants (Williams *et al.*, 1990) and prolonged survival of heterotopic fetal mouse skin or pancreas were obtained in animals which were treated systemically with *cis*-UCA for seven days after transplantation (Guymer *et al.*, 1990).

Antigen presentation is a pivotal event in the induction of CH, or DH to viruses or tumour antigens. *Cis*-UCA, but not *trans*-UCA, has been shown to affect the morphology, phenotype and function of APC in murine models. Culturing epidermal sheets in the presence of *cis*-UCA for three days resulted in a reduction in Ia^+ cells but had no effect on the number of $ATPase^+$ (Noonan *et al.*, 1985). Treatment with *cis*-UCA, but not *trans- in vivo* resulted in the reduction of Ia^+ cells at the site of application but no effect on the number of $I\text{-}J^+$ cells was observed (Ross *et al.*, 1987/88). The morphology of the cells which remained was exactly the same as that after UVR i.e. the dendrites had retracted and the network of cells was no longer apparent (Kurimoto and Streilein, 1992). Human epidermal cells from suction blister roofs were also found to have reduced expression of HLA-DR following a three day incubation *in vitro* with *cis*-UCA (Räsänen *et al.*, 1987).

Functionally, human epidermal cells and peripheral blood monocytes were also found to have a reduced ability to produce IL-1 in addition to a reduction in HLA-DR expression following incubation with *cis*-UCA (Räsänen *et al.*, 1987; Räsänen *et al.*, 1989). UVR is associated with a reduced antigen presenting function by splenic DC (Noonan *et al.*, 1988), similarly *cis*-UCA injected intravenously resulted in an impaired ability of purified splenic dendritic cells to present antigen in an *in vitro* lymphoproliferation assay seven days after injection (Noonan *et al.*, 1988). However simply adding *cis*-UCA to proliferation assays had no effect on the uptake of ^3H-thymidine (Noonan *et al.*, 1988; Higaki *et al.*, 1986). The mechanism of the APC defect remains to be elucidated.

Histamine and *trans*-UCA induce the production of cAMP (adenosine3', 5'-cyclic

monophosphate) by dermal fibroblasts *in vitro* which *cis*-UCA down regulates in a dose dependent manner (Palaszynski *et al.*, 1992). This may indicate a role for the dermal fibrobast in UV-induced immunosuppression, but more importantly it indicates that *cis*-UCA may be capable of modulating second messenger systems which play a pivotal role in cell signaling.

1.5 Aims of Thesis

Previously in this laboratory pre-treatment with *cis*-UCA had been shown to result in suppression of DH responses to HSV in a murine model of infection (Ross *et al.*, 1986; Ross *et al.*, 1987) in a very similar manner to UVB irradiation. Histamine, which is linked metabolically, and is structurally very similar to UCA (Fig 1.3) was also found to result in suppression of the DH to HSV (Norval *et al.*, 1989b). Therefore, *cis*-UCA may have induced suppression of the response through histamine-like receptors, which are found on cells of the immune system (reviewed Falus and Meretey, 1992; Hill, 1990). A primary aim of the present study was to confirm this hypothesis by investigating the effect of known histamine receptor antagonists on *cis*-UCA induced modulation of immune responses. The results are outlined in Chapter 3. In addition, CH responses had previously been shown to be biphasic, with an early response two hours following antigen challenge in immune mice, which was subsequently followed by the classical CH response 24-48 h later (reviewed by Askenase *et al.*, 1991). It was aimed in the present study to determine whether the elicitation phase of DH to HSV in mice followed similar kinetics. If so, it was aimed to establish what effect pre-treatment with UV or UCA isomers had on phases of the elicitation of the response. The results from this study may be found in Chapter 4.

As discussed in the Introduction UVB results in profound changes in epidermal LC and altered antigen presentation. It was therefore of interest to establish what effect *in vivo* or *in vitro* treatment with UCA isomers had on the ability of murine APC to present HSV antigen or Con A to T cells in an *in vitro* lymphoproliferation assay. These experiments are outlined in Chapter 5.

Phototherapy is commonly employed in the management of a number of dermatoses. Its use is largely empirical, and little is known of its mechanism of action. It is

possible that it may act, at least in part, by modulation of immune responses. Most previous investigations of the immunological effects of UV irradiation have been conducted either in experimental animals or in humans using regimens bearing little resemblance to those used therapeutically. It was aimed in the present study to examine the effects of standard courses of UVB and PUVA therapy on various parameters of systemic and cutaneous immunity in patients with psoriasis. The results were compared with two control groups: the first consisted of normal subjects who were given a standard course of UVB phototherapy and, the second, patients with psoriasis who received topical coal tar paste treatment instead of UV irradiation. Assays included several humoral components, phenotypic profiles and counts of peripheral blood mononuclear cells (PBMC), UCA isomers and TNF-α analyses, and *in vitro* lymphoproliferative responses (LPR) to Con A and HSV. The results are given in Chapter 6, and in Chapter 7 studies on the modulation of natural killer cell (NK) activity are reported.

CHAPTER 2

MATERIALS AND METHODS

2.1 GENERAL MATERIALS AND METHODS COMMON TO MURINE AND HUMAN STUDIES

2.1.1 HSV Culture

The virus used to infect mice and also as an inactivated antigen, used in lymphoproliferation and enzyme-linked immunosorbent assays (ELISA), was an isolate of HSV-1 from a clinical case, which was plaque-purified in Vero cells. Vero cells were cultured in Eagle's minimal essential medium pH 7.4 (Northumbria Biologicals Limited, UK) supplemented with 5% new born calf serum, 2 mM L-glutamine, 100 IU ml^{-1} penicillin and 200 $\mu g\ ml^{-1}$ streptomycin. Plaque assays for infectious virus were performed using monolayers of Vero cells in microtitre plates with 0.25% Seaplaque agarose (FMC Corporation, USA) in the overlay. After 2-3 days incubation at 37°C the cells were fixed in formol saline, stained with Giemsa, and the plaques counted under the low power of a light microscope. The virus was stored at -70°C in small aliquots for upto one year, after which the viability of the virus diminished. Three different batches of HSV were used through out the study which varied from 4×10^7 to 10^8 plaque forming units (pfu) ml^{-1}.

2.1.2 Preparation of HSV Antigens

UV-inactivated HSV-1 was prepared from stock HSV-1 by irradiation of a thin film of sonicated virus with 300 mJ cm^{-2} UVB under 2 Philips TL 20W/12 lamps. UV-inactivated antigen was used in proliferation assays and DH experiments. The same batch of HSV antigen was used for all assays for a particular individual in Chapter 6. Mock infected sonicated Vero cells were used as the control antigen.

The antigen used for the HSV ELISA were glycine extracted antigens. Monolayers of Vero cells in Roux flasks were infected with HSV at a multiplicity of 2-3 pfu per cell and incubated at 37°C in 4% CO_2 in a humidified incubator. The cells were

harvested 24 h post infection with gentle agitation with glass beads. Glycine extracts of the infected cells were prepared using the method of Booth *et al.*, (1979). Briefly, cells were suspended in buffer prepared from five parts 0.1 M aqueous solution of glycine and three parts 0.1 M aqueous sodium hydroxide. The cells were disrupted by three freeze/thaw cycles and ultrasonicated for one minute and vigorously agitated between each cycle. Uninfected Vero cells were treated in exactly the same manner and used as control antigens. The protein concentration of the extracts were determined by the Lowry method (Lowery *et al.*, 1951). The extracts were stored at -70°C in small aliquots.

2.1.3 UCA and Histamine Receptor Antagonists and Agonists

Trans-UCA was purchased from Sigma UK and contained no detectable *cis*-UCA as measured by HPLC (employing the method outline by Norval *et al.*, 1988). Purified *cis*-UCA was prepared by thin layer chromatography, by J. Crosby (Bristol University) as previously described (Norval *et al.*, 1989). In some initial experiments, both UCA isomers were dissolved in dimethyl sulfoxide (DMSO) at 10 mg ml^{-1} and subsequently diluted in ethanol for skin painting (topical application) or 0.01M phosphate buffered saline pH 7.2 (PBS) for use in all other experiments. In more recent experiments *cis*-UCA was dissolved at 10^{-1} M and *trans*-UCA at 10^{-2} M in PBS respectively.

Cimetidine (H2 receptor antagonist) and terfenadine (H1 receptor antagonist) were obtained from Sigma UK Ltd. They were dissolved at 10 mg ml^{-1} in DMSO. Each was diluted just before use in ethanol for skin painting. Cimetidine was dissolved in PBS for subcutaneous injections or tissue culture. DMSO was used as the diluent for terfenadine when it was injected subcutaneously alone or together with UCA. 4-methylhistamine, a known H2 agonist, was a kind gift from Dr T. G. Payne, Sandoz Pharma Ltd, Basel, Switzerland. This was dissolved at 20 mg ml^{-1} in DMSO and further diluted in PBS. Thioperamide (H3 receptor antagonist) and (R)-α-

methylhistamine (H3 agonist) were gifts from Dr M. Clark, Schering Plough Corporation, New Jersey, USA. Both substances were soluble in PBS at 10 mg ml^{-1}.

2.2 MURINE MATERIALS AND METHODS

2.2.1 Mice

C3Hf Bu/Kam female mice, aged 8-12 weeks, were used throughout. They were bred and maintained in the Department of Medical Microbiology animal house.

2.2.2 UV Irradiation

Mice had their backs shaved and were placed in a perspex box with narrow chambers to prevent it turning around or climbing up towards the UV-source. They were irradiated under a bank of two Philips TL20W/12 UV lamps with a output range of 270-320 nm (the relative output spectrum was very similar to that of the Philips UV 6 lamp illustrated in Fig 2.1). The irradiance was 80 $\mu W\ cm^{-2}$, 10 cm from the source, measured inside the perspex box using an Industrial Research Radiometer (Dr J D Simpson, Radiation Protection Services, Edinburgh). In all experiments the mice were irradiated with a single suberythemal dose of 96 $mJ\ cm^{-2}$. The UVA source employed was a single VL-6L lamp (Viber Lourmat, Marne La Vallée, France) which emits over the range 310-400nm (similar to the UVA source illustrated in Figure 2.1). The output of the lamp, measured using the same meter used to measure the output of the PUVA lamps (2.3.3), was 1 $mW\ cm^{-2}$ at a distance of 9 cm from the source (approximate distance of the back of the mouse from the source). The mice were irradiated with a single dose of 200 $mJ\ cm^{-2}$ of UVA.

2.2.3 ATPase Staining of Epidermal Sheets

For enumeration of LC in murine epidermis, 20 µl volumes containing 20 µg *cis*- or 20 µg *trans*-UCA alone or 20 µg *cis*-UCA with 20 µg of the histamine receptor antagonists were painted on each ear surface of the mice after tape-stripping twice. Control mice received the vehicle alone after tape stripping twice. A further control group was not tape stripped before being painted with the vehicle. The mice were

killed 24 h later, the ears removed, ear surfaces split and epidermal sheets prepared by floating ear surfaces dosal side down in 0.76% tetrasodium ethylenediamine tetra-acetic acid (EDTA) (pH 7.2) at 37°C for two hours (Juhlin and Shelley, 1977). The epidermal sheets were fixed in 1.3% sodium cacodylate/formaldehyde buffer and stained for ATPase using ADP as a substrate (Chaker *et al.*, 1984). One group of mice was irradiated for 20 min under 2 Philips TL20W/12 lamps giving a dose of 96 mJ cm^{-2} of UV in the range 270-350 nm. Epidermal sheets from the dorsal surface only of the ears were prepared 3 days later. The specimens were coded at this stage and examined "blind" microscopically. The number of $ATPase^{+}$ cells was counted in at least 40 fields of view of 0.14 mm^{-2} (using a graticule) chosen randomly for each sheet. The significance of the counts was determined by Student's t-test.

2.2.4 HSV Infection of Mice

Two methods of HSV infection were employed; (a) epidermal or (b) subcutaneous.

(a) The dorsal side of mice were shaved and the shaved area was tape stripped six times, followed by 4×10^4 pfu of HSV in 10 µl being rubbed into the tape stripped site. Approximately two thirds of the mice developed lesions between day four and eight following infection (Fig 1.b). Mice were selected on the basis of a visible clinical lesion, and those which did not develop lesions were discarded. In a few instances mice developed zosteriform lesions which result in hind leg paralysis and these mice were killed.

(b) Mice were infected subcutaneously with 5×10^5 pfu of HSV in 100 µl of PBS.

2.2.5 Lymph Node and Spleen Cell Preparations

After killing the mice with ether inhalation, LN and spleens were removed aseptically and collected into RPMI 1640 (Gibco Ltd., Paisley, UK) supplemented with 5 mM 2-mercaptoethanol, 2 mM L-glutamine, 100 IU ml^{-1} penicillin,

200 μg ml^{-1} streptomycin, 100 μg ml^{-1} gentamicin and 10 μg ml^{-1} fungizone (tissue culture medium, TCM) plus 10% FCS (foetal calf serum) (Gibco Ltd, Paisley, UK). They were disaggregated by grinding them through a fine wire mesh and the cells were washed twice in TCM plus 10% FCS. The cells were finally resuspended at a concentration of 1 x 10^6 ml^{-1} in TCM plus 10% FCS and viable cell counts determined by trypan blue exclusion; usually > 90%. T enriched cells (TEC) were prepared by passage over nylon wool columns (see section 2.3.8).

2.2.6 Preparation of Antigen Presenting Cells and T Enriched Cells

Peritoneal macrophages (PM) were derived by lavage of the peritoneum with 10 mls of PBS per mouse. Viability was determined by trypan blue exclusion and was > 95%.

Epidermal sheets were prepared from mouse ears. The mouse was killed, the ears removed and split into two surfaces. These were floated dermis down in 1% trypsin (Sigma UK Ltd, Poole) in PBS for 45 min at 37°C. The ear surface was then placed in a dish containing TCM supplemented with 10% FCS to stop further action of the trypsin. Using fine forceps the epidermal sheet was pulled off allowing the epidermal cells to float into the medium. The dermis was discarded and the epidermis pulled apart in the medium followed by filtration through fine wire mesh. The single cell suspension was washed twice in TCM and resuspended at 1 x 10^6 cells ml^{-1}.

T enriched cells (TEC) were prepared from spleen cell suspensions in an identical manner to that employed for human peripheral blood mononuclear cells (see section 2.3.8). Nylon wool columns only were used to enrich T cells from LN cell suspensions only (as outlined in 2.3.8)

2.2.7 Lymphoproliferation Assay

The mice were given a primary infection with HSV epidermally. At least two weeks later the mice were inoculated with HSV subcutaneously (section 2.2.4). Eight to ten days following the secondary infection spleens and LN were collected and single cell suspensions prepared (section 2.2.5). Two hundred µl of the cell suspensions or TEC were placed into each well of a 96 well plate of varying dimensions (Falcon, Oxnard, USA). To quintuplicate wells 0, 0.3%, 3% or 10% of either PM or epidermal cells (EC) were added. The plates were either incubated without any further additions, 5 µg ml^{-1} Con A, 1 pfu $cell^{-1}$ of HSV antigen or an equivalent number of mock infected Vero cells (10^2 ml^{-1}). The cells were incubated for five days at 37°C in 5% CO_2 in a humidified incubator, and pulsed with 0.75 µCi ^{3}H-thymidine (Amersham, Little Chalfont, UK) per well over the final 24 h of culture. The cells were then harvested onto glass fibre paper and the uptake of ^{3}H-thymidine measured using a Packard liquid scintillation counter and the results calculated as in section 2.3.12.

2.2.8. The Effect of UCA on DH to HSV.

This protocol has been described previously (Howie *et al.*, 1986) and is illustrated in Fig 3.3. Briefly, 100 µl volumes containing the appropriate concentrations of *cis*-UCA alone, or with the histamine receptor antagonists, were either painted on the shaved backs of the mice after tape-stripping twice or injected subcutaneously into the flank 5 h before subcutaneous infection of the mice with 5 x 10^5 pfu HSV. To act as control groups, mice were injected subcutaneously with the vehicle alone before being infected with HSV or being sensitized with an equivalent number (10^3) of sonicated, uninfected Vero cells. There were normally 8 mice in each group. Ten days later, the thickness of each ear was measured; then the mice were challenged by injecting 10 µl (4-10 x 10^5 pfu) inactivated HSV into each ear pinna

using a 31 gauge needle. Succesful intradermal injections were characterized by the formation of a "blip" which was visible by eye. The ears were re-measured 24 h later and DH calculated as an average ear increase per mouse.

$$\text{Percentage suppression of DH} = 100 - \left(\frac{100 \times \text{net increase experimental mice}}{\text{net increase control mice}} \right)$$

Concentrations of *cis*-UCA used lay within a physiological range (Noonan *et al.*, 1988). The significance of the suppression was calculated by Student's t test.

2.2.9 TNF-α Specific Antibodies

The protocol followed was based on that of Vermeer and Streilein (1991). Polyclonal rabbit anti-mouse serum from hyperimmune New Zealand rabbits immunized with recombinant TNF-α was purchased (NBS Biologicals, Hatfield, Herts, UK). The neutralizing activity was 1×10^6 units ml^{-1}. The serum was preservative free and had been sterilized using a 0.22 μm filter. Mice received 2×10^4 units of anti-TNF in 100 μl intraperitoneally two hours before treatment with *cis*-UCA or a single dose of UVB (96 mJ cm^{-2}). Control mice received 100 μl of PBS intraperitoneally.

2.3 HUMAN MATERIALS AND METHODS

2.3.1 Patients

A total of 44 patients with chronic plaque psoriasis and six with guttate psoriasis were studied. Healthy individuals acted as controls. The subjects were recruited in two cohorts; one for the studies outlined in Chapter 6 and the other for the studies in Chapter 7. Patient details are outlined in Table 6.1 and 7.1 at the begining of these chapters. All subjects were attending the Department of Dermatology, Royal Infirmary of Edinburgh with the exception of group C and D in Chapter 7, who were attending the Photobiology Unit, Ninewells Hospital, Dundee. Normal healthy voluteers were recruited from hospital or university staff in Edinburgh. All subjects were in good general health, taking no other immunosuppressive medication, and were of skin types II or III. Liquid paraffin/white soft parafin (50/50) or emulsifying ointment were used as non-UV absorbing emollients before and during therapy by all psoriatic patients, including those receiving topical coal tar.

The aim of the study described in Chapter 6 was to examine the effect of UVB phototherapy or PUVA therapy on immune function in patients with psoriasis, and the possible role of UCA in any immune modulation. Chapter 7 aimed to examine the effect of various UV-based treatments on NK cell function. Details of the samples taken and the time course of sampling are outlined in Chapter 6 and 7. The UV-treatment regimens employed are outlined below. In both studies psoriatic subjects who were treated with increasing concentrations of coal tar paste (1-4%) under stockinette dressings acted as one control. In addition, where possible normal, healthy individuals also acted as controls.

2.3.2 UVB Therapy

Subjects received the UVB regimen normally employed for psoriasis in Edinburgh. They were irradiated with incremental doses of UVB starting with 37 mJ cm^{-2} in an upright Waldmann 1000 UVB cabinet (Fig 6.2 illustrates a Waldmann upright cabinet used for phototherapy) containing 26 Sylvania tubes, emitting 42% in the UVA waveband, 57.6% in the UVB and approximately 0.3% in the UVC. The relative output spectrum from the lamps is illustrated in Fig 2.1. Irradiance was determined using a filtered photodiode meter (Jagger, 1967) which was calibrated against measurements made with a UV-visible spectroradiometer (model 742, Optronic Lab Inc, UK), and the total output (250-400 nm) was 1.4 mW cm^{-2}. They were treated three times a week until clear (4-6 weeks).

2.3.3 PUVA Therapy (Edinburgh)

Patients in Edinburgh who received PUVA therapy (Chapter 6) were irradiated twice weekly in a Waldmann 6001 PUVA cabinet containing UVA lamps emitting 10.5 mW cm^{-2} UVA and 0.3 mW cm^{-2} UVB. Irradiance was monitored using a Waldmann PUVA meter calibrated at the regional Medical Physics Department, Durham. The relative output of the lamps is illustrated in Fig 2.1. Patients received 0.8 mg kg^{-1} body weight of 8-methoxypsoralen orally 2 h before each irradiation. The minimal phototoxic dose for each patient was determined and, at the first treatment they received 70% of that dose followed by doses increased by 20% at each subsequent treatment, up to a total of 20 treatments. The relative output spectrum of these lamps is illustrated in Fig 2.1.

2.3.4 PUVA Therapy (Dundee)

Patients studied in Chapter 7 received PUVA therapy in Dundee. A Waldmann 6000 cabinet incorporating 62 Sylvania FR74T12 fluorescent lamps (UVB = 0.4 mW cm^{-2},

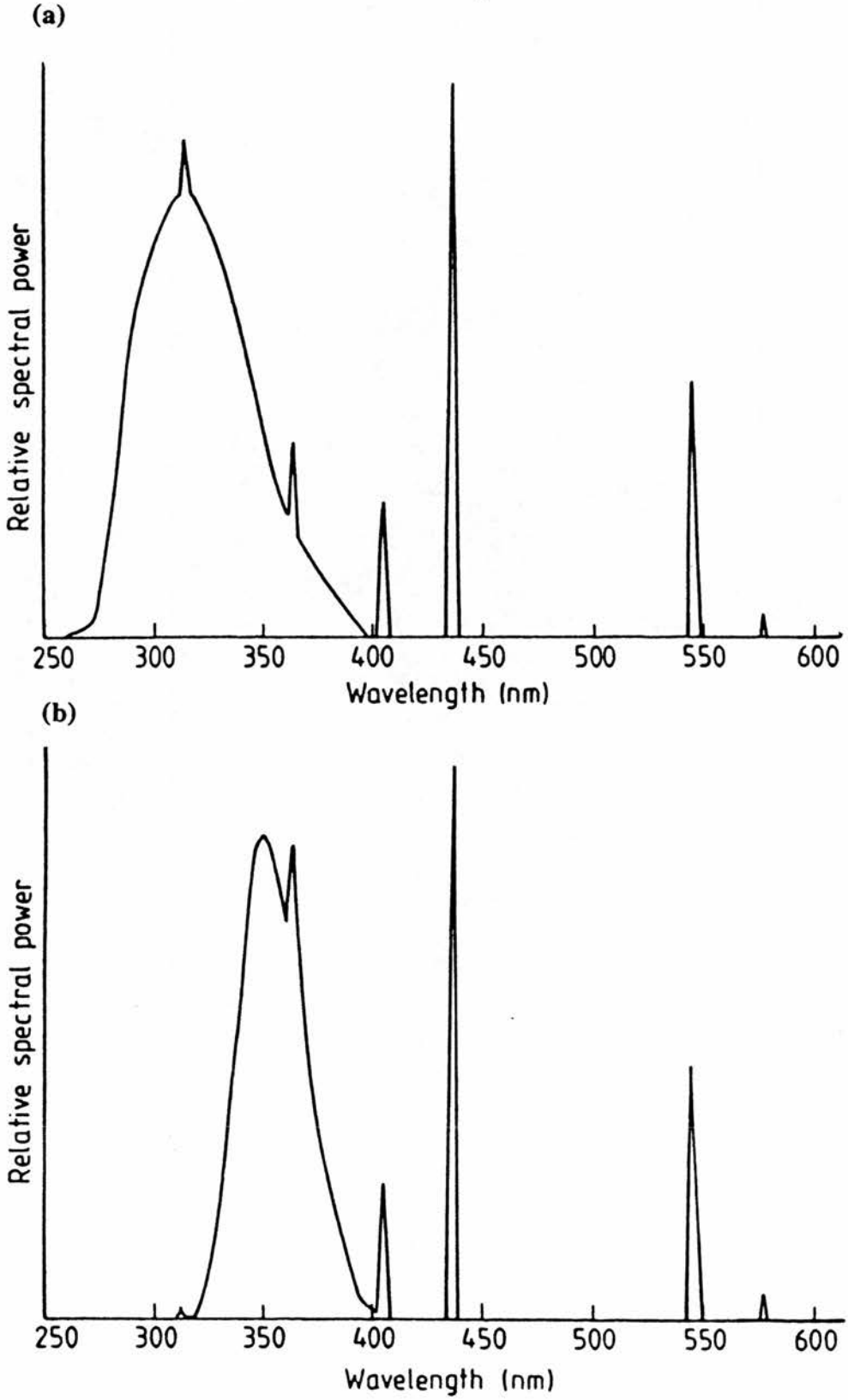
UVA = 9.8 mW cm^{-2}) was used, and irradiance assessed using the same meter as outlined above. All patients had their minimal phototoxic dose determined, and the first irradiation dose was 70% of this. Treatment was then given twice weekly with 40% incremental doses (less if erythema occurred) at each visit until minimal residual activity was achieved (15-20 treatments). The relative output spectrum of these lamps is very similar to that of the PUVA lamps used in Edinburgh (Fig 2.1).

2.3.5 Narrow Band UVB (TLO1) Therapy

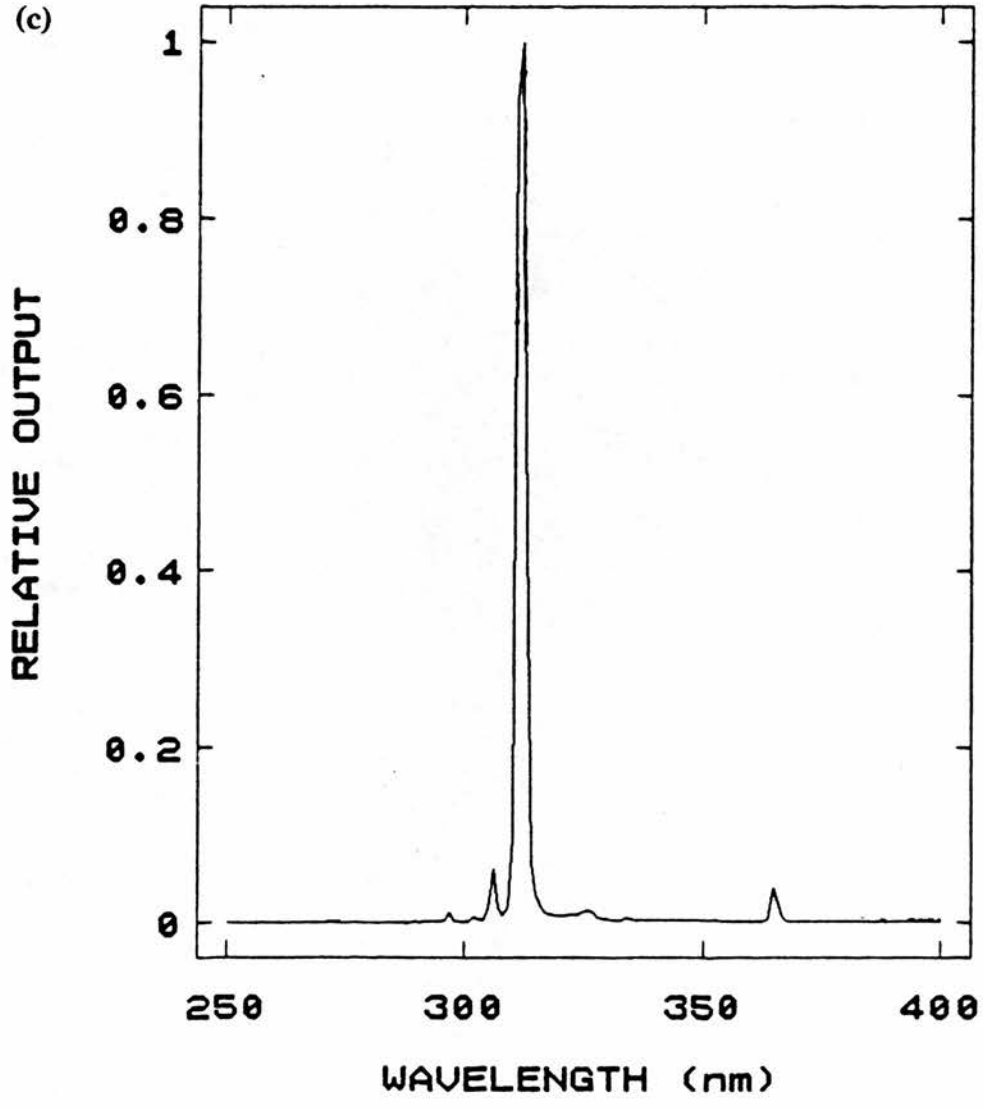
Patients had their minimal erythema dose (MED) determined and an initial dose of 70% of MED was given. They were treated three times weekly and at each visit the dose was increased by 40%; the increment was reduced or omitted depending on the occurrence and severity of erythema. Treatment was stopped when psoriatic plaques had resolved to the point of impalpability over all affected areas, excepting minimal residual activity on knees or elbows (15-20 treatments). The irradiation, determined as for the UV6 lamps, was given in an upright cubicle incorporating 50 Philips 100W fluorescent lamps and was 4.1 mW cm^{-2}. The relative output of the TLO1 lamp is illustrated in Fig 2.1.

Figure 2.1

The relative output spectra of (a) the UV6 lamp , (b) PUVA lamp and (c) the TLO1 lamp



(c)



2.3.6 HSV Isolation

Oropharyngeal swabs were collected into 5 mls of Eagle's minimal essential medium plus 5% new born calf serum (supplemented as in section 2.1.1). The samples were stored at -70°C until being assayed for plaque forming units on monolayers of Vero cells.

2.3.7 Preparation of Peripheral Blood Mononuclear Cells

Venous blood was collected into preservative-free heparin (10 U ml^{-1} final concentration) and PBMC isolated by centrifugation (1500g for 25 min) on lymphopaque (Nyegaard Ltd., Birmingham, UK). The cells were washed three times in PBS containing 5 U ml^{-1} of heparin and two percent autologous plasma, and resuspended in RPMI 1640 (Gibco Ltd., Paisley, UK) supplemented with 5 mM 2-mercaptoethanol, 2 mM L-glutamine, 100 IU ml^{-1} penicillin, 200 µg ml^{-1} streptomycin, 100 µg ml^{-1} gentamicin and 10 µg ml^{-1} fungizone (TCM) plus 15% autologous plasma. In the case of NK cell activity assays 10% FCS (Gibco Ltd., Paisley, UK) was added instead of autologous plasma. Viability was determined by trypan blue exclusion, and was found to be greater than 95%.

2.3.8 Preparation of Adherent Cells and T Enriched Cells

The PBMC were incubated in 50 ml tissue culture flasks (Falcon, Oxnard, USA) for 1h at 37°C (5 ml of cells containing up to 3 x 10^7 cells). Non-adherent cells were washed off and the adherent cells (AC) scraped off using a cell scraper (Costar Corporation, Cambridge, USA). AC were resuspended in TCM at 5 x 10^5 ml^{-1}. The non-AC were then enriched for T cells by passage over a nylon wool column (Julius *et al.*, 1973). Briefly 2g of teased nylon wool was placed in the barrel of a 10 ml syringe. The column was equilibrated with TCM plus 15% autologous plasma at 37°C for at least 1 h before adding 2 mls of non-AC to the column (the number of

cells added to the column never exceeded 6 x 10^7) for 90 mins at 37°C. The cells were eluted from the column with TCM plus 15% autologous plasma (warmed to 37°C), washed and finally resuspended in TCM plus 15% autologous plasma at 5 x 10^5 cells ml^{-1} (TEC). The viability of all cell populations was greater than 95%, determined by trypan blue exclusion.

2.3.9 Preparation of Epidermal Cell Suspensions

The method employed was that of Vestey *et al.*, (1990). In brief, suction blister roofs were raised under negative pressure from uninvolved areas of the forearm (illustrated in Fig 2.2), the blister fluid was collected and stored at -70°C and the roofs placed into 0.5% trypsin (T2021) (Sigma UK Ltd, Poole). They were incubated at 37°C for 1h with gentle agitation to prepare a single cell suspension. Fifteen percent autologous plasma was added, the cells were then washed twice and resuspended at 5 x 10^5 cells ml^{-1} in TCM.

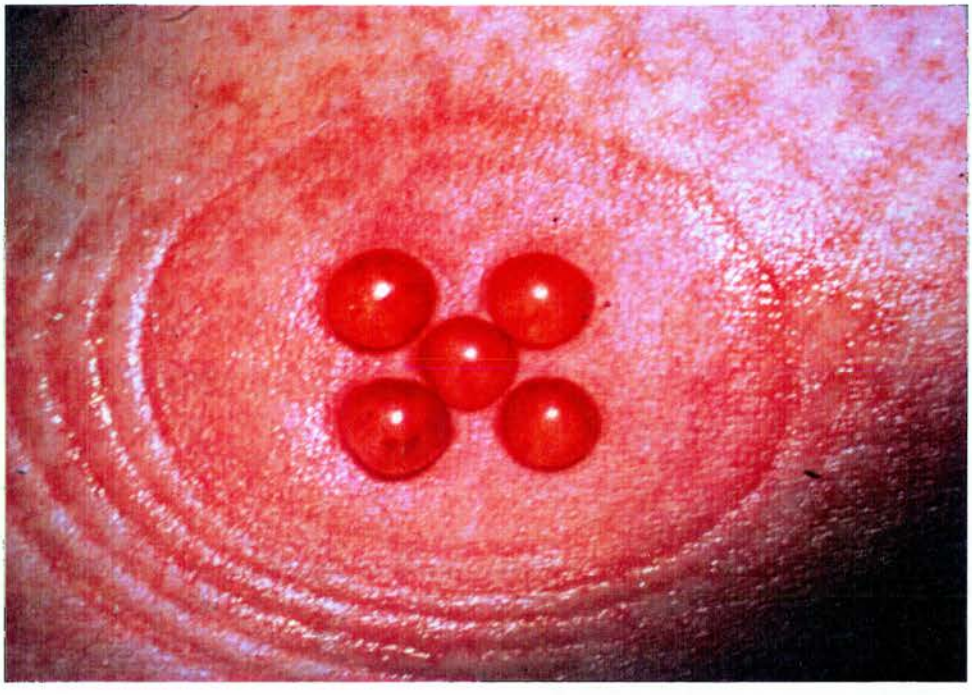
2.3.10 HSV-Specific ELISA

The ELISA method employed that of Vestey *et al.*, (1988) (based on that of Booth *et al.*, (1979)). Briefly, HSV or control glycine extracted antigen (described in section 2.1.2) was used to coat Immunoplates (Nunc, Gibco Ltd, Paisley, UK) at 10 µg protein ml^{-1} in sodium carbonate/bicarbonate buffer (pH 9.6) overnight at 4°C. After washing with PBS containing 0.1 % bovine serum albumin (BSA; Sigma UK Ltd, Poole) and 0.05% Tween 20 (wash buffer), 100 µl of doubling dilutions of test serum, in PBS plus 1% BSA, were added to the wells and incubated at room temperature for 2-3 h. The plates were then washed with wash buffer and incubated for 2-3 h at room temperature with a 1:500 dilution of goat anti-human IgG antibody conjugated to alkaline phosphatase (Sigma UK Ltd, Poole). The plates were washed again and 100 µl of diethanolamine buffer (pH 9.8) containing *p*-nitrophenyl phosphate substrate (Sigma UK Ltd, Poole) added for for approximately 20 min,

until a strong colour change had developed in the wells containing a pooled HSV positive control serum. The reaction was then stopped by the addition of 50 µl of 3M aqueous sodium hydroxide. The plates were then read on a spectrophotometer at 405 nm. The antibody titre was estimated as the dilution of serum which gave an absorbence greater than the mean plus three standard deviations of eight wells of pooled negative serum at 1:100 dilution.

Figure 2.2

Suction Blister Roofs. Suction blister cups were held in place by means of an elasticised bandage and a negative pressure of -150 mm Hg applied using a hand operated pump. Suction blisters were raised in 45-60 min. The blister roofs were removed under sterile conditions using scissors and the fluid collected. Epidermal cell suspensions were prepared as outlined in 2.3.9.



2.3.11 Fluorescent Flow Cytometry

The method employed was that used by Vestey *et al.*, (1988). Briefly 5 x 10^5 PBMC were stained with the murine monoclonal antibodies outlined in Table 2.1 over night at 4°C. An irrelevant antibody was used to determine the background nonspecific binding (approximately 1.1% of the total histogram). The cells were then washed in PBS with 1% BSA (Sigma UK Ltd, Poole) and incubated with sheep anti-mouse IgG $F(ab')_2$ fraction conjugated to FITC (Sigma UK Ltd, Poole) at a 1:40 dilution at 4°C for 45 mins (with the exception of those cells stained with anti-CD16 which was directly labelled with phycoerythrin). The cells were then washed in PBS plus 1% BSA, resuspended in 1ml of cold PBS plus 1% BSA and fixed by the addition of 1% formaldehyde solution. Approximately 10^4 cells were analysed on a Coulter EPICS 'C' flow cytometer (output 150 mW at 488 nm, flow rate 400-500 cells s^{-1}). The percentage of cells exhibiting fluorescent levels higher than the background was recorded. All flow cytometric analysis was carried out by W Neill.

Table 2.1

Monoclonal Antibodies Employed in this Study

Cell Surface Marker	Target cell	Monoclonal antibody	Source
CD3	T cells	DAKO-T3	Dakopatts
CD4	T-helper cells	DAKO-T4	Dakopatts
CD8	T-suppressor/ cytotoxic cells	DAKO-T8	Dakopatts
CD1a	Thymocytes Dendritic cells	DAKO-T6	Dakopatts
MHC II	Monocytes/macrophages B cells Activated T cells	DA6.231	Dr K Guy¥
CD57	LGL* NK cells$^{\Delta}$ K cells$^{\Diamond}$	Leu 7	Beckton Dickinson
CD16	IgG Fc Receptors NK cells Granulocytes	Leu 11	Beckton Dickinson
CD56	NK cells	Leu 19	Beckton Dickinson

*: large granular lymphocytes
$^{\Delta}$: natural killer cells
$^{\Diamond}$: killer cells
¥: Gift from Dr K Guy
Dakopatts, High Wycombe, UK
Beckton-Dickison, California, USA

2.3.12 Lymphoproliferation Assay

Two hundred µl of human PBMC or TEC were plated into each well of a 96 well round bottom plate (Falcon, Oxnard, USA). To quintuplicate wells 0, 0.3%, 3% or 10% of either AC or EC were added. The cells were either incubated without any further additions, 5µg ml^{-1} Con A, 1 pfu $cell^{-1}$ of HSV antigen or an equivalent number of mock infected Vero cells (10^2 ml^{-1}). The cells were incubated for six days at 37°C in 5% CO_2 in a humidified incubator, and pulsed with 0.75 µCi ^{3}H-

thymidine (Amersham, Little Chalfont, UK) per well over the final 24 h of culture. The cells were then harvested onto glass fibre paper and the uptake of ^{3}H-thymidine measured using a Packard liquid scintillation counter. The stimulation index (SI) for each culture was calculated as:

$$SI = \frac{\text{mean counts min}^{-1}\text{ (cpm) test}}{\text{mean counts min}^{-1}\text{ (cpm) control}}$$

Arithmetic mean cpm had previously been employed in this laboratory for several studies involving HSV responses and for the sake of direct comparison with previous work, arithmetic means were used in preference to geometric means in the present study.

2.3.13 Determination of Natural Killer Cell Activity

K562 erythroleukaemic cells, which are sensitive to lysis by NK cells (Lozzio and Lozzio, 1975) were employed as target cells in all experiments. They were cultured in TCM plus 10% FCS. 10^6 cells were labelled with 100 μCi of sodium chromate (^{51}Cr; Amersham, Little Chalfont, UK) for 4h at 37°C. The cells were washed three times with PBS and resuspended in TCM plus 10% FCS at 10^5 cells ml^{-1}. One hundred μl of ^{51}Cr-labeled cells were placed in each well of a round bottomed 96 well plate (Falcon, Oxnard, USA).

PBMC suspensions at 4 x 10^6 cells ml^{-1} were doubly diluted in TCM and 100 μl of each dilution was added to quadruplicate wells containing the labelled target cells, thus resulting in effector (E) to target cell ratios (E:T) of 40:1 through to 1.25:1. Spontaneous ^{51}Cr release was determined by adding 100 μl of TCM to quadruplicate wells and maximum release by adding 100 μl of 2% acetic acid to quadruplicate wells. The cells were incubated for 18h at 37°C in 5% CO_2 in a humidified incubator. The plates were then spun at 500 g for five minutes before removing 120 μl of the supernatant for determination of radioactivity (mean cpm) using a Packard liquid scintillation counter. The standard errors at each E:T cell ratio were

always less than 10% of the mean value. The % specific ^{51}Cr release was determined by:

$$\% \text{ Specific } ^{51}\text{Cr Release} = \left(\frac{\text{Test cpm - Spontaneous cpm}}{\text{Maximum cpm-Spontaneous cpm}} \right) \text{x100}$$

2.3.14 The Effect of *in vitro* Irradiation of PBMC on NK Cell Activity

PBMC were washed and resuspended in indicator free PBS at 2 x 10^7 cell ml^{-1}. Fifty µl of the suspension was placed in single wells of a 96 well flat-bottomed plate (Falcon, Oxnard, USA). Adjacent wells remained empty to remove the possibility that cells in these wells may be irradiated with reflected UV-from the neighbouring well. The plate was spun at 500 g for 5 min (before irradiation) to form a monolayer of cells in each well.

(i) Irradiation of cells with TLO1 (311-313 nm).

A single TLO1 lamp (Philips) was used to irradiate the cells. The output of the lamp was measured using the meter employed in 2.3.2. The plate containing the cells was placed on the bench at a distance of 20 cm from the lamp. Two glass sheets (UV opaque) were placed over the plate and pulled back at known time intervals to expose cells to shorter time intervals.

(ii) Irradiation of cells with monochromatic light.

A single grating monochromator (Rank Hilger D330, UK) with a 450 W xenon arc source was used. Wavelength and band width (2.6nm) calibrations had been performed using a medium pressure mercury lamp and an Instaspec photodiode array spectograph (Oriel Corporation, UK) respectively, by N Gibbs. A 5 mm diameter liquid light guide (Oriel Corporation, UK) was employed to direct the light onto single wells of cells chosen randomly. The cells were irradiated with incremental wavelengths and the irradiance was measured using a Higer-Watts FT17 thermopile calibrated by the National Physical Laboratory, UK.

Following irradiation the cells were resuspended in 1 ml of TCM plus 10% FCS. The viability of the cells was determined by trypan blue exclusion before serially doubly diluting the cells and determining the NK cell activity of the irradiated cells. The 40:1 E:T cell ratio was attained by incubating 100 µl of PBMC at 2 x 10^6 with 100 µl of ^{51}Cr-labelled target cells at 5 x 10^4.

2.3.15 The Effect of UCA Isomers on NK Cell Activity *in vitro*

Serial tenfold dilutions of *cis* - (10^{-1}M to 10^{-8} M) or *trans* - (10^{-2} to 10^{-8}) UCA were carried out in PBS. Ten µl of the appropriate concentration of UCA were added to 0.5 ml of E cells. The cells were then added to wells of 96 well round bottomed plate (Falcon) and approximately 15 min later labelled target cells were added at an E:T cell ratio of 40:1. This resulted in a final concentration when the target cells had been added of 10^{-3} M to 10^{-10} M *cis*- or 10^{-4} M to 10^{-10} M *trans* -UCA. One hundred µl of 10^{-1} M *cis*- or 10^{-2} M *trans*-UCA were added to 0.4 ml of cells, containing the same number of cells as 0.5 ml above, resulting in the final concentrations of 10^{-2} and 10^{-3} M respectively. NK cell activity was determined following an 18 h incubation as usual.

2.3.16 TNF-α Quantification

The amount of TNF-α present in the suction blister fluid was determined using an ELISA kit (purchased from NBS Biologicals, Hatfiel Herts, UK). One hundred µl of each sample or standard was added to each well.

2.3.17 Urocanic Acid Analysis

Epidermal UCA was initially quantified from 4 successive tape strips taken from the buttock (Norval *et al.*, 1988). The UCA was extracted from each strip with 0.1 M KOH and analysed for UCA. The values for the four strips were then pooled. More recently, six filter paper discs soaked in 0.1 M KOH which were held in place with

Finn Chambers for 30 min were applied to the inner-fore arm (Jansen *et al* 1991). The filter discs were placed in 0.1 M KOH and stored in the dark at room temperature for 24 h, to extract the UCA, before storage. Twenty µl of suction blister fluid was also analysed.

All samples were taken from uninvolved sites. They were stored in the dark at -70°C until analysis. Total UCA and relative amount of each isomer were determined by reverse phase HPLC by J Crosby (Bristol University) as reported by Norval *et al.*, (1988).

CHAPTER 3

THE ROLE OF HISTAMINE-LIKE RECEPTORS IN IMMUNOSUPPRESSION INDUCED BY *CIS*-UCA

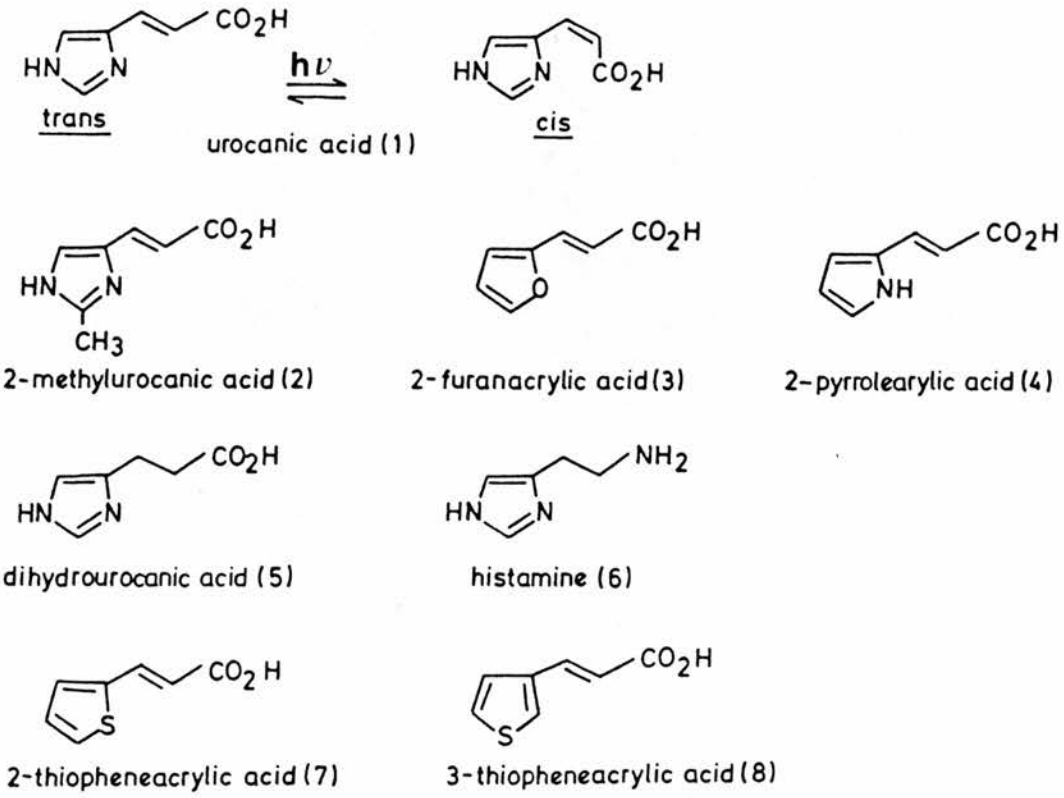
3.1. INTRODUCTION

LC are pivotal in presentation of HSV antigen to T lymphocytes and thus in the generation of DH responses to the virus. UVR reduces the surface marker expression by LC in the epidermis and also their ability to present antigen (discussed in section 1.3). In the absence of a monoclonal antibody specific for murine LC, membrane ATPase staining was employed to study the effect of UCA isomers on their numbers and morphology.

Previously in the laboratory a single sub-erythemal dose of UVB prior to infection of mice with HSV resulted in an antigen-specific suppression in the DH response to the virus on subsequent challenge (see section 1.3.3). *Cis*-UCA was found to mimic the effects of UVB in this system (outlined in section 1.4.2). A number of structural analogues of UCA were synthesised and the ability of these compounds to induce immunosuppression of DH to HSV tested (Norval *et al.*, 1989 b). The structures of these compounds are outlined in Fig 3.1. It was of interest to find that histamine, which like UCA is formed from histidine in a one step process, was suppressive. These results indicated that a histamine-like receptor may be involved in *cis*-UCA induced immunosuppression of DH to HSV. Therefore the effect of histamine receptor type 1 (H1) and 2 antagonists on epidermal LC and DH responses in mice treated with *cis*-UCA was examined. Subsequent experiments employed a H2 receptor and an H3 receptor agonist on the DH response to HSV. A number of additional UCA analogues were tested for their ability to suppress the DH response to HSV to extend previous studies and in order to deduce the molecular structure required by UCA to induce suppression.

Figure 3.1

Urocanic Acid Analogues Previously Tested for Immunosuppression of the DH Response to HSV. Cis- and trans-isomers of 2-furanacrylic acid, 2-pyrroleacrylic acid and 2-thiopheneacrylic acid effectively suppressed the DH response to HSV at levels of 1 μg per mouse, the cis-form being more effective generally than the trans-isomer, and 2-pyrroleacrylic acid was particularly potent. Both isomers of 3-thiopheneacrylic acid were only marginally immunosuppressive. Neither isomer of 2-methlyurocanic acid had any suppressive activity. Dihydrourocanic acid and histamine were also found to be suppressive.



3.2. RESULTS

3.2 1 The Effect of UCA Isomers on Epidermal ATPase$^+$ Cells

Following two light tape strips of both ear surfaces, mice were painted on both ear surfaces with *cis*- or *trans*-UCA, or with *cis*- or *trans*-UCA together with cimetidine or terfenadine or the histamine receptor antagonists alone, and epidermal sheets prepared 24 h later. In addition, some mice had received a suberythemal dose of UVB (96 mJ cm^{-2}) three days before preparing the epidermal sheets from the dorsal ear surfaces. The sheets were stained for ATPase activity and the number of ATPase$^+$ cells determined by light microscopy.

In the control epidermal sheets, tape stripped twice and painted with the vehicle, the LC formed a complete network (Fig 3.2 (a)) and had a very characteristic dendritic morphology (Fig 3.2 (b)). Following painting with *cis*-UCA the picture was very similar to that following UVR. The number of cells had decreased significantly, thus the complete network had been disrupted (Fig 3.2 (c)) and the cells which remained did not have the dendritic morphology (Fig 3.2 (d)). In some areas, there were none of these cells left, but rounded cells with some scattered granular ATPase staining were present (Fig 3.2 (e)).

The number of ATPase$^+$ cells in the control mice which had been tape stripped prior to painting with the vehicle was 1136 mm^{-2} (sem ± 25) and this compared to 1068 mm^{-2} (sem ± 19) in mice which had not been tape stripped; this was not statistically significant by Student's t-test. The number of ATPase$^+$ cells declined following UVB irradiation and *cis*-UCA application compared with the control group painted with the vehicle, but there was only a slight decrease in the cell numbers following treatment with *trans*-UCA or the histamine receptor antagonists alone (table 3.1). If *cis*-UCA was applied together with cimetidine and terfenadine, a decrese in the number of cells occurred. The number of ATPase$^+$ cells after either *cis*-UCA plus

cimetidine or *cis*-UCA plus terfenadine alone was considerably greater than *cis*-UCA alone.

Table 3.1

Number of ATPase$^+$ Cells in Epidermal Sheets from Murine Ears

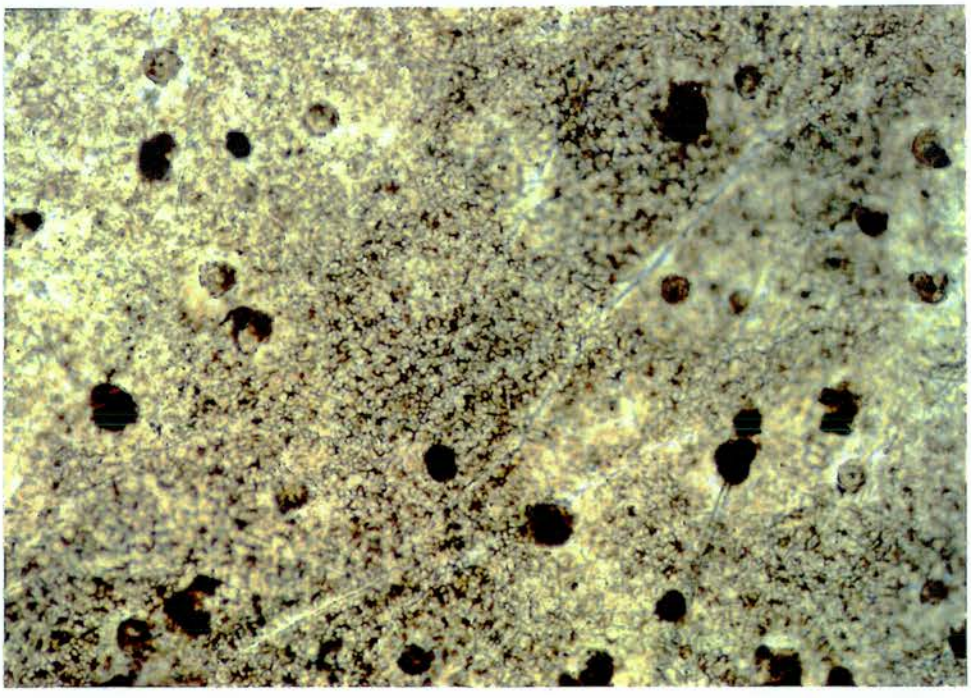
Treatment	No. Fields Counted	Mean No. ATPase$^+$ Cells (mm^{-2})	% Reduction in ATPase$^+$ Cells *
Vehicle	240	1068 ± 19	—
20 µg *cis*-UCA	80	408 ± 31	62
20 µg *trans*-UCA	80	933 ± 36	13
20 µg cimetidine	80	1095 ± 27	-3
20 µg terfenadine	80	1156 ± 22	-8
20 µg *cis*-UCA + 20 µg cimetidine	76	1028 ± 42	4
20 µg *cis*-UCA + 20 µg terfenadine	80	892 ± 22	17
96 mJ cm^{-2} UVB	40	426 ± 40	61

No.: number

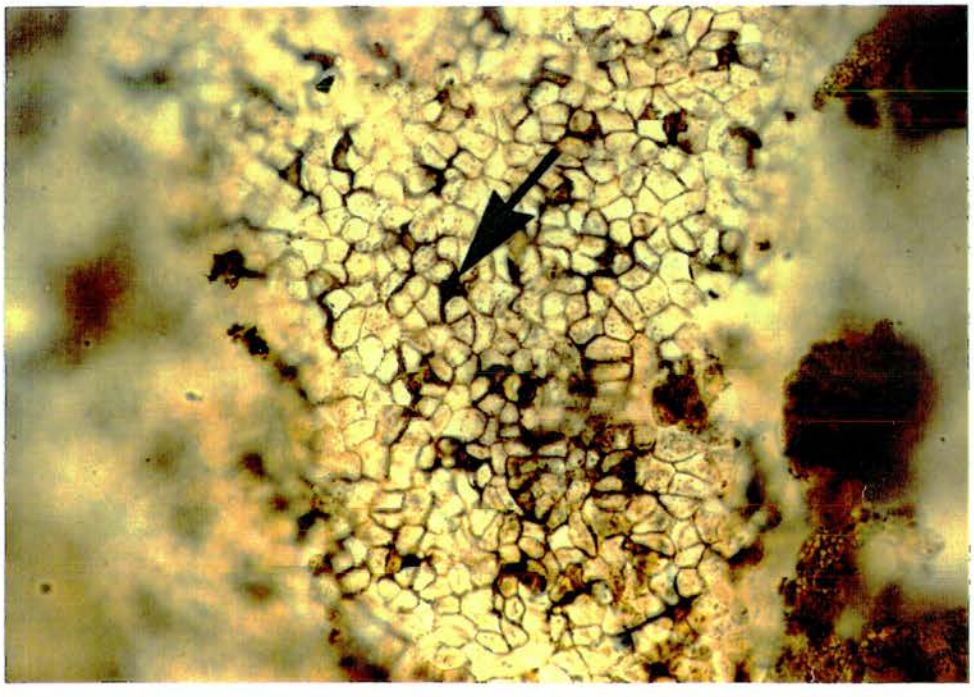
Figure 3. 2

ATPase Staining of Epidermal Sheets from Murine Ears 24 h After Tape Stripping and Painting with (a) vehicle (x 10), (b) vehicle (x 40), (c) 20 μg cis-*UCA (x 10), (d) 20 μg* cis-*UCA (x 40), (e) 20 μg* cis-*UCA (x 40). Arrows show ATPase$^+$ cells in (b) and (d), and cells with ATPase$^+$ granules in (e).*

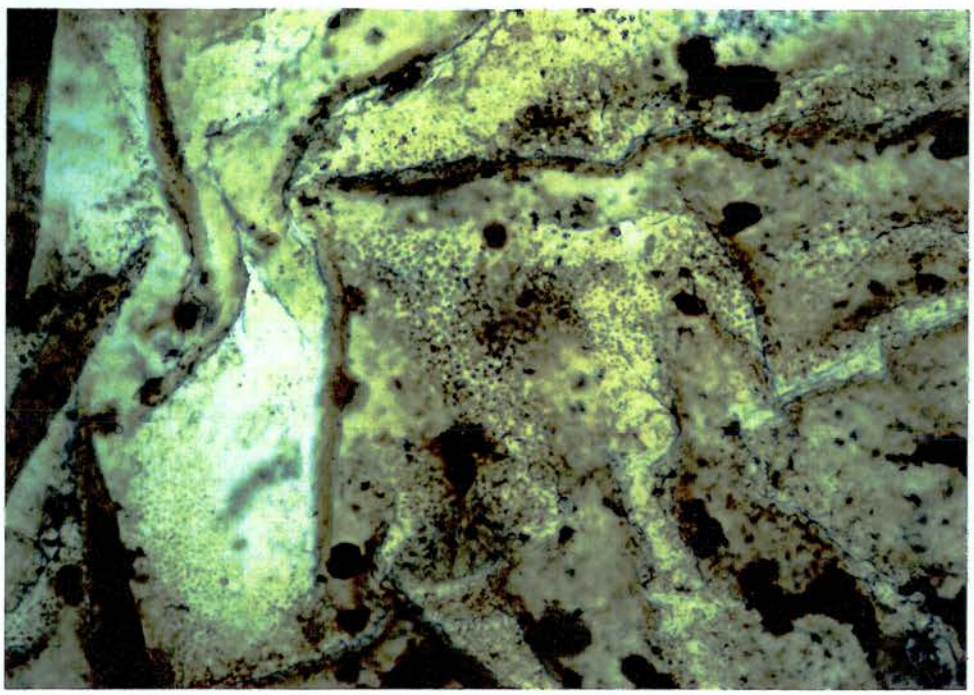
(a)



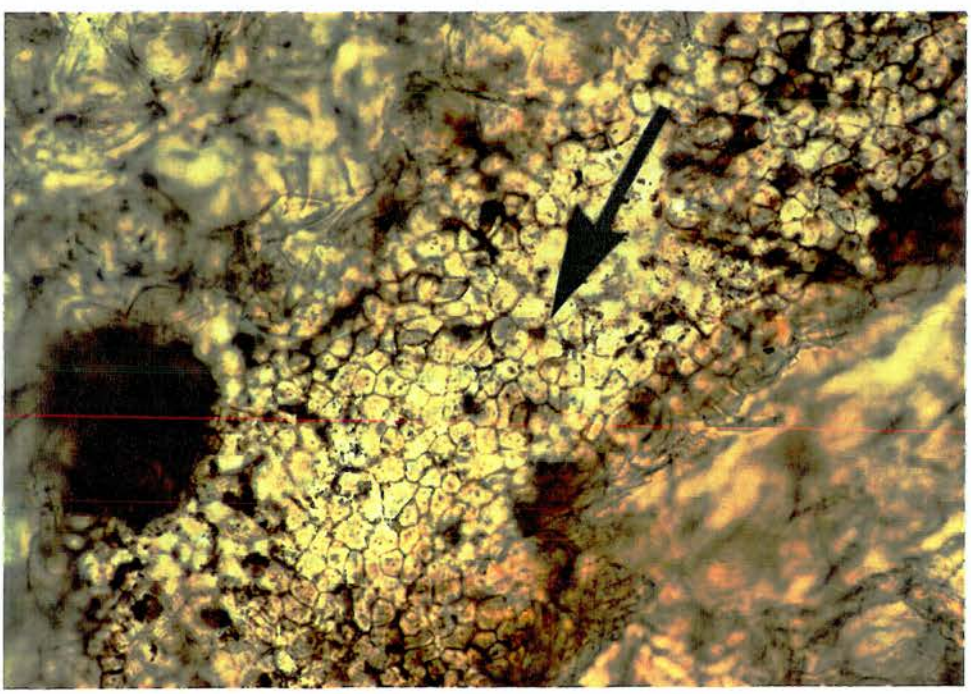
(b)



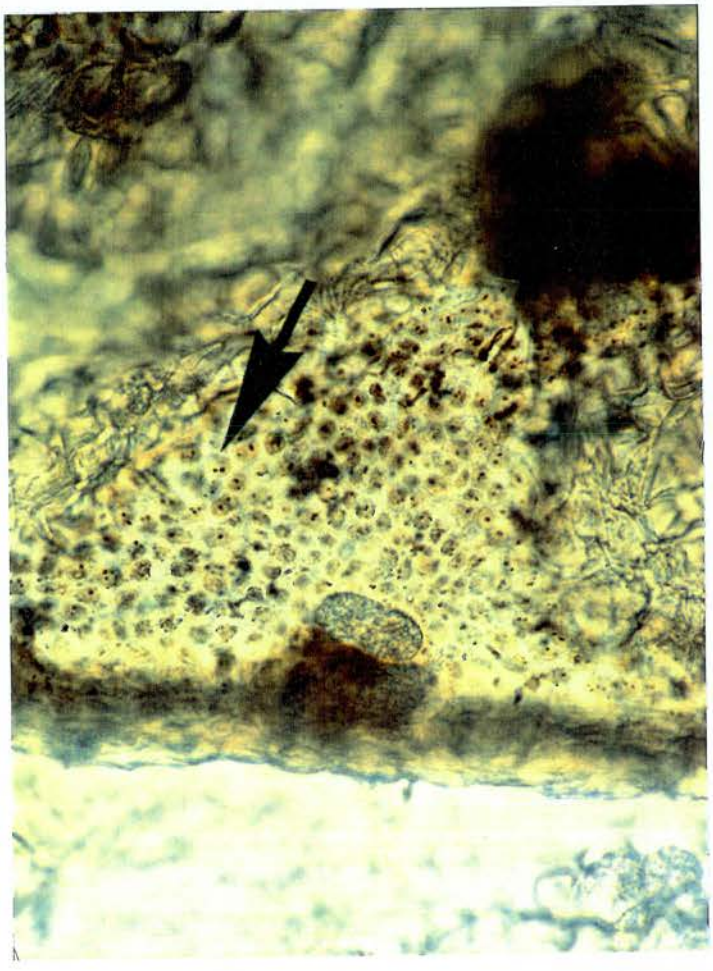
(c)



(d)



(e)



3.2.2 DH Response to HSV

The DH protocol is outlined in Fig 3.3. Mice were pretreated with a single dose of UVB (96 mJ cm^{-2}) three days before, or a single dose of UCA five hours before being infected subcutaneously with HSV. Application of UCA epidermally or subcutaneously had a similar effect on DH. Ten days later each mouse was individually marked and the thickness of each ear measured before challenging with inactivated HSV antigen. Twenty four hours after challenge the thickness of each ear was measured again and the increase in thickness taken as a measure of DH to the virus. The average increase per mouse was calculated and the mean value for each group (consisting of six to eight mice) calculated. Table 3.2 shows the effect of UVB or UCA isomers alone on the DH response to HSV.

Figure 3.3

The Protocol Employed to Assess the DH Response to HSV. Mice were pre-treated with UVB or cis-UCA (three days or five hours) before infection with HSV. Ten days later the ears of each mouse were measured before challenging with inactivated HSV antigen. 24 h later the increase in ear thickness was assessed and taken as a measure of the DH response.

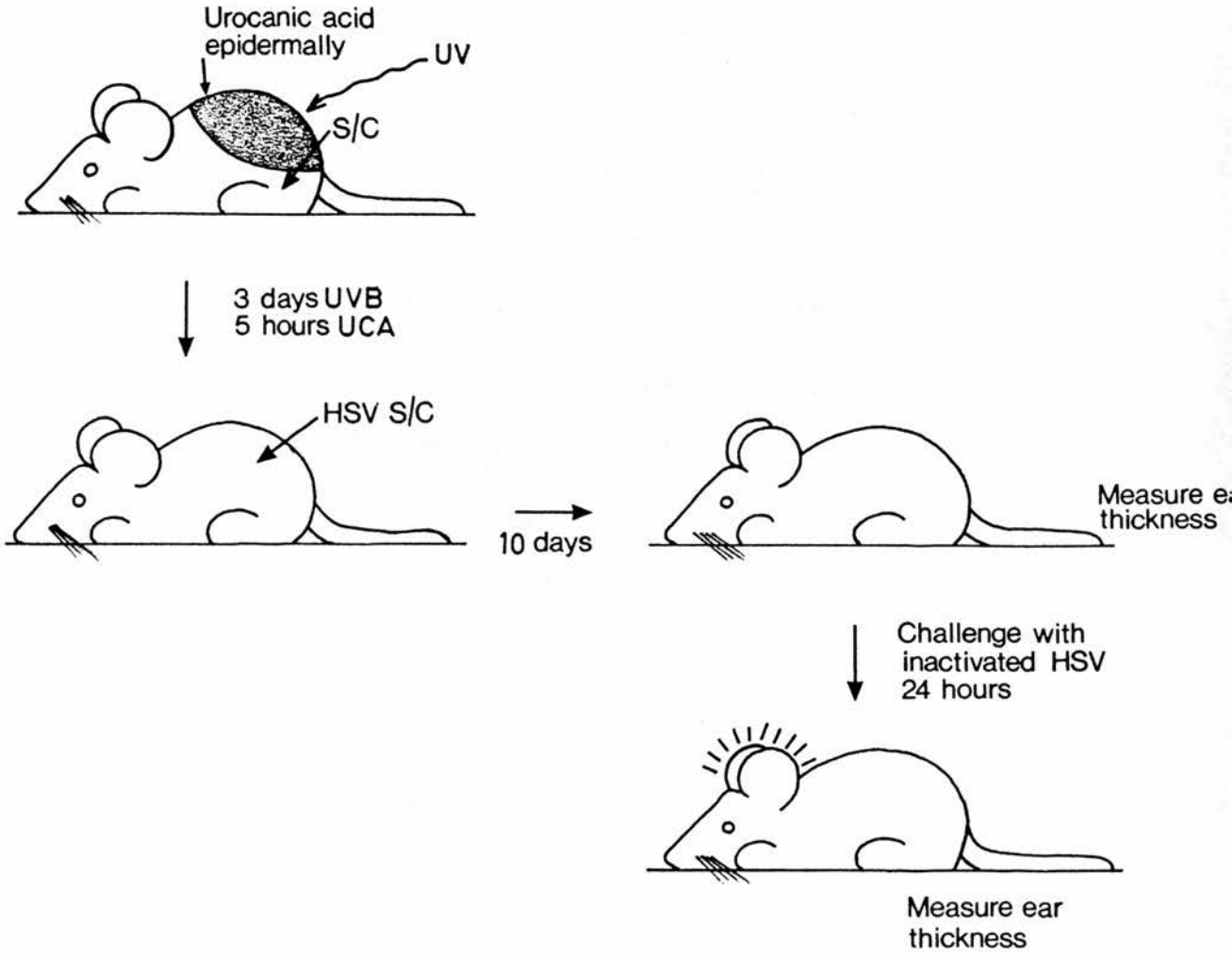


Table 3.2

The Effect of cis-UCA and Histamine Receptor Antagonists on the DH Response to HSV

Pre-treated with	Applied	Sensitise (5h later)	EXPERIMENT 1 Increase in ear thickness ($mm^{-2} \pm sem$)	p	% suppr-ession	EXPERIMENT 2 Increase in ear thickness ($mm^{-2} \pm sem$)	p	% suppr-ession
Vehicle alone	S/C	Vero cells	1.0 ± 0.2	—	—	1.2 ± 0.4	—	—
Vehicle alone		HSV	16.2 ± 0.7	—	—	14.4 ± 1.0	—	—
100 µg *cis*-UCA	S/C	HSV	3.5 ± 0.8	< 0.001	84	5.7 ± 0.7	< 0.001	66
100 µg cimetidine		HSV	13.9 ± 0.7	NS	15	13.8 ± 0.7	NS	5
100 µg terfenadine		HSV	13.4 ± 0.4	< 0.01	18	11.6 ± 0.6	NS	21
100 µg *cis*-UCA + 100 µg cimetidine		HSV	13.6 ± 0.9	< 0.01	17	14.3 ± 0.6	NS	1
100 µg *cis*-UCA + 100µg terfenadine		HSV	14.0 ± 0.3	< 0.01	14	15.3 ± 0.5	NS	0
100 µg *cis*-UCA + 10 µg cimetidine		HSV	ND	—	—	14.3 ± 0.8	NS	1
100 µg *cis*-UCA + 10 µg terfenadine		HSV	ND	—	—	11.4 ± 0.6	< 0.01	23
100 µg *cis*-UCA	Epid'	HSV	7.3 ± 0.7	< 0.001	59	7.1 ± 0.3	< 0.01	55
100 µg cimetidine		HSV	13.1 ± 0.6	< 0.01	20	11.9 ± 0.5	NS	19
100 µg terfenadine		HSV	12.9 ± 0.6	< 0.01	22	13.4 ± 0.7	NS	8
100 µg *cis*-UCA + 100 µg cimetidine		HSV	11.4 ± 0.7	< 0.001	32	13.7 ± 0.6	NS	5
100 µg *cis*-UCA + 100µg terfenadine		HSV	13.6 ± 0.8	< 0.01	17	14.9 ± 0.5	NS	0

Epid': epidermally; ND: not done; NS: not significant ($p > 0.05$; Student's t-test); p: probability compared with positive control; S/C: subcutaneously; .

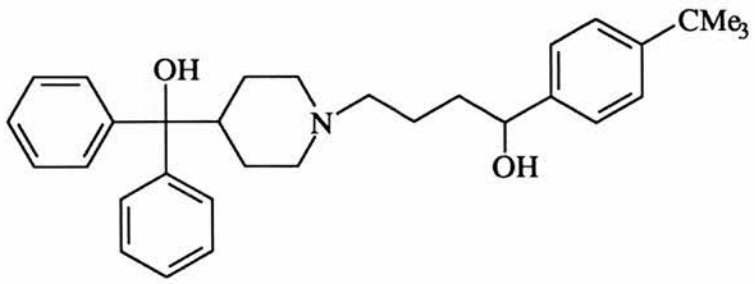
Cis-UCA was applied together with either cimetidine or terfenadine (Fig 3.4 illustrates the structures of these compounds), or with each of the antagonists alone five hours before infection with HSV. *Cis*-UCA caused a significant suppression in the DH response when given alone, but when it was given together with the H1 or H2 receptor antagonists, there was only a small depression of the DH response. Cimetidine or terfenadine alone had a similar effect, or no effect at all. Therefore, the antagonists appeared to negate the effects of *cis*-UCA on DH responses. *Trans*-UCA had been shown to induce only a small suppression of the DH response to HSV (Ross *et al.*, 1986). Cimetidine or terfenadine had little effect on this (data not shown).

At a later date thioperamide, a highly specific H3 receptor antagonist (Arrang *et al.*, 1987) became available, the molecular structure of which is illustrated in Fig 3.4. The ability of this compound to inhibit the suppression of the DH response to HSV induced by *cis*-UCA was tested in the same manner as the H1 and H2 antagonists. The results, outlined in Fig 3.5, revealed that thioperamide alone did cause a suppression in the DH response, but this did not reach statistical significance. No significant difference between mice which were treated with *cis*-UCA alone or with *cis*-UCA plus thioperamide was measured ($p > 0.05$ by Students t-test).

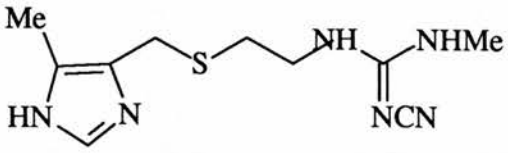
These results indicated that a histamine-like receptor may indeed be involved in immunosuppression induced by *cis*-UCA. Therefore, known histamine receptor agonists should induce suppression of the DH response to HSV. The hypothesis was tested using 4-methylhistamine, a known H2 agonist, and (R)-α-methylhistamine, a known H3 receptor agonist (Arrang *et al.*, 1987). The structures of these compounds are illustrated in Fig 3.4. The mice were pretreated with 100 μg of the H-receptor agonist in 100 μl of PBS subcutaneously five hours before infection with HSV. The results illustrated in Fig 3.6 show that pretreatment with the H2 agonist

Figure 3.4

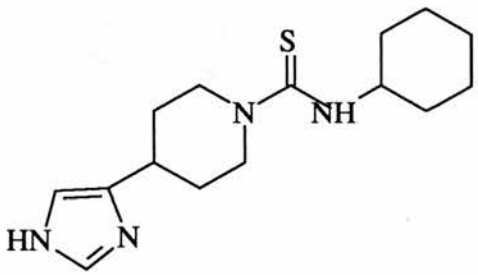
The Chemical Structure of Histamine Receptor Antagonists and Agonists Employed in this Study



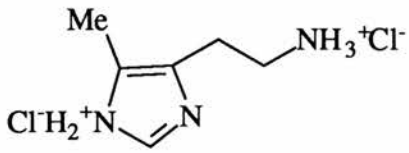
Terfenadine (H - 1 receptor antagonist)



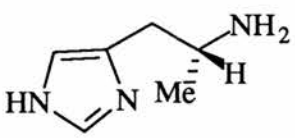
Cimetidine (H - 2 receptor antagonist)



Thioperamide (H - 3 receptor antagonist)



4 - Methylhistamine dihydrochloride (H - 2 receptor agonist)



(R) - α - Methylhistamine (H - 3 receptor agonist)

resulted in a significant suppression of the DH response, but the H3 agonist had no effect at all. This result mirrors those obtained with the histamine receptor antagonists; the H2 antagonist blocked the effects of *cis*-UCA whereas the H3 had no effect on *cis*-UCA.

Fig 3.5

The Effect of a Selective H3 Receptor Antagonist (thioperamide) on the Immunosuppression of the DH Response to HSV induced by cis-UCA. (: $p < 0.05$ compared with the positive group, by Students t-test).*

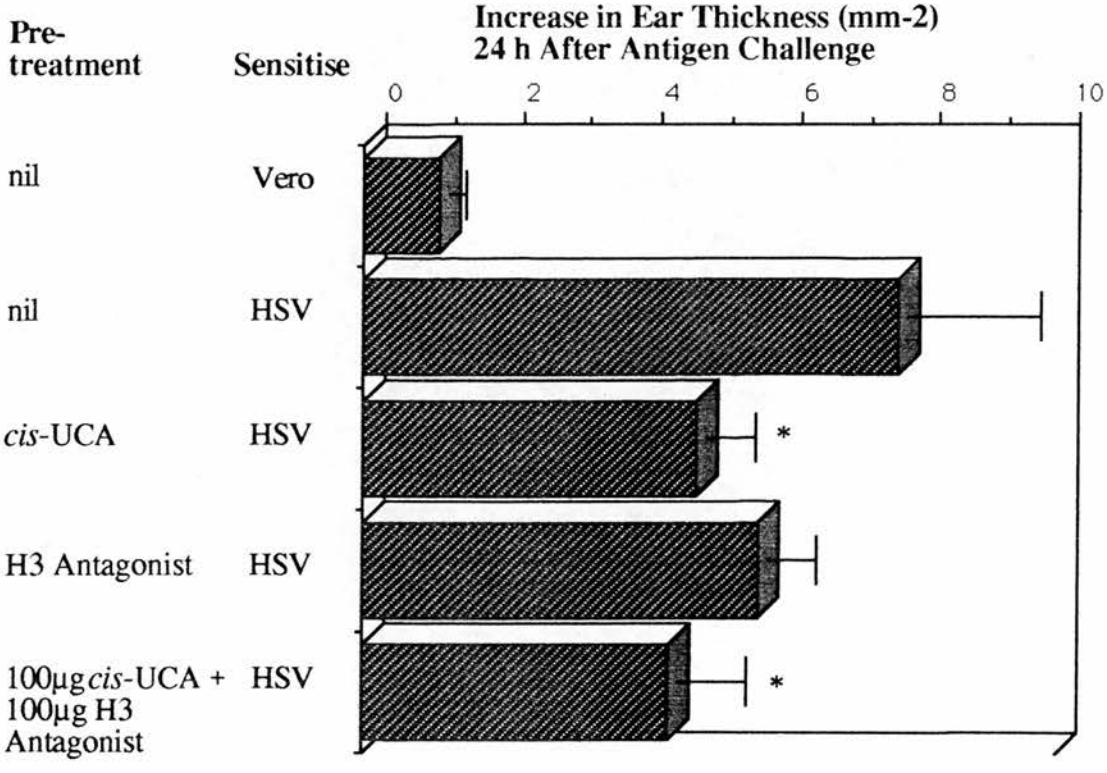
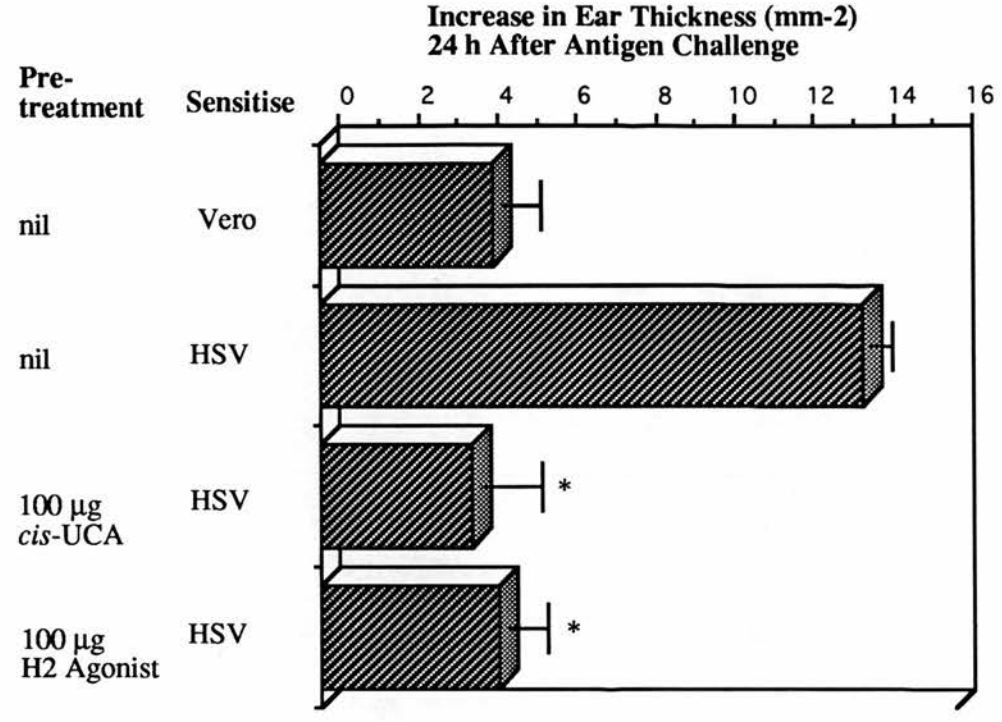
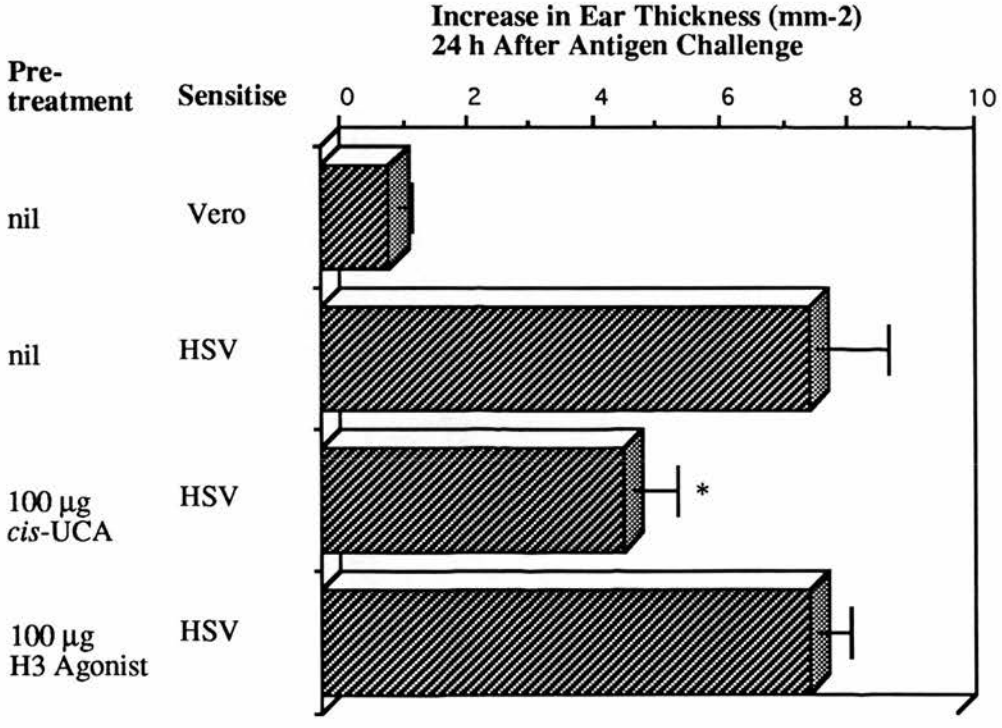


Fig 3.6

The Effect of Histamine Receptor Agonists on DH to HSV. Mice were pretreated 4-methylhistamine dihydrocloride (H2 agonist), Fig(a), or (R)-α-methylhistamine (H3 agonist), Fig (b), subcutaneously prior to infection with HSV and DH measurement. (: $p < 0.05$ compared with positive control group)*





3.2.3 The Effect of UCA Analogues on DH to HSV

Four new UCA analogues became available for testing in addition to the ones already studied (Fig 3.1). Their immunosuppressive potential was tested by DH following the same protocol as used with UCA isomers. The first two analogues (A and B) were prepared by Dr Perti (University of Turku, Finland). In these compounds the position of the acrylic acid side chain had been moved on the imidazol ring. The structures are illustrated in Fig 3.7. They were dissolved at 20 mg ml^{-1} in DMSO and then diluted 1:20 before injecting 100 µl subcutaneously five hours before infection with HSV. Neither compound had any significant effect on the DH response as shown in Table 3.3.

Table 3.3

The Effect of UCA Analogues on the DH Response to HSV.

Pre-treated With Subcutaneously	**Sensitize (5 h later)**	**Increase in Ear Thickness (mm x 10^{-2}) ± sem**	**p* Compared with Control**	**% Suppression**
Vehicle	Vero	1.4 ± 0.9	—	—
Vehicle	HSV	10.9 ± 0.9	—	—
100 µg *trans*-UCA	HSV	10.0 ± 1.2	$p > 0.5$	9.4
100 µg *cis*-UCA	HSV	5.1 ± 0.9	$p < 0.001$	61.1
100 µg *trans*- A	HSV	9.9 ± 1.3	$p > 0.5$	10.5
100 µg *cis*- A	HSV	11.3 ± 0.9	$p > 0.5$	-4.2
100 µg *trans*- B	HSV	10.2 ± 2.2	$p > 0.5$	7.4
100 µg *cis*- B	HSV	9.2 ± 2.2	$p > 0.5$	17.9

* Student's t-test

The third and fourth analogues (C and D) were prepared by Dr Bardshiri (University of Bristol) and consisted of six membered rings unlike the five membered ring of UCA. The structure of these compounds is shown in Fig 3.7. These compounds were dissolved in DMSO at 10 mg ml^{-1} and then diluted to 1 mg ml^{-1} in PBS prior to

injecting the mice with 100 µl subcutaneously five hours before infecting the mice with HSV. The experiment was carried out on two separate occasions and the results are shown in table 3.4. The *cis*-isomer of compound three induced a consistent suppression of the DH response whereas compound 4 appeared to be relatively ineffective at inducing suppression. The *trans*-isomer of neither compound had a significant effect.

Table 3.4

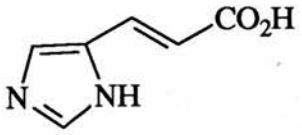
The Effect of UCA Analogues with a Six Membered Ring

		EXPERIMENT 1		
Pre-treated With Subcutaneously	**Sensitize (5 h later)**	**Increase in Ear Thickness (mm x 10^{-2}) ± sem**	**p Compared With Control**	**% Suppression**
Vehicle	Vero	1.5 ± 0.3	—	—
Vehicle	HSV	11.7 ± 1.2	—	—
100 µg *trans*-UCA	HSV	11.1 ± 1.3	NS	5.9
100 µg *cis*-UCA	HSV	6.4 ± 1.1	< 0.01	52.0
100 µg *trans*- C	HSV	13.1 ± 1.5	NS	-13.7
100 µg *cis*-3- C	HSV	7.9 ± 0.8	< 0.02	37.7
100 µg *trans*- D	HSV	10.4 ± 0.7	NS	12.7
100 µg *cis*- D	HSV	10.3 ± 1.2	NS	13.7
		EXPERIMENT 2		
Vehicle	Vero	4.4 ± 0.5	—	—
Vehicle	HSV	14.4 ± 0.5	—	—
100 µg *cis*-UCA	HSV	8.1 ± 0.5	p < 0.001	62.6
100 µg *cis*- C	HSV	10.5 ± 0.5	p < 0.001	39.0
100 µg *cis*- D	HSV	13.5 ± 0.3	p < 0.02	9.0

NS: not significant

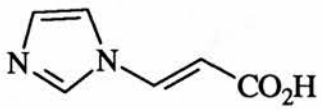
Figure 3.7

The Structure of UCA Analogues Used in this Study



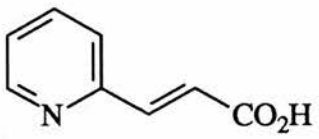
Trans **- UCA**

(A)



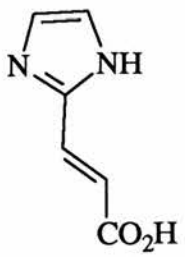
Trans **- 1- imidazoyl - acrylic acid**

(C)



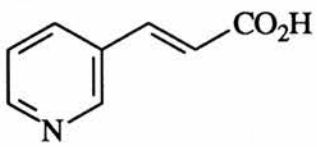
Trans **- 2 - pyridyl - acrylic acid**

(B)



Trans **- 2 - imidazoyl - acrylic acid**

(D)



Trans **- 3 - pyridyl - acrylic acid**

3.3 DISCUSSION

There is increasing evidence that *cis*-UCA may be a mediator of at least some of the effects of UVB irradiation in inducing immunosuppression (reviewed in section 1.4). In this chapter the effect of skin painting murine ears with *cis*-UCA on ATPase$^+$ cells in epidermal sheets has been described. The number of ATPase$^+$ cells declined markedly to a level approximately the same as that found after a low dose of UVB irradiation. In some areas ATPase$^+$ cells remained but they had lost most of their dendritic morphology, no longer forming a network across the epidermal sheet; in other areas, granular staining was noted associated with round cells which were not characterised further. *Trans*-UCA had no significant effect on LC morphology or numbers. Iacobelli *et al.* (1985) reported that exposure of guinea pig skin to low doses of UVB altered the linear distribution of LC membrane ATPase resulting in aggregation of the marker. In contrast higher doses caused actual cytomembrane damage. Therefore, the effect of *cis*-UCA is remarkably like that described following UVB irradiation, where it is possible that membrane surface markers of LC are susceptible to UVR and also suffer some morphological damage (Aberer *et al.*, 1981; Koulu *et al.*, 1985; Toews *et al.*, 1980). Alternatively loss of LC from the epidermis may occur (Ashworth *et al.*, 1989; Moodycliffe *et al.*, 1992; Spencer *et al.*, in press). There is the possibility that *cis*-UCA may affect the ATPase enzyme activity which is extremely sensitive to the effects of UVB (Aberer *et al.*, 1981; Lynch *et al.*, 1981). A recent report by Moodycliffe *et al.*, (1992) suggested that UVB irradiation resulted in accumulation of DC in the DLN but *cis*-UCA did not. However *cis*-UCA, but not *trans*-UCA, has been shown in a number of experimental systems to affect the morphology, phenotype and function of antigen presenting cells, as discussed in section 1.4.2 (Kurimoto and Streilein, 1992; Noonan *et al.*, 1985; Räsänen *et al.*, 1987; Ross *et al.*, 1987/88). *Cis*-UCA administered intradermally (Kurimoto and Streilein, 1992) resulted in a significant reduction of

Ia^+ epidermal cells as well as altered dendritic morphology, and in this study the ears were lightly tape stripped to allow better absorption of UCA. No such measures were taken by Moodycliffe *et al.*, (1991) and it is therefore possible that *cis*-UCA needs to percolate through to the dermis where it exerts its effects on cells such as the DD by inducing, for example, the production of TNF-α (Kurimoto and Streilein 1992). TNF-α also reduces the number of Ia^+ cells in murine epidermal sheets and induces the DC of the epidermis to migrate to the DLN (Cumberbatch and Kimber, 1992).

A preliminary experiment suggested that *cis*-UCA painted for example, onto the left ear of a mouse significantly reduced the number of $ATPase^+$ cells in epidermal sheets prepared from the right ear (909 ± 29.9 $ATPase^+$ cells mm^{-2} from left ear compared with 1068 ± 31 mm^{-2} from an untreated ear surface; mean of 20 fields of view ± sem). Subcutaneous or intradermal injection of physiological doses of *cis*-UCA also reduced the number of $ATPase^+$ cells in epidermal sheets prepared from murine ear surfaces 24 h following injection (709 ± 30.5 or 500 ± 24.4 from mice treated subcutaneously or intradermally respectively; mean of 20 fields of view ± sem). This study needs to be repeated but may indicate that *cis*-UCA itself leaves the epidermis, or induces another mediator in the epidermis, such as TNF-α, which may have an effect at a distant site. In contrast to the results presented in this chapter, Noonan *et al* (1985) reported that culturing epidermal sheets in the presence of *cis*-UCA for three days resulted in a reduction in Ia^+ cells but had no effect on the number of ATPase cells.

Previously it had been shown that suppression of DH to HSV resulted if mice were skin painted with *cis*-UCA before being infected with virus (Ross *et al.*, 1986). This effect was dose dependent, as little as 1 μg *cis*- UCA per mouse inducing reproducible suppression. To obtain information on the structural features necessary to generate suppression, a series of structural analogues of UCA isomers were

prepared and tested (Norval *et al.*, 1989). An unsubstituted 5-membered ring was required for suppressor activity while the acrylic acid side chain could be modified considerably or even replaced, as occurs in histamine, while still maintaining suppressor activity. Moving the position of the acrylic acid side chain abrogated the suppressive activity (Table 3.3), while the six membered rings were capable of inducing suppression (Table 3.4).

It is also of interest that histamine and UCA are closely linked metabolically, each being formed in a single step from histidine. It seemed possible, therefore, that UCA may occupy receptor sites similar to histamine. To begin testing this hypothesis, two histamine receptor antagonists (an H1 and an H2 antagonist) were used to find out whether they might alter the effects of *cis*-UCA on ATPase$^+$ cells in the epidermis or on the suppression of DH responses. Clearly both antagonists substantially reduce the effects of *cis*-UCA. The highly specific H3 receptor antagonist thioperamide had no effect on *cis*-UCA induced suppression of the DH response. Thus *cis*-UCA may act through histamine receptors on the surface of particular cells in the epidermis and elsewhere. This theory was substantiated by employing an H2 and an H3 agonist. No highly selective H1 receptor agonist exists (Hill 1990). As predicted, 4-methylhistamine, an H2 agonist, induced suppression of the DH response but (R)-α-methylhistamine, an H3 agonist, had no effect at all (Fig 3.6). Thus the effects of *cis*-UCA on immunity appear to be mediated through a histamine-like receptor, but it does not appear to bear any resemblence to the H3 receptor.

Previously, Reeve *et al.* (1989) reported that mice which were treated epidermally with UCA plus chronic UVR produced a greater number and more invasive UV-induced tumors than those chronically irradiated without UCA application. Further work by this group showed that mice treated with cimetidine which were chronically UV-irradiated suffered from fewer UV-induced tumours than unirradiated

control mice (Matheson and Reeve, 1991). These results also suggests that a histamine receptor may play an important role in UV-induced immunosuppression and that *cis*-UCA may interact with the receptor. It would be interesting to examine the effects of H receptor antagonists on UV-induced suppression of the DH response to HSV and reduction in epidermal ATPase cells.

Most preformed histamine is stored in the cytoplasm of mast cells and basophils. It is also produced *de novo* by histidine decarboxylase in adherent peritoneal cells and T cells. For many years histamine was mainly regarded as a vasoactive mediator which initiated inflammatory reactions, but it has become evident more recently that histamine is capable of regulating a variety of immune effector cells especially T cells and monocytes /macrophages by interacting with cell receptors or by recruiting them to particular local sites. Most effects are suppressive, resulting for example, in decreased antigen and mitogen induced lymphoproliferation, cytotoxic T cell activity and inhibition of IL-1, IL-2, IFN and TNF-α production (reviewed Falus and Meretey, 1992; Beer and Rocklin, 1987; Melmon and Khan 1987). The effect is likely to be dependent on the type of receptors and cells as well as on the local concentration of histamine in given circumstances.

In addition to the suppression of the DH response to HSV in the model presented in this chapter, histamine has been implicated in the suppression of DH responses in other circumstances. Serum histamine is increased in patients with active dermatitis (Ring 1989) and several investigators have reported that a suppression of CH responses in subjects with active atopic dermatitis (Jones *et al.*, 1973; Masami and Takayuk, 1989).

Some studies with cimetidine indicate its immunomodulatory properties. In addition to its inhibitory effect on gastric acid secretion, cimetidine increased DH responses in patients with duodenal ulcers, perhaps by preventing the normal inhibitory role of histamine acting via H2 receptors on T lymphocytes (Avella *et al.*, 1978). T

suppressor cells have been reported to produce a suppressor factor in response to histamine (Avella *et al.*, 1978) and cimetidine may block H2 receptors on such suppressor cells, thus augmenting DH responses (Jorizzo *et al.*, 1980). Recently, after oral administration to healthy volunteers, cimetidine has been shown to increase mitogen-induced proliferation of lymphocytes, possibly due to an increase of CD4 subset numbers and an increase in the functional ability of macrophages (Brockmeyer *et al.*, 1989). Furthermore, Belsito *et al.* have found that cimetidine enhanced the allergic contact hypersensitivity response in mice, if given at the time of sensitization (Belisto *et al.*, 1990). The increase was not due to effects on mast cells or epidermal antigen presenting cells (Langerhans cells or $Thy1^+$ dendritic epidermal cells), but to an inhibition of the induction of T suppressor cells at sensitization.

It was of interest in the present study that both an H1 and H2 receptor antagonist were able to block the activity of *cis*-UCA. This may be an indication that *cis*-UCA acts in more than one way, through more than one type of receptor. Indeed, there is already some indication of this possibility as *cis*-UCA applied epidermally before HSV infection induced two subclasses of T cells ($L3T4^+$ $Ly2^-$ and L3T4 $Ly2^+$) which suppressed the DH response to the virus, while transfer of epidermal cells from mice skin-painted with *cis*-UCA to naive mice at the time of HSV infection induced only one subclass of T suppressor cell ($L3T4^+$ $Ly2^-$) (Ross *et al.*, 1986, Ross *et al.*, 1987/88). One interaction could be directly through H2 receptors on T suppressor cells, as in the study of Belsito *et al.* (1990), which may be blocked by cimetidine. The second is likely to be local, through the epidermal antigen presenting cells, altering perhaps trafficking of cells, expression of surface markers or release of cytokines. Alternatively, a receptor is only as specific as the antagonists employed to characterise it, and it is conceivable that a distinct UCA receptor exists towards which the cimetidine and terfenadine have some degree of specificity.

In order to test this hypothesis, *in vitro* ligand binding assays need to be performed. Binding studies would require a population of cells which are known to express histamine and/or UCA receptors, preferably from the skin. Preliminary experiments indicate that *cis*-UCA treatment of purified murine LC abrogates their ability to present antigen to Th1 cells (Dr P D Cruz, personal communication). Little is known about the distribution of histamine receptors in the skin, particularly whether they are present on LC or not. It may be possible to select epidermal cells according to their receptor expression by for example, passing them through histamine-sepharose or UCA-sepharose colums. Alternatively, UCA isomers or histamine could be covalently linked to magnetic beads. It would be interesting to see if histamine and UCA columns or beads selected the same population of cells. In addition the relative potencies of the ligands used in the binding studies could be compared with their biological activity in an *in vitro* model of immunomodulation by *cis*-UCA. However, the mechanism of action of UCA appears to be complex and it is difficult to produce an *in vitro* model of suppression using *cis*-UCA. A sensitive reproducible system of *in vitro* modulation by *cis*-UCA would permit competition studies to be carried out.

A range of concentrations of antagonists, agonists and *cis*-UCA may have been employed in the experiments described in this chapter to further evaluate the role of a histamine receptor in *cis*-UCA induced immunomodulation.

CHAPTER 4

FURTHER STUDIES OF THE MECHANISM OF UVR OR *CIS*-UCA-INDUCED SUPPRESSION OF THE DH RESPONSE TO HSV

4.1 INTRODUCTION

Inbred strains of mice display varying susceptibilities to the immunosuppressive effects of UVR. Thus UVB-susceptible strains, such as C57 BL, display impaired CH responses when exposed to UVR prior to sensitization whereas other strains, such as BALB/c, when treated in exactly the same way, develop normal CH responses (Streilein and Bergstresser, 1988). There may also be a variation in genetic susceptibility to the immunosuppressive effects of UVR in humans (Yoshikawa *et al.*, 1990). In the mouse, susceptibility is restricted to polymorphisms at the *Tnfa* and *Lps* loci (Yoshikawa and Streilein 1990), although there may be at least one other locus involved. On UV-irradiation it is thought that UV susceptible strains produce TNF-α whereas resistant strains do not.

TNF-α, injected intradermally in mice, reduces the number of Ia^+ cells at the site of injection (Vermeer and Streilein 1990). In addition when TNF-α is injected intradermally at the site of hapten application, the effect of low dose UVB irradiation is again mimicked and a reduced CH response is subsequently produced on antigen challenge (Yoshikawa and Streilein, 1990). This phenomenon is strictly local and could be reversed by administering neutralizing TNF-α antibodies, as could the effect of UVB (Yoshikawa and Streilein 1990). It was therefore of interest in the present study to examine the role of TNF-α in UVB and *cis*-UCA induced suppression of the DH response to HSV.

As discussed in section 1.1.5, the elicitation phase of a CH response is biphasic with a substantial ear swelling occurring only two hours after antigen challenge, followed by the classical CH swelling 24-48 h later. In the following chapter the time course of the DH response to HSV was studied. Further experiments were conducted to determine whether UVR or *cis*-UCA had any effect on different phases of the elicitation response.

4.2 RESULTS

4.2.1 The Role of TNF-α in the Suppression of the DH Response to HSV Induced by UVB or *cis*-UCA

TNF-α has been implicated as a mediator of major importance in UV-induced modulation of immunity. The experiments in this section were designed to determine whether TNF-α played a role in UV-induced suppression of the DH response to HSV, and similarly whether it was important in the modulation induced by *cis*-UCA. Mice were pre-treated intraperitoneally with neutralizing anti-TNF-α antibodies two hours before either a single dose of UVB (96 mJ cm^{-2}) or subcutaneous injection of 100 μg *cis*-UCA (dissolved in PBS). Control mice received PBS intraperitoneally prior to *cis*-UCA or UVB. The mice were then infected subcutaneously with HSV three days after UVB or five hours after *cis*-UCA treatment and the DH response measured in the usual way (Fig 3.3). The mean data per group is outlined in Table 4.1 below. Pre-treatment with neutralizing TNF-α antibodies had no effect on the suppression of DH to HSV induced by *cis*-UCA but significantly reduced that induced by UVB.

Cis-UCA was injected subcutaneously in these experiments and as it is possible that the local concentration of *cis*-UCA within the epidermis is of importance. The effect of pre-treatment with anti-TNF-α on *cis*-UCA applied epidermally was tested in the DH model. The results are outlined in Table 4.2. It may be seen that UV-induced suppression of the initiation of the DH response was again abrogated if neutralising TNF-α was given prior to irradiation. Once more there was no effect on *cis*-UCA induced modulation, even when UCA was painted onto the epidermis.

Table 4.1

The Effect of Neutralising Antibodies to TNF-α on the Suppression of the DH Response to HSV Induced by UVB or cis-UCA. Mice were pretreated with neutralizing anti-TNF-α antibodies two hours before UVB or subcutaneous injection of cis-UCA. Mice were sensitized with HSV three days after UVB or five hours after cis-UCA and challenged 10 days later with inactivated HSV antigen. The mean data for groups of 6-8 mice are shown.

			EXPERIMENT 1			EXPERIMENT 2		
Neutra -lising TNF-α Abs	**Two hours later: treated with**	**Sensitized with**	**Increase in ear thickness (mm^{-2}) ± sem**	**p Compared with +ve**	**% suppre -ssion**	**Increase in ear thickness (mm^{-2}) ± sem**	**p Compared with +ve**	**% suppre -ssion**
—	PBS	Vero	4.4 ± 1.0	—	—	2.7 ± 0.8	—	—
—	PBS	HSV	13.7 ± 0.5	—	—	9.8 ± 1.1	—	—
—	96 mJ cm^{-2} UVB	HSV	4.4 ± 1.2	< 0.001	100	6.4 ± 0.9	< 0.05	47.9
—	100 μg *cis*-UCA	HSV	3.8 ± 1.5	< 0.001	100	4.0 ± 0.8	< 0.01	81.7
+	PBS	HSV	11.6 ± 1.1	NS	23.4	10.9 ± 0.7	NS	8.2
+	96 mJ cm^{-2} UVB	HSV	7.8 ± 0.8	< 0.001*	63.8	8.3 ± 1.2	NS	5.6
+	100 μg *cis*-UCA	HSV	3.9 ± 1.0	< 0.001$^{\Delta}$	100	4.8 ± 0.9$^{\Delta}$	< 0.01	70.4

* Significantly different from UVB alone ($p < 0.05$; paired t-test)

$^{\Delta}$ Not significantly different from *cis*-UCA alone ($p > 0.05$; paired t-test)

Abs: antibodies

Table 4.2

The Effect of Neutralising Anti-TNF-α Antibodies on cis-UCA Induced Suppression of the DH response to HSV. Following treatment with anti-TNF-α, mice were UV-irradiated, injected with 100 μg or skin painted with 100 μg of cis-UCA (in 100 μl of ethanol on a shaved back). The results represent the mean increase in ear thickness per group

Neutra-lising TNF-α Abs	Two hours later: Treated with	Sensitized with	Increase in ear thickness (mm x 10^{-2}) ± sem	p	% Suppre-ssion
—	PBS	Vero	1.5 ± 0.4	—	—
—	PBS	HSV	7.4 ± 0.8	—	—
—	96 mJ cm^{-2} UVB	HSV	3.3 ± 0.6	< 0.02	69.5
—	100 μg *cis*-UCA (s/c)	HSV	3.8 ± 0.6	< 0.01	61.0
—	100 μg *cis*-UCA (epid')	HSV	3.1 ± 0.6	< 0.001	72.9
+	PBS	HSV	6.8 ± 2.4	NS	11.0
+	96 mJ cm^{-2} UVB	HSV	6.4 ± 0.9	NS *	16.9
+	100 μg *cis*-UCA (s/c)	HSV	2.9 ± 1.0	< 0.01$^{\Delta}$	76.3
+	100 μg *cis*-UCA (epid')	HSV	1.4 ± 0.8	< 0.001$^{\Delta}$	100

s/c: injected subcutaneously. epid': applied to epidermis
* Significantly different from UVB alone ($p < 0.05$; paired t-test).
$^{\Delta}$ Not significantly different from *cis*-UCA alone ($p > 0.05$; paired t-test)
Abs: antibodies

Cis-UCA had previously been shown to produce a dose dependent suppression of the DH response to HSV, even at a dose as low as 1-10 μg per mouse. It was therefore possible that the dose of *cis*-UCA employed in the experiments above was too high for the anti-TNF-α to be effective. The following experiment was designed to confirm that anti-TNF-α had no effect on high or low doses of *cis*-UCA, applied subcutaneously or epidermally. High doses of UCA (100 μg per mouse) or low doses (10 μg per mouse) were applied subcutaneously or epidermally following treatment with a vehicle or TNF-α antibodies prior to infection. The results in Table 4.3 reveal that all groups were significantly suppressed relative to the positive control group, and in addition, prior treatment with TNF-α antibodies had no effect on *cis*-UCA induced suppression of the DH response.

Table 4.3

The Effect of Neutralising Anti-TNF-α Antibodies on Suppression of the DH Response to HSV Induced by Various Doses of cis-UCA

Neutra -lising TNF-α Abs	Two hours later: Treated With	Sensitize	Increase in ear thickness (mm x 10^{-2}) ± sem	p Compared with +ve	% suppre-ssion
—	PBS	Vero	0.4 ± 0.2	—	—
—	PBS	HSV	8.3 ± 0.9	—	—
—	10 μg *cis*-UCA (epid')	HSV	5.2 ± 0.9	< 0.05	39.2
—	10 μg *cis*-UCA (s/c)	HSV	3.8 ± 1.0	< 0.01	57.0
—	100 μg *cis*-UCA (epid')	HSV	3.2 ± 0.4	< 0.001	64.6
—	100 μg *cis*-UCA (s/c)	HSV	2.6 ± 0.4	< 0.001	72.2
+	PBS	HSV	8.9 ± 0.8	NS	-7.6
+	10 μg *cis*-UCA (epid')	HSV	3.8 ± 1.0	< 0.01$^{\Delta}$	57.0
+	10 μg *cis*-UCA (s/c)	HSV	1.1 ± 0.2	< 0.001$^{\Delta}$	91.1
+	100 μg *cis*-UCA (epid')	HSV	4.1 ± 1.1	< 0.02$^{\Delta}$	53.1
+	100 μg *cis*-UCA (s/c)	HSV	1.0 ± 0.4	< 0.001$^{\Delta}$	92.4

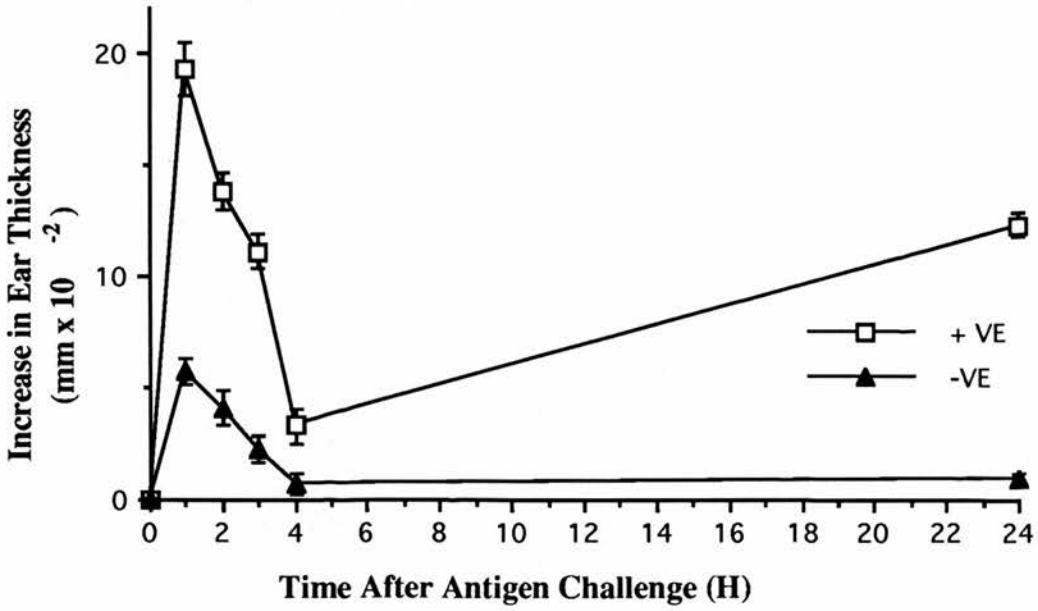
s/c: injected subcutaneously. epid': applied to epidermis
$^{\Delta}$ not significantly different from *cis*-UCA alone

4.2.2 The Time Course of the DH Response to HSV in Mice

The CH response in mice has been reported to be biphasic, with an early initial ear swelling response two hours after antigen challenge (Van Loveren *et al.*, 1983; Lavaud *et al.*, 1991). It is possible that a CH response to a sensitizing chemical and a DH response to a replicating virus are different and therefore the time course of a DH response to HSV was examined, taking ear measurements every hour following antigen challenge. The time course was carried out on three occasions and produced similar results on each occasion. The results from one experiment are shown in Fig 4.1.

Figure 4.1

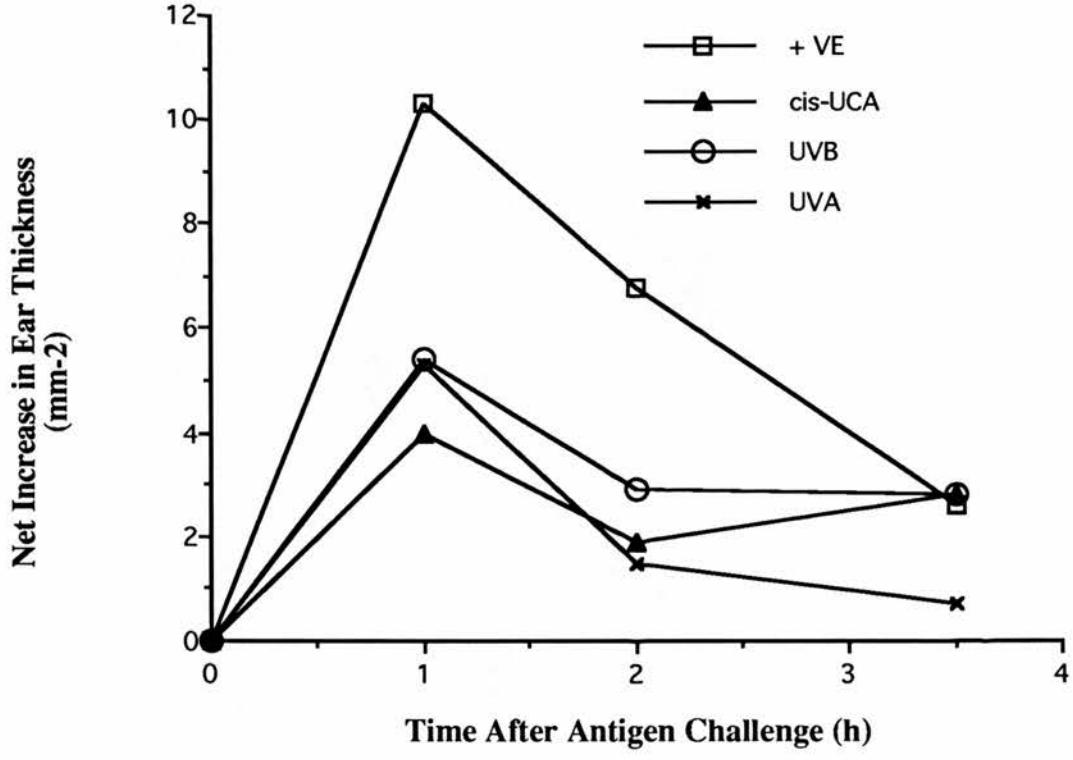
Time Course of the DH Response to HSV. Non-immune (sensitized with uninfected Vero cells) and HSV infected mice were challenged with inactivated HSV antigen 10 days after sensitization. Ear measurements were recorded for each mouse every hour for 4 h and also 24 h after challenge. The mean increase in ear thickness (± sem) for each group at each time is shown below.



A primary ear swelling response occurred one hour following antigen challenge during the DH response to HSV in mice. It was therefore of interest to determine whether UVR or *cis*-UCA treatment before infection with the virus suppressed this response in addition to the classical DH response noted previously. Mice were irradiated with 96 mJ cm^{-2} of UVB, 200 mJ cm^{-2} UVA or injected subcutaneously with 100 μg of *cis*-UCA prior to infection with HSV. Ten days later the ears were challenged with HSV antigen and ear measurements taken at 1, 2, 3.5 and 24 h after challenge. The full time course was determined on two occasions with UVA, twice with UVB and three times with *cis*-UCA. The results were similar on each occasion. The results from one such experiment are shown in Fig 4.2.

Figure 4.2

The Effect of UVR or cis-UCA on the Primary Ear Swelling in the DH Response to HSV. The graph represents the mean net ear swelling (test group minus the negative control group) for each group in the first 3.5 h following antigen challenge.



During subsequent DH experiments the ear measurements were determined 1h and 24 h following antigen challenge. The mean data from these experiments are illustrated in Table 4.4.

Table 4.4

The Effect of UVB or cis-UCA on the DH Response to HSV. The data illustrated in the table represent the mean net increase in ear thickness (test group minus back ground swelling in non-immune mice). The significance of differences from the positive group were tested by Student's t-test. In the case of the UVB group the UVB values were compared with the corresponding 5 positive controls within the same experiment.

Treatment	No. Experiments	1 h After Antigen Challenge: Increase in Ear Thickness (mm x 10^{-2})	p	24 h After Antigen Challenge: Increase in Ear Thickness (mm x10^{-2})	p
+ ve control	7	9.1 ± 1.3	—	8.6 ± 0.7	—
UVB	5	5.6 ± 1.2	0.012	3.6 ± 0.7	0.002
cis-UCA	7	4.5 ± 1.0	0.002	3.5 ± 0.6	< 0.0001
UVA	2	7.7 ± 2.4	ND	3.8 ± 3.1	ND

The results clearly indicate that both *cis*-UCA and UVB significantly suppress the one hour as well as 24 h ear swelling response to HSV.

Having established that UV and *cis*-UCA suppressed the one hour swelling response it would have been of interest to determine what cell populations were present at the site of antigen challenge in each of the groups at each stage of the DH response. Time did not permit detailed studies to be undertaken. One preliminary experiment was carried out using only two mice from one experiment to determine the number of $ATPase^+$ cells one hour and 24 h following antigen challenge. Having measured the thickness of the ear the mouse was killed, the ears removed, epidermal sheets were prepared and stained for ATPase activity. The samples were coded and 40 fields, chosen at random, were counted per mouse. The results are shown in Table 4.5.

Following antigen challenge there was a drop in the number of $ATPase^+$ cells in the epidermis at one hour which was more pronounced at 24 h. A greater number of $ATPase^+$ cells were found in the epidermis of mice which exhibited a suppressed DH

response i.e. those mice which have received UVA, UVB or *cis*-UCA prior to infection. These results merit further investigation.

Table 4.5

The number of ATPase$^+$ Cells Present in the Epidermis During a DH Response. Mice were pre-treated with 96 mJ cm^{-2} of UVB, 200 mJ cm^{-2} UVA or 100 µg of cis-UCA subcutaneously before infection with HSV. Ten days later the ears were challenged with HSV antigen. The mouse was killed, the ears removed and epidermal sheets stained for ATPase activity.

Pre-treated with	Sensitize (5h later)	ATPase$^+$ Cells (mm^{-2}) ± sem 1h After Challenge	% Reduction	ATPase$^+$ Cells (mm^{-2}) ± sem 24h After Challenge	% Reduction
PBS	Vero	926 ± 48.0	—	672 ± 26.4	—
PBS	HSV	731 ± 29.7	21.1	433 ± 34.0	35.5
UVA	HSV	696 ± 41.1	24.8	553 ± 36.5	17.7
UVB	HSV	804 ± 42.6	13.1	527 ± 37.2	21.6
cis-UCA	HSV	804 ± 40.8	13.1	587 ± 35.8	12.6

4.3 DISCUSSION

4.3.1 The Role of TNF-α in UVB and *cis*-UCA-Induced Suppression of the DH Response to HSV

The results outlined in Tables 4.1-4.3 demonstrate that neutralising anti-TNF-α antibodies administered to mice prior to UVR abrogate the suppression of DH to HSV induced by UVR. It was therefore surprising that the same treatment had no effect on *cis*-UCA induced suppression, which had been shown previously to be remarkably similar to UV-induced suppression of the DH response to the HSV (Ross *et al.*, 1986; Ross *et al.*, 1987; Ross *et al.*, 1987/88).

It is possible that the dose, the timing or route of application of *cis*-UCA relative to that of the anti-TNF-α administration in these experiments did not exactly correspond to the induction of *cis*-UCA produced following UVR *in vivo*. Two different doses of *cis*-UCA were administered via two different routes, epidermally and subcutaneously, but this had no effect on the outcome of the DH response.

It is also possible that UCA is not the only mediator of UV-induced immunosuppression, and may not act via a TNF-α dependent mechanism. Genetic susceptibility to UVR in inbred strains of mice is restricted to polymorphisms in the *Lps* and *Tnfa* loci (Yoshikawa and Streilein, 1990), therefore if *cis*-UCA was solely responsible for the immunomodulatory effects of UVR one might expect *cis*-UCA to have no effect on the induction of CH responses in UV-resistant strains of mice. However it was recently reported that intradermal injection of UV-irradiated UCA (containing approximately 50% of the *cis*-isomer) resulted in suppressed CH responses to dinitroflurobenzene (DNFB) in both UV-susceptible (C57BL and C3H/HeN) and UV-resistant strains (BALB/c and C3H/HeJ). *Cis*-UCA was more effective at producing suppression of the response in the UV-susceptible strains but did cause significant suppression in the UV-resistant mice at concentrations of 10 μg

of *cis*-UCA per mouse (Kurimoto and Streilein, 1992). Comparing the total UCA content of the ears of different strains of mice, housed in the departmental animal house, it was found that C3H (UV-susceptible strain), BALB/c and C57Bl contained 371, 396 and 182 ng mg^{-1} weight weight of UCA respectively. Therefore the UV resistant strain (BALB/c) did not have less UCA than the susceptible strains (M. Norval personal communication). It would be of interest to know if the rate of isomerization of UCA in UV-resistant and UV-susceptible strains of mice is the same following UVR.

It was also recently reported that UVB induced DC migration from the epidermis to the DLN but UCA did not (Moodycliffe *et al.*, 1992). Injection of recombinant TNF-α has also been shown to induce DC migration (Cumberbatch and Kimber, 1992) and pre-treatment with neutralising anti-TNF-α antibodies suppressed UV-induced migration of DC from the epidermis (A Moodycliffe personal communication). Thus it seems reasonable to suggest that UVR induces TNF-α production (Köck *et al.*, 1990; Oxenholm *et al.*, 1988) which induces DC migration and this in turn leads to altered antigen presentation and induction of suppressed immune responses to antigens encountered in the skin shortly thereafter. *Cis*-UCA, however may act through histamine-like receptors, as demonstrated in Chapter 3, which also results in suppressed immunity. This mechanism may be dependent on the dose or wavelength of UVR.

Kurimoto and Streilein (1992) recently reported that pre-treatment of C57BL/6 mice with neutralising anti-TNF-α antibodies partially reduced the suppression of CH responses to DNFB by *cis*-UCA. The value of the mice pre-treated with anti-TNF-α antibodies followed by treatment with *cis*-UCA was not significantly different from the positive control, but the statistics were not included to compare the value with mice which were treated with *cis*-UCA alone. A previous report indicated a complete recovery of the CH response in C3H/HeN mice which had been pre-treated

with anti-TNF-α prior to UVB-irradiation (Yoshikawa and Streilein, 1990). *Cis*-UCA was given intradermally in the study of Kurimoto and Streilein (1992) and either that or the different strains of mice may account for the difference in the results of that study and the present one. Alternatively there may be a difference between the DH response to HSV and a CH response, and the mechanism of UV-induced suppression of the different responses (Kripke and Morison, 1986). Further evidence in support of this theory was reported by Kim *et al.* (1990). In that study supernatants produced by UVB or UVA irradiated KC were selective in their ability to suppress CH and DH responses, suggesting that the two responses were indeed different, and the mechanism of UV-induced suppression of the two responses may occur by different mechanisms.

Both intradermal injection of TNF-α and UVB irradiation were shown to result in a reduction of the number of Ia^+ cells in the epidermis (Vermeer and Streilein, 1991). The effects of both UVB and TNF-α could be reversed be pre-treating the mice with neutralising anti-TNF-α antibodies (Vermeer and Streilein, 1991). Similarly intradermal injection of *cis*-UCA resulted in a reduction in the number of Ia^+ cells and those which remained had lost their dendritic processes (Kurimoto and Streilein, 1992). TNF-α antibodies blocked the reduction in the number of epidermal Ia^+ cells but had no effect on the retraction of the dendrites (Kurimoto and Streilein, 1992). Therefore UCA may have two distinct effects on epidermal cells: the first, the loss of Ia bearing cells from the epidermis, dependent on the production of TNF-α, and the second, being the retraction of the dendritic cell processes, which is independent of TNF-α. Thus UCA may have effects on LC which are independent of TNF-α and it is these effects which may have important implications for the induction of a DH response to a replicating virus.

Simon *et al* (1991) reported that TNF-α was capable of specifically suppressing the antigen presentation of keyhole limpet hemocyanin (KLH) by enriched epidermal LC

to KLH specific $CD4^+$ Th1 cells *in vitro* in a similar manner to UVB (Simon *et al.*, 1992). However anti-TNF-α did not protect LC from the loss of antigen presenting cell function induced by UVB or restore the ability of the cells to present antigen to KLH specific Th1 cells. Thus the authors suggested that the mechanism of impairment in the APC capacity of LC by TNF-α and UVB are different. Preliminary data indicates that *cis*-UCA is also capable of selectively abrogating the ability of purified LC to present KLH to Th1 cells (Dr P D Cruz, personal communication). It would be of interest to examine whether anti-TNF-α had any effect on the altered APC function induced by *cis*-UCA.

In addition it would be of importance to examine what effects, if any, UCA isomers have on TNF-α production. As outlined in Chapter 3 there is evidence that *cis*-UCA may act through a histamine-like receptor. Histamine has been reported to suppress the production of TNF-α via H2 receptors (Vannier *et al.*, 1991) and therefore it is possible that *cis*-UCA may also suppress the production of TNF-α by certain cell populations. Thus, within the skin, there may exist a balance between one effect of UVR, the induction of TNF-α, and another, the production of *cis*-UCA which under certain circumstances may suppress the production of TNF-α. Thus, UVB may mediate some of its suppressive effects via TNF-α but this is unlikely to be the only pathway by which UVR induces suppressed immune responses.

4.3.2 The Time Course of the DH Response to HSV

Mice which had been infected with HSV or sensitized with uninfected Vero cells ten days earlier were challenged with 10 µl of inactivated HSV antigen by intradermal injection of each ear pinna. Ear measurements, taken every hour thereafter, revealed that there was a significant ear swelling within one hour of antigen challenge. Ear measurements were not taken at earlier times as the 10 µl 'blip' formed by the injection of the HSV antigen may affect the background

measurements. One hour after antigen challenge there was no longer any visible evidence of the injected fluid.

These results are very similar to those reported by Van Loveren *et al.* (1983) who found an initial ear swelling response two hours following antigen challenge with a range of contact sensitizers in immune mice. However the peak response was detected earlier, one hour after challenge with HSV antigen compared with the CH response, which may indicate that there is a difference in the mechanisms involved in CH and DH responses. The initiator cells, responsible for the one hour swelling, produce antigen-specific non-IgE factors which sensitize mast cells for the release of the vasoactive amine serotonin at the local site of antigen challenge (Van Loveren *et al.*, 1983). 5HT initiates DH by acting on endothelial cells which allows the recruitment of the DH effector cells, responsible for the swelling at 24-48 h.

Examination of the initiator population, responsible for the one hour swelling, revealed that the cells were antigen specific but not MHC restricted, irradiation insensitive and induced within one day of the initial sensitization. This is in contrast to the later acting effector cell (at 24 h) which is antigen specific and also MHC restricted, irradiation sensitive and induced three to four days following initial sensitization (Van Lovern *et al.*, 1984).

Further studies revealed that the initiator cells were an extremely unusual phenotype for an antigen specific cell; Thy-1^+, $CD5^+$ (Ly-1^+), $CD4^-$, $CD8^-$, $CD3^-$, sIg^-, $CD45RA^+$ (not found on mature T cells), $Mac1^+$, IL-$2R^-$ and Il-$3R^+$ (present on pre-B and pre-T cells) (Herzog *et al.*, 1989). The recruited effector cells (at 24 h), in contrast to the initiator population were $Thy1^+$, $CD5^+$, $CD4^+$, $CD8^-$ and $CD3^+$ (Herzog *et al.*, 1989). Therefore, it has been proposed that the DH response consists of at least two separate, but connected cascades, with local 5HT release being the connecting factor. It is possible that the DH initiating cells are activated locally and do not circulate.

Mukherjee *et al.*, 1986 reported that mice challenged with purified protein derivative of tuberculin (PPD) four days after sensitization also exhibited a DH-initiating response one hour following antigen challenge, which was followed by the classical DH response 24 h later. These authors also found that splenic DC which were pulsed with PPD did not sensitize for DH. PPD-pulsed splenic DC induced antigen specific/MHC-restricted late acting T cells, but not the DH-initiating cells. When mice were sensitized with PPD-pulsed DC followed by PPD-pulsed macrophages PPD-specific DH initiating cells were induced within one day which permitted the expression of the late acting DH cells induced by the DC.

It was therefore interesting to find that *cis*-UCA and UVB suppressed the initial one hour peak and also the 24 h peak . Thus UVR or *cis*-UCA prior to infection with HSV reduces the number of functioning DH initiating cells in the skin. It may be that UV or *cis*-UCA alters the macrophage/DC population of the epidermis resulting in modified sensitization of the DH initiating cell. In humans there is evidence that the epidermis is re-populated with a macrophage population following UV-irradiation (Baadsgaard *et al.*, 1990c; Cooper *et al.*, 1985) and there is also preliminary evidence that this may also occur in the mouse (Duraiswamy *et al.*, 1992). It is possible that there is an alteration in the migration of T cells to and from the skin following UVB or *cis*-UCA treatment which may be due to an altered expression of adhesion molecules or homing receptors or other mechanisms, perhaps as a result of altered cytokine production.

Further work is required to determine whether UV or *cis*-UCA alters the number of DH initiator cells present at the time of infection and subsequently the number of sensitized T cells following the primary HSV infection, or activated on subsequent antigen challenge. In the model of DH response to HSV employed in this study, infection was subcutaneous. Therefore it would be of interest to determine whether the DH initiator cell populations are found in the LN, whether they are actively

recruited to this site and if they preferentially home to the skin. The results in Table 4.5 indicate that there may be a reduced number of ATPase$^+$ cells which migrate from the epidermis on antigen challenge in the groups which exhibited a suppressed DH response i.e. mice which had been UV-irradiated or treated with *cis*-UCA prior to HSV infection. It would also be of interest to examine other cell populations present during the elicitation phase of the DH response in mice which had been pre-treated with UVR or UCA isomers. These populations might include T cell subsets, macrophages and mast cells.

CHAPTER 5

THE EFFECT OF UCA ISOMERS ON ANTIGEN PRESENTATION BY MURINE EPIDERMAL CELLS OR PERITONEAL MACROPHAGES

5.1 INTRODUCTION

Having established that *cis*-UCA reduced the number of epidermal ATPase$^+$ cells (Chapter 3), and to complement the studies examining the effects of phototherapy on epidermal antigen presentation in humans reported in Chapter 6, the following study aimed to examine the effect of UCA isomers on antigen presentation of Con A and HSV by macrophages and LC to T lymphocytes in a murine model. The initial experiments were designed to establish the optimal conditions for murine lymphoproliferation assay and to examine the effect of treatment *in vitro* or *in vivo* with UCA isomers on responses to Con A or HSV. Subsequently, a model of antigen presentation of HSV or Con A to T cells was developed. Peritoneal macrophages (PM), splenic adherent cells (AC) or epidermal cells (EC) were tried as antigen presenting cells (APC) with the ultimate aim of determining the effect of UCA isomers *in vitro* and *in vivo* on the ability of the cells to present antigen.

5.2 RESULTS

5.2.1 Optimization of the Lymphoproliferation Assay

Initial experiments were performed to optimize the conditions for *in vitro* LPR using Con A as a mitogen in whole LN or spleen cell suspensions. Mice were killed, LN and spleens collected into PBS and single cell suspensions prepared. The cells were finally resuspended at a concentration of 5 x 10^5 cells ml^{-1}. Two hundred µl of cells were placed in each well of a flat bottomed 96 well microtitre plate. To quintuplicate wells Con A was added at concentrations ranging from 0 to 20 µg ml^{-1} (final concentration). The plates were incubated for three to five days pulsing with ^{3}H-thymidine over the final 24 h of culture. Variations of this experiment were set up on five different occasions and suggested that in the spleen cell cultures 5-7 µg ml^{-1} of Con A and four days of culture resulted in optimal uptake of ^{3}H-thymidine. However results were highly variable, and significant proliferation of LN cells was measured on only one occasion. Collecting the LN into TCM plus 10% FCS did improve the responses of the cells.

Further manipulations of the system involved using different dimensions of plates, sources of serum in the TCM and cell numbers (Table 5.1). The number of cells appeared to be crucial to the proliferation; thus 10^5 cells per well was not sufficient but 2 x 10^5 was. Proliferation was much greater in round bottomed plates compared with flat bottomed (Table 5.1). A number of different serum sources, at varying concentrations, were employed some of which are outlined in Table 5.1. The results shown in Table 5.1 are those of LN cells but a similar pattern was obtained using splenic cells.

In addition to the experiments using Con A mice were infected with HSV epidermally, followed by a secondary subcutaneous infection. Ten days after the secondary, infection spleens and LN were removed and the lymphoproliferative

response (LPR) *in vitro* to UV-inactivated HSV measured. Similar results to those outlined in Table 5.1 were obtained. The assay was very sensitive to the source of serum; FCS at 10% resulted in much more efficient proliferation than newborn calf serum (NBCS) or horse serum (HS) which had previously been employed in the laboratory. Different batches of serum from the same company also varied in their ability to support a proliferative response. Thus a batch of FCS which resulted in optimal incorporation of ^{3}H-thymidine was selected (Gibco Ltd., Paisley, UK).

Table 5.1

Optimization of the Conditions for the Mitogenic Response of LN Cells to Con A

Serum Concentration	**Dimension of Plate**	**Number of Cell per Well**	**Nothing Added Mean cpm (± sem)**	**7 μg ml^{-1} Con A Mean cpm (± sem)**
5% FCS (1)	Flat	1 x 10^5	182 ± 21.3	1994 ± 101
10% FCS (1)	Flat	1 x10^5	304 ± 125	350 ± 92.7
5% FCS (1)	Flat	2 x 10^5	416 ± 44.3	353 ± 43.9
10% FCS (1)	Flat	2 x 10^5	313 ± 41.2	11 635 ± 2887
5 % FCS (1)	Round	2 x 10^5	90.6 ± 17.6	634 ± 169
10 % FCS (1)	Round	2 x 10^5	771 ± 167	127 669 ± 17807
5% FCS (2)	Flat	2 x 10^5	203 ± 48.6	884 ± 119
10 % FCS (2)	Flat	2 x 10^5	443 ± 62.1	146 863 ± 4 598
5% HS (1)	Flat	2 x 10^5	174 ± 27.7	2 998 ± 1465
10% HS (1)	Flat	2 x 10^5	97.6 ± 11.4	13 548 ± 4 254
5% HS (2)	Flat	2 x 10^5	122 ± 25.5	237 ± 79
10% HS (2)	Flat	2 x 10^5	236 ± 32.0	319 ± 119
5% NBCS	Flat	2 x 10^5	106 ± 17.7	22 585 ± 4 704
10% NBCS	Flat	2 x 10^5	178 ± 21.4	73 616± 15 529

(1): serum from Northumbria Biologicals, UK
(2): serum from Gibco Ltd., Paisley, UK

However the assay system did give highly variable results and was plagued by contamination of cultures. The contaminating organism was identified as a *Pseudomonas* species which was resistant to penicillin, streptomycin and gentamicin,

which was likely to have originated from the mice. It was sensitive to carbenicillin, and this antibiotic was subsequently incorporated into the TCM (100 μg ml^{-1}) used for all murine assays. This was sufficient in most experiments to inhibit the growth of the organism.

5.2.2 The Effect of *cis*-UCA *in vitro* on Lymphoproliferative Responses to HSV or Con A.

UCA isomers at varying concentrations were added to lymphoproliferation assays for the duration of the culture and the effect on the response to Con A or HSV determined. *Cis*- and *trans*-UCA were both dissolved in DMSO at 10 mg ml^{-1}, and diluted further in TCM. An appropriate volume of DMSO in TCM was always added to parallel cultures as a control. This experiment was carried out on four separate occasions using both LN and spleen cells. The addition of 1, 10 or 100 μg ml^{-1} of either UCA isomer to either LN or spleen cells did not result in consistent reproducible effect on the *in vitro* lymphoproliferative response to Con A or HSV. Table 5.2 illustrates the data from two experiments using spleen cells.

Table 5.2

The Effect of cis-*UCA* in vitro *on Lymphoproliferative Responses Using Spleen Cells*

Stimulus	Additions to Culture	Experiment 1 Mean cpm (±sem)	Experiment 2 Mean cpm (±sem)
Vero	DMSO Vehicle (2 µl)	11 117 ± 910	8 098 ± 893
Con A	DMSO Vehicle (2 µl)	89 597 ± 13 326	87 003 ± 18 922
HSV	DMSO Vehicle (2 µl)	62 549 ± 9 056	76 232 ± 5 080
Vero	1 µg ml^{-1} *cis*-UCA	25 952 ± 2 111	8 452 ± 1 356
Con A	1 µg ml^{-1} *cis*-UCA	79 924 ± 5 429	80 268 ± 5 908
HSV	1 µg ml^{-1} *cis*-UCA	49 558 ± 4 575	89 027 ± 2 627
Vero	DMSO Vehicle (10 µl)	15 300 ± 1 956	8 231 ± 521
Con A	DMSO Vehicle (10 µl)	104 634 ± 3 492	83 527 ± 9 074
HSV	DMSO Vehicle (10 µl)	45 501 ± 12 057	45 801 ± 2 641
Vero	10 µg ml^{-1} *cis*-UCA	12 342 ± 2 182	4 026 ± 518
Con A	10 µg ml^{-1} *cis*-UCA	100 930 ± 3 891	79 627 ± 5 123
HSV	10 µg ml^{-1} *cis*-UCA	12 314 ± 9 385	68 209 ± 5 807

5.2.3 The Effect of Treatment with UCA Isomers *In Vivo* on Lymphoproliferative Responses

Mice were injected subcutaneously with 100 µg of *cis*- UCA five hours before subcutaneous infection with HSV. Control mice were pre-treated with the appropriate dilution of DMSO. Ten days after infection the mice were killed, LN and spleens removed and single cell suspensions prepared. The effect of pre-treatment with *cis*-UCA on LPR to HSV using LN cells is shown in Table 5.3; similar results were obtained with spleen cells. Experiment one indicated that pre-treatment with *cis*-UCA *in vivo* suppressed LPR. However this experiment was conducted on four further occasions and the result was not consistent as shown in Experiment 2 below.

Table 5.3

The Effect of cis-*UCA on LPR to HSV or Con A. Mice were injected with* cis-*UCA five hours before infection with HSV. Ten days later the LN and spleens were removed and the* in vitro *PLR to HSV or Con A measured. The results below represent the mean value of ten wells using LN cells.*

Stimulus	Pre-treatment	Experiment 1		Experiment 2	
		Mean cpm (± sem)	% Suppr-ession	Mean cpm (± sem)	% Suppr-ession
Vero	Vehicle	6968 ± 463	—	729 ± 122	—
Con A	Vehicle	41237 ± 1812	—	44 180 ± 9 579	—
HSV	Vehicle	10527 ± 659	—	1 803 ± 345	—
Vero	100µg *cis*-UCA	3803 ± 236	45.4	1 193 ± 481	0
Con A	100 µg *cis*-UCA	12443 ± 849	69.8	21 706 ± 11 133	50.9
HSV	100 µg cis-UCA	3458 ± 286	67.2	2 648 ± 526	0

5.2.4 Antigen Presentation of HSV or Con A by Epidermal Cells

A number of experiments were conducted to examine the effects of *cis*-UCA *in vitro* and *in vivo* on antigen presentation. These experiments were highly variable, largely due to contamination problems and inconsistent LPR of whole spleen and LN preparations. The inconsistency of the LPR made it impossible to examine suppression of the response, or antigen presentation to TEC derived from whole preparations which themselves did not respond to the antigen. The mice suffered from eye infections during this time period and it is possible that many of the problems encountered in this section was due to the poor condition of the animals used in the system. (Parallel cultures of human PBMC populations, employing the same culture medium were free of contamination and responded to the antigen preparations as shown in Chapter 6). Therefore despite desperate attempts (at least 15 experiments) to study the effects of *cis*-UCA on antigen presentation in an *in vitro* LPR no reproducible results were obtained.

5.3 DISCUSSION

As discussed in section 1.3.4, UVR causes profound changes in the morphology and numbers of epidermal LC. This may result in altered antigen presentation, which may in turn, lead to suppression of selected immune responses to antigens encountered shortly after irradiation. The results outlined in Chapter 3 indicate that *cis*-UCA induces similar morphological damage of epidermal ATPase$^+$ cells as that after irradiation, and therefore it was of interest to determine whether similar functional changes in epidermal antigen presentation take place following treatment with *cis*-UCA. Unfortunately the question remains unanswered, as enormous difficulties were experienced in developing a consistent and sensitive assay which were not overcome.

If a reliable *in vitro* lymphoproliferation assay system in response to HSV could have been established, a number of important questions could have been answered. It would be of interest, for example, to establish whether APC treated (*in vitro* or *in vivo*) with *cis*-UCA have the same capacity to present antigen as APC treated with *trans*-UCA. Intravenous injection of mice with *cis*-UCA (50-100 μg) resulted in suppressed antigen presentation by splenic DC seven days after injection (Noonan *et al.*, 1988). However, after *in vivo* treatment with *cis*-UCA it would be possible to find out whether any effect on antigen presentation was restricted to the site of *cis*-UCA application, or if it was systemic. It was planned to study not only the total proliferative response of T cells but also to investigate which subsets of cells responded. This could be done by phenotyping the proliferating cells with monoclonal antibodies to CD4 or CD8 and analysing by flow cytometry. In addition by assaying the cytokines produced by the cells *in vitro*, it would be possible to distinguish Th1 (which produce Il-2 and IFN-γ) from Th2 cells (which produce IL-4).

It has been reported that purified hapten derivatised LC, which are irradiated *in vitro,* induce anergy of Th1 cells, such that on subsequent stimulation with hapten derivatised LC which have not been UV-irradiated, no proliferation was detected (Simon *et al.,* 1992). *Cis*-UCA may also induce anergy and preliminary evidence from P. Cruz (personal communication) indicates that this may be the case. In these experiments FACS purified, hapten derivatised LC which were pulsed with *cis*-UCA *in vitro* preferentially induced proliferation of Th2 clones, and induced anergy in Th1 clones.

This area of study merits further investigation.

CHAPTER 6

THE EFFECT OF PHOTOTHERAPY ON IMMUNE FUNCTION IN PATIENTS WITH PSORIASIS

6.1 INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder which is characterised by hyperproliferation of the epidermis and accumulation of inflammatory cells, particularly T lymphocytes, monocytes and neutrophils. There are many clinical forms of the disease, chronic plaque psoriasis being the most common (Fig 6.3). With few exceptions the patients studied in this chapter, and also Chapter 7, were suffering from chronic plaque psoriasis. Details in this introduction are confined to chronic plaque psoriasis.

Psoriasis is a multifactorial disease which affects approximately two percent of the population of Western countries. It affects all races, males and females equally. The exact aetiology of the disease remains a controversial issue but there is good evidence that it is an inherited disorder. It is significantly more prevalent amongst relatives of psoriatics, and twin studies reveal a 72% concordance between monozygotic twins (Brandrup *et al.,* 1978). A census study carried out in a closed community in the Faroe Isles reported a 91% family occurrence in patients with psoriasis (Baker and Fry, 1992). HLA typing of psoriasis patients revealed an increased expression of certain class I antigens including B13, B17, B37 and CW6, and also the class II antigen DR7 compared with non-psoriatic individuals (Russell *et al.,* 1972). As well as a genetic predisposition to psoriasis a number of environmental factors may be also involved. These include epidermal injury (Koebner phenomenon), streptococcal infections, stress and certain drugs. Circumstantial evidence indicates that the immune system plays an important role in the pathogenesis of psoriasis (reviewed by Baadsgaard *et al.,* 1990a; Baker and Fry, 1992; Barker, 1991; Nickoloff, 1991a).

Activated T lymphocytes (ie IL-2 receptor$^+$ HLA-DR$^+$) are found both in the epidermis and dermis of active psoriatic plaques (Baker *et al.,* 1984; Gottlieb *et al.,*

1986; Gottlieb, 1988). Clinically uninvolved psoriatic skin also contains an increased number of activated T cells. Resolution of plaques has been associated with the appearance of HLA-DR$^+$ CD8$^+$ T cells in the epidermis (Baker *et al.*, 1984). A population of T cells which bear the UM4D4 surface marker has been isolated from psoriatic plaques; this molecule is expressed on a minority (20%) of peripheral blood T cells but on the majority (75%) of T cells in lesional skin. The monoclonal antibody to UM4D4 consistently induces proliferation of UM4D4 T cell clones (Baadsgaard *et al.*, 1990c). The mechanism of T cell activation in psoriasis remains unknown.

The interaction between lymphocytes and epidermal cells, mediated by cytokine production and/or intercellular adhesion, is likely to play a key role in the induction and perpetuation of psoriatic lesions. Interleukin-8 protein, which is not found in normal epidermis, is present within the psoriatic scale and epidermis (Nickoloff *et al.*, 1991). Il-6 is also increased in psoriatic skin (Grossman *et al.*, 1989). Il-6 and Il-8 are both stimulatory for KC proliferation *in vitro* (Grossman *et al.*, 1989), and in addition IL-8 is a potent T cell and neutrophil chemoattractant (Nickoloff *et al.*, 1991). It is also interesting to note that Il-1α activity is decreased and nonfunctional Il-1β is increased in psoriatic epidermis compared with normal skin (Cooper *et al.*, 1990). TGF-α and its receptor are also enhanced in psoriatic epidermis (Elder *et al.*, 1989; Nanney *et al.*, 1986). TGF-α is produced by, and is required for growth by, epithelial cells, and therefore may also be important in stimulating growth of the epidermis in psoriasis.

IFN-γ, a product of activated T cells, has been identified in the serum and suction blister fluid from psoriatic lesions (Gomi *et al.*, 1991). IFN-γ has a range of immunological activities. Of particular interest in psoriasis is its ability to induce ICAM-1 expression on KC. The presence of epidermal T lymphocytes, which express the LFA-1 ligand, correlates well with the expression of ICAM-1 and

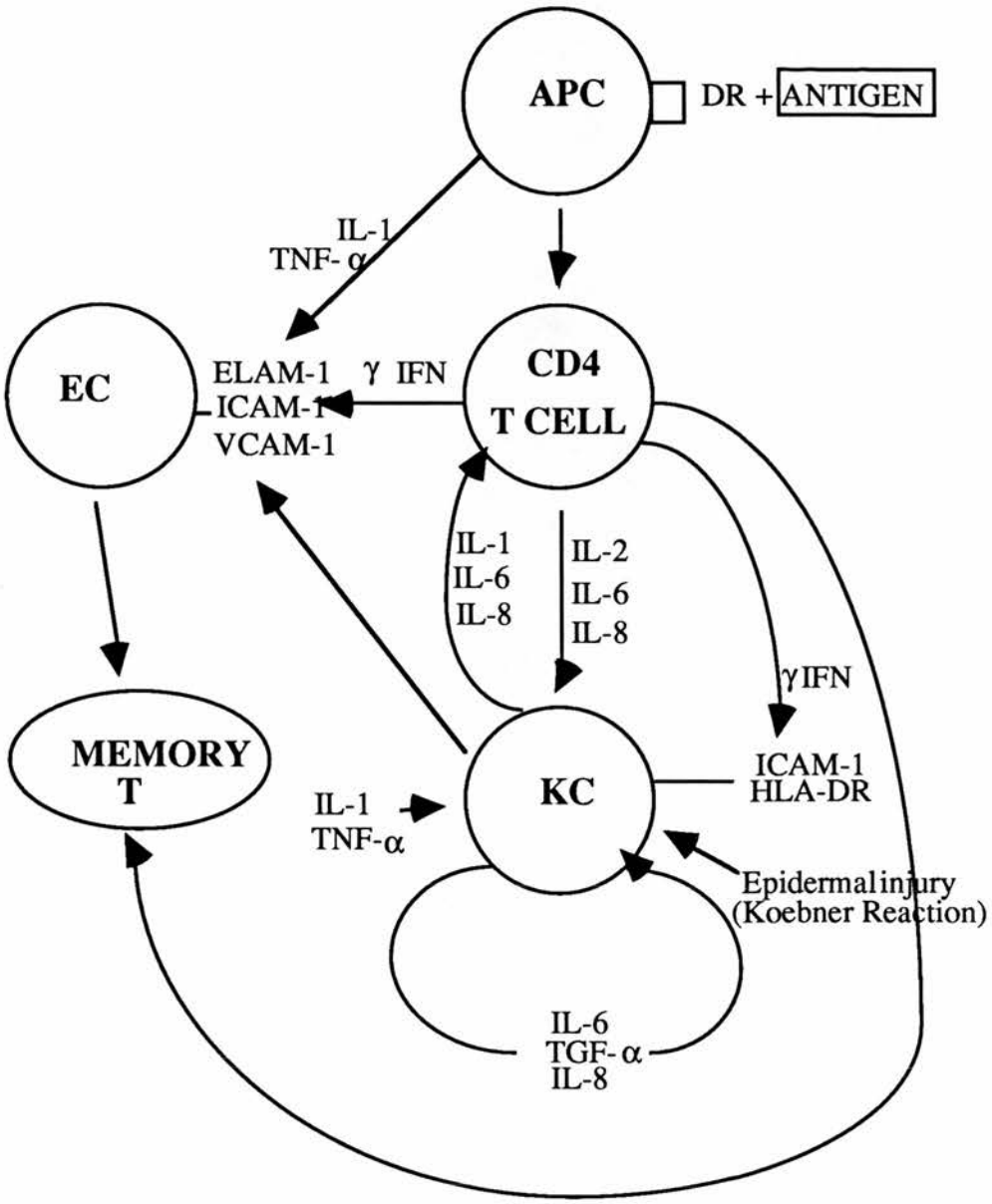
therefore IFN-γ may be important in the trafficking of T cells into the lesional epidermis (Griffiths *et al.*, 1989). Psoriatic KC may have a decreased response to locally produced IFN-γ. For example, psoriatic KC do not appear to be as susceptible to the growth inhibitory effects of IFN-γ (Baker *et al.*, 1988; Barker *et al.*, 1991) or the expression of HLA-DR (Barker *et al.*, 1991; Terui *et al.*, 1987) as normal KC.

Nickoloff *et al.*, (1991) proposed TNF-α as a key mediator in psoriasis. It is produced by dermal dendrocytes, keratinocytes and epidermal LC, and induces IL-8, ICAM-1 and TGF-α production by KC. Nickoloff *et al.* (1991) further demonstrated the expression of IL-8 on KC immediately above the TNF-α positive dermal dendrocytes. However TNF-α is inhibitory for normal KC proliferation (Detmar and Orfanos, 1990; Pillai *et al.*, 1989) and injection of TNF-α into the mouse tail model of psoriasis resulted in a reduction of the mitotic index and formation of the granular layer (Nagano *et al.*, 1980). Furthermore in a recent study, systemic administration of TNF in three psoriasis patients resulted in a substantial resolution in two patients who had extensive psoriasis recalcitrant to therapy (Takematsu *et al.*, 1991).

It remains to be determined whether any dysregulation in cytokine production is a primary cause or a result of the disease process. Fig 6.1. (adapted from Baker and Fry, 1992) represents a possible model for the role of the immune system in the pathogenesis of psoriasis. However it is also possible that the defect lies in the response of KC to stimulation by cytokines other than IFN-γ or that altered antigen presentation by psoriatic EC may result in epidermal T cell activation and the epidermal hyperproliferation characteristic of the disease (Baadsgaard *et al.*, 1989; Demidem *et al.*, 1991).

Figure 6.1

A Model of the Immunopathology of Psoriasis. Presentation of antigen by LC, for example, to T cells leads to the synthesis of ctytokines which stimulate KC proliferation and expression of adhesion molecules by KC and endothelial cells. KC in turn are stimulated to secrete their own cytokines, which may maintain the psoriatic process.



UVR is widely employed by dermatologists in the treatment of a number of dermatosis, one of the most common being psoriasis. Patients usually receive whole body irradiation with either UVB or UVA (plus oral psoralens; PUVA) in cabinets shown in Fig 6.2. UVB and PUVA therapy can both be very effective in the treatment of psoriasis as illustrated in Fig 6.3 (Henseler *et al.*, 1981, Melski *et al.*, 1977). These treatments are largely empirical and little is known of their mechanism of action. It is possible that they may act, at least in part, by modulating immune responses.

As discussed in Chapter 1, in animal models, UVR results in suppression of selected immune responses to antigens encountered shortly after exposure. The immunological consequences of UV irradiation in man are much less well characterized (reviewed by Baadsgaard, 1991; Morison, 1989) and have been confined mainly to suppression of contact sensitivity responses at UV-exposed sites (Friedman *et al.*, 1989; Halprin *et al.*, 1981; Hersey *et al.*, 1983a; Sjoval and Christensen, 1986; Yoshikawa *et al.*, 1990). However, a recent report indicates that theUVR-induced suppression of CH may be systemic, if the dose is erythemogenic (Cooper *et al.*, 1992). It is well recognised that UV irradiation produces transient alterations in the numbers, ultrastructure and morphology of LC (reviewed by Cruz and Bergstresser, 1991). This is associated with suppressed *in vitro* alloactivation by epidermal cells (Cooper *et al.*, 1985) and reduced antigen presentation (Ashworth *et al,* 1989; Austad and Braathen, 1985). There is evidence for re-population of human skin with APC which preferentially activate suppressor cells (Baadsgaard *et al.*, 1990b). In addition to its effects on APC, UV irradiation may alter circulating human T cell subsets temporarily, but the nature and magnitude of such changes are variable. The majority of work in humans has also employed UV regimens which bear little resemblance to those used therapeutically.

Fig 6.2

A Waldmann Upright Cabinet as Used For Whole Body Irradiation in Phototherapy

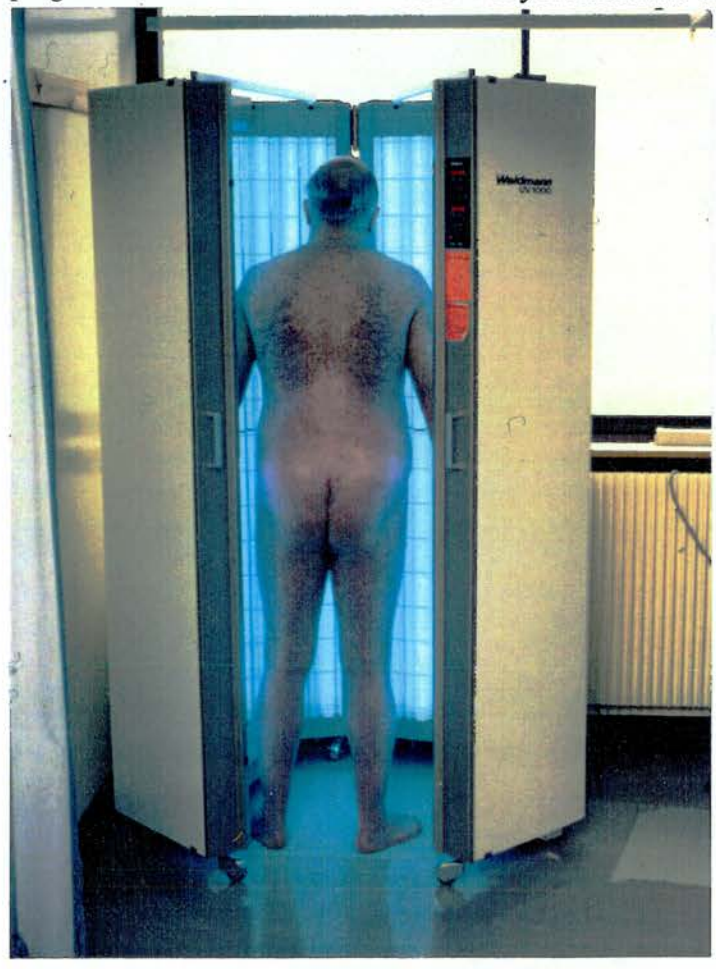
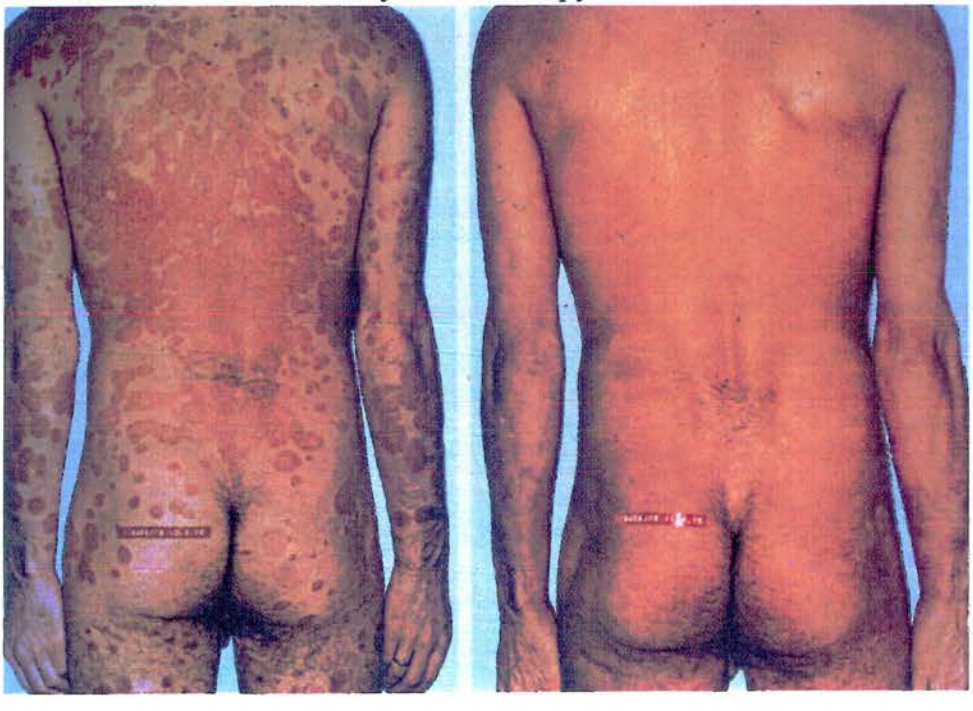


Fig 6.3

The Efficacy of Phototherapy. The right panel shows a patient with chronic plaque psoriasis before therapy and the left panel shows the same subject following a course of PUVA therapy



The aim of the work described in this chapter was to examine the effects of standard courses of UVB or PUVA therapy on various parameters of systemic and cutaneous immunity in patients with psoriasis. These were compared with psoriasis patients receiving topical coal tar dressings. In addition, where possible immune parameters assayed in psoriatic subjects were compared with those in normal individuals. The immunological parameters monitored included:

(i) several humoral components : circulating autoantibody profiles, immunoglobulin isotypes and specific HSV antibody titres, complement C3, C4 levels and activity (CH50)
(ii) full blood counts and the relative percentages of circulating PBMC subsets
(iii) the LPR to the mitogen Con A and to HSV using AC from peripheral blood and EC from suction blister roofs as antigen presenting cell (APC)
(iv) the total UCA content and the relative % of *cis*-UCA in the epidermis and suction blister fluid
(v) TNF-α content of suction blister fluid.

HSV was chosen as a convenient test antigen since about 80% of adults show acquired immunity and, in addition, exposure to UV is an important factor in HSV pathogenesis in a high proportion of latently infected subjects (Spruance *et al.*, 1991; Vestey *et al.*, 1989).

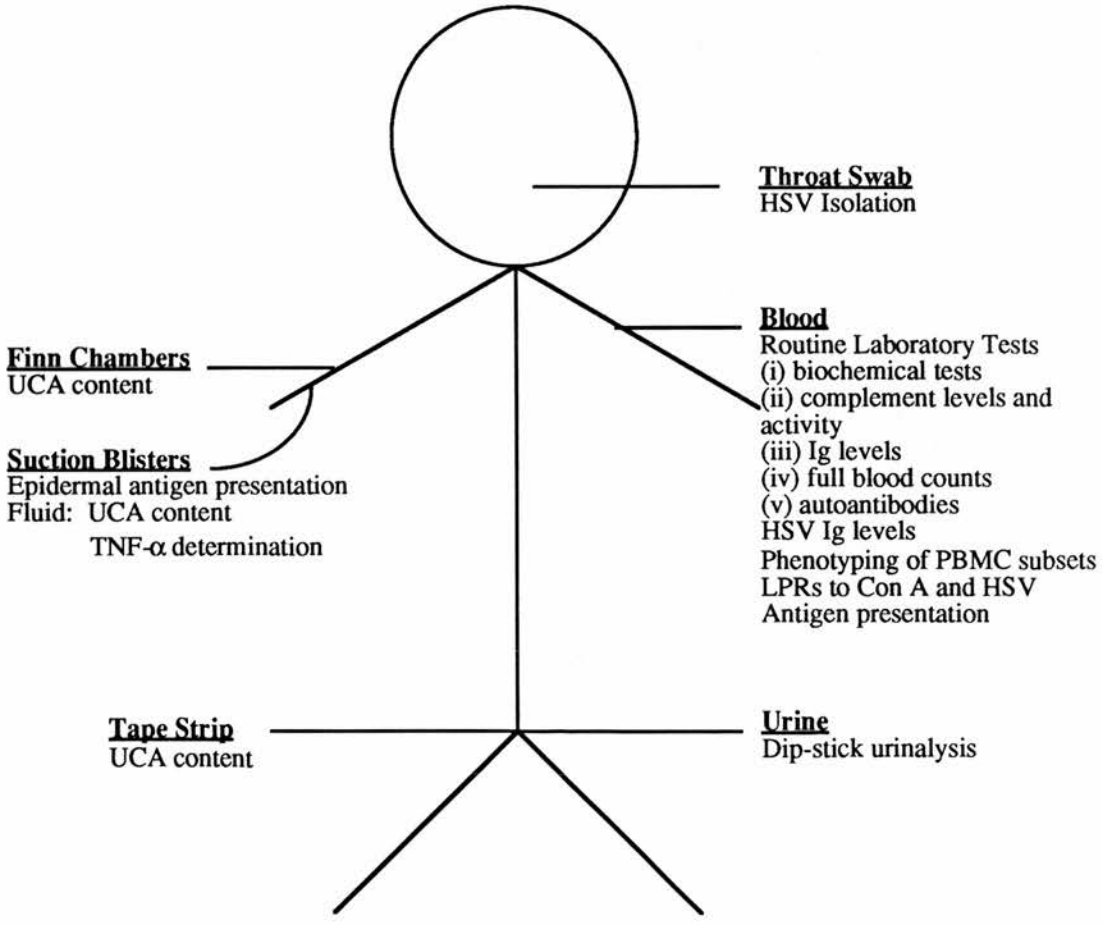
6.2 RESULTS

6.2.1 Study Design and Clinical Details

Three groups of patients with chronic plaque psoriasis were studied, one group received UVB therapy, one group received PUVA therapy and the control group consisted of subjects receiving topical coal tar dressings. In most instances samples of blood, urine, epidermis and oropharyngeal secretions were collected. The sample times and the assays performed are illustrated in Fig 6.4.

Figure 6.4.

Study Design. Patients were recruited on the basis of a positive LPR to HSV. The samples outlined below were collected in the early morning, immediately before commencing treatment, following four weeks of treatment (48 h after irradiation) and in patients receiving UVB or PUVA again four weeks after the final irradiation. Throat swabs, plasma, suction blister fluid, Finn Chambers and tape strips were placed at -70 ℃ and stored there until analysis.



At each visit the patients were assessed and the Psoriasis Area and Severity Index (PASI) calculated. In addition at each visit routine tests, including full blood counts, biochemical profiles, liver function and dip stick urinalysis, serum immunoglobulin isotypes, complement levels (C3, C4, and CH50) and auto antibody profiles were determined in the Royal Infirmary of Edinburgh hospital laboratories. The presence of HSV-specific antibodies in plasma were determined by ELISA (see section 2.3.10).

In addition, the relative percentages of PBMC subsets was also assessed in the patients described in Chapter 7 (28 subjects in total). The subjects discussed in Chapter 7 who attended in Edinburgh also underwent the same routine laboratory tests and epidermal UCA analysis studies carried out as those patients described here (ie group A, five patients receiving coal tar treatment; group B, six subjects receiving UVB; group F, four normal individuals receiving a standard course of UVB received by psoriasis subjects, with the exception that only their arms were exposed and the rest of their bodies remained draped). The normal subjects receiving a standard course of UVB had blister roofs raised on their arms before commencing UV and 48 h after the last irradiation (approximately 6 weeks) as part of another study but it was possible to collect blister fluid for UCA and TNF-α analysis from them for use in this study. The data obtained from these subjects are also included in the relevant sections in this chapter and only their NK cell study results are described in Chapter 7.

Table 6.1 summarizes the clinical details of the subjects investigated in this chapter. Although the absolute dose of UVR received varied between patients, a clinical improvement in psoriasis and a reduction in PASI occurred during treatment in every case. The routine laboratory profiles performed at each visit (full blood counts, plasma urea, glucose and electrolytes, liver function, dip-stick urinalysis,

autoantibodies) were within normal limits for each subject and their values were not influenced by treatment (data not shown).

Serum immunoglobulin isotypes and complement were also meaured; two subjects had slightly elevated IgE levels (between 150-180 IU ml^{-1}) and several had slightly raised concentrations of serum immunoglobulin isotypes and/or complement components. These abnormalities appeared to be consistent for that individual and neither the extent of the psoriasis nor the type of therapy influenced them significantly (Fig 6.5). At no time did any subject have a positive autoantibody test. C3 and C4 levels and CH 50 activity were found to be within normal limits and again UV therapy had no effect on these parameters (Table 6.5). This was also the case for the total numbers of white cells and lymphocytes (Table 6.5), and also the total numbers of monocytes, eosinophils, basophils, myeloid and promyeloid cells as well as red blood cells and platelets (data not shown).

Table 6.1

Clinical Details of Psoriasis Patients Studied in Chapter 6. Twenty four patients with chronic plaque psoriasis were investigated. Subjects receiving UVB or PUVA therapy had samples taken prior to the first irradiation, following four weeks of irradiation, and four weeks after the last treatment. Control subjects receiving coal tar paste had samples taken before treatment and four weeks later.

Group	No Patients	Mean Age	Age Range	Sex	Therapy	UV At Sample Point *	Total UV*	PASI Before	PASI at Sample Point	PASI 4 Weeks After
A	10	51.3 ± 5.7	22-69	4M/6F	Coal Tar	NA	NA	18.1 ± 0.7	2.9 ± 0.7	ND
B	10	37.8 ± 5.2	21-66	2M/8F	UVB	1.2 ± 0.2	2.2 ± 0.4	18.7 ± 1.1	5.9 ±1.5	4.1 ± 1.9
C	4	42.2 ± 7.9	26-63	2M/2F	PUVA	14.2 ± 4.2	119.3 ± 39.0	18.1 ± 1.7	8.5 ± 2.6	2.9 ± 1.1

* J cm^{-2}

Figure 6.5

The Effect of Phototherapy on Immunoglobulin Levels. The graphs represent the mean data from (a) 15 psoriasiasis subjects receiving topical coal tar (b) 17 psoriasis patients receiving UVB (c) 4 psoriasis patients receiving PUVA and (d) 4 normal individuals who received a standard course of UVB therapy, before treatment (B), following four weeks of treatment (D) and four weeks after the last treatment (A).

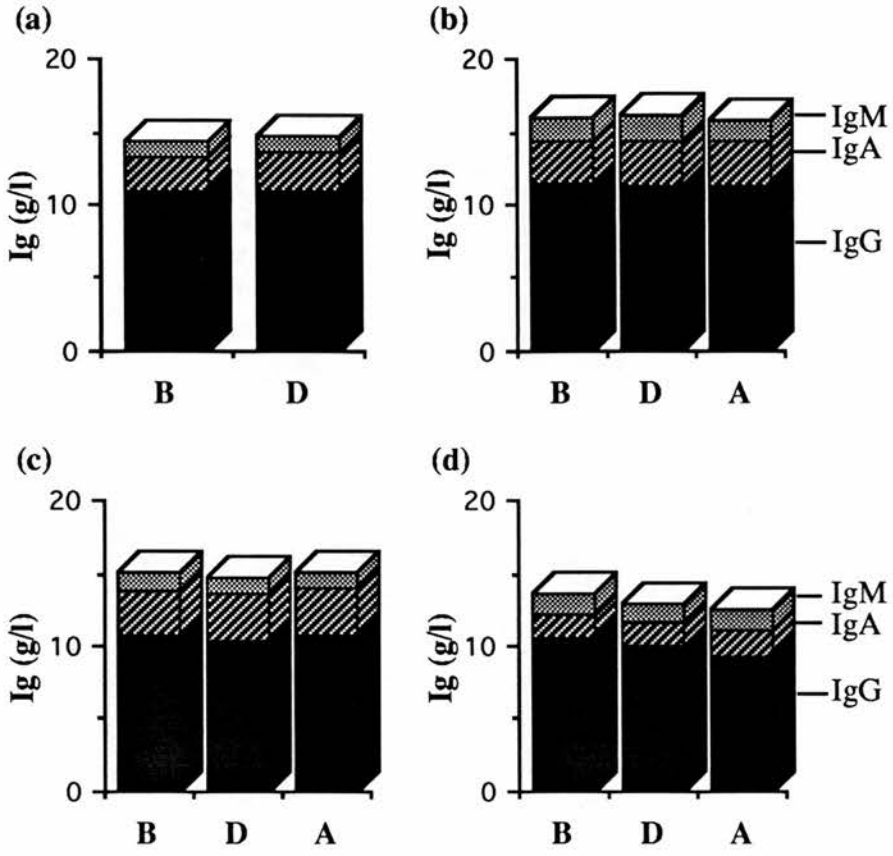


Table 6.2

The Effect of Phototherapy on Complement and Full Blood Counts. Complement levels and activity (C50) were determined before, during and after UVB treatment (group B and F) and also PUVA therapy (group C). Psoriatic subjects who received coal tar dressings had samples taken before treatment and also four weeks later. The results shown are mean ± sem for the group.

	n	COMPLEMENT			BLOOD COUNT	
		C3 ($g\ l^{-1}$)	C4 ($g\ l^{-1}$)	C50 (% HNP)	WC ($x\ 10^9$)	LC ($x\ 10^9$)
Group A						
Before	15	1.22 ± 0.04	0.26 ± 0.02	136 ± 26.8	7.30 ± 0.44	1.97 ± 0.21
During	15	1.16 ± 0.04	0.26 ± 0.01	128 ± 15.7	6.81 ± 0.54	1.93 ± 0.19
Group B						
Before	16	1.17 ± 0.06	0.25 ± 0.01	123 ± 13.6	6.40 ± 0.34	1.92 ± 0.10
During	16	1.19 ± 0.06	0.26 ± 0.16	112 ± 9.2	6.31 ± 0.39	1.88 ± 0.14
After	16	1.21 ± 0.06	0.29 ± 0.02	123 ± 12.4	6.50 ± 0.39	2.06 ± 0.15
Group C						
Before	4	1.23 ± 0.10	0.28 ± 0.05	165 ± 21.6	5.32 ± 0.16	3.22 ± 0.99
During	4	1.12 ± 0.10	0.24 ± 0.06	101 ± 10.7	5.50 ± 0.07	1.91 ± 0.18
After	4	1.27 ± 0.14	0.24 ± 0.05	92.8 ± 0.05	5.04 ± 1.50	2.04 ± 0.10
Group F*						
Before	4	1.00 ± 0.08	0.20 ± 0.03	106 ± 7.52	5.20 ± 0.35	1.32 ± 0.12
During	4	0.93 ± 0.04	1.17 ± 0.02	150 ± 47.4	4.95 ± 0.80	1.56 ± 0.14
After	4	0.95 ± 0.04	0.19 ± 0.02	132 ± 30.4	5.88 ± 2.00	1.35 ± 0.12

n: number in group
* Normal individuals who received a course of UVB identical to that received by psoriasis subjects.

6.2.2 HSV Infection

The patients described in this chapter were selected on the basis of a positive LPR to HSV, with an SI greater than three (24 patients in total). Only two of the patients suffered recrudescent perioral HSV infections during UVB (group B) and none during PUVA (group C) therapy. One recrudescence during UVB was associated with a β-haemolytic streptococcal infection which was the probable trigger, the other followed a mild UVB-induced facial burn a few days after commencing therapy. Three subjects from group A suffered recrudescent perioral infections: two were

associated with mild upper respiratory tract infections and the third occurred when the patient was "tired and run down". One subject was found to be shedding HSV asymptomatically, by virus isolation from the oropharynx, prior to the commencing UVB therapy. HSV antibody titres remained high in all subjects and varied little during the therapy (data not shown). (LPRs to HSV are outlined in table 6.4).

6.2.3 Phenotyping of PBMC

Preliminary experiments were carried out to determine the the optimal concentration of each antibody, which gave the highest and most consistant staining of PBMC. It was found that 10 µl neat or 50 µl of 1:10 dilution of the antibody per pellet of 5 x 10^5 cells was optimal for all the antibodies used, with the exception of DA6.231 which still gave consistent results at 1:100. The anti-IgG FITC conjugated antibody had been previously titrated (co-workers in lab) and 50 µl (per 5 x 10^5 cells) of a 1:40 dilution reduced the background non-specific binding while maintaining the positive staining.

At each visit PBMC were stained with a range of monclonal antibodies and the relative percentage of each subset was determined by florescence flow cytometry. No significant differences in the percentages of PBMC subsets between patients with psoriasis and normal controls were observed (Table 6.3). As there were only four normal subjects, data from a further nine controls were taken from a concurrent study in which the same monoclonal antobodies, staining protocol and flow cyometer were used (Charleson *et al.*, 1992). These show similar values to those shown in Table 6.3, i.e. percentages of positive cells (mean ± 1 standard deviation) CD3 45 ± 7, CD4 33± 11, CD8 24 ± 7, CD 1a 3± 1.9, CD 57 16 ± 9, MHC II 21 ± 6. No significant alterations occurred during therapy, although there was a slight decrease in the percentage of $CD3^+$ and $CD4^+$ cells in groups B and C ($p > 0.05$; paired t test). CD19 staining was performed in four of the subjects in group B only, but no modulation in the percentage of $CD19^+$ cells was observed.

Table 6.3

The Effect of UV Therapy On The Percentage of PBMC Subsets Assessed by Flow Cytometry .

Group	No Subjects (n)	Marker	Cell Subset	% Positive Cells Before Treatment	% Positive Cells After 4 Weeks of Treatment	% Positive Cells 4 Weeks Post-Treatment
A	3	CD1a	LC	4.5 ± 0.8	3.7 ± 0.5	ND
B	7			6.0 ± 0.4	5.3 ± 1.0	6.6 ± 2.0
C	4			4.8 ± 1.0	4.2 ± 1.4	5.2 ± 1.2
F	ND			ND	ND	ND
A	10	CD 3	Pan T	55.5 ± 3.7	56.1 ± 3.2	ND
B	9			54.2 ± 3.9	47.3 ± 3.3	53.8 ± 3.3
C	9			50.9 ± 3.5	54.4 ± 3.8	52.7 ± 5.4 $^{\Delta}$
F	4			46.7 ± 14.8	53.6 ± 13.4	48.1 ± 16.6
A	10	CD 4	T_h	38.8 ± 3.6	39.7 ± 4.9	ND
B	9			41.5 ± 7.2	35.4 ± 4.5	42.3 ± 4.9
C	9			36.3 ± 2.4	36.1 ± 2.9	27.6 ± 3.2 *
F	4			35.5 ± 3.7	40.0 ± 13.4	28.9 ± 4.9
A	10	CD 8	T_c	15.7 ± 1.4	17.4 ± 1.4	ND
B	9			18.1 ± 1.7	16.4 ± 2.1	18.1 ± 2.1
C	9			22.0 ± 2.7	25.1 ± 3.4	25.7 ± 5.5 *
F	4			15.8 ± 3.8	18.2 ± 1.3	14.4 ± 2.4
A	11	CD 57	NK	9.17 ± 1.3	12.5 ± 2.6	ND
B	9			11.4 ± 1.5	13.0 ± 1.9	10.1 ± 1.2
C	9			17.7 ± 3.6	19.2 ± 2.7	15.9 ± 2.9
F	4			10.7 ± 1.5	11.6 ± 2.6	11.3 ± 1.8
A	10	DP,DQ,	MHC	14.9 ± 1.6	15.2 ± 3.3	ND
B	9	DR	II	17.7 ± 2.9	16.3 ± 1.8	14.8 ± 2.1
C	9			15.9 ± 1.7	17.3 ± 2.5	17.5 ± 5.6 *
F	4			12.7 ± 5.0	15.6 ± 2.4	20.6 ± 4.1

Group A; psoriasis patients receiving coal tar
Group B; psoriasis patient s receiving UVB therapy
Group C; psoriasis patients receiving PUVA therapy
Group F; normals receiving a course of UVB
$^{\Delta}$ n – 1 * n – 3

6.2.4 *In vitro* Lymphoproliferative Responses to HSV

Prior to treatment 10 ml of blood was collected, the PBMC isolated and screened for LPRs to HSV; the patients were selected on the basis of a positive test (SI > 3). LPRs to Con A and HSV were monitored during treatment in seven patients from group A (coal tar paste), six from group B (UVB) and three from group C (PUVA). The mean data for each group (± sem) are outlined in in table 6.4.

Table 6.4

The Effect of Phototherapy on LPRs to Con A and HSV. The table shows the mean cpm (± sem) and stimulation indices (SI) for each group.

	Nil	Con A	SI	Vero	HSV	SI
Group A	(n=7)					
Before	1572$^{\Delta}$± 451	35682 ± 7 647	31.5 ± 11.7	1361 ± 429	10093 ± 2495	7.1 ± 1.9
During	1248$^{\Delta}$± 242	38747 ± 5.7	37.6 ± 5.7	1057 ± 195	7587 ± 1 491	8.0 ± 1.4
Group B	(n = 6)					
Before	1650 ± 341	50134 ± 9341	35.8 ± 11.8	2017 ± 370	15403 ± 2017	8.6 ± 1.2
During	3866 ± 1381	42376 ± 8 539	12.2*± 3.2	3644 ± 1 043	23027 ± 8 003	6.2 ± 0.76
After	1 677 ± 1 381	67 861 ± 12287	49.7 ± 18.4	2563 ± 427	18030 ± 4545	7.7 ± 1.2
Group C	(n = 3)					
Before	2610 ± 1337	46618 ± 1136	28.8 ± 14.5	1292 ± 526	8171 ± 816	8.5 ± 1.8
During	1075 ± 302	41684 ± 10 044	52.2 ± 27.2	831 ± 294	8152 ± 2294	11.2 ± 6.6
After	1608 ± 628	39906 ± 13291	25.7 ± 2.3	1757 ± 99.7	12920 ± 5981	10.4 ± 4.0

* p = 0.023 compared with before therapy (Wilcoxon signed ranks test)
$^{\Delta}$ n = 3

There was no statistically significant modulation of *in vitro* LPR to HSV or Con A during therapy in any group on comparison of the mean cpm (p > 0.05; Wilcoxon

signed ranks test). However the background counts of unstimulated PBMC increased in five of the six UVB treated patients (mean cpm for the group of six 1650 ± 341 increasing to 3866 ± 1381) during therapy, and returned to normal (1677 ± 428) four weeks after the last irradiation. This resulted in a significant suppression in the SI to Con A ($p = 0.023$; Wilcoxon signed ranks test) and was not found in patients in group A or C.

6.2.5 Antigen Presentation of HSV by EC or AC

The aim of the following section was to examine the effect of phototherapy on the ability of EC and AC to present HSV antigen to TEC. The initial experiments attempted to generate a standard T cell population prior to the onset of therapy for each patient and then to examine the effects of UV treatment on antigen presentation to these cells. Initially TEC were prepared and stored frozen, then thawed and their ability to proliferate when stimulated with AC or EC plus antigen measured. This approach had only limited success as outlined below, and therefore an attempt to generate T cell clones from each patient was attempted. Finally it was resolved to study the ability of APC to stimulate TEC prepared prepared on the same day.

Initially TEC were resuspended in 10% FCS and 10% DMSO in RPMI at 2×10^6 cells ml^{-1} and aliquoted into 1 ml samples. They were placed in a polystyrene box at -70°C for approximately 1h, until frozen, and then placed under liquid N_2. The cells were rapidly thawed at 37°C and placed on ice. To 1 ml of cells, 0.2 ml of ice cold medium was added every 30 seconds for 3 min. This was repeated with 0.4 ml and then 0.8 ml of medium. The cells were washed twice, a viable cell count performed and a lymphoproliferation assay performed with either no further additions or with Con A, mock infected Vero cells or HSV antigen. Autologous AC from blood were added to the TEC at cell numbers of 1, 3 and 10% of the TEC and incubated without antigen or with HSV or Vero control antigen as described in 2.3.12. The uptake of ^{3}H-thymidine was measured over the final 24 h of a six day

2.3.12. The uptake of ^{3}H-thymidine was measured over the final 24 h of a six day culture and compared with the whole PBMC population or fresh TEC cells. In an attempt to achieve consistent results, a number of freezing mixtures were used including 15, 50 and 75% autologous plasma or FCS in 10% DMSO and TCM. It was found that 75% FCS in TCM and 10% DMSO resulted in the most efficient recovery of cells following freezing. However it was still not sufficient to yield consistent stimulation of the frozen TEC (fTEC) by adherent cells in the lymphoproliferation asay. A number of different thawing techniques was also employed. The proliferation assay conditions were also manipulated; fTEC were subsequently cultured in round or flat bottomed plates, pulsed or harvested over a range of different time courses. In total, variations of this experiment were carried out on sixteen different occasions using cells from seven psoriatic and two normal subjects. Satisfactory antigen presentation by AC to fTEC cells was obtained on only six occasions. However, even in these instances, if fTEC were thawed at a later date and fresh autologous AC used to present HSV or Con A, highly variable results were obtained; this included two normal healthy individuals who were receiving no medication.

On five separate occasions a programmed cell freezer (Planar Products Ltd, London) was employed to freeze the cells. The temperature was lowered by 1°C min^{-1} until -40°C was reached. The cells were placed under liquid N_2. On three occasions this yielded satisfactory results on the day of freezing, but the results were variable when AC were added at various times thereafter. In addition it was found using various culture conditions, despite satifactory antigen presentation by AC, EC would not function as APCs to fTEC despite satifactory presentation of HSV or Con A to fresh TEC. It was finally decided to abandon this approach.

The second approach to generate a constant population of T cells prior to the patients commencing therapy was to generate T cell clones for each patient. The method

this was not a practical proposition. Often only two weeks notice was given that a patient was to commence UV therapy, and this was not sufficient time to generate HSV T cell clones and to conduct the first antigen presenting function experiment. This method was also therefore abandoned.

Finally, EC suspensions were prepared from suction blister roofs of patients in groups B and C. The ability of EC, and also AC purified from PBMC, to present HSV antigen to fresh autologous TEC was assessed. The purification procedure employed (see 2.3.8) was sufficient to abrogate the antigen presenting function of the TEC on most, although not all occasions. Since the background cpm for the TEC varied slightly between samples obtained from the same patient on different occasions, this value was subtracted from the cpm generated by the TEC stimulated by EC or AC to determine the stimulation attributable to the APC.

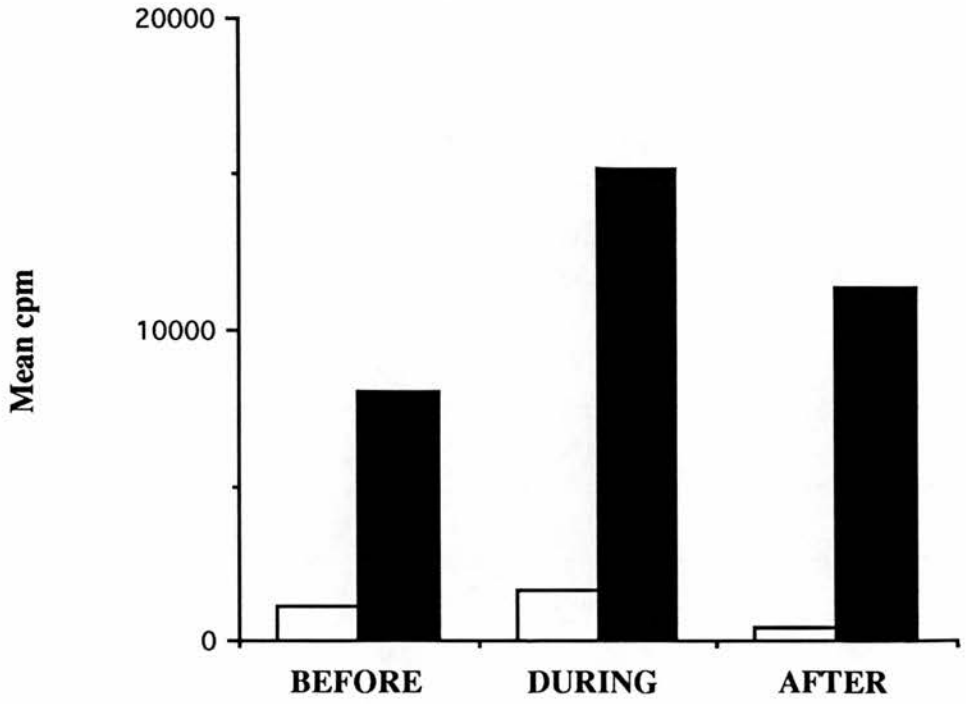
Satisfactory and consistent functional depletions of T cell responses were achieved in four of the group B patients on all three occasions. The ability of AC and EC to present HSV before, during and after UVB was compared and the results from one such experiment are shown in Fig 6.6. AC were unaffected in their ability to present HSV to TEC by UVB radiation but epidermal antigen presentation was abrogated during UVB therapy, recovering 4 weeks after the last treatment. This was also true of the other three patients monitored in the same way in this group, with the exception of EC from one subject which did not recover completely four weeks after therapy was completed.

The same experiment was carried out with four group C patients but no consistent effect on HSV presentation by AC or EC was obtained. However it was clear from the results of two of the patients that, four weeks after PUVA therapy had finished, the EC had not regained their ability to present HSV (data not shown).

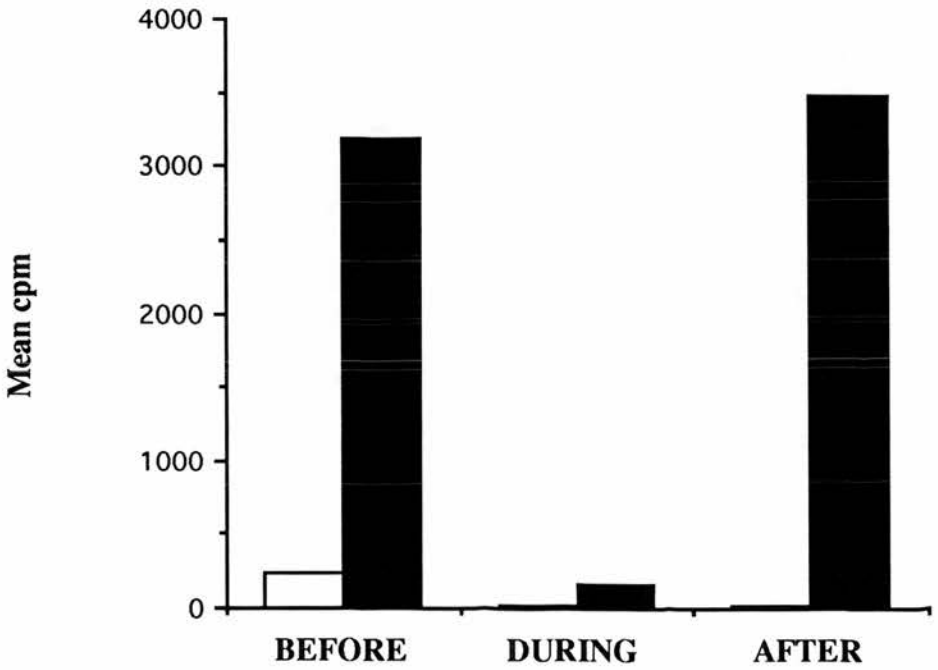
Figure 6.6

The Effect of UVB Irradiation on Antigen Presentation of HSV by (a) adherent cells from blood or (b) epidermal cells. LPRs of one patient with psoriasis to Vero control antigen (open bars) and HSV antigen (closed bars) before, during and after therapy. TEC were constituted with either 10% AC from blood or 10 % EC.

(a)



(b)



6.2.6 UCA Quantification

Initially, 4 successive tape strips were taken from the buttock (assumed to be unirradiated prior to therapy). However this method was later abandoned in preferance to Finn Chamber sampling because the latter was less invasive, not prone to the same degree of sampling error and removes the total epidermal UCA content. The samples were analysed by HPLC, and representative traces are shown in Fig 6.7. The epidermal UCA results outlined below represent Finn Chamber sampling unless otherwise stated.

A preliminary experiment was conducted to compare the UCA content, and the percentage of the *cis*-isomer, in the epidermis and suction blister fluid obtained from uninvolved forearm skin of untreated psoriasis patients with that from normal subjects (Table 6.5). The mean UCA content and the relative percentage of the *cis*-isomer in suction blister fluid was not significantly different between the two groups, although it should be noted that nine out of twelve psoriatic patients had no detectable *cis*-UCA, whereas six out of seven of the normal individuals did. The epidermal content of the psoriasis patients was significantly elevated compared with the normal subjects (Table 6.5). The relative percentage of each isomer did not differ between the two groups.

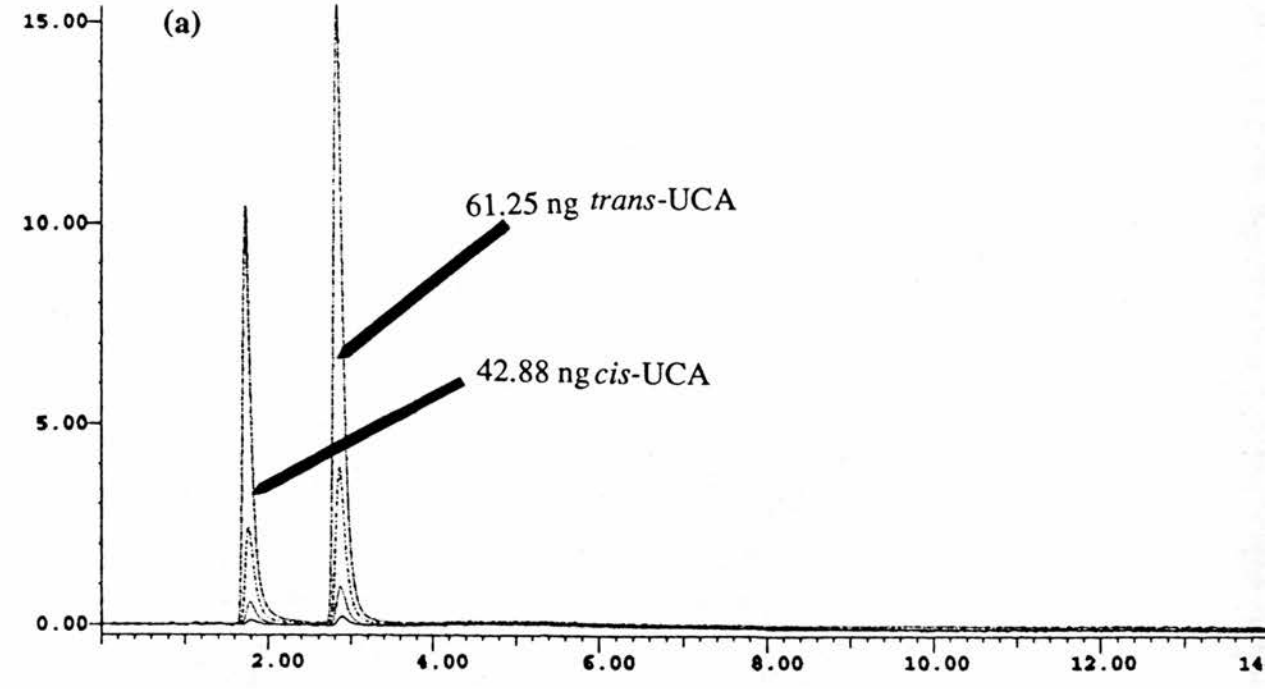
Table 6.5
UCA Content of Epidermis and Suction Blister Fluid in Normal Skin and Uninvolved Psoriatic Skin

	Normal			Psoriatic		
	n	**Total UCA (mean ± sem)**	**% *cis*-UCA**	**n**	**Total UCA (mean ± sem)**	**% *cis*-UCA**
Epidermis (nM cm^{-2})	11	2.3 ± 0.3	17.9 ± 2.8	22	7.6 ± 1.5*	21.9 ± 4.7
Blister Fluid (μM)	7	97.8 ± 11.6	3.2 ± 1.0	12	106.5 ±14.5	2.1 ± 1.2

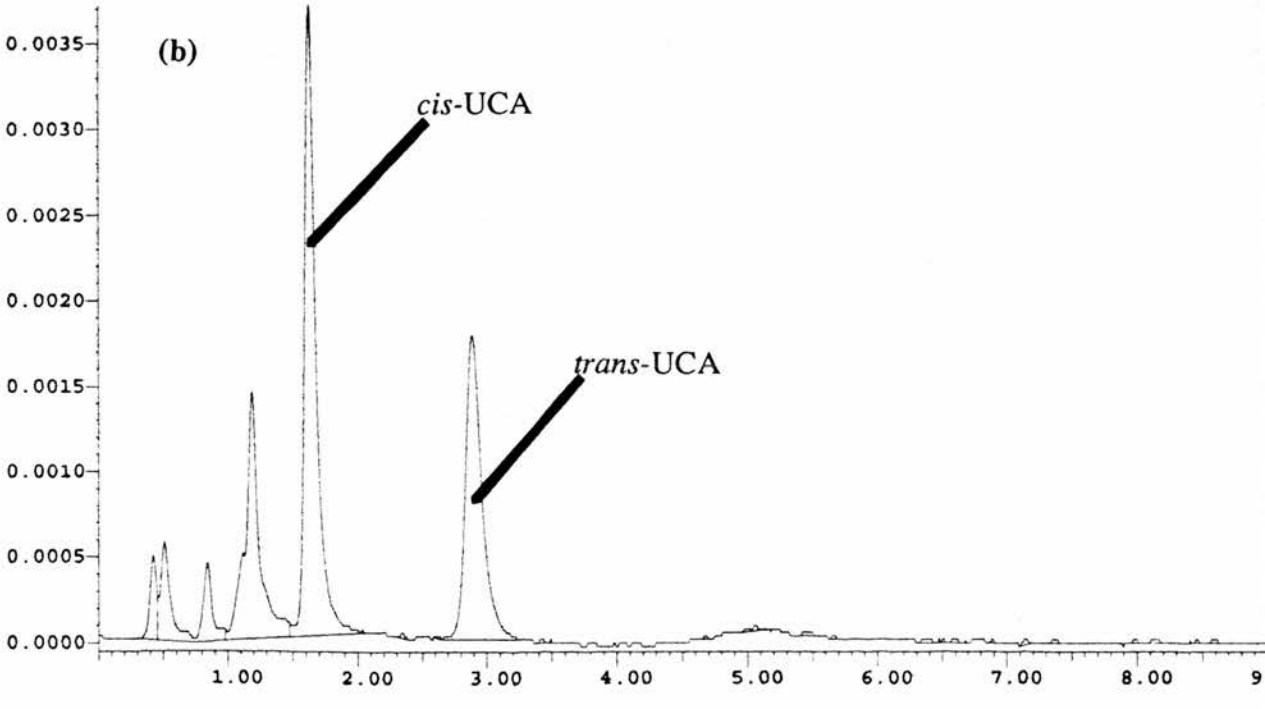
* significantly different from normal epidermis (Student's unpaired t test, $p < 0.01$)
n = number of patients

Figure 6.7

Chromatograms of (a) standard UCA solution and (b) Finn chambers



UCA_CAL.018\DAT00101.DTI 270 nm 0.02 AUF
cis/trans 0.67/0.96ng
UCA_CAL.018\DAT00202.DTI 270 nm 0.02 AUF
cis/trans 2.68/3.83ng
UCA_CAL.018\DAT00303.DTI 270 nm 0.02 AUF
cis/trans 10.72/15.31ng
UCA_CAL.018\DAT00404.DTI 270 nm 0.02 AUF
cis/trans 42.88/61.25ng



The effect of UV-therapy on UCA content of the epidermis and blister fluid is shown in Table 6.6. Finn Chambers were collected from a group of normal individuals (F) who received a standard course of UVB, on their arms, in Edinburgh before commencing treatment, following four weeks of irradiation and four weeks after the last irradiation. Blister fluid was also collected from these subjects before treatment and 48 h after the last irradiation (approximately six weeks). In both groups B (psoriasis patients receiving UVB) and F (normal subjects receiving UVB irradiation) UV exposure tended to increase the total UCA content of the epidermis, but this was not statistically significant ($p > 0.05$; paired t test). After four weeks of therapy the percentage of *cis*-UCA had increased significantly in both groups. Similar results were obtained from four successive tape strips as shown in the table below.

Table 6.7

The Effect of UVB Therapy on Epidermal UCA Content as Sampled by Tape stripping. The values equal the mean ± sem for each group.

	n	Before	During	After
Group A				
Total UCA ($nM\ cm^{-2}$)	4	0.11 ± 0.04	0.15 ± 0.01	ND
% Cis UCA		10.2 ± 3.0	2.8 ± 1.9	ND
Group B				
Total UCA ($nM\ cm^{-2}$)	10	0.14 ± 70.3	0.15 ± 0.041	0.09 ± 0.03
% *Cis*-UCA	15	16.7 ± 3.0	52.5 ± 4.0	13.3 ± 4.0
Group C*				
% *Cis*-UCA	4	15.7 ± 11.5	46.8 ± 11.8	23.0 ± 19

n: number of subjects per group
* tape strips from two of the subjects in this group had folded onto each other, and therefore it was impossible to extract the total UCA from these.

As shown in Table 6.6 the concentration of UCA in suction blister fluid remained relatively constant throughout the study. The percentage of *cis*-UCA increased during therapy and remained elevated in group B four weeks after the last irradiation.

Table 6.6
Effect of Therapy on UCA Content of Epidermis and Suction Blister Fluid

Sample/group	Before Treatment			During Treatment			After Treatment		
	n	Total UCA (mean ± sem)	% *cis*-UCA (mean ± sem)	n	Total UCA (mean ± sem)	% *cis*-UCA (mean ± sem)	n	Total UCA (mean ± sem)	% *cis*-UCA (mean ± sem)
Epidermis ($nM\ cm^{-2}$)									
A	6	7.8 ± 3.0	10.5 ± 6.2	6	11.0 ± 2.7	4.5 ± 1.8	-	ND	ND
B	13	6.7 ± 1.3	24.1 ± 5.9	13	7.1 ± 1.1	54.3 ± 5.5*	11	8.1 ± 1.2	11.0 ± 8.0
F	4	2.9 ± 0.7	13.7 ± 7.0	4	5.9 ± 2.3	54.4 ± 13.3$^{\Delta}$	4	3.6 ± 1.1	5.9 ± 3.3
Blister Fluid (μM)									
A	3	120.0 ± 18.8	0	6	110.9 ± 23.9	0	-	ND	ND
B	7	100.7 ± 20.3	2.9 ± 2.0	7	121.0 ± 10.1	28.7 ± 7.3*	5	120.0 ± 19.6	22.5 ± 9.9$^{\Diamond}$
F	4	89.9 ± 10.9	4.1 ± 1.1	4	73.3 ± 23.9	23.4 ± 8.2$^{\bullet}$	-	ND	ND

* $p < 0.01$ compared with samples before UV
$^{\Delta}$ $p < 0.05$ compared with samples before UV
$^{\bullet}$ $p < 0.001$ compared with samples before UV
$^{\Diamond}$ $p < 0.05$ compared with same 5 samples before therapy

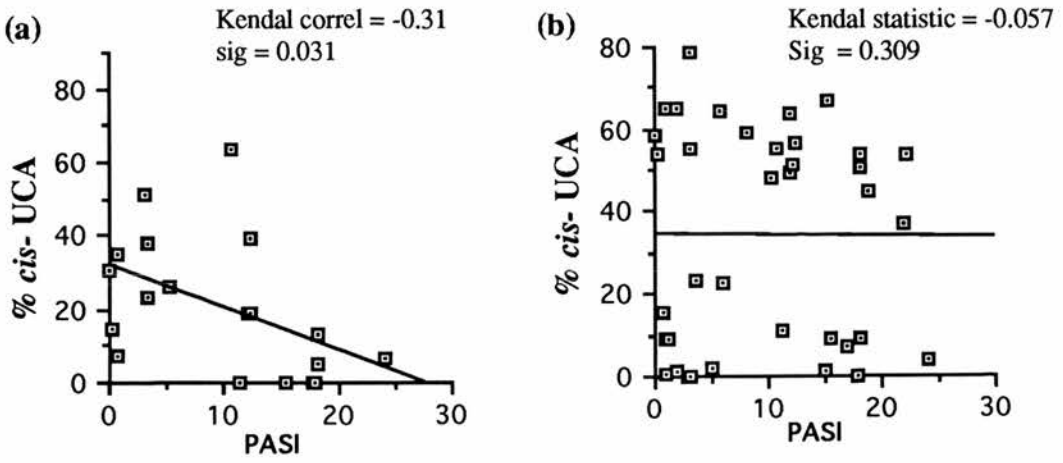
n: number of subjects
ND: not done

Only a few samples were available for UCA analysis from group C (PUVA) . Two out of three subjects showed a slight increase in epidermal UCA during therapy, with the percentage of *cis*-UCA increasing in all three subjects after four weeks of therapy (from 35.4% ± 14.2 to 46.1 % ± 12.5; mean ± sem). Two of these subjects had elevated percentages of *cis*-UCA before irradiation commenced (44.95 and 53.5% respectively). Suction blister fluid from two of the PUVA patients showed no alteration in the total UCA content during treatment but an increased percentage of *cis*-UCA was detected following four weeks of therapy and, as with the psoriasis subjects receiving UVB treatment, this remained elevated four weeks after the last treatment.

The correlation (Kendall's coefficient of concordance) between total epidermal UCA (or *cis*-UCA) and suction blister fluid UCA (or *cis*-UCA) compared with the PASI was calculated. No correlation between the severity of psorasis and the epidermal UCA was found but there was a negative correlation between the PASI and the % *cis*-UCA in the suction blister fluid (Fig 6.8).

Figure 6.8

The % of cis-UCA present in (a) suction blister fluid and (b) the epidemis in psoriasis patients treated with UVB or PUVA therapy and its correlation with the PASI score



6.2.7 TNF-α Analysis

Due to the limited volume of the sample available from each subject, only 100 µl of blister fluid or the standard was added to each well of the ELISA plate instead of the recomended 200 µl (section 2.3.16). This lowered the sensitivity of the test from 7.5 pg ml^{-1} to approximately 16 pg ml^{-1}.

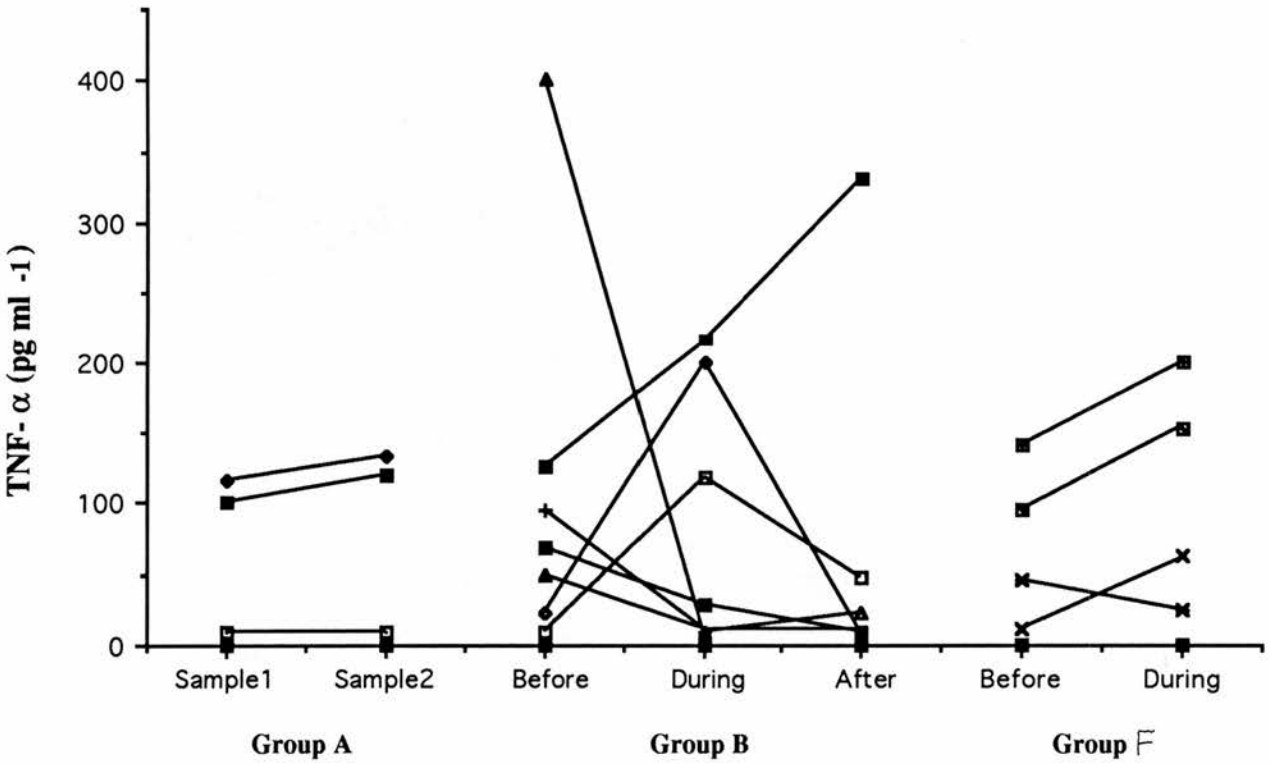
TNF-α was detected in the majority of samples. Patients with psoriasis from groups A-C had slightly elevated levels (95.6 ± 10.1 pg ml^{-1}; mean ± sem; n=9) compared with normal subjects (58.7 ± 15.3 pg ml^{-1}; n=7; four group F subjects and 3 additional normal subjects), but this did not reach statistical significance ($p > 0.05$; Student's t-test).

As shown in Fig 6.9, there was little change in TNF-α concentration during coal tar therapy (group A) but three patients from group B showed increased TNF-α concentrations during UVB therapy although four showed a decrease. Three out of four normal subjects (group F) showed increased TNF-α in blister fluid during therapy. No paired samples from group C were analysed.

There was no correlation between the concentration of *cis*-UCA and TNF-α in the blister fluid ($p > 0.05$; Kendall's coefficient of concordance).

Figure 6.9

The Effect of UVB Therapy on TNF-α Concentration in Suction Blister Fluid. Samples were taken before, during and after therapy for group B (7 patients with psoriasis) and before and during UVB therapy for group F (4 normal subjects from Chapter 7). For group A (3 patient with psoriasis) one sample was taken before coal tar therapy and the second 4 weeks after starting it. The detection limit was 16 pg ml^{-1}.



6.3 DISCUSSION

In this study several immunological parameters were assessed in patients suffering from chronic plaque psoriasis and, where possible, compared with those in normal subjects. The effect of phototherapy or coal tar therapy was then measured by comparing samples from the patients taken before, during and after treatment.

6.3.1 Humoral Immunity

Since certain reports, although not all, have indicated that abnormalities in complement components (Acevedo and Hammer, 1989; Rosenberg *et al.*, 1990) and immunoglobulin levels, particularly IgA (Guilhou et al., 1976) may occur in patients with psoriasis, patients were tested before therapy for circulating complement components and immunoglobulin levels, as well as haematological, autoantibody, urinary and biochemical profiles. All of these routine measurements were within normal limits with the exception of a small number of psoriatic subjects who had slightly elevated immunoglobulin or complement levels which did not vary with disease severity. Although deposition of complement and immunoglobulins appears to occur in established lesions and may be involved in recruiting polymorphs into plaques, they appear to be secondary phenomena (reviewed Bos 1988). It is possible that elevation of complement levels relate to particular types of psoriasis, while the lack of correlation between the clinical improvement of psoriasis and increased levels of circulating immunoglobulin argues against their being primarily related to the development of skin lesions (Baker and Fry 1992).

Neither UVB nor PUVA therapy had a measurable effect on any of these parameters. Hersey *et al.*, (1983a) reported that exposure to sunlight had no effect on circulating complement or immunoglobulin levels, although Livden *et al.*, (1977) detected slightly decreased circulating IgG titres in normal subjects exposed to four weeks of

UVB or UVA radiation, and a slight increase in IgM and C3 levels following UVB irradiation.

6.3.2 Circulating PBMC

Although some reports have indicated abnormalities in circulating lymphocyte subsets in patients with psoriasis, others found no differences from normal subjects (David *et al.*, 1990; De Pietro *et al.*, 1981; Kokelj *et al.*, 1984). This was also the case in the present study in which analysis was by flow cytometry using monoclonal antibodies to T cells (CD3, CD4, CD8), LC (CD1a), B cells (CD19), natural killer cells (CD57) and MHC Class II antigens (Table 6.5); the patients full blood count also remained normal and did not fluctuate during the study.

The effect of UV irradiation on circulating blood cells is controversial and, indeed, it is possible that any detected changes are only transient. Thus some authors reported a temporary decrease in circulating T cells after UVB or PUVA therapy but others found no modulation in circulating PBMC following a variety of UV regimens (see section 1.3.4 and discussed by Mutzas *et al.*, 1991). Differences in the type of UV source, wavelength, duration or dose of UV radiation, as well as the time of sampling may explain these apparent differences. The results of this study demonstrated an insignificant reduction in the percentage of circulating $CD3^+$ and $CD4^+$ cells following UVB or PUVA therapy but no alteration in any of the other subsets. No changes were noted in the normal subjects receiving UVB, or in patients receiving tar.

6.3.3. HSV Infection

Most studies of the immunosuppressive effects of UV irradiation in man or experimental animals have focussed on immune responses to antigens, usually contact sensitizers, which are encountered for the first time shortly after irradiation. The present study was different in that subjects were already infected with HSV

before starting therapy. In addition, although erythemal exposure to UV is a common triggering factor for orofacial recrudescent HSV infection (Spruance *et al.*, 1991; Vestey *et al.*, 1989), the role of suberythemal doses of UV, such as those employed in phototherapy, in HSV pathogenesis is unknown.

Volunteers were observed for signs of orofacial HSV recrudescences at each visit and had oropharyngeal swabs taken for virus isolation before, during and after therapy. Ten patients receiving coal tar (group A), ten receiving UVB (group B) and 4 receiving PUVA (group C) were found to be HSV carriers on the basis of lymphoproliferative responses *in vitro*. One subject from group B was shedding virus asymptomatically before therapy and only two others suffered recrudescent lesions during therapy; one following a mild burn produced by UVB irradiation but the other was not associated with therapy. Thus suberythemal phototherapy was not generally a triggering factor for HSV recrudescences nor for inducing reactivation of latent virus with asymptomatic shedding into the oropharynx. However the oropharyngeal swabs were taken at one time point only during therapy and, as virus is detectable for about two to five days in each recurrent episodes, it is possible that shedding may have occurred outwith the sample points.

It has already been reported that patients with psoriasis develop normal lymphoproliferative responses to HSV (Kapp *et al.*, 1988). Four weeks of UVB or PUVA therapy caused no significant modulation in the lymphoproliferative response to HSV, or in HSV-specific antibody titres in any individual. However PBMC cultured alone tended to show an increased ^{3}H-thymidine uptake during UV irradiation which may indicate some non-specific lymphocyte activation due to phototherapy.

6.3.4 Antigen Presentation

When peripheral blood AC were used as accessory cells in the lymphoproliferation assays, no alteration in antigen presenting ability as a result of UVB or PUVA therapy was detected. However, this was not the case when EC were used as accessory cells. This resulted in markedly lowered proliferative responses in patients during UVB (6.6) or PUVA therapy. The responses were restored in most individuals four weeks after UVB irradiation had finished although they remained depressed four weeks after PUVA therapy.

Few studies have examined the effects of UV irradiation on human epidermal antigen presentating function and none following such typical regimens used for the treatment of psoriasis. *In vitro* irradiation of human EC suspensions with UVB (Austad and Braathen., 1985; Czernielewski *et al.*, 1985) UVA or PUVA (Mork *et al.*, 1987) resulted in suppressed allo-activation and antigen presentation by the cells. Cooper *et al.* (1985) demonstrated a depressed antigen presentation by LC immediately after irradiation of normal subjects with 2-4 MEDs of UVB but this was followed by an enhancement in antigen presentation due to an influx of $T6^{-}Dr^{+}$ cells into the epidermis. Similarly PUVA therapy has been reported to suppress the antigen presenting capacity of human epidermal cells, (Ashworth *et al.*, 1989), with loss of LC, although on a per cell basis those which remain function normally.

6.3.5 UCA Determination

Cis-UCA is formed from the naturally occurring *trans*-isomer on UV irradiation of skin, up to a maximum value of about 60% of the total UCA which represents a photostationary state (Norval *et al.*, 1989a). In mice, the percentage of *cis*-UCA declines steadily following a single UVB exposure until a background level is reached after 14 days (Norval *et al.*, 1988). An analysis of the UCA content of

human epidermis and suction blister fluid and percentage of the *cis*-isomer during and after phototherapy had not been undertaken before. The total epidermal UCA content was measured using Finn Chamber samples, which extracts all the UCA from human epidermis and gives an equivalent value to successive tape strips (Jansen *et al.*, 1991).

Patients with psoriasis had approximately three times more UCA in their epidermis as normal subjects (Table 6.5). The value obtained for psoriatic epidermis was similar to that reported recently by Snellman *et al.*, (1992). It is possible that the increased UCA detected reflects the mild epidermal thickening which occurs even in "uninvolved" skin sites in psoriasis. Alternatively, since UCA is formed in one step from histidine by the action of histidase, increased histidine may be available in psoriasis due, perhaps, to increased epidermal cell turnover or keratohyalin granule degradation which would account for increased UCA. In addition abnormalities in histidase activity have been detected in patients with psoriasis (Reavan *et al.*, 1965).

During and after UVB exposure, the total epidermal UCA content of the psoriasis patients remained relatively constant while that of normal subjects doubled during irradiation, returning to near the starting value 4 weeks after the last dose. All irradiated groups showed an increase in the percentage of *cis*-UCA in the epidermis which returned to normal 4 weeks after the last treatment. In a previous study four weeks of heliotherapy (sunbathing in the Canary Islands) for psoriasis produced similar effects on epidermal UCA despite using very different UV regimens (Snellman *et al.*, 1992). An increase in the % of *cis*-UCA occurred in the small number of PUVA patients studied, during therapy.

It has been proposed that photosensitizing agents may play a role in the isomerization of UCA (De Fabo *et al*, 1991; Schwarz *et al* ., 1987). It is not known whether 8-methoxypsoralen will affect the isomerization of UCA *in vivo*. However in this study, *in vitro* irradiation of a solution of *trans*-UCA, in the presence of 8-

methoxypsoralen, in the PUVA cabinet had little effect on the production of *cis*-UCA over a range of irradiances. For example following irradiation for 10 minutes in the PUVA cabinet 13.9, 11.7, 11.7 and 6.3 % of *cis*-UCA was formed in the presence of 0, 0.1%, 0.01% and 0.001% of 8-methoxpsoralen respectively. Others have found that UVA irradiation of *trans*-UCA in the presence of psoralens had little or a slightly protective effect on the isomerization (H. Morrison personal communication).

Although *cis*-UCA has been suggested as a mediator of the immunosuppressive effects of UV irradiation, it is not known if the formation of *cis*-UCA in the epidermis following UV irradiation contributes either directly or indirectly to the impaired presentation of HSV by EC during phototherapy. Evidence to support a direct effect comes from the measurement of the number of dendritic cells in murine epidermis following *cis*-UCA treatment (discussed in Chapter 3).

UCA was detected in suction blister fluid from both psoriatic and normal subjects, at similar concentrations. This differs from a previous report of reduced levels of UCA in suction blister fluid from psoriasis patients (Juhlin *et al.*, 1986). Phototherapy did not affect the total concentration of UCA although the percentage of *cis*-UCA increased. In contrast to the epidermis, this increase was maintained four weeks after the last treatment in both groups B and C. These results are similar to those of Pasanen *et al.*, (1990). UCA is synthesized in the stratum corneum on activation of histidase (Scott, 1981), and it is interesting that UCA isomers are found in blister fluid which is likely to be formed mainly from a dermal exudate. The persistence of an increased percentage of *cis*-UCA in blister fluid after it had returned to a low percentage in the epidermis might indicate that *cis*-UCA persists in the dermis or near the dermo-epidermal junction. It is possible that it may play a significant role in immunomodulation there and, indeed, a comparison of UCA and the percentage of *cis*-UCA in the epidermis and blister fluid samples from the psoriatic patients

revealed an inverse correlation between percentage of *cis*-UCA in blister fluid and the PASI ($p = 0.031$), while there was no correlation between the percentage of epidermal *cis*-UCA and UCA, or of blister fluid UCA, with the PASI ($p > 0.308$).

6.3.6 TNF-α Analysis

Finally, a small number of blister fluid samples were analysed for TNF-α. It was present in measurable quantities in most samples before irradiation but UVB therapy had no consistent effect on its concentration (Fig. 6.9). However it is possible that any changes may occur rapidly after the start of phototherapy and may not be sustained until our samples were taken, at least 48 h after irradiation. TNF-α is one of the cytokines released by UV-irradiated KC which has important properties in mediating the effects of UVB on the immune system, at least in mice. It has been shown, for example, to mediate the suppression of contact hypersensitivity induction through local release in the skin but also to be involved in the enhanced expression of contact hypersensitivity in UV-susceptible mice (Yoshikawa *et al.*, 1992) It is not known whether human skin responds similarly during phototherapy. TNF-α alters adhesion molecule expression on KC and in the dermal vasculature, thus influencing recruitment of inflammatory cells into both the epidermal and dermal compartments. In psoriasis there is a characteristic T cell infiltrate and KC hyperproliferation, in which dermal dendrocytes may play a central role, possibly mediated by the production of TNF-α (Nickoloff *et al.*, 1991).

Whether TNF-α and *cis*-UCA production in the skin are linked is unknown at present, although Kurimoto and Streilein (1992) have reported recently that *cis*-UCA may cause the local release of TNF-α within the murine epidermis with consequences for the induction of CH. In addition, *cis*-UCA has been shown to down-regulate the induction of cAMP by *trans*-UCA or histamine in dermal fibroblasts *in vitro* and thus it is possible that *cis*-UCA, formed on irradiation, may regulate the activity of cytokines through a secondary signalling system (Palaszynski *et al.*, 1992).

Although changes have been noted in this study, predominantly in epidermal APC function and in *cis*-UCA content as a result of phototherapy, it remains uncertain whether these modulations contribute to the effectiveness of the UV-based treatment for psoriasis or if they are related to the disease process itself.

CHAPTER 7

THE EFFECT OF UV-THERAPY, AND UCA-ISOMERS *in vitro*, ON NK CELL FUNCTION IN NORMAL AND PSORIATIC SUBJECTS

7.1 INTRODUCTION

Natural killer (NK) cells represent an extremely heterogeneous population of predominantly large granular lymphocytes (LGL). They have not, as yet, been formally assigned to a single lineage or a distinct anatomical location of maturation. However it has been suggested that NK cells are dependent on intact bone marrow, and not on thymus for their differentiation (Lanier *et al.*, 1986; Trinchieri and Perussia, 1984). NK cells express both T cell markers eg CD2 and myeloid markers eg CD11b (LFA-1) or CD16 (FcγR). The majority of NK cells in peripheral blood in humans are $CD56^+$ $CD16^+$ $CD2^+$ $CD11b^+$ $CD3^-$. Other markers such as CD57, CD3 and CD8 are only expressed on sub-sets of NK cells (Mingari *et al.*, 1987). Figure 7.1 (adapted from Trinchieri, 1989) illustrates the range of markers found on NK cells.

NK cells have therefore tended to be defined on a functional basis. Classically they are considered to be spontaneously cytotoxic cells which lyse target cells in a non-MHC restricted manner at the level of the target cell. Unlike specific cytotoxic T cells, NK cells require no prior sensitization and do not appear to exhibit any immunologic memory (reviewed by Trinchieri, 1989). Until recently it had not been possible to demonstrate any clonally distributed specificity.

Despite this apparent lack of specificity, NK cells have been implicated in a number of immunological processes. These functions include cytotoxicity against tumour cells and virally infected cells (Herberman and Ortaldo, 1981), and resistance to some microbial, fungal and parasitic agents (Hatcher and Kuhn, 1982; Murphy and McDaniel, 1982; Nenecioni *et al.*, 1983). NK cells also provide resistance against allogeneic grafts (Kiessling *et al.*, 1977; Warner and Dennert, 1982) and they may play a role in immune regulation through the secretion of a number of cytokines. For

example, stimulation of purified NK cells with IL-2 and CD16 ligands induced high levels of mRNA accumulation, and release of IL-2 receptor (CD25) (Anegón *et al.*; 1988), IFN γ (Kobayaski et al., 1989), TNFα (Anegón *et al.*, 1988), GM-CSF and CSF-1 (Anegón *et al.*, 1988; Cuturi *et al.*, 1989).

Figure 7.1.

Cell Markers Found On Human NK Cells Compared With B Cells, T Cells, Monocytes and Polymorphonuclear Neutrophils (PMN). ■ *positive cells,* □ *low density positive cells and* ▩ *activated cells only. The length of each filled bar within each population indicates the approximate proportion of cells expressing the antigens. The position of the bar does not represent the true overlapping or exclusive expression of the antigens on different subsets.*

Cluster	Antibodies	B	T Cells	NK	Monocytes	PMN
MHC-I	Class I MHC					
MHC-II	Class II MHC					
CD2	OKT11, Leu-5b					
CD3	OKT3, Leu4					
CD4	OKT4, Leu11					
CD7	Leu-1					
CD8	OKT8, Leu-2					
CD11a	LFA-1					
CD11b	OKM1					
CD11c	Leu-M5					
CD16	Leu-11(FcR)					
CD18	β-chain CD11					
CD25	IL-2R(TAC)					
CD38	OKT10, Leu-17					
CD56	NKH-1, Leu-19*					
CD57	HNK-1, Leu-7					

* NCAM

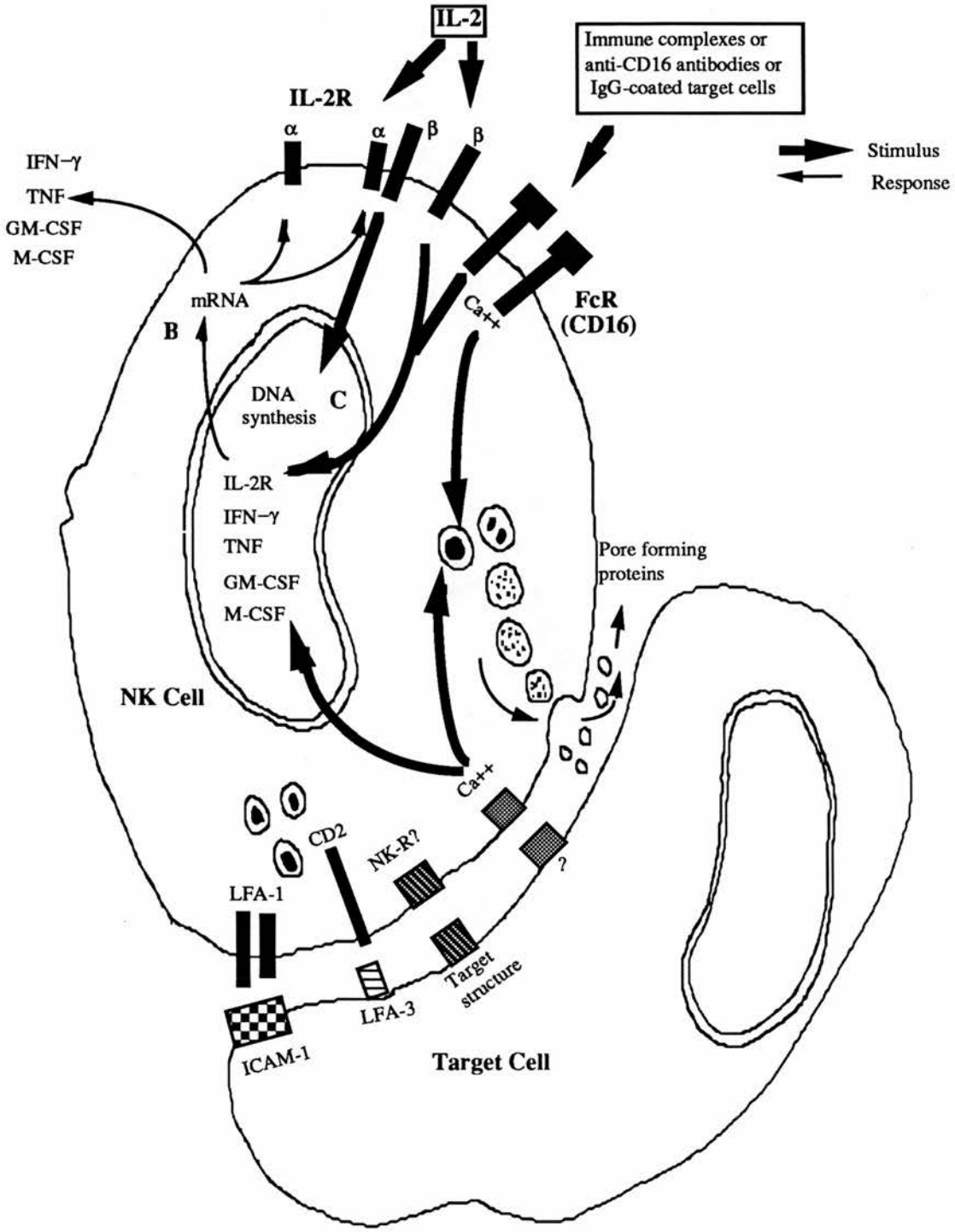
The response of NK cells can be broken down into three sequential phases (Fig 7.2. adapted from Trinchieri, 1989). The first is a rapid response on the interaction of NK cells with the appropriate target cell (1-10 min) and is associated with

cytotoxicity and release of granule contents. The second phase (10 min to 2 h) involves the transcription and expression of genes encoding lymphokines and surface activation markers including CD25. In the presence of IL-2, NK cells proceed to the third phase which involves DNA synthesis, blast formation and proliferation. Il-2 and IFN are both potent enhancers of NK cell activity (Trinchieri *et al.*, 1984; Trinchieri and Santoli, 1978a). Il-2 is widely employed to induce the lytic effect of NK cells from, for example, bone marrow cultures. However antibodies directed against TNF-α and IFN abrogate the IL-2 dependent maturation. This suggests that IL-2 may act through the induction of another cytokine (Kärre *et al.*, 1991).

The receptors involved in NK cell target cell recognition and interaction or the signal transduction mechanism responsible for E (effector) cell activation remain unknown. Interaction of E and target cells induces an increase in intracellular Ca^{+} which is in approximate proportion to the sensitivity of the target cells to lysis, and thought to be regulated by cAMP (Windebank *et al.*, 1988). Both CD16 and CD2 have been shown to act directly in the signal transduction and activation of the cytotoxic mechanism (Cassatella *et al.*, 1989; Schmidt *et al.*, 1988). CD11 (LFA-1) and CD18 (LFA-β sub-unit) are also thought to be important in the NK cell cytotoxicity; patients who suffer from a severe deficiency in the CD18 chain are deficient in NK cell activity (Kahl *et al.*, 1984; Ross *et al.*, 1985) and antibodies directed against CD11a block NK cell mediated cytotoxicity. It has also been reported that antibodies directed against CD45 will block NK cell mediated cytoxicity, but they act at the post-binding stage (Pawlec *et al.*, 1985; Starling *et al.*, 1987).

Figure 7.2

A Model of Natural Killer Cell Activity Following Interaction with Target Cells or Immune Complexes



The possibility exists that there is a distinct NK cell receptor which as yet remains elusive. Evidence of education and clonal specificity of NK cells is now emerging (reviewed by Moretta, 1992; Versteeg, 1992). Initially it was found that purified alloreactive $CD56^+$ $CD3^-$ precursors could be induced in allogeneic, but not in autologous MLR (Ciccone *et al.*, 1988). Further studies at a clonal level indicated that clones which lysed autologous cells could not be generated and also that in a given individual, NK cells were heterogeneous, since only a proportion of clones could lyse normal target cells derived from any given donor (Ciconne *et al.*, 1990b; Suzuki *et al.*, 1990). Large numbers of NK cell clones ($CD3^-$) from one donor were assessed for their ability to lyse a panel of target cells derived from a range of allogeneic donors. This study revealed the existence of four groups of alloreactive clones, within which a homogeneous pattern of cytotoxic activity was found (Ciccone *et al.*, 1992). More than 60% of the clones did not lyse any of the target cells tested which may indicate that a wider repertoire may exist. It may also be that not all NK cells will recognize allogeneic cells. It is conceivable that the NK cell repertoire may be somewhat limited in comparison to that of T or B cells.

A bank of target cells were derived from various members of different families. It was found that the resistance to killing by NK cells was dominantly inherited by target cells and co-segregated with the MHC class I region (Ciconne *et al.*, 1990a) which suggested that a particular MHC class I allele product protects against the NK cell clone. Further evidence in support of the protective role of MHC class I expression by target cells includes the observations that treatment of target cells with IFN-γ, which increases the expression of MHC class I, protects against NK cell cytotoxicity, or that many tumors express high levels of *c-myc* or N-*myc* oncogenes, which switch off class I MHC expression, and render cells susceptible to NK cell lysis. This was confirmed by the transfection of human MHC class I genes into an HLA class I negative mutant cell line which restored the NK-resistant phenotype

(Storkus, *et al.*, 1989). It was found that transfection with seven out of eight genes conferred resistance to NK cell lysis. Examination of the seven proteins identified an epitope which was accessible to perhaps a protective peptide but this groove was not accessible to peptides in the eigth product (Garette *et al.*, 1989; Storkus *et al.*, 1991). It is therefore possible that MHC I plus a protective peptide, fitting this groove, are recognized by the NK cell, and on recognition of this structure the signal sent to the NK cell is "no lysis" and if this structure is altered in any way the signal is "lysis" (Moretta *et al.*, 1992; Versteeg, 1992).

A variety of target cell types, with varying degrees of susceptibility, have been employed to measure NK cytotoxicity. The K 562 cell line employed in the following study was derived from a patient with chronic myeloid leukemia and is highly sensitive to NK lysis. This line lacks both MHC class I and II antigens.

UV irradiation results in a transient suppression in immune responses to antigens which are encountered shortly thereafter (reviewed in section 1.3), including a number of agents where NK cell activity may play a role in immunity, for example, viruses such as HSV (Howie *et al.*, 1986a) or MAIDS (Brozek *et al.*, 1992), and also tumor antigens (Fisher and Kripke, 1977) and allografts. *In vivo* exposure to UV is directly correlated to the incidence of non-melanoma skin carcinomas both in humans and also in animal models and is also thought to be a contributory factor in the development of malignant melanoma. It is therefore of interest that *in vitro* UV-irradiation of human PBMC results in a dose dependent suppression of NK cell activity (Elmets *et al.*, 1987; Schacter *et al.*, 1983; Toda *et al.*, 1986; Weitzen and Bonavida, 1984). In addition, there is an indication that exposure to UV *in vivo* may result in suppressed NK cell activity (Hersey, 1986; Hersey *et al.*, 1987; Hersey *et al.*, 1988; Viander *et al.*, 1984).

As discussed in Chapter 6 psoriasis is a common hyperproliferative skin disorder in which the immune system may play a critical role. A number of UV

based regimens, UVB phototherapy or PUVA photochemotherapy, are employed successfully in the management of psoriasis without their effects on immunity being known clearly. Conventional broad-band UV sources such as the Sylvania UV6 emit wavelengths from 270 nm to 370 nm. Wavelengths less than 300 nm are highly erythemogenic, and as with those over 315 nm, are relatively ineffective therapeutically (Young and van der Leun, 1975; Parrish and Jaenicke, 1981). Therefore a narrow band (311-313 nm) source was developed (Philips TLO1) (Van Weelden and van der Leun, 1988) which is very effective in the mangement of psoriasis (Green *et al.*, 1988; Larkö, 1989; van Weelden *et al.*, 1990). The relative output spectrum of these lamps are illustrated in Fig 2.1.

In this chapter the effect of therapeutic regimens of UV6 (broad-band), TLO1 (311-313 nm) or PUVA therapy on NK cell activity in patients with psoriasis was examined. In addition, NK cell activity was determined in a group of psoriasis patients who were receiving coal tar therapy. A group of 4 normal individuals who received a course of UV6 identical to that received by psoriasis patients were assessed in a similar fashion. NK activity was measured before irradiation, after 4 weeks of treatment and four weeks after the last irradiation. Control groups who received no UV-irradiation had samples taken at four week intervals. The effect of UCA isomers on NK cell activity *in vitro* was also determined.

7.2. RESULTS

7.2.1. Patients and Study Design

The clinical details of the subjects examined in the following study are outlined in Table 7.1. A total of twenty six psoriasis patients were recruited; twenty were suffering from chronic plaque psoriasis and the other six had guttate psoriasis. Five in group A receiving coal tar dressings, six in group B receiving broad band UVB therapy (UV6), were attending the Department of Dermatology, Royal Infirmary Edinburgh. The nine patients in group C receiving narrow band UVB (311-312 nm, TLO1), and six in group D receiving PUVA were attending the Photobiology Clinic, Ninewells Hospital, Dundee. A further two control groups were recruited in Edinburgh. These consisted of four normal individuals who received no treatment (group E) and four individuals who received a standard course of UVB treatment, identical to that given psoriatic patients attending in Edinburgh (as part of another study), except that only their arms were exposed, while the remainder of their bodies were draped (Group F). At each visit the severity of the psoriasis was assessed, NK cell activity determined and phenotyping of PBMC subsets carried out. All subjects receiving a UV-based treatment in this cohort had blood samples taken before commencing treatment, after four weeks of treatment and four weeks after the last irradiation. (Patients attending in Edinburgh had the same routine laboratory tests carried out as those described in Chapter 6 and also Finn Chamber sampling of the epidermis for UCA was carried out.) Subjects who received no UV had samples taken on two occasions at an interval of four weeks. In group A the first sample was taken immediately before commencing coal tar and the second four weeks later.

Table 7.1

Clinical Details of Subjects Studied in Chapter 7

Group	No Patients	Mean Age (± sem)	Age Range	Sex	Skin Condition	Therapy	Mean Number Of Treatments At Sample Point (± sem)	Mean UV Dose At Sample Point During Therapy J cm^{-2} (± sem)	Mean Number Of Treatments In Total (± sem)	Mean Total UV Dose (J cm^{-2} (± sem)
A	5	41 ± 8.1	19-61	1M/4F	Psoriasis	Coal Tar	NA	NA	NA	NA
B	6	37 ± 5.1	31-45	4M/2F	Psoriasis	UVB	11.4 ± 1.8	1.81 ± 0.3	18.9 ± 2.3	5.46 ± 1.28
C	6	60 ± 4.1	45-72	6M	Psoriasis	PUVA	7.0 ± 0.3	21.6 ± 4.0	15.5 ± 1.5	88.6 ± 18.1
D	9	41 ± 6.0	22-72	4M/5F	Psoriasis*	TLO1	11.7 ± 0.5	6.55 ± 8.7	21.5 ± 1.5	19.2 ± 02.7
E	7	33 ± 4.4	21-55	2M/5F	Normal	Nil	NA	NA	NA	NA
F	4	38 ± 10	32-53	2M/2F	Normal	UVB	13.0 ± 1.1	3.40 ± 0.6	16.5 ± 0.6	6.3 ± 1.3

*All psoriasis patients were suffering from chronic plaque psoriasis with the exception of 6 patients in group C who had guttate psoriasis.
NA: not applicable

7.2.2. Typical Results Obtained in NK Cell Assay

With few exceptions in the experiments described the standard error of the mean (sem) of cpm for quadruplicate wells in NK cell assays was always less than 10% of the mean cpm. The maximum release ranged from 125 760-21 500 cpm and the spontaneous ^{51}Cr-release was less than 30% of the maximum release. Table 7.2 shows representative data from two typical experiments carried out on one of the group F patients (normal subjects receiving UVB) before (experiment 1) and during UVB therapy (experiment 2). The % specific release of ^{51}Cr was calculated as outlined in the Materials and Methods (section 2.3.13) and this was taken as a measure of NK activity. Despite a marked difference in the maximum and spontaneous release in Table 7.2, the relative % specific release was of the same order.

7.2.3. NK Activity In Normal And Psoriatic Subjects

On comparing the NK cell activity of normal subjects (11 subjects) with those of untreated psoriasis patients (26 patients) there was no statistical difference at any E:T (effector to target cell) ratio (unpaired t-test). The mean data are shown in Table 7.3.

Table 7.2.

Representative Data From Two Experiments Carried Out To Determine NK Cell Activity on a Single Patient in Group F on Two Separate Occasions 4 weeks Apart. E cells were incubated with 51Cr-labeled K562 target cells at a range of E:T (effector to target) cell ratios (40:1 to 1.3:1) in quadruplicate wells for 18h. The radioactivity released into the culture medium was measured and the specific release induced by E cells determined.

	EXPERIMENT 1			EXPERIMENT 2		
E:T	**Mean cpm**	**sem**	**% Specific ^{51}Cr-Release**	**Mean cpm**	**sem**	**% Specific ^{51}Cr-Release**
1.3	11 827	555	14.4	24 338	842	10.1
2.5	14 198	98	22.9	25 322	852	11.4
5.0	14 904	317	25.4	31 758	1 375	19.5
10	17 880	258	36.0	41 905	1 183	32.3
20	22 162	594	51.2	54 647	1 842	48.5
40	27 652	812	70.8	64 342	895	60.7
Spont	7 773	419	NA	16 346	1 142	NA
Max	35 863	382	NA	95 378	2 068	NA

NA: not applicable
Spont: spontaneous release of ^{51}Cr
Max: maximum release of ^{51}Cr

Table 7.3.

Mean NK Cell Activity (% Specific ^{51}Cr-release) in Normal and Untreated Psoriatic Subjects at Different Effector : Target (E:T) cell ratios.

	NORMALS *			PSORIATICS **			
E:T	**Mean NK Activity**	**sem**	**Range**	**Mean NK Activity**	**sem**	**Range**	**p**
40	52.2	5.4	25.9 - 70.0	61.7	3.8	33.8 - 100	0.105
20	42.7	5.0	13.2 - 58.4	52.8	3.6	26.1 - 95.1	0.139
10	32.8	4.8	7.1 - 52.0	38.9	3.8	15.4 - 76.4	0.231
5.0	21.4	3.4	5.2 - 29.9	29.8	3.7	9.7 - 89.4	0.213
2.5	15.6	2.4	3.0 - 25.2	23.2	3.5	4.2 - 61.0	0.200
1.3	13.2	2.4	2.3 -16.1	17.2	2.8	2.0 - 45.3	0.322

* (n=11)
** (n=26)
p: determined by unpaired Student's t test

7.2.4. Effect Of Phototherapy On NK Cell Activity In Psoriasis Patients.

All patients showed a marked clinical improvement (to minimal residual activity) in the severity of psoriasis irrespective of the treatment used. It should be noted that, although all samples were collected after four weeks of therapy, in most instances patients required six weeks of therapy to achieve complete, or almost complete, clearance of psoriasis.

Fig 7.3 illustrates the mean NK activity before, during and after therapy in the four psoriasis groups. Coal tar treatment (group A) had no significant effect on NK cell activity (Fig 7.3 (a)). Patients receiving broad band UV6 treatment (group B) showed no significant alteration in NK activity after 4 weeks of therapy. However, there was a marked reduction in activity 4 weeks after the last treatment in five out of the six patients (Fig 7.3 (b)), which was statistically significant for the whole group at all E:T ratios ($p = 0.046$; Wilcoxon signed ranks test).

Six patients receiving TLO1(group C) showed a marked suppression in NK cell activity following four weeks of therapy (Fig 7.3.(c)) eg the mean value for the group falling from 66.8 ± 7.1 to 42.0 ± 6.6 at an E:T ratio of 40:1. This was statistically significant at all E:T cell ratios ($p = 0.028$; Wilcoxon signed rank test). Four weeks after the last irradiation the mean NK value for the group had recovered significantly (eg 50.9 ± 7.0 at the 40:1 E:T ratio; $p = 0.043$ Wilcoxon signed rank). However, it still remained significantly suppressed compared with the original value prior to irradiation in 5/6 patients ($p = 0.043$; Wilcoxon signed ranks test). Samples were collected from 2 of the patients a further 4 weeks later, i.e. 8 weeks after the last irradiation. The NK activity was again increased but had still not returned to the original level. The individual data for these two patients are outlined in Table 7.4.

Table 7.4.

The Effect Of TLO1 Therapy on NK Activity In 2 Psoriasis Patients. NK activity was assessed before therapy, following 4 weeks of therapy and subsequently four and eight weeks after the last treatment.

	No. Treatments	Dose (J cm^{-2})	E:T 40	20	10	5.0	2.5	1.3
PATIENT 1								
Before	0	0	85.6	79.5	76.4	62.5	61.0	45.3
During	11	6.13	52.8	42.8	24.4	13.4	8.8	7.0
4 Weeks After	NA	NA	57.8	49.1	45.8	34.5	31.8	20.1
8 Weeks After	NA	NA	76.0	67.9	61.1	50.1	42.6	27.9
PATIENT 2								
Before	0	0	78.6	64.0	43.1	25.4	16.0	10.6
During	14	11.01	42.0	43.0	23.0	11.7	5.2	4.9
4 Weeks After	NA	NA	54.4	39.5	24.5	18.0	17.0	8.2
8 Weeks After	NA	NA	58.9	48.6	36.7	23.4	12.0	7.2

E:T: effector to target cell ratio
NA: not applicable
Patient 1 received a total of 17 treatments (12.91 J cm^{-2})
Patient 2 received a total of 18 treatments (17.67 J cm^{-2})

Figure 7.3.

The Effect of UV Therapy on NK cell activity in Patients with Psoriasis. NK activity was determined before (⊟), during (Δ) and after (o) either broad band UV6 (b), narrow band TLO1 (c) or PUVA (d) therapy. Patients receiving coal tar dressings had samples taken on two occasions, before (⊟) and during (Δ) treatment (a). The graphs represent the mean of 5 patients in (a) and 6 patients in (b) - (d). The sem shown for each group prior to treatment was approximately the same on subsequent occasions for the group.

Fig. 7.3.(a)

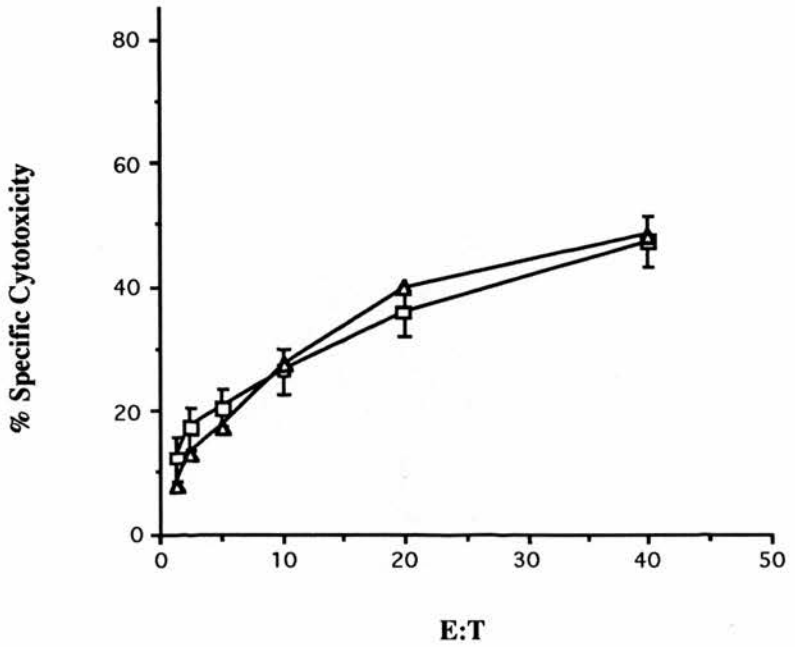


Fig. 7.3.(b)

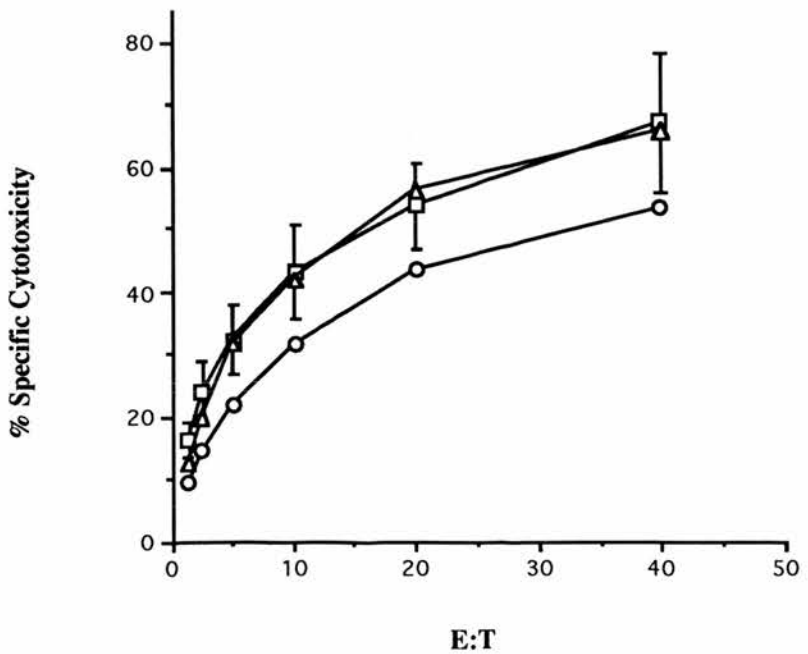


Fig. 7.3.(c)

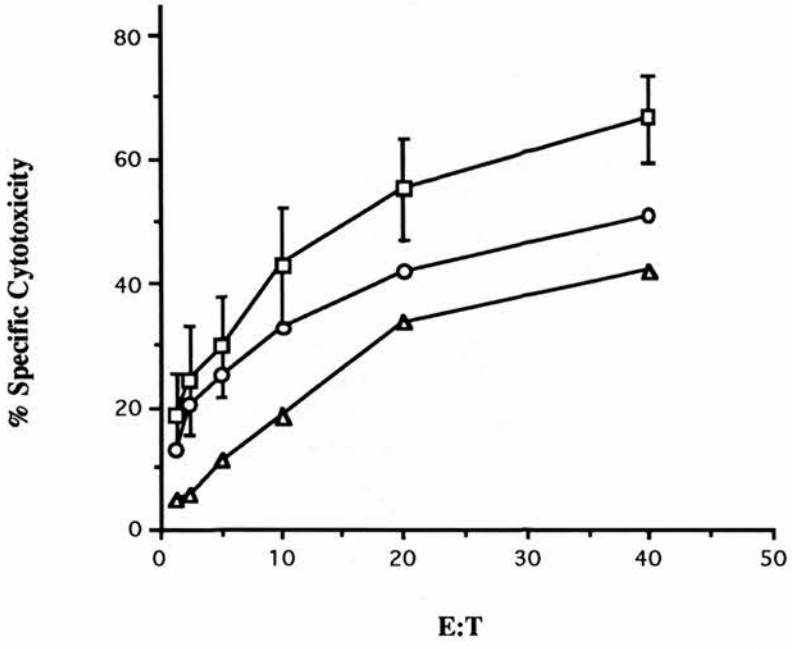
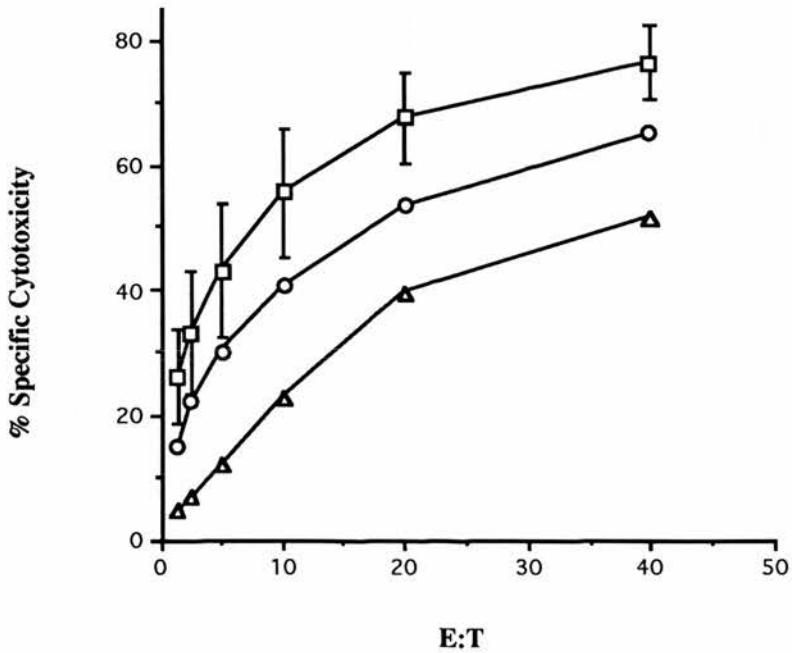


Fig. 7.3. (d)



A further three patients were recruited into this group, but the results were not as clear cut with these patients, as illustrated in Table 7.5.

Table 7.5.

The Effect Of TLO1 Therapy In Three Psoriasis Patients Who Did Not Show Suppressed NK Cell Activity Following Four Weeks Of Therapy.

	No. Treatments	Dose (J cm^{-2})	E:T 40	20	10	5.0	2.5	1.3
PATIENT 7								
Before	NA	0	48.7	3.5	18.5	7.9	4.2	2.0
During[1]	11	3.88	65.6	51.6	35.8	23.7	16.1	12.8
During[2]	18	9.76	56.8	43.1	38.9	27.6	15.4	14.2
After	NA	NA	74.9	62.9	54.2	36.7	27.3	16.0
PATIENT 8								
Before	NA	NA	59.7	56.0	33.6	17.1	10.7	5.0
During[1]	8	2.72	64.4	52.6	42.2	27.3	18.6	12.1
During[2]	15	10.7	55.8	46.9	37.6	22.7	20.1	14.2
After	NA	NA	Did not attend					
PATIENT 9								
Before	NA	NA	42.4	44.1	30.2	16.8	10.9	8.4
During[1]	12	4.60	54.5	39.3	30.7	17.2	12.4	8.2
After	NA	NA	63.5	52.6	52.9	41.8	25.0	20.0

[1] at four weeks of TLO1 therapy
[2] at six weeks of TLO1 therapy
E:T: effector to target cell ratio
NA: not applicable

It should be noted that patients 7-9 had all their samples taken and analysed on the same day at each sample point. All three of these patients were suffering from guttate psoriasis which was relatively mild in comparison to the other patients in the study. Following 4 weeks of therapy there was no suppression in NK cell activity, in fact it tended to increase. It was possible that these patients simply had not received sufficiently high doses of UV to induce suppression. All three patients had received less than the mean dose of 8.0 J cm^{-2} UV received by the other six patients

in this group, and therefore a further sample was taken from patient 7 and 8 two weeks later when the patients had received six weeks of therapy. (Patient 9's skin was completely clear following four weeks of treatment and therefore the NK activity determined at four weeks of therapy was this subject's last treatment). As illustrated in Table 7.5, both patients exhibited a reduced NK cell activity compared to the four week sample point, but this was still not suppressed compared with the starting value in patient 7. The final sample taken four weeks after the last treatment revealed an enhanced NK cell activity compared with the original value assessed before the therapy had commenced. Patient 8 had failed to attend a number of treatments in the first four weeks of the therapy and therefore had a reduced number of exposures, and subsequently a lower total irradiation dose. This patient also failed to attend on two subsequent dates following the last treatment and therefore no further samples were obtained. It should also be noted that at the first sample point during therapy no significant improvement in the severity of the patient's psoriasis was observed.

Following four weeks of PUVA therapy, all six patients exhibited a marked reduction in NK cell activity eg the mean NK cell activity for the group at the 40:1 E:T fell from 76.4 ± 6.1 to 51.6 ± 8.0 (Fig 7.3.(d)). This was statistically significant at all E:T cell ratios but more so at E:T ratios < 40:1 ($p = 0.046$ at 40:1 and $p = 0.028$ at E:T cell ratios < 40:1; Wilcoxon sign ranks test). Four weeks after the last treatment the activity had recovered significantly ($p= 0.028$ at E:T ratios < 40:1; Wilcoxon sign ranks test) but remained significantly suppressed relative to the original value at E:T ratios less than 40:1 ($p = 0.028$; Wilcoxon sign ranks test). Three patients had their NK cell activity assessed four weeks later when they had almost returned to normal (Table 7.6).

Table 7.6.

The Effect of PUVA Therapy on NK Activity In 3 Psoriasis Patients. NK activity was assessed before therapy, following 4 weeks of therapy and subsequently 4 and 8 weeks after the last treatment.

	No. Treatments	Dose (J cm^{-2})	E:T 40	20	10	5.0	2.5	1.3
PATIENT 1								
Before	0	0	57.8	50.0	31.3	24.8	10.7	7.8
During	7	33.39	29.7	23.3	12.3	6.5	0	0
4 Weeks After	NA	NA	46.2	34.0	24.7	13.4	7.1	4.8
8 Weeks After	NA	NA	53.1	39.7	24.0	20.0	10.0	5.5
PATIENT 2								
Before	0	0	67.6	46.8	28.8	20.1	18.1	18.5
During	8	29.70	32.0	29.3	3.2	2.7	0.7	0
4 Weeks After	NA	NA	41.9	33.4	24.0	19.7	15.3	10.6
8 Weeks After	NA	NA	53.8	41.8	26.2	14.6	9.9	5.1
PATIENT 3								
Before	0	0	69.0	64.6	42.3	27.1	17.7	9.5
During	6	25.53	71.4	40.1	27.0	8.0	8.0	6.4
4 Weeks After	NA	NA	56.2	49.4	37.1	21.7	14.8	12.3
8 Weeks After	NA	NA	71.4	66.6	46.8	38.9	17.7	9.8

E:T: effector to target cell ratio
NA: not applicable
Patient 1 received a total of 19 treatments (166.12 J cm^{-2})
Patient 2 received a total of 13 treatments (71.30 J cm^{-2})
Patient 3 received a total of 19 treatments (166.12 J cm^{-2})

7.2.5 Effect of UVB Phototherapy on NK cell Activity in Normal Subjects

Four normal subjects received a course of UV6 phototherapy identical to that received by psoriasis patients attending in Edinburgh, exposing only their arms. Control subjects were normal individuals who received no UV-irradiation. NK activity was assessed in the same manner in this group as for group A and B; psoriasis patients receiving UV6 or coal tar therapy in Edinburgh respectively. Fig 7.4. illustrates that the data for the non-psoriatic group were similar to those of the psoriasis patients. Four weeks of UV6 treatment had little or no effect on NK cell activity. However four weeks after the last irradiation the NK cell activity was suppressed in three out of the four subjects but this did not reach statistical significance ($p > 0.05$; Student's paired t test).

Figure 7.4.

The Effect of UV6 Treatment on NK Cell Activity in Normal Subjects. 4 control subjects, receiving no treatment, had samples taken on two occasions at time 0 (□) and four weeks later (Δ), Fig (a). 4 subjects received a standard course of UV6 treatment. NK activity was determined before (□), during (Δ) and after (o) treatment; Fig (b). The figures represent the mean value for each group, the sem shown was approximately the same on each occassion in both groups.

Fig. 7.4. (a)

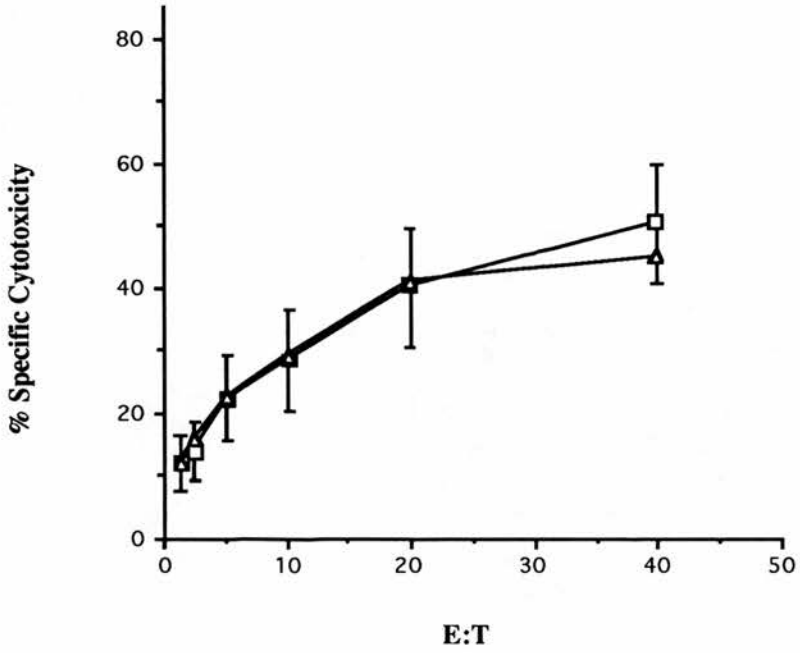
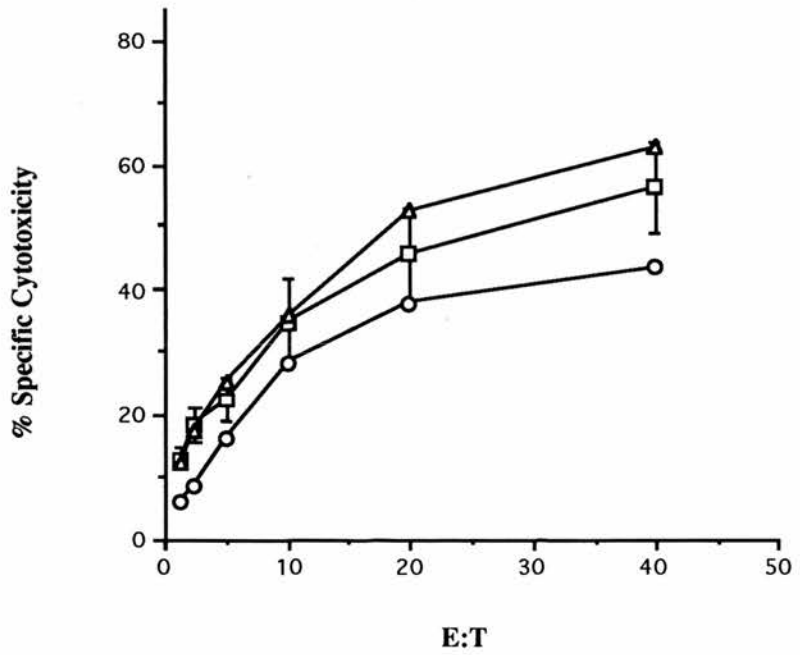


Fig. 7.4. (b)



7.2.6 Phenotyping

PBMC were stained with the same monoclonal antibodies employed in Chapter 6 which were directed against CD1a, CD3, CD4, CD8, CD57 and MHC class II. No significant alteration in any of the markers occurred as a result of therapy (Table 6.3), including patients receiving TLO1 therapy (data not shown).

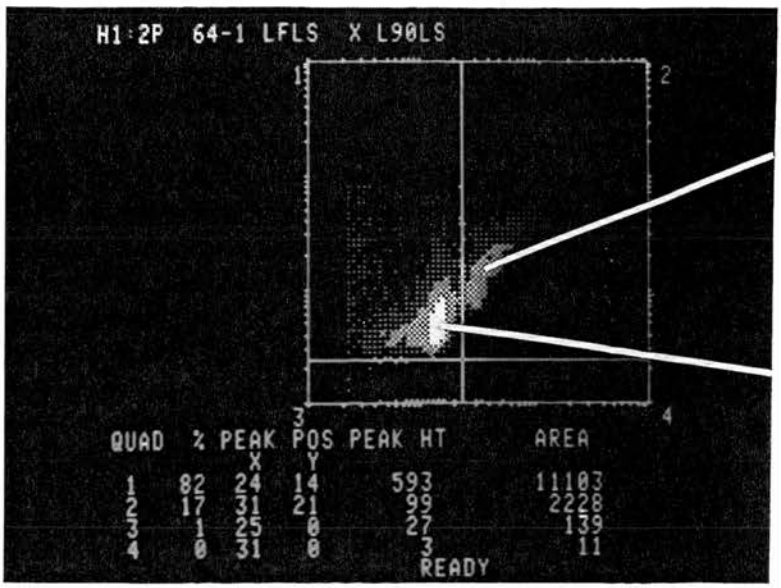
In addition to these antibodies, a further two monoclonal markers directed against CD16 and CD56 were employed to determine the number of NK cells present in three of the TLO1 patients and four of the PUVA patients. An irrelevant antibody was used to determine the background non-specific staining, which was approximately 1.1% of the total histogram (Fig 7.5 (b)).

Data were originally collected on a two parameter histogram measuring forward angle light and 90° light scatter (Fig 7.5 (a)). Bit maps were drawn around both the lymphocyte (H3) and monocyte/LGL (H4) populations and the percentage of cells exhibiting fluorescent levels higher than the background were recorded for these populations independently as well as the for the total cell population (H2). The pattern of staining for patient 3 (Table7.7) in the the TLO1 patient group with the CD 56 antibody before and during therapy are shown in Fig 7.5 (c) - (f). The percentage of cells stained with CD16 and CD56 for each patient are shown in Table 7.7. TLO1 and PUVA treatment resulted in a reduced number of $CD56^+$ cells in the H4 histogram (which contains the LGLs) and a corresponding drop in the H2 histogram. The reduced number of large granular cells expressing CD56 corresponded well with reduced NK cell activity, with the exception of patient 3 in the TLO1 group (Table 7.7). The three PUVA patients also displayed an increase in the number of cells expressing the CD 16 marker when examining the H4 histogram. This was not observed with the TLO1 patients.

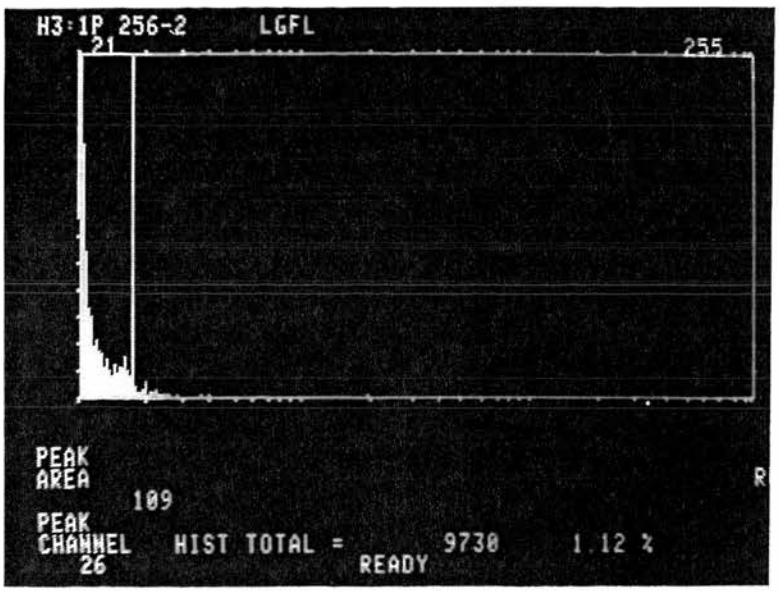
Figure 7.5

Fluorescent Flow Cytometry Analysis of PBMC from Patient 3 in the TLO1 Group Stained with the anti-CD56 Antibody. Data were originally collected on a two parameter histogram measuring forward angle light and 90° light scatter (Fig 7.5 (a)) Bit maps were drawn around both the lymphocyte (H3) and monocyte/LGL (H4) populations and the percentage of cells exhibiting fluorescent levels higher than the background were recorded for these populations independently as well as the for the total cell population (H2). An irrelevant antibody was used to determine the background non-specific staining, which was approximately 1.1% of the total histogram (Fig 7.5 (b); cell number Y axis, plotted against log fluorescence X axis. Fig (c)-(f) show the cell number plotted against log fluorescence; (c) and (d) represent the pattern of staining before and during therapy in the H2 histogram and (e) and (f) represent the pattern of the H4 before and during therapy (respectively).

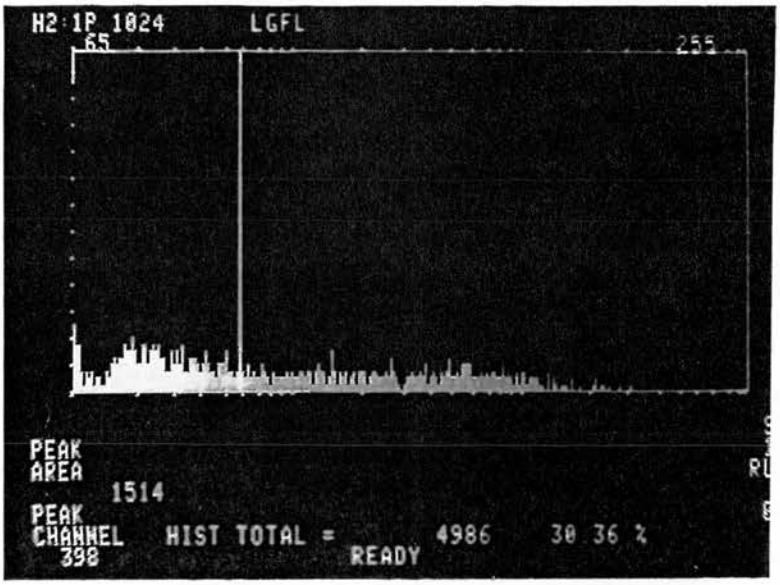
(a)



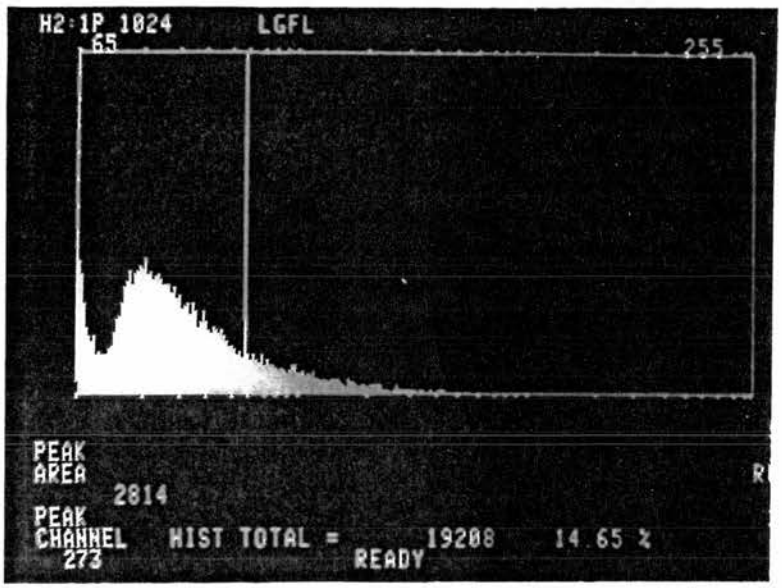
(b)



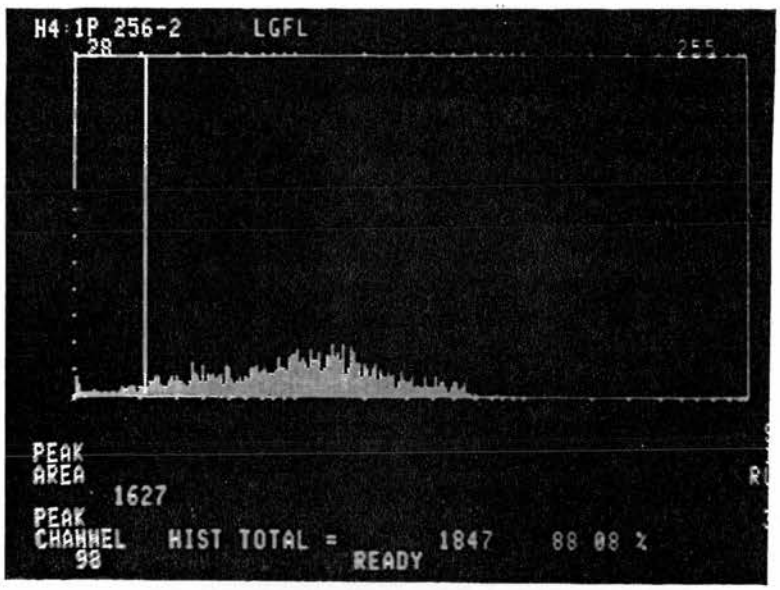
(c)


H2:1P 1024
LGFL
65
255
PEAK
AREA
1514
PEAK
CHANNEL
398
HIST TOTAL = 4986
30 36 %
READY

(d)


H2:1P 1024
LGFL
65
255
PEAK
AREA
2814
PEAK
CHANNEL
273
HIST TOTAL = 19208
14 65 %
READY

(e)


H4:1P 256-2 LGFL
28
255
PEAK
AREA
1627
PEAK
CHANNEL
98
HIST TOTAL = 1847 88 08 %
READY

(f)

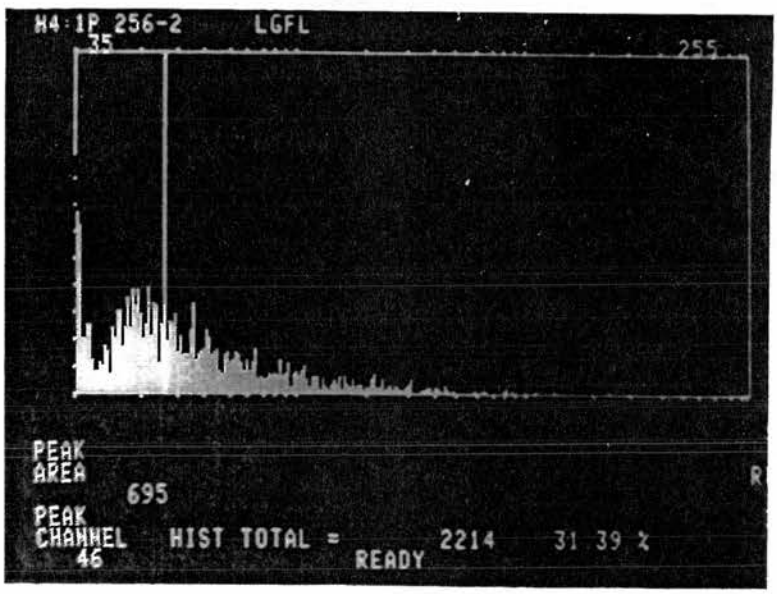

H4:1P 256-2 LGFL
35
255
PEAK
AREA
695
PEAK
CHANNEL
46
HIST TOTAL = 2214 31 39 %
READY

Table 7.7.

The Effect of TLO1 (Group D) or PUVA (Group C) Therapy on Circulating NK Cells Assessed by Flow Cytometry. The % of CD56 and CD16 cells was determined before, during and after therapy. The H2 histogram contains all PBMC while the H4 gates the larger granular cells.

	CD56		CD16		% NK Cell
	H2	H4	H2	H4	Activity*
GROUP D					
Patient 1					
Before	13.4	49.9	44.7	72.0	79.5
During	3.5	7.8	34.9	64.4	42.8
After	32.7	57.1	17.8	24.9	49.1
Patient 3					
Before	30.0	88.1	21.1	43.2	70.5
During	14.7	31.5	24.2	45.6	47.7
After	12.0	18.9	13.0	13.4	63.7
Patient 5					
Before	ND	ND	ND	ND	28.6
During	5.2	8.3	12.64	ND	5.1
After	9.3	62.7	21.4	ND	31.0
GROUP C					
Patient 2					
Before	ND	ND	ND	ND	46.8
During	20.6	33.3	13.5	ND	29.3
After	34.8	78.7	17.3	19.5	33.4
Patient 3					
Before	15.0	79.5	13.3	14.5	73.8
During	5.2	25.0	17.9	29.3	27.6
After	16.7	55.6	8.9	11.9	30.0
Patient 4					
Before	12.0	83.4	18.7	18.5	75.6
During	13.3	33.3	30.5	52.8	41.3
After	20.2	66.7	8.6	14.7	86.2
Patient 5					
Before	13.2	84.0	34.1	33.6	95.1
During	6.4	26.2	36.1	57.4	76.6
After	28.6	88.0	28.3	35.6	86.6

*NK activity at E:T ratio of 20:1

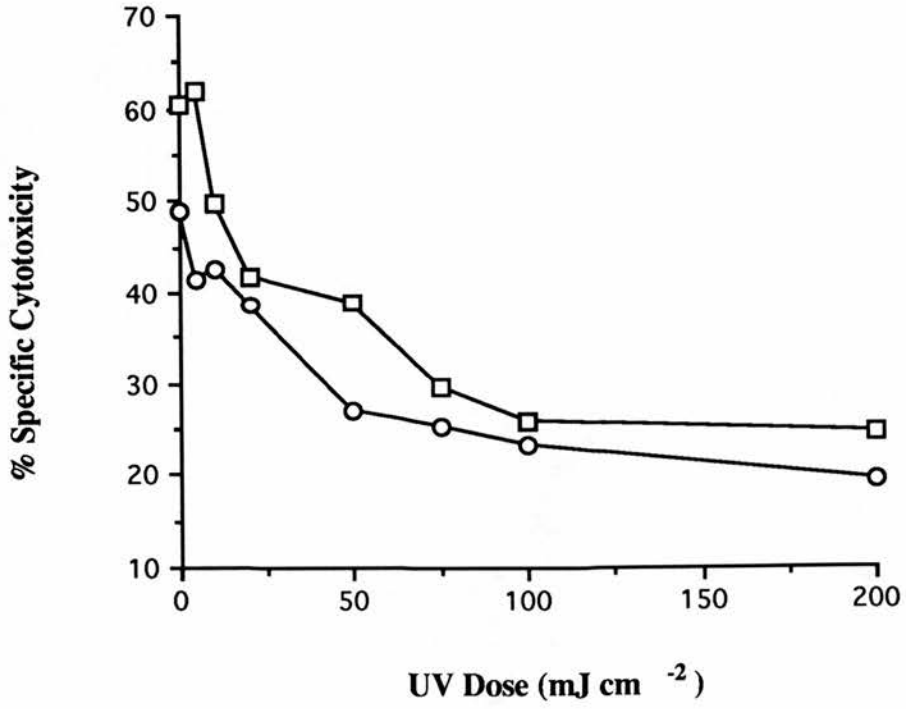
7.2.7 The Effect of *in vitro* UV-Irradiation of PBMC on NK Cell Activity.

The results in section 7.2.4.-7.2.5. demonstrated that four weeks of both PUVA and TLO1, but not UV6 therapy resulted in a significant modulation of NK activity and possibly the numbers of circulating NK cells. However it was impossible to say definitively whether this was a dose dependent or a wavelength dependent phenomenon. The aim of the following experiments was to compare the effect of *in vitro* irradiation of PBMC with different wavelengths of UV on NK cell activity to determine whether any effects observed were wavelength dependent.

In a preliminary experiment, PBMC were isolated from a normal healthy donor, washed and resuspended in PBS with no indicator. Monolayers of the cells (10^6 cells per well of a flat bottomed 96 well plate) were then irradiated with increasing doses of UVB from a single TLO1 lamp *in vitro* as outlined in section 2.3.14. Cell viability remained between 95-100%, as determined by trypan blue exclusion, following irradiation. The ability of these cells to lyse labelled K562 cells at an E:T cell ratio of 40:1 and 20:1 was subsequently quantified. The results of this experiment are illustrated in Fig 7.6. Irradiation of PBMC with UVB of 311-313 nm resulted in a dose dependent suppression of NK cell activity.

Figure 7.6.

The Effect of in vitro Irradiation of PBMC With Narrow Band TLO1 (311-312 nm) on NK Cell Activity. PBMCs were irradiated with increasing doses of TLO1 and NK activity measured at E:T ratios of 40:1 (□) *or 20:1* (o).



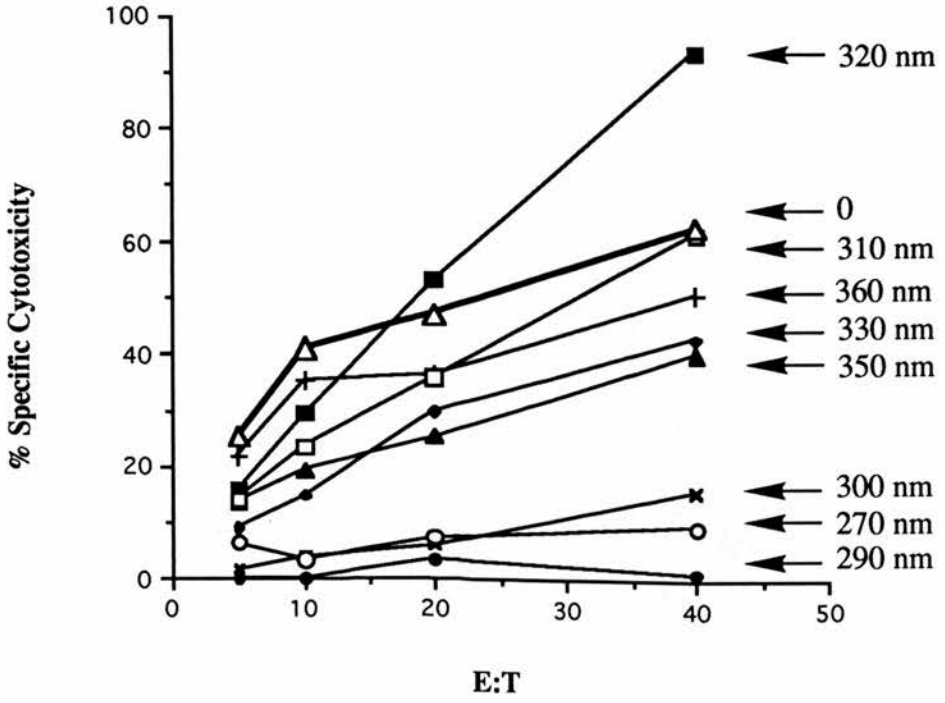
The effect of irradiation with monochromatic UV radiation ranging from 270-360 nm was examined. This experiment was carried out twice with PBMC isolated from two healthy donors on each occasion. The results obtained in one experiment, treating cells with either 50 or 100 mJ cm^{-2} of monochromatic UV, are outlined below. Similar results were obtained with PBMC from the other donors. The NK cell activities measured at each wavelength over a range of E:T cell ratios are shown in Fig 7.7.

The percent residual NK activity following irradiation (percentage NK activity of irradiated cells expressed as a percentage of the activity of unirradiated cells) for this subject at E:T cell ratios of 40:1 and 20:1 are illustrated in Fig 7.8; the results at the lower E:T ratios were similar. It can be concluded from Fig 7.7 and Fig 7.8 that the lower UV wavelengths, below 310 nm in this instance are the most potent at suppressing NK cell activity *in vitro,* and 100mJ cm^{-2} of a given wavelength did not appear to induce a significantly greater suppression in NK cell activity than 50 mJ cm^{-2}.

To determine whether the suppressive effect of UV-irradiation was specific for NK cells, UV irradiated PBMC from two subjects were cultured for three days in the presence of 5 μg ml^{-1} of Con A, a T cell mitogen, and the control cells without Con A. The cultures were pulsed with ^{3}H-thymidine during the final 24 h of culture and the uptake determined. The stimulation indices were calculated and the results from donor three are outlined in Fig 7.9.

Figure 7.7.
The Effect of Monochromatic UV-Irradiation of PBMCs on NK Cell Activity. PBMC were irradiated with (a) 50 mJ cm^{-2} or (b) 100 mJ cm^{-2} of monochromatic UV ranging from 270-360 nm before assaying NK cell activity

(a)



(b)

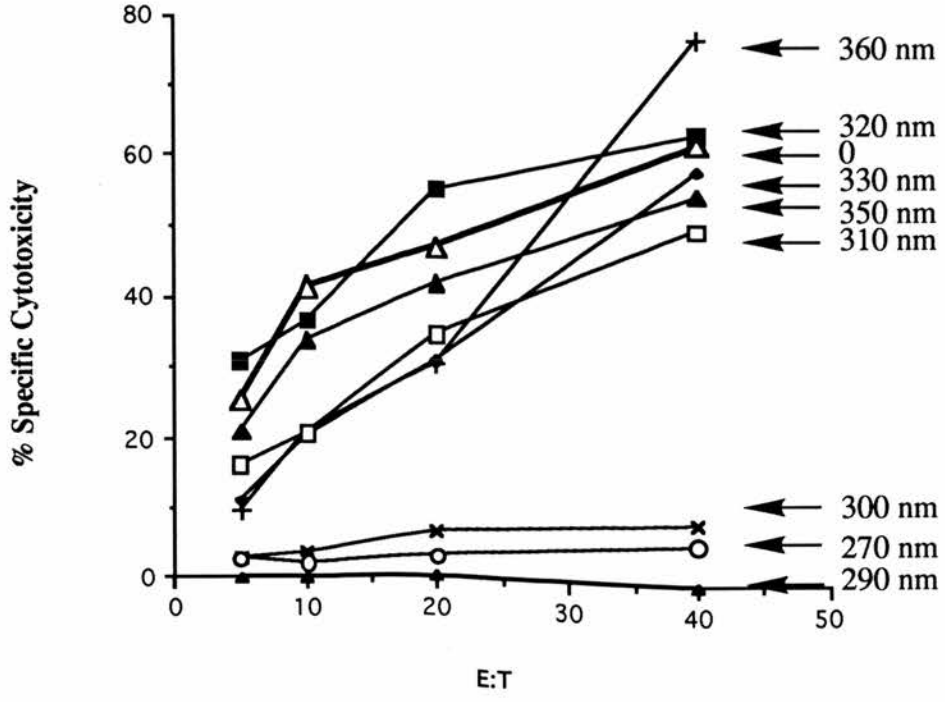


Figure 7.8.

Residual NK Cell Activity Following Irradiation With (a) 50 mJ cm^{-2} or (b) 100 mJ cm^{-2} of Monochromatic UV. The residual NK cell activity is shown at E:T cell ratios of 40:1 (■) and 20:1 (o).

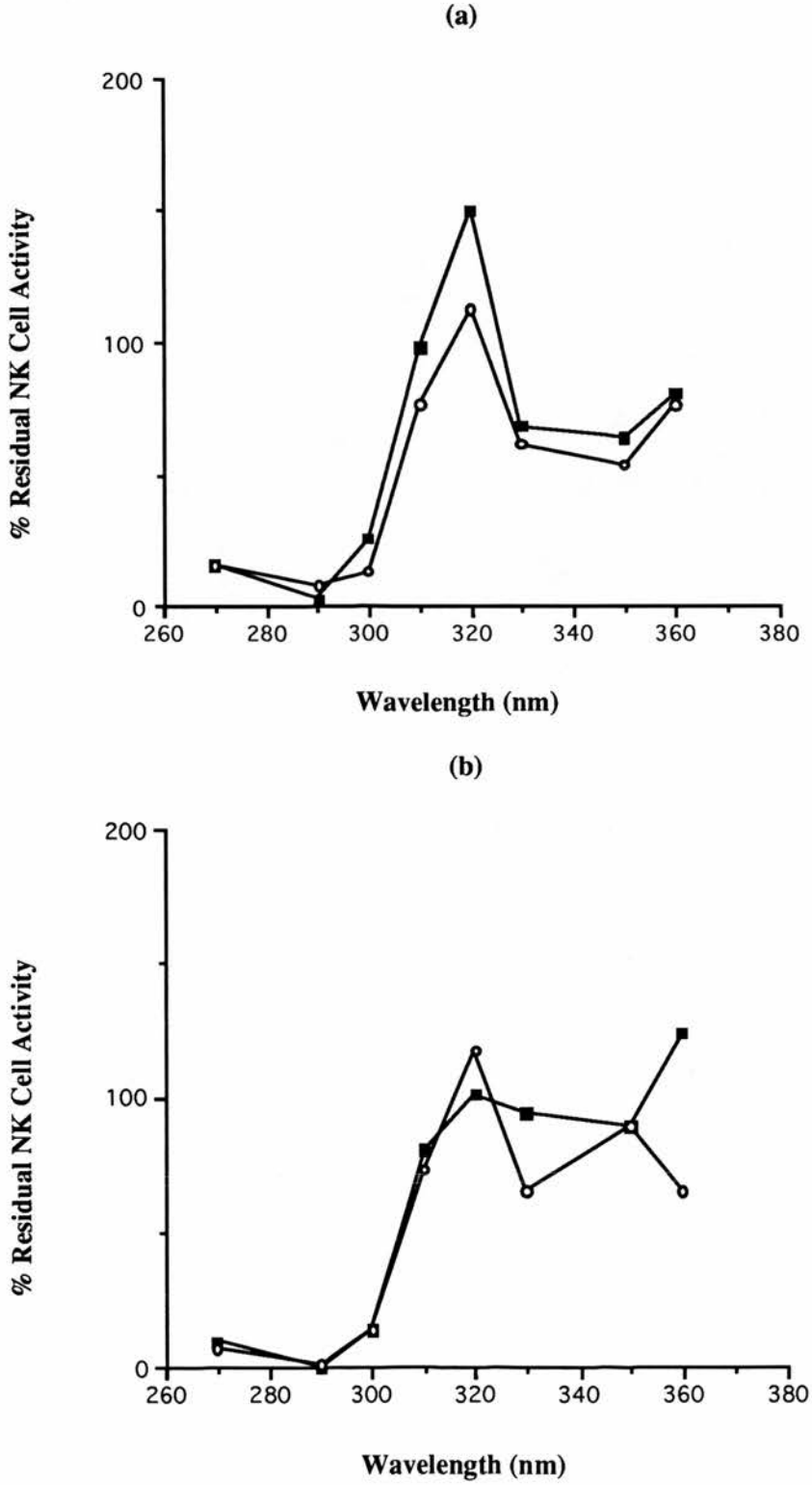
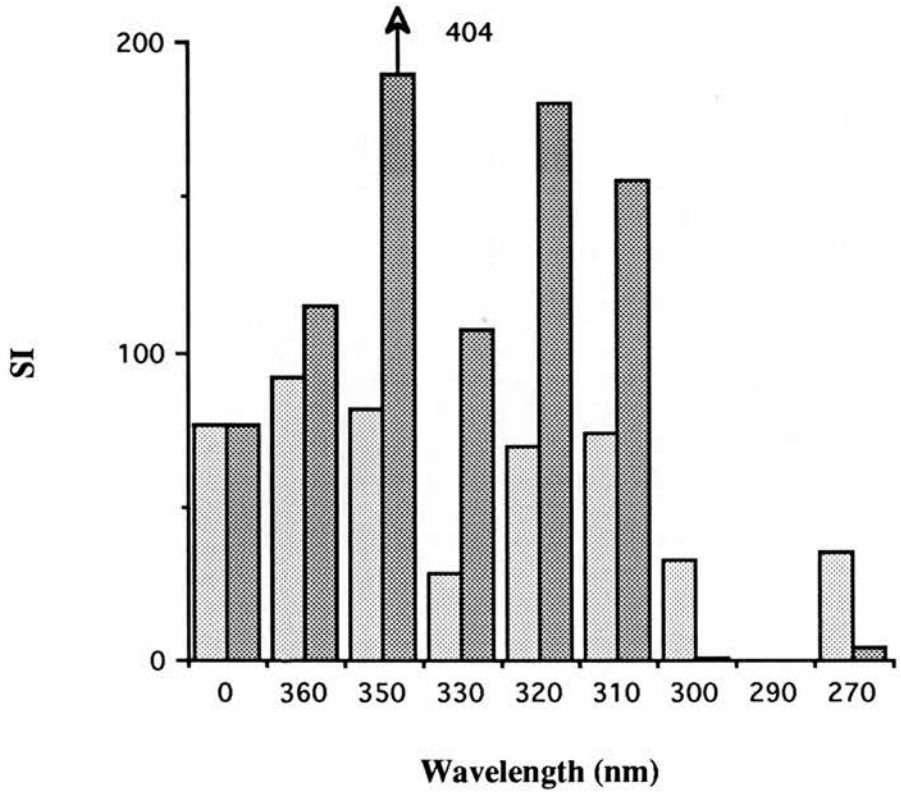


Figure 7.9.

The Effect of Monochromatic UVR on in vitro LPR to Con A. PBMC were irradiated with 50 (□) or 100 (■) mJ cm^{-2} of monochromatic light and the uptake of ^{3}H-thymidine determined over the final 24h of a three days culture with nil or 5 μg ml^{-1} Con A. The stimulation indices (SI) for one subject at each wavelength are illustrated below.



7.2.8 The Effect of UCA isomers on NK Cell Activity *in vitro*

The results outlined previously in this chapter demonstrated that UVR *in vitro* and also *in vivo* resulted in suppression of NK cell activity. The following series of experiments was undertaken to determine the effects of UCA, a proposed mediator of UV-induced immunosuppression, on NK cell activity *in vitro*. It was important to establish that incubation of labelled target cells with UCA isomers had no effect on the background release of ^{51}Cr from the cells. The concentrations of UCA isomers employed were relatively high, up to 10^{-2} M *cis*-UCA, and therefore it was also necessary to also establish that any suppression in NK cell activity was not due to toxicity. This was determined by trypan blue exclusion. The LPR to Con A and HSV were also measured to test whether any suppression was specific to NK cell activity.

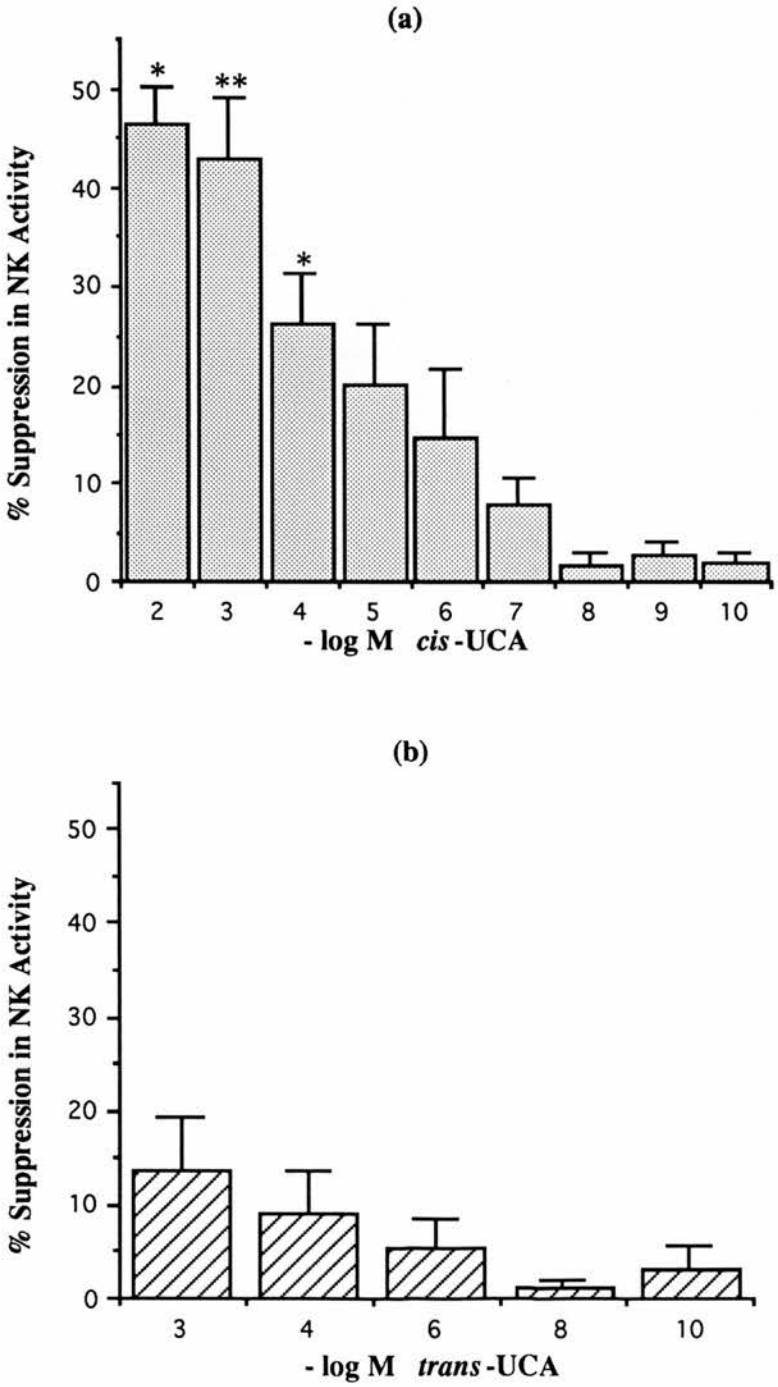
Serial tenfold dilutions of *cis* (10^{-1}M to 10^{-8} M) or *trans* (10^{-2} to 10^{-8}) UCA in PBS were prepared. Ten µl of the appropriate concentration of UCA were added to 0.5 ml of E cells. The cells were then added to wells of 96 well plate and approximately 15 minutes later, labelled target cells were added at an E:T cell ratio of 40:1. This resulted in a final concentration of 10^{-3}M to 10^{-10} M *cis* or 10^{-4} M to 10^{-10} M *trans* -UCA when the target cells were added. One hundred µl of 10^{-1} M *cis*- or 10^{-2} M *trans*-UCA were added to 0.4 ml of cells, containing the same number of cells as 0.5 ml above, resulting in the final concentrations of 10^{-2} and 10^{-3} M respectively.

The percentage suppression in NK cell activity induced by *cis*-UCA was determined over a range of concentrations for 12 subjects; seven normal individuals and five untreated psoriasis patients. UCA-isomers had the same effect on PBMC from psoriatic or normal subjects and therefore Fig 7.10. represents the mean (± sem) suppression in NK activity of groups of six to eleven of these individuals . The

effect of *trans*-UCA was determined in four normal subjects in the same fashion (Fig 7.10.).

Figure 7.10.

*The Effect of (a) cis-UCA or (b) trans-UCA on NK Cell Activity in vitro. Graph (a) represents the mean ± sem of 6-11 subjects and (b) represents the mean ± sem for four subjects. *p<0.05 and **p<0.01 compared with the mean of the same individuals with no UCA added, paired t test.*

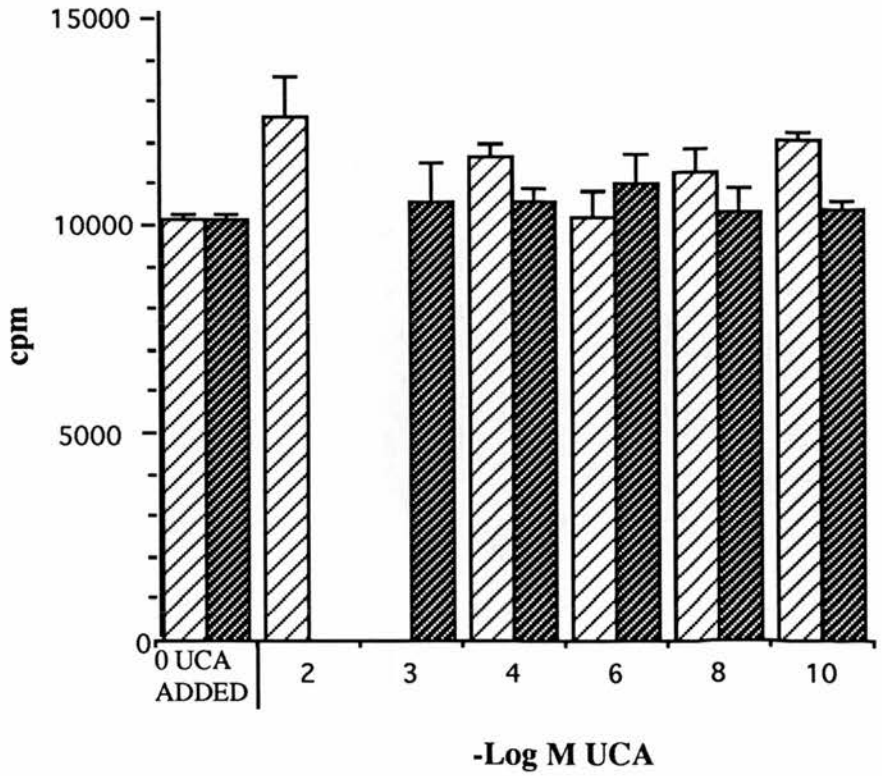


Treatment with *cis*-UCA resulted in a dose dependent suppression of NK cell activity which was statistically significant at the higher concentrations of 10^{-2}M to 10^{-4} M ($p < 0.05$ at 10^{-2} and 10^{-4} and $p < 0.01$ at 10^{-3} M: paired t-test). *Trans*-UCA was slightly suppressive at higher concentrations but this was minimal in comparison to *cis*-UCA, and was not statistically significant.

To test that the addition of UCA-isomers during the incubation of E and target cells did not effect the spontaneous release of ^{51}Cr from the target cells on four separate occasions, one hundred µl of medium containing the appropriate concentration of UCA isomer was added to ten wells containing the labelled targets, resulting in a final concentration of 10^{-2} M to 10^{-10} M. The cells were incubated for 18 h and the supernatant assayed for ^{51}Cr. Control cultures of PBMC with or without UCA isomers were added to labeled target cells in parallel to confirm that the target cells were lysed as normal. The result from one experiment is shown in Fig 7.11. E cells induced normal target cell lysis and incubation of target cells with UCA isomers caused no significant alteration in the background release of ^{51}Cr.

Figure 7.11

The Effect of UCA-isomers on Spontaneous ^{51}Cr-Release from Target Cells. Labeled target cells were cultured for 18h with medium containing cis (▨) or trans (■) UCA from 10^{-2} and 10^{-3} M (respectively) to 10^{-10} M. The mean cpm ± sem of ^{51}Cr-released into the medium at each concentration from one experiment are shown. (10^{-3} M cis and 10^{-2} trans not done)

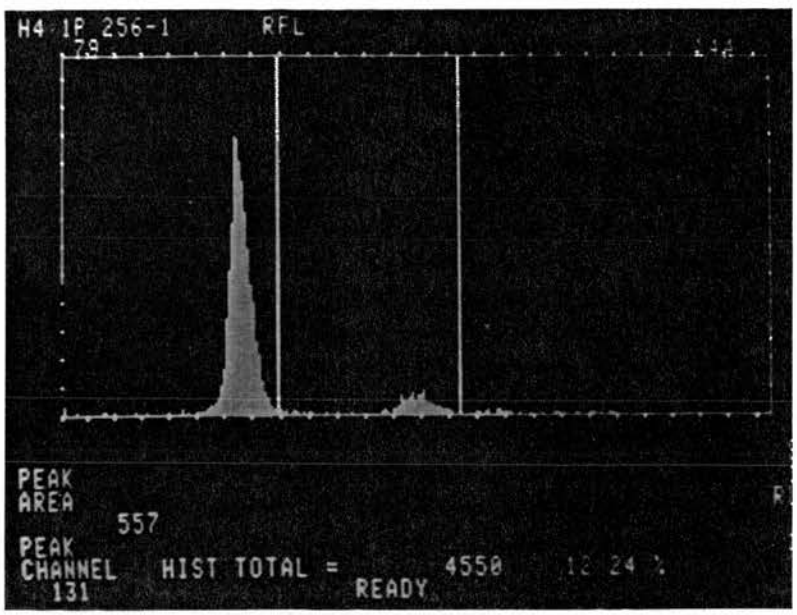


Cis-UCA at relatively high doses resulted in suppression of NK cell activity. To determine whether this was merely a toxic effect on E cells they were incubated with UCA isomers and the number of viable E cells present determined by trypan blue exclusion. Viability was between 90-100%. The cells were also stained overnight with propidium iodide and the number of cells in the S + G_2M stage of division quantitated by flow cytometry using the S-fit DNA analysis programe (Coulter Electronics Ltd, Luton, UK). Fig 7.12. illustrates the typical staining pattern. There was no difference in the number of dividing cells (Table 7.8.). Therefore

Fig 7.12

Propidium Iodide Staining. Fig (a) represents PBMC cultured for 18h with no UCA and Fig (b) represents cells incubated with 10^{-3} M cis-*UCA*

(a)



(b)

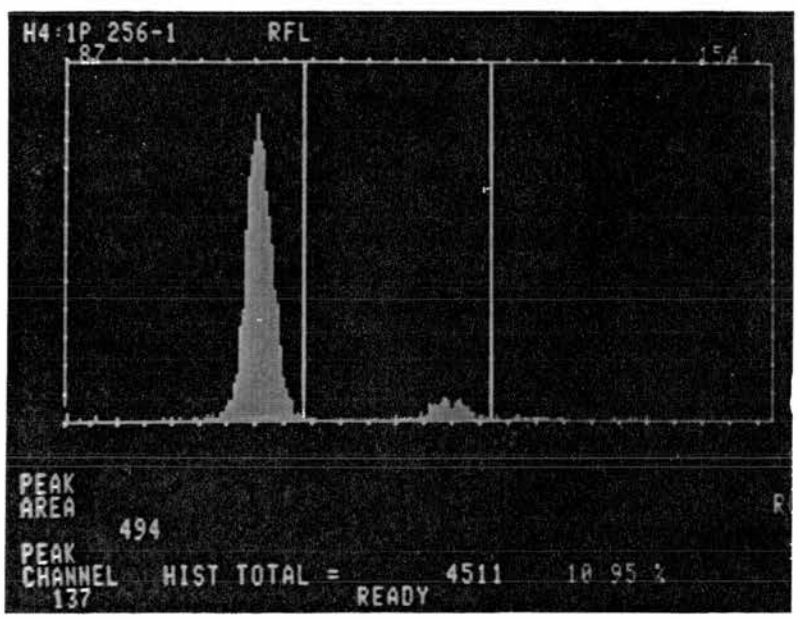


Table 7.8.

The Effect of UCA Isomers on The Percentage of Cells In S + G_2M Determined by Propidium Iodide Staining and Annalysis by Flow Cytometry.

Concentration (-Log M)	% Cells in S + G_2M	
	Trans	*Cis*
Nil	**17.59**	**17.59**
9	13.82	16.00
6	15.96	15.27
3	16.73	15.87

neither *cis*- or *trans*-UCA, even at relatively high concentrations, were lethal to the cells.

Having established that neither UCA isomer was toxic to the cells the next aim was to determine whether UCA affected the function of cell populations other than NK cells. The effect of UCA on lymphoproliferation was examined. UCA isomers, in similar concentrations to those used in the NK assay, were added to quintuplicate wells of PBMC along with sub-optimal concentrations of Con A (1.5 $\mu g\ ml^{-1}$) or HSV antigen (0.1 pfu $cell^{-1}$) and the effect on proliferation determined. The experiment was carried out on two separate occasions using PBMC from two normal healthy donors known to be HSV carriers. It was found on both occasions that neither UCA isomer produced any consistent, or dose dependent effect on the number of cells taking up ^{3}H-thymidine. (The results of one such experiment are shown in Table 7.9.) This again suggests that the *in vitro* dose dependent suppression of NK cell activity by *cis*-UCA is not merely a toxic effect, and appears to be specific to the function of NK cells.

Table 7.9.

The Effect Of UCA Isomers on Lymphoproliferative Responses to Sub-optimal Concentrations of Con A or HSV. The sem of cpm for quintuplicate wells was always less than 10% of the mean

Concentration (-Log M)	Con A		HSV	
	cpm	SI	cpm	SI
Nil	**9023**	**14.5**	**12 998**	**4.3**
***Trans*-UCA**				
14	8 172	13.1	10 458	3.5
12	8 205	13.1	11 750	3.9
10	8 606	13.9	11 184	3.7
8	7 041	11.3	11 328	3.8
6	7 960	12.8	8 623	2.9
4	7 351	11.8	10 665	3.6
***Cis*-UCA**				
14	7 824	12.5	13 206	4.4
12	7 572	12.1	10 528	3.5
10	7 512	12.0	10 457	3.5
8	7 278	11.7	10 459	3.5
6	3 098	5.0	10 038	3.3
4	8 960	14.4	10 007	3.3

No Con A, cpm = 624
Vero control antigen, cpm = 2 999

From the results outlined it appears that *cis*-UCA selectively affects NK cell activity. UCA was added to both the E and target cells for the duration of the assay and therefore it was impossible to establish whether it was the effector or target cells which were directly affected by UCA. It is possible that UCA may alter the target cells resulting in resistance to NK cell lysis. On three occasions ^{51}Cr-labelled target cells were cultured for 30 min at 37°C with 0, 10^{-9}, 10^{-6} or 10^{-3} M *cis*-UCA. The cells were washed three times in PBS and their susceptibility to lysis by PBMC at an E:T cell ratio of 40:1 tested. The results from one such experiment are outlined in Table 7.10.

Pulsing target cells with *cis*-UCA for 30 min had no significant effect on their susceptibility to NK cell mediated lysis. A similar experiment was conducted pulsing the E cells rather than target cells with *cis*-UCA. PBMC pulsed with 0, 10^{-3}, 10^{-6} and 10^{-9} M *cis*-UCA produced 37.1, 35.9, 43.0 and 35.8 % specific cytotoxicity repectively when assayed. Therefore no significant effect on NK cell activity was observed when E or target cells were pulsed with *cis*-UCA.

Table 7.10

The Effect of Pulsing ^{51}Cr-Labelled K562 cells with cis-UCA on their Susceptibility to NK cell Mediated Lysis

UCA (-log M)	^{51}Cr-release Spontaneous	Maximum	E+T cells (40:1)	% Specific NK Cytotoxicity
Nil	4 366	21 614	12 106	47.6
9	4 307	19 665	12 157	51.1
6	5 595	21 251	13 869	52.8
3	4 383	20 817	13 231	53.8

7.2.9 The Effect of Histamine on NK cell Activity *in vitro*

As outlined in Chapter 3, UCA isomers appear to act through histamine-like receptors. As *cis*-UCA produces a dose dependent suppression of NK cell activity *in vitro* it was therefore of interest to determine whether (a) histamine had any effect on NK cell activity *in vitro*, and (b) whether histamine receptors were involved in the *cis*-UCA induced suppression. Several preliminary experiments were carried out to investigate this possibility.

Addition of histamine to NK assays, using the same protocol as that employed to examine the effects of UCA on NK cell activity, produced highly variable results. This experiment was carried out using PBMC from four healthy donors. Any

suppression observed was minimal and was not always dose dependent. The mean results are illustrated in Table 7.11. Histamine had no effect on the spontaneous or maximum ^{51}Cr-release either.

Table 7.11.

The Effect of Histamine on NK Cell Activity in vitro.

Concentration (-Log M)	Number of Subjects	% Suppression NK Cell Activity	sem
6	3	19.9	11.3
5	3	12.4	10.2
4	4	11.2	2.3
3	2	30.2	5.5

The effect of histamine on LPR to sub-optimal concentrations of Con A and HSV was also examined in two of the subjects, known to be seropositive for HSV, in exactly the same way as UCA in section 7.2.6. The results from one such experiment are shown inTable 7.12. As found with UCA, no significant ($p > 0.05$; Student's t test), or dose dependent suppression in LPR to either Con A or HSV was observed.

Table 7.12.

The Effect Of Histamine on Lymphoproliferative Responses to Sub-optimal Concentrations of Con A or HSV. The sem of cpm for quintuplicate wells was always less than 10% of the mean.

	Con A		HSV	
Concentration (-Log M)	**cpm**	**SI**	**cpm**	**SI**
14	8 566	13.7	13 146	4.4
12	8 298	13.3	12 423	4.1
10	8 967	14.4	14 222	4.7
8	9 213	14.8	8 971	3.0
6	6 585	10.6	11 839	3.9
4	7 788	12.5	12 278	4.1

No Con A, cpm = 624
Vero Control Antigen, cpm = 2 999

The possibility remained that *cis*-UCA might act upon NK cells through a histamine-like receptor. Therefore the possibility to blocking the effects of *cis*-UCA by the addition of either an H1, H2 or H3 antagonist to the NK assay was tested. PBMC were pre-incubated for 30 min at 37°C with the antagonist before adding *cis*-UCA and the labelled target cells. Both the antagonist and UCA were present for the duration of the assay. Terfenadine (H1 antagonist) alone (10^{-4} M) was toxic to K562 cells, resulting in 83.1% release of ^{51}Cr, with no PBMC present. Thioperamide (highly specific H3 antagonist, Arrang *et al.*, 1987) had no effect on the spontaneous ^{51}Cr-release by the K562 (data not shown), but suppressed NK cell activity at higher concentrations (Table 7.13.). (R)-α-methylhistamine (H3 agonist, Arrang *et al.*, 1987) had no effect on spontaneous ^{51}Cr-release (data not shown), it also suppressed NK cell activity (Table 7.13.). Since these compounds appear to inhibit NK cell activity directly they were not suitable for use in competion assays with UCA.

Table 7.13.

The Effect of Thioperamide (H3 antagonist) and (R)-α-methylhistamine (H3 agonist) on NK Cell activity in vitro

Concentration (-Log M)	% Specific Cytotoxicity	
	Thioperamide	(R)-α-methylhistamine
9	61.6	52.7
8	69.6	49.6
7	59.6	48.3
6	56.6	50.1
5	55.7	46.1
4	55.7	46.9
3	24.7	38.0
Nil	**63.2**	**63.2**

7.3. DISCUSSION

7.3.1. The Effect of UVR on NK Cell Function *in vivo*

Suppressed NK cell activity has been reported in patients with generalized pustular psoriasis (von Zunbusch type) and to a lesser degree, psoriasis vulgaris (Kaminski *et al.*, 1984). On the other hand Hunyadi *et al.*, (1981) also reported normal NK cell activity in a group of patients with psoriasis vulgaris. In the present study no statistically significant difference in NK cell activity was observed in twenty patients with chronic plaque psoriasis, and six patients with guttate psoriasis, compared with eleven normal healthy individuals.

Three highly effective therapeutic UV-based regimens used in the management of psoriasis resulted in significant suppression of NK cell activity. In addition, *in vitro* treatment with *cis-*, but not *trans*-UCA, resulted in a dose dependent suppression in NK cell activity.

All patients showed a marked clinical improvement in the severity of psoriasis irrespective of the treatment employed. However only subjects receiving UV-based treatments exhibited any suppression in NK cell activity (Fig 7.3. and 7.4.). This suggested that the modulation in NK activity resulted from UV-exposure and not merely as a consequence of an improvement in psoriasis.

All six patients receiving PUVA therapy exhibited a marked suppression in NK cell activity as did the majority of patients receiving TLO1 treatment (Fig 7.3(d) and (c) respectively). Three patients in the TLO1 group did not follow the pattern displayed by the other subjects in this group (Table 7.5.). All three had guttate psoriasis which was relatively mild in comparison to the other patients in the study, which may have been an important factor. In addition it was possible that those patients had not received a sufficiently high dose of UV to induce suppression of NK activity.

Therefore two of the patients had samples taken on the last day of their treatment, after six weeks of therapy. The third patient had only four weeks of treatment. UV therapy is given in incremental doses and therefore the total dose was considerably greater than that received following four weeks of treatment (Table 7.5.). The NK activity at the six week sample point was significantly suppressed compared with that at the four weeks in both subjects, but not compared to the value obtained prior to treatment. It is tempting to speculate that there was something odd about the NK assay prior to treatment which yielded low NK values for these subjects. It has also been suggested that humans, as well as animals have a genetic predisposition to UV-induced immunosuppresion (Yoshikawa and Streilein, 1990), and it is possible that these subjects are UV-resistant. However the samples taken from patients 7 and 8 after four and six weeks of therapy suggested the effects on UV-induced suppression of NK cell activity was dose dependent.

Follow up samples taken from the TLO1 and PUVA groups four weeks after the last treatment revealed that the NK activity had not returned to normal. Therefore samples were obtained from two of the TLO1 and three of the PUVA patients four weeks later. Eight weeks post-irradiation both, TLO1 patients displayed a marked increase in NK activity compared with the previous sample point. However, it had not yet been completely restored to the original value. Similarly, two of the three PUVA patients still had slightly suppressed NK cell activities relative to the starting value.

7.3.2 UV-induced Immunosuppression of NK Cell Activity May Be Dependent on the Dose or Wavelength of Irradiation

A dose dependent suppression in NK cell activity following solarium exposure was reported by Hersey (1986). Normal subjects were exposed to 30 min of solarium radiation (approximately 1 MED) on two, six and twelve consecutive days. At least six consecutive days exposure was required to induce suppression of NK activity,

and the suppression was more pronounced following twelve days. Follow up samples were taken up to twenty one days after the last irradiation. The NK activity of the subjects who had received twelve whole body exposures remained suppressed at this time point. Subjects who only received UV on their back exhibited no significant suppression in NK cell activity.

One previous study (Viander *et al.*, 1984) looked at the effects of PUVA therapy on NK cell activity in a group of patients with a number of skin disorders, 50% of whom had psoriasis vulgaris. A diminution in NK cell activity was observed during PUVA therapy however this tended to return to normal at the higher E:T cell ratios if the therapy was continued beyond 25 irradiations.

Given that UV-induced suppression of NK activity is possibly dose dependent and that the recovery time could be up to eight weeks, it was of interest in the present study to find that both psoriasis patients and normal subjects receiving broad band UVB (UV6) treatment exhibited no significant modulation in NK cell activity following four weeks of therapy but did four weeks after completion of the six week course (Fig 7.3(b) and 7.4(b) respectively). It is therefore possible that a dose sufficient to induce NK cell suppression had not been received following four weeks of treatment but had after six weeks especially since the patients received incremental doses. The TLO1 and UVA sources do not emit the highly erythemogenic wavelengths (less than 300 nm) and therefore patients treated with them receive higher doses of UV in a shorter period of time than those receiving the broad band UVB. For example the mean cumulative UV6 dose in psoriasis patients following four weeks of therapy in this study was 1.8 J cm^{-2} compared with 8.0 J cm^{-2} of TLO1 or 21.6 J cm^{-2} of UVA. It is therefore possible that the dose of UV required to suppress NK cell activity *in vivo* was only attained following five or six weeks of UV6 exposure. Furthermore this is suggested by the results from group F (normal subjects undergoing broad band UVB irradiation of their arms only) as, although a

reduction in NK cell activity was recorded in most individuals four weeks after the last irradiation, it did not reach statistical significance.

Suppression in NK activity following UVR *in vivo* was more marked when the melanoma target cell line MM200 was employed in the studies of Hersey (Hersey, 1986; Hersey *et al.*, 1987), in comparison with the K562 cell line (used in this study). It would be of interest to test the NK activity of patients following four weeks of UV6 therapy against the MM200 cell line.

In addition to the total dose of UV received, it is possible that the incident wavelengths are also of significance in producing suppression of NK cell function. In this respect Hersey *et al.* (1983a) reported that twelve one hour exposures of sunlight exposure, in a two week period, had only minimal effects on NK cell activity. Further studies by this group revealed that two weeks irradiation of healthy volunteers with solarium lamps resulted in suppression of NK cell activity (Hersey, *et al.*, 1983b). The proportion of UVA to UVB emitted by solarium lamps is higher than that of natural sunlight. It is estimated that subjects exposed to solarium lamps receive approximately three times more UVA than those exposed to natural sunlight (Hersey, *et al.*, 1983a). Solarium exposure remained suppressive even if subjects applied a sunscreen which blocked UVB (Hersey *et al.*, 1987) or if Mylar sheeting, which inhibits the transmission of 99% of UV with a wavelength below 315-317 nm, was placed between the source and the subject (Hersey *et al.*, 1988). Hence it was hypothesized that wavelengths in the UVA range (320-400 nm) were solely responsible for the observed suppression in NK cell activity. It is possible that this is a direct effect since UVA radiation can penetrate the epidermis and reach the dermis, where in theory it may directly affect NK cells as they circulate through the capillaries of the skin.

However, the TLO1 source used in this study, which emits narrow-band UV (311-313 nm), also resulted in significant suppression of NK cell activity. Therefore, it is

not only UVA which is responsible for the suppression. Indeed, *in vitro,* it is the shorter UV wavelengths which cause maximum suppression in NK cell activity (Figs 7.7. and 7.8.; Schacter *et al.*, 1983). *In vivo* a balance between the longer wavelengths which penetrate the epidermis but are relatively inefficient at suppressing NK activity and the relatively non-penetrating shorter wavelengths which suppress NK cell activity may exist. *In vitro* irradiation of PBMC with shorter wavelengths of UV results in suppressed LPRs as well as NK cell activity (Fig. 7.9.; Schacter *et al.*, 1983) and therefore if UV acts directly on NK cells as they pass through the skin T cell activity may also be affected. Unfortunately LPR were not assayed in PUVA or TLO1 patients in this study. However the patients receiving UV6 or PUVA therapy in Chapter 6 exhibited no significant alteration in LPR to the mitogen Con A or HSV antigen. This may indicate that the effect of UV-irradiation on NK cells *in vivo* is not a direct effect on cells circulating through the skin.

It would be of interest to test the dose dependency theory by taking samples more frequently from the patients receiving TLO1 or PUVA therapy during the first four weeks of therapy and also to obtain samples from subjects receiving UV6 treatment during the final stages of treatment. TLO1 and PUVA patients were recruited in Dundee and therefore this was not practical and unfortunately time did not permit a second cohort of UV patients to be recruited.

7.3.3 Possible Mechanisms of UV-Induced Suppression of NK Cell Activity

The suppression in NK cell activity took at least four to eight weeks to recover in this study. Others have reported that the suppression is still detectable at 14 (Hersey, *et al.*, 1988) or 21 days (Hersey, 1986). This may suggest that UV-irradiation induces permanent functional damage to circulating cells.

Alternatively, it is possible that a soluble mediator is released from epidermal cells on UV-irradiation, which enters the circulation and results in suppressed NK cell mediated cytotoxicity. Kim *et al.*, (1990) and Brodie and Halliday (1991) have both reported soluble suppressive factors produced in mice on UV irradiation, which are potential candidates. *Cis*-UCA is another possible candidate. As discussed in Chapter 6 it is not confined to the stratum-corneum and elevated levels persisted for four weeks following irradiation in suction blister fluid. PGs, which are produced on UV irradiation have also been shown to inhibit human NK cell activity (Koren and Leung, 1983). Epidermal cells release a factor which augments NK cell activity *in vitro* , EC-derived NK cell activity-augmenting factor (ENKAF) (Luger *et al.*, 1985), thought to be IL-6. UVR has been shown to increases the expression of IL-6 which enhances NK cell activity. It would be of interest to examine whether increased IL-6 is found sytemically following UVR and how long it persists if so. The effect of UVR on the production of other cytokines may be of relevance. IL-2 and IFN-γ are both potent NK cell augmenting factors. In mice, Th-1 cells are characterized by their ability to produce Il-2 and IFN-γ, whereas Th-2 cells produce IL-4. In murine models there is very good evidence to support the selective stimulation of Th-2 in preference to Th-1 cells following UVR (Araneo, *et al.*, 1989, Cruz and Bergstresser, 1991) and preferential induction of Th-2 cells could lead to a reduction in the production of IL-2 and IFN-γ by activated T cells. Il-1 production is elevated following UVR in both mice (Gahring, *et al.*, 1984) and humans (Granstein and Sauder, 1987; Oxholm, *et al.*, 1988), and has been implicated in UVR-induced changes in immune responses (Robertson, *et al* 1987; Krutmann *et al.*, 1990). Araneo *et al.*, (1989) also found that administration of recombinant IL-1β facilitated a similar change in cytokine production to UVR. Therefore, it is possible that such a switch in cytokine production following UVR may have implications for NK cell maturation or activity. It would certainly be of interest to examine the cytokine production profile from epidermal cells and stimulated peripheral blood T cells from

patients receiving UV treatment. It is possible that one or a combination of these factors may be responsible for UV-induced suppression of NK cell activity.

Whether the defect in NK cell function *in vivo* is reversible remains unknown. It would be of interest to see if treatment of PBMC derived from patients with suppressed NK cell activity as a result of UVR with cytokines such as IL-2, IFN, TNF-α would restore the NK cell activity. Culturing UVB or PUVA irradiated PBMC with IL-2 partially reversed the suppression in NK activity of (Toda *et al.*, 1986). UVB or PUVA are known to induce reactive oxygen species (ROS) which cause tissue damage; ROS produced by KC contributes to the formation of sunburn cells. Cells treated with UVB or PUVA in the presence of superoxide dismutase (SOD), an antioxidant which scavenges superoxide anions, were partially protected from UV-induced suppression of NK cell activity.

It is possible that UVR affects the expression of the adhesion molecules or receptors involved in the binding of E and target cells, or possibly E cells and monocytes which may regulate NK cell activity. However the suppression of NK cell activity induced by *in vitro* irradiation of human PBMCs occurs at the post binding stage (Elmets *et al.*, 1987; Weitzen and Bonavida, 1984). It would be interesting to determine whether the suppressive effect of UV on NK cell function *in vivo* also occurs at the post-binding stage. The number of E and target cell conjugates formed before and during irradiation could be determined using fixed numbers of PBMC and target cells in a single cell binding assay as employed by Elmets *et al.*, (1987) or by two colour staining of E and T cells and analysis by flow cytometry (Cavarec *et al.*, 1990).

Hersey, *et al.*, (1988) reported minimal changes in the number of circulating CD16 positive cells in the whole PBMC population following solarium exposure. No change in circulating CD57 cells was observed in this study which implies that the total number of circulating NK cells remains unaltered. However preliminary data

indicated that the number of LGLs expressing CD56 correlates with the measured NK cell activity in patients receiving TLO1 or PUVA therapy (Table 7.7.). CD56 is thought to be an activation marker expressed on some NK cells (very few T cells express this molecule) (Trinchieri, 1989). CD45 also appears to be required for NK lytic activity and antibodies directed against CD45 block NK cell function at the post binding stage of activity (Pawelec *et al.*, 1985; Starling *et al.*, 1987). It would therefore be of interest to examine NK cell surface proteins in more depth in these patients.

In murine models both macrophages and T cells which suppress NK cell activity have been described (Trinchieri, 1989). Monocytes have been demonstrated to play a regulatory role in NK cell activity *in vitro* in humans through a direct cell to cell interaction (Hellstrand and Hermodsson, 1990). It has also been suggested that NK cells themselves may also function as immunoregulators which may regulate their own activity (D'Amore and Golub, 1985). It is therefore possible that the UV-induced suppression of NK cell activity observed is induced indirectly by another cell population.

7.3.4 The Effect of UCA Isomers on NK Cell Activity *in vitro*

Treatment with *cis*- but not the *trans*-UCA resulted in a dose dependent suppression of NK cell activity (Fig 7.4.). However this effect was statistically significant only at relatively high concentrations (10^{-2} to 10^{-4} M). If the effects of *cis*-UCA are receptor mediated, a significant effect on NK cell activity may be expected at concentrations less than 10^{-4} M. It is possible that an 18 h assay, as employed in this study, is too long an incubation period. This time course may be sufficient to cause desensitization of the receptor. Alternatively enrichment of NK cells may be required to reveal the effects of *cis*-UCA at lower concentrations. Trypan blue exclusion and propidium iodide staining demonstrated that such high concentrations of UCA did not cause cell death, and these findings were confirmed by the discovery

that UCA isomers did not cause suppression of LPR to Con A or HSV (Table 7.9.). Other groups have also reported that *cis*-UCA has no effect on *in vitro* lymphoproliferative responses (Noonan *et al.*, 1988; Higaki *et al.*, 1986). Despite the apparent lack of effect on LPR it is possible that different populations of cells are induced in the presence of UCA isomers. It would therefore be of interest to phenotype the responding cells in the presence of each UCA isomer in comparison to those with no UCA, and also to assay the cytokines produced by these cells.

The second messenger system(s) involved in NK cell mediated lysis of NK cells remains to be identified. An increase in intracellular Ca^{2+} is associated with NK cell activation and this is thought to be regulated by c-AMP (Windebank *et al.*, 1988). It is therefore sigificant that *cis*-UCA, but not the *trans*-isomer, results in a dose dependent suppression of NK cell activity *in vitro* (Fig 7.4.), as *cis*-UCA has been shown to down-regulate the induction of c-AMP by *trans*-UCA or histamine. It is possible that *cis*-UCA may down regulate the second mesenger system involved in NK cell activation.

Another alternative is that *cis*-UCA does not act directly on the NK cell but does so via another cell present in the PBMC population. As discussed earlier, monocytes and T cells have been shown to exert suppressive effects on NK cells and enrichment for NK cells might indicate whether *cis*-UCA, or indeed UVR acts directly on NK cells or if the effects are mediated by another cell population. It is impossible to say definitively whether UCA, which is present in the culture medium throughout the incubation, affects the target cells or the E cells. However pulsing the target cells with UCA did not result in suppressed NK cell activity. It is possible that the timing or temperature at which the pulsing was carried out was not optimal. The negative result obtained in this experiment should have been complemented by the converse experiment, i.e. pulsing the E cells, to see if this resulted in suppression of NK cell

activity. It is also possible that *cis*-UCA must be present through out the incubation to have an effect.

Finally, the relevance of these findings for the *in vivo* situation should be considered. The concentrations of UCA employed in the *in vitro* assay appear to bear little resemblance to those which might be expected physiologically. However, as discussed previously, the conditions in the *in vitro* assay may not been optimal for the suppression of NK cell activity. Epidermal *cis*-UCA appears to migrate from the epidermis to the dermo-epidermal junction where it can be detected in blister fluid. *Cis*-UCA is retained in this compartment at least four weeks after UVR (Fig 6.8) indicating that it may remain in the circulation certainly as long as NK cell activity is suppressed. It would be possible to test the effect of *cis*-UCA on NK cell activity *in vivo* in a murine model. Animals could be treated with a range of doses of UCA-isomers, including those which might be expected following UVR, epidermally and subcutaneously as in the DTH system and the ability of LN or spleen cells to lyse an NK susceptible target cell eg YAK cells determined. Alternatively the mice could be treated with a monoclonal antibody which specifically recognizes *cis*-UCA and not *trans*- before UVR and assaying NK cell activity. In addition UCA possibly acts through histamine receptors (discussed in Chapter 3). Matheson and Reeve, (1991) also reported that mice treated with cimetidine (H2 receptor antagonist) had a reduced number of UV-induced tumours compared to untreated mice. It would therefore be of interest to pre-treat either mice or humans with H-1 or H-2 blockers before UV-irradiation and assessment of NK cell activity.

CHAPTER 8

OVERVIEW

Man is exposed to greater amounts of solar UVR as world travel increases and ozone depletion occurs. In addition, exposure to artificial UV sources such as those used therapeutically in the management of a number of skin disorders and for cosmetic reasons in tanning parlours, make it imperative to understand what effects this may have biologically.

It is known that UV-irradiation results in suppression of selected immune responses. The suppression may be local or systemic depending on the dose and wavelength of the irradiation and the site of subsequent antigen challenge. Variation in the host response also appears to be important as genetic susceptibility of the immunosuppressive effects of UVR has been documented in inbred strains of mice, and has been suggested that such variation may also occur in man. Thus the effects of UV on immune responses are complex and it is difficult to distinguish initiating events from those occurring secondarily. One result of UV-irradiation is isomerization of the naturally occurring *trans*-isomer of UCA to *cis*-UCA, which mimics many of the effects of UV on the immune responses. The mechanism of action of UCA is not clearly understood. The approaches used in the present study add to the current knowledge of the mechanism of *cis*-UCA induced immunosuppression, and indicate directions for further studies. Much of what is known about the effects of UV on the immune system is derived from experimental animals, and equivalent studies in man have been few, thus far.

The results outlined in Chapter 3 indicate that *cis*-UCA may act through histamine-like receptors. Both the histamine H1 and H2 receptor antagonists abrogated the *cis*-UCA-induced reduction in epidermal ATPase$^+$ cells and also the suppression of the DH response to HSV. Previously it had been indicated that *cis*-UCA may have more than one effect; *cis*-UCA applied epidermally before HSV infection induced two subsets of T cells (L3T4$^+$ Ly2$^-$ and L3T4 Ly2$^+$) which suppressed the DH response to the virus, while transfer of epidermal cells from mice skin-painted with *cis*-UCA

to naive mice at the time of HSV infection induced only one subset of T suppressor cells ($L3T4^+$ $Ly2^-$). Thus *cis*-UCA may act through two different receptors, for example one possibly located locally in the skin (eg H1) and the other (eg H2) systemically (as discussed in section 3.3). Alternatively there may be a distinct UCA receptor, which cimetidine and terfenadine (H2 and H1 receptor antagonists employed in this study) partially antagonise. A key direction for future work is to identify the cell populations which carry UCA/histamine receptors, and to distinguish by binding studies whether UCA acts through a receptor which is distinct from a characterized histamine receptor. To determine the cell signaling mechanism which *cis*-UCA may affect would also be worthy of further study. *Cis*-UCA was found to result in a dose dependent suppression of NK cell activity of human PBMC (Chapter 7) and it is therefore possible that an NK cell enriched cell population may be a suitable source of cells for such a study.

A time course study of the DH response to HSV in immune animals revealed that the kinetics of the responses were similar to the CH response reported by Van Lovern *et al.*, (1983) (Chapter 4). However, during the CH response, the initial response peaked two hours following antigen challenge but the primary peak during the DH response in the present study occurred one hour after antigen challenge. The slightly different kinetics of the response may indicate a difference between a CH and DH response, as previously suggested (Kim *et al.*, 1991). It may be that the immune response to a replicating virus is different to a single exposure to a sensitizing chemical applied epicutaneously. It would be of interest to determine if similar kinetics of the DH response were measured if the mice were initially sensitized with inactivated virus.

It was even more intriguing to discover that pre-treatment of the mice with *cis*-UCA or UVR, prior to the primary infection with HSV, resulted in suppression of both phases of the elicitation of the DH response when the mice were subsequently

challenged. Thus *cis*-UCA or UVB may alter the induction or subsequent activation of the early acting DH initiating cell. It has been reported that UV-irradiated LC induce anergic responses of Th1 cells. It may be that a similar phenomenon occurs with the DH initiating cells. In the experiments outlined in Chapter 4 the primary infection with live HSV was subcutaneous and therefore sensitized cells would require to migrate to the skin where they are activated locally on antigen challenge. Alternatively *cis*-UCA or UVB may affect the migration of these cells to the skin following sensitization. Further work is required to understand the mechanism of suppression of the initiating DH response (one hour after antigen challenge in immune animals).

It has been reported that UVR of sensitized animals results in an enhanced elicitation of the CH response. All experiments conducted in the present study, and previously in the laboratory, focused on the effects of UVR and UCA-isomers on primary immune responses. Another area for examination would be to study immunomodulation by UVR or UCA isomers in mice which were already infected with HSV. In humans latently infected with the HSV, sub-erythemal doses of UV employed therapeutically had little effect on LPR or antibody responses to the virus (Chapter 6). In addition there did not appear to be any effect on reactivation of the virus, unlike erythemal doses of UV which frequently cause HSV reactivation (Spruance *et al.*, 1991). It is possible that only erythemal doses of UV result in systemic suppression of CH responses in man (Cooper *et al.*, 1992).

The results in Chapter 4 which focus on the role of TNF-a in UV or *cis*-UCA induced suppression of DH responses to HSV indicate that UCA is not the sole mediator of UV-induced immunosuppression. Pre-treatment of mice with neutralising

anti-TNF-α antibodies prior to UVB irradiation blocked the subsequent suppression of DH responses to HSV, but had no effect on suppression induced by *cis*-UCA. As discussed in Chapter 4, it is possible that the timing or dose of *cis*-UCA administered does not mimic that of the production of *cis*-UCA following UVB *in vivo*. UVB-irradiation of mice, however, has been shown to induce DC migration from the epidermis to the DLN but *cis*-UCA did not (Moodycliffe *et al.*, 1992). TNF-a has been shown to induce DC migration (Cumberbatch and Kimber, 1992) and a genetic susceptibility to the effects of UVR in mice is associated with polymorphism in the *Tnfa* loci. Therefore a primary pathway of induction of immunosuppression may be through the induction of the production of TNF-α following UVR. *Cis*-UCA however does not appear to act through such a mechanism and it appears that it is more likely to act through a histamine-like receptor. It cannot be ruled out that such an interaction would not result in altered cytokine production by these cells and therefore another possible area of investigation would be the effect of UCA isomers on cytokine production, particularly TNF-a, which may resolve this debate.

As raised in Chapter 5 it would be interesting to examine the effect of UCA-isomers on antigen presentation, particularly that of epidermal cells. These experiments should be infinitely easier to study in mice than in man. However recently a method to culture human LC from cord blood has been reported (Caux *et al.*, 1992). As phototherapy of patients with psoriasis was shown to result in the abrogation of antigen presentation by epidermal cells, and as the percentage of *cis*-UCA also increased, it is possible that *cis*-UCA may play a role in down regulating antigen presentation. It would be of interest to determine the effect of *in vitro* treatment of cultured LC with UCA-isomers on their APC function and cell surface marker expression.

Phototherapy resulted in suppression of NK cell activity. NK cells play an important role in tumour rejection and also in controlling the outcome of infection with viruses.

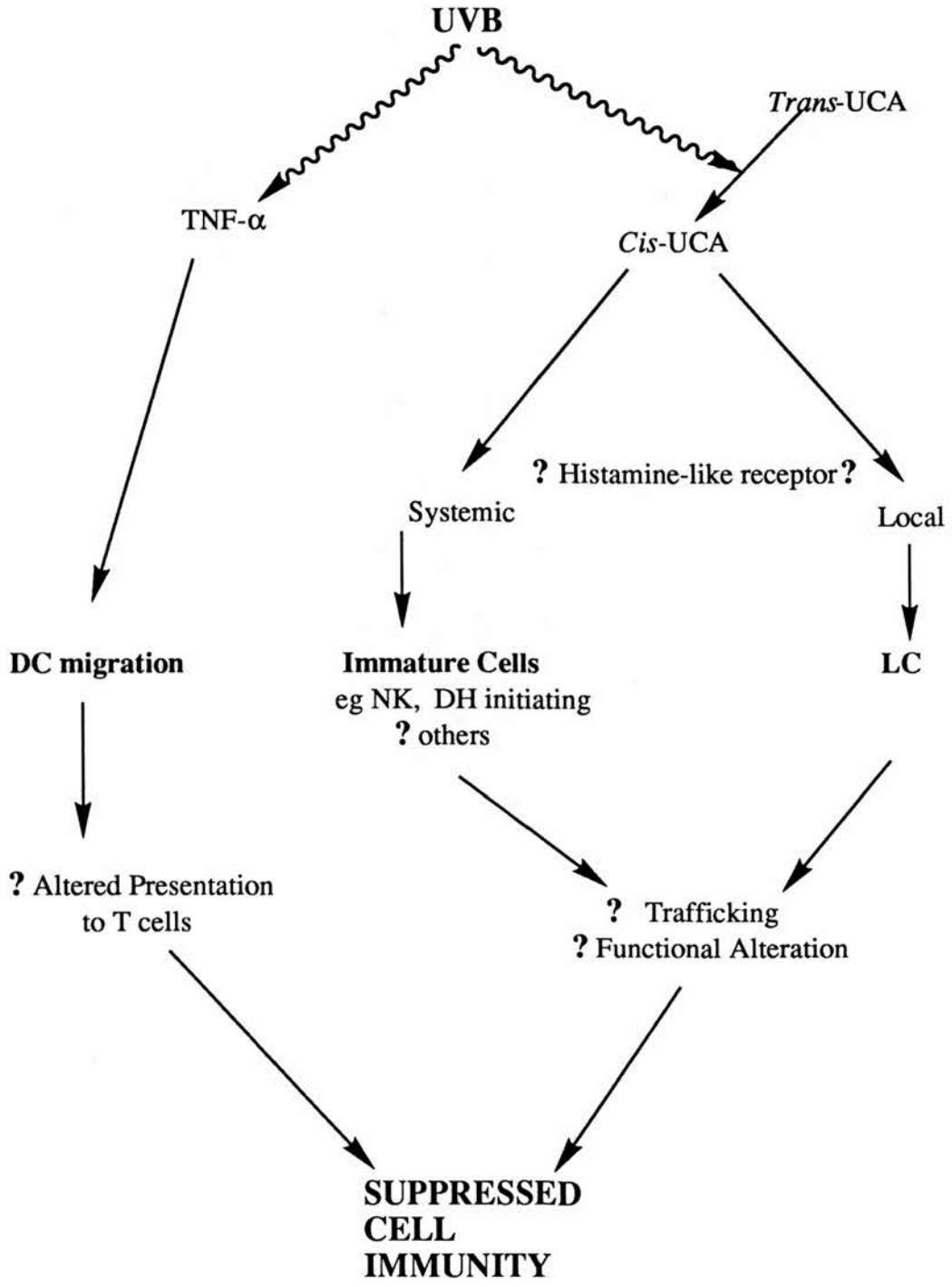
It should therefore be of concern that UV efficiently suppresses their activity. *Cis*-UCA suppresses the activity of human NK cells *in vitro*, but it would be interesting to establish whether this interaction happens *in vivo*. Such experiments may be conducted in experimental animals, by studying the effects of treatment of mice *in vivo* with UCA isomers. One other possibility would be to investigate the effect of phototherapy on NK cell activity of subjects who were taking histamine receptor blockers, assuming that *cis*-UCA acts through a histamine receptor.

Fig 8.1 illustrates a proposed model of UV-induced immunosuppression. UV-irradiation of the epidermis is likely to have multiple effects on immune responses. One effect may be the induction of TNF-αwhich induces DC migration to the DLN. This may result in altered antigen presentation by the DC, resulting in suppressed cellular immunity. One other effect is the isomerization of UCA resulting in the formation of the immunosuppressive *cis*-isomer. *Cis*-UCA may then act locally resulting a reduction and possibly altered function of $ATPase^+$ cells, as demonstrated in section 3.2.1. *Cis*-UCA may also have systemic effects. It has been demonstrated that there is suppression of the initiation phase during the elicitation of the DH response to HSV. The early acting initiating cell must be present at the local site of antigen challenge and therefore *cis*-UCA or UVR may alter the activation or recirculation of this population of T cells. This may be indirectly through APC or a direct receptor mediated effect on the cells themselves. It is possible that TNF–α and *cis*-UCA interact with different APC populations. It has been suggested that monocyte/macrophages are the APC which induce sensitization of the DH initiating cell population and monocytes may also regulate NK cell function. Therefore the effect of *cis*-UCA on monocyte/macrophage function should be examined.

The results of this study have added to the elucidation of the mechanisms of UV-induced immunosuppression. However many questions remain to be answered.

Figure 8.1

A Model of UV-induced Immunosuppression and the Role of UCA



REFERENCES

Aberer W, Schuler G, Stingl G, Honigsmann H and Wolff K (1981). Ultraviolet light depletes surface markers of Langerhans cells. J Invest Dermatol 76: 202-210.

Acevedo F and Hammar H (1989). Complement C_3 proteins in psoriasis. Br J Dermatol 121: 329-335.

Albelda SM and Buck CA (1990). Integrins and other cell adhesion molecules. FASEB J 4 : 2868-2880.

Anegón I, Cuturi MC, Trinchieri G and Perussia B (1988). Interaction of Fc receptor (CD16) ligands induces transcription of interleukin-2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells. J Exp Med 167: 452-472, 1988.

Ansel J, Perry P, Brown J, Damn D, Phan T and Hart C (1990). Cytokine modulation of keratinocyte cytokines. J Invest Dermatol 94: 101S-107S.

Araneo BA, Dowell T, Moon HB and Daynes R (1989). Regulation of murine lymphokine production in vivo. Ultraviolet radiation exposure depresses IL-2 and enhances IL-4 production by T cells through an IL-1-dependent mechanism. J Immunol 143: 1737-1744.

Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W and Schwartz JC (1987). Highly potent and selective ligands for histamine H_3-receptors. Nature 327: 117-123.

Ashworth JA, Kahan MC and Breathnach SM (1989). PUVA therapy decreases $HLA^-Dr^+CD1a^+$ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA Dr^+CD1a^+ Langerhans cells exhibit normal alloantigen-presenting function. Br J Dermatol 120: 329-39.

Askenase PW, Herzog WR, Millet I, Paliwal V, Ramashadran R, Rochester C, Geba G and Ptak W (1991). Serotonin initiation of delayed-type hypersensitivity: mediation by a primitive $Thy\text{-}1^+$ antigen-specific clone or by specific monoclonal IgE antibody. Skin Pharmcol 4 (Suppl 1): 25-42.

Austad J and Braathen LR (1985). Effect of UVB on alloactivating and antigen-presenting capacity of human epidermal Langerhans cells. Scand J Immunol 21: 417-423.

Avella J, Binder HGJ, Madsen JE and Askenase PW (1978). Effect of histamine H2-receptor antagonists on delayed hypersensitivity. Lancet i: 624-626.

Baadsgaard O, Gupta KA, Taylor RS, Ellis CN, Voorhes JJ and Cooper KD (1989). Psoriatic epidermal cells demonstrate increased numbers and function of non-Langerhans antigen-presenting cells. J Invest Dermatol 92: 190-195.

Baadsgaard O, Fisher G, Voorhes JJ and Cooper KD (1990a). The role of the immune system in the pathogenesis of psoriasis. J Invest Dermatol 95: 32S-34S.

Baadsgaard O, Salivo B, Mannie A, Dass B, Fox DA and Cooper KD (1990b). In vivo ultraviolet-exposed human epidermal cells activate T suppressor cell pathways that involve $CD4^+$ and $CD45RA^+$ suppressor-inducer T cells. J Immunol 445: 2854-2861.

Baadsgaard O, Tong P, Elder JT, Hansen ER, Ho V, Hammerberg C, Small W, Vejlsgaard GL, Fox DA, Fisher G, Voorhes JJ and Cooper KD (1990c). $UM4D4^+$ (CDw 60) T-cells are compartmentalised into psoriatic skin and release lymphokines that induce a keratinocyte phenotype expressed in psoriatic lesions. J Invest Dermatol 95: 275-287.

Baadsgaard O (1991). In vivo ultraviolet irradiation of human skin results in profound perturbation of the immune system. Arch Dermatol, 127: 99-109.

Baden HP and Pathak MA (1967). The metabolism and function of urocanic acid in skin. J Invest Dermatol 48: 11-17.

Baker BS, Swain AF, Fry L and Valdimarsson H (1984). Epidermal T lymphocytes and HLA-DR expression in psoriasis. Br J Dermatol 11: 555-564.

Baker BS, Powles AV, Valdimarsson H and Fry L (1988). An altered response by psoriatic keratinocytes to gamma interferon. Scand J Immunol 28: 735-740.

Baker BS and Fry L (1992). The immunology of psoriasis. Br J Dermatol, 126: 1-9.

Barker CF and Billingham RE (1968). The role of afferent lymphatics in the rejection of skin homografts. J Exp Med 128: 197-220.

Barker JNWN, Karabin GD, Stoot TS, Sarma VJ, Dixit VM and Nickoloff BJ (1991). Detection of interferon-gamma mRNA in psoriatic epidermis by polymerase chain reaction. J Dermatol Sci 2: 106-111.

Barker JNWN (1991). The pathophysiology of psoriasis. The Lancet i: 227-230.

Basham TY, Nickoloff BJ, Merigan TC and Morhenn VB (1985). Recombinant gamma-interferon differentially regulates class II antigen expression and biosynthesis on cultured normal human keratinocytes. J Interferon Res 5: 23-32.

Beer DJ and Rocklin RE (1987). Histamine modulation of lymphocyte biology: membrane receptors, signal transduction, and functions. Crit Rev Immunol 7: 55-91.

Belsito DV, Kerden FA, Potozkin J and Soter NA (1990). Cimetidine-induced augmentation of allergic contact hypersensitivity reactions in mice. J Invest Dermatol 94: 441-445.

Bergstresser PR, Tigelaar RE, Dees JH and Streilein JW (1983). Thy-1 antigen bearing dendritic cells populate murine epidermis. J Invest Dermatol 81: 286-288.

Bergstresser PR, Toews GB and Streilein JW (1980). Natural and perturbed distributions of Langerhans cells: responses to ultraviolet light, heterotropic skin grafting, and dinitrofluorobenzene sensitisation. J Invest Dermatol 75: 73-77.

Blum HF (1959). Carcinogenesis by ultraviolet light. Princeton University Press. Princeton, N.J. USA.

Booth JC, Hannington G, Aziz TA and Stern H (1979). Comparison of enzyme-linked immunosorbent assay (ELISA) technique and complement fixation test for estimation of cytomegalovirus IgG antibody. J Clin Pathol 32: 122-127.

Bos JD (1988). The pathomechanisms of psoriasis; the skin immune system and cylosporin. Br J Dermatol 118: 141-155.

Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ and Kapsenberg ML (1989). Predominance of 'memory' T cells ($CD4^+$, $CD29^+$) over 'naive' T cells ($CD4^+$, $CD45R^+$) in both normal and diseased human skin. Arch Dermatol Res 281: 24-30.

Bos JD and Kapsenberg ML (1986). The skin immune system (SIS): its cellular constituents and their interactions. Immunol Today 7: 235-240.

Bos JD, Teunissen MBM, van der Kraan J, Krieg SR, Kapsenberg ML, Peters PJ and Borst J (1988). Gamma/delta receptor bearing lymphocytes in normal human epidermis: Analogues of murine Thy-1^+ dendritic cells? J Invest Dermatol 91: 382.

Bos JD, Zonneveld I, Das PK, Krieg SR, van der Loos Ch.M and Kapsenberg ML (1987). The skin immune system (SIS): Distribution and immunophenotype of lymphocyte subpopulations in normal human skin. J Invest Dermatol 88: 569-573.

Botham PA, Rattray NJ, Walsh ST and Riley EJ (1987). Control of the immune response to contact sensitizing chemicals by cutaneous antigen-presenting cells. Br J Dermatol 117: 1-9.

Brandrup F, Hauge M, Henningsen K and Eriksen B (1978). Psoriasis in an unselected series of twins. Arch Dermatol 114: 874-878.

Breathnach SM, Shimada S, Kovac and Katz SI (1986). Immunological aspects of acute cutaneous graft-versus-host disease: Decreased density and antigen presenting function of Ia^+ Langerhans cells and absent antigen presenting capacity of Ia^+ keratinocytes. J Invest Dermatol. 86: 226-234.

Brockmeyer NH, Kreuzfelder E, Guttmann W, Mertins L, Goos M and Ohnhaus EE (1989). Cimetidine and the immuno-response in healthy volunteers. J Invest Dermatol 93: 757-761.

Brodie AM and Halliday WJ (1991). Suppressor cells and soluble factors from the spleens of UV-irradiated mice. Immunol Cell Biol 69: 199-204.

Brozek CM, Shopp GM, Ryan SL, Gillespie PM, Kuseivitt DF, Rajagopalan MS, Ley KD and Ley RD (1992). In vivo exposure to ultraviolet radiation enhances pathogenic effects of murine leukemia virus, LP-BM5, in murine acquired immunodeficiency syndrome. Photochem Photobiol 56: 287-296.

Bujdoso R, Young P, Harkiss GD and McConnell FI (1989). Antigen presentation in sheep: generation of antigen-specific T-cell lines. Immunology 66: 559-564.

Cassatella MA, Anegón I, Cuturi MC, Griskey P, Trinchieri G and Perussia B (1989). FcR (CD16) interaction with ligand induces Ca^{2+} mobilization and phosphoinositide turnover in human natural killer cells. Differential role of Ca^{2+} in FcR (CD16) and IL-2-induced transcription and expression of lymphokine genes. J Exp Med 169: 549-567.

Caux C, Dezutter-Dambuyant C, Schmitt D and Banchereau J (1992). GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. Nature (London) 360: 258-261.

Cavarec L, Quillet-Mary A, Fradelizi M and Conjeaud H (1990). An improved double fluorescence flow cytometry method for the quantification of killer cell/target cell conjugate formation. J Immunol Meth 132: 251-261.

Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ and Headington JT (1989). Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. Br J Dermatol. 121: 421-431.

Chaker MB, Tharp MD and Bergstresser PR (1984). Rodent epidermal Langerhans cells demonstrate greater histochemical specificity for ADP than for ATP and AMP. J Invest Dermatol 82: 496-500.

Charleson FC, Norval M, Benton EC and Hunter JAA (1992). Lymphoproliferative responses to human papillomaviruses in patients with cutaneous warts. Br J Dermatol 127: 551-559.

Chung H-T, Burnham DK, Roberts LK and Daynes RA (1986). Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. J Immunol 137: 2478-2484.

Chung H-T, Lee D C, Im SY and Daynes RA (1988). UVB-exposed animals exhibit an enhanced susceptibility to bacterial and fungal infections. J Invest Dermatol 90 (Abstr.): 552.

Ciccone E, Viale O, Pende D, Malnati M, Biassoni R, Mellioli G, Moretta A, Long EO and Morreta L (1988). Specific lysis of allogeneic cells after activation of $CD3^-$ lymphocytes in mixed lymphocyte culture. J Exp Med 168: 24-3-2408.

Ciccone E, Colonna M, Viale O, Pende D, Di Donato C, Reinharz D, Amoroso A, Jeannet M, Guardiota J, Moretta A, Spies T, Strominger J and Moretta L (1990a). Susceptibility or resistance to lysis by alloreactive natural killer cells is governed by a gene in the human major histocompatibility complex between BF and HLA-B. Proc Natl Acad Sci USA 87: 9794-9797.

Ciccone E, Pende D, Viale O, Tambussi G, Ferrini S, Biassoni R, Longo A, Guardiola J, Moretta A and Moretta L (1990b). Specific recognition of human $CD3^-$ $CD16^+$ natural killer cells requires the expression of an autosomic recessive gene on target cells. J Exp Med 172: 47-52.

Ciccone E, Pende D, Viale O, Di Donato C, Tripodi G, Orengo AM. Guarddiola J, Moretta A and Moretta L (1992). Evidence of a natural killer (NK) cell repertoire for (allo) antigen recognition: definition of five distinct NK-determined allospecificities in humans. J Exp Med 145: 709-718.

Cohen PI and Katz SI (1992). Cultured human Langerhans cells process and present intact protein antigens. J Invest Dermatol 99: 331-336.

Cooper KD, Fox P, Neises CR and Katz SI (1985). Effects of ultraviolet radiation on human epidermal cell alloantigen presentation: initial depression of Langerhans cell-dependent function is followed by the appearance of $T6^{-}Dr^{+}$ cells that enhance epidermal alloantigen presentation. J Immunol 134: 129-137.

Cooper KD, Hammerberg C, Baadsgaard O, Edler JT, Chan LS, Sauder DN, Voorhes JJ and Fisher G (1990). IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased non-functional IL-1β. J Immunol 144: 4593-4603.

Cooper KD, Oberhelman L, Hamilton TA, Baadsgaard O, Terhune M, Le Vee G, Anderson T and Koren H (1992). UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: Relationship to dose, $CD1a^{-}DR^{+}$ epidermal macrophage induction, and Langerhans cell depletion. Proc Natl Acad Sci USA 89: 8497-501.

Cormane RH, Hamerlinck F and Siddiqui AH (1979). Immunogenic implications of PUVA therapy in psoriasis vulgaris. Arch Derm 263: 245-267.

Cruz PD and Bergstresser PR (1991). The influence of ultraviolet radiation and other physical and chemical agents on epidermal Langerhans cells. In: Epidermal Langerhans Cells. Ed. Gerald Schuler, CRC Press pp. 253-271.

Cruz PD, Nixon-Fulton JL, Tigelaar RE and Bergstresser PR (1989). Disparate effects of in vitro low-dose UVB irradiation on intravenous immunization with purified epidermal cell subpopulations for the induction of contact hypersensitivity. J Invest Dermatol 92: 160-165.

Cullen JJ, Neale PJ and Lesser MP (1992). Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. Science 258: 646-650.

Cumberbatch M and Kimber I (1992). Dermal tumour necrosis factor-α induces dendritic cell migration to draining lymph nodes, and possibly provides the stimulus for Langerhans cell migration. Immunology 75: 257-263.

Cuturi MC, Anegón I, Sherman F, Loudon R, Clark SC, Perussia B and Trinchieri G (1989). Production of hematopoietic colony-stimulating factors by human natural killer cells. J Exp Med 169: 569-583.

Czernielewski J, Juhlin L, Shroot S and Brun P (1985). Langerhans cells in patients with psoriasis: Effect of treatment with PUVA, PUVA bath, etretinate and anthralin. Acta Derm Venereol (Stockh) 65: 97-101.

Czernielewski JM and Bagot M (1986). Class II MHC antigen expression by human keratinocytes results from lymphoepidermal interactions and interferon production. Clin Exp Immunol 66: 295-302.

Czernielewski J, Vaigot P, Asselinou D and Prunierad M (1984). In vitro effect of UV radiation on immune function and membrane markers of human Langerhans cells. J Invest Dermatol 83: 62-65.

D'Amore PJ and Golub SH (1985). Suppression of human NK cytotoxicity by an MLC-generated cell population. J Immunol 134: 272-279.

Danno K, Todd K and Horio J (1986). Ultraviolet-B radiation suppresses mast cell degranulation induced by compound 48/80. J Invest Dermatol 87: 775-778.

David M, Shohat B, Hodack E and Sandbank M (1990). Effect of etretinate on peripheral T lymphocytes in psoriasis patients before, during and after 6 months of therapy. Dermatologica 180: 86-9.

Daynes RA and Spellman CW (1977). Evidence for the generation of suppressor cells by ultraviolet radiation. Cell Immunol 31: 182-187.

De Fabo EC and Noonan FP (1983a). Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp Med 158: 84-98.

De Fabo EC, Noonan FP, Fisher M, Burns J and Kacser H (1983b). Further evidence that the photoreceptor mediating UV-induced systemic immune suppression is urocanic acid. J Invest Dermatol 80: 319 (abstr.).

De Fabo E, Reilly DC and Noonan FP (1991). Mechanism of UVA effects on immune function: preliminary studies. In: Biological Responses to Ultraviolet A Radiation. Ed. F. Urbach. 2nd edition. Kansas, Vladenmar Publishing Company, pp 227-237.

De Gruijl FT and van de Leun (1991). Action spectra for carcinogenesis. In: Biological responses to Ultraviolet A Radiation. F. Urbach (ed). 2nd edition. Pennsylvania, Valdenmar Publishing Company, pp 91-97.

Demidem A, Taylor JR, Grammer SF and Streilein JW (1991). T-lymphocyte-activating properties of epidermal antigen-presenting cells from normal and psoriatic skin: evidence that psoriatic epidermal antigen-presenting cells resemble cultured normal Langerhans cells. J Invest Darmatol 97: 454-460.

Denkins Y, Fidler IJ and Kripke ML (1989). Exposure of mice to UV-B radiation suppresses delayed hypersensitivity to Candida albicans. Photochem Photobiol 49: 615-619.

De Pietro WP, Berger CL, Harker LC and Edelson RL (1981). Normal numbers of phenotypic helper, suppressor, and total T cell populations in psoriasis vulgaris: quantitation by monoclonal antibodies. J Am Acad Dermatol 5: 304-307.

Detmar M and Orfanos CE (1990). Tumor necrosis factor-alpha inhibits cell proliferation and induces class II antigens and cell adhesion molecules in cultured normal human keratinocytes in vitro. Arch Dermatol Res 282: 238-245.

Duraiswamy N, Hammerberg C and Cooper KD (1992). Induction of tolerance to DNFB by sunburned skin is dependent upon infiltrating CD11b$^+$ leukocytes. J Invest Dermatol 98: 578 (abst).

Dustin ML, Singer KH, Tuck DT and Springer TA (1988). Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med 167: 1323-1340.

Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Cottey RJ, Ellingsworth L, Derynck R and Voorhes JJ (1989). Over-expression of transforming growth factor alpha in psoriatic epidermis. Science 243: 811-814.

Elmets CA, Bergstresser PR, Tigelaar RE, Wood PJ and Streilein JW (1983). Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. J Exp Med 158: 781-794.

Elmets CA, Larson K, Urda B and Schacter B (1987). Inhibition of postbinding target cell lysis of lymphokine enhancement of human natural killer cell activity by in vitro exposure to ultraviolet B radiation. Cell Immunol 104: 47-58.

Falus A and Merétey (1992). Histamine: an early messenger in inflammatory and immune reactions. Immunol Today 13: 154-156.

Farman JC, Gardiner BG and Shanklin JD (1985). Large losses of total ozone in Antarctica reveal seasonal C10x/WOx interaction. Nature 315: 207-209.

Fischer T, Alsons J and Berne B (1984). Ultraviolet action spectrum and evaluation of ultraviolet lamps for psoriasis healing. Int J Dermatol 23: 633-637.

Fisher MS and Kripke MC (1977). Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc Natl Acad Sci USA 74: 1688-1692.

Flegg PJ (1990). Potential risks of ultraviolet radiation in HIV infection. Int J STD and AIDS 1: 46-48.

Foster CA, Yokozeki H, Groh V, Rieger A, Kofler G, Koning F and Stingl G (1988). Human epidermis harbours two phenotypically distinct populations of CD3$^+$ cells. J Invest Dermatol 90: 560.

Friedmann PS, White SI, Parker S, Moss C and Matthews JNS (1989). Antigenic stimulation during ultraviolet therapy in man does not result in immunological tolerance. Clin Exp Immunol 76: 68-72.

Gahring LC, Baltz M, Peypys MB and Daynes RA (1984). Effect of ultraviolet radiation on production of epidermal cell thymocyte-activating factor/interleukin-1 in vivo and in vitro. Proc Natl Acad Sci USA 81: 1198-1202.

Gallatin WM, Weissman IL and Butcher EC (1983). A cell surface molecule involved in organ-specific homing of lymphocytes. Nature (London) 304: 30-34.

Gallo RL, Stoszewski R and Granstein R (1990). Physiology and pathology of skin photoimmunology. In: Skin Immune System. Ed. Jan D. Bos. Florida, CRC Press, pp 381-401.

Garrett TPS, Saper MA, Bjorkman PS, Stominger JL and Wiley DC (1989). Specificity pockets for the side chains of peptide antigens in HLA-Aw68. Nature 342: 692-696.

Giannini MSH (1986). Suppression of pathogenesis in cutaneous leishmaniasis by UV-irradiation. Infect Immun 51: 838-846.

Gomi T, Shiohara T, Munakata T, Imanishi K and Nageshuma M (1991). Interleukin 1α, tumour necrosis factor α, and interferon-alpha in psoriasis. Arch Dermatol 127: 827-830.

Gottleib AB, Lifshitz B, Fu SM, Staino-Coico L, Wang CY and Carter DM (1986). Expression of HLA-DR molecules by keratinocytes and presence of Langerhans cells in the dermal infiltrate of active psoriatic plaques. J Exp Med 164: 1013-1028.

Gottlieb AB (1988). Immunologic mechanisms in psoriasis. J Am Acad Dermatol 18: 1376-1380.

Granstein RD and Sauder DN (1987). Whole body exposure to ultraviolet radiation results in increased serum interleukin-1 activity in humans. Lymphokine Res 6: 187-193.

Green C, Ferguson J, Lakshimipathi T and Johnson BE (1988). 311 nm UVB phototherapy - an effective treatment for psoriasis. Br J Dermatol 119: 691-696.

Griffiths CEM, Vorhees JJ and Nickoloff BJ (1989). Characterisation of intercellular adhesin molecule-1 and HLA-DR expression in normal and inflamed skin: Modulation by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol 20: 617-629.

Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB and Gottlieb AB (1989). Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86: 6367-6371.

Gruner S, Diezel W, Stoppe H, Oesterwitz H and Henke W (1992). Inhibition of skin allograft rejection and acute graft-versus-host disease by cis-urocanic acid. J Invest Dermatol 98: 459-462.

Guilhou JJ, Clot J, Meynadier J and Lapinski H (1976). Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. Br J Dermatol 94: 501-7.

Guymer RH and Mandel TG (1990). Urocanic acid in allotransplantation. Transplant Proc 22: 2119-2120.

Haftek M, Glinski W, Jablonska S and Obalek S (1979). T lymphocyte E rosette function during photochemotherapy (PUVA) of psoriasis. J Invest Dermatol 72: 214-218.

Halprin KM, Comerford M, Pressor SE and Taylor JR (1981). Ultraviolet treatment delays contact sensitization to nitrogen mustard. Br J Dermatol 109: 71-76.

Harper RA, Tam DW, Vanderheid EC and Urbach F (1979). Normal T-lymphocyte function in psoriatic patients undergoing methoxsalen photochemotherapy. J Invest Dermatol 72: 323-325.

Harriott-Smith TH and Halliday WJ (1988). Suppression of contact hypersensitivity by short-term ultraviolet radiation. II. The role of urocanic acid. Clin Exp Immunol 72: 174-177.

Hatcher FM AND Kuhn RE (1982) Destruction of Trypanosoma cruzi by natural killer cells. Science 218: 295-296.

Hellstrand K and Hermodsson S (1990). A cell-to-cell mediated interaction involving monocytes and non-T/CD16$^+$ natural killer (NK) cells is required for histamine H_2-receptor-mediated NK-cell activation. Scand J Immunol 31: 631-644.

Heng MCY, Allen SG, Heng SY, Matsuyman R and Fazier J (1989). An electron microscopic study of the epidermis infiltrate in recurrent herpes simplex. Clin Exp Dermatol 14: 199-202.

Hensler T, Wolff K, Hënigsmann H and Christophers E (1981). Oral 8-methoxypsoralen photochemotherapy of psoriasis. Lancet i: 853-857.

Herberman RB and Ortaldo JR (1981) Natural killer cells: their role in defences against disease. Science 214 : 24-30

Hersey P, Haran G, Hasic E and Edwards A (1983a). Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. J Immunol 131: 171-174.

Hersey P, Bradley M, Hasic E, Haran G, Edwards E and McCarthy WH (1983b). Immunological effects of solarium exposure in human subjects. Lancet i: 545-548.

Hersey P (1986). Effects of electromagnetic radiation on the immune system in mice and man. J Immunol Immunopharmacol 5: 216-222.

Hersey P, MacDonald M, Burns C, Schibeci S, Matthews H and Wilkinson FJ (1987). Analysis of the effect of a sunscreen agent on the suppression of natural killer cell activity induced in human subjects by radiation from solarium lamps. J Invest Dermatol 88: 271-276.

Hersey P, MacDonald M, Henderson C, Schibeci S, D'Alessandro G, Pryor M, Wilkinson FJ (1988). Suppression of natural killer cell activity in humans from solarium lamps depleted of UVB. J Invest Dermatol 90: 305-310.

Herzog WR, Ferreri N, Ptak W and Askenase PW (1989). The DTH initiating Thy-1$^+$ cell is double negative (CD4$^-$, CD8$^-$) and CD3$^-$, and expresses IL-3 but no IL-2 receptors. J. Immunol 143: 3125-3133.

Heufler C, Koch F and Schuler G (1988). Granulocyte/macrophage colony-stimulating factor and interleukin-1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med 167: 700-705.

Higaki Y, Hauser C, Siegenthaler G and Saurat JH (1986). Cis-urocanic acid does not inhibit mitogen induced lymphocyte transformation in man. Acta Derm Venerol (Stockh) 66: 523-526.

Hill SJ (1990). Distribution properties and functional characteristics of three classes of histamine receptor. Pharmacol Rev 42: 45-83.

Howie S, Norval M and Maingay J (1986a). Exposure to low dose ultraviolet B light suppresses delayed type hypersensitivity to herpes simplex virus in mice. J. Invest Dermatol 86: 125-128.

Howie SEM, Norval M and Maingay JP (1986b). Alterations in epidermal handling of HSV-1 antigens in vitro induced by in vivo exposure to UV-B light. Immunology 57: 225-230.

Howie SEM, Ross JA, Norval M and Maingay JP (1987). In vivo modulation of antigen presentation generates T_s rather than T_{DH} in HSV-1 infection. Immunology 60: 419-423.

Hunyadi J, Szegedi G and Nigra TP (1981). Natural killer cell activity in psoriasis vulgaris. Arch Dermatol Res 271: 107-109.

Iacobelli D, Hashimoto K and Takahashi S (1985). Effect of ultraviolet radiation on guinea pig epidermal Langerhans cell cytomembrane: light and electron microscopic studies. Photodermatology 2: 132-143.

Ikai K and Kanauchi H (1991). Effect of ultraviolet irradiation on Langerhans cells in mast-cell deficient W/Wv mice. Photodermatol Photoimmunol Photomed 7: 258-265.

Jaffe M (1984). Concerning a new constituent in the urine of dogs. Ber Deut Chem Ges 7: 1669-1673.

Jalkanen S, Bargatze R, Herron L and Butcher EC (1986). A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. Eur J Immunol 16: 1195-1202.

Jansen CT, Lammintausta K, Pasanen P, Neuvonen J, Varjonen E, Kalimo K and Äyras P (1991). A non-invasive chamber sampling technique for HPLC analysis of human epidermal urocanic acid. Acta Derm Venereol (Stockh) 71: 143-145.

Jeevan A and Kripke ML (1989). Alteration of the immune responses to Mycobacterium bovis BCG in mice exposed chronically to low doses of UV irradiation. Cell Immunol 145: 2087-2091.

Jeevan A, Evans R, Brown EL and Kripke ML (1992a). Effect of local ultraviolet irradiation on infections of mice with Candida albicans, Mycobacterium bovis BCG, and Schistosoma mansoni. J Invest Dermatol 99: 59-64.

Jeevan A, Ullrich SE, Ducan V and Kripke ML (1992b). Supernatants from ultraviolet-irradiated keratinocytes decrease the resistance and delayed-type hypersensitivity response to Mycobacterium bovis Bacillus Calmette-Guerin in mice and impair the phagocytic ability of macrophages. Photodermatol Photoimmunol Photomed 9: 255-263.

Jones HE, Lewis CW and McMartin SL (1973). Allergic contact sensitivity in atopic dermatitis. Arch Dermatol 107: 217-22.

Jorizzo JL, Sams MW Jr, Jegasothy BV and Olanski AJ (1980). Cimetidine as an immunomodulator: Chronic mucocutaneous candidiasis as a model. Ann Int Med 92: 192-195.

Juhlin L and Shelley WB (1977). New staining method for the Langerhans cell. Acta Derm Venereol (Stockh) 57: 289-296.

Juhlin L, Shroot B, Martin B and Caron JC (1986). Reduced levels of histidine and urocanic acid in suction blister fluids from patients with psoriasis. Acta Derm Venereol (Stockh) 66: 295-300.

Julius MH, Simpson E and Herzenberg LA (1973). A rapid methof for the isolation of functional thymus-derived murine lymphocytes. Eur J Immunol 3: 645-649.

Kaleta EW, Applegate LA and Ley RD (1991). Photoreactivation of ultraviolet radiation-induced release of arachidonic acid from marsupial cells. Photochem Photobiol 54: 747-752.

Kaminski M, Szmurlo A, Pawinska M and Jablonska S (1984). Decreased natural killer cell activity in generalized pustular psoriasis (von Zumbusch type). Br J Dermatol 110: 565-568.

Kapp A, Gillitzer R, Kirchner H and Schopf E (1988). Decreased production of interferon in whole blood cultures derived from patients with psoriasis. J Invest Dermatol 90: 511-14.

Kapsenberg ML, Teunissen MB and Bos JD (1990). Langerhans cells: A unique subpopulation of antigen presenting dendritic cells. In: Skin Immune System. Boss JD (ed). Florida, CRC Press, pp 109-124.

Kärre K, Hansson M and Kiessling (1991). Multiple interaction at the natural killer workshop. Immunol Today 12: 343-345.

Katz SI, Tamaki K and Sachs DH (1979). Epidermal Langerhans cells are derived from cells originating in the bone marrow. Nature (London) 262: 324-326.

Kiessling R, Hochman PS, Hallwer O, Shearer GM, Wigzell H and Cudkowicz G (1977). Evidence for a similar or common mechanism for natural killer cell activity and resistance to haemopoietic grafts. Eur J Immunol 7: 655-663.

Kim T-Y, Kripke ML and Ullrich SE (1990). Immunosuppression by factors released from UV-irradiated epidermal cells: selective effects on the generation of contact and delayed hypersensitivity after exposure to UVA or UVB radiation. J Invest Dermatol 94: 26-32.

Kinnaird A, Peters SW, Foster JR and Kimber I (1989). Dendritic cell accumulation in draining lymph nodes during the induction phase of contact allergy. Int Arch Allergy App Immun 89: 202-210.

Kirnbauer R, Kock A, Neuner P, Förster E, Krutmann K, Urbanski A, Schauer E, Ansel JC, Schwarz T and Luger TA (1991). Regulation of epidermal cell interleukin-6 production by UV light and corticosteroids. J Invest Dermatol 96: 484-489.

Kligman AM (1979). Perspectives and problems in cutaneous gerontology. J Invest Dermatol 73: 39-46.

Kligman LH (1989). Photoageing: manifestations, prevention and treatment. Clin Geriatr Med 5: 235-251.

Knight SC, Bedford P and Hunt R (1985a). The role of dendritic cells in the initiation of immune responses to contact sensitizers. II. Studies in nude mice. Cell Immunol 94: 435-439.

Knight SC, Krejci J, Malkovsky M, Colizzi V, Gautam A and Asherson GL (1985b). The role of dendritic cells in the initiation of immune responses to contact sensitizers. I. In vivo exposure to antigen. Cell Immunol 94: 427-434.

Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B and Trinchieri G (1989). Identification and purification of natural killer cell stimulatory factor (NKSF) a cytokine with multiple biological effects on human lymphocytes. J Exp Med 170: 827-845.

Koch F, Heufler C, Kampgen E, Schneeweiss D, Bock G and Schuler G (1990). Tumor necrosis factor α maintains the viability of murine epidermal Langerhans cells in culture, but in contrast to granulocyte/macrophage colony-stimulating factor, without inducing their functional maturation. J Exp Med 171: 159-171.

Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC and Luger TA (1990). Human keratinocytes are a source for tumor necrosis factor α. Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 172: 1609-1614.

Kohl S, Springer TA, Schmalstieg FC, Loo LS and Anderson DC (1984). Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency. J Immunol 133: 2972-2978.

Kohl S (1991). Role of antibody-dependent cellular cytotoxicity in defence against herpes simplex virus infections. Rev Infect Dis 13: 108-114.

Koning F, Stingl G, Yokoyama WM, Yamada H, Maloy WL, Tschachler E, Shevach EM and Coligan JE (1987). Identification of a T3-associated gamma/delta T cell receptor on Thy-1$^+$ dendritic epidermal cell lines. Science 236: 834-837.

Korelj F, Presani G, Petricarari S and Trevisan G (1984). Blood lymphocyte subpopulations in psoriasis. Acta Derm Venereol (Stockh) 113 (Suppl): 17-19.

Koren HS and Leung KH (1982). Modulation of human NK cells by interferon and prostaglandin E2. Mol Immunol 19: 1341-1346.

Koulu L, Jansen CT and Viander M (1985). Effects of UVA and UVB irradiation on human epidermal Langerhans cell membrane markers defined by ATPase activity and monoclonal antibodies (OKT6 and anti-Ia). Photodermatol 2: 339-346.

Kripke ML and Morison WL (1986). Studies on the mechanism of systemic suppression of contact hypersensitivity in UVB radiation. II. Differences in suppression of delayed and contact hypersensitivity in mice. J Invest Dermatol 86: 543-549.

Kripke ML, Munn CG, Jeevan A, Tang J and Bucana C (1990). Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. J Immunol 145: 2833-2838.

Kripke ML (1991). Immunological effects of ultraviolet radiation. J Dermatol 18: 429-433.

Kripke ML, Cox P and Yaroch DB (1991). Effect of T4WS liposome-enhanced DNA repair on UV-induced immune suppression in the mouse. Photochem Photobiol 53: 295 (Abst).

Krutmann J, Köck A, Schauer E, Parlow F, Möller A, Kapp A, Forster E, Schöpf E and Luger T (1990). Tumor necrosis factor β and ultraviolet radiation are potent regulators of human keratinocyte ICAM-1 expression. J Invest Dermatol 95: 127-131.

Krutmann J, Kammer GM, Toosi Z, Waller RL, Ellner JJ and Elmets CA (1990). UVB radiation and human monocyte accessory function: Differential effects on pre-mitotic events in T-cell activation. J Invest Dermatol 94: 204-209.

Kupper TS, Ballard DW, Chua AE, McGuire JS, Flood PM, Horowitz ML, Langdon J, Lightfoot L and Gubler U (1986). Human keratinocytes contain mRNA indistinguishable from monocyte interleukin-1 alpha and beta mRNA. Keratinocyte epidermal cell-derived thymocyte activating factor is identical to interleukin-1. J Exp Med 164: 2095-2100.

Kupper TS, Chua AE, Flood P, McGuire JS and Gubler U (1987). Interleukin-1 gene expression in cultured human keratinocytes is augmented by ultraviolet irradiation. J Clin Invest 80: 430-436.

Kurimoto I and Streilein JW (1992). Cis-urocanic acid suppression of contact hypersensitivity induction is mediated via tumor necrosis factor-alpha. J Immunol 148: 3072-3078.

Kuziel WA, Takashima A, Bonyhadi M, Bergstresser PR, Allison JP, Tigelaar RE and Tucker PW (1987). Regulation of T-cell receptor gamma-chain RNA expression in murine Thy-1$^+$ dendritic epidermal cells. Nature (London) 328: 263-266.

Lampert IA (1984). Expression of HLA-DR (Ia-like) antigens on epidermal keratinocytes in human dermatoses. Clin Exp Immunol 57: 93-100.

Lampert IA, Smitters AJ and Chisholm PM (1981). Expression of Ia antigen in epidermal keratinocytes on graft-versus-host disease. Nature (London) 293: 149-150.

Langerhans P (1868). Ueber die neruen der menschlichen haut. Virchows Arch Pathol Anat Physiol 44: 325-335.

Lanier LL, Phillips J, Hackett J, Tutt M and Kumar V (1986). Natural killer cells: Definition of a cell type rather than function. J Immunol 137: 2735-2739.

Larkö O (1989). Treatment of psoriasis with a new UVB-lamp. Acta Derm Venereol (Stockh) 69: 357-359.

Lavaud P, Rodrigue F, Carré C, Touvay C, Mencice-Huerta J and Braquet P (1991). Pharmacological modulation of picryl chloride-induced contact dermatitis in the mouse. J Invest Dermatol 97: 101-105.

Livden JK, Bjere JR, Degre M and Matre R (1977). Effect of UV irradiation on interferon, immunoglobulins, and complement components in serum from healthy individuals. Photodermatol 4: 296-301.

Lowry OH, Rosenborough NJ, Farr AL and Randall RJ (1951). Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265-275.

Lozzio CB and Lozzio BB (1975). Human chronic myelogenous leukaemia cell-line with positive Philadelphia chromosome. Blood 45: 321-334.

Luger TA, Stadler BM, Katz SI and Oppenheim JJ (1981). Epidermal cell (keratinocyte) derived thymocyte activating factor (ETAF). J Immunol 127: 1493-1498.

Luger TA, Stadler BM, Luger BM, Sztein MB, Schmidt JA, Hawley-Nelson P, Grabner G and Oppenheim JJ (1983a). Characteristics of an epidermal thymocyte-activating factor (ETAF) produced by human epidermal cells and human squamous cell carcinoma cell line. J Invest Dermatol 81: 187-193.

Luger TA, Sztein MB, Schmidt JA, Murphy P, Grabner G and Oppenheim JJ (1983b). Properties of murine and human epidermal cell-derived thymocyte-activating factor. Fed Proc 47: 2772-2776.

Luger TA, Uchida A, Kock A, Colot M and Micksche M (1985). Human epidermal cells and squamous carcinoma cells synthesize a cytokine that augments natural killer cell activity. J Immunol 134: 2477-2483.

Luger TA and Schwarz T (1991). Epidermal cell derived secretory regulins. In: Schuler G (ed). Epidermal Langerhans Cells. CRC Press, Boca Raton, Florida. pp 217-251.

Lynch DH, Gurish MF and Daynes RA (1981). Relationship between epidermal Langerhans cell density, ATPase activity and the induction of contact hypersensitivity. J Immunol 126: 1892-1897.

Macatonia SE, Edwards AJ and Knight SC (1986). Dendritic cells and the activation of contact sensitivity to fluorescein isothiocyanate. Immunology 59: 509-514.

Mackie R, Hunter JAA, Aitchison TC, Hole D, McLaren K, Rankin R, Blessing K, Evans AT, Hutcheon AW, Jones DFH, Soutar DS, Watson ACH, Cornbleet MA and Smyth JF (1992). Cutaneous malignant melanoma, Scotland 1979-89. The Lancet i: 971-975.

Martin S and Rouse BT (1987). The mechanisms of antiviral immunity induced by a vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D: clearance of local infection. J Immunol 138: 3431-3437.

Masami U and Takayuki S (1983). A longitudinal study of contact sensitivity in patients with atopic dermatitis. Arch Dermatol 125: 366-368.

Matheson MJ and Reeve VE (1991). The effect of the antihistamine cimetidine on ultraviolet-radiation-induced tumorigenesis in the hairless mouse. Photochem Photobiol 53: 639-642.

Melmon KL and Khan MM (1987). Histamine and its lymphocyte-selective derivatives as immune modulators. Trends Pharmacol Sci 8: 437-441.

Melski JW, Tanenbaum L, Parrish JA, Fitzpatrick TB, Bleich HL and 28 participating investigators (1977). Oral methoxsalen photochemotherapy for the treatment of psoriasis: A cooperative clinical trial. J Invest Dermatol 68: 328-335.

Mingari MC, Ferrini S, Pende D, Bottino C, Prigione I, Moretta A, Moretta L (1987) Phenotypic and functional analysis of human CD3+ and CD3- clones with "lymphoproliferative-activated killer" (LAK) activity. Frequent occurrence of CD3+ LAK clones which produce interleukin-2. Int J Cancer 40: 495-498.

Mitsuhashi M and Payan DG (1992). Functional diversity of histamine and histamine receptors. J Invest Dermatol 98 85-115.

Möller A, Grabbe J and Czarnetcki BM (1991). Mast cells and their mediators in immediate and delayed immune reactions. Skin Pharmacol 4 (Suppl 1): 56-63.

Moodycliffe AM, Kimber I and Norval M (1992). The effect of ultraviolet B irradiation and urocanic acid isomers on dendritic cell migration. Immunology 77: 294-399.

Morahan PS, Connor JR and Leary KR (1985). Viruses and the versatile macrophage. Br Med Bull 41: 15-21.

Moretta L, Ciccone R, Moretta A, Hoglund P, Ohlen C, Karre K (1992). Allorecognition by NK cells: nonself or no self? Immunol Today 13: 300-306.

Morison WL, Parrish JA, Bloch KJ and Krugler JI (1979a). Transient impairment of peripheral blood lymphocyte function during PUVA therapy. Br J Dermatol 101: 391-397.

Morison WL, Parrish JA, Bloch KJ and Krugler JI (1979b). In vivo effect of UV-B on lymphocyte function. Br J Dermatol 101: 513-519.

Morison WL (1989). Effects of ultraviolet radiation on the immune system in humans. Photochem Photobiol, 50: 515-24.

Mork NJ, Gaudernack G and Braathen LR (1987). Effect of UVA and PUVA on allo-activating and antigen-presenting capacity of human epidermal Langerhans cells. Photodermatol 4: 66-72.

Morrison HC, Bernascani and Pandy G (1984). A wavelength effect of urocanic acid E-Z photoisomerization. Photochem Photobiol 40: 549-550.

Morrison H (1985). Photochemistry and photobiology of urocanic acid. Photodermatol 2: 158-165.

Mukherjee S, Katz DR and Rook GAW (1986). Differing role of dendritic cells and macrophages in the induction of delayed-type hypersensitivity responses to PPD. Immunology 59: 229-234.

Murphy JW and McDaniel DO (1982). In vitro reactivity of natural killer (NK) cells against Cryptococcus neoformans. J Immunol 128: 1577-1583.

Murphy GM, Dowd PM, Hudspith BN, Brostoff J and Greaves MW (1989). Local increase in interleukin-1-like activity following UVB irradiation of human skin in vivo. Photodermatol 6: 268-274.

Mutzas M, van Arnim B, Hentschel H, Volger E and Stickel HA (1991). Effects of UV-A1-light on immune status in man. In: Biological Responses to Ultraviolet A Radiation. Urbach FJ (ed). 2nd edn. Kansan, Vladenmar Publishing Company, 2: 249-256.

Nagano K, Hori K, Nagane T, Snugawara T, Oh-ishi J, Hayashi H, Watanabe N and Niitsu Y (1990). Effect of tumour necrosis factor in the mouse-tail model of psoriasis. Arch Dermatol Res 282: 459-462.

Nanney LB, Stoscheck CM, Magid M and King LE (1986). Altered epidermal growth factor binding and receptor distribution inpsoriasis. J Invest Dermatol 86: 260-265.

National Aeronautics and Space Administration (NASA) (1988). Executive summary of the ozone trends panel. Washington DC, USA, March.

Nencioni L, Villa L, Boraschi D, Berti D, Tagliabue A (1983). Natural and antibody-dependent cell-mediated activity against Salmonella typhimurium by peripheral and intestinal lymphoid cells in mice. J Immunol 130: 903-907.

Nickoloff BJ, Griffiths CE and Barker JN (1990). The role of adhesion molecules, chemotactic factors and cytokines in inflammatory and neoplastic disease - 1990 update. J Invest Dermatol 94: 151S-157S.

Nickoloff BJ, Karabin GD, Barker JNWN, Griffiths CEM, Sarma V, Mitra RS, Elder JT, Kuntzel SL and Dixit VM (1991). Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am J Pathol 138: 129-40.

Nickoloff BJ (1991a). The cytokine network in psoriasis. Arch Dermatol, 127: 99-109.

Nickoloff BJ (1991b). Adhesion molecules and inflammatory cell migration pathways in the skin. In: Epidermal Langerhans Cells. Schuler G (ed). CRC Press, Boston, pp 49-71.

Noonan FP, De Fabo EC and Morrison H (1985). Cis-urocanate, a UV irradiation product, decreases the number of Ia-positive Langerhans cells in cultured epidermal sheets. J Invest Dermatol 84: 342.

Noonan FP, De Fabo EC and Morrison H (1988). Cis-urocanic acid, a product formed by ultraviolet B irradiation of the skin, initiates an antigen presentation defect of splenic dendritic cells in vivo. J Invest Dermatol 90: 92-99.

Noonan FP and De Fabo EC (1990). Ultraviolet-B dose-response curves for local and systemic immunosuppression are identical. Photochem Photobiol 52: 801-810.

Noonan FP and De Fabo EC (1992). Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. Immunol Today 13: 250-254.

Norlund JJ, Ackles AE and Lerner AB (1981). The effects of ultraviolet light and certain drugs in Ia-bearing Langerhans cells in murine epidermis. Cell Immunol 60: 50-63.

Norris DA, Bradley L, Middleton MH, Yohn JJ and Kashihara-Sauami M (1990). Ultraviolet radiation can either suppress or induce expression of intercellular adhesion molecule 1 (ICAM-1) on the surface of cultured human keratinocytes. J Invest Dermatol 95: 132-138.

Norval M, McIntyre CR, Simpson TJ, Howie SEM and Bardshiri E (1988). Quantification of urocanic acid isomers in murine skin during development and after irradiation with ultraviolet B light. Photodermatol 5: 179-86.

Norval M, Simpson TJ, Bardshiri E and Crosby J (1989a). Quantification of urocanic acid isomers in human strateum corneum. Photodermatol Photoimmunol Photomed 6: 142-145.

Norval M, Simpson TJ, Bardshiri E and Howie SEM (1989b). Urocanic acid analogues and the suppression of the delayed type hypersensitivity response to herpes simplex virus. Photochem Photobiol 49: 633-639.

Norval M, Simpson TJ and Ross JA (1989c). Urocanic acid and immunosuppression. Photochem Photobiol 50: 267-275.

Oesterwitz H, Gruner S, Diezel W and Schneider W (1990). Inhibition of rat heart allograft rejection by a PUVA treatment of the graft recipient. Role of cis-urocanic acid. Transplant Int 3: 8-11.

Okamoto H and Kripke ML (1987). Effector and suppressor circuits of the immune response are activated in vivo by different mechanisms. Proc Natl Acad Sci USA 84: 3841-3845.

Oxholm AP, Staberg B and Bendtzen K (1988). Immunohistological detection of interleukin-1-like molecules and tumor necrosis factor in human epidermis before and after UVB-irradiation in vivo. Br J Dermatol 118: 369-376.

Palaszynski EW, Noonan FP and De Fabo EC (1992). Cis-urocanic acid down regulates the induction of adenosine 3',5'-cyclic monophosphate by either trans-urocanic acid or histamine in human dermal fibroblasts in vitro. Photochem Photobiol 55: 165-171.

Pamphilon DH, Alnaudy AA and Wallington TB (1991). Immunomodulation by ultraviolet light: clinical studies and biological effects. Immunol Today 12: 119-123.

Parrish JA and Jaenicke KF (1981). Action spectrum for phototherapy of psoriasis. J Invest Dermatol 76: 359-362.29

Pasanen P, Reunala T, Jansen CT, Rasanen L, Neuvonen K and Äyräs P (1990). Urocanic acid isomers in epidermal samples and suction blister fluid of nonirradiated and UVB-irradiated human skin. Photodermatol Photoimmunol Photomed 7: 40-2.

Pawlec G, Newman W, Schwulera U and Wernet P (1985). Heterogeneity of human natural killer recognition demonstrated by cloned effector cells and differential blocking of cytotoxicity with monoclonal antibodies. Cell Immunol 92: 31-40.

Perna JJ, Mannix ML, Rooney JF, Notkins AL and Straus SE (1987). Reactivation of latent herpes simplex virus infection by ultraviolet light: a human model. J Am Acad Dermatol 17: 473-478.

Pillai S, Bikle DD, Eessalu TE, Aggarwal BB and Elias PM (1989). Binding and biological effects of tumor necrosis factor alpha on cultured human neonatal foreskin keratinocytes. J Clin Invest 83: 816-821.

Pitts DG, Cameron LL, Jose JG, Lerman S, Moss E, Varma SD, Zigler S, Zigman S and Zuchlich J (1986). Optical radiation and cataracts. In: Optical radiation and visual health. Waxler M and Hitchings VM (eds). CRC Press, Inc., Boca Raton, Florida USA.

Pitzalis C, Kingsley G, Haskard D and Panayi G (1988). The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: Evidence for regulation by selective endothelial and homotypic adhesion. Eur J Immunol 18: 1397-1404.

Polla L, Margolis R, Goulsten C, Parrish JA and Granstein RD (1986). Enhancement of the elicitation phase of the murine contact hypersensitivity response by prior exposure to local ultraviolet radiation. J Invest Dermatol 86: 13-17.

Pollock Shea C (1988). Worldwatch Paper 87. Protecting life on earth: steps to save the ozone layer. Worldwatch Paper 13-19.

Ptak W, Geba G and Askenase PW (1991). Incubation of delayed-type hypersensitivity by low doses of monoclonal IgG antibody: Mediation by serotonin and inhibition by histamine. J Immunol 146: 3929-3936.

Räsänen L, Jansen CT, Hyoty H, Reunala T and Morrison H (1989). Cis-urocanic acid stereospecifically modulates human monocyte IL-1 production and surface HLA-DR antigen expression, T-cell IL-2 production and CD4/CD8 ratio. Photodermatol 6: 287-292.

Räsänen L, Jansen CT, Reunala T and Morrison H (1987). Stereospecific inhibition of human epidermal cell interleukin-1 secretion of HLA-DR expression by cis-urocanic acid. Photodermatol 4: 182-186.

Reavan EP and Cox AJ (1965). Histidine and keratinization. J Invest Dermatol 45: 422-431.

Reeve VG, Bosnic WG, Reilly WG and Ley RD (1991). Urocanic acid photobiology in the hairless mouse. Photochem Photobiol 53: 88S-89S.

Reeve VG and Mitchell LE (1991). Hazards of urocanic acid as a cosmetic ingredient. Photodermatol Photoimmunol Photomed 8: 176-180.

Reeve E, Greenoak GE, Canfield PJ, Boehm-Wilcox C and Gallagher CH (1989). Topical urocanic acid enhanced UV-induced tumour yield and malignancy in the hairless mouse. Photochem Photobiol 49: 459-464.

Reilly SK and De Fabo EC (1991). Dietary histidine increases mouse skin urocanic acid levels and enhances UVB-induced immune suppression of contact hypersensitivity. Photochem Photobiol 53: 431-438.

Ring J (1983). Plasma histamine concentration in atopic eczema. Clin Allergy 13: 545-552.

Ring J and Thomas P (1989). Histamine and atopic eczema. Acta Derm Venereol 144: 570-577.

Rivers JK, Norris PG, Murphy GM, Chu AC, Midgeley G, Morris J, Morris RW, Young AR and Hawk JLM (1989). UVA sunbeds, tanning, photoprotection, active adverse effects and immunological changes. Br J Dermatol 120: 767-777.

Robertson B, Garhing L, Newton R and Daynes RA (1987). In vivo administration of interleukin-1 to normal mice depresses their capacity to elicit contact hypersensitivity responses: prostaglandins are involved in this modification of immune function. J Invest Dermatol 88: 380-387.

Romani N, Koide S, Crowley M, Witmer-Pack M, Livingstone HM, Fathman CG, Inaba K and Steinman RM (1989). Presentation of exogeneous protein antigens by dendritic cells to T cell clones. Intact protein is presented best by immature, epidermal Langerhans cells. J Exp Med 169: 1169-1178.

Romani N, Schuler G and Fritsch P (1991). Identification and phenotype of epidermal Langherans cells. In: Epidermal Langerhans cells. Schuler G (ed). Boston: CRC Press, pp 49-86.

Rosenberg EW, Noah PW, Wyatt RJ, Jones RM and Kolb WP (1990). Complement activation in psoriasis. Clin Exp Dermatol 15: 16-20.

Ross GD, Thompson RA, Walport MJ, Springer TA, Watson JV, Ward RH, Lidd J, Newman SL, Harrison RA and Lachman PJ (1985). Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type 3 and the related membrane antigen LFA-1. Blood 66: 882-890.

Ross JA, Howie SEM, Norval M, Maingay J and Simpson TJ (1986). Ultraviolet-irradiated urocanic acid suppressed delayed-type hypersensitivity to herpes simplex virus. J Invest Dermatol 87, 630-633.

Ross JA, Howie SEM, Norval M and Maingay JP (1987). Two phenotypically distinct T cells involved in UV-irradiated urocanic acid induced suppression of the efferent DTH response to HSV-1 in vitro. J Invest Dermatol 89: 230-233.

Ross JA, Howie SEM, Norval M, and Maingay JP (1987/88). Induction of suppression of delayed type hypersensitivity to herpes simplex virus by epidermal cells exposed to UV-irradiated urocanic acid in vivo. Viral Immunol 1: 191-198.

Rücker BU, Haberle M, Koch HU, Bouchek P, Schriever KH and Hornstein OP (1991). Ultraviolet light hardening vs polymorphous light eruption - a controlled study comparing different emission spectra. Photodermatol Photoimmunol Photomed 8: 73-78.

Russell TJ, Schultes LM and Kuban DJ (1972). Histocompatibility (HLA) antigens associated with psoriasis. N Engl J Med 287: 738-740.

Sauder DN, Carter C, Katz SI and Oppenheim JJ (1982). Epidermal cell production of thymocyte activating factor (ETAF). J Invest Dermatol 79: 34-39.

Schacter B, Lederman MM, LeVine MJ, Ellner JJ (1983). Ultraviolet radiation inhibits human natural killer cell activity and lymphocyte proliferation. J Immunol 130: 2484-2487.

Schmidt D, Faure M, Dambuyant-Dezutter C and Thivolet J (1984). The semi-quantitative distribution of T4 and T6 surface antigens on human Langerhans cells. Br J Dermatol 111: 655-661.

Schmidt RE, Caulfield JP, Michon J, Heun A, Kamada MM, MacDermott RP, Stevens RL and Ritz J (1988). T11/CD2 activation of cloned human natural killer cells results in increased conjugate formation and exocytosis of cytolytic granules. J Immunol 140: 991-1002.

Schwarz W, Langer K, Schell H and Schonberger A (1986a). Distribution of urocanic acid in human stratum corneum. Photodermatol 3: 239-240.

Schwarz T, Urbanski A Gschnait F and Luger TA (1986b). Inhibition of the induction of contact hypersensitivity by a UV-mediated epidermal cytokine. J Invest Dermatol 87: 289-291.

Schwarz T, Urbanski A, Gschnait F and Luger TA (1987). UV-irradiated epidermal cells produce a specific inhibitor of interleukin-1 activity. J Immunol 138: 1457-1463.

Schwarz T, Urbanski A, Kirnbauer R, Köch A, Gschnait F and Luger TA (1988). Detection of a specific inhibitor of interleukin-1 in sera of UVB-treated mice. J Invest Dermatol 91: 536-540.

Schwarz W, Schell H, Hüttinger G, Wasmeir H and Diepgen T (1987). Effect of UVA on human stratum corneum histidine and urocanic acid isomers. Photodermatol 4: 269-271.

Schuler G (ed) (1991). Epidermal Langerhans cells. CRC Press, Boca Raton, Florida.

Scott IR (1981). Factors controlling the expressed activity of histidine ammonia-lyase in the epidermis and the resulting accumulation of urocanic acid. Biochem J 194: 829-838.

Shimada S, Caughman SW, Sharrow SO, Stephany D and Katz SI (1987). Enhanced antigen-presenting capacity of cultured Langerhans cells is associated with markedly increased expression of Ia antigen. J Immunol 139: 2551-2555.

Shimizu Y, Newman W, Tanaka Y and Shaw S (1992). Lymphocyte interactions with endothelial cells. Immunol Today 13: 106-112.

Silberberg-Sunakin I, Thorbecke GJ, Baer RL, Rosenthal SA and Berezowsky V (1976). Antigen-bearing Langerhans cells in skin, dermal lymphatics and in lymph nodes. Cell Immunol 25: 137-151.

Simon JC, Girdomoni G, Edelbaum D, Bergstresser PR, Schopf E and Cruz PD (1991). Epidermal Langerhans cells express both ICAM-1 and LFA-1, whereas spleen dendritic cells express LFA-1: these molecules identify disparate requirements for activation of CD4 Th1 and Th2 cells. Clin Res 96: 626-631.

Simon JC, Edelbaum D, Bergstresser PR and Cruz PD (1992). Distorted antigen-presenting function of Langerhans cells induced by tumor necrosis factor α via a mechanism that appears different from that induced by ultraviolet B irradiation. Photochem Photobiol 8: 190-194.

Simon JC, Krutmann J, Elmets CA, Bergstresser PR and Cruz PD (1992). Ultraviolet B-irriadiated antigen-presenting cells display altered accessory signalling for T-cell activation: relevance to immune responses initiated in the skin. J Invest Dermatol 98: 66S-69S.

Simon M and Hunyadi J (1987). Expression of OKM5 antigen on human keratinocytes in positive intracutaneous tests for delayed type hypersensitivity. Dermatologica 175: 121-125.

Sjovall P and Christensen OB (1986). Local and systemic effects of ultraviolet irradiation (UVB and UVA) on human allergic contact dermatitis. Acta Derm Venereol 66: 290-294.

Snellman E, Koulu L, Pasanen P, Lammintausta K, Neuvonen K, Äyräs P asnd Jansen CT (1992). Effect of psoriasis heliotherapy on epidermal urocanic acid isomer concentrations. Acta Derm Venereol (Stockh) 72: 231-3.

Sontheimer RD, Matsubara T and Seeling LL Jr (1989). A macrophage phenotype for a constitutive, class II antigen-expressing, human dermal perivascular dendritic cell. J Invest Dermatol 93: 154-159.

Spangrude GJ, Berhard EJ, Ajoika RS and Daynes RA (1983). Alterations on lymphocyte homing patterns within mice exposed to ultraviolet radiation. J Immunol 130: 2974-2981.

Spencer M-J, Vestey JP, Tidman MJ, McVittie E and Hunter JAA (1992). Major histo-compatibility class II antigen expression on the surface of epidermal cells from normal and ultraviolet B irradiated subjects. J Invest Dermatol (in press).

Sprecher E and Becker Y (1987). Herpes simplex virus type 1 pathogenicity in footpad and ear skin of mice depends on Langerhans cell diversity, mouse genetics, and virus strain. J Virol 61: 2515-2522.

Sprecher E and Becker Y (1989). Langherans cell density and activity in mouse skin and lymph nodes affect herpes simplex type 1 (HSV-1) pathogenicity. Arch Virol 107: 191-205.

Spruance SL (1985). Pathogenesis of herpes simplex labialis: experimental induction of lesions with ultraviolet light. J Clin Microbiol 22: 366-368.

Spruance SL, Freeman DJ, Stewart JCB, <cKeough MB, Wenerstrom LG, Krueger GG, Piepkorn MW, Stroop WG and Rowe N (1991). The natural history of ultraviolet radiation-induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. J Infect Dis 163: 728-34.

Starling GC, Davidson SE, McKenzie JL and Hart DN (1987). Inhibition of natural killer cell mediated cytolysis with monoclonal antibodies and restricted and non-restricted epitopes of the leucocyte common antigen. Immunology 61: 351-356.

Steiner G, Koning F, Elbe A, Tschachler E, Yokoyama WM, Shevach EM, Stingl G and Coligan JE (1988). Characterisation of T cell receptors on resident murine dendritic epidermal T cells. Eur J Immunol 18: 1323-1328.

Steiner G, Taschachler E, Tani M, Malek TR, Shevach EM, Holter W, Knapp W, Wolff K and Stingl G (1986). Interleukin 2 receptors on cultured murine epidermal Langerhans cells. J Immunol 137: 155-159.

Stingl G, Gazze-Stingl LA, Aberer W and Wolff K (1981). Antigen presentation by murine epidermal Langerhans cells and its alteration by ultraviolet B light. J Immunol 127: 1707-1713.

Stingl G, Koning F, Yamada H, Yokoyama WM, Tschachler E, Bluestone JA, Steiner G, Samelson IE, Lew AM, Cougan JE and Shevach EM (1987). Thy-1$^+$ dendritic epidermal cells express T3 antigen and the T-cell receptor gamma chain. Proc Natl Acad Sci USA 84: 4586-4590.

Stingl G and Shevach EM (1991). Langerhans cells as antigen-presenting cells. In: Schuler G (ed). Epidermal Langerhans Cells. Boca Raton, CRC Press, Chapter 6, pp 159-190.

Storkus WJ, Alexander J, Payne JA, Cresswell P and Dawson JR (1989). The $\alpha 1/\alpha 2$ domains of class I HLA molecules confer resistance to natural killing. J Immunol 143: 3853-3857.

Storkus WJ, Salter RD, Alexander S, Ward FE, Ruiz RE, Cresswell P and Dawson JR (1991). Class I-indiuced resistance to natural killing: Identification of non-permissive residues in HLA-A2. Proc Natl Acad Sci USA 88: 3989-3992.

Streilein JW (1983). Skin-associated lymphoid tissues (SALT): origins and functions. J Invest Dermatol 80 (Suppl): 12S-16S.

Streilein JW and Bergstresser PR (1988). Genetic basis of ultraviolet-B effects on contact hypersensitivity. Immunogen 27: 252-258.

Suzuki N, Biachi E, Bass H, Suzuki T, Bender J, Pardi R, Brenner LA, Larrik JW and Engleman EL (1990). Natural killer lines and clones with apparent antigen specificity. J Exp Med 172: 457-462.

Tabachnick J (1957). Urocanic acid, the major acid soluble, UV-absorbing compound in guinea pig epidermis. Arch Biochem Biophys 70: 295-298.

Takematsu H, Ozawa H, Yoshimura T, Hara M, Sakakibara A, Oyama J and Tagami H (1991). Systemic TNF administration in psoriatic patients: a promising therapeutic modality for severe psoriasis. Br J Dermatol 124: 209-10.

Tang A and Udey MC (1991). Inhibition of epidermal Langerhans cell function by low dose ultraviolet B radiation. J Immunol 146: 3347-3355.

Taylor HR, West SK, Rosenthal FS, Munoz B, Newland HS, Abbey H and Emmett EA (1988). Effect of ultraviolet radiation on cataract formation. New Engl J Med 319: 1429-1433.

Terui T, Aiba S and Kato T (1987). HLA-DR antigen expression on keratinocytes in highly inflamed parts of psoriatic lesions. Br J Dermatol 116: 87-93.

Teunissen MBM, Wormmeester J, Kapsenberg ML and Bos JD (1988). Enrichment of unlabelled human Langerhans cells from epidermal cell suspensions by discontinuous density gradient centrifugation. J Invest Dermatol 91: 358-361.

Teunissen MB, Wormmester J, Krieg SR, Peters PJ, Vogels IM, Kapsenberg ML and Bos JD (1990). Human epidermal Langerhans cells undergo profound morphological and phenotypical changes during in vitro culture. J Invest Dermatol 94: 166-173.

Toda K, Miyachi Y, Nesumi N, Konishi J and Imamura S (1986). UVB/PUVA-induced suppression of human natural killer cell activity is reduced by superoxide dismutase and/or interleukin-2 in vitro. J Invest Dermatol 86: 519-522.

Toews GB, Bergstresser PR and Streilein JW (1980). Epidermal Langerhans cell density determined whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124: 445-453.

Trinchieri G and Santoli D (1978a). Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of target cells to lysis. J Exp Med 147: 1314-1333.

Trinchieri G and Santoli D (1978b). Spontaneous cell-mediated cytotoxicity in humans. J Immunol 120: 1849-1855.

Trinchieri G, Matsumoto-Kobayashi M, Clark SC, Sheehra J, Landon L and Perussia B (1984). Response of resting human peripheral blood natural killer cells to interleukin-2. J Exp Med 160: 1147-1169.

Trinchieri G and Perussia B (1984). Human natural killer cells: biologic and pathologic aspects. Lab Invest 50: 489-513.

Trinchieri G (1989). Biology of natural killer cells. Adv Immunol 47: 187-376.

Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K and Stingl G (1983). Expression of Thy-1 antigen by murine epidermal cells. J Invest Dermatol 81: 283-285.

Tse Y and Cooper KD (1990). Cutaneous dermal Ia^+ cells are capable of initiating delayed type hypersensitivity responses. J Invest Dermatol 94: 267-272.

Urbanski A, Schwarz T, Neuner P, Krutmann J, Kirnbauer R, Köch A and Luger TA (1990). Ultraviolet light induces increased circulating interleukin-6 in humans. J Invest Dermatol 94: 808-811.

Van Lovern H, Meade R and Askenase PW (1983). An early component of delayed hypersensitivity mediated by T cells and mast cells. J Exp Med 157: 1604-1617.

Van Lovern H, Kato K, Meade R, Green DR, Horowitz M, Ptak W and Askenase PW (1984). Characterization of two different $Ly1^+$ T cell populations that mediate delayed-type hypersensitivity. J Immunol 133: 2402-2411.

Van Weelden H, van der Leun JC (1984). Improving the effectiveness of phototherapy for psoriasis. Br J Dermatol 111: 484.

Van Weelden H, Baart de la Faille H, Young E, van der Leun JC (1990). comparison of narrow-band UV-B phototherapy and PUVA photochemotherapy in the treatment of psoriasis. Acta Derm Venereol (Stockh) 70: 212-215.

Vannier E, Miller LC and Dinarello (1991). Histamine suppresses gene expression and synthesis of tumor necrosis factor alpha via histamine H_2 receptors. J Exp Med 174: 281-284.

Vermeer M and Streilein JW (1991). Ultraviolet B light-induced alterations in epidermal Langerhans cells are mediated in part by tumor necrosis factor-alpha. Photoderm Photoimmunol Photomed 7: 258-265.

Versteeg R (1992). NK cells and T cells: mirror images? Immunol Today 13: 244-247.

Vestey JP, Howie SEM, Norval M, Maingay JP and Neill WA (1988). Immune responses to herpes simplex virus in patients with facial herpes simplex and those with eczema herpeticum. Br J Dermatol 118: 775-82.

Vestey JP, Norval M, Howie S, Maingay J and Neill WA (1989). Variation in lympho-proliferative responses during recrudescent orofacial herpes simplex virus infections. Clin Exp Immunol 77: 384-390.

Vestey JP, Norval M, Howie SEM, Maingay JP and Neill WA (1990). Antigen presentation in patients with recrudescent orofacial herpes simplex virus infection. Br J Dermatol 122: 33-42.

Viander M, Jansen CT, Eskold J (1984). Influence of whole-body PUVA treatments on human peripheral blood NK activity. Photodermatology 1: 23-29.

Waldorf HA, Walsh JL, Schechter NM and Murphy GF (1991). Early cellular events in evolving cutaneous delayed hypersensitivity in humans. Am J Pathol 138: 477-486.

Warner SF and Dennert G (1982). Effects of a cloned cell line with NK activity on bone marrow transplants, tumour development and metastasis in vivo. Nature (London) 300: 31-34.

Weitzen ML and Bonavida B (1984). Mechanism of inhibition of human natural killer activity by ultraviolet radiation. J Immunol 133: 3128-3132.

Wildy P and Gell PGH (1985). The host response to herpes simplex virus. Br Med Bull 41: 86-91.

Williams NA, Hill TJ and Hooper DC (1991). Murine epidermal antigen-presenting cells in primary and secondary T-cell proliferative responses to herpes simplex virus in vitro. Immunology 72: 34-39.

Williams KA, Lubeck D, Noonan FP and Coster DJ (1990). Prolongation of rabbit corneal graft survival following systemic administration of urocanic acid. In: Ocular Immunology Today, Usui M, Ohno S and Aoki K (eds). Elsevier Science Publishers, pp 103-106.

Windebank KP, Abraham RT, Powis G, Olsen RA, Barna TJ (1992). Signal transduction during human natural killer cell activation: inositol phosphate generation and regulation by cyclic AMP. J Immunol 141: 3951-3957.

Yoshikawa T and Streilein JW (1990). Genetic basis of the effects of ultraviolet light B on cutaneous immunity. Evidence that polymorphism at the Tnfa and Lps loci governs susceptibility. Immunogenetics 32: 398-405.

Yoshikawa T, Rae V, Bruins-Slot W, Van den Berg J, Taylor JR and Streilein JW (1990). Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. J Invest Dermatol 95: 530-536.

Yoshikawa T, Kurimoto I and Streilein JW (1992). Tumour necrosis factor-alpha mediates ultraviolet light B-enhanced expression of contact hypersensitivity. Immunology 76: 264-271.

Young E and van der Leun JC (1975) Treatment of psoriasis with longwave ultraviolet light. Dermatologica 150: 352-354.

Zambruno G, Mori L, Marconi A, Mangiardo N, De Rienzo, Bertazzoni U and Giannetti A (1991). Detection of HIV-1 in epidermal Langerhans cells of HIV-infected patients using polymerase chain reaction. J Invest Dermatol 96: 979-982.

Zamudzka BZ and Beer JZ (1990). Yearly review. Activation of human immunodeficiency virus by ultraviolet radiation. Photochem Photobiol 52: 1153-1162.

Zannoni VG and La Du BN (1963). Determination of histidine alphadeaminase in human stratum corneum and its absence in histidinaemia. Biochem J 88: 160-162.

APPENDIX

PUBLICATIONS ARISING FROM THE WORK CONTAINED IN THIS THESIS

Publications

The publications listed below were derived from the work carried out in this study. Copies of the manuscripts are found below.

Gilmour JW and Norval M (1991). Urocanic acid may act through histamine like receptors in the skin. Photochem Photobiol **53**: 24s

Gilmour JW, Vestey JP, and Norval M (1992). The effect of UV therapy on immune function in patients with psoriasis. (In press) Br J Dermatol

Gilmour JW, Vestey JP, George S and Norval M (1992). The effect of phototherapy and urocanic acid isomers on natural killer cell activity. Submitted to J Invest Dermatol

Norval M, Gilmour JW and Simpson TJ (1990). The effect of histamine receptor antagonists on immunosuppression induced by the *cis* isomer of urocanic acid. Photochem Photobiol Photomed **7**: 243-248

S/pm-C1

UROCANIC ACID MAY ACT THROUGH A HISTAMINE-LIKE RECEPTOR IN THE SKIN. J. W. Gilmour* and M. Norval*, Department of Medical Microbiology, University of Edinburgh Medical School, Edinburgh EH8 9AG, and T.J. Simpson, School of Chemistry, University of Bristol.

Ultraviolet radiation (UV) transiently suppresses the immune response. The putative mediator of this suppression is urocanic acid (UCA), a major constituent of the stratum corneum, formed by a one step deamination from histidine (as is histamine). On UV-B irradiation, isomerisation of the naturally occurring trans-isomer to the cis-isomer takes place. Cis-UCA has been shown to mimic some of the effects of UV in inducing suppressed immune responses. In this study we have shown that skin painting with cis-UCA significantly reduced the number of murine epidermal ATPase$^+$ cells, adding to our previous finding that cis-UCA administered before herpes simplex virus (HSV) infection suppressed the delayed hypersensitivity (DH) response to the virus in a murine model. The effects of two histamine receptor antagonists, cimetidine (H-2 receptor antagonist) and terfenadine (H-1 receptor antagonist), on the action of cis-UCA were then examined. When ears were painted with cis-UCA together with either histamine receptor antagonist, no significant reduction in ATPase$^+$ cells was observed. In addition, if cis-UCA was administered epidermally or subcutaneously together with one of the histamine receptor antagonists before HSV infection, little suppression of the DH response to the virus was observed. Thus histamine receptor antagonists reduced or abrogated two of the suppressive effects of cis-UCA. These data therefore suggest that cis-UCA may act through a histamine-like receptor in the skin.

S/pm-C2

UVB INDUCES SUPPRESSION OF THE EARLY INITIATING PHASE OF CONTACT HYPERSENSITIVITY TO PICRYLCHLORIDE. Yvonne Sontag[+*], Johan Garssen[*], Frank R. de Gruijl[+*], Jan C. van der Leun[+], Willem A. van Vloten[+*] and Henk van Loveren[*], [+]) Dept. of Dermatology, Utrecht University, Utrecht and [*]) RIVM, Bilthoven, The Netherlands.

Murine type IV reactions depend on the sequential activities of 2 different antigen(Ag)-specific T cells (H. van Loveren et al., Lymphokines 14:405-429, 1987). The early acting T cell produces an Ag-specific, non-MHC restricted factor that mediates Ag-dependent local serotonin release from mast cells. The resulting increased vascular permeability -measurable as a swelling 2 hrs after challenge- enables the influx of circulating, late acting Ag-specific, MHC-restricted T cells. These "classical" T_{DTH} cells produce lymphokines that attract the 24-hr perivascular infiltrate of non-specific inflammatory cells. Thus, the 2-hr response is an obligatory step towards the 24-hr response. The question is whether UVB can suppress the initiating phase of Type IV reactions. In the present study the influence of different doses of UVB radiation on the early and late phases of contact hypersensitivity (CHS) to picrylchloride (PCl) was studied. In duplicate experiments DBA/2 mice (5 per group) were irradiated for 16 or 32 seconds (suberythemal doses) on the shaved backs with a Kromayer UVB lamp for 4 consecutive days. Four days later mice were sensitized on the shaved abdomens with 5% PCl. Animals were challenged with 0.8% PCl on the ears 4 days after sensitization, and ear swelling was measured 2, 4, 24 and 48 hrs after challenge. There is a biphasic response, with maximum swellings at 2 and 24 hr. The 2-hr swelling was almost completely suppressed and the 24-hr swelling by about 50 % in UVB-treated mice. Dose-dependency was indicated. These results suggest that the known suppression of CHS responses by UVB may be due to suppression of the early initiating phase of CHS.

S/pm-C3

MODULATION OF ICAM-1 EXPRESSION ON HUMAN ANTIGEN PRESENTING CELLS BY UV RADIATION. CA Elmets, WS Mirando and J Krutmann, Depts of Dermatology, Case Western Reserve Univ, Cleveland, Ohio and Univ of Freiburg, Germany.

UVB radiation inhibits the capacity of antigen presenting cells (APC) to initiate helper T cell mediated immune responses. The mechanism by which this occurs has not been fully elucidated, although recent evidence suggests that a reduction in the expression of the adhesion molecule ICAM-1 plays an important role in the deleterious effects of UV radiation on APC. The purpose of this study was to examine this issue further by attempting to correlate the effect of UV radiation on antigen presentation with its effect on surface expression of ICAM-1 among different cell populations. Both monocytes and freshly prepared Langerhans cells lost their capacity to stimulate autologous T cells in in vitro T cell proliferation assays following exposure to 100 J/m^2 UVB. Similar UV doses inhibited ICAM-1 expression by >60% on these cell types. In contrast, the antigen presenting function of B-lymphoblastoid cell lines was resistant to UVB doses up to 400 J/m^2. In this cell type, surface expression of ICAM-1 was not reduced by UV radiation. In monocytes, an increase in ICAM-1 mRNA was not observed in unirradiated or in UV-irradiated cells, indicating that the decrease in ICAM-1 expression was not mediated by transcriptional mechanisms. These results support the hypothesis that UV radiation acts to inhibit the function of APC by reducing the surface expression of ICAM-1. At least in monocytes, this effect appears to be through post-transcriptional mechanisms.

THE EFFECT OF UV THERAPY ON IMMUNE FUNCTION IN PATIENTS WITH PSORIASIS

J.W. Gilmour[1], J.P. Vestey[2], M. Norval[1*]

[1] Department of Medical Microbiology, University of Edinburgh Medical School, Edinburgh

[2] University Department of Dermatology, Royal Infirmary of Edinburgh

*Correspondence to Dr M. Norval, Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

ref: mn/mc/tc/uvpsor

ABSTRACT

Ultraviolet radiation (UVR) is known to suppress some cell-mediated immune responses to antigens encountered during or soon after exposure. Phototherapy is widely used in psoriasis and this study was undertaken to monitor changes in a range of immunological parameters during standard courses of treatment with the aim of ascertaining whether such modulations contribute to the effectiveness of therapy. In this study the responses of 17 patients with psoriasis undergoing UVB and 4 PUVA were compared with 15 patients receiving coal tar treatment and 4 normal subjects undergoing UVB irradiation. In each case samples were taken before starting therapy, after 4 weeks of therapy and finally 4 weeks after completing it. Serum immunoglobulin isotypes and complement components were within normal ranges in most of the psoriasis patients and remained unchanged throughout therapy. Similarly, percentages of subsets of peripheral blood mononuclear cells (PBMC) were normal and were unaltered by treatment. Patients, already infected with herpes simplex virus (HSV) as demonstrated by a positive lymphoproliferation test in vitro, were monitored for asymptomatic HSV shedding and HSV recrudescences during therapy. There was little evidence that phototherapy caused reactivation of the virus. No significant alteration in lymphoproliferative response to HSV and to the mitogen, concanavalin A, was observed during therapy. Epidermal cells and blood adherent cells were used to present HSV to PBMC, depleted of adherent cells and enriched for T cells, in a lymphoproliferative assay. The functional antigen presenting ability of adherent cells remained unchanged throughout therapy while that of epidermal cells was suppressed during UVB irradiation and recovered, in most instances, after UVB therapy had been completed. The epidermis of patients with psoriasis contained about three times the quantity of urocanic acid (UCA) of normal subjects while the UCA concentration in suction blister fluid did not differ between the two groups. During UVB irradiation, the percentage of cis-UCA rose in both the epidermis and suction blister fluid of all subjects and it remained elevated in the blister fluid after therapy had finished. Tumour necrosis factor-α was measured in suction blister fluid and its concentration did not alter consistently as a result of therapy. Whether any of the

immunological parameters measured and the changes noted contribute to the effectiveness of phototherapy in the treatment of psoriasis remain uncertain.

INTRODUCTION

Psoriasis is a multifactorial, genetically determined disease which affects approximately 2% of the population of Western countries. Its aetiology is controversial but there is evidence that immunological mechanisms play an important role[1,2]. Ultraviolet-B (UVB) irradiation and psoralen photochemotherapy (PUVA) are both widely employed, effective treatments for psoriasis. However their use is largely empirical and little is known of their mechanism of action. It is possible that they may act, at least in part, by modulating cutaneous immune responses.

In animal models, UV irradiation results in suppression of selected immune responses to antigens encountered within a short period after exposure. The immunological consequences of UV irradiation in man are less well characterized and have been confined mainly to suppression of contact sensitivity responses in UV-exposed sites[3-5]. A recent report indicates that the suppression may be systemic, if the dose of UV is erythemagenic[6]. range

It is well recognised that UV irradiation produces transient alterations in the numbers, ultrastructure and morphology of Langerhans cells (LC), the main antigen presenting cell (APC) of the epidermis[6,7]. This is associated with suppressed in vitro alloactivation by epidermal cells[8] and reduced antigen presentation[9,10]. There is evidence for re-population of human skin with APC which preferentially activate suppressor cells[11]. In addition to its effects on APC, UV irradiation may alter circulating human T cell subsets temporarily, but reports of the nature and magnitude of such changes are variable.

UV irradiation also modulates the expression of various cytokines which are likely to be important in the pathogenesis of psoriasis. Tumour necrosis factor-α (TNF-α) may be of particular interest in this context since human keratinocytes produce it during inflammatory reactions and its synthesis is enhanced by UV irradiation[12]. In one preliminary study,

systemic administration of TNF-α resulted in resolution of lesions in 2 out of 3 patients with psoriasis[13]. In mice there is evidence for a genetic susceptibility to the immunosuppressive effects of UV irradiation; polymorphism at the Tnfa and Lps loci may govern susceptibility to UV[14]. Recently a genetic susceptibility to UV induced immunosuppression in man has also been suggested[15].

A further mechanism for UV-induced immunosuppression involves urocanic acid (UCA). UCA is a major component of the epidermis and undergoes isomerization from the naturally occurring trans to the cis-isomer on UV irradiation. Cis-UCA was proposed as a mediator of immunosuppression in 1983[16] and, since then, has been shown to mimic many of the effects of UV irradiation in a variety of experimental animal models[17].

The present study was designed to examine the effects of standard courses of UVB and PUVA therapy on a range of parameters of systemic and cutaneous immunity in patients with psoriasis in an attempt to ascertain whether the success of phototherapy could be attributed to modulations in immune responses. The results were compared with two control groups: the first consisted of normal subjects who were given a standard course of UVB phototherapy and, the second, patients with psoriasis who received topical coal tar paste treatment instead of UV irradiation. Assays included several humoral components, phenotypic profiles and counts of peripheral blood mononuclear cells (PBMC), UCA isomers and TNF-α analyses, and in vitro lymphoproliferative responses to concanavalin A (Con A) and herpes simplex virus (HSV). HSV was selected as a convenient test antigen for study as about 80% of adults show evidence of acquired immunity and, in addition, exposure to UV is an important factor in HSV pathogenesis in a proportion of latently infected subjects[18].

MATERIALS AND METHODS

Patients

Thirty-six patients with chronic plaque psoriasis who were attending the Dermatology Department at the Royal Infirmary of Edinburgh were investigated. Otherwise all subjects were in good general health and taking no medication. Fifteen of the patients did not receive UV therapy and acted as controls (group A). They were treated with increasing concentrations (1-4%) of topical coal tar paste under stockinette dressings. Seventeen subjects received UVB phototherapy (group B) and four PUVA (group C). One other control group D consisted of 4 non-psoriatic individuals who were receiving UVB as part of another study.

At each visit the patients were assessed and the Psoriasis Area and Severity Index (PASI) calculated. Samples were taken from group A subjects on two occasions: once immediately before starting treatment and secondly four weeks later. Samples were taken from subjects in groups B and C on three occasions: before starting treatment, four weeks after starting treatment (48h after irradiation) and finally four weeks after the last irradiation. In most instances samples consisted of blood, oropharyngeal secretions, epidermis and urine. Group D subjects had only blood and epidermal samples taken, before irradiation and 48h after the last irradiation. All samples were collected in the early morning and at each visit routine tests, including full blood counts, biochemical profiles, liver function and dip stick urinalysis were undertaken. Serum immunoglobulin and complement levels (C3, C4 and CH50) and autoantibody profiles were determined and HSV-specific antibodies measured in plasma by ELISA, as described previously[19].

UV Irradiation

All subjects were of skin types II or III. Patients in groups B and C received the UVB regimen normally used for psoriasis in Edinburgh. They were irradiated with incremental doses of UVB starting with 37 mJ cm^{-2} in a Waldmann 1000 UVB cabinet containing 26

Sylvania UV6 tubes, emitting 0.93 mW/cm^2 UVB and 0.03 mW/cm^2 UVA thrice weekly until clear (4-6 weeks). Group C subjects were irradiated twice weekly in a Waldmann 6001 PUVA cabinet with bulbs emitting 2 mW/cm^2 UVA and 0.3 mW/cm^2 UVB. They received 0.8 mg kg^{-1} body weight of 8-methoxypsoralen orally 2 h before each irradiation. The minimal phototoxic dose for each patient was calculated and, at the first treatment, they received 70% of that dose followed by doses increased by 20% at each subsequent treatment, up to a total of 20 treatments or until their skin was clear.

HSV Isolation

Oropharyngeal swabs were collected into 5 ml Eagle's minimal essential medium containing 5% newborn calf serum. The samples were stored at -70°C until being assayed for plaque forming units (pfu) in monolayers of Vero cells, as described previously[19].

HSV Antigen

HSV was cultured in Vero cells and UV-inactivated virus prepared as the test antigen, as described previously[20]. Mock infected Vero cells were used as the control antigen.

Preparation of PBMC, Adherent Cells (AC) and T Enriched Cells (TEC)

This method has been described previously[19,20]. Briefly, 50 ml venous blood was collected into preservative-free heparin and PBMC isolated by centrifugation on lymphopaque (Nyegard Ltd., Birmingham, UK). The cells were washed three times and resuspended in RPMI 1640 medium (Gibco Ltd., Paisley, UK) supplemented with antibiotics and 15% autologous plasma (tissue culture medium, TCM). The PBMC were incubated in tissue culture flasks (Becton Dickinson, Plymouth, UK) for 1h at 37°C and the non-adherent cells washed off. AC were scraped off using a cell scraper (Costar Corporation, Cambridge, MA, USA) and resuspended in TCM at 5×10^5 ml^{-1}. TEC were prepared by passage of the non-adherent cells over a nylon wool column. They were washed and resuspended in TCM at 5×10^5 ml^{-1}.

Preparation of Epidermal cell (EC) Suspensions and Suction Blister Fluid

The method employed has been described previously[20]. In brief, suction blisters were raised under a negative pressure of 150 mm Hg from uninvolved skin on the medial aspect of the forearm. The blister fluid was collected and stored at -70°C for UCA and TNF-α analyses. The roofs were removed and incubated in 0.5% trypsin (T2021, Sigma, UK) at 37°C for 1h and gently agitated to prepare a single cell suspension. Autologous plasma was added to 15%, and the cells were then washed and resuspended at 5×10^5 ml^{-1} in TCM.

Fluorescent Flow Cytometry

The method for staining and analysis has been described previously[19]. Murine monoclonal antibodies were directed against CD1a, CD3, CD4, CD8, CD19 (Dakopatts, High Wycombe, UK), CD57 (Beckton-Dickinson, California, USA) and MHC Class II antigens (DP, DQ and DR; DA6.231, a gift from Dr K. Guy). CD1a is expressed on cortical thymocytes, LC, a B cell subset and some dendritic cells; CD19 on all B cells; and CD57 on natural killer cells, T and B cell subsets.

Lymphoproliferation Assay

PBMC or TEC were plated at 200μl/well into 96-well round bottomed tissue culture plates (Falcon) and, either AC or EC were added to groups of five adjacent wells containing TEC to form 0, 0.3%, 3% or 10% of the total cell number. The cells were either incubated alone or with 5μg ml^{-1} Con A, 1 pfu/cell inactivated HSV, or an equivalent number of mock infected Vero cells (10^2 ml^{-1}). The cells were incubated for six days at 37°C in 5% CO_2 in a humidified incubator, pulsed with 0.75 μCi ^{3}H-thymidine per well during the final 24h of culture, harvested onto glass fibre paper and counted in toluene-based scintillation fluid. The stimulation index (SI) for each culture was calculated as:

$$\frac{\text{geometric mean count per min (cpm) test}}{\text{geometric mean cpm control}}$$

UCA Analysis

Filter paper discs soaked in 0.1 M KOH were applied to uninvolved skin on the inner forearm for 30 min and held in place under Finn Chambers[21]. The samples were stored in the dark at -70°C and used for the quantification of total epidermal UCA and the relative concentrations of UCA isomers by HPLC[22]. Suction blister fluid was also analysed by HPLC.

TNF-α Quantification

The amount of TNF-α in suction blister fluid was determined using an ELISA kit (Research and Diagnostic Systems, Minneapolis, USA) with 100μl sample or standard per well.

Statistical Analysis

Data were analysed using parametric or non-parametric tests, Student's t test and Wilcoxon's signed-ranks test respectively, where appropriate. All errors quoted are standard errors of the mean. Kendall's coefficient of concordance was used to calculate the correlation of bivariate data.

RESULTS

Clinical Details

Table 1 summarizes the clinical details of the subjects investigated. Although the dose of UV irradiation received after four weeks of therapy varied between patients, a clinical improvement in psoriasis and a reduced PASI occurred in every case. The routine laboratory profiles performed at each visit (full blood counts, plasma urea, glucose and electrolytes, liver function, dip-stick urinalysis, autoantibodies) were within normal limits for each subject and their values were not influenced by treatment (data not shown). Serum immunoglobulin and complement levels were also measured; two patients with psoriasis had slightly elevated IgE levels (between 150-180 IU ml^{-1}) and several had slightly raised concentrations of serum immunoglobulin isotypes and/or complement components. These abnormalities appeared to be consistent for the individual and neither the type of therapy given, nor the extent of the psoriasis influenced them significantly (data not shown).

HSV Infection

For this part of the study, 22 patients from Groups A, B and C were selected on the basis of a positive lymphoproliferative response to HSV with an SI greater than 3 (9 group A, 9 group B and 4 group C). Only two patients suffered recrudescent perioral HSV infections during UVB (group B) and none during PUVA (group C) therapy. One recrudescence was associated with a β-haemolytic streptococcal throat infection which was probably the triggering factor. The other followed a mild UVB-induced facial burn a few days after starting therapy. Three subjects from group A suffered recrudescent perioral HSV infections: two were associated with mild upper respiratory tract infections and the third when "tired and run down". One patient was found to be shedding HSV asymptomatically prior to UVB therapy by virus isolation from the oropharynx. HSV antibody titres remained high and varied little in individual subjects during the study (data not shown).

Phenotyping of PBMC

No significant differences between the percentages of PBMC subsets in patients with psoriasis and normal controls were observed (Table 2). As there were only 4 normal subjects, data from a further 9 controls were taken from a previous study in which the same monoclonal antibodies and flow cytometer were used[23]. These show similar values to the ones in Table 2, i.e. percentages of positive cells (mean ± 1 standard derivation) CD3 45 ± 7, CD4 33 ± 11, CD8 24 ± 7, CD1a 3 ± 1.9, CD57 16 ± 9, MHC Class II 21 ± 6. No major alterations occurred during treatment, although there were slight reductions in percentages of $CD3^+$ and $CD4^+$ cells in groups B and C which were not statistically significant ($p > 0.05$; paired sample t test). CD19 staining was performed in four of the patients in group B only, but no modulation in the percentage of $CD19^+$ cells during therapy was observed.

In vitro Lymphoproliferation Responses to HSV

Proliferative responses of PBMC to HSV and Con A were monitored during treatment in 7 patients from group A, 6 from group B and 3 from group C, all of whom had positive lymphoproliferative responses to HSV before starting therapy. There was no consistent or statistically significant modulation of in vitro lymphoproliferative responses to HSV during therapy, and Con A-induced responses were high throughout (SI > 10) (data not shown). However the background counts of unstimulated PBMC increased in five of the six UVB treated patients from group B (mean cpm 1650 ± 341 increasing to 3866 ± 1381) during therapy, and returned to normal (1667 ± 428) four weeks after the last treatment. This was not found in the patients from groups A and C.

Antigen Presentation of HSV by EC or AC

EC suspensions were prepared from suction blister roofs of patients in groups B and C. The ability of EC, and also AC purified from PBMC, to present HSV antigen to autologous TEC, depleted of APC, was assessed. The purification procedure employed was sufficient to abrogate the antigen presenting function of the TEC on most, although not all, occasions. Since the background cpm for TEC varied slightly between samples from the same patient,

this value was subtracted from the cpm generated by the TEC stimulated by EC or AC to determine the stimulation attributable to the APC.

Satisfactory and consistent functional depletions were achieved with four of the group B patients on all three occasions of sampling. The ability of AC and EC to present HSV before, during and after UVB therapy was compared and the results from one subject are illustrated in Fig. 1. AC were unaffected in their ability to present HSV to TEC by UVB but epidermal antigen presentation was abrogated during UVB therapy, recovering 4 weeks after the last treatment. This was also true of the 3 other patients monitored in the same way except that EC from one patient did not recover completely four weeks after therapy was completed.

The same experiment was attempted with four group C patients but no consistent effect on HSV presentation by AC or EC to TEC during PUVA therapy was obtained. However it was clear from the results of two patients that, four weeks after PUVA therapy had finished, the EC had not regained the ability to present HSV (data not shown).

UCA Quantification

A preliminary experiment was undertaken to compare the total amount of UCA and the percentage of cis-UCA in the epidermis and suction blister fluid obtained from uninvolved forearm skin of untreated psoriasis patients with that of normal subjects (Table 3). The mean UCA content and % cis-UCA in blister fluid did not differ between psoriasis patients and normal individuals, although 9 out of 12 psoriasis patients had no detectable cis-UCA, whereas 6 out of 7 normal subjects had low levels. The epidermal UCA content of psoriasis patients was significantly higher than that of normal subjects. However the relative percentage of each isomer did not differ between the two groups.

The effect of therapy on the UCA content of the epidermis and suction blister fluid is shown in Table 4. In both groups B (UVB-treated psoriasis patients) and D (normal subjects receiving UVB), UV exposure tended to increase the total epidermal UCA content, but the

difference was not statistically significant ($p > 0.05$, paired sample t test). However after 4 weeks of UVB therapy, the percentage of cis-UCA within the epidermis increased significantly in both groups B and D. The concentration of UCA in suction blister fluid remained relatively constant in each group throughout the study (Table 4). There was a significant increase in the percentage of cis-UCA in blister fluid of groups B and D during UVB therapy. This remained significantly elevated four weeks after the last irradiation in Group B; no equivalent samples were available from group D subjects.

Only a few samples were available for UCA analysis from group C (PUVA cohort). Two out of three subjects showed a slight increase in epidermal UCA during therapy, with the percentage of cis-UCA increasing in all three subjects after four weeks of irradiation (from 35.4% $\pm$ 14.2 to 46.1% $\pm$ 12.5; mean $\pm$ SEM). Two of the group had relatively high percentages of cis-UCA before irradiation started (44.9% and 53.8% respectively). Suction blister fluid from two PUVA patients showed no significant alteration in total UCA concentration following PUVA but an increased percentage of cis-UCA which remained elevated four weeks after the last treatment, as found in group B (Table 4).

TNF-α Analysis

Due to the limited volume of suction blister fluid available from each subject, only 100μl of the sample or standard was added to each well of the ELISA plate instead of the recommended 200μl. This lowered the sensitivity of the test from 7.5 pg ml^{-1} to approximately 16 pg ml^{-1}.

TNF-α was detected in the majority of the samples. Patients with psoriasis from groups A-C had slightly elevated levels (95.6 pg ml^{-1} $\pm$ 10.1; mean $\pm$ SEM; $n = 9$) compared with normal subjects (58.7 $\pm$ 15.3 pg ml^{-1}; $n = 7$; 4 group D subjects and 3 additional normal subjects), but this difference did not reach statistical significance ($P > 0.05$).

As shown in Fig.2, there was little change in TNF-α concentration during coal tar therapy (group A) but three patients from group B showed increased TNF-α concentrations during

UVB therapy while four showed a decrease. Three out of four normal controls receiving UVB (group D) showed increased TNF-α in blister fluid during the therapy. No samples from group C were analysed.

There was no correlation between the concentrations of cis-UCA and TNF-α in blister fluid.

DISCUSSION

In this study several immunological parameters were assessed in patients suffering from chronic plaque psoriasis and, where possible, compared with those in normal subjects. The effect of phototherapy or coal tar therapy was then measured by comparing samples from the patients taken before, during and after treatment.

As certain reports, although not all, have indicated that abnormalities in complement components[24] and immunoglobulin levels, particularly IgA[25] may occur in patients with psoriasis, patients were tested before therapy for circulating complement components and immunoglobulin levels, as well as haematological, autoantibody, urinary and biochemical profiles. All of these routine measurements were within normal limits with the exception of a small number of psoriatic subjects who had slightly elevated immunoglobulin or complement levels which did not vary with disease severity. It is possible that high complement levels relate to paricular types of psoriasis, while the lack of correlation between clinical improvement of psoriasis and increased levels of circulating immunoglobulin argues against their being primarily related to cutaneous manifestations of the disease[1].

Neither UVB nor PUVA therapy had a measurable effect on any of these parameters. Hersey et al.[26] reported that exposure to sunlight had no effect on circulating complement or immunoglobulin levels, although Livden et al.[27] detected slightly decreased circulating IgG titres in normal subjects exposed to four weeks of UVB or UVA radiation, and a slight increase in IgM and C3 levels following UVB irradiation.

Although some reports have indicated abnormalities in circulating lymphocyte subsets in patients with psoriasis, others found no differences from normal subjects, for example[28]. This was also the case in the present study in which analysis was by flow cytometry using monoclonal antibodies to T cells (CD3, CD4, CD8), dendritic cells (CD1a), B cells (CD19), natural killer cells (CD57) and MHC Class II antigens (Table 2). The effect of UV

irradiation on circulating blood cells is controversial and, indeed, it is possible that any detected changes are only transient. Thus some authors reported a temporary decrease in circulating T cells after UVB or PUVA therapy but others found no modulation in circulating PBMC following a variety of UV regimens (reviewed in[29]). Differences in the source, wavelengths, duration or dose of UV, as well as the time of sampling may explain these apparent differences. Our results demonstrated an insignificant reduction in the percentage of circulating $CD3^+$ and $CD4^+$ cells following UVB or PUVA therapy but no alteration in any of the other subsets. No changes were noted in the normal subjects receiving UVB, or in patients receiving tar.

Most studies of the immunosuppressive effects of UV irradiation in man or experimental animals have focussed on immune responses to antigens, commonly contact sensitizers, which are encountered for the first time shortly after irradiation. The present study was different in that subjects were already infected with HSV before starting therapy. In addition, although erythemal exposure to UV is a common triggering factor for orofacial recrudescent HSV infection[18], the role of suberythemal doses of UV, such as those employed in phototherapy, in HSV pathogenesis is unknown.

Volunteers were observed for signs of orofacial HSV recrudescences at each visit and had oropharyngeal swabs taken for virus isolation before, during and after therapy. Nine patients receiving coal tar (group A), nine receiving UVB (group B) and 4 receiving PUVA (group C) were found to be HSV carriers on the basis of lymphoproliferative responses in vitro. One subject from group B was shedding virus asymptomatically before therapy and only two others suffered recrudescent lesions during therapy; one following a mild burn produced by UVB irradiation but the other was not associated with therapy. Thus phototherapy was not generally a triggering factor for HSV recrudescences nor for inducing reactivation of latent virus with asymptomatic shedding into the oropharynx. However the oropharyngeal swabs were taken at one time point only during therapy and, as virus is detectable for about 2–4 days in each recurrent episode, it is possible that shedding may have occurred outwith the

sample point.

It has already been reported that patients with psoriasis develop normal lymphoproliferative responses to HSV in vitro[30]. Four weeks of UVB or PUVA therapy caused no significant modulation in the lymphoproliferative response to HSV, or in HSV-specific antibody titres in any individual. However PBMC cultured alone tended to show an increased ^{3}H-thymidine uptake during UV irradiation which may indicate some non-specific activation due to phototherapy.

When peripheral blood AC were used as accessory cells in lymphoproliferation assays, no alteration in antigen presenting ability was found as a result of UVB or PUVA therapy. However, this was not the case when EC were used as accessory cells. This resulted in markedly lowered proliferative responses in patients during UVB (Fig.1) or PUVA therapy. The responses were restored in most individuals four weeks after UVB irradiation had finished although they remained depressed four weeks after PUVA therapy. Few studies have examined the effects of in vivo UV irradiation on human epidermal antigen presentation in vitro and none following typical regimens used for the treatment of psoriasis. In vitro irradiation of human EC suspensions with UVB[10], UVA or PUVA[31] resulted in suppressed allo-activation and antigen presentation by the cells. Cooper et al.[8] demonstrated a suppression of antigen presentation by LC immediately after irradiation of normal subjects with 2-4 MEDs of UVB but this was followed by an enhancement in antigen presentation caused by an influx of $T6^{-}Dr^{+}$ cells into the epidermis. Similarly PUVA therapy has been reported to suppress the antigen presenting capacity of human skin[9].

Cis-UCA is formed from the naturally occurring trans-isomer on UV irradiation of skin, up to a maximum value of about 60% of the total UCA which represents a photostationary state[32]. In mice the percentage of cis-UCA declines steadily following a single UVB exposure until a background level is reached after 14 days[22]. An analysis of the UCA content of human

epidermis and suction blister fluid and percentage of the cis-isomer during and after phototherapy had not been undertaken before. Total epidermal UCA content was measured in Finn Chamber samples which extract all the UCA from human epidermis and give an equivalent value to successive tape strips[21].

Patients with psoriasis had approximately three times more UCA in their epidermis than normal subjects (Table 3). The value obtained for psoriatic epidermis was similar to that reported recently by Snellman et al[33]. It is possible that the increased UCA reflects the mild epidermal thickening of "uninvolved" skin sites in psoriasis. Alternatively, since UCA is formed in one step from histidine by the enzyme histidase, increased histidine may be available in psoriasis due, perhaps, to increased epidermal cell turnover or keratohyalin granule degradation which would account for increased UCA. In addition abnormalities in histidase activity have been detected in patients with psoriasis[34].

During and after UVB exposure, the epidermal UCA content of the psoriasis patients remained relatively constant while that of normal subjects doubled during irradiation, returning to near the starting value 4 weeks after the last dose. All irradiated groups showed an increase in the percentage of cis-UCA in the epidermis which returned to normal 4 weeks after the last treatment. In a previous study four weeks of heliotherapy (sunbathing at southern resorts) for psoriasis produced similar effects on epidermal UCA despite very different UV regimens[33]. Although cis-UCA has been suggested as a mediator of the immunosuppressive effects of UV irradiation, it is not known if the formation of cis-UCA in the epidermis following UV irradiation contributes either directly or indirectly to the impaired presentation of HSV by EC during phototherapy. Evidence to support a direct effect comes from the measurement of the number of dendritic cells in murine epidermis following cis-UCA treatment where a significant reduction was observed by analysis of $ATPase^{+}$[35] or Ia^{+} cells[36].

UCA was detected in suction blister fluid from both psoriatic and normal subjects, at similar concentrations. This differs from a previous report of reduced levels of UCA in suction blister fluid from psoriasis patients[37]. Phototherapy did not affect the total concentration of UCA although the percentage of cis-UCA increased. In contrast to the epidermis, this increase was maintained four weeks after the last treatment in both groups B and C. These results are similar to those of Pasanen et al[38]. UCA is synthesized in the stratum corneum on activation of histidase[39] and it is interesting that UCA isomers are found in blister fluid which is likely to be formed mainly from a dermal exudate. The persistence of an increased percentage of cis-UCA in blister fluid after it had returned to a low percentage in the epidermis might indicate that cis-UCA persists in the dermis or near the dermo-epidermal junction. It is possible that it may play a significant role in immunomodulation there and, indeed, a comparison of UCA and the percentage of cis-UCA in the epidermis and blister fluid samples from the psoriatic patients revealed an inverse correlation between percentage of cis-UCA in blister fluid and the PASI ($p = 0.031$) while there was no correlation between the percentage of epidermal cis-UCA and UCA, or of blister fluid UCA, with the PASI ($p > 0.308$).

Finally a small number of blister fluid samples were analysed for TNF-α. It was present in measurable quantities in most samples before irradiation but UVB therapy had no consistent effect on its concentration (Fig. 2). However it is possible that such changes may occur rapidly after the start of phototherapy and may not be sustained until our sampling points, at least 48 h after irradiation. TNF-α is one of the factors released from UV-irradiated keratinocytes which has important properties in mediating the effects of UVB on the immune system, at least in mice. It has been shown, for example, to mediate the suppression of contact hypersensitivity induction through local release in the skin but also to be involved in the enhanced expression of contact hypersensitivity in UV-susceptible mice[40]. It is not known whether human skin responds similarly during phototherapy. TNF-α alters adhesion molecule expression on keratinocytes and in the dermal vasculature, thus influencing recruitment of inflammatory cells into both the epidermal/dermal compartments. In psoriasis

there is a characteristic T cell infiltrate and keratinocyte hyperproliferation, in which dermal dendrocytes may play a central role, possibly mediated by the production of TNF-α[41].

Whether TNF-α and cis-UCA production in the skin are linked is unknown at present, although Kurimoto and Streilein[36] have reported recently that cis-UCA may cause the local release of TNF-α within the murine epidermis with consequences for the induction of contact hypersensitivity. In addition cis-UCA has been shown to down-regulate the induction of cAMP by trans-UCA or histamine in dermal fibroblasts in vitro and thus it is possible that cis-UCA, formed on irradiation, may regulate the activity of cytokines through a secondary signalling system[42]

Although changes have been noted in this study, predominantly in epidermal APC function and in cis-UCA content as a result of phototherapy, it remains uncertain whether these modulations contribute to the effectiveness of the treatment for psoriasis or are related to the disease process itself.

ACKNOWLEDGEMENT

J.W.G. was in receipt of an MRC Studentship.

REFERENCES

1. Baker BS, Fry L. The Immunology of Psoriasis. Br J Dermatol 1992; **126**: 1-9.
2. Nickoloff BJ. The cytokine network in psoriasis. Arch Dermatol 1991; **127**: 99-109.
3. Baadsgaard O. In vivo ultraviolet irradiation of human skin results in profound perturbation of the immune system. Arch Dermatol 1991; **127**: 99-109.
4. Morison WL. Effects of ultraviolet radiation on the immune system in humans. Photochem Photobiol 1989; **50**: 515-24.
5. Friedmann PS, White SI, Parker S, Moss C, Matthews JNS. Antigenic stimulation during ultraviolet therapy in man does not result in immunological tolerance. Clin Exp Immunol 1989; **76**: 68-72.
6. Cooper KD, Oberhelman L, Hamilton TA et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: Relationship to dose, $CD1a^{-}DR^{+}$ epidermal macrophage induction, and Langerhans cell depletion. Proc Natl Acad Sci USA 1982; **89**: 8497-501.
7. Cruz PD, Bergstresser PR. The influence of ultraviolet radiation and other physical and chemical agents on epidermal Langerhans cells. In "Epidermal Langerhans cells", (G. Schuler, ed.) Florida, CRC Press, 1991; 253-71.
8. Cooper KD, Fox P, Neises G, Katz SI. Effects of ultraviolet radiation on human epidermal cell alloantigen presentation: initial depression of Langerhans cell-dependent function is followed by the appearance of $T6^{-}DR^{+}$ cells that enhance epidermal alloantigen presentation. J Immunol 1985; **134**: 129-37.
9. Ashworth JA, Kahan MC, Breathnach SM. PUVA therapy decreases $HLA^{-}Dr^{+}CD1a^{+}$ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA $Dr^{+}CD1a^{+}$ Langerhans cells exhibit normal alloantigen-presenting function. Br J Dermatol 1989; **120**: 329-39.
10. Austad J, Braathen LR. Effect of UVB on alloactivating and antigen-presenting capacity of human epidermal Langerhans cells. Scand J Immunol 1985; **21**: 417-23.

11. Baadsgaard O, Salvo B, Mannie A et al. In vivo ultraviolet-exposed human epidermal cells activate T suppressor cell pathways that involve $CD4^{+}CD45RA^{+}$ suppressor-inducer T cells. J Immunol 1990; **445**: 2854-61.

12. Kock A, Schwarz T, Kirnbauer R et al. Human keratinocytes are a source for tumor necrosis factor α. Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 1990; **172**: 1609-14.

13. Takematsu H, Ozawa H, Yoshimura T et al. Systemic TNF administration in psoriatic patients: a promising therapeutic modality for severe psoriasis. Br J Dermatol 1991: **124**: 209-10.

14. Yoshikawa T, Streilein JW. Genetic basis of the effects of ultraviolet light B on cutaneous immunity. Evidence that polymorphism at the Tnfa and Lps loci governs susceptibility. Immunogenetics 1990; **32**: 398-405.

15. Yoshikawa T, Rae V, Bruins-Slot W et al. Susceptibilty to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. J Invest Dermatol 1990; 95: **530-6.**

16. De Fabo EC, Noonan FP. Mechanism of immune suppression by UV irradiation in vivo. I Evidence for the existence of a unique photoreceptor in the skin and its role in photoimmunology. J Exp Med 1983; **158**: 84-98.

17. Noonan FP, De Fabo EC. Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. Immunol Today 1992; **13**: 250-4.

18. Spruance SL, Freeman DJ, Stewart JCB et al. The natural history of ultraviolet radiation-induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. J Infect Dis 1991; **163**: 728-34.

19. Vestey JP, Howie SEM, Norval M et al. Immune responses to herpes simplex virus in patients with facial herpes simplex and those with eczema herpeticum. Br J Dermatol 1988; **118**: 775-82.

20. Vestey JP, Norval M, Howie SEM et al. Antigen presentation in patients with recrudescent orofacial herpes simplex virus infection. Br J Dermatol 1990; **122**: 33-42.

21. Jansen CT, Lammintausta K, Pasanen P et al. A non-invasive chamber sampling technique for HPLC analysis of human epidermal urocanic acid. Acta Derm Venereol (Stockh) 1991; **71**: 143-5.

22. Norval M, McIntyre CR, Simpson TJ et al. Quantification of urocanic acid isomers in murine skin during development and after iorradiation with ultraviolet B light. Photodermatol 1988; **5**: 179-86.

23. Charleson FC, Norval M, Benton EC, Hunter JAA. Lymphoproliferative responses to human papillomaviruses in patients with cutaneous warts. Br J Dermatol, in press.

24. Rosenberg EW, Noah PW, Wyatt RJ et al. Complement activation in psoriasis. Clin Exp Dermatol 1990; **15**: 16-20.

25. Guilhou JJ, Clot J, Meynadier J, Lapinski H. Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. Br J Dermatol 1976; **94**: 501-7.

26. Hersey P, Haran G, Hasic E, Edwards A. Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. J Immunol 1983: **131**: 171-4.

27. Livden JK, Bjere JR, Degre M, Matre R. Effect of UV irradiation on interferon immunoglobulins, and complement components in serum from healthy individuals. Photodermatol 1987; **4**: 296-301.

28. David M, Shohat B, Hodack E, Sandbank M. Effect of etretinate on peripheral T lymphocytes in psoriasis patients before, during and after 6 months of therapy. Dermatologica 1990; **180**: 86-9.

29. Mutzas M, von Arnim B, Hentschel H et al. Effects of UV-A1-light on immune status in man. In: Biological Responses to Ultraviolet A Radiation (Urbach F. ed), 2nd edn., Kansas, Vladenmar Publishing Company, 1991; **2**: 249-56.

30. Kapp A, Gillitzer R, Kirchner H, Schopf E. Decreased production of interferon in whole blood cultures derived from patients with psoriasis. J Invest Dermatol 1988; **90**: 511-14.

31. Mork NJ, Gaudernack G, Braathen LR. Effect of UVA and PUVA on allo-activating and antigen-presenting capacity of human epidermal Langerhans cells. Photodermatol 1987; **4**: 66-72.

32. Norval M, Simpson TJ, Bardshiri E, Crosby J. Quantification of urocanic acid isomers in human stratum corneum. Photodermatol 1989; **6**: 142-5.

33. Snellman E, Koulu L, Pasanen P et al. Effect of psoriasis heliotherapy on epidermal urocanic acid isomer concentrations. Acta Derm Venereol (Stockh) 1992; **72**: 231-3.

34. Reavan EP, Cox AJ. Histidine and keratinization. J Invest Dermatol 1965; **45**: 422-31.

35. Norval M, Gilmour JW, Simpson TJ. The effect of histamine receptor antagonists on immunosuppression induced by the cis-isomer of urocanic acid. Photodermatol Photoimmunol Photomed 1990; **7**: 243-8.

36. Kurimoto I. Streilein JW. Cis-urocanic acid suppression of contact hypersensitivity induction is mediated via tumor necrosis factor-alpha. J. Immunol 1992; **148**: 3072-8.

37. Juhlin L, Shroot B, Martin B, Caron JC. Reduced levels of histidine and urocanic acid in suction blister fluids from patients with psoriasis. Acta Derm Venereol (Stockh) 1986; **66**: 295-300.

38. Pasanen P, Reunala T, Jansen CT et al. Urocanic acid isomers in epidermal samples and suction blister fluid of nonirradiated and UVB-irradiated human skin. Photodermatol Photoimmunol Photomed 1990; **7**: 40-2.

39. Scott IR. Factors controlling the expressed activity of histidine ammonia-lyase in the epidermis and the resulting accumulation of urocanic acid. Biochem J 1981; **194**: 829-38.

40. Yoshikawa T, Kurimoto I, Streilein JW. Tumour necrosis factor-alpha mediates ultra-violet light B-enhanced expression of contact hypersensitivity. Immunology 1992; **76**: 264-71.

41. Nickoloff BJ, Karabin GD, Barker JNWN et al. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am J Pathol 1991; **138**: 129-40.

42. Palaszynski EW, Noonan FP, De Fabo EC. Cis-urocanic acid down regulates the induction of adenosine 3',5'-cyclic monophosphate by either trans-urocanic acid or histamine in human dermal fibroblasts in vitro. Photochem Photobiol 1992; **55**: 165-71.

LEGENDS FOR FIGURES

Fig.1. The effect of UVB irradiation on antigen presentation of HSV by (a) adherent cells from blood or (b) epidermal cells. Lymphoproliferative responses (^{3}H-thymidine incorporation, geometric mean cpm) of one patient with psoriasis to Vero control antigen (open bars) and HSV antigen (closed bars) before, during and after UVB therapy. T enriched cells were constituted with either 10% adherent cells from blood or 10% epidermal cells.

Fig.2. The effect of UVB irradiation on TNF-α concentration in suction blister fluid. Samples were taken before, during and after UVB therapy for group B (7 patients with psoriasis) and before and during UVB therapy for group D (4 normal subjects). For group A (3 patients with psoriasis) one sample was taken before coal tar therapy and the second 4 weeks after starting it. The detection limit was 16 pg ml^{-1}.

Figure 1 (a)

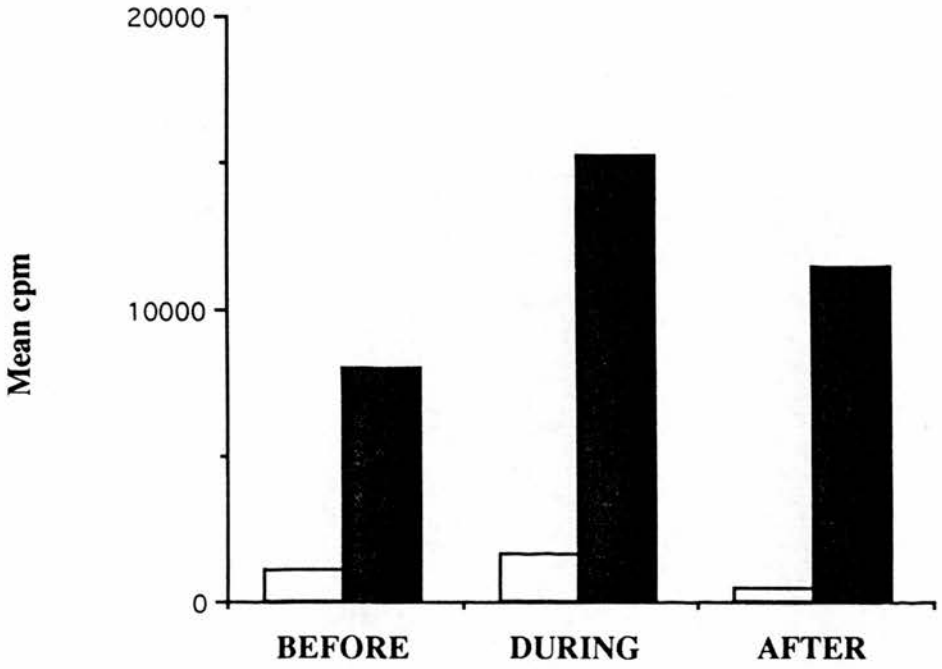


Figure 1 (b)

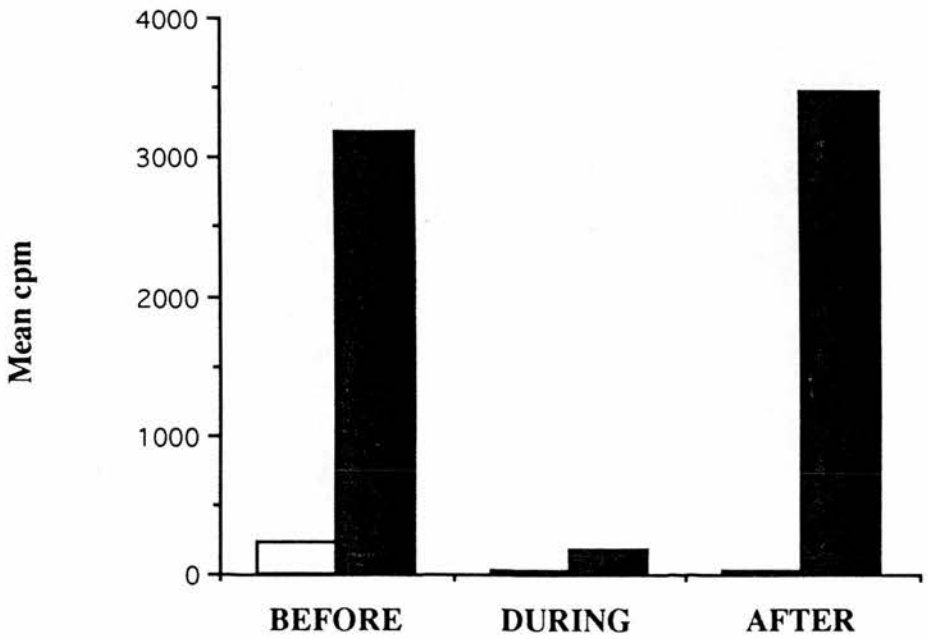
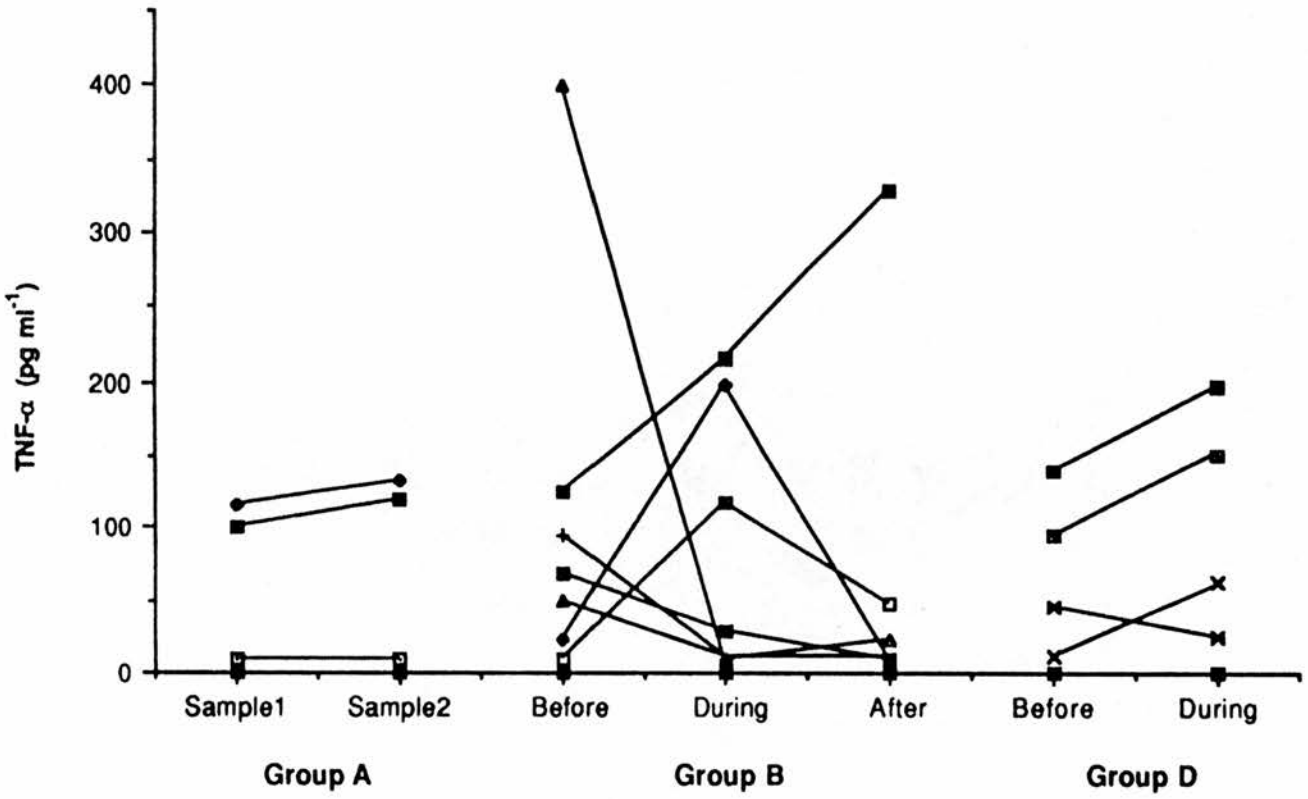


Figure 2


400
300
200
100
0
TNF-α (pg ml-1)
Sample1
Sample2
Before
During
After
Before
During
Group A
Group B
Group D

Group	Therapy	Number of Patients	Mean Age	Age Range	Sex	UV Dose at sample point during therapy ($J\ cm^{-2}$)	Total UV dose at end of therapy ($J\ cm^{-2}$)	PASI before therapy	PASI at sample point	PASI after therapy
A	Coal Tar	15	42.8 ± 4.2	19-69	5M/10F	0	0	17.5 ± 0.9	2.2 ± 0.6	NA
B	UVB	17	37.5 ± 3.1	21-66	6M/11F	1.4 ± 0.01	3.8 ± 0.7	17.8 ± 0.9	6.2 ± 1.1	3.5 ± 1.1
C	PUVA	4	42.2 ± 7.9	26-63	2M/2F	14.2 ± 4.2	119.3 ± 39.0	18.1 ± 1.7	8.5 ± 2.6	2.9 ± 1.1
D*	UVB	4	38.0 ± 5.0	32-53	2M/2F	3.4 ± 1.7	3.4 ± 1.7	NA	NA	NA

* Non-psoriatic control subjects

NA = not applicable

Table 2. The effect of UV therapy on percentages of peripheral blood mononuclear cell subsets

Patient Group	Marker	% positive cells before treatment	% positive cells after 4 weeks of treatment	% positive cells 4 weeks post-treatment
A	CD1a	4.5 ± 0.8	3.7 ± 0.5	NA
B		6.0 ± 0.4	5.3 ± 1.0	6.6 ± 2.0
C		4.4 ± 1.3	2.3 ± 2.2	4.0 ± 0.5
D		ND	ND	ND
A	CD3	55.5 ± 3.7	56.1 ± 3.2	NA
B		54.2 ± 3.9	47.3 ± 3.3	53.8 ± 3.3
C		58.8 ± 2.7	54.1 ± 6.5	52.3 ± 8.6
D		46.7 ±14.8	53.6 ±13.4	48.1 ±16.6
A	CD4	38.8 ± 3.6	39.7 ± 4.9	NA
B		41.5 ± 7.2	35.4 ± 4.5	42.3 ± 4.9
C		42.1 ± 2.4	38.2 ± 5.0	31.7 ± 2.9
D		35.5 ± 3.7	40.0 ±13.4	28.9 ± 4.9
A	CD8	15.7 ± 1.4	17.4 ± 1.4	NA
B		18.1 ± 1.7	16.4 ± 2.1	18.1 ± 2.1
C		26.5 ± 1.6	25.5 ± 3.8	29.4 ± 7.6
D		15.8 ± 3.8	18.2 ± 1.3	14.4 ± 2.4
A	CD57	9.2 ± 1.3	12.5 ± 2.6	NA
B		11.4 ± 1.5	13.0 ± 1.9	10.1 ± 1.2
C		10.8 ± 1.5	14.3 ± 1.5	11.1 ± 3.0
D		10.7 ± 1.5	11.6 ± 2.6	11.3 ± 1.8
A	MHC	14.9 ± 1.6	15.2 ± 3.3	NA
B	class II	17.7 ± 2.9	16.3 ± 1.8	14.8 ± 2.1
C		12.9 ± 0.2	16.8 ± 1.3	10.3 ± 4.9
D		12.7 ± 5.0	15.6 ± 2.4	20.6 ± 4.1

A n = 9; B n = 9; C n = 4; D n = 4

NA = not applicable

ND = not done

Table 3. UCA content of epidermis and suction blister fluid in normal and uninvolved psoriatic skin

	Normals			Psoriatics		
	n	Total UCA (mean ± SEM)	% *cis*-UCA (mean ± SEM)	n	Total UCA (mean ± SEM)	% *cis*-UCA (mean ± SEM)
Epidermis ($nM\ cm^{-2}$)	11	2.3 ± 0.3	17.9 ± 2.8	22	7.6 ± 1.5*	21.9 ± 4.7
Blister fluid (μM)	7	97.8 ± 11.6	3.2 ± 1.0	12	106.5 ±14.5	2.1 ± 1.2

* significantly different from normal epidermis (student's t test, $p < 0.01$)

n = number of patients

Sample/group	Before treatment			During treatment			After treatment		
	n	Total UCA (mean ± SEM)	% cis-UCA (mean ± SEM)	n	Total UCA (mean ± SEM)	% cis-UCA (mean ± SEM)	n	Total UCA (mean ± sem)	% cis-UCA (mean ± SEM)
Epidermis ($nM\ cm^{-2}$)									
A	6	7.8 ± 3.0	10.5 ± 6.2	6	11.0 ± 2.7	4.5 ± 1.8		ND	ND
B	13	6.7 ± 1.3	24.1 ± 5.9	13	7.1 ± 1.1	54.3 ± 5.5*	11	8.1 ± 1.2	11.0 ± 8.0
D	4	2.9 ± 0.7	13.7 ± 7.0	4	5.9 ± 2.3	54.4 ± 13.3#	4	3.6 ± 1.1	5.9 ± 3.3
Blister fluid (μM)									
A	3	120.0 ± 18.8	0	6	110.9 ± 23.9	0		ND	ND
B	7	100.7 ± 20.3	2.9 ± 2.0	7	121.0 ± 10.1	28.7 ± 7.3*	5	120.0 ± 19.6	22.5 ± 9.9**
D	4	89.9 ± 10.9	4.1 ± 1.1	4	73.2 ± 23.9	23.4 ± 8.2@		ND	ND

* $p < 0.01$ compared with samples before UV
\# $p < 0.05$ compared with samples before UV
@ $p < 0.001$ compared with samples before UV
** $p < 0.05$ compared with same 5 samples before UV

n = number of patients
ND = not done

THE EFFECT OF PHOTOTHERAPY AND UROCANIC ACID ISOMERS ON NATURAL KILLER CELL FUNCTION

Jill W. Gilmour[1], James P. Vestey[2], Sheru George[3] and Mary Norval[1*]

[1] Department of Medical Microbiology, University of Edinburgh Medical School, Edinburgh, Scotland

[2] University Department of Dermatology, Royal Infirmary, Edinburgh, Scotland

[3] Photobiology Unit, Ninewells Hospital, University of Dundee, Scotland

Running title : UV and UCA alteration of NK cell activity

Key words : UV irradiation/psoriasis/cis-urocanic acid

Abbreviations : CPM counts per minute, E effector cell, HSV herpes simplex virus, MED minimal erythemal dose, NK natural killer, PBMC peripheral blood mononuclear cells, PUVA psoralen plus UVA, SEM standard error of the mean, T target cell, TCM tissue culture medium, UCA urocanic acid, UV ultraviolet, UVA ultraviolet A, UVB ultraviolet B

* Correspondence to Dr Mary Norval, Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland

ref: mn/mc/uca-nk

ABSTRACT

Ultraviolet (UV) radiation suppresses a variety of immune responses but it is uncertain whether this action contributes to the effectiveness of phototherapy for psoriasis. Urocanic acid (UCA) has been proposed as a mediator of the immunological effects of UV. On exposure the naturally occurring trans-isomer of UCA in the skin changes into the cis-isomer which has been demonstrated to mimic many of the immunomodulatory effects of UV irradiation. Natural killer (NK) cells play an important role in several immunological processes and published evidence indicates that their activity is altered by UV irradiation. To ascertain the effect on NK cells of phototherapy used in the treatment of psoriasis, modulation of NK activity in psoriatic patients undergoing broad band UVB, narrow band UVB or PUVA regimens was examined. This was compared with NK cell activity in psoriatic patients treated with topical coal tar and in normal subjects receiving broad band UVB.

The NK cell activity of psoriatic and normal subjects was the same over a wide range of effector to target cell ratios. Almost all patients undergoing phototherapy exhibited depressed NK cell activity during or after irradiation, although the timing of the depression varied between the lamps used and may be related to dose. However patients treated with topical coal tar showed unchanged NK cell activity throughout the therapy. The effect of UCA isomers on NK cell activity in vitro was also determined. It was found that cis-UCA induced a dose dependent suppression of NK cell activity while trans-UCA had hardly any effect.

Thus it is possible that there may be a correlation between cis-UCA formation in the epidermis and the modulation of NK cell activity which occurs during phototherapy for psoriasis.

INTRODUCTION

Natural killer (NK) cells represent a heterogeneous population of large granular lymphocytes which have not been assigned to a single lineage or distinct anatomical location of maturation, and tend to be defined on a functional basis. The majority of NK cells in human peripheral blood are $CD56^+$ $CD16^+$ $CD2^+$ $CD11b^+$ $CD3^-$; other markers such as CD57 are only expressed on certain NK subsets (1). They are cytotoxic cells which detect and lyse target cells in a non-MHC restricted manner, although MHC Class I alleles have been implicated recently in target cell recognition (2).

There is evidence that NK cells play a substantial role in a number of immunological processes. These functions include cytotoxic action against tumour cells and virally infected cells (3) and resistance to some bacteria, fungal and parasitic agents (4,5). In addition they may be involved in rejection of allogeneic grafts (6), and in immune regulation through the secretion of a number of cytokines (7,8). The receptors involved in target cell recognition by NK cells or the signal transduction mechanism responsible for effector cell activation remain largely undefined.

In addition to the well recognized association between in vivo UV exposure and the incidence of squamous cell carcinomas, a number of experimental models show that UV irradiation results in transient suppression of immune responses to antigens which are encountered shortly thereafter. These include systems where NK cell activity is thought to be important in immunity, such as infections with herpes simplex virus (HSV) (9) and murine acquired immunodeficiency virus (10), tumour development (11) and allograft rejection. It is of interest that in vitro UV irradiation of human peripheral blood mononuclear cells (PBMC) results in dose dependent suppression of NK cell activity (12-15). In addition, there is an indication that exposure to UV and PUVA in vivo may result in suppressed NK cell activity (16,17). Urocanic acid (UCA), found in the stratum corneum, has been proposed as a photoreceptor and mediator of the immunomodulatory effects of UV radiation (18). On UV

exposure, the naturally occurring trans-isomer of UCA undergoes a dose dependent isomerization to cis-UCA which has been shown to mimic many of the effects of UV on the immune system (reviewed in 19).

Psoriasis is a common, genetically determined, hyperproliferative skin disorder in which the immune system is thought to play a critical role (20,21). UVB phototherapy and photochemotherapy (PUVA) are successfully employed in the management of psoriasis. However their use is largely empirical and little is known of their mechanisms of action or of their possible effects on the immune system. Conventional broad band UV sources, such as Sylvania UV6 tubes, emit wavelengths from 270-370 nm. Wavelengths less than 300 nm are highly erythemogenic and, in common with those over 315 nm, are relatively ineffective therapeutically (22,23). Therefore a narrow band (311-313 nm) source was developed (Philips TL01) (24) which has proved effective in the management of psoriasis (25,26).

In the present study the effect of therapeutic regimens employing UV6, TL01 or PUVA therapy on NK cell activity in patients with psoriasis was examined. This was compared with the effect of coal tar therapy in psoriatic patients and with a course of UV6 irradiation in normal subjects. In addition modulation of NK cell activity by UCA isomers in vitro was determined.

MATERIALS AND METHODS

Patients

Details of patients and normal subjects are summarized in Table 1: groups A, B, E and F attended the Department of Dermatology, Royal Infirmary of Edinburgh, while groups C and D attended the Photobiology Unit, Ninewells Hospital, Dundee. All subjects were in good general health and taking no other photoactive or immunosuppressive medication. Those with psoriasis had chronic plaque psoriasis except for three patients in group C who had guttate psoriasis. Liquid paraffin/white soft paraffin (50/50) or emulsifying ointment were used as non-UV absorbing emollients before and during therapy by all psoriatic patients including group A.

Thirty mls venous blood was collected into preservative-free heparin in the early morning. Samples were taken from subjects receiving UV-based therapy (groups B,C,D and F) immediately before treatment started, after four weeks of therapy and four weeks after the last irradiation. Group A patients were treated with increasing concentrations (1-4%) of topical coal tar paste under stockinette dressings. Samples were taken from them before treatment commenced and after four weeks of therapy. Group E consisted of normal subjects who received no therapy: samples were taken from four of this group on two occasions, four weeks apart.

UV irradiation

All subjects were of skin types II or III. Those in group B received the UVB regimen normally used for treatment of psoriasis in Edinburgh. They were irradiated in a Waldmann 1000 UVB cabinet containing 26 Sylvania UV6 tubes emitting 42% in the UVA waveband, 57.6% UVB and approximately 0.3% UVC. Irradiance of the tubes was measured using a filtered photodiode meter (27) which was calibrated against a spectroradiometer (model 742,

Optronic Lab Inc), and the total output (250-400 nm) was 1.4 mW cm^{-2}. Patients were irradiated with incremental doses starting with 37 mJ cm^{-2}, thrice weekly until clear (4-6 weeks). Those in group F were normal volunteers who received the same regimen for six weeks except that only their arms were exposed while the remainder of their bodies were draped.

Group C patients had their minimal erythema doses (MED) determined and an initial dose of 70% of MED was given. They were treated 3 times weekly and at each visit the irradiation dose was increased by 40%; the increment was reduced or omitted depending on the occurrence and severity of erythema. Treatment was stopped when psoriatic plaques had resolved to the point of impalpability over all affected sites, excepting minimal residual activity on knees or elbows (15-20 treatments). The irradiation, determined as for the UV6 lamps, was given in an upright cubicle incorporating 50 Philips TL01 100W fluorescent lamps, and was 4.1 mW cm^{-2}.

Group D patients received PUVA in a Waldmann 6000 cabinet incorporating 62 Sylvania FR74T12 fluorescent lamps (UVB = 0.2 mW cm^{-2}, UVA = 11.7 mW cm^{-2}). Irradiance was monitored using a Waldmann PUVA meter calibrated at the Regional Medical Physics Department, Durham. All patients had their minimal phototoxic dose determined, and the first irradiation dose was 70% of this. Treatment was then given twice weekly with 40% incremental doses (less in case of erythema) at each visit until minimal residual activity was achieved (15-20 treatments).

Preparation of peripheral blood mononuclear cells (PBMC) and phenotyping

PBMC were isolated by centrifugation on lymphopaque (Nyegard Ltd, Oslo, Norway). The cells were washed three times and suspended in RPMI 1640 supplemented with antibiotics (28) plus 10% fetal calf serum (tissue culture medium, TCM) at 4 x 10^6 ml^{-1}.

The method for phenotyping of PBMC using a Coulter EPICS "C" flow cytometer has been described previously (29). The cells were stained with murine monoclonal antibodies to CD1a, CD3, CD4, CD8 (Dakopatts, High Wycombe, UK), CD56, CD57 (Beckton-Dickinson, California, USA) and pan MHC Class II (DP, DR, DQ : DA6.231, a gift from Dr K. Guy). An irrelevant antibody was used to determine the background non-specific staining which was set to approximately 1% of the total histogram. Data were initially collected on a 2-parameter histogram measuring forward light scatter. Bit maps were drawn around both the lymphocyte and the monocyte/large granular lymphocyte populations and the percentage of cells exhibiting fluorescent levels higher than background were recorded for these two populations independently.

NK cell assay

K562 cells were employed as targets in all experiments (29). One million cells were labelled with 100 μC_i sodium chromate (^{51}Cr, Amersham International Ltd, England) for 4 h at 37°C. They were washed three times in 0.01M phosphate buffered saline pH 7.2, suspended in TCM at 10^5 cells ml^{-1} and plated at 100μl/well in round bottomed microtitre plates (Falcon, Becton Dickinson, Oxford, U.K). Doubling dilutions of PBMC were carried out in TCM and 100μl of each dilution added to quadruplicate wells resulting in effector to target cell (E:T) ratios from 40:1 to 1.25:1. Spontaneous ^{51}Cr release was determined by adding 100μl TCM to quadruplicate wells and maximum release by adding 100μl 2% acetic acid to quadruplicate wells. The plates were incubated for 18 h at 37°C in 5% CO_2 in a humidified incubator, then spun at 300 g for 5 min before removing 120μl supernatant for determination of counts per minute (CPM) using a Packard liquid scintillation counter. The standard error of the mean at each ratio was always less than 10% of the mean value.

$$\% \text{ specific } ^{51}Cr \text{ release was determined by } 100X \frac{\text{Test CPM - spontaneous CPM}}{\text{Maximum CPM - spontaneous CPM}}$$

Urocanic acid (UCA)

Trans-UCA was purchased from Sigma (Poole, Dorset). Cis-UCA was prepared and purified after UV irradiation of a solution of trans-UCA as already described (31). Cis and trans-UCA were dissolved in phosphate buffered saline at concentrations of 10^{-1}M and 10^{-2}M respectively and serial tenfold dilutions to 10^{-8}M carried out in phosphate buffered saline. 10μl of each dilution was added to 0.5 ml PBMC at 10^5 cells ml^{-1}. In addition 100μl of 10^{-1}M cis or 10^{-2}M trans-UCA were added to 0.4 ml PBMC at 1.25 x 10^5 ml^{-1}. 100μl UCA-treated PBMC were then added to the wells of a microtitre plate and 15 min later 100μl labelled target cells added at an E:T ratio of 40:1. This resulted in a final concentration of cis-UCA of 10^{-2}M to 10^{-10}M, and trans-UCA of of 10^{-3} to 10^{-10}M. Target cells were also incubated alone with the UCA isomers.

Statistical analysis

Differences in NK cell activity of patients were determined by the Wilcoxon signed-ranks test or Student's t test was used, where appropriate.

RESULTS

NK cell activity in normal subjects and patients with psoriasis

Before any treatment commenced, all 23 patients with psoriasis and 11 normal subjects detailed in Table 1 were tested for NK cell activity. A comparison of the two groups showed no statistical difference at any E:T ratio. For example, at an E:T ratio of 40:1, NK cell activity of normal volunteers was 52.2 ± 5.4 (mean ± standard error of the mean, SEM) compared with psoriatics 64.1 ± 4.2 ($p = 0.11$; Wilcoxon signed-ranks test).

Effect of therapy on NK cell activity in normal subjects and patients with psoriasis

All patients showed a marked clinical improvement (to at least minimal residual activity) in the degree of psoriasis irrespective of the treatment used. It should be noted that, although a set of samples was collected after four weeks of therapy, in most instances patients required six weeks of UV therapy to achieve complete or almost complete clearance of psoriasis.

Fig. 1 illustrates the mean NK cell activity before, during and after therapy in all subjects. Coal tar therapy (group A) had no significant effect on NK cell activity (Fig 1a). Patients receiving broad band UV6 treatment (group B) showed no significant alteration in NK cell activity after four weeks of therapy (Fig. 1b). However there was some reduction in activity four weeks after the last treatment in five of the six patients. This reduction was statistically significant for the group at all E:T ratios ($p = 0.046$, Wilcoxon signed-ranks test). Group C patients, receiving narrow band TL01 therapy, showed a marked depression in NK cell activity after four weeks of therapy (Fig. 1c); for example the mean value (± SEM) for the group fell from 66.8 ± 7.1 to 42.0 ± 6.6 at E:T ratio of 40:1. This reduction was statistically significant at all E:T ratios ($p = 0.028$, Wilcoxon signed rank test). Four weeks after the last irradiation the mean NK value for group C had recovered significantly;

for example to 50.9 ± 7.0 at E:T of 40:1 ($p = 0.043$; Wilcoxon signed-ranks test). However it still remained significantly depressed compared with the original value prior to irradiation in five out of six patients ($p = 0.043$ Wilcoxon signed-ranks test). Samples were collected from two of the patients a further four weeks later, i.e. eight weeks after the last irradiation. NK cell activity was again increased and had almost returned to the original value. A similar pattern was observed in the six patients receiving PUVA therapy (group D, Fig 1d). Following four weeks of therapy, all exhibited a marked reduction in NK cell activity; for example at E:T of 40:1 the activity fell from a mean (± SEM) of 76.4 ± 6.1 to 51.6 ± 8.0. The reduction was statistically significant at all E:T ratios but more so if the E:T was less than 40:1 ($p = 0.046$ at E:T of 40:1, and $p = 0.028$ at E:T less than 40:1). Four weeks after the last treatment the activity had recovered but it remained significantly suppressed relative to the original values at E:T less than 40:1 ($p = 0.028$). Three patients had their NK cell activity assessed a further four weeks later when it had almost returned to normal.

Two groups of normal subjects were also analysed. The first, part of group E, consisted of four individuals who received no treatment. The second, group F, consisted of four individuals who received a course of UV6 identical to that received by the psoriasis patients in group B, except that only their arms were irradiated. Fig. 1e shows that there was no fluctuation in NK cell activity in the group E subjects while Fig. 1f demonstrates that four weeks of UV6 therapy had no significant effect on NK cell activity in group F subjects. However four weeks after the last irradiation NK cell activity was suppressed in three individuals. This suppression did not reach statistical significance ($p > 0.05$; paired sample t test).

Phenotyping

PBMC from all the normal subjects and psoriasis patients were stained with monoclonal antibodies against CD1a, CD3, CD4, CD8, CD57 and MHC Class II antigens. There was no

difference in any of the markers between normal subjects and patients, and no significant alteration of the markers occurred as a result of therapy (data not shown).

In addition to these antibodies, a further monoclonal against CD56 was employed to determine the number of NK cells present in three patients in Group C and four in Group D. In the population of cells gated to include large granular lymphocytes and to exclude smaller cells, there was a reduced percentage of $CD56^+$ cells during therapy which rose after therapy had finished [% $CD56^+$ cells before therapy = 77.0 ± 6.5 (mean ± SEM), during therapy = 22.4 ± 3.9, after therapy = 59.7 ± 8.3].

Effect of UCA isomers on NK cell activity in vitro

The results above indicate that UV irradiation in vivo suppresses NK cell activity and the following experiments were undertaken to determine the effects of UCA on NK cell activity in vitro. Since the concentrations of UCA isomers used were relatively high various tests were carried out to ensure that the isomers were not toxic. In the first place, on four separate occasions, UCA isomers ranging from 10^{-2} to 10^{-10}M were added to wells containing labelled target cells which were then incubated for 18 h and the supernatant assayed for release of ^{51}Cr. No alteration in background release of ^{51}Cr occurred at any concentration of cis or trans-UCA. Secondly, effector cells were incubated with UCA isomers for 18 h and the number of viable cells determined by trypan blue exclusion. This was always between 90–100%. Thirdly, the effector cells were incubated for 18 h with 10^{-3}M 10^{-6}M and 10^{-9}M cis or trans-UCA and then stained overnight with propidium iodide. The number of cells in the S + G_2M stage of cell division were quantitated by flow cytometry using the S-Fit DNA analysis programme (Coulter Electronics Ltd, Luton, U.K.). Approximately 18% of cells were in S + G_2M, and this figure was not altered by the presence of UCA isomers.

The percentage suppression of NK cell activity induced by a range of concentrations of cis-UCA in vitro at an E:T ratio of 20:1 was determined for 12 subjects; 7 normal volunteers

and 5 untreated patients with psoriasis. Cis-UCA had the same effect on PBMC from all of these subjects and Fig. 2a represents the mean suppression of NK activity in these individuals. The effect of trans-UCA was measured in four normal subjects in the same manner (Fig. 2b). Treatment with cis-UCA resulted in a dose dependent suppression of NK cell activity which was statistically significant at concentrations between 10^{-2} and 10^{-4}M. Trans-UCA was slightly suppressive at higher concentrations but this was minimal in comparison with cis-UCA and was not statistically significant.

Finally the effect of UCA isomers on the function of lymphocytes other than NK cells was examined. A lymphoproliferation test was carried out in which UCA isomers, in concentrations similar to those used in the NK assay, were added to quintuplicate wells containing PBMC together with sub-optimal concentrations of concanavalin A (1.5 μg/ml) or HSV antigen (0.1 plaque forming unit/cell, UV inactivated), as already outlined (28). The experiment was done on two separate occasions using PBMC from two healthy donors known to be HSV carriers. In both cases, neither UCA isomer had any consistent or dose dependent effect on lymphoproliferation (data not shown).

DISCUSSION

Suppressed NK cell activity has been reported in patients with generalised pustular psoriasis and to a lesser extent, with psoriasis vulgaris (31). In contrast, Hunyadi et al (32) found normal NK cell activity in a group of subjects with chronic plaque psoriasis. Our study included twenty patients with chronic plaque psoriasis and three with guttate psoriasis. No significant difference in NK cell activity was observed in comparison with eleven normal healthy subjects.

Coal tar treatment as well as three UV irradiation regimens commonly used in the management of psoriasis caused a marked improvement in the severity of psoriasis in all cases. However, only patients receiving UV exhibited any suppression in NK cell activity. This suggested that the modulation in NK cell function resulted from UV irradiation and was not secondary to clinical improvement.

Suppressed NK cell activity was found in all patients after four weeks of TL01 (group C) or PUVA (group D) therapy. Four weeks after treatment had stopped, NK cell activity had begun to recover although in neither group had it regained its initial level. The kinetics of NK cell modulation were different when the broad band UVB (UV6) lamp was used (group B). In this case, there was significant change in NK cell activity following four weeks of therapy but suppression had occured four weeks after the completion of the course (Fig. 1). It is possible that the suppression of NK cell activity by UV irradiation may be dose dependent. As the TL01 and UVA sources do not emit the highly erythemogenic wavelengths, patients received higher doses of UV in a short period of time compared with group B (Table 1). Furthermore, this is suggested by the results from group F (normal subjects undergoing broad band UVB irradiation of their arms only) as, although a reduction in NK cell activity was recorded in most individuals four weeks after the last irradiation, it did not reach statistical significance (Fig. 1f). A dose dependent suppression in NK cell activity following solarium exposure has been reported (33). In that study normal subjects

were irradiated with approximately one minimal erythemal dose for 2, 6 or 12 consecutive days. At least 6 consecutive days of exposure were required to induce suppression of NK cell activity and the suppression was more pronounced following 12 days. Those patients who received 12 whole body exposures still demonstrated suppressed NK cell function 21 days after the last irradiation. Another study examined the effects of PUVA therapy on NK cells in a group of patients with a number of skin disorders, 50% of whom had psoriasis vulgaris (17). A reduction in NK cell activity was observed during therapy although this tended to return to normal at the higher E:T ratio, if the therapy was continued beyond 25 irradiations.

In addition to the total UV dose, it is possible that the incident wavelengths of UV are important. In this respect Hersey et al (34) reported that 12 one hour exposures to sunlight in a 2 week period had only a minimal effect on NK cell activity. However further studies revealed that 2 weeks of irradiation with solarium lamps resulted in suppressed activity (35). The ratio of UVA to UVB emitted by solarium lamps was estimated to be three times higher than that of natural sunlight, and it was shown that the suppression of NK cell function still occurred even if wavelengths in the UVB range were excluded (16,36). Hence it was concluded that UVA wavelengths (320 - 400 nm) were solely responsible for the effects on NK cells. However our present studies do not support this view since the TL01 source which emits narrow band UVB (311 - 313 nm) produced suppression of NK cell activity during therapy. In vitro, shorter UV wavelengths have been demonstrated to cause more suppression of NK cell activity than longer wavelengths (14).

Hersey et al (36) reported minimal changes in the number of circulating $CD16^+$ cells as a result of irradiation and we observed no change in $CD57^+$ cells, suggesting that the total number of circulating NK cells remains unaltered. However preliminary tests indicated that the percentage of large granular lymphocytes expressing CD56 was reduced in patients receiving TL01 or PUVA therapy and this correlated with reduced NK cell activity. CD56 is thought to be an activation marker expressed on NK cells and is found on very few T cells (37).

The mechanism whereby UV irradiation affects NK function is unknown. Possibly there may be a direct effect on NK cells as they circulate through the dermal capillaries which results in permanent functional damage. The recovery of activity may then be dependent on the regeneration of cells from the bone marrow. It is also possible that there may be modulation by another cell type, such as monocytes (38) or NK cells themselves (39). Alternatively a soluble mediator may be released from epidermal cells on irradiation which enters the circulation and affects NK cell function. Kim et al (40) and Brodie and Halliday (42) have reported soluble suppressive factors in murine serum following UV irradiation which are potential candidates. Prostaglandins, induced by UV exposure, are other possibilities as they have been shown to inhibit NK cell activity (42). In vitro epidermal cells release an interleukin-1-like factor which augments NK cell activity. This is called epidermal cell-derived NK cell activity-augmenting factor and may be relevant in vivo (43). Finally there may be altered regulation of the function of other cells through modified cytokine secretion by NK cells themselves.

One further factor which merits consideration is cis-UCA, formed in the epidermis from trans-UCA on UV irradiation. We have shown in the present study that cis-UCA treatment in vitro resulted in a dose-dependent suppression of NK cell activity while trans-UCA had little effect. The concentration of cis-UCA required to modulate the activity was relatively high (10^{-2} to 10^{-4}M). However trans-UCA is present in considerable amounts in the human epidermis (about 8 μgcm^{-2}; 44) and, on UV exposure, up to approximately 60% converts to the cis-isomer in a dose dependent fashion. We have shown that cis-UCA is present in suction blister fluid and it is retained in this compartment for at least four weeks after irradiation (Gilmour JW, Vestey JP and Norval M: submitted for publication). Strenuous efforts were made to ensure that the conditions were not toxic for the effector or target cells in the NK assay. Trypan blue exclusion and propidium iodide staining indicated no demonstrable cell death and PBMC were not suppressed in a lymphoproliferation test.

While the second messenger system involved in NK cell lysis remains to be identified, an increase in intracellular Ca^{++} is associated with NK cell activation and this is thought to be regulated by cyclic adenosine monophosphate (45). Significantly cis-UCA has been shown recently to down-regulate the induction of cyclic adenosine monophosphate by trans-UCA or histamine (46) and therefore it is possible that cis-UCA may down-regulate the second messenger system of the NK cell.

Finally, because NK cells are thought to play a major role in tumour surveillance, it is of concern that currently employed phototherapies result in significant suppression of NK cell function during treatment with some UV sources and for several weeks following treatment with all UV sources.

ACKNOWLEDGEMENT

JWG was supported by a studentship from the Medical Research Council.

REFERENCES

1. Mingari MC, Ferrini S, Pende D, Bottino C, Prigione I, Moretta A, Moretta L: Phenotypic and functional analysis of human CD3+ and CD3- clones with "lymphoproliferative-activated killer" (LAK) activity. Frequent occurrence of CD3+ LAK clones which produce interleukin-2. Int J Cancer 40: 495-498, 1987.

2. Moretta L, Ciccone R, Moretta A, Hoglund P, Ohlen C, Karre K: Allorecognition by NK cells: nonself or no self? Immunol Today 13: 300-306, 1992.

3. Herberman RB, Ortaldo JR: Natural killer cells: their role in defences against disease. Science 214 : 24-30, 1981.

4. Hatcher FM, Kuhn RE: Destruction of Trypanosoma cruzi by natural killer cells. Science 218: 295-296, 1982.

5. Nencioni L, Villa L, Boraschi D, Berti D, Tagliabue A: Natural and antibody-dependent cell-mediated activity against Salmonella typhimurium by peripheral and intestinal lymphoid cells in mice. J Immunol 130: 903-907, 1983.

6. Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G: Evidence for a similar or common mechanism for natural killer cell activity and resistance to haemopoietic grafts. Eur J Immunol 7: 655-663, 1977.

7. Anegon I, Cuturi MC, Trinchieri G, Perussia B: Interaction of Fc receptor (CD16) ligands induces transcription of interleukin-2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells. J Exp Med 167: 452-472, 1988.

8. Cuturi MC, Anegon I, Sherman F, Loudon R, Clark SC, Perussia B, Trinchieri G: Production of hematopoietic colony-stimulating factors by human natural killer cells. J Exp Med 169: 569-583, 1989.

9. Howie S, Norval M, Maingay J: Exposure to low dose ultra-violet B light suppresses delayed type hypersensitivity to herpes simplex virus in mice. J Invest Dermatol 86: 125-127, 1986.

10. Brozek CM, Shopp GM, Ryan SL, Gillespie PM, Kusewitt DF, Rajagopalen MS, Ley KD, Ley RD: In vivo exposure to ultraviolet radiation enhances pathogenic effects of murine leukemia virus, LP-BM5, in murine acquired immunodeficiency syndrome. Photochem Photobiol 56: 287-296, 1992.

11. Fisher MS, Kripke ML: Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc Natl Acad Sci USA 74: 1688-1692, 1977.

12. Elmets CA, Larson K, Urda B, Schacter B: Inhibition of postbinding target cell lysis of lymphokine enhancement of human natural killer cell activity by in vitro exposure to ultraviolet B radiation. Cell Immunol 104: 47-58, 1987.

13. Toda K, Miyachi Y, Nesumi N, Konishi J, Imamura S: UVB/PUVA-induced suppression of human natural killer cell activity is reduced by superoxide dismutase and/or interleukin-2 in vitro. J Invest Dermatol 86: 519-522, 1986.

14. Schacter B, Lederman MM, LeVine MJ, Ellner JJ: Ultraviolet radiation inhibits human natural killer cell activity and lymphocyte proliferation. J Immunol 130: 2484-2487, 1983.

15. Weitzen ML, Bonavida B: Mechanism of inhibition of human natural killer activity by ultraviolet radiation. J Immunol 133, 3128-3132, 1984.

16. Hersey P, MacDonald M, Burns C, Schibeci S, Matthews H, Wilkinson FJ: Analysis of the effect of a sunscreen agent on the suppression of natural killer cell activity induced in human subjects by radiation from solarium lamps. J Invest Dermatol 88: 271-276, 1987.

17. Viander M, Jansen CT, Eskold J: Influence of whole-body PUVA treatments on human peripheral blood NK activity. Photodermatology 1: 23-29, 1984.

18. De Fabo E, Noonan FP: Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp med 158: 84-98, 1983.

19. Noonan FP, De Fabo EC: Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. Immunol Today 13: 250-254, 1992.

20. Baker BS, Fry L: The immunology of psoriasis. Br J Dermatol 126: 1-9, 1992.

21. Nickoloff BJ: The cytokine network in psoriasis. Arch Dermatol 127: 871-884, 1991.

22. Young E, van der Leun JC: Treatment of psoriasis with longwave ultraviolet light. Dermatologica 150: 352-354, 1975.

23. Parrish JA, Jaenicke KF: Action spectrum for phototherapy of psoriasis. J Invest Dermatol 76: 359-362, 1981.

24. Van Weelden H, van der Leun JC: Improving the effectiveness of phototherapy for psoriasis. Br J Dermatol 111: 484, 1984.

25. Van Weelden H, Baart de la Faille H, Young E, van der Leun JC: comparison of narrow-band UV-B phototherapy and PUVA photochemotherapy in the treatment of psoriasis. Acta Derm Venereol (Stockh) 70: 212-215, 1990.

26. Green C, Ferguson J, Lakshimipathi T, Johnson BE: 311 nm UVB phototherapy - an effective treatment for psoriasis. Br J Dermatol 119: 691-696, 1988.

27. Jagger J: Introduction to research in ultraviolet photobiology. Prentice-Hall, New Jersey, 1967.

28. Vestey JP, Howie SEM, Norval M, Maingay JP, Neill WA: Immune responses to herpes simplex virus in patients with facial herpes simplex and those with eczema herpeticum. Br J Dermatol 118: 775-782, 1988.

29. Lozzio CB, Lozzio BB: Human chronic myelogenous leukaemia cell-line with positive Philadelphia chromosome. Blood 45: 321-334, 1975.

30. Norval M, Simpson TJ, Bardshiri E, Howie SEM: Urocanic acid analogues and the suppression of the delayed type hypersensitivity response to herpes simplex virus. Photochem Photobiol 49: 633-639, 1989.

31. Kaminski M, Szmurlo A, Pawinska M, Jablonska S: Decreased natural killer cell activity in generalized pustular psoriasis (von Zumbusch type). Br J Dermatol 110: 565-568, 1984.

32. Hunyadi J, Szegedi G, Nigra TP: Natural killer cell activity in psoriasis vulgaris. Arch Dermatol Res 271: 107-109, 1981.

33. Hersey PH: Effects of electromagnetic radiation on the immune system in mice and man. J Immunol Immunopharmacol 5: 216-222, 1986.

34. Hersey P, Haran G, Hasic E, Edwards A: Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. J Immunol 31: 171-174, 1983.

35. Hersey P, Bradley M, Hasic E, Haran G, Edwards E, McCarthy WH: Immunological effects of solarium exposure in human subjects. Lancet i: 545-548, 1983.

36. Hersey P, MacDonald M, Henderson C, Schibeci S, D'Alessandro G, Pryor M, Wilkinson FJ: Suppression of natural killer cell activity in humans from solarium lamps depleted of UVB. J Invest Dermatol 90: 305-310, 1988.

37. Trinchieri G: Biology of natural killer cells. Adv Immunol 47: 187-376, 1989.

38. Hellstrand K, Hermodsson S : A cell-to-cell mediated interaction involving monocytes and non-T/CD16$^+$ natural killer (NK) cells is required for histamine H_2-receptor-mediated NK-cell activation. Scand J Immunol 31: 631-644, 1990.

39. D'Amore PJ, Golub SH: Suppression of human NK cytotoxicity by an MLC-generated cell population. J Immunol 134: 272-279, 1985.

40. Kim T-Y, Kripke ML, Ullrich SE: Immunosuppression by factors released from UV-irradiated epidermal cells: selective effects on the generation of contact and delayed hypersensitivity after exposure to UVA or UVB radiation. J Invest Dermatol 94: 26-32, 1990.

41. Brodie AM, Halliday WJ: Suppressor cells and soluble factors from the spleens of UV-irradiated mice. Immunol Cell Biol 69: 199-204, 1991.

42. Koren HS, Leung KH: Modulation of human NK cells by interferon and prostaglandin E2. Mol Immunol 19: 1341-1346, 1982.

43. Luger TA, Uchida A, Kock A, Colot M, Micksche M: Human epidermal cells and squamous carcinoma cells synthesize a cytokine that augments natural killer cell activity. J Immunol 134: 2477-2483, 1985.

44. Norval M, Simpson TJ, Bardshiri E, Crosby J: Quantification of urocanic acid isomers in human stratum corneum. Photodermatology 6: 142-145, 1989.

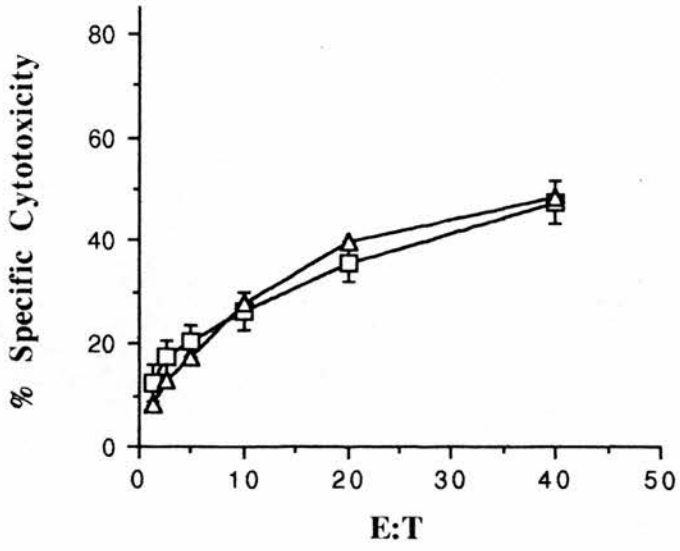
45. Windebank KP, Abraham RT, Powis G, Olsen RA, Barna TJ: Signal transduction during human natural killer cell activation: inositol phosphate generation and regulation by cyclic AMP. J Immunol 141: 3951-3957, 1988.

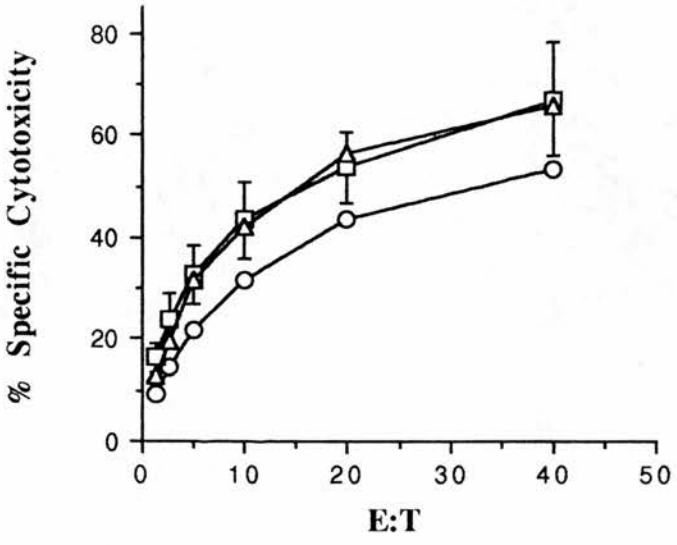
46. Palaszynski EW, Noonan FP, De Fabo EC: Cis-urocanic acid down-regulates the induction of adenosine 3'5'-cyclic monophosphate by either trans-urocanic acid or histamine in human dermal fibroblasts in vitro. Photochem Photobiol 55: 165-172, 1992.

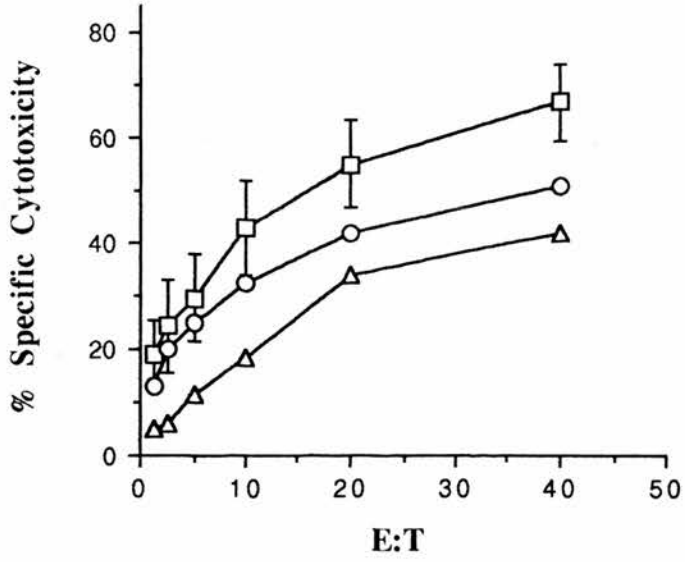
LEGEND FOR FIGURES

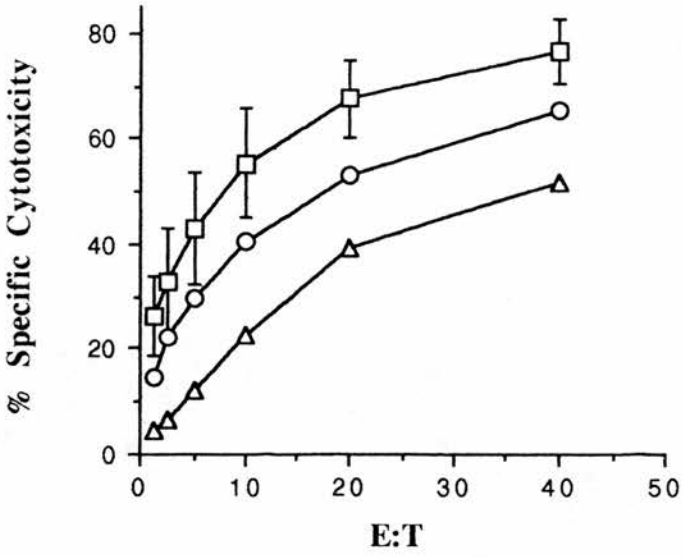
Figure 1. The effect of therapy on NK cell activity in patients with psoriasis and normal subjects. Psoriasis patients receiving coal tar dressing had samples taken on 2 occasions, before (□) and during (Δ) treatment (a). Assays were performed before (□) during (Δ) and after (○) broad band UV6 (b), narrow band TL01 (c) or PUVA (d) therapy of psoriasis patients. Normal subjects, receiving no treatment, had samples taken on 2 occasions 4 weeks apart (e, □ and Δ). Normal subjects were assayed before (□) during (Δ) and after (○) UV6 therapy (f). The figures represent the mean of 5 patients in (a), 6 in (b-d) and 4 in (e,f). The SEM shown for each group prior to treatment was approximately the same on subsequent occasions.

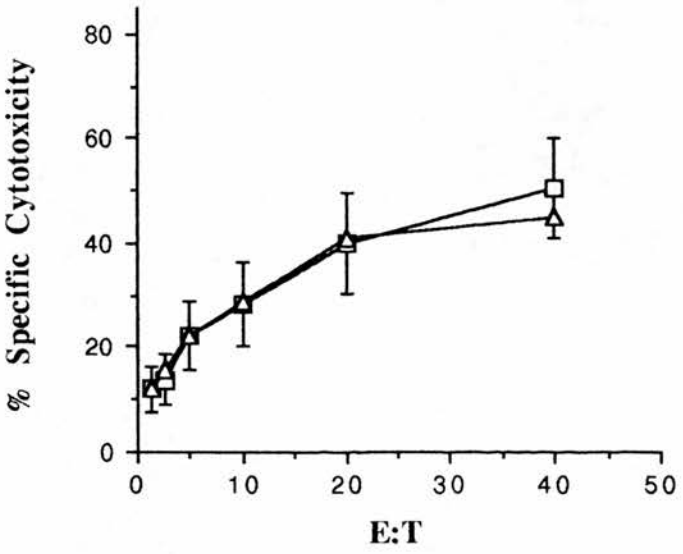
Figure 2. The effect of cis-UCA (a) and trans-UCA (b) on NK cell activity in vitro. Fig.2a represents the mean ± SEM of 6-11 subjects at each UCA concentration and Fig. 2b represents the mean ± SEM of 4 subjects. * $p < 0.05$ and ** $p < 0.01$ compared with the mean of the same individuals with no UCA added, paired sample t test.

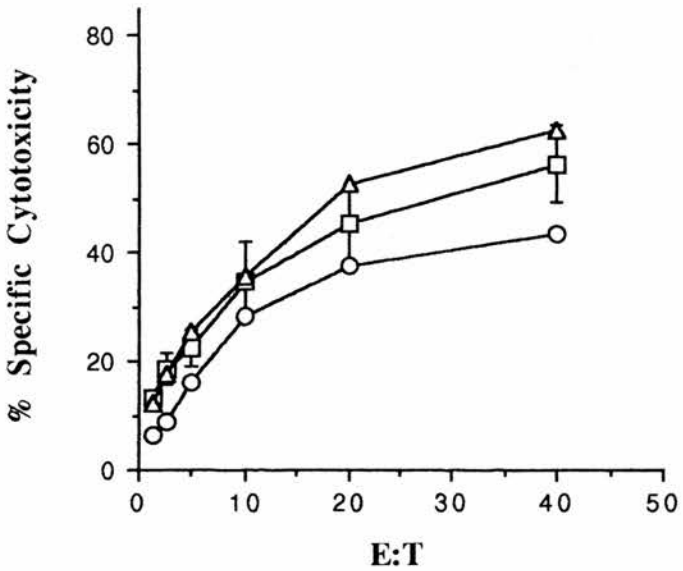

% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T

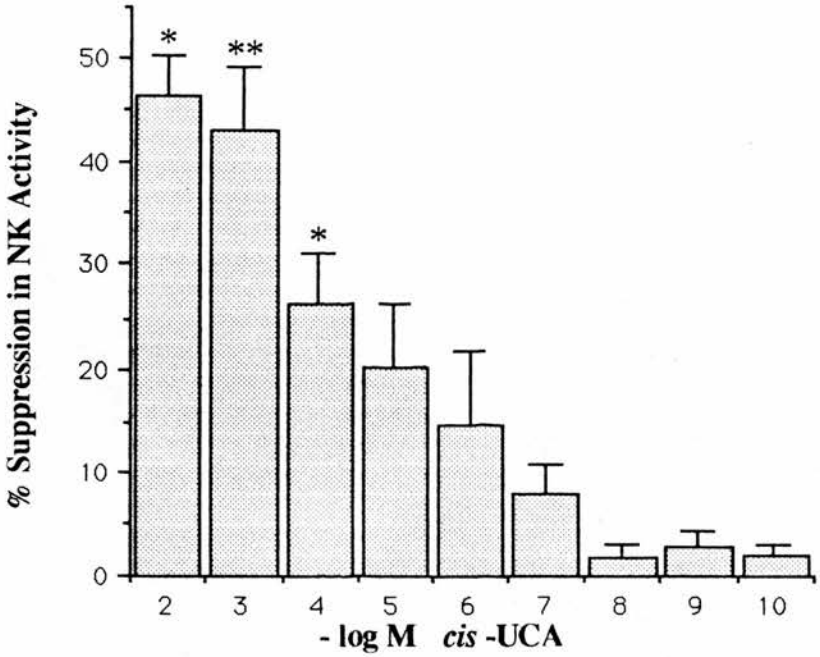

% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T

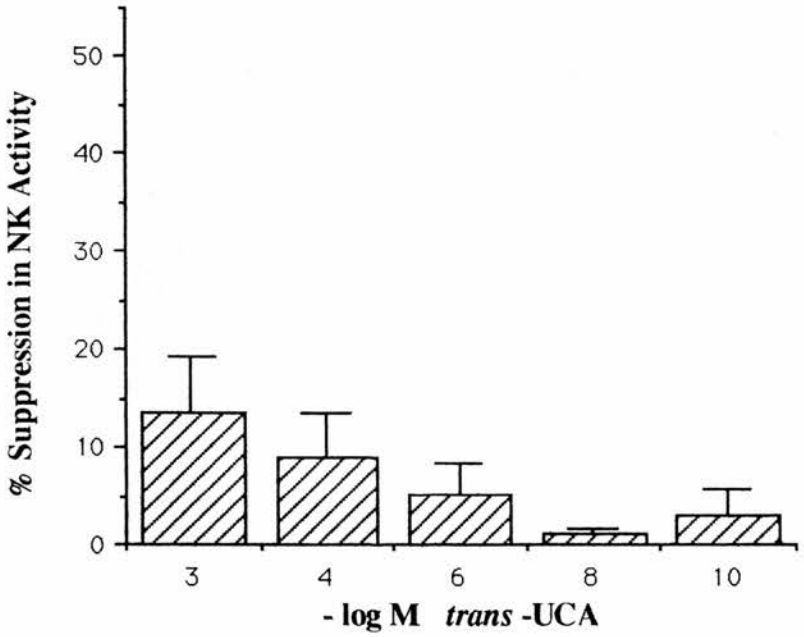

% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T


% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T


% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T


% Specific Cytotoxicity
80
60
40
20
0
0
10
20
30
40
50
E:T


% Suppression in NK Activity
50
40
30
20
10
0
*
**
*
2
3
4
5
6
7
8
9
10
- log M cis -UCA


% Suppression in NK Activity
50
40
30
20
10
0
3
4
6
8
10
- log M trans -UCA