POLYCLONAL AND MONOCLONAL ANALYSIS OF THE HUMAN T LYMPHOCYTE IMMUNE RESPONSE TO DERMATOPHAGOIDES SPP.

by

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To my parents

ABSTRACT

Immunoglobulin E (IgE) is the main immunoglobulin associated with allergic hypersensitivity. Although IgE synthesis is T cell dependent, the specificity and regulatory role of T lymphocytes in the immune responses to allergens in either atopic or non-atopic individuals are poorly understood. The aim of this work was to investigate T cell immunity to the most common mite species inducing allergy, Dermatophagoides spp. (house dust mite).

Antigen specificity was examined using polyclonal and cloned CD4+ T lymphocytes. Responses to soluble crude allergen extracts, nitrocellulose immunoblots of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) fractionated Dermatophagoides spp. preparations and affinity-purified allergens were analysed and compared. T cell populations reactive with allergen could be identified and expanded from both allergic and non-allergic subjects. Cross-reactive and species-specific T cell clones recognising the serologically-defined group I (24 kD) and II (12.5 kD) allergens were isolated; the predominant response being species-specific.

In order to assess the contribution of different subsets of molecules encoded by the major histocompatibility gene complex (MHC) in T cell recognition of Dermatophagoides farinae, the restriction specificity of a panel of house dust mite specific T cell clones from an atopic individual was examined. Serological inhibition of antigen-dependent proliferation suggested that all the clones were restricted by HLA-DR molecules. Using MHC-typed allogeneic presenting cells the major proportion of the clones appeared to be restricted by beta III (DRw52), as opposed to beta I gene

products. This was confirmed using murine L cells transfected with human MHC class II molecules.

An allergen-dependent model of in vitro IgE synthesis by autologous human B cells was developed to investigate functional differences in IgE regulation. Using this model, polyclonal and house dust mite specific cloned CD4+ T cells from an atopic individual with house dust mite allergy could support IgE synthesis that was interleukin-4 (IL-4) dependent. In contrast, neither population isolated from a non-atopic individual could support IgE production even in the presence of exogenous IL-4 although both subjects could support IgG synthesis.

Finally, in vitro models of hyposensitisation have been developed. Incubation with escalating concentrations of antigen rendered cloned T cells less responsive to an immunogenic challenge with specific antigen but not to interleukin-2 (IL-2). Furthermore, the addition of an anti-IL-4 receptor antibody abrogated IgE synthesis.

Taken together, this work provides models for investigating T cell responses to aeroallergens which may allow logical examination of mechanisms of hyposensitisation for potential in vivo use.

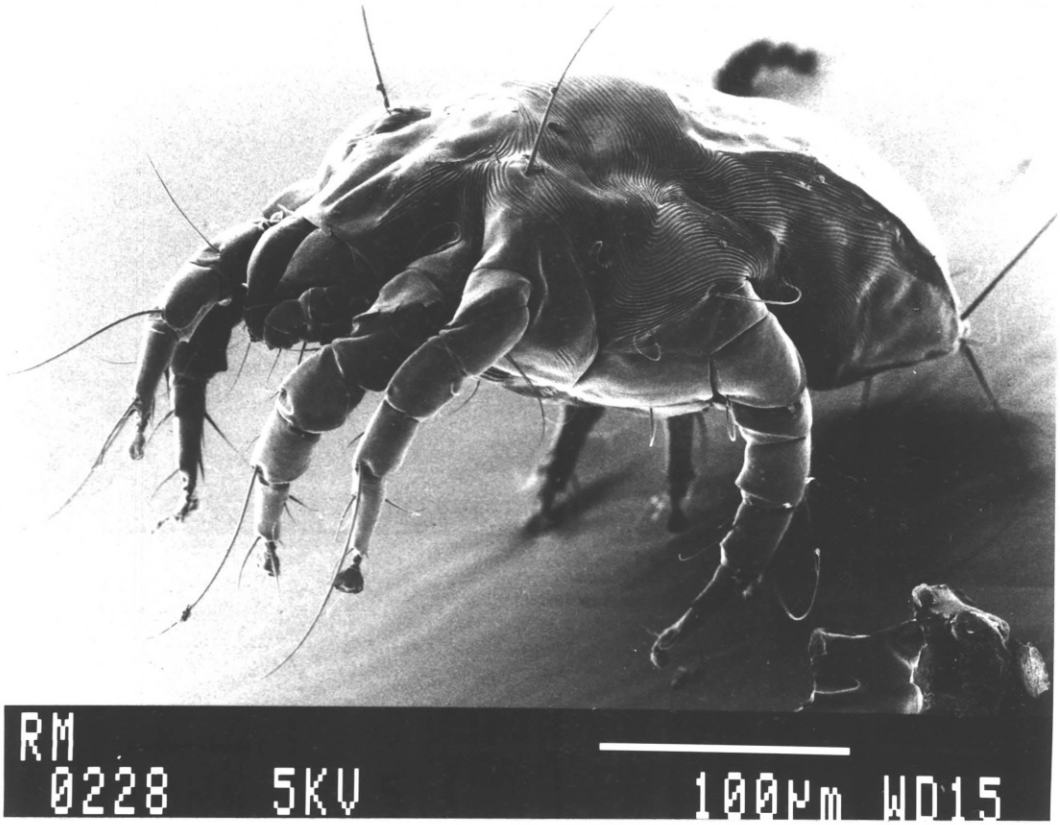

Plate 1: **Dermatophagoides pteronyssinus**

Scanning electron micrograph of a house dust mite. (Kind gift of Drs. Reinhardt Mobjerg Kristensen and Carsten Schou, A.L.K., Denmark).

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ABBREVIATIONS

AC	accessory cell
AET	aminoethylisothiouronium bromide hydrobromide
APC	antigen presenting cell
B cell	immunoglobulin bearing bone marrow-derived lymphocyte
BSA	bovine serum albumin
B.U.	Biological Units
CNBr	cyanogen bromide
c.p.m.	counts per minute
D. farinae	Dermatophagoides farinae
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
D. pteronyssinus	Dermatophagoides pteronyssinus
EBV	Epstein-Barr virus
E^+ cells	E-rosette forming lymphocytes
E^- cells	non-E-rosette forming lymphocytes
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence activated cell sorter
FCS	foetal calf serum
HDM	house dust mite
HLA	human MHC
[^{3}H]TdR	tritiated methyl thymidine
IFN-gamma	interferon gamma
Ig	immunoglobulin
IgE	immunoglobulin E
IgG	immunoglobulin G
IL-2	interleukin-2
IL-4	interleukin-4
Ir	Immune response
I.U.	International Units
kD	kilodaltons
mAb	monoclonal antibody
MHC	major histocompatibility complex
MW	molecular weight
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PHA	phytohaemagglutinin

PWM	pokeweed mitogen
RAST	radioallergosorbent test
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
SRBC	sheep erythrocytes
T cell	non-immunoglobulin bearing thymus-derived lymphocyte
T_H	helper T cell
V	volts

Amino acids - single letter code

A	alanine
C	cysteine
D	aspartic acid
E	glutamic acid
F	phenylalanine
G	glycine
H	histidine
I	isoleucine
K	lysine
L	leucine
M	methionine
N	asparagine
P	proline
Q	glutamine
R	arginine
S	serine
T	threonine
V	valine
W	tryptophan
Y	tyrosine

INTRODUCTION

The humoral response to aeroallergens and the role of effector cells such as mast cells and eosinophils in mediating allergic responses in atopic diseases have been extensively investigated. In contrast, the antigen specificity and functional role of T lymphocytes in the induction and regulation of allergic responses are ill-defined. This has been due in part to research efforts concentrating on the effector mechanisms and to a lack of understanding of the growth requirements for the long-term culture of allergen-specific T lymphocytes. However, with the advent of technologies for the maintenance of monoclonal populations of antigen-specific T cells it is now possible to analyse the specificity and functional activities of allergen-reactive T cells in detail.

The aims of this investigation were:-

1) To identify and isolate T cell populations reactive with the most common aeroallergen Dermatophagoides spp. (house dust mite) from both atopic and non-atopic individuals.

2) To define the species-specificity and cross-reactivity of T cell recognition of Dermatophagoides spp. at the polyclonal and monoclonal levels and to use fractionated nitrocellulose-bound antigens, affinity-purified allergen and synthetic peptides to map the antigenic determinants recognised by Dermatophagoides spp. reactive T cells.

3) To investigate the MHC restriction specificity of Dermatophagoides spp. reactive T cell clones and lines using serological inhibition, allogeneic

presenting cell panels and murine fibroblasts expressing HLA-D region proteins.

4) To examine the functional activity of Dermatophagoides spp. reactive polyclonal and monoclonal T cells in the induction of allergen-dependent immunoglobulin synthesis by autologous B cells in vitro.

5) To modulate the functional expression of house dust mite reactive T cells by:-

a) repeated and escalating low doses of specific antigen as a model for functional clonal deletion.

b) a putative monoclonal antibody directed against the IL-4 receptor.

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1.1 ALLERGIC INFLAMMATION

Atopy is the predisposition for the formation of specific IgE antibody to common environmental allergens and occurs in 25-30% of the total population. The reason why some individuals mount an IgE response to these allergens and other individuals fail to do so is unclear. Of the atopic subjects, 10-15% demonstrate manifestations of allergic diseases, namely extrinsic asthma, allergic rhinitis and/or atopic dermatitis.

Since the discovery of human IgE in 1966 (Ishizaka, Ishizaka and Hornbrook, 1966) it has been generally accepted that common forms of immediate hypersensitivity are mediated primarily by specific IgE antibodies. When sensitised airways or skin of these individuals are provoked by exposure to the specific allergen a biphasic response is often observed. Cross-linking of IgE receptors on the primary effector cells, mast cells or basophils, results in an immediate or early reaction, due to the release of primary mediators. In contrast, the subsequent secondary or late-phase response is largely of an inflammatory nature (reviewed in Kay, 1988). The early response occurs within minutes of allergen exposure and is ascribed mainly to the release of both membrane-derived (newly-generated) and granule-associated (preformed) mediators. The major preformed mediators are histamine, heparin, tryptase and exoglycosidases with leukotriene C_4 (LTC_4), prostaglandin D_2 and thromboxane A_2 being the predominant newly-generated mediators (reviewed in Cromwell, 1988). The late response starts after 4-6 hours and terminates within 24-48 hours. During this response there is cellular infiltration by eosinophils, neutrophils, macrophages, lymphocytes and basophils, presumably attracted to the site of provocation by chemotactic agents, with the subsequent release of secondary mediators including prostaglandins, leukotrienes B_4 and C_4 and platelet activating

factor. Until quite recently, the role of the CD4+ T lymphocyte in this infiltrate was thought to be unimportant. However, it has now been shown that activated T lymphocytes are involved in allergic inflammation via a number of secreted lymphokine products. These include: histamine release enhancing factor (Sedgwick, Holt and Turner, 1981; McDonald et al., 1987; Theuson et al., 1979); IL-3, shown to possess mast cell and basophil growth stimulating activity (Schrader, 1986); IL-4, capable of enhancing IgE production (Paul and Ohara, 1987); IL-5, required for eosinophil differentiation (Kinashi et al., 1986; Mosmann and Coffman, 1987); interferon-gamma, capable of enhancing antigen-induced histamine chemotactic release (Ida et al., 1977) and 10kD neutrophil chemotactic factor (Maestrelli et al., in press).

A diverse range of allergenic stimuli are able to evoke the same chain of events. Commonly encountered allergens include house dust mite, grass pollens, cat dander, dog dander and moulds. Of these, the house dust mite, Dermatophagoides spp., is the most common and the human T lymphocyte immune response to this allergen forms the basis for this study.

1.2 HOUSE DUST MITE AND ALLERGIC DISEASES

An association between house dust and episodic attacks of breathlessness was first described by Kern (1921). This was followed in 1928 by the conclusion of Decker that mites were the source of this allergic manifestation. His study, however, was ignored until 1964 when Voorhorst et al. (1967) suggested that the pyroglyphid mite Dermatophagoides pteronyssinus was the main source of the "house dust allergen". These authors documented a good correlation between house dust allergenicity and mite content and asserted that mite allergens were secreted or excreted,

increasing when mites actually grew in house dust (Voorhorst, Spieksma and Varekamp, 1969). Confirmation followed from all over the world, particularly in humid areas in Europe, South America, Australia and Japan (van Bronswijk and Sinha, 1971; Miyamoto et al., 1968; Bullock and Frick, 1972; Romagnani et al., 1972; Murray, Ferguson and Morrison, 1980).

Although difficult to study, perennial rhinitis due to house dust mite allergy is also recognised, often associated with early morning sneezing, exacerbation on bed-making and improvement outside the house. Recent evidence supports a role for house dust mite in atopic dermatitis (Mitchell et al., 1982; 1984; 1986).

Population surveys in many different countries have shown a high prevalence of mite allergy among asthmatic subjects; 45-85% among asthmatics and 5-30% among controls (Smith et al., 1969; Witt et al., 1986). A recent study described the introduction of asthma, in association with house dust mite allergy and specific serum IgE, to a group of highland villages in New Guinea, by the introduction of blankets (Dowse et al., 1985). Dust from the blankets contained an average of 1300 mites/gram whereas dust from other parts of their houses contained <100 mites/gram.

Asthma and rhinitis are multifactorial conditions with their expression depending on both genetic and environmental controls. However, no clear mode of inheritance is recognised. It is generally felt that a predisposition to allergic disease together with a specific liability to asthma or rhinitis is inherited (Blumenthal, Mendell and Yunis, 1980; Meyers and Marsh, 1981). Although boys are more often affected by asthma than girls, family studies show asthma is neither a sex-influenced nor a sex-linked condition. The

mode of inheritance varies in different studies from polygenic inheritance to dominant inheritance with incomplete penetrance (Sibbald, 1986).

Studies of IgE levels in unrelated persons and small families suggested that high IgE levels were inherited by two recessive alleles (Wilcox and Marsh, 1978). The absence of one or the other resulted in high levels of IgE. Gerrard, Rao and Morton (1978), studying high IgE in nuclear families, found significant polygenic inheritance and evidence for a major locus with a recessive homozygote maintaining persistently high levels of IgE. However, other groups failed to confirm these observations (Blumenthal et al., 1981). They observed heterogeneity among families.

1.3 HOUSE DUST MITE

I Dust mite biology (acarology)

Dust mites are acarids related to ticks and spiders and closely related to the mites that cause scabies. They are sightless, eight-legged arthropods just invisible to the naked eye (<1/3 mm in length; Plate 1). The main house dust mites are of the family Pyroglyphidae which includes the genus Dermatophagoides with the main species being D. pteronyssinus, D. farinae and D. microceras. They feed on human skin scales or fungi growing on such scales with the major secretory product of the mites being their faecal pellet (10-30μ diameter; Plate 1), which is enveloped by a peritrophic membrane. (Platts-Mills and Chapman, 1987).

Humidity and temperature are important determinants of mite infestation, with bedding, upholstered furniture or carpeting being the preferred locations. They are ubiquitous allergens found throughout the world but D.

pteronyssinus is more prevalent in humid, warm areas and D. farinae is more common in dry, cooler regions.

II Dust mite allergens

Improved methods for the laboratory culture of mites have enabled the preparation of mite extracts without growth media contamination in sufficiently large quantities to allow the isolation and purification of allergen preparations. The antigenic and allergenic complexity of mite extracts have been investigated using preparative fractionation, ultrafiltration, gel permeation chromatography and isoelectric focusing (reviewed by Lowenstein, 1978). Using crossed radioimmunoelectrophoresis at least nineteen allergens could be identified but, using sera from patients with house dust mite allergy and a protein blotting technique (Towbin, Staehelin and Gordon, 1979), only four appeared to be clinically of major importance (Lind and Lowenstein, 1983; Krilis, Baldo and Basten, 1984; Stewart et al., 1986; Tovey and Baldo, 1987). The definition of two major groups of allergens from the genus Dermatophagoides (Table I.1) has subsequently followed. The Group I allergens (Der p I, Der f I and Der m I) are heat labile glycoproteins of MW 24,000 which are excreted in faeces (Der p I: Chapman and Platts-Mills, 1980; Lind, 1985; Stewart and Turner 1980; Der f I: Heymann, Chapman and Platts-Mills, 1986; Dandeu et al., 1982; Lind, 1986). The Group II allergens (Der p II and Der f II) are proteins of MW 12,500-15,000 (Der p II: Lind, 1985, Der f II: Holck, Dale and Sletten, 1986; Yasueda et al., 1986; Heymann et al., 1987). Although there is marked structural homology between members of each allergen group (Table I.2) there is no evidence for structural similarity or cross-reactivity between groups. Mite-allergic subjects, however, elicit IgE antibody responses to these allergens in the majority of cases (>90%). Recently, Group III

allergen, Der f III, has been identified but its allergenic importance is not yet clearly established (Heymann et al., 1987).

The gene encoding Der p I has been cloned and expressed in lambda gt11 with subsequent identification of the full amino acid sequence from the cDNA (Chua et al., 1988). Cloning and sequencing of the other major allergens is currently underway. Murine monoclonal antibodies have been raised in several laboratories against the Group I and Group II allergens (reviewed in Chapman, 1988; Chapman, Heymann and Platts-Mills, 1987; Horn and Lind, 1987). These have allowed allergen purification in single-step, affinity-chromatography procedures from aqueous mite culture extracts (Heymann et al., 1987), the development of radioimmunoassays for allergen quantification (Chapman et al., 1987) and the identification of B cell epitopes (Chapman, Sutherland and Platts-Mills, 1984; Chapman, Heymann and Platts-Mills, 1987; Horn and Lind, 1987).

1.4 T LYMPHOCYTES AND ALLERGY

Unlike the detailed studies evaluating effector cell mechanisms, such as the mast cell, basophil and eosinophil, and the sophisticated analyses using monoclonal antibodies, culminating in affinity-purified allergen preparations and B cell epitope mapping, only limited research has addressed the interactions of T cells and allergens. Furthermore, most research has focused on polyclonal T cell analysis, although it has been shown that the incidence of allergen-immune T cells in such a population may be only 0.02% (Halvorsen, Bosnes and Thorsby, 1986). The development of technology for the isolation and maintenance in culture of cloned T cell populations

provides the means for detailed analysis of T cell immune responses to allergens.

I Phenotypic studies of T cell subsets in atopic diseases

Conflicting reports have been obtained in studies addressing the lymphocyte subpopulations in atopic diseases. Several studies have reported decreased T cell numbers, as determined by sheep erythrocyte rosette formation, in patients with allergic rhinitis (Valverde et al., 1984; Strannegard and Strannegard, 1978) or extrinsic asthma (Gupta et al., 1975) as compared to age-matched non-atopic controls. However, other studies using monoclonal antibodies and indirect immunofluorescence challenged these findings (Schuyler, Gerblich and Urda, 1985), reporting no significant difference in the numbers of CD3+ cells or the percentage of T cells expressing CD4, CD8 and HLA-DR antigens.

More recently, studies have utilised highly-purified monoclonal antibodies and improved immunocytochemical techniques and enumerated circulating lymphocyte populations and those obtained from skin biopsies and bronchoalveolar lavage. Again the results have proven conflicting. Several groups have reported increased CD4+/CD8+ ratios, however, in three studies (Leung, Rhodes and Geha, 1981; Butler, Atherton and Levinsky, 1982; Zachary and MacDonald, 1983) this was attributable to lowered CD8+ percentages and in another (Joffe, Kew and Robson, 1983) an increased CD4+ percentage was the explanation. In contrast, most studies have reported no peripheral T cell subset abnormalities in patients with mild asthma or allergic rhinitis (Leung, Rhodes and Geha, 1981; Schuyler et al., 1985).

Several groups have used the late-phase allergic skin reaction as an experimental model in which to study the cellular components of allergic inflammation. Intradermal injections of allergen or control saline injections have been followed by serial punch skin biopsies and immunocytochemical analysis using monoclonal antibodies. An infiltration of T lymphocytes, predominantly CD4+, persisted for at least 48 hr with some showing evidence of activation by staining positive for the IL-2 receptor (IL-2R; Frew and Kay, in press; Sillevis Smitt et al., 1986; Leung et al., 1983). Further evidence for the role of the activated T lymphocyte in allergic diseases was obtained in a study of patients with acute severe asthma (Corrigan, Hartnell and Kay, 1988). Using monoclonal antibodies and flow cytometric analysis they detected significantly elevated markers of T cell activation (IL-2R, HLA-DR and VLA-1) in the CD4+ blood lymphocytes of asthmatics as compared to normal controls. Bruijnzeel et al. (1987) detected a small but significant decrease in the number of CD8+ T cells in intrinsic asthmatics as compared to controls (normals, extrinsic asthmatics and intrinsic asthmatics treated with systemic corticosteroid therapy).

Quite recently, bronchoprovocation testing has been used experimentally to examine changes in T cell subsets in the peripheral blood (Gerblich, Campbell and Schuyler, 1984), in bronchoalveolar lavage fluid (Metzger et al., 1987) or in both sites (Gonzalez et al., 1987). Gerblich detected a fall in circulating CD4+ cells immediately after challenge with a clinically-relevant allergen, which lasted $\geq$72 hr. Levels of activated T cells as correlated with HLA-DR expression increased 48 hr after challenge. These observations only occurred with a relevant allergenic challenge. Metzger et al. (1987) demonstrated a selective increase in CD4+ cells in lavage fluid 48hr after segmental bronchial allergen challenge. Together these studies

suggested a selective recruitment and retention of CD4+ T lymphocytes in the lungs during the late asthmatic reaction. In contrast, Gonzalez reported a significant increase in the percentage of circulating CD4+ cells with an associated decrease of these in bronchoalveolar lavage fluid and a relative increase of CD8+ cells in the lavage fluid of single early asthmatic responders as compared to late-phase responders.

II Suppressor cell function in allergic disorders

Numerous investigators have hypothesised that patients with atopic disorders have defects in their non-specific suppressor cell function. Again, studies have proven confusing with some investigators reporting a defect in concanavalin A-induced suppressor cell function in atopic patients (Rola-Pleszczynski and Blanchard, 1981; Saxon, Morrow and Stevens, 1980; Harper et al., 1980; Hwang et al., 1985) and others failing to confirm this finding (Martinez et al., 1979; Schuster et al., 1979). Treatment with oral colchicine was able to correct the observed defect in a group of eleven extrinsic asthmatic subjects (Schwarz et al., 1988).

Rocklin (1976) investigated histamine-induced suppressor activity in atopic and non-atopic subjects and observed a specific defect in suppressor cell response for histamine. It was observed that atopic patients had a corresponding decrease in the number of H_2-receptor-bearing T lymphocytes. He postulated that chronic in vivo exposure to histamine could result in the observed down-regulation of the H_2-receptor expression, rather than the presence of a primary defect. Beer et al. (1982) examined this hypothesis by studying the histamine-responsive suppressor cells in a group of non-atopic patients with systemic mastocytosis. They, however,

demonstrated normal suppressor cell responses to histamine and normal expression of H_1- and H_2-receptors on the lymphocytes. These findings tend to suggest a primary defect associated with the atopic state.

III Antigen specificity

In vitro proliferation by T lymphocytes cultured in the presence of relevant allergens as correlated with tritiated thymidine ([^{3}H]TdR) incorporation into cellular DNA or the detection of soluble lymphokines such as migration inhibitory factor (MIF) or, more recently, interleukin-2 (IL-2) has been described since the early 1960s (Slavin et al., 1963; Zeitz, Van Arsdel and McClure, 1966; Girard et al., 1967; Richter and Naspitz, 1968; Young, Zimmerman and Smithwick, 1968; Brostoff and Roitt, 1969; Go, Michaels and Ellis, 1970; Maini et al., 1971; Massie and Wutanasupta, 1972; Anderson et al., 1974). Clearly, specific allergens induce proliferation of peripheral blood mononuclear cells (PBMC) from allergic individuals (Rocklin et al., 1974; Geha et al., 1975; Evans et al., 1976; Eisenbrey et al., 1985; Gatien, Merler and Colten, 1975; Rawle, Mitchell and Platts-Mills, 1984). However, controversy surrounds whether this response is peculiar to allergic subjects or whether similar proliferation is observable in non-allergic subjects (Buckley et al., 1977; Halvorsen et al., 1986). The proliferative response may be specific to an allergen or a mitogenic effect of contaminants in the allergen preparation (Ownby and Buckley, 1979). Halvorsen et al. (1986) excluded the presence of a mitogenic effect in their D. farinae extract by screening on PBMC from umbilical cord blood of six healthy neonates. They could detect no significant differences in T cell proliferation and T cell surface markers between mite-allergic individuals and healthy controls. Furthermore, the frequencies of allergen-reactive T cells as determined by

limiting dilution were comparable.

Detailed study of the fine antigen specificity of these responses has been limited by the lack of purified major allergen extracts (Chapman and Platts-Mills, 1980; King, Norman and Connell, 1964) and knowledge of the amino-acid sequence of these antigens. In fact, the identification of these major allergens was based on serology. However, supporting the serologically-determined identification of major allergens, studies by (Rawle, Mitchell and Platts-Mills, 1984) and Phillips et al. (1987) have suggested that the polyclonal T cell responses to these major allergens predominantly occur in atopic individuals. Rawle et al. (Rawle, Mitchell and Platts-Mills, 1984) demonstrated T helper cell proliferation to Der p I in 46 of 67 house dust mite allergic patients whereas 14 of the 15 non-sensitive control patients made no significant response. Phillips et al. (1987) found that by extending their cultures for a longer period with a second in vitro boost with ragweed antigen E (AgE) they could demonstrate specific proliferation only in PBMC from the ragweed-allergic subjects. They were then able to expand the cultures as a ragweed AgE- specific line and isolate a number of CD4+ ragweed AgE-reactive T cell clones. However, with time in culture the line became autoreactive. AgE-responsive T cells could only be cloned from ragweed-allergic donors. Lanzavecchia et al. (1983) had previously used 15-25,000 MW D. pteronyssinus allergen to isolate from allergic individuals HLA-DR-restricted CD4+ T cell lines and clones reactive with D. pteronyssinus and others reactive with Rye group I allergen.

These studies have been limited by the use of relatively poorly defined antigen, such as ragweed AgE for which the primary amino acid structure is unknown. Gurka et al. (Gurka, Ohman and Rosenwasser, 1987) have isolated

human T cell clones specific for a mouse urinary protein allergen (mouse allergen-1). They subsequently identified a dominant antigenic epitope (RA2uG 80-110) using a cDNA probe, however it appeared to activate T cells irregardless of the source or HLA specificity of the T cell clone (Gurka et al., 1988), raising the possibility of a mitogenic effect. Experiments in the mouse (Kuisaki et al., 1986) and man (Rothbard et al., 1988) have demonstrated T cells reactive with residues 51-65 of Amb a III.

1.5 T CELL RECOGNITION OF ANTIGEN AND MHC GENE PRODUCTS

As knowledge on the diversity of the antigen combining site and the molecular genetics of antibody molecules evolved it was anticipated that the diversity of antigen recognition by T cells would operate by a similar, if not identical, mechanism. Indeed, cloning revealed that the genetic organisation and sequence of the antigen receptor on T cells and immunoglobulin were similar (Hedrick et al., 1984; Yanagi et al., 1984). However, a major difference between B and T cell recognition exists in that B cells are able to bind free antigen, whereas T cells recognise protein antigens only in association with MHC gene products expressed on the surface of accessory cells (Shevach and Rosenthal, 1973; Zinkernagel and Doherty, 1974). In the last few years the molecular basis of antigen-MHC complexes and their recognition by antigen-reactive, MHC restricted T cells has advanced significantly. This has been the result of the use of synthetic peptides as antigens (Schwartz, 1985; Livingstone and Fathman, 1987; Rothbard and Taylor, 1988; Lamb et al., 1988), direct binding studies (Babbitt et al., 1985; Buus et al., 1987; Moller, 1987), the genetic manipulation and expression of MHC genes (Malissen et al., 1984; Lechler et al., 1986; Germain and Malissen, 1986) and the resolution of the crystal structure of HLA-A2 (Bjorkman et al., 1987a and b). It now appears that

cell surface molecules additional to MHC proteins and the T cell receptor complex (CD3-Ti; reviewed in Weiss et al., 1986; Allison and Lanier, 1987) are involved in antigen recognition. The results of recent experiments suggested that the "accessory molecules", CD4 and CD8, may actually form components of the antigen recognition structure of T cells (Saizawa, Rojo and Janeway, 1987) and participate in the signal transduction during activation, lowering the concentration of ligand required by as much as 100-fold (Janeway, 1988; Janeway et al., 1988). This extended the functional role of CD4 and CD8 beyond that of adhesion molecules merely forming contact with MHC proteins and suggested that they increase the affinity of the T cell receptor for antigen (Biddison et al., 1983; Martz, 1987; Doyle and Strominger, 1987).

I Recognition of processed antigen

The capacity of macrophages to "process" and present antigen to T cells has been extensively investigated (Unanue, 1984). Ziegler and Unanue (1981; 1982) in studies using the bacterial antigen Listeria monocytogenes identified two major features of antigen processing. They demonstrated that a processing period was required to allow functional presentation, for if the antigen presenting cells were fixed before the processing period they were unable to present antigen. However, if the presenting cells were fixed after the obligatory processing interval they were still able to present antigen to T cells. Secondly, they observed that treatment of antigen presenting cells soon after exposure to antigen with paraformaldehyde or lysosomotropic drugs such as chloroquine or ammonium chloride would inhibit T cell activation. Therefore, it was concluded that processing involving proteolytic cleavage of foreign antigens was required, not just binding of native antigen to the macrophage membrane. These conclusions

have subsequently been confirmed using other antigen systems, including lysozyme (Allen and Unanue, 1984), ovalbumin (Shimonkevitz et al., 1983), myoglobin (Streicher et al., 1984), cytochrome C (Schwartz, 1985) and insulin (Falo, Benacerraf and Rock, 1986). Shimonkevitz et al. (1983) demonstrated that following glutaraldehyde fixation, B cell lymphomas were able to present tryptic digests of ovalbumin, but not the intact or native protein, to T cell hybrids. Therefore, cell-free antigen processing was achieved by the use of a proteolytic enzyme. Other groups have confirmed these findings (Lee, Wong and Spitzer, 1982; Falo et al., 1985; Buus and Werdelin, 1986). Streicher et al. (1984) demonstrated that an unfolded form of myoglobin, S-methyl apomyoglobin, induced by chemical modification in the presence of accessory cells was able to activate specific T cell clones as effectively as the parent peptide, suggesting that conformational disruption exposing hydrophobic residues may be as important as proteolysis in antigen processing (Allen, 1987). In these experiments the requirements for antigen recognition were determined for MHC class II restricted CD4+ T cells.

Current evidence suggests that foreign antigen can be processed and presented as peptides in association with MHC class I gene products for recognition by cytotoxic (CD8+) T cells. The results of experiments investigating the cytolytic response to influenza nucleoprotein demonstrated that overlapping genes transfected into fibroblasts were able to stimulate cytotoxic T cells and suggested that short peptide fragments were recognised (Townsend, Gotch and Davey, 1985). This was subsequently confirmed with synthetic peptides (Townsend et al., 1986). In similar studies on the human cytolytic immune response to influenza virus (McMichael, Gotch and Rothbard, 1986) MHC class I restricted T cells were reported to recognise synthetic peptides. Class I restricted recognition of peptides by

cytolytic T cells was not limited to the influenza viral model and has been reported for MHC peptides (Maryanski et al., 1986).

Since it appeared that the majority of both helper and cytotoxic T cells recognised short linear peptides, the sequences of T cell epitopes have been analysed in an attempt to define common features which would allow the prediction of T cell epitopes to be made in different proteins (DeLisi and Berzofsky, 1986; Rothbard, 1986; Rothbard and Taylor, 1988). It has been proposed that the regions of proteins containing defined T cell epitopes could be modelled with amphipathic alpha helices (DeLisi and Berzofsky, 1986). An alternative algorithm based on primary sequence suggested that a distinctive pattern of amino acids commencing with a charged or glycine residue followed by two or three hydrophobic residues and terminating in a polar amino acid was characteristic of a T cell epitope (Rothbard, 1986; Rothbard and Taylor, 1988).

II Immune response (Ir) genes as MHC gene products

A genetic influence in the tendency of individuals to mount quantitatively different immune responses has been recognised for many years (Benacerraf, 1981). However, only with the availability of synthetic polypeptides with relatively restricted structural heterogeneity was it possible to analyse systematically the genetic basis of immunogenicity (Levine, Ojeda and Benacerraf, 1963; Bluestein, Green and Benacerraf, 1971; McDevitt and Benacerraf, 1969; Benacerraf and McDevitt, 1972). Investigation of the responder status to haptenated conjugates of the poly-L-lysine (DNP-PLL) revealed that strain 2 guinea pigs were high responders to DNP-PLL with strain 13 guinea pigs being non-responders, whereas F1 (2x13) were also responders, suggesting specific immune responses were regulated by a single

dominant autosomal gene. These observations were subsequently extended to other species and for other copolymers, thus the linkage of Ir genes with those of the MHC was established (Lieberman et al., 1972; Green and Benacerraf, 1971; McDevitt and Sela, 1965; Martin, Maurer and Benacerraf, 1971). This was confirmed by the experiments of Shevach et al. (1972) using inbred guinea pigs in which strain 2 and 13 animals were high responders to the dinitrophenylated copolymer L-glutamic acid^{60}-L-lysine^{40} (DNP-GL) and the copolymer L-glutamic acid^{60}-L-tyrosine^{50} (GT) respectively. These responses could be blocked by only the appropriate anti-strain 2 or 13 antisera. Furthermore, anti-strain 2 antisera inhibited the response of F1 (2x13)-derived lymphocytes to DNP-GL but not GT and the reciprocal effects were observed with anti-strain 13 antibody.

Extending these studies in the guinea pig it was observed that F1 lymphocytes responded to strain 2 or strain 13 macrophages pulsed with DNP-GL and GT respectively, whereas GT pulsed strain 2 or DNP-GL pulsed strain 13 macrophages were unable to activate the F1 lymphocytes (Shevach and Rosenthal, 1973). The responses to the copolymers were inhibited by the appropriate anti-strain 2 or 13 alloantisera. In contrast, using purified protein derivative of Mycobacterium tuberculosis (PPD) that was not under Ir gene control in strain 2 and 13 guinea pigs, PPD-pulsed parental macrophages induced a response in F1 (2x13) pre-immune lymphocytes equal to 50% of that observed with PPD-pulsed F1 (2x13) macrophages (Rosenthal and Shevach, 1973).

From these experiments taken collectively it was concluded that the products of Ir genes were MHC proteins expressed on the surface of macrophages and that they were associated with T cell antigen recognition.

III The formation of antigen-MHC class II protein complexes

Evidence derived from both functional (Berzofsky, 1987; Schwartz, 1985) and structural (Babbitt et al., 1985; Moller, 1987) studies indicated that peptide fragments of antigen interacted directly with MHC class II proteins. The most extensively studied of the functional models was the murine immune response to cytochrome C. It was observed that T cell clones isolated from B10.A (E^kalpha E^kbeta) mice immunised with pigeon cytochrome C recognised moth but not pigeon cytochrome C in the presence of B10.A(5R) (E^kalpha E^bbeta) accessory cells (Heber-Katz et al., 1982). These experiments were extended using combinations of cytochrome C from different species and allelic forms of class II molecules (Matis et al., 1983; Schwartz, 1985). In summary, the conclusion drawn from the results was that by altering the MHC class II proteins of the accessory cells the fine antigen specificity of a given T cell clone could be changed.

An alternative functional approach designed to demonstrate specific interactions between antigen and MHC class II proteins was to examine the ability of non-stimulatory antigen analogues to compete with the immunogenic antigen at the level of the accessory cell (Werdelin, 1982). Although strain 2 guinea pigs were able to respond to both dinitrophenyl-poly-L-lysine (DNP-PLL) and L-glutamic $acid^{60}$-$L\text{-}lysine^{40}$ (GL) the T cell population induced by DNP-PLL failed to cross-react on GL and vice versa. However, pulsing the accessory cells with GL at the same time as DNP-PLL, it was possible to inhibit the subsequent activation of DNP-PLL but not ovalbumin-specific T cells. These experiments were refined by Rock and Benacerraf (1983) using I-A^d restricted murine T cell hybridomas reactive with the copolymer L-$glutamic\ acid^{60}$-L-$alanine^{40}$-L-$tyrosine^{10}$ (GAT), who

demonstrated that by pulsing F1 ($I\text{-}A^d$ x $I\text{-}A^b$) accessory cells with the related copolymer GT only the $I\text{-}A^d$ restricted GAT response was inhibited. The results of the experiments suggested that antigen fragments formed specific interactions with MHC class II proteins. The ability to demonstrate peptide competition using purified class II proteins (Buus et al., 1987) confirmed these earlier observations.

In an attempt to define those residues critical for binding to MHC class II proteins, hybrid peptides were constructed between two peptides recognised by T cell clones of different antigen specificity but restricted by the same MHC class II protein (HLA-DR1; Rothbard et al., 1988). Indeed, the hybrid peptides were able to stimulate the T cell clones specific for each parental peptide. This result suggested that the peptides bound in the same location and orientation, and thus shared residues in each peptide capable of interacting with MHC class II could be identified. Using truncated and substituted peptides of ovalbumin (322-339) in both proliferation and binding assays it was observed that residues 327-333 were required for T cell activation of which only the amino acid at position 327 formed a unique contact with MHC class II proteins (Sette et al., 1987). Examination of peptide analogues of hen egg lysozyme, residues 52-61 with sequential alanine substitutions, for their ability to competitively inhibit or induce T cell proliferation revealed that residues 52, 58 and 61 formed contacts with the MHC class II proteins (Allen et al., 1987). Structural analysis indicated that this peptide formed an alpha helical conformation when complexed with class II molecules whereas Sette et al. (1987) concluded that the ovalbumin peptide formed a beta sheet.

Early studies on the characterisation of antigen and MHC class II protein

complexes focused on macrophage products (Erb and Feldmann, 1975a,b,c and D). They observed that supernatants derived from antigen-stimulated macrophages were able to substitute for the parental cells displaying the same requirements of MHC restriction and antigen specificity in the activation of helper T cells.

Formal biochemical evidence of the existence of peptide-MHC class II protein complexes was first demonstrated by Babbitt et al. (1985). Using equilibrium dialysis it was observed that a fluorescenated peptide of hen egg lysozyme (residues 46-61) bound strongly to affinity-purified I-A^k from the high responder haplotype but not to I-A^d. This binding was inhibitable by unlabelled peptide.

The high affinity of peptide-MHC class II protein complexes and their resultant stability enabled them to be isolated from excess free peptide and uncomplexed class II by gel filtration (Buus et al., 1986). Using gel filtration the binding of ovalbumin peptide (residues 323-339) to I-A^d was analysed in detail. The cross-linking of ovalbumin, haemagglutinin (111-120) and myoglobin (132-153) peptides complexed to I-A^d with glutaraldehyde suggested that binding could occur to the alpha, the beta or to both chains of the MHC class II molecule (Buus et al., 1986). Analysis of the kinetics of binding revealed that the association rate constant was slow, 1.2 $M^{-1}s^{-1}$, as compared to 10^5 $M^{-1}s^{-1}$ for antibody/antigen complex formation, whereas the dissociation rate constants were similar, 2.8 x $10^{-6}s^{-1}$ at room temperature with a half life of 30 hr. Fluorescence energy transfer studies suggested that the presence of the T cell receptor promoted the association of peptide-MHC class II proteins, hence stabilising a low affinity interaction (Watts, Gaub and McConnell, 1986). The slow association rate has been

interpreted as reflecting that the peptide has to assume a certain preferred conformation for binding (Watts et al., 1986; Buus et al., 1986). Alternatively, it may result from the foreign peptide having to displace endogenous peptides.

IV T cell receptor and peptide-MHC protein interactions

The recent resolution of the crystal structure of MHC class I, HLA-A2 (Bjorkman et al., 1987a and b) has greatly aided the understanding of the molecular interactions between MHC, T cell receptor and antigen. Therefore, it is relevant at the outset of this section to describe the putative antigen combining site of MHC molecules. There appears to be a single antigen binding site present as a cleft on the outer surface of the molecule located between two alpha helical walls. The alpha 1 domain forms the first four beta strands from the amino terminal and an alpha helical wall (residues 58-84). The alpha 2 domain forms the remaining four beta strands and the second alpha helical wall. Thus the antigen combining site is composed of eight beta strands, four from each domain, that form the floor of the cleft with two helical walls, one from each domain. The polymorphic residues of the different MHC class I alleles are located almost entirely in the antigen combining site. From structural homology based on sequence and mutation experiments the structure of the antigen combining site of MHC class II molecules can be modelled on class I (Brown et al., 1988). In this model the residues predicted to form contact with the T cell receptor are those that project upward and outward from the alpha helices, while those pointing inward, in combination with the residues of the alpha 1 and beta 1 domains form the strands on the floor of the cleft determining peptide binding. The overall size of the antigen binding site could accommodate peptides of 8-20 amino acids in an alpha helical or extended

chain conformation (Allen et al., 1987; Sette et al., 1987).

A variety of functional experiments have been designed to examine those residues in antigen that form critical contacts with the T cell receptor (Schwartz, 1985; Berzofsky, 1987; Allen et al., 1987; Sette et al., 1987; Rothbard et al., 1988). Investigating the response to cytochrome C it was observed that B10.A T cells, primed with intact moth cytochrome C or to peptide residues (88-103) with a lysine at position 99, responded very weakly to an analogue of 88-103 with glutamine at 99. However, B10.A T cells primed with 88-103 with glutamine at 99 responded well to this peptide but not to the analogue with lysine-99 (Hansburg et al., 1983). This was confirmed with T cell clones. As the same accessory cells were able to present the different peptides to both T cell populations these results suggested that the effects of the substitutions at residue 99 reflected the presence of T cells of different specificities and not the failure of MHC class II proteins to bind the peptides.

The experiments described in the previous section (Sect. 1.5.III) analysing the effects of amino acid substitutions on T cell recognition of peptides such as ovalbumin (323-339; Sette et al., 1987), hen egg lysozyme (52-61; Allen et al., 1987) and hybrid peptides of influenza viral antigens (Rothbard et al., 1988) also provided information on the residues that may interact with the T cell receptor. The experimental models of Allen et al. (1987) and Rothbard et al. (1988) suggested that antigen adopted an alpha helical conformation with opposing facades contacting MHC class II and the T cell receptor respectively. Alternatively, it appeared from binding and proliferation studies that ovalbumin (323-339) bound as a beta sheet with residues at positions 326, 329, 330, 331 and 334 interacting with only the T cell receptor

and shared recognition of residues 328, 332 and 333 by both T cell receptor and MHC class II protein (Sette et al., 1987).

Support for the concept that the formation of a stable interaction between the T cell receptor and peptide-MHC class II protein complexes were influenced by direct contacts between MHC class II and T cell receptor molecules was drawn from both functional and structural evidence. The B10.A mouse strain responded to both pigeon and moth cytochrome C, whereas the B10.A(5R) recognised only the latter, yet accessory cells from the B10.A(5R) were able to present pigeon cytochrome C to B10.A T cells. Furthermore, approximately 50% of the moth cytochrome C reactive B10.A(5R) T cells recognised antigen in association with B10.A accessory cells. Thus T cells from two different strains of mice were able to recognise antigen by MHC class II proteins of different specificity suggesting that they shared a common sequence that contacted the T cell receptor. From comparison of the primary sequences (Mengle-Gaw and McDevitt, 1983; Widera and Flavell, 1984) and modelling of E^{k}beta (B10.A) and E^{b}beta (B10.A(5R)) on the proposed structure of MHC class II protein (Brown et al., 1988), it would appear that the residues of the beta I domain forming the upper facade of the alpha helical wall (58-84) of the antigen combining site and thus most likely to contact the T cell receptor, were conserved for these two class II molecules. Site specific mutagenesis in both MHC class I (H-$2K^{b}$; Ajitkurmar et al., 1988) and class II (A^{bm12}beta; Ronchese, Brown and Germain, 1987) indicated that the alpha helices of MHC proteins were recognised by the T cell receptor. In the case of the A^{bm12}beta mutant this differed from the wild type A^{b}beta by only three amino acids at positions 67, 70 and 71. Substitution of any one of these amino acids in the parent A^{b}beta with the corresponding residue of the

bm12 abrogated the majority of T cell responses irrespective of antigen specificity. These findings suggested that these residues were important in MHC-T cell receptor interaction.

More recently, studies have suggested that Vbeta17a+ receptors may bind to the I-Ealpha chain (Kappler et al., 1987). Evidence for this was obtained in studies using Vbeta17a+ T cell hybridomas which were observed to bind to I-E of all haplotypes. As it is known that marked polymorphism resides in the beta chains the implication was that the binding was to the highly conserved alpha chains. Fink et al. (1986) reported that mouse T cell receptor molecules specific for cytochrome C and restricted by I-E MHC class II proteins preferentially used a single Valpha gene segment. Since the alpha chain of I-E molecules are conserved this suggested that the T cell receptor might contact the alpha chain of class II proteins.

V Non-responder status

Not all individuals of a species are able to generate an immune response to a given antigen and to explain Ir gene regulation of "non-responsiveness" two main hypotheses have been proposed. The first suggested a possible lack of appropriate T cells (holes in the repertoire) and the second suggested a lack of binding to MHC molecules (determinant selection).

i. Holes in the repertoire

In its simplest form this hypothesis suggested that during selection of the T cell repertoire in thymic development many potential receptor combinations were deleted. As a consequence of this, the functional T cell repertoire was limited so the appropriate T cell receptors for certain antigen-MHC combinations were not present and therefore no immune response resulted

when the antigen was encountered. In a series of experiments (Guillet et al., 1987), it was demonstrated that although the lambda cI peptide (residues 12-26) was able to bind to both I-A^d and I-E^d it was not restricted by I-E^d in the H-2^d haplotype. It had been suggested that the sharing of three residues between the lambda repressor peptide and the hypervariable beta I domain of I-E^d might have resulted in clonal deletion of T cells capable of recognising the lambda repressor peptide during the development of tolerance to self I-E^d, hence explaining the observed non-responsiveness to lambda repressor peptide-I-E^d. In other experiments using a small number of T cell clones, clonal activation by combinations of MHC class II molecules from low responder haplotypes and antigen had been demonstrated (Clark and Shevach, 1982; Kimoto, Krenz and Fathman, 1981).

Ishii et al. (1981) extended these observations using larger populations of allogeneic, alloreactive-depleted T cells and confirmed that T cells from both responder and non-responder strains were able to proliferate to antigen in combination with MHC class II of allogeneic non-responder macrophages in all strain combinations studied. Only the combination of T cells from a non-responder haplotype with antigen in the presence of syngeneic or F1 hybrids with the same non-responder strain as one of the parents failed to elicit a response. Together these observations favoured a hole in the T cell repertoire as the defect in the non-responder.

ii. Determinant selection

The second hypothesis suggested that non-responder status resulted in the inability of antigen to bind to appropriate MHC molecules, thus T cells expressing the relevant T cell receptor were not activated. Support for this theory came from functional studies (Schwartz, 1985) in which T cells from

a low responder animal, B10.A(5R) mice, were able to respond to a peptide of pigeon cytochrome C (81-104) when it was presented by accessory cells from a high responder strain, B10.A. This suggested that the defect lay with the accessory cells from the low responder animals, as the two strains differed only in the haplotype of MHC. Further support for this hypothesis was obtained using another peptide of pigeon cytochrome C (43-58) and the B10.A mouse. Using peptide analogues with substitutions only at positions which interacted with the T cell receptor and not the MHC, it was demonstrated that all the analogues were immunogenic, arguing against holes in the T cell repertoire (Ogasawara, Maloy and Schwartz, 1987). More elegant evidence has arisen from the availability of direct binding assays using purified MHC class II molecules (Buus et al., 1987). In general, the ability of the class II molecules to bind antigen corresponded to the responder status of the haplotype. In competitive peptide binding experiments, they demonstrated successful competition for accessory cell presentation with the original peptide.

VI HLA associations with immune responsiveness to allergens

Levine, Stember and Fotino (1972) first described a relationship between the HLA gene complex and the allergic response to ragweed antigen. Subsequent studies of atopic humans with positive skin testing to pollen allergens have revealed significant associations between particular HLA-D specificities and specific immune responsiveness toward each of several highly-purified pollen allergens (Marsh, Meyers and Bias, 1981). Marsh et al. (1982a and b) reported an association between HLA-Dw2 and immune responsiveness to Amb a V, a highly-purified component of ragweed pollen. Moreover, Dw2 Amb a V-positive subjects responded better to allergen immunotherapy with Amb a V. They and several other groups (Goodfriend et

al., 1985; Roebber et al., 1985; Blumenthal et al., 1985) have shown that 90-95% of individuals with specific IgE for Amb a V expressed DR2/Dw2 class II proteins whereas among ragweed-allergic subjects with no specific IgE to Amb a V only 20-25% were DR2/Dw2 positive, the frequency observed in the normal population. More recently, Marsh et al. (1987) assessed specific immune responsiveness to Amb a VI (short ragweed allergen Ra6) by measuring serum IgE and IgG antibodies by double antibody radioimmunoassay. They demonstrated a positive association between IgE antibody responsiveness to Amb a VI and the HLA-DR5 haplotype. Freidhoff et al. (1986) and Ansari et al. (1987) demonstrated an association between immune responsiveness to rye grass pollen Lol p II and Lol p III and the HLA-DR3/Dw3 class II specificity.

1.6 TOLERANCE

The interaction of antigen with immunologically competent cells may lead to the induction of an immune response or to a state of complete or partial antigen-specific unresponsiveness. This state of "tolerance" is thought to play a major role in the discrimination between self and non-self which exists in health; autoimmunity ensuing when a functional defect is present (Smith and Steinberg, 1983; Nossal, 1983; Chiller and Weigle, 1972). The results of current experiments analysing T cell phenotype (CD4/8) and specific receptor expression in transgenic mice in which T cell receptor (alpha and beta chain) genes have been introduced demonstrate that both positive and negative selection can occur in thymic development and lead to the induction of T cell tolerance (Teh et al., 1988). In contrast to the B cell, cellular and molecular mechanisms regulating the development of antigen-specific tolerance in mature peripheral T cells have remained

undefined until relatively recently due to the lack of suitable models. The advent of T cell cloning has allowed these mechanisms to be examined.

I "Low zone" versus "high zone" tolerance

Historically, two types of tolerance have been recognised; one which affects T lymphocytes only, induced by repeated administration of subimmunogenic concentrations of antigen (low zone) and the other (high zone) requiring a single administration of supraimmunogenic concentrations of antigen and involving both T and B lymphocytes (Mitchison, 1971; Chiller and Weigle, 1972).

It has been demonstrated that the lack of antigen-specific responsiveness following immunisation of mice with large doses of sheep erythrocytes could be induced in syngeneic recipients by the adoptive transfer of lymphocytes from tolerant donors (Gershon and Kondo, 1971; 1972). The cells capable of mediating this phenomenon of "infectious tolerance" were termed "suppressor" T cells (Gershon, 1974) and have subsequently been reported to belong to the CD8+ T cell population (Reinherz et al., 1980). This was followed by the demonstration that membrane extracts (Takemori and Tada, 1975; Taniguchi, Hayakawa and Tada, 1976) and cell-free supernatants (Feldmann 1973; Kontiainen and Feldmann, 1977; Germain and Benacerraf, 1980) derived from antigen-induced suppressor T cells were able to mimic the activity of the parent cells from which they were derived. Both T and B lymphocytes have been reported to be the targets of suppressor T cells and factors (Kontiainen and Feldmann, 1978; Taussig et al., 1979). Since these early descriptions a large wealth of literature has been generated on the role of suppressor T cells in tolerance induction (for example, Lancaster, Chui and Batchelor, 1985). However, more relevant to this thesis is the

second model whereby functionally mature lymphocytes are able to be tolerised directly by incomplete activation and this is discussed below.

II Models of tolerance induction *in vitro*

Analysis of T cell tolerance induction and attempts to distinguish between suppression and direct inactivation have been facilitated by the development of techniques for propagating homogeneous cloned cell populations *in vitro*, rather than using the complex mixture of cell types of different function and specificity observed *in vivo*. Lamb *et al.* (1984) were able to induce unresponsiveness *in vitro* in human influenza A virus-immune T cell clones by exposing the cells to synthetic peptides over a wide range of concentrations, in the presence of antigen presenting cells. With supraoptimal stimuli the proliferative responses were decreased by 70-80% compared with control cultures. In the absence of accessory cells the degree of antigen-mediated unresponsiveness observed in the T cells was greatly increased (>90%) and long-lasting; at least 7 days after induction. This differs in the kinetics of induction and recovery from the anergy-induced in dinitrophenyl (DNP)-specific delayed type hypersensitivity cells that could be blocked by pre-exposure to free DNP-lysine, which is rapid and short-lasting (one hour) (Moorhead, 1981). The tolerised T cells responded to IL-2 suggesting that the lack of responsiveness was not due to clonal deletion. As unresponsiveness could be induced in a CD4+ helper T cell clone by direct exposure to antigen, the influence of exogenous suppressor T cells (CD8+) was excluded. Moreover, the induction of tolerance in the absence of accessory cells raised the possibility that the T cell receptors were recognising peptide alone and therefore not receiving the complete signals required for activation (Cohn and Epstein, 1978). Alternatively, the

T cell clones which characteristically express MHC class II determinants (Lamb et al., 1982a), were presenting the peptide antigen in association with class II determinants on the T cells and again in the absence of additional signals such as lymphokines, activation was incomplete. Serological inhibition studies (Lamb and Feldmann, 1984), revealed that murine monoclonal anti-MHC class II antibodies were capable of blocking both the immunogenic recognition of antigen and the induction of tolerance in vitro. Therefore, as shown in vivo, T cell unresponsiveness in vitro appears to be MHC-restricted (Groves and Singer, 1983; Matzinger, Zamoyska and Waldmann, 1984; Rammensee and Bevan, 1984). Phenotypic analysis using monoclonal antibodies demonstrated extensive loss of the surface CD3-Ti receptor complex (Zanders et al., 1984). The enhanced expression of IL-2-receptor and CD2 during the induction of functional unresponsivenss (Zanders et al., 1984) was paralleled by an increase in the mRNA levels for both IL-2 receptor and CD2 while the transcripts for CD3-Ti were unaltered (Lamb et al., 1987). In contrast, no IL-2 mRNA transcripts were detectable after exposure to a tolerising concentration of peptide (Lamb et al., 1987). Thus the mechanism of tolerance induction in this model would appear to be a loss of surface CD3-Ti concomitant with down-regulation of the IL-2 gene.

Jenkins et al. (1987) examined the antigen specificity and presentation requirements for the induction of T cell unresponsiveness in vitro and in vivo, using the well-defined proliferative response of B10.A mice to pigeon cytochrome C as their model. After incubation with chemically-modified antigen presenting cells, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, (ECDI)-treated splenocytes, it was observed that the T cells were rendered anergic to a subsequent immunogenic challenge, using normal accessory cells. This model of unresponsiveness was applied both in vitro and in vivo,

with the induced anergy revealing the same requirements of antigen and Ia specificity as the T cell activation. These findings also argued against T suppressor cells being the only inducers of unresponsiveness.

Decreased transcription of the IL-2 gene accompanied by increased intracellular calcium (Jenkins et al., 1987) was demonstrable in this model. A central role for IL-2 in T cell tolerance was also suggested by the experiments in which the introduction of IL-2 into skin grafted neonatally-tolerised mice resulted in graft rejection (Malkorsky et al., 1985). Normally, the interaction of the T cell receptor with antigen occupying the combining site of MHC class II molecules induces lymphokine production and other "second signals" required for activation. In the absence of these second signals the formation of a ternary complex of T cell receptor, antigen and MHC may be insufficient for full activation culminating in an inability to transcribe the IL-2 gene which would occur under normal conditions of antigen presentation. The result would be the induction of T cell anergy.

Further evidence was provided by studies demonstrating that normal T cell clones do not proliferate in response to antigen presented by purified Ia molecules impregnated into planar membranes, but instead are then rendered anergic to a normal immunogenic challenge (Quill and Schwartz, 1987). Therefore, the T cell receptor occupancy by antigen complexed with MHC class II in the absence of the uncharacterised "second signals" deliver a negative signal. It would appear that other ligand/receptor interactions such as the CD2 (Lamb et al., 1987) and IL-2 receptor (Wilde and Fitch, 1987) are also able to induce antigen-specific unresponsiveness. In the latter case, IL-2 induced antigen-specific anergy by inhibiting calcium-

dependent signalling (Otten, Herold and Fitch, 1987). The T cell clones investigated by Jenkins et al. (1987) were of the T_H1 subset, however, the ability to tolerise the antigen-dependent helper activity of both murine (Levich, Parks and Weigle, 1985) and human (Lamb et al., 1985) T cell clones capable of supporting B cell help has been described. In the latter case, the ability of antigen to tolerise both proliferation and help was compared and significantly lower concentrations of antigen were required to tolerise help.

III Clinical tolerance induction with respect to allergy

Although allergen hyposensitisation is practised widely little information is available on the immunological mechanisms by which it may modify allergic responses. Several studies have examined the effects of conventional low dose allergen immunotherapy on the in vitro responses of T cells isolated from atopic patients. During immunotherapy T lymphocyte proliferation to the specific allergen was decreased (Gatien, Merler and Colter, 1975; Evans et al., 1976; Anderson et al., 1974; Hsieh, 1982, 1984). Possible explanations that were considered were the generation of allergen-specific suppressor cells (Rocklin et al., 1980; Hsieh, 1984), decreased antigen-specific sensitivity of helper T cells (Hsieh, 1985a), diminished IL-2 production (Evans et al., 1976; Hsieh, 1985b) and decreased expression of high-affinity IL-2 receptors (IL-2R; Hsieh, 1987). Hsieh (1988) recently demonstrated an increase in the serum IL-2R in both new and hyposensitised asthmatic patients as compared to normal controls, postulating that there may be persistent activation of lymphocytes by allergen in vivo. In contrast, comparable levels of soluble IL-2R were produced in all groups using the mitogen phytohaemagglutinin (PHA). However, when the specific house dust mite allergen was used as a stimulant, lymphocytes from new asthmatic

patients produced a much greater amount of soluble IL-2R than did those of normal subjects, and this heightened response in patients was decreased after immunotherapy.

It has been postulated that overproduction of specific IgE by atopic individuals with allergic disease might be due to altered regulatory function affecting non-specific and/or antigen-specific suppressor function. Canonica et al. (1979) suggested that atopic subjects pre-immunotherapy had a decreased number of Fc gamma T cells, which were increased to normal levels after successful immunotherapy. Cells within this population have been reported to suppress T-dependent B cell differentiation. Additionally, Fc gamma T cells bearing H_2-receptors have been reported to exhibit histamine-triggered suppressor activity. Supporting the suppression theory, Rocklin et al. (1980), in a study of ragweed-allergic subjects during immunotherapy for 6-12 months, demonstrated the appearance of ragweed antigen-specific suppressor T cells, not detectable prior to immunotherapy. This population could be deleted by passage of the T cells over a column containing insolubilised histamine. These findings suggested that H_2-receptor positive T cells were generated during ragweed desensitisation.

Nagaya (1985) studied a group of rye-grass allergic subjects and reported that immunotherapy induced suppressor T cells. The cells adhered to allergen-coated plates and were capable of suppressing specific allergen-dependent proliferation. However, the suppression was reversible by pretreatment of the adherent T cells with anti-CD8 plus complement. Immunotherapy has been reported to increase the number of suppressor T cells, their potency and to induce their de novo generation (Tamir, Castracane and Rocklin, 1987). In a study involving five ragweed-allergic

individuals before and after allergen immunotherapy, they investigated whether the suppressor T cell population that was generated during immunotherapy could modulate IgE production. They observed that depletion of the CD8+ T cells generated during immunotherapy resulted in enhancement of in vitro IgE production. This effect was only demonstrated after one year of immunotherapy, with no effect being observed prior to immunotherapy. Furthermore, the effect observed was antigen-specific as the addition of ragweed antigen E to the cultures did not alter IgE synthesis before immunotherapy whereas a decrease in IgE production was triggered one year after therapy. Elimination of CD8+ cells from the culture could abrogate the ragweed antigen E-induced suppression of IgE synthesis.

Following immunotherapy, the immediate skin reaction to injected allergen (the standard subcutaneous skin prick test) is decreased to a variable degree but does not correlate with the clinical success of the immunotherapy. The cutaneous late-phase reaction is substantially attenuated or even abrogated in patients who have had successful hyposensitisation (Metzger et al., 1987). Levels of allergen-specific IgE however, usually show an initial rise and then decrease over a period of 2-3 years. Blunting of the normal seasonal rise in IgE observed in pollen-sensitive patients also occurs. (Levy et al., 1971; Gleich et al., 1977; 1982; Evans et al., 1976).

A possible reason for the lack of good clinical response to house dust mite immunotherapy could be a result of complex mixtures of allergen present in the extracts used. Unpurified mite preparations have been demonstrated to contain as many as nineteen allergens yet only four of these appear to be of major clinical significance (Krilis, Baldo and Basten, 1984; Lind and Lowenstein, 1983; Stewart et al., 1986). On this basis, Stewart and Holt

(1987) examined the nature of the humoral immune response (IgE and IgG isotypes) to the D. pteronyssinus-derived allergen Der p I using rats and mice, attempting to manipulate the immune response at the molecular level. They demonstrated that the purified allergen was tolerogenic in animals exposed to this allergen via a mucosal route, in particular the respiratory system. Primary immunisation of different inbred strains of mice with Der p I absorbed to adjuvant revealed that certain strains (CBA and C57 Black) were high IgE responders, whereas other strains (C3H, AKR and Balb/c) were poor responders. These responses were adjuvant-independent and could be adoptively-transferred using immune spleen cells. Repeated intranasal administration of soluble-allergen induced IgE tolerance in rats, however, this was restricted to the low IgE responder phenotype.

1.7 HUMORAL IMMUNE RESPONSES

The mechanisms whereby antigen-specific T lymphocytes are able to regulate IgE synthesis have attracted much research over the twenty year period since the discovery of IgE (reviewed in Leung and Geha, 1987; Ishizaka, 1984).

I Humoral responses to house dust mite allergens

The humoral immune response to the major mite allergen Der p I has been extensively studied in patients suffering from perennial rhinitis, perennial atopic asthma and eczema and in patients undergoing allergen immunotherapy. Most patients with positive skin prick tests to D. pteronyssinus have detectable anti-Der p I of IgE, IgG and IgA isotypes as measured by radioimmunoassays. In contrast, most skin test negative

control subjects have been reported to have no detectable serum antibody of any isotype, 20-30% producing low levels of IgG and IgE antibody. IgE antibody was never detected in the absence of IgG antibody with a good quantitative correlation existing between the levels of IgG and IgE antibody. Ratios of IgG antibody:IgE antibody ranged from 2:1 to 15:1. (Chapman and Platts-Mills, 1980; Chapman and Platts-Mills, 1978; Chapman et al., 1980; Chapman et al., 1983) RAST absorption studies demonstrated that in some patients IgE-anti-Der p I antibody comprised 40-75% of IgE anti-mite antibody and $\leq$20% of the total IgE (Chapman and Platts-Mills, 1980). A similar correlation between IgG and IgE antibody has been observed with purified pollen allergens and so raises the possibility that the two isotypes are under common genetic and immunoregulatory control.

Prospective studies on U.K. children born to at least one atopic parent have demonstrated the presence of IgG and IgE anti-Der p I antibody from the age of two years, increasing in both prevalence and mean concentration of both isotypes up to five years of age (Rowntree et al., 1985). Levels of antibody tend to fall with increasing age (Rawle, Burr and Platts-Mills, 1983; Hill et al., 1981). A broad correlation has been shown to exist between the level of antibody present and the severity of the allergic manifestations, patients with perennial rhinitis tending to have lower levels than those with severe asthma and/or eczema (Chapman et al., 1983).

Several studies of allergen immunotherapy using aqueous D. pteronyssinus extracts, have shown significant increases in IgG anti-Der p I antibody with either no change or a slight decrease in IgE antibody (Chapman et al., 1983; Bousquet et al., 1985). Although patients tended to report clinical improvement no correlation was observed quantitatively with changes in IgG

levels.

More recent studies using other purified mite major allergens have demonstrated similar patterns of response. In particular, humoral responses to allergens within the same group appeared cross-reactive, with most Der p I sensitive patients producing IgG and IgE antibody to Der f I and Der m I, and similarly, cross-reactive IgG and IgE antibody responses for Der p II and Der f II (Heymann et al., 1987).

II Animal models of allergy

The localisation of B cell sites on Der p I and Der f I has been studied with human, murine and rabbit antibodies in immunoabsorption and epitope-mapping experiments. It was demonstrated using cross-absorption studies that 80-90% of human IgE antibody is directed against shared epitopes on Der p I and Der f I (Heymann et al., 1986). Generally the IgE antibody response was cross-reactive, however, in some patients a preponderance of antibody was directed against species-specific sites (Lind, Ingemann and Brouvez, 1987). In contrast, polyclonal and murine monoclonal antibodies predominantly recognised species-specific epitopes on either allergen (Chapman, Sutherland and Platts-Mills, 1984; Chapman, et al., 1987; Horn and Lind, 1987; Heymann et al., 1986). These epitope-mapping studies have highlighted marked differences in the repertoires of human IgE and murine IgG antibody responses to Der p I and Der f I.

Animal studies with mice and rats employing the repeated inhalation of aerosols containing ovalbumin or ragweed antigen induced transient IgE responses which spontaneously "switched off" despite continuing aerosol

exposure (Fox and Siraganian, 1981). Subsequent challenge of the animals revealed that aerosol exposure had selectively suppressed responsiveness to these antigens in the IgE antibody class. Tolerance lasted three months after cessation of the aerosol exposure (Holt and Leivers, 1982). Furthermore, the antigen and IgE-isotype-specific suppression was readily transferable with splenic and lymph node lymphocytes of suppressor phenotype (Sedgwick and Holt, 1984). Helper T cells were shown to be the targets for suppression in this model with specific B cell function unaffected (Sedgwick and Holt, 1985) The suppression of immune responsiveness to the antigen appeared restricted to the IgE-isotype in mouse strains of high IgE responder phenotype (Holt, Batty and Turner, 1981; Holt and Leivers 1982) and under most circumstances in high IgE responder rat strains (Sedgwick and Holt, 1983; Sedgwick and Holt, 1985). However, using nanogram amounts of antigen as would be encountered by non-atopic subjects in the normal environment it was possible to induce tolerance in low IgE responder animal strains (Sedgwick and Holt, 1984); one thousand-fold greater concentrations being required to achieve the same effect in high IgE responder rat strains.

In other murine studies of the IgE antibody response to mite antigens (Kudo et al., 1978) suppression of the IgE antibody response was obtained using chemically-modified antigen. More recently, studies of IgG and IgE-responsiveness of inbred strains of rats and mice have examined the immunogenicity and tolerogenicity of highly-purified Der p I (Stewart and Holt, 1987). Repeated intranasal administration of soluble allergen induced IgE tolerance in the low IgE responder phenotype strain of rats.

III Models for studying the IgE antibody response using human lymphocytes

The observation of an association between patients with primary immunodeficiency and excessive IgE antibody production (Buckley and Becker, 1978; Buckley and Fiscus, 1975; Buckley and Sampson, 1981) has provided a model for studying the mechanisms of IgE regulation in man. Wiskott-Aldrich syndrome, in particular, combines a form of atopic dermatitis, partial impairment of both B and T cell function and an increase of IgE antibody synthesis (Buckley and Fiscus, 1975; Polmar, Waldmann and Terry, 1972). Selective IgA deficiency is also associated with increased IgE synthesis, a high frequency of atopy and the increased susceptibility to infection (Buckley and Fiscus, 1975). In addition, infants with thymic aplasia (Nezelof syndrome; Kikkawa et al., 1973) and thymic hypoplasia (DiGeorge syndrome; Polmar et al., 1972) have elevated IgE antibody synthesis. Taken together, these observations suggest a relationship between cellular abnormalities and allergic diseases associated with elevated IgE antibody levels. Furthermore, in patients with acute graft-versus-host disease, elevated serum IgE levels develop in association with the severe imbalance in circulating T cell phenotypes (Saryan et al., 1983; Geha et al., 1980). Such hyper-IgE patients have provided the means for propagating T cell lines and clones to allow examination of T cell phenotypes and secreted IgE binding factors (Saryan, Leung and Geha, 1983; Romagnani et al., 1983; Young, Leung and Geha, 1984; Geha and Leung, 1986). Problems arise, however, with the extrapolation of findings from pathological variants to the pattern of IgE regulation observed in normal individuals.

Apart from conditions predisposing to hyper-IgE states the study of human IgE synthesis has, until recently, been limited to the spontaneous release of IgE antibody by B cells from allergic individuals (Hemady et al., 1983; Sampson and Buckley, 1981). Studies using polyclonal activators such as pokeweed mitogen were notoriously unreliable in inducing IgE synthesis in peripheral blood B cells from both non-atopic and atopic subjects (Hemady et al., 1983; Saryan, Leung and Geha, 1983). Several groups then focused on the possibility of inducing a polyclonal IgE response in vitro using cloned T lymphocytes, with reports that human alloreactive T cell clones could induce IgE secretion by human B cells (Lanzavecchia and Parodi, 1984; Lanzavecchia, 1983; Umetsu et al., 1985). Lanzavecchia (1983) reported that one out of five peripheral blood lymphocytes could be activated to high-rate immunoglobulin production by human alloreactive T cell clones. However, the allergic status of the B cell donors used in this study was not assessed and it has been well-documented that B cells from allergic individuals are able to spontaneously secrete IgE (Hemady et al., 1983; Sampson and Buckley, 1981; Tjio, Hull and Gleich, 1979). Umetsu et al. (1985) demonstrated that an alloreactive T cell clone was able to induce IgE synthesis in B cells from both allergic and non-allergic individuals. They observed that IgE synthesis could be induced in "non-allergic" B cells only when cognate interactions with T cells occurred, with the B cells expressing alloantigen recognised by the T cells. In contrast, IgE synthesis in "allergic" B cells occurred both via cognate interactions and under bystander conditions. In other studies (Leung, Young and Geha, 1986) IgE and IgG synthesis were induced in normal autologous B cells by autoreactive T cell clones which followed the same pattern of HLA-DR restriction as observed in the proliferative responses of the clones.

Using filarial parasite-specific T cell lines or their supernatants (Nutman et al., 1985), IgE synthesis has been demonstrated in "non-atopic" B cells. Interestingly, when using T cell lines against a non-parasite antigen, IgE synthesis could not be induced. Similarly, Umetsu et al. (1985) could not induce IgE synthesis in normal B cells using tetanus toxoid-specific clones.

In a more recent model (Maggi et al., 1988) phytohaemagglutinin-driven T cell clones were used to stimulate marked IgE synthesis from "atopic" B cells, with much lower levels of IgE synthesis from "non-atopic" B cells. However, culture supernatants from all the T cell clones were able to induce comparable levels of IgG and IgM synthesis in B cells from both sources. The IgE helper activity described was compatible with IL-4, possibly acting in combination with other lymphokines.

IV Lymphokines involved in IgE synthesis

Current experimental models of the human B cell response suggest that B cells undergo a series of transformations; activation, proliferation and differentiation into immunoglobulin-secreting plasma cells (reviewed in Howard and Paul, 1983; Abbas, 1988; Gordon and Guy, 1987). Initially the lymphokines involved in the process were described as B cell growth factors and B cell differentiation factors. However, with the recent availability of recombinant lymphokines, a better understanding of this process has been facilitated. It has become clear that the same factor may influence all stages whereas other factors may operate at one particular stage.

The well-characterised lymphokines involved in B cell function and activation, which have been cloned to date, are IL-1, IL-2 and IFN-gamma

(Roehm, Marrack and Kappler, 1983; Howard and Paul, 1983), IL-4 (B cell stimulatory factor-1, BCSF-1; Noma et al., 1986; Lee et al., 1986), IL-5 (B cell growth factor-II or BCGF-II, T cell replacing factor or TCRF; Kinashi et al., 1986) and IL-6 (B cell stimulatory factor-2, BCSF-2, or interferon-beta$_2$; Hirano et al., 1986). These lymphokines are involved in both B cell growth and their differentiation into antibody-secreting cells. They have also been shown to selectively or preferentially influence isotype switching, for example, IL-4 has been shown to selectively enhance IgG_1 and IgE synthesis in murine B cells stimulated with lipopolysaccharide, suppressing IgG_{2b} and IgG_3 (Isakson et al., 1982; Coffman and Carty, 1986; Coffman et al., 1986; Snapper, Finkelman and Paul, 1988). Subsequent experiments performed in vivo supported these observations. Finkelman et al. (1986) showed that helminthic-infected mice had a 100-fold increase in serum IgE concentrations two weeks after inoculation with N. brasiliensis larvae. The administration of antibody directed against IL-4 blunted this rise. Kinetic studies have demonstrated that IL-4 acts both at the early and late phases of the IgG_1 and IgE responses (Snapper et al., 1988). The early phase of both these responses appeared to be concentration independent, whereas the late phase required a high concentration of IL-4 for IgE production to be expressed and the addition of IL-4 late in the culture period was inhibitory for IgG_1 production. Furthermore, anti-IL-4 antibody could abrogate these effects. In addition, IL-4 has been shown to be a growth factor for mast cells and T lymphocytes (reviewed in Paul and Ohara, 1987). IL-5, in contrast, has been demonstrated to preferentially induce IgA production (Mosmann and Coffman, 1987) with a further role in eosinophil differentiation (O'Garra et al., 1986).

The actual obligatory roles of these lymphokines in T-dependent B cell

activation is unclear. In the murine model, two distinct T cell populations (T_H1 and T_H2) have been described (Mosmann and Coffman, 1987), with the former secreting IL-2 and IFN-gamma and the latter secreting IL-4 and IL-5. Studies have demonstrated that efficient B cell activation can occur by T_H2 cells in the absence of IL-2 and IFN-gamma. Furthermore, it has been shown that IFN-gamma may regulate immunoglobulin synthesis by inhibiting most B cell responses, but selectively promoting switching to IgG_{2a} isotypes (Snapper and Paul, 1987). As most of these studies were performed in the mouse model their accuracy in the human system remains to be demonstrated. Other studies have used T helper clones with a defective capacity to support immunoglobulin synthesis. The addition of selected purified lymphokines to restore functional activity provided an insight into the role of these factors. In this way it was concluded that IL-1, IL-2 and IFN-gamma were important for the induction of antibody synthesis (Roehm et al., 1983).

V Regulation of IgE by IgE binding factors

Studies examining specific IgE isotype regulation have been the focus of a large volume of research (reviewed in Ishizaka, 1984; Katz, 1982; Kishimoto, 1982; Gaveriaux, Payne and Loor, 1988). Opinion was divided as to whether high or low IgE responsiveness was regulated by T (Ishizaka, 1984; Kishimoto, 1982) or B (Katz, 1982) cells.

Ishizaka (Ishizaka, 1983; Ishizaka et al., 1983) reported that IgE-specific regulatory T cells, which were Lyt-1+ cells possessing Fc receptors for IgE ($Fc_{epsilon}$ R+ T cells), secreted low molecular weight glycoproteins which bound to IgE, IgE binding factors. They described rat IgE-potentiating

factors and IgE-suppressive factors, both of 15,000 MW but differing in their carbohydrate content. Both could bind to B cell membranous IgE and modulate the ability of the B lymphocytes to be activated to form IgE plasma cells, thus regulating serum IgE production. The level of glycosylation of the IgE binding factors were reported to be under the control of at least two other factors, glycosylation inhibiting factor (GIF), released by Lyt-2+ T cells and glycosylation enhancing factor (GEF), for Lyt-1+ T cells. It has been suggested that the ratio of these two products is determined by the type, concentration and mode of antigen administration and in turn determines the form of binding factor synthesised.

In the model described by Katz (1982), the central cells were low $Fc_{epsilon}R+$ B cells, which on exposure to serum IgE produced a factor (IgE induced regulant of B cell origin) which in turn increased the expression of $Fc_{epsilon}R+$ on B cells. These B cells then interacted with the Lyt-1+ subset of T cells to produce suppressive factor of anaphylaxis (SFA) or with the Lyt-2+ subset to produce IgE-induced regulant of T cell origin (EIR_T). The SFA in turn acted on Lyt-1+ T cells to induce synthesis of the suppressor effector molecule (SEM) which suppressed IgE synthesis in vitro and in vivo. An additional factor, enhancing factor of anaphylaxis (EFA), was reported to be synthesised by ill-defined T cells with an action on the new $Fc_{epsilon}R+$ B cells, stimulating the production of the enhancing effector molecule (EEM) which enhanced IgE synthesis in vitro and in vivo. Both EEM and SEM were IgE binding factors.

Young et al. (1986) isolated a human factor reported to have IgE potentiating activity and affinity for IgE from the supernatants of $Fc_{epsilon}R+$ T cell lines derived from individuals with the hyper-IgE

syndrome. The factor(s), which were not isotype-specific, did not bind to immobilised IgE, suggesting a possible role as a differentiation signal for B cells. Human IgE binding factors have also been identified, characterised and shown to regulate IgE synthesis in an isotype-specific fashion (Leung et al., 1984; 1986; Ishizaka and Sandberg, 1981; Young, Leung and Geha, 1984).

Both major models of IgE regulation describe complex cascades of positive and negative regulatory cells and factors, in particular IgE binding factors, rather than a direct antigen effect on IgE-bearing B cells. Subsequent gene cloning and expression are needed to clarify the role of such factors and the applicability of such models in vivo.

TABLE I.1

Highly purified allergens from pyroglyphid mites (*Dermatophagoides* spp.)

Allergen	Previous nomenclature	MW
Group I		
Der p I	P1, Dp 42, Dpt 12	24,000
Der f I	F1, Ag 11, Df 6	24,000
Der m I	Dm6	24,000
Group II		
Der p II	Dp X	15,000
Der f II	Ag 19/20, DF 2	15,000
Der m II		15,000
Group III		
Der f III		29,000

(after Platts-Mills and Chapman, 1987)

TABLE L2

Structural homology of house dust mite major allergens: N-terminal sequences.

Group I allergens:

	1																			20									29
Der f I:	T	S	A	C	R	I	N	S	V	N	V	P	S	E	L	D	L	R	S	L	M	R	T	V	T	P	I	R	M
Der m I:	T	S	A	-	R	I	N	S	* G	N	V	P	S	E	L	D	L	* R	S	L	R	T	V	T	P	I	R	M	Q
Der p I:	-	N	A	-	-	I	N	G	N	A	P	A	E	I	D	L	R	Q	M	R	T	V	T	P	I	R	M	Q	G

Group II allergens:

	1							10												20
Der p II:	D	Q	V	D	V	K	D	-	A	N	H	E	I	K	K	V	L	V	P	G
Der f II:	D	Q	V	D	V	K	D	-	A	N	* N	E	I	K	K	V	* M	V	* D	G

	30										35									40
Der p II:	-	H	G	S	E	P	-	I	I	H	R	G	K	P	F	Q	L	E	A	V
Der f II:	-	H	G	S	* D	P	-	I	I	H	R	G	K	P	F					

Group III allergens;

	1																			20
Der f III:	I	V	G	G	V	K	A	L	A	G	D	-	P	Y	E	I	S	L	E	V

(after Chapman, Heymann and Platts-Mills, 1988)

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2.1 ANTIGENS

I Dermatophagoides farinae (D. farinae)

A purified, freeze-dried preparation, Spectralgen D. farinae, was generously supplied by Pharmacia (Uppsala, Sweden). Spectralgen D. farinae was produced from isolated and crushed mites grown on a daphnia and yeast medium, free from human dander contaminants. After extraction, a purification procedure was performed removing material of both high molecular weight (MW >70,000 daltons) on a sepharose column and low MW (<5,000 daltons) by hollow fibre (Dale and Landmark, 1984). Lot number GK 23605 was used throughout this work. Possible mitogenicity of the preparation was excluded by proliferation assays using peripheral blood mononuclear cells (PBMC) from newborn infants which failed to respond to the allergen preparation (Halvorsen et al., 1986). Allergen content was expressed in biological units/ml (B.U./ml), being an arbitrary unit used to express the allergenic activity of an allergen extract. The median histamine equivalent concentration Ch_1 (response corresponding to histamine hydrochloride 1 mg/ml, in a skin prick test) obtained in at least twenty patients fulfilling the inclusion criteria is defined as having an allergenic activity of 1,000 B.U./ml (Dreborg, Einarrson and Longbottom, 1987).

10^3 Biological Units = 2.5 µg dry weight

= 1 µg protein /ml

Antigens were reconstituted with RPMI-1640 medium to stock solutions of 10^5 B.U./ml, filtered with a 0.2 micron Millipore filter and stored at -20°C or at 4°C for short periods.

II Dermatophagoides pteronyssinus (D. pteronyssinus)

a. Pharmalgen D. pteronyssinus

A lyophilised preparation of Pharmalgen D. pteronyssinus was kindly provided by Pharmacia, containing mite bodies, culture medium and faecal particles. Materials of low molecular weight (MW <5,000 daltons) were removed by hollow fibre (Ewan et al., 1988). The extract contained 15% dry weight of the major allergen Der p I with 10^5 B.U. being equivalent to approx. 260,000 IU of the international standard of D. pteronyssinus when assessed by RAST inhibition (Pharmacia in-house data). Lot number KB 31373 was used. Reconstitution was as detailed in 2.1.I.

b. Bencard D. pteronyssinus

A lyophilised whole mite culture extract of D. pteronyssinus was generously provided by Bencard (Brentford, Middlesex). Lot number W 2176 was used. Stock solutions of 1 mg/ml were prepared in RPMI-1640 medium and filtered and stored as in 2.1.I.

III Affinity-purified major allergens of D. farinae and D. pteronyssinus

Aqueous preparations of the house dust mite major allergens were kindly provided by Drs. M.D. Chapman and P.W. Heymann (University of Virginia, U.S.A.).

a. Antigen Der f I

D. farinae culture (Hollister-Stier, Spokane, Washington) was extracted with borate buffered saline and precipitated with 50% saturated ammonium sulphate and S200 gel filtration (Heymann et al., 1986).

b. Antigen Der p I

Der p I was purified from D. pteronyssinus culture (Bencard) by aqueous extraction for 4 hours and precipitation with 50% saturated ammonium sulphate. Preparative electrophoresis was on Pevikon and Sephacryl S200 gel filtration (Chapman and Platts-Mills, 1980). On SDS-PAGE the purified Der p I migrates as a single band at 24kD.

c. Antigen Der f II

A second major allergen of D. farinae has been purified to homogeneity from 50-80% saturated ammonium sulphate fractions of aqueous D. farinae extract (Heymann et al., 1987). Der f II was purified by affinity chromatography using a monoclonal antibody specific for Der p II which showed cross-reactive binding with D. farinae protein. On SDS-PAGE the purified Der f II migrates as a single band at 12.5 kD.

IV Peptides of Der p I

Peptides of selected sequences of Der p I (Chua et al., 1988) were synthesised using solid phase techniques on an Applied Biosystems 430A peptide synthesiser. They were the kind gift of Dr. J.B. Rothbard (I.C.R.F., London; Rothbard, 1986). The sequences were selected for synthesis based on the presence of a distinctive pattern consisting of a charged or glycine residue followed by two or three hydrophobic residues and terminating in a polar amino acid. The following peptides were synthesised:

Der p I 12-25	AEIDLRQMRTVTPI
Der p I 51-64	RNQSLDLAEQELVD
Der p I 81-102	EYIQHNGVVQESYYRYVAREQS
Der p I 93-108	YYRYVAREQSARRPNA

Der p I 123-136	NANKIREALAQPQR
Der p I 135-149	QRYSRHYWTIKDLDA
Der p I 156-168	RTIIQRDNGYQPN
Der p I 165-179	YQPNYHAVNIVGYSN
Der p I 185-196	YWIVRNSWDTNWG
Der p I 197-212	GDNGYGYFAANIDLMM

Cyanogen bromide (CNBr) treatment, which cleaves at the methionine residues, successfully inactivated the Der p I preparation (kindly performed by Dr. J.B. Rothbard). Two subsequent peptides containing methionine residues were synthesised:

Der p I 12-34	AEIDLRQMRTYTPIRMQGGSGSS
Der p I 209-222	DLMMIEEYPYVVIL

Peptides were solubilised in saline to form a stock solution of 1 mg/ml, filtered with a 0.2 micron Millipore filter and stored at -20°C until use.

V Parietaria judaica, Timothy grass and mixed grass pollen

Pharmalgen and Spectralgen lyophilised preparations of these allergens were provided for use as control antigens; lot number LH 36274 for Pharmalgen Parietaria judaica, lot number MA 36351 for Spectralgen Timothy grass and lot number KA 31359 for Spectralgen grass-mix. Reconstitution and storage were as described for Dermatophagoides spp. preparations.

VI Influenza haemagglutinin peptide

The synthetic peptide, residues 306-324 (HA306-324) of the carboxyl terminus of the HA1 molecule of influenza haemagglutinin, was the kind gift of Dr. J.R. Rothbard, Imperial Cancer Research Fund, London.

VII Phytohaemagglutinin (PHA-P)

The lectin PHA-P (salt-free, lyophilised) was purchased from Sigma Pharmaceuticals.

VIII Immunoblotted antigen preparations

Antigen insolubilised on nitrocellulose is able to activate T lymphocytes following presentation by appropriate accessory cells. Furthermore, complex mixtures of antigens can be fractionated by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE), transferred to nitrocellulose membrane and then added to T cell proliferation assays (Young and Lamb, 1986).

a. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

i. Buffers and gels

1. <u>Running gel (12%)</u>

acrylamide	0.4 ml

distilled water	1.4 ml
0.5M Tris pH 6.8	0.6 ml
SDS	25 µl
N'N'N'N' tetramethylethylene-diamine (TEMED)	3.5 µl
ammonium persulphate-(APS)	35 µl

2. Stacking gel

acrylamide	2.0 ml
distilled water	1.7 ml
1M Tris pH 8.8	1.2 ml
10% SDS	50 µl
TEMED	7.5 µl
APS (100 mg/ml)	75 µl

3. Acrylamide stock

acrylamide	73 g
bis-acrylamide	2 g

4. Gel buffer

glycine	14.4 g/l
Tris	3 g/l
SDS	1 g/l

5. Sample buffer

glycerol	20% (v/v)
2 mercaptoethanol	10% (v/v)

SDS	4.6% (w/v)
0.125M Tris HCl pH 6.8	
0.1% bromophenol blue	a few crystals

ii. **Procedure**

Proteins were separated by polyacrylamide gel electrophoresis in the presence of SDS after denaturation of the sample under reducing conditions by boiling in the presence of SDS and 2-mercaptoethanol described by Laemmli (1970), using an acrylamide concentration of 12-15% (w/v). Gels were prepared on the small scale "mini-gel" apparatus developed by Hoeffer Scientific Instruments (San Francisco, CA), with dimensions (8cm x 5cm). To allow multiple assays to be carried out a blank comb having a single reference well for molecular weight markers was used with a sample loading of 0.1-0.2 mg protein per gel (Plate 2).

b. Transfer to nitrocellulose

i. Buffer

1. Blot buffer

Tris	3 g/l	]	
		]	
glycine	14.4 g/l	]	2 litre
		]	
methanol	20%	]	

ii. **Procedure**

Separated proteins were transferred from the gel to a nitrocellulose membrane by electrophoresis for one hour at 50V in blot buffer (Towbin, Staehelin and Gordon, 1979). Blots were reversibly stained by dipping for a

few seconds in Amido black (0.01% in 0.5% acetic acid) followed by washing with distilled water for localisation of particular proteins or to check for successful transfer.

To remove residual dye and SDS, blots were washed for one hour with three changes of PBS containing 0.2% Triton X-100, and then for a further one hour with PBS alone.

c. Use in T cell proliferation assays

For addition to proliferation assays, immunoblots (0.5 x 4 cm) were cut into 20 sections of 2mm each to cover the 4cm length of the running gel. These sections were then solubilised using dimethylsulphoxide (DMSO; BDH Chemicals, Poole; Abou-Zeid et al., 1987). Each section was incubated with DMSO (1 ml) and intermittently vortexed for 1 hr at room temperature then reprecipitated by the addition of an equal volume of bicarbonate/carbonate buffer, pH 9.6, to allow reprecipitation. The preparations were then collected by centrifuging at 10,000 rpm, washed four times with complete medium and resuspended to a final volume of 0.5 ml, of which 20 µl were added to each well in proliferation assays. A cycle of freeze-thawing was used to decrease the tendency for clumping of the particulate material. Molecular weights were determined from a standard curve obtained using molecular weight markers (Sigma, Poole, Dorset).

2.2 Antibodies

I Anti-human lymphocyte surface antigens

Leu 2a (CD8), IgG_1, culture supernatant (Becton-Dickinson, Sunnyvale, CA, U.S.A.).

Leu 3a (CD4), IgG_1, culture supernatant, serum or ascitic fluid of tumour-bearing BALB/c mice (Becton-Dickinson).

Leu 4 (CD3), IgG_1, culture supernatant (Becton-Dickinson).

Leu 8, IgG_{2a}, serum or ascitic fluid of tumour-bearing BALB/c mice (Becton-Dickinson).

Anti-IL2R (CD25), IgG_1, culture supernatant (Becton-Dickinson).

UCHL1 (CD45, 180 kD chain), helper-inducer subset of $CD4^+$ T lymphocytes IgG_{2a}, (Smith et al., 1986), (Dr. P. Beverley, University College Hospital, London).

OKT3 (CD3), IgG_1, culture supernatant (Ortho Diagnostic Systems, Raritan, NJ, U.S.A.).

II Anti-human MHC class II gene products

L243, anti-HLA-DR (alpha beta I and alpha beta III), IgG_{2a}, from serum and/or ascitic fluid of BALB/c mice immunised with the human lymphoblastoid B cell line, RPMI 8866, (Becton-Dickinson).

Leu 10, anti-HLA-DQ (DQw1 and DQw3), IgG_1, culture supernatant, (Becton-Dickinson).

B7/21, anti-HLA-DP, IgG_1, culture supernatant, (Becton-Dickinson).

III Miscellaneous

a. **Anti-IL4 receptor** (Larche, Lamb and Ritter, 1988)

b. **Anti-human placental alkaline phosphatase, H17E2** (Travers and Bodmer, 1974).

c. **Anti-human B lymphoma idiotype, 2118** (Unilever, Bedford)

d. **Anti-interferon-gamma**

Lot number 73A-E10 generously provided by Dr. G. Garotta, (Hoffman-Le Roche, Basle).

2.3 Lymphokines

I Interleukin 2 (IL-2)

IL-2 was obtained from commercial sources. Batches were screened in proliferation assays over a range of concentrations to select optimal conditions for culture supplementation (Fig 2.1).

1. Lymphocult-T (Biotest Folex, Frankfurt, FRG)
2. Recombinant IL-2 (Boehringer, Mannheim, FRG).

II Interleukin 4 (IL-4)

Genzyme recombinant IL-4 was the generous gift of Glaxo (Greenford, Middlesex, U.K.). It was used at a final concentration of 100 U/ml; diluted in RPMI-1640 medium and filtered with a 0.2 micron Millipore filter prior to use.

2.4 Lymphocyte culture

I. Media

Culture medium used was RPMI-1640 with 2 g/l sodium bicarbonate, (Flow Laboratories, Irvine, U.K.) supplemented with 2mM L-glutamine (Flow), 100 IU/ml penicillin and 100 µg/ml streptomycin (Flow) (complete medium). This was supplemented with 5-10% heat-inactivated, pooled human A^+, AB^- or foetal calf serum (FCS) which were screened in proliferation assays to exclude the presence of non-specific inhibitors and to determine the optimal concentration (Fig. 2.2). Sera were filtered with a 0.2 micron Millipore filter prior to use. The use of FCS or A^+ serum to supplement media is indicated in the text.

II Lymphocyte preparation

a. Donors

i. Atopic individuals

Atopic status was defined by the presence of positive skin prick tests to the common environmental aeroallergens (D. pteronyssinus, D. farinae, mixed grass pollen, cat dander, dog dander, horse dander, Aspergillus fumigatus, Cladosporium herbarum, Alternaria), positive radioallergosorbent tests (RAST) to common aeroallergens (D. pteronyssinus, D. farinae, mixed grass pollen, Parietaria officinalis and Timothy grass) and the correlation of exposure to known allergens with the development of allergic symptoms (Table II.1).

House dust mite allergy was defined by a positive skin prick test and positive RAST to D. pteronyssinus or D. farinae extracts and the presence of symptoms clinically referable to these allergens, namely perennial rhinitis

or perennial asthma.

ii. Non-atopic individuals

Non-atopic status was defined in an asymptomatic subject by the absence of positive skin prick tests to common environmental aeroallergens in the presence of a positive wheal response to histamine hydrochloride (1mg/ml), and the presence of negative RASTs to the common aeroallergens with a total IgE within the accepted normal range of 1-180 U/ml (Table II.2).

b. Separation

i. Ficoll-Paque discontinuous gradients

Peripheral blood mononuclear cells (PBMC) were isolated from heparinised peripheral venous blood by centrifugation on a discontinuous gradient of Ficoll-Paque (Pharmacia). Whole blood was mixed with an equal volume of complete medium containing 5% FCS and 20 ml was layered onto 20 ml of Ficoll-Paque in polypropylene-glycol tubes (Falcon 2070 Blue Max, Becton-Dickinson) before spinning at 1200 x G for 20 minutes. The PBMC were removed from the plasma density gradient interface, transferred to a polystyrene universal bottle and washed with complete medium supplemented with 5% FCS and 1% additive-free heparin (Paines and Byrne Ltd., Greenford, U.K.), spinning at 400 x G for 15 minutes. After a second wash the PBMC were resuspended in complete medium supplemented with 5% human serum. The number of viable cells was determined by trypan blue exclusion. Cell preparations and centrifugation were carried out at 20°C.

ii. Sheep erythrocyte rosette formation

T cell depleted (B cell enriched) and T cell enriched preparations were obtained by utilisation of the tendency for T cells to form spontaneous

rosettes with sheep erythrocytes (SRBC), (Kaplan and Clark, 1974). The SRBC stored in Alsever's solution were washed three times in MEM-Hepes (1500 x G for 10 min at room temperature). Then one volume SRBC was mixed with five volumes 40.2 mg/ml S 2-aminoethylisothiouronium bromide hydrobromide (AET) in distilled water (pH 9) for 20 min at 37°C. After three washes in MEM-Hepes, the coupled SRBC were stored at 4°C. Equal volumes of coupled SRBC (4%) and PBMC (10^7/ml) were mixed together with 0.5 volumes FCS. The suspension was centrifuged at 300 x G for 10 min and placed on ice for 60 min. After resuspension of the pellet by gentle rotation, the separation of E-rosette forming (E^+) from non-rosetting (E^-) lymphocytes was achieved by centrifugation (1500 x G, 20 min) over Percoll (density 1.080 g/ml; Pharmacia) at room temperature. The interface containing the T-depleted non-rosette forming (E^-) mononuclear cells was washed three times. Lysis of contaminating red cells in the pellet of E^+ cells was obtained using 0.85% ammonium chloride buffered to pH 7.4 (lysis buffer) followed by washing. Purity of E^+ and E^- populations was determined using an endogenous peroxidase stain, followed by Giemsa.

c. Cryopreservation and thawing

For storage, cells were resuspended at the required concentration in complete medium containing 5% FCS. A 15% solution of DMSO in FCS was prepared, chilled and added dropwise to an equal volume of cell suspension mixing throughout at 4°C. One ml aliquots of the final cell suspension were added to sterile liquid nitrogen ampoules and frozen slowly in a polystyrene box which was sealed and placed in a -70°C freezer overnight. The ampoules were then transferred to the vapour phase of a liquid nitrogen freezer and stored at -180°C until required.

Frozen vials of cells were removed from -180°C storage and rapidly thawed over 1-2 min in a 37°C waterbath. The cell suspension was then added to a universal bottle and 1 ml of complete medium/5% FCS was added dropwise with continuous mixing. A further 20 ml of medium was then added to remove the DMSO from the cells which were spun at 300 x G for 10min. The pellet was resuspended to the required concentration in complete medium/5% A^+ serum for use in tissue culture.

III T cell proliferative assays

a. Polyclonal responses

PBMC (5 x 10^4/well) were cultured with antigen over a dose range in 96-well round bottom microtitre plates (Nunc) in an incubator with 95% humidity and 5% CO_2/air at 37°C. Optimal conditions for culture were determined by incubating over a concentration range for varying periods of time. In experiments where nitrocellulose antigen preparations were used as antigen, cells were cultured for 7 days in 96-well flat bottom plates. Cultures were pulsed with 1 µCi of tritiated methyl thymidine ([^{3}H]TdR, Amersham), then harvested on to glass fibre filters. Proliferation as correlated with [^{3}H]TdR incorporation was measured by liquid scintillation spectroscopy. The results were expressed as the mean counts per minute of triplicate cultures (cpm) with the standard error of the mean, expressed as a percentage, shown in brackets.

b. T cell lines and clones

T cells from the long term lines and clones (10^4/well) were cultured with antigen in 96-well round bottom microtitre plates (Nunc) in the presence of antigen presenting cells (2.5 x 10^4 irradiated PBMC/well or 1 x 10^4 irradiated EBV transformed B cells/well). In experiments where

nitrocellulose antigen preparations were used, cultures were performed in 96 well flat bottom plates in the presence of two-fold the number of antigen presenting cells (5 x 10^4 cells/well). After incubation for 48 hours (soluble antigen) or 72 hours (particulate antigen) the cultures were pulsed and harvested as described in 2.4 IIIa.

c. Antibody inhibition

T cell clones (10^4/well) were cultured with the inducing antigen D. farinae at optimal concentration in the presence of autologous accessory cells. Antibodies were added over a concentration range at the initiation of the culture (Chapter 4; O'Hehir et al., 1988). Proliferation was determined as before (2.4 IIIa).

IV Accessory cells for antigen presentation

a. Peripheral blood mononuclear cells

Autologous and allogeneic irradiated (2500 Rads; Xray) PBMC were used as a source of accessory cells.

b. Epstein Barr virus (EBV) transformed B cells

Human B cells can be transformed into immortalised cell lines expressing Ia antigens with the Epstein Barr virus (EBV), thus providing an alternative autologous source of accessory cell. EBV was obtained from the cell line B95-8 which is derived from cotton-topped marmoset PBMC infected with EBV obtained from a cell line derived from a patient with infectious mononucleosis (Miller and Lipman, 1973). The cells were grown to confluence and the culture supernatant harvested and passed through a 0.45 micron filter. Infection of B cells was carried out by suspending up to 10^7 PBMC in 1 ml B95-8 culture supernatant in complete medium, serum-free,

for 1 hr at 37°C with gentle agitation. The cells were then pelleted, the supernatant removed and the cells cultured. Immortalised EBV-infected B cell lines were obtained from infected cells seeded at 5 x 10^5 cells/ml in complete medium / 10% FCS in 24-well plates. The problem of T cell cytolysis was minimised by the addition of cyclosporin-A (Sandoz; 1 µg/ml) for the first 2-3 weeks of culture. Cultures were fed by weekly replacement of half the medium without disturbing the cell layer. Using an inverted microscope, foci of proliferating transformed B cells were identified after 1-2 weeks. As transformed cells approached confluence (10^6 cells/ml) they were diluted to 10^5 cells/ml.

For use as antigen presenting cells, EBV-transformed B cells were resuspended in complete medium/5% A^+ serum and irradiated by an X-ray source (5000 rad).

c. Transfected murine fibroblasts (L cells)

Because of the microheterogeneity of the HLA-D region gene products, murine fibroblast lines, L cells, expressing the products of transfected human MHC class II genes can be used to present antigen to T cells. This approach allows the MHC class II product, encoded by the transfected genes to be studied in isolation as the L cells have no innate MHC class II molecules. L cells were prepared in serum-free complete medium by treatment with mitomycin-C (50µg/ml for 45' at 37°C), washed three times and then resuspended in complete medium supplemented with 5% human serum for use in assays. (Rothbard et al., 1988) The transfected L cells used were the generous gifts of:- Drs. R. Lechler (DR1), B. Mach (DRw52b and c), J. Trowsdale (DRw53), D. Wilkinson (DR7) and H. Ikeda (DR4).

V Isolation of IL-2 dependent T cell lines and clones

a. Primary culture

PBMC prepared at 2.5 x 10^5 cells/ml in complete medium/5% A^+ serum, were stimulated with Dermatophagoides spp. extract. The concentration of allergen required to produce optimal stimulation of the PBMC, assayed by the incorporation of [^{3}H]TdR varied for the different allergen preparations but was always in the range of 10^3 -10^4 B.U./ml or 1-50 µg/ml. After mixing, 200 µl aliquots of the cell suspensions were plated into 96-well round bottom tissue culture plates and incubated for 6-8 days (as selected from proliferative assay kinetics studies).

b. T cell lines

After primary culture under the optimal conditions, antigen concentration and duration of culture, the T blast cells were recovered by density centrifugation over Ficoll-Paque for 20 min at 1200 x G. Blast-enriched cell suspensions at the interface were diluted to 1 x 10^5 cells/ml of complete medium/5% A^+ serum supplemented with 10% IL-2, 5 x 10^5/ml autologous PBMC x-irradiated (2500 rad) as antigen presenting cells and optimal concentrations of D. farinae. Cultures were aliquoted in 10ml volumes in tissue culture flasks and incubated in humidified chambers at 37°C in 5% CO_2/air.

c. T cell clones

i. Cloning from a long term T cell line

The D. farinae-specific T cell lines were used to isolate allergen-specific T cell clones. The T blasts were recovered by Ficoll-Paque density centrifugation, washed in complete medium/5% FCS and then resuspended in

complete medium/5% A^+ serum. They were plated by limiting dilution in microtest-II trays with 0.3 viable cells/well, 10^4 APC and D. farinae in the presence of 10% v/v IL-2 with a total volume of 20µl/well. Cultures were incubated for 7 days after which growing cells were transferred to fresh medium (200µl) containing 10% IL-2, APC (10^5/well) and D. farinae in 96-well flat bottom plates. After 7 additional days of culture, the clones were transferred to 24-well plates containing the same concentration of IL-2, APC and D. farinae in a total volume of 2 mls. (Lamb et al., 1982a)

ii. Cloning from a primary culture

After 7 days primary culture, cells were harvested by centrifugation over Ficoll-Paque. Blast-enriched suspensions were diluted to 30 cells/ml of complete medium/5% A^+ serum containing 10% IL-2, 5 x 10^5 APC/ml and D. farinae and added to microtest II plates in 20µl aliquots as detailed in 2.4 Vci. (Ratcliffe and Lamb, 1988).

d. Screening of clones for antigen reactivity

Screening panels of newly-generated clones for antigen reactivity was performed one week after transfer of growing clones to 24-well plates. T cells were cultured at 2-10 x 10^4 cells/ml with antigen and autologous APC (1-5 x 10^6 cells/ml). Control triplicates of cloned T lymphocytes plus APC and cloned T lymphocytes plus medium were cultured in parallel. Proliferation was assessed after 2-3 days by the addition of 1 µCi of [^{3}H]TdR for the final 8-16 hr of culture, after which the cells were harvested for scintillation counting of incorporated radioactivity.

e. Expansion and maintenance

Lines and clones were expanded and maintained by the addition of fresh

medium and IL-2 every 3-4 days and irradiated accessory cells and antigen every 7 days. Cell cultures were tested at regular intervals for mycoplasma contamination using fluorescein DNA staining.

VI Culture conditions for in vitro antibody production

a. Lymphocyte separation

The preparation of E^+ (T cell enriched) and E^- (monocyte and B cell enriched) populations was as described in 2.4 IIb.

b. Culture conditions

Polyclonal (E^+; 1.5 x 10^6/ml), or cloned T cells (2.5 x 10^5/ml) were cultured with antigen (D. farinae or mixed grass pollen; 5 x 10^3 B.U./ml) and autologous E^- cells (2.5 x 10^5/ml) in complete medium/10% FCS, with or without the addition of recombinant interleukin 4 (Genzyme IL-4; 100 U/ml, Glaxo). In control experiments, allogeneic E^+ cells (1.5 x 10^6/ml) were cultured with E^- cells (2.5 x 10^5/ml). The supernatants were harvested at 10-12 days and the total levels of IgG and IgE determined by ELISA (2.4 VIc).

c. ELISAs for immunoglobulin determination

Total IgG and IgE were assayed using solid phase enzyme-linked immunosorbent assays (Quint et al., submitted). Standard curves were performed for both IgG and IgE. The limits of detection were 8-3,000 ng/ml and 40-12,000 pg/ml for IgG and IgE respectively. IgG and IgM at concentrations of 50 µg/well were not detected in the IgE assay.

i. **Buffers**

1. **Coating buffer: pH9.6**

Na_2CO_3 1.59g

$NaHCO_3$ 2.93g

Dissolved in 1 litre distilled water and pH adjusted to 9.6.

2. **Washing buffer**

PBS

0.05% Tween 20 (Sigma)

0.02% sodium azide (BDH Ltd., Poole, U.K.)

3. **Diluent for antibodies**

PBS

0.05% Tween 20

1% BSA (Sigma)

4. **0.05M carbonate buffer: pH9.8**

(Buffer for alkaline phosphatase substrate)

1M $NaHCO_3$ (solution A)

1M Na_2CO_3 (solution B)

The buffer was prepared by mixing 9 parts of solution A with 1 part solution B, titrating the pH to 9.8 and diluting the buffer to 0.05M with saline.

5. **0.15M citrate/phosphate buffer: pH5.0**

(buffer for horseradish peroxidase substrate)

0.1M citric acid (solution A)

0.2M $Na_2HPO_4.H_2O$ (solution B)

The buffer was prepared by mixing 49ml solution A with 51 ml solution B.

6. Substrate for alkaline phosphatase.

4 x Sigma 104 phosphatase substrate tablets (p-Nitrophenyl phosphate disodium, 5mg per tablet) were dissolved in 11ml 0.05M carbonate buffered pH9.8, containing 34 µl 300mM $MgCl_2$.

7. Substrate for horseradish peroxidase

4 x 1,2, O-phenylenediamine dihydrochloride (OPD) tablets, (2mg per tablet Dakopatts, Glostrup, Denmark) were dissolved in 12ml 0.15M citrate/phosphate buffered pH5.0 by frequent shaking. 5µl of 30% hydrogen peroxide was added and the solution was protected from light until used (within 1 hour).

ii. IgG ELISA

Ninety-six well flat bottom plates (Nunc Immunoplates Type I) were incubated overnight at room temperature with 50µl of goat antibody to human IgG (1:500 in coating buffer, Miles Scientific, Slough, U.K.). After three washes the plates were blocked with 1% BSA in PBS for 30 minutes at 37°C and then incubated with test samples and standards (Human IgG, Sigma). For detection of IgG, alkaline phosphatase labelled goat anti-human IgG (1:500, Sigma) conjugate was used for 1 hour at 37°C. 50µl of the alkaline phosphatase substrate solution (NPP; 2.4 VIc.i.6) was added and the absorbance measured at 405nm. Concentrations of IgG in the test

supernatants were determined from the standard curve prepared.

iii. IgE ELISA

Ninety-six well flat bottom plates (Nunc Immunoplates Type I) were incubated overnight at room temperature with 50µl of mouse antibody to human IgE (1:500 in coating buffer, Serotec, U.K.). The plates were washed and blocked with 1% BSA and then incubated with test samples and standards (a polyclonal human IgE preparation from the National Institute of Biological Standards, South Mimms, U.K.) overnight at room temperature. A second blocking step was used at this stage to lower the background further, then after washing three times, 50µl of biotinylated goat anti-human IgE (1:250, Vector, Peterborough, U.K.) was used as a second antibody for 1 hour at 37°C. The next layer, after three washes, was a streptavidin biotinylated horseradish peroxidase conjugate (1:500), for a further hour at 37°C. The plates were washed as before then incubated in the dark at 37°C with the horseradish peroxidase substrate solution (2.4 VIc.i.7). Absorbance was measured at 450nm.

VII Induction of hyporesponsiveness

a. Direct inactivation with specific antigen

Cloned T cells (5 x 10^4/ml) were activated with autologous irradiated PBMC (1.25 x 10^5/ml) and D. farinae (10^3 B.U./ml) with added IL-2 (10% v/v) in 2 mls of complete medium / 5% A^+ serum in 5 ml Falcon tissue culture tubes. After 18 hours incubation the cells were washed and D. farinae added at 10, 10^2, 10^3, 10^3 B.U./ml consecutively over four days. Control cultures were maintained in medium supplemented with IL-2 alone. The pretreated clones were washed and viable cells (5 x 10^4/ml) were

stimulated with an immunogenic concentration of D. farinae (10^4 B.U./ml) or mixed grass pollen as a control in the presence of autologous irradiated PBMC (1.25 x 10^5/ml) or in IL-2 alone. Proliferation as correlated with $[^3]$HTdR incorporation was determined after 72 hours.

b. Anti-IL-4 receptor antibody inhibition

The monoclonal antibody (MR6; 1.25 or 2.5µg/ml) was added at the initiation and again after 5 days to cultures containing cloned T cells (2 x 10^5/ml) autologous E^- cells (2.5 x 10^5/ml) and D. farinae (5 x 10^3B.U./ml). After 10 days incubation the supernatants were harvested and the presence of IgG and IgE antibodies determined as described in section 2.4 VIc. In control cultures, mixed grass pollen and isotype-matched antibodies (H17E2 or 2118) were added. Additional controls of E^- cells with and without the addition of antigen and cloned T cells were performed.

2.5 Cytofluorimetric analysis

Samples of 2.5 x 10^6 cells were resuspended in 50µl of ice-cold phosphate buffered saline (PBS) containing 0.1% NaN_3 and 0.5% BSA (PAB) and incubated with saturating amounts of the monoclonal antibodies for 30 mins at 4°C. After washing once with 4ml of cold PAB, FITC-conjugated $F(ab')_2$ fragments of rabbit antibody to mouse immunoglobulins were added in excess and the cells incubated for a further 30 mins at 4°C. After washing, the cells were resuspended in PBS containing 0.1% NaN_3 and kept on ice until analysed. Propidium iodide was added to the cell suspension to identify dead cells. Background fluorescence was determined by incubating cells with the second antibody only. Fluorescence intensity was determined on 20,000 cells from each sample by a Becton Dickinson FACS Analyser,

standardised with fluorescent microbeads, using logarithmic amplification which was converted to the linear equivalent by a Hewlett-Packard Consort 30 computer. The cell population was analysed by gating on the volume and 90° light scatter characteristics to exclude debris, and the percentage of positive cells was determined by setting a marker on the background fluorescence such that less than 1% of cells were positive.

2.6 Western blotting and autoradiography

Nitrocellulose-transferred SDS-PAGE immunoblots of D. farinae and D. pteronyssinus were prepared as described (2.1 VIII). Western blotting and autoradiography were performed using the modification of Tee et al. (1988).

I. Buffers

a. Blocking buffer

phosphate buffered saline

human serum albumin 2%

b. Blot wash buffer

Tris	2.42g)
NaCl	29.24g) pH 7.5
distilled water	1 litre)

II. Procedure

Non-specific binding was blocked by immersing the nitrocellulose sheets in blocking buffer for 1 hr at 39°C. After washing three times with blot wash buffer a range of concentrations of autologous serum (undiluted, 1:2, 1:5; diluted with blocking buffer) were allowed to react over the nitrocellulose

sheets for 16 hr at room temperature. After washing again with blot buffer and distilled water, a further 16-hr incubation with the tracer ^{125}I anti-IgE (Pharmacia; 100 µl/approx. 10^4 cpm; diluted with blocking buffer) followed. The blots were then washed, dried and exposed to X-OMAT L Kodak film with DuPont Quanta III intensifying screens at -70°C for 48-70 hr, and then developed. Negative controls using serum (1:2, 1:5) from a non-atopic individual were processed in parallel.

2.7 Dot Blot analysis for mRNA

– performed with the kind assistance of Dr. Vineeta Bal.

I. Oligonucleotide probes and cDNA

a. IL-2 cDNA

Human IL-2 cDNA was the kind gift of Dr. T.Taniguchi (Taniguchi et al., 1983). The cDNA was labelled using a DNA labelling kit (Boehringer Mannheim).

b. IL-4 oligonucleotides

Two partially overlapping cDNA strands (Yokota et al., 1986):

i. 5'GTGCGACTGCACAGCAGTTCCACAGGCACA3'

ii. 3'GTGTCCGTGTTCGTCGACTAGGCTAAGGAC5'

were hybridised and labelled using ^{32}PdCTP (Amersham) and three cold triphosphate nucleotides in the presence of Klenow enzyme.

II Procedure

Cloned T lymphocytes were activated on solid phase anti-CD3 antibody

(OKT3, Ortho Pharmaceutical Corp., Raritan, N.J.) for 6 hr in the presence of 5% IL-2 (Lymphocult-T), collected, washed in PBS and resuspended in PBS containing cycloheximide (50 µg/ml) and vanadyl ribonucleoside complex (10mM). Selective mRNA immobilisation on the nylon membrane (Hybond-N, Amersham) was as described by (Bresser, Doering and Gillespie, 1983). Cells were lysed using pronase digestion followed by detergent treatment, the lysate was saturated with sodium iodide and put onto the membrane. After washing, the membrane was exposed to UV light before storage. Prehybridisation (4 hr) and hybridisation (overnight) were carried out at 42°C in 50% formamide and 6 x SSC (20 x SSC = 3M NaCl + 0.3M Na citrate). Gifts of cDNA sequences were hybridised and radiolabelled. Blots were exposed to Kodak XAR films at -70°C.

2.8 PRESENTATION OF RESULTS AND STATISTICAL METHODS

I Presentation of results

The data expressed either in graphical or tabular form are the results of one experiment, but each experiment has been repeated at least twice and in the majority of cases three or more times. The results are expressed as the arithmetic mean $\pm$ SEM expressed as a percentage of triplicate cultures in counts per minute (cpm).

II. Statistical analysis

Negative and positive responses were objectively classified by employing the T-max analysis suggested by Mendell et al. (1977). For each clone in the genetic restriction panel experiments cpm data were first normalised by $\log_{10}$ transformation, ranked and partitioned into high (positive) and low (negative) groups. This approach has been used successfully to analyse the response patterns of alloreactive T cells (Rosen-Bronson et al., 1986).

Table II.1 Summary of clinical data of atopic donors

	RM	TB	IC	AH	DSt
clinical rhinitis	+	+	+	+	+
" asthma	-	+	+	-	-
" eczema	-	-	+	-	-
skin prick testing	+	+	+	+	+
*RAST score D.farinae	3	3	4	4	2
" " D.pteronyssinus	4	4	4	4	3
" " Parietaria officinalis	0	0	1	0	0
" " Timothy grass	0	1	2	3	0
" " mixed grass pollen	0	1	2	3	0
**total IgE (IU/ml)	150	220	3,720	628	135
age	39	21	29	24	24
sex	M	F	F	F	M

*RAST results as graded 0,1,2,3,4 where 0 is negative and 4 is strongly positive - 1 is borderline.

**total IgE normal range for non-atopic U.K. adults is 1-180 u/ml : geometric mean = 21 u/ml.

Table IL1 atopic donors continued

	AD	CR	AK	GL	DY
clinical rhinitis	+	+	+	+	+
" asthma	+	+	-	-	-
" eczema	-	-	-	-	-
skin prick testing	+	+	+	+	+
*RAST score D.farinae	3	4	3	3	4
" " D.pteronyssinus	3	4	4	4	4
" " Parietaria officinalis	0	0	0	2	2
" " Timothy grass	0	0	0	2	0
" " mixed grass pollen	1	1	0	1	0
**total IgE (IU/ml)	98	303	130	215	230
age	21	26	49	42	34
sex	M	F	M	M	M

Table II.2 Summary of clinical data of non-atopic donors

	CC	JT	RO	AC	DS	JL
clinical symptoms	-	-	-	-	-	-
skin prick testing	-	-	-	-	-	-
*RAST score D.farinae	0	0	0	0	0	0
" " D.pteronyssinus	0	0	0	0	0	0
" " Parietaria officinalis	0	0	0	0	0	0
" " Timothy grass	0	0	0	0	0	0
" " mixed grass pollen	0	0	0	0	0	0
**total IgE (IU/ml)	64	33	<5	<5	35	28
age	29	31	32	24	22	35
sex	M	M	F	F	F	M

*RAST results as graded 0,1,2,3,4 where 0 is negative and 4 is strongly positive - 1 is borderline.

**total IgE normal range for non-atopic U.K. adults is 1-180 u/ml : geometric mean = 21 u/ml.

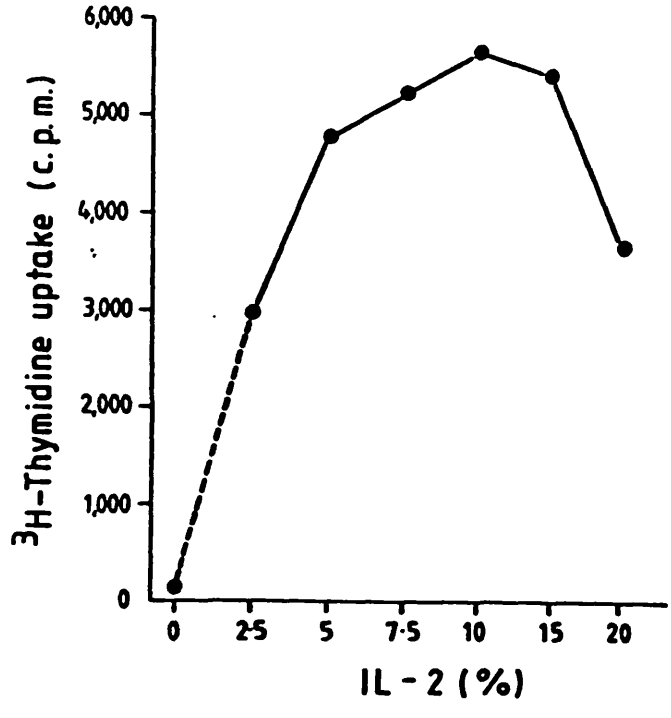

Figure 2.1: Screening of IL-2.

Cloned T lymphocytes (10^4/well) were cultured in complete medium supplemented with 5% human A+ serum in the presence of IL-2 (Lymphocult T, Biotest Folex) at a final concentration of 0, 2.5, 5, 7.5 10, 15 and 20%. [^{3}H]TdR incorporation was determined at 72 hrs. Background response of T cells in the absence of IL-2 was 143 c.p.m.

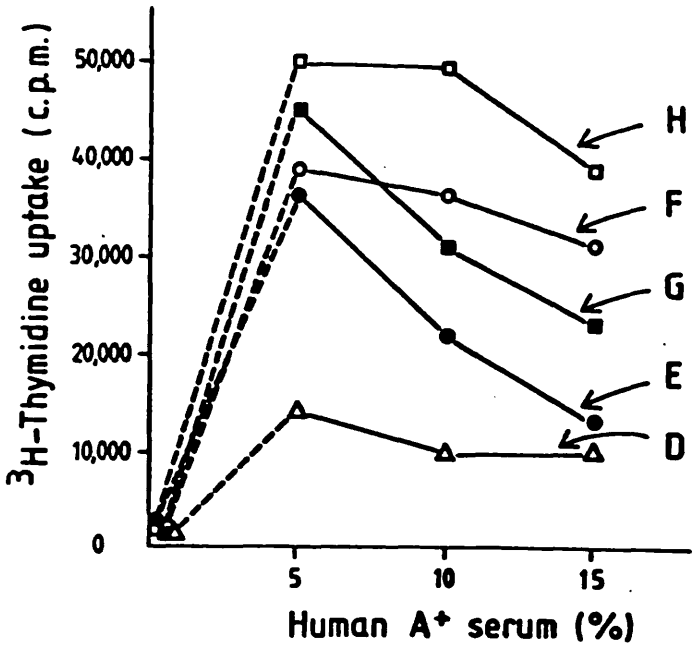

Figure 2.2: Screening of human serum.

PBMC (5 x 10^4/well) were stimulated with suboptimal concentrations of PHA (2.5 µg/ml) in complete medium supplemented with 5, 10 or 15% of heat-inactivated human serum (batch numbers D, E, F, G, H). [^{3}H]TdR incorporation was determined at 72 hrs. Background response of PBMC in the absence of PHA was 115 c.p.m. Batches such as D which proved inhibitory to T cell proliferation were discarded.

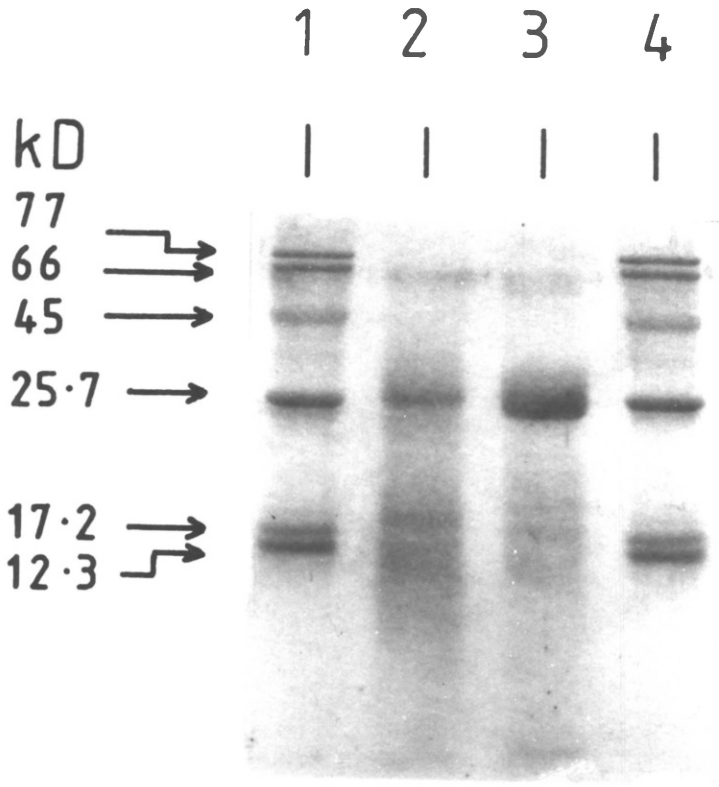

Plate 2: SDS-polyacrylamide gel of D. farinae and D. pteronyssinus extracts. D. farinae extract (lane 2) and D. pteronyssinus extract (lane 3) (10^5 B.U. protein per lane) were prepared and run on SDS-polyacrylamide gel, and proteins were blotted onto nitrocellulose. A representative gel stained with Coomassie blue is shown with molecular weight markers run in parallel (lanes 1 and 4).

CHAPTER 3 INVESTIGATION OF ANTIGEN SPECIFICITY OF T CELL REPERTOIRE REACTIVE WITH *DERMATOPHAGOIDES* SPP. IN ATOPIC AND NON-ATOPIC SUBJECTS

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3.1 INTRODUCTION

Although the T cell dependence and reaginic activity of IgE in clinical allergy is well documented (Ishizaka, 1984) little is known about the antigen specificity and functional role of T lymphocytes in the induction and regulation of allergic respiratory diseases. Recognition of allergens such as ragweed (Meuer et al., 1983), pollen (Brostoff, Greaves and Roitt, 1969) and Dermatophagoides spp. (Rawle, Mitchell and Platts-Mills, 1984; Lanzavecchia et al., 1983) by the T cells of atopic individuals has been reported but controversy surrounds whether the T cell repertoire of non-atopic individuals includes recognition of aeroallergens.

For advances to be made in the current immunological approaches used in the treatment and prevention of allergic responses to Dermatophagoides spp. detailed knowledge of the specificity of the T cell repertoire of atopic subjects is required. Furthermore, the identification of any differences in the antigen specificity of T cells observed in atopic and non-atopic subjects may allow "immunodominant" or unique epitopes to be identified that are recognised by T cells from only the atopic individuals.

To address these questions, the antigen specificity of polyclonal T cell populations and a panel of long term T cell lines and CD4+ T cell clones derived from both atopic and non-atopic subjects has been examined in detail.

3.2 RESULTS

I POLYCLONAL T CELL POPULATIONS

A. T cell proliferation in response to soluble aeroallergen extracts

PBMC from both atopic and non-atopic individuals proliferated in

a dose-dependent manner when stimulated with soluble aeroallergen extracts (Table III.1). The data shown is the count of maximum proliferation over a dose-response curve for each antigen. Full dose response curves are plotted for two non-atopic subjects (DS and CC) and one atopic subject (RM; Fig. 3.1). On the basis of the marked proliferative responses observed in these three individuals, T cell lines and/or CD4+ T cell clones were subsequently isolated.

B. T cell proliferation in response to affinity-purified preparations of Der f I and Der p I.

In order to identify antigenic determinants involved in T cell antigen recognition, PBMC from selected atopic and non-atopic individuals were cultured with affinity-purified preparations of two of the major allergens of Dermatophagoides spp., Der p I and Der f I, over a concentration range. At the polyclonal level, marked proliferative responses were observed with the atopic individual (RM) and from two of the non-atopic subjects (DS and CC); weaker responses being observed in the third non-atopic subject (JL; Table III.2).

C. Reactivity pattern of PBMC to SDS-PAGE immunoblots of D. farinae

The identification of "major" allergens of Dermatophagoides spp. has been based on monoclonal antibodies and since T and B cells may preferentially recognise different components of an antigen mixture, the serological isolation should be supplemented by a direct approach for the analysis of T cell antigen recognition. Antigen insolubilised on nitrocellulose is able to activate T lymphocytes following presentation by appropriate accessory cells. Using the SDS-PAGE immunoblot assay

system for T cell antigen recognition (Young and Lamb, 1986) the fine specificity was further examined. At the polyclonal level, PBMC from the atopic donor (RM) showed four small peaks of activity at molecular weight 10, 22, 39 and 58 kD but proliferative responses obtained were much lower than that obtained with the entire D. farinae preparation (Fig. 3.2).

D. T cell proliferation in response to synthetic peptides of Der p I

In order to map the epitopes recognised by house dust mite immune T cells, synthetic peptides were used in proliferation assays. On the basis of the published sequence of the Der p I major allergen of D. pteronyssinus (Chua et al., 1988; Figure 3.3), amino acid sequences were selected and peptides synthesised. These were tested on PBMC from the house dust mite allergic individual RM (Table III.3). Proliferation was consistently observed in response to stimulation with peptide 81-102. None of the other peptides tested were able to stimulate the peripheral T cells. Analysis of the response of polyclonal T cells from an additional group of 5 atopic individuals to the panel of peptides revealed that donors DY and DSt both recognised peptide 81-102 although the response of the former donor was weak (Stimulation Index = 1.7; Table III.4). Interestingly, T cells of donor DSt proliferated weakly in response to stimulation with the other peptides. To obtain the approximate location of the epitope recognised by the Der p I specific T cell clone, DE9, (Sect. 3.2IIG), an affinity-purified preparation of Der p I was treated with cyanogen bromide (CNBr) to cleave at the methionine residues and the preparation was subsequently used in a proliferation study with PBMC from the allergic subject, RM, (Table III.5). Although marked proliferation was observed with the control Der p I preparation,

immunogenicity was abrogated by CNBr treatment. Using this information, two further peptides were synthesised containing the methionine residues, peptide 12-34 (AEIDLRQMRTYTPIRMQGGSGSS) and peptide 209-222 (DLMIEEYPYVVIL). Neither peptide was able to induce proliferation of PBMC from the allergic subject, RM, (Table III.6). The results of the clone DE9 stimulated with these peptides is described later (Sect. 3.2IIG).

II OLIGOCLONAL AND MONOCLONAL T CELL POPULATIONS

A. Primary screen of antigen reactivity

i. T cell clones

A panel of CD4+ T cell clones was isolated from the atopic subject, RM, and the non-atopic subjects, DS and CC, by the limiting dilution method (Chap. 2.4V; Lamb et al., 1982a). Preliminary screening of the house dust mite reactivity of the clones is shown (Fig. 3.4).

ii. T cell lines

A panel of long term T cell lines was also isolated from the atopic subject, RM. The antigen dependence of a selection of these lines is shown (Fig. 3.5).

B. Phenotypic analysis of cloned T lymphocytes

The clones from both the atopic and non-atopic donors which were studied in detail were analysed using monoclonal antibodies and cytofluorimetry. All expressed CD4, being negative for CD8. Evidence of activation was present by the expression of IL2-R and MHC class II determinants. Representative data for clone DD11 is shown (Fig. 3.6). All clones tested also stained positive for the monoclonal antibody

UCHL1, implying that they are of the helper/inducer subset of T cells (Table III.7).

C. T cell proliferation in response to soluble aeroallergen extracts

i. Atopic donor

One long term T cell line and ten of the T cell clones from the atopic donor RM were studied in greater detail. The cloned T cells proliferated in response to the inducing antigen D. farinae but failed to recognise the unrelated allergens mixed grass pollen and Parietaria judaica over a range of concentrations (Table III.8a). Clones DE9 and DE26 gave marked proliferative responses to the closely related member of the same genus, D. pteronyssinus. The other clones (DE5, DE12, DD11, DE41, DE28 and DE43) were species-specific, proliferating only to the inducing antigen, D. farinae. The T cell line, DX, gave a limited proliferative response to D. pteronyssinus, but also failed to recognise the unrelated allergens (Table III.8a). In contrast, T cell clones DH12 and DH15 induced with D. pteronyssinus were cross-reactive on both D. pteronyssinus and D. farinae (Table III.8b).

ii. Non-atopic donor

Two Dermatophagoides spp. specific T cell clones were isolated from non-atopic individuals, clone DF1 from subject DS and clone DG2 from subject CC. Both clones proliferated strongly to the inducing antigen D. farinae and failed to respond to D. pteronyssinus or the unrelated allergens Parietaria judaica or mixed grass pollen (Table III.9).

D. T cell proliferation in response to affinity-purified Der f I and Der p I

i. Atopic donor

In order to further characterise the specificity of the T cell clones, responses to affinity-purified preparations of Der f I and Der p I were examined. The cross-reactive T cell clone DE9 proliferated in response to both preparations, however, the species-specific T cell clones tested failed to respond to either of the group I allergens (Table III.10). Clones DH15 and DH12 raised with the inducing antigen D. pteronyssinus both also responded to Der p I. In addition, DH15 recognised a cross-reactive determinant present in Der f I.

ii. Non-atopic donor

Clones DF1 and DG2 from two non-atopic subjects which were specifically reactive with D. farinae both failed to respond to either of the group I allergens when tested over a concentration range (Table III.11).

E. Response of T cell line to affinity-purified Der f II and Der p II

Affinity-purified Der f II and Der p II (gifts of Drs. Heymann and Schou respectively) allowed further analysis of the antigenic determinants recognised by the T cells. The long term line DX responded markedly to the Der f II allergen with a diminished response to the Der p II preparation (Table III.12). Responses obtained to the soluble extracts of D. farinae and D. pteronyssinus were again consistent with the predominant recognition of species-specific determinant(s) of D. farinae by the T cells of this individual.

F. Reactivity pattern of cloned T lymphocytes to SDS-PAGE

immunoblots of *D. farinae*

T lymphocytes from the long term line DX and clone DD11, in the presence of accessory cells, were observed to proliferate in response to *D. farinae* supplied in particulate form bound to nitrocellulose after fractionation on SDS-PAGE (Fig. 3.7). The T cell line predominantly recognised antigenic determinants within the molecular weight range 9,000-13,000 (Fig. 3.7a) which contained the only determinant recognised by the clone DD11 (Fig. 3.7b) In addition, the line showed minimal recognition of the protein band (24,000-29,000) that would correspond to the *Der f* I and *Der f* III allergens.

To control for the possibility that proliferation was a non-specific mitogenic effect, all 20 fractions were added in the presence of accessory cells to cloned T cells reactive with influenza haemagglutinin (residues 306-324) (Lamb *et al.*, 1982b) with the result that no proliferation was observed (Fig 3.8). Neither were any of the fractions able to inhibit the response of this clone to the HA peptide thus eliminating the presence of non-specific inhibitors (Fig. 3.8). These results would suggest that the proteins in the molecular weight range 9,000-13,000 constitute the major component of T cell recognition for this individual.

Cross-reactive T cell clone DE9 was tested for its reactivity pattern to SDS-PAGE immunoblots of both *D. farinae* (Fig. 3.9) and *D. pteronyssinus* (Fig. 3.10). Consistent with the observed response to the affinity-purified group I allergens of both species of *Dermatophagoides*, the clone was observed to recognise proteins in the molecular weight range of 24-36 kD which comigrate with the group I allergens. The

recognised fractions of the D. pteronyssinus immunoblots spread over a greater number of fractions, consistent with the greater concentration of Der p I in the D. pteronyssinus extract (Plate 2). Responses observed were reproducible with subsequent SDS-PAGE fractionations of the Dermatophagoides spp. extracts (Fig. 3.11).

However, not all the T cell clones recognised SDS-PAGE immunoblots of house dust mite antigen extract. Although capable of marked responses to soluble D. farinae extracts, clones DE5 and DE12 from the atopic subject and clones DF1 and DG2 from the non-atopic subjects failed to recognise immunoblotted antigen (data not shown).

G. Proliferative responses of cloned T lymphocytes to synthetic peptides of Der p I

The fine antigen specificity of the cross-reactive T cell clone DE9 which recognises the group I allergens of Dermatophagoides spp., giving a marked response to both nitrocellulose-bound antigen preparations and affinity-purified Der f I and Der p I was further investigated using a panel of synthetic peptides (Table III.13). The peptides were selected on the basis that they contained a similar pattern of amino acids to those identified in known T cell epitopes (Rothbard and Taylor, 1988). None of these peptides tested over a concentration range stimulated the T cell clone, despite strong responses to the D. pteronyssinus extract. As described in Sect. 3.2ID, cyanogen bromide (CNBr) treated extracts of Der p I were then prepared, to cleave at the methionine residues. The treatment successfully inactivated the Der p I preparation (Table III.14). The CNBr-Der p I was not toxic to the T cells as evidenced by control cultures where CNBr-Der p I was added to the Der p I preparation, as no

inhibition was observed.

Two subsequent peptides were synthesised containing the methionine residues. Proliferative responses of clone DE9 to these peptides are shown (Table III.15).

III Comparison of B cell and T cell responses to Dermatophagoides spp.

Nitrocellulose-transferred SDS-PAGE immunoblots of D. farinae and D. pteronyssinus were prepared and Western blotting and autoradiography were performed to show the IgE response of serum from the house dust mite allergic individual RM and control non-atopic allogeneic serum.

The autoradiographs using the autologous serum showed strong IgE binding at the 13,000 fraction corresponding to the major site of T cell recognition (Plate 3). Fainter bands were demonstrable at 26,000, 29,000 and 42,000 molecular weight. These first two bands correspond to the molecular weights of Der f I and Der f III and reflect the minor recognition site of the T cell line DX. Similarly, the pattern of binding to D. pteronyssinus was predominantly against the 13,500 molecular weight band, with additional bands at 41,000 and 31,500 (Plate 4). Interestingly, the major protein band of the gel was consistent with Der p I (Plate 2). None of these antigens was recognised by the control sera.

3.3 DISCUSSION

Our knowledge of the antigen specificity of the T cell repertoire reactive with aeroallergens is limited. Research in the field of allergy

has generally focused on effector cells such as mast cells and eosinophils or serological analysis. The latter has resulted in the specificity of the immune response to a number of aeroallergens being considered only in terms of those allergenic components recognised by the humoral response (Chapman and Platts-Mills, 1980; Dandeu et al., 1982; Stewart and Turner, 1980). However, the diversity of antigen specificities of the T and B cell repertoires do not necessarily overlap (Hurwitz et al., 1984). Therefore, to examine the specificity of T cells activated by house dust mite, analysis using only antigens preselected by antibody may prove incomplete and, to control for this, alternative approaches have been applied.

Only restricted information is available on T cell recognition of the genus Dermatophagoides spp. Rawle et al. (Rawle, Mitchell and Platts-Mills, 1984) described polyclonal T cell proliferation to the Der p I antigen of D. pteronyssinus in atopic house dust mite allergic individuals but possible cross-reactivity with the closely-related mite species D. farinae was not investigated. Lanzavecchia et al. (1983) isolated HLA-DR restricted T cell lines and clones reactive with the aeroallergen D. pteronyssinus. However, their subsequent studies investigated alloreactive T helper clones for the in vitro stimulation of IgE production (Lanzavecchia and Parodi, 1984).

In this study, the antigen specificity of both polyclonal and monoclonal T cells from atopic house dust mite allergic and non-atopic individuals to Dermatophagoides spp. has been analysed. At the polyclonal level, the T cell repertoires of both groups of subjects were shown to include cells capable of recognition of a panel of soluble aeroallergens, including both

species of Dermatophagoides, the weed Parietaria judaica, Timothy grass and mixed grass pollen. These responses were not attributable to non-specific mitogenic effects of the allergen extracts as with each preparation not all individuals responded. The observation that non-atopic individuals respond to house dust mite is contrary to the findings of Rawle et al. (Rawle, Mitchell and Platts-Mills, 1984) who reported that only the T cells of atopic subjects proliferated to Der p I. Furthermore, the ability of some non-atopic subjects to recognise both Der p I and Der f I suggest that differences in atopic and non-atopic individuals may reside in the quality of help induced rather than in the specificity of the T cell repertoires.

In order to investigate the antigenic determinants of D. farinae which were evoking the observed T cell response, polyclonal populations were then analysed using affinity-purified preparations of the major group I allergens of Dermatophagoides spp. (Der p I and Der f I). The responses observed to these preparations suggested that although identification of these allergens had been performed by serological analysis, important T cell epitopes were also present. The greatest response observed was with PBMC from the atopic house dust mite allergic subject, supporting the "major allergen" status attributed to these proteins.

In view of the possibility that antigenic determinants capable of eliciting a T cell response independently of antibody-antigen recognition exist, the D. farinae mite body extract was fractionated by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE), transferred to nitrocellulose membranes and then added to T cell proliferation assays (Lamb, O'Hehir and Young, 1988). This method allows a direct screening

for the recognition of individual polypeptides by monoclonal and polyclonal T cell populations without the necessity for extensive biochemical purification and in the absence of initial antibody preselection of antigen. At the polyclonal level, analysis of the PBMC response from the atopic mite allergic subject RM showed a number of small peaks of activity. Responses observed were 10-15% of the response obtained to the soluble D. farinae extract. However, two of the peaks of proliferation that corresponded to known major allergens of the Der f I (MW 24kD) and Der f II (MW 12kD) were observed reproducibly. Responses obtained in assays using immunoblotted antigen preparations were several-fold less than that observed with the unfractionated extract. This may result from accessory cells being unable to process nitrocellulose-bound antigen as efficiently as soluble antigen. In particular, responses observed at the polyclonal level were low-grade, reflecting the low numbers of sensitised house dust mite immune T cells present in the peripheral blood of a mite-allergic subject (Halvorsen et al., 1986). The wide variation in [^{3}H]TdR uptake for triplicate cultures observed at the polyclonal level provided further evidence of the low and variable numbers of antigen-reactive T cells in the peripheral blood.

In view of the wide variability observed in analyses of polyclonal antigen specific responses, it was necessary to positively select for antigen reactivity and this was achieved by T cell cloning. Therefore, in order to further characterise the antigen specificity, a panel of lines and clones was isolated using the house dust mite allergen D. farinae. As only 12-20% of the population suffers from clinical allergy it was important to study both atopic and non-atopic individuals and identify possible differences between them with respect to the antigenic determinants recognised.

Interestingly, T cell clones and lines could be isolated from two non-atopic subjects in addition to an atopic mite-allergic subject who all gave similar patterns of proliferative response to a number of aeroallergens including house dust mite. D. farinae-reactive T cell clones could be isolated from all three subjects. The predominant pattern of T cell recognition for the clones and the line that were characterised fully was directed mainly to species-specific determinant(s). Only a minor component of the responsiveness of the line was directed towards the closely related D. pteronyssinus. Of the eight clones from the atopic donor and the two clones from the non-atopic donor induced with D. farinae, only two recognised cross-reactive determinants. Subsequently, to identify the T cell epitopes recognised by a number of these clones affinity-purified preparations and SDS-PAGE-separated immunoblots were used. Only the T cell clones cross-reactive for both species of Dermatophagoides spp. and the two clones isolated using D. pteronyssinus as the inducing agent proliferated in response to the affinity-purified group I allergens. Subsequently, assaying affinity-purified group II allergens revealed that the line DX recognised Der f II and Der p II, however, the proliferative response to the latter was decreased. In agreement with this observation, the determinant(s) recognised by both the T cell line and clone using nitrocellulose immunoblots of SDS-PAGE-separated antigen could be mapped in the molecular range of 9,000-13,000. This comigrates with Der f II (MW 12,500) recently proposed by Heymann et al. (1987) to be a major allergen of D. farinae in addition to the 24,000 MW protein Der f I (Heymann et al., 1986). Interestingly, the results reported would suggest that the T cell response of this individual is directed predominantly against a species-specific determinant(s) in Der f II. In contrast, analysis of one of the cross-reactive clones, DE9, using immunoblots of D.

farinae and D. pteronyssinus confirmed the response observed to affinity-purified extracts of Der f I and Der p I. A single peak of activity corresponding to the group I allergens was evident (Fig. 3.9 and 3.10). The profile of response to SDS-PAGE-separated immunoblots was reproducible with different immunoblot preparations (Fig. 3.11). Not all of the clones tested responded to immunoblotted antigen which may be the result of technical problems such as inadequate antigen binding to nitrocellulose or the denaturation of determinants by the solubilisation procedure. Alternative explanations include inherent differences in the T cell repertoire, or that some T cells may recognise conformational determinants (Glimcher et al., 1983) which are no longer present after immunoblotting. The patterns of proliferation observed to immunoblots of fractionated Dermatophagoides spp. were specific since non-specific inhibition and mitogenesis were excluded by testing on influenza haemagglutinin immune clone, HA1.7.

The subsequent availability of affinity-purified Der f II allowed confirmation that the line DX predominantly recognised this determinant with only a minor recognition of Der p II, a response analagous to that observed with the immunoblotted antigen and supporting the contention that species-specific determinants of Der f II are a predominant cellular response in this individual.

Whether or not Der f II, as opposed to Der f I, for example, is an immunodominant allergen for the haplotype of this individual, as has been described for the cytotoxic T cell response to the internal components of influenza (Gotch et al, 1987), will need to be established. Alternatively, as suggested by the polyclonal response, T cell recognition

is neither limited to species-specific determinants, nor indeed to Der f II, with the clonal response merely reflecting the restriction element usage for a specific epitope.

In analysing the specificity of the D. farinae IgE antibody response, similar to the T cells, recognition was predominantly of a protein that migrates with the same molecular weight as the Der f II allergen (12,500), although antibodies reactive with determinants that migrate with identical molecular weights to the Der f I and Der f III allergens (26,000 and 29,000) respectively, were also observed. These results suggest that while T cell recognition is mainly of the Der f II allergen, the IgE antibody response is directed towards both the major and minor allergens. The human IgE antibody to Der p I and Der f I appears to be 80-95% cross-reactive (Chapman et al., 1987), therefore, it is possible that T cells primed to D. pteronyssinus are providing the helper activity for the cross-reactive B cell response. Indeed, the PBMC of this individual were able to respond to D. pteronyssinus. Furthermore, the T helper mechanisms involved in the induction of antibody reactive with Der f II and Der f III are unclear. Alternatively, the Der f II-reactive T cells may provide not only helper activity for the anti-f II antibody response, but also the anti-f I and anti-f III B cell responses in a similar manner to the "intermolecular" help described by Lake and Mitchison (1977). These same arguments may apply to the immune response to D. pteronyssinus.

The recent availability of the sequence of the Der p I allergen (Chua et al., 1988) offers the opportunity to investigate the specificity of antigen recognition at the epitope level for both polyclonal and monoclonal T

cell populations from non-atopic and house dust mite allergic subjects using synthetic peptides. The observation that T cell determinants usually comprise short linear peptide sequences has encouraged the development of predictive theories for T cell epitopes that are based on analysis of amino acid sequence (DeLisi and Berzofsky, 1985; Rothbard and Taylor, 1988). Potential T cell epitopes of Der p I were selected based on the presence of specific patterns of amino acids similar to those identified in known T cell epitopes. In many instances, this pattern consisted of a charged, or a glycine residue, followed by two or three consecutive hydrophobic residues, and terminating with a polar amino acid. Interestingly, the Der p I peptide 81-102 was able to evoke a polyclonal response in three of the five house dust mite allergic donors tested, however, the T cell clone DE9 isolated from one of the atopic subjects (RM) failed to recognise this peptide. The cleavage of Der p I extract with cyanogen bromide abrogated the immunogenicity and this was not due to toxicity following enzymatic treatment of the preparation. The subsequent synthesis of two peptides based on the location of methionine residues also failed to identify a T cell epitope recognised by either polyclonal T cells or the T cell clone from the donor. If the epitope recognised by the T cells of this donor was located in the carboxyl terminal portion of Der p I and associated with the methionine residues it is possible that peptide 209-222 contains insufficient flanking sequences on the amino terminal sides of the methionine residues. It has been documented that the reduction of the length of a peptide by a single amino acid can completely abrogate T cell recognition (Benjamin et al., 1984).

The possibility that the T cell clone DE9 recognises a contaminant

cannot be excluded, although this is unlikely. The contaminant would have to be present in all the antigen preparations demonstrated capable of stimulating the clone, D. pteronyssinus (Pharmalgen and Bencard), D. farinae (Pharmalgen and Bencard), affinity-purified Der p I and affinity-purified Der f I and to comigrate with the same molecular weight as the group I allergens on the immunoblots. Furthermore, clone DE9 is able to induce IgE synthesis (Chapter 5) and thus if it recognised a contaminant this would be of interest.

The results of the experiments reported in this chapter demonstrate that the T cell repertoires of both atopic and non-atopic subjects contain cells reactive with house dust mite. From the investigation of the specificity of T cell clones both cross-reactive and species-specific epitopes could be identified suggesting that the T cell response is heterogeneous. Clearly, information such as this is necessary if new approaches to immunotherapy based on desensitisation are to be developed.

Table III.1 **Polyclonal response to soluble extracts of *Dermatophagoides* spp.**

	D. farinae	*D. pteronyssinus*	Medium
ATOPIC			
RM	31,690 (14)	24,220 (11)	335 (13)
DY	9,660 (30)	8,081 (35)	577 (8)
GL	12,017 (28)	9,314 (11)	257 (10)
NON-ATOPIC			
DS	37,238 (4)	36,509 (15)	115 (31)
CC	18,063 (37)	4,245 (22)	86 (18)
AC	16,608 (20)	3,952 (40)	956 (50)
RO	1,794 (33)	9,646 (14)	344 (9)

PBMC (5 x 10^4/well) were cultured with soluble extracts of *D. farinae* and *D. pteronyssinus* over a concentration range. Proliferation as correlated with [^{3}H]TdR uptake was determined after 6 days. Results shown are the maximum response expressed as the mean c.p.m. (%SEM) for triplicate cultures.

Table III.2 Polyclonal T cell proliferation to affinity-purified Der f I and Der p I

	RM	DS	CC	JL
No antigen	349 (17)	214 (24)	208 (21)	517 (22)
Soluble D. far.	20,843 (45)	16,178 (16)	3,319 (32)	637 (30)
Der f I:				
0.2µg/ml	1,392 (46)	305 (17)	217 (29)	117 (15)
2.0µg/ml	6,817 (36)	1,002 (10)	4,621 (48)	388 (27)
20.0µg/ml	6,529 (15)	4,723 (43)	2,888 (32)	2,093 (45)
Soluble D. pter.	13,530 (8)	7,213 (4)	3,530 (10)	1,414 (37)
Der p I:				
0.2µg/ml	5,741 (17)	2,099 (21)	2,810 (53)	633 (48)
2.0µg/ml	14,365 (18)	2,315 (34)	10,377 (48)	2,145 (40)
20.0µg.ml	20,967 (9)	3,973 (25)	7,108 (36)	2,177 (39)
PHA 2.5µg/ml	31,318 (3)	21,534 (12)	30,153 (10	12,019 (7)

PBMC (5 x 10^4/well) were cultured with affinity-purified preparations of Der f I and Der p I over a concentration range. Proliferation was determined as previously after 6 days. Results are expressed as the mean c.p.m. for triplicate cultures (%SEM).

Table III.3 Response of PBMC to synthetic peptides of Der p I

Peptide	PBMC	Medium	Soluble D. pter.
P 51- 64	540 (26)	423 (10)	19,655 (4)
P 81-102	8,432 (33)	729 (30)	15,733 (23)
P 135-149	265 (12)	290 (10)	11,278 (13)
P 165-179	581 (25)	729 (30)	15,733 (23)
P 197-212	246 (50)	229 (10)	8,481 (31)
P 12- 25	580 (44)	360 (1)	7,426 (9)
P 185-196	894 (23)	263 (13)	28,582 (7)

PBMC (5 x 10^4/well) from the atopic donor RM were cultured with synthetic peptides of the Der p I allergen over a concentration range. Proliferation was determined as previously after 6 days. Results shown are the count of maximum proliferation, expressed as the mean c.p.m. for triplicate cultures (%SEM).

Table III.4 Response of PBMC from house dust mite allergic individuals to synthetic peptides of Der p I

PBMC	No Ag	D.pter.	51-64	81-102	165-179	135-149	197-212
CW	492	11,390	467	735	904	601	683
CR	253	2,691	207	985	440	943	539
DY	2,810	12,615	429	4,783	947	502	1,649
DSt	905	9,489	2,469	6,102	1,318	2,503	3,281
AD	313	9,049	222	381	670	1,733	1,569

PBMC (5×10^4/well) from a panel of house dust mite allergic individuals were tested for their proliferative responses to a number of synthetic peptides of the Der p I allergen over a concentration range. $^{[3]}$HTdR uptake was determined after 6 days. Results shown give the maximum response observed, expressed as previously.

Table III.5 Response of PBMC to CNBr-cleaved Der p I

	PBMC (RM)
No antigen	162 (27)
D. pter. 50µg/ml	10,535 (13)
Der p I 10µg/ml	7,873 (13)
Der p I 30µg/ml	13,449 (1)
CNBr-Der p I:	
0.03µg/ml	148 (19)
0.1µg/ml	316 (7)
0.3µg/ml	117 (15)
1.0µg/ml	675 (28)
3.0µg/ml	111 (23)
10.0µg/ml	329 (19)
30.0µg/ml	185 (28)

PBMC from the house dust mite allergic subject, RM, ($5x10^4$/well) were cultured with a CNBr-cleaved preparation of Der p I over a concentration range. $^{[3]}$HTdR incorporation was determined at day 7 as previously.

Table IIL6 Response of PBMC to synthetic peptides containing methionine residues

	PBMC (RM)	Controls	
		Medium	Soluble *D. pter.*
P 12-34	473 (20)	340 (22)	17,340 (12)
P 209-222	389 (17)	423 (17)	8,927 (15)

PBMC ($5x10^4$/well) from the allergic subject RM were cultured with two methionine-containing synthetic peptides of *Der p* I over a concentration range. Proliferative responses at day 7 were determined as previously.

Table III.7 Indirect immunofluorescent cytofluorimetric analysis of house dust mite specific T cell clones

Clone	CD4	CD8	UCHL1	control
atopic donor				
DX	89.9%	2.2%	NT	0.6%
DD11	97.8	0.8	97.9	0.5
DE9	99.8	0.7	99.5	0.5
DE5	99.5	0.4	99.7	0.5
DE41	99.8	0.8	99.1	0.5
DE12	99.3	0.6	99.3	0.5
DE26	99.8	0.9	99.7	0.6
DH15	98.2	1.5	NT	1.6
DE43	98.9	1.2	NT	0.5
non-atopic donor				
DF1	99.9	0.6	99.3	0.4
DG2	99.6	0.3	NT	0.5

Cloned T lymphocytes were incubated with monoclonal antibodies Leu 2a, Leu 3a and UCHL1 followed by FITC-conjugated rabbit anti-mouse immunoglobulin, before analysis for fluorescence on a FACS-analyser cytofluorimeter. Percentage positivity is shown. NT: not tested.

Table III.8 Proliferation of T cell clones to soluble allergen preparations

	D. far.	D. pter.	Grass	Pariet. jud.	No antigen
a)					
DX	52,940 (5)	2,482 (5)	78 (35)	241 (18)	87 (22)
DD11	24,476 (5)	319 (81)	65 (49)	58 (16)	85 (32)
DE9	24,212 (6)	19,255 (2)	245 (39)	561 (41)	849 (38)
DE5	16,279 (9)	401 (28)	1,441 (21)	835 (12)	1,659 (10)
DE41	6,435 (7)	197 (40)	324 (8)	325 (9)	152 (44)
DE12	12,845 (6)	290 (19)	169 (27)	247 (6)	444 (24)
DE26	12,801 (3)	4,492 (6)	95 (13)	147 (47)	50 (13)
DE28	6,572 (20)	422 (25)	204 (30)	336 (16)	392 (12)
DE43	4,376 (15)	386 (26)	84 (27)	306 (40)	384 (22)
b)					
DH12	7,521 (18)	7,525 (15)	181 (19)	316 (7)	217 (19)
DH15	13,521 (3)	18,168 (7)	383 (21)	287 (10)	978 (18)

Cloned T cells ($5x10^4$/ml) were stimulated with a panel of soluble aeroallergens together with autologous irradiated PBMC (1.25×10^5/ml). $^{[3]}$HTdR incorporation was determined at 72hr. Results are the maximum proliferative response observed over a concentration range and expressed as mean c.p.m. (%SEM).

Table III.9 Proliferation of T cell clones from non-atopic donors to soluble allergen preparations

	D. far.	D. pter.	Grass	Pariet. jud.	No antigen
DF1	30,780 (3)	222 (29)	101 (40)	1280 (7)	40 (46)
DG2	10,931 (3)	315 (12)	131 (30)	120 (10)	66 (22)

Cloned T cells ($5x10^4$/ml) from two house dust mite reactive T cell clones isolated from non-atopic subjects were cultured with a panel of soluble allergen extracts in the presence of autologous irradiated PBMC (1.25×10^5ml) as accessory cells. $^{[3]}$HTdR incorporation was determined at 72 hr and results expressed as for Table III.8.

Table III.10 Proliferation of T cell clones from atopic donor to affinity-purified Der f I and Der p I

	DX	DE9	DE5	DE12	DH12	DH15
No antigen	587 (31)	836 (9)	184 (11)	235 (24)	217 (19)	978 (18)
Soluble D. farinae	9,105 (12)	6,627 (2)	4,595 (17)	10,823 (4)	7,521 (18)	13,521 (3)
Der f I: 0.2μg/ml	687 (15)	821 (16)	263 (38)	129 (16)	253 (36)	679 (26)
2μg/ml	3,037 (3)	3,148 (8)	217 (18)	199 (19)	281 (15)	3,447 (16)
20μg/ml	4,738 (13)	4,546 (1)	149 (14)	473 (39)	325 (25)	6,223 (9)
Soluble D. pteronyssinus	2,043 (28)	2,738 (7)	324 (22)	180 (38)	7,525 (15)	18,168 (7)
Dter p I: 0.2μg/ml	242 (18)	199 (17)	142 (36)	604 (30)	215 (47)	167 (29)
2μg/ml	468 (35)	1,149 (8)	198 (18)	428 (16)	707 (11)	678 (30)
20μg/ml	402 (22)	2,595 (8)	421 (28)	118 (24)	1,257 (19)	5,876 (10)

T cells of line DX and cloned T lymphocytes from a panel of clones isolated from the house dust mite allergic subject RM (10^4/well) were stimulated with affinity-purified preparations of Der f I and Der p I in the presence of autologous accessory cells (2.5 x 10^4/well). Proliferation was determined at 72 hr as previously.

Table III.11 **Proliferation of T cell clones from non-atopic donors to affinity-purified Der f I and Der p I**

	DF1	DG2
No antigen	275 (29)	117 (20)
Soluble D. far.	30,780 (3)	4,115 (16)
Der f I:		
0.2µg/ml	69 (14)	117 (20)
2.0µg/ml	91 (26)	141 (15)
10.0µg.ml	61 (6)	122 (27)
Soluble D. pter.	222 (29)	217 (17)
Der p I:		
0.2µg/ml	87 (14)	84 (34)
2.0µg/ml	60 (10)	232 (20)
20.0µg/ml	79 (23)	117 (44)

Cloned T cells (10^4/well) from two non-atopic subjects were cultured with affinity-purified preparations of Der f I and Der p I in the presence of autologous PBMC (2.5 x 10^4/well) as accessory cells. Proliferative responses were determined as previously at 72 hr.

Table III.12 Response of T cell line to affinity-purified Der f II and Der p II

No antigen	729 (29)
Soluble D. farinae	23,482 (4)
Der f II: 0.3μg/ml	1,340 (10)
3.0μg/ml	3,895 (9)
30.0μg/ml	11,874 (4)
Soluble D. pteronyssinus	4,260 (15)
Der p II: 0.3μg/ml	848 (17)
3.0μg/ml	1,529 (17)
30.0μg/ml	1,558 (23)

T cells from long term line DX (10^4/well) were cultured with affinity-purified preparations of Der f II and Der p II in the presence of autologous irradiated PBMC (2.5 x 10^4/well) as accessory cells. Proliferative responses were determined as previously at 72 hr.

Table III.13 Response of T cell clone DE9 to synthetic peptides

	clone DE9	Controls	
		no antigen	Soluble D. pter.
P 51-64	1,063 (18)	481 (10)	44,949 (1)
P 81-102	758 (23)	481 (10)	44,949 (1)
P 135-149	225 (16)	142 (5)	17,108 (5)
P 165-179	2,799 (24)	2,646 (19)	33,992 (9)
P 197-212	479 (30)	167 (17)	26,301 (8)
P 12-25	738 (25)	619 (26)	28,582 (7)
P 185-196	894 (23)	619 (26)	28,582 (7)
P 156-168	546 (30)	775 (25)	29,453 (4)
P 93-108	790 (15)	173 (40)	7,235 (8)
P 123-136	441 (12)	173 (40)	7,235 (8)

Cloned T cells from clone DE9 (5×10^4/ml) were stimulated by synthetic peptides of Der p I in the presence of autologous irradiated accessory cells. $^{[3]}$HTdR uptake was determined at 72 hr and the result of maximum proliferation expressed as before.

Table III.14 Response of T cell clone DE9 to CNBr-cleaved Der p I

	clone DE9
No antigen	724 (8)
D. pter. 50μg/ml	110,738 (1)
Der p I 10μg/ml	55,087 (1)
Der p I 10μg/ml + CNBr-Der p I 12μg/ml	56,165 (1)
Der p I 30μg/ml	57,247 (1)
Der p I 30μg/ml + CNBr-Der p I 12μg/ml	64,422 (1)
CNBr-Der p I:	
0.03μg/ml	81 (17)
0.1 μg/ml	124 (15)
0.3 μg/ml	76 (8)
1.0 μg/ml	206 (18)
3.0 μg/ml	75 (10)
10.0μg/ml	130 (30)
30.0μg/ml	63 (13)

Cloned T cells from clone DE9 ($2x10^4$/well) were stimulated with CNBr-cleaved preparations of Der p I over a concentration range in the presences of irradiated autologous accessory cells (2.5×10^4/well). Incorporation of $^{[3]}$HTdR was determined at 72 hr as before.

Table III.15 Response of T cell clone DE9 to synthetic peptides containing methionine residues.

	DE9	Controls	
		No Antigen	Soluble D. pter.
P 12-34	374 (18)	534 (9)	28,048 (12)
P 209-222	401 (22)	462 (18)	32,526 (10)

Cloned T cells (10^4/well) were cultured with two methionine-containing synthetic peptides of Der p I over a concentration range in the presence of irradiated autologous PBMC ($2.5x10^4$/well) as accessory cells. Proliferative responses were determined as previously at 72 hrs.

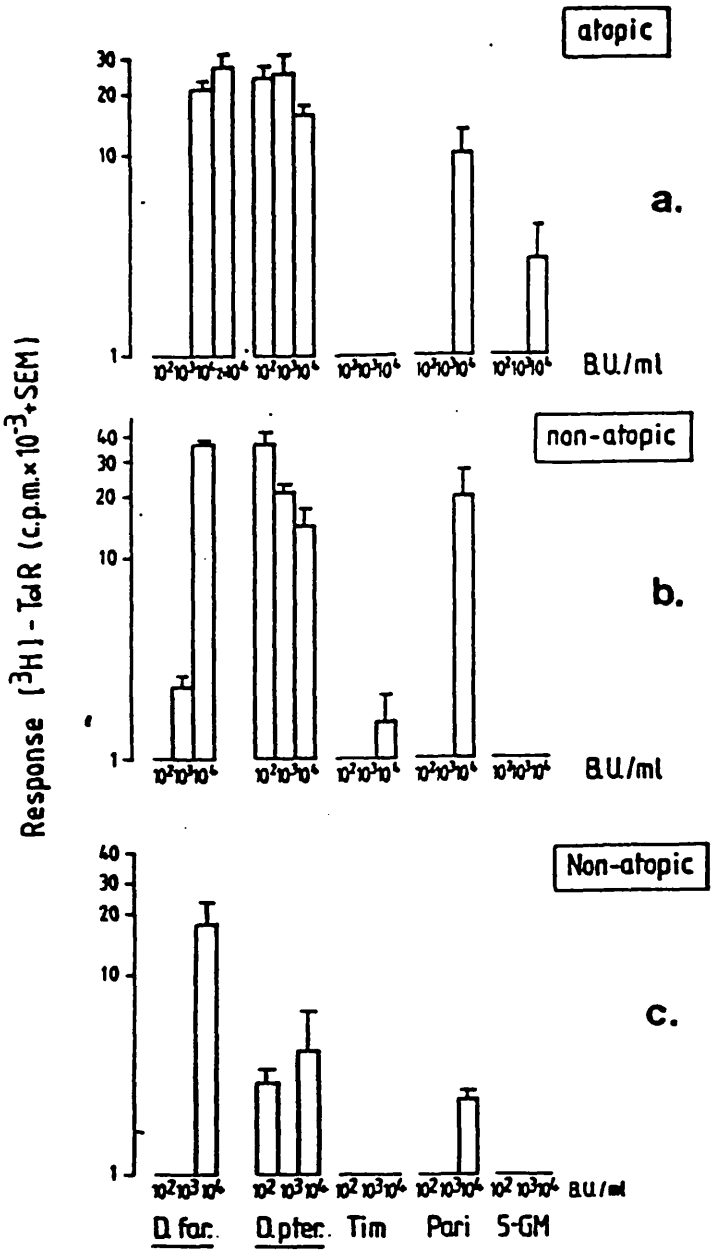

Figure 3.1: Polyclonal T cell proliferative responses to a panel of soluble aeroallergens. PBMC (5 x 10^4/well) from atopic donor RM (a), non-atopic donor DS (b) and non-atopic donor CC (c) were cultured with soluble D. farinae, D. pteronyssinus, Timothy grass, Parietaria judaica and mixed grass pollen over a concentration range. Proliferation was determined by [^{3}H]TdR incorporation in a 7-day assay. The results are expressed as counts per minute (c.p.m.) + SEM of triplicate cultures. Background responses of PBMC in the absence of antigen were less than 500 c.p.m.

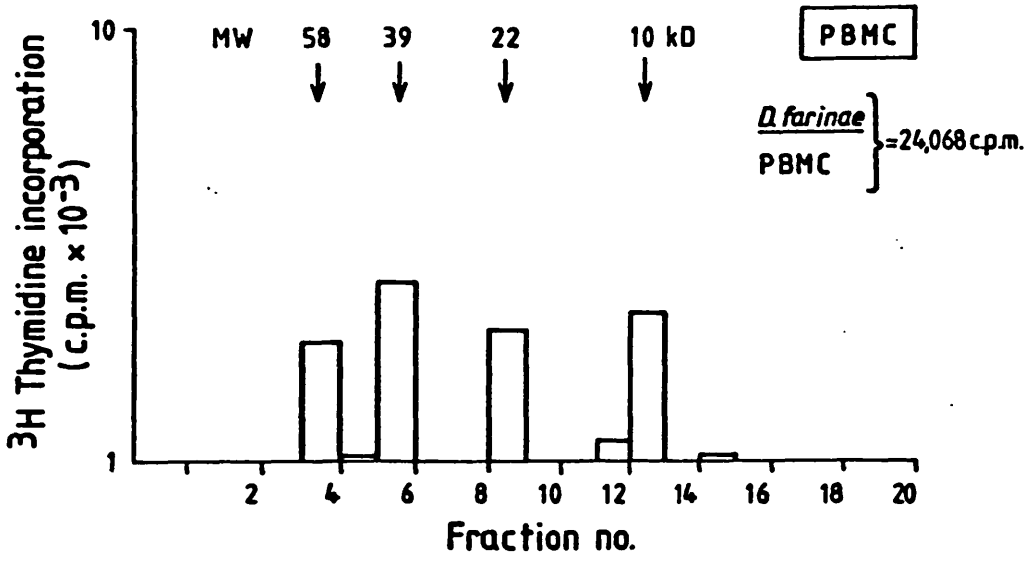

Figure 3.2: Pattern of reactivity of PBMC to SDS-PAGE antigens solubilised from immunoblots of D. farinae. PBMC from atopic donor RM (10^5/well) were cultured with SDS-PAGE-separated immunoblots (20 fractions) after solubilisation with DMSO and assayed as described in the legend to Fig. 3.1. Background responses of PBMC in the absence of antigen were 355 $\pm$ 30 c.p.m.

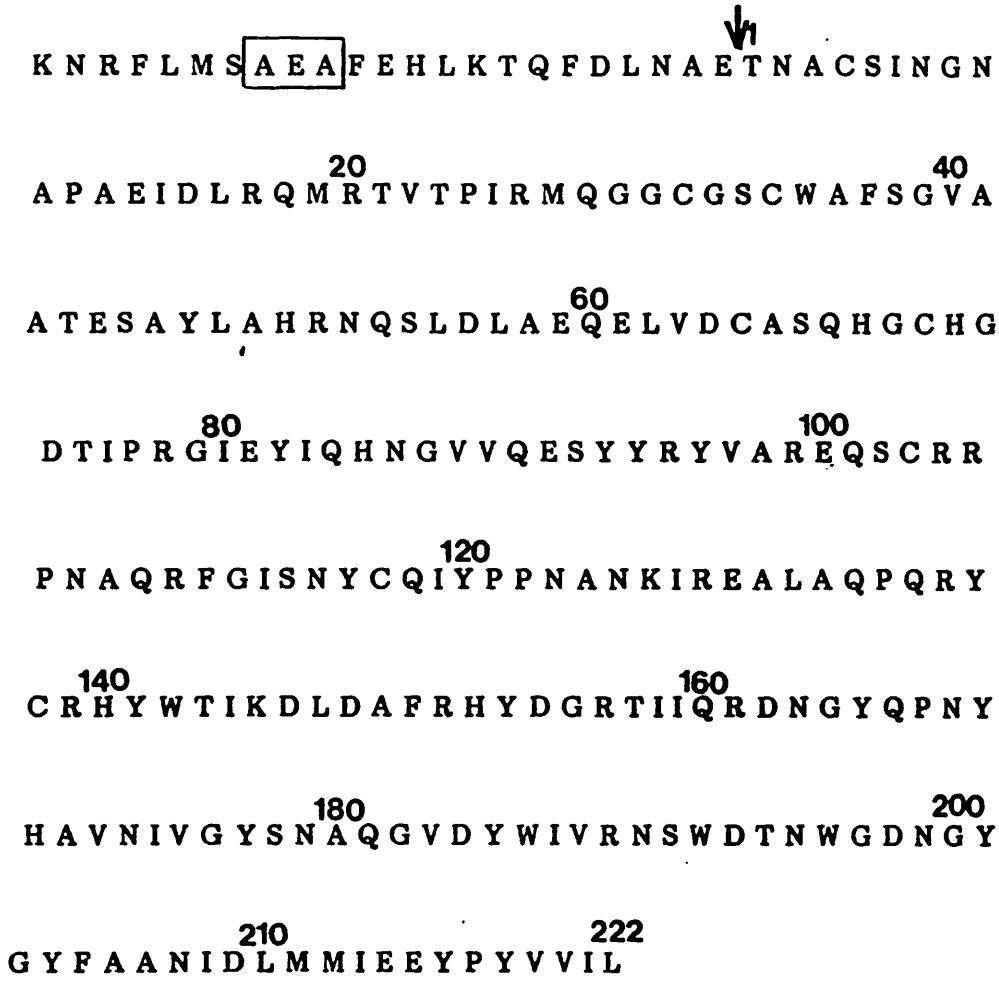

Figure 3.3: Amino acid sequence of Der p I (Chua et al., 1988).

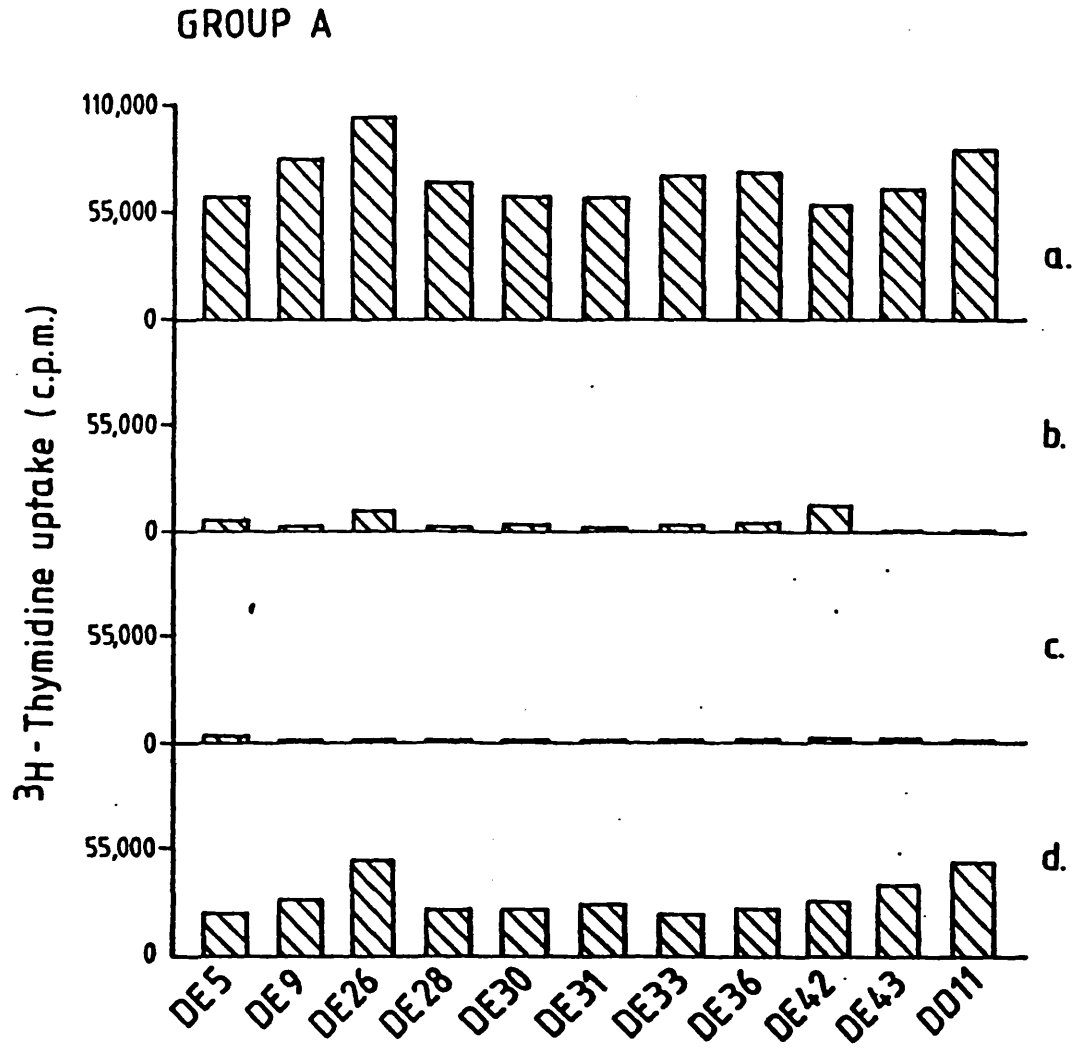

Figure 3.4a: Primary screen of the reactivity of a panel of CD4+ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr. Group A: proliferative responses to D. farinae of 60-110,000 c.p.m.

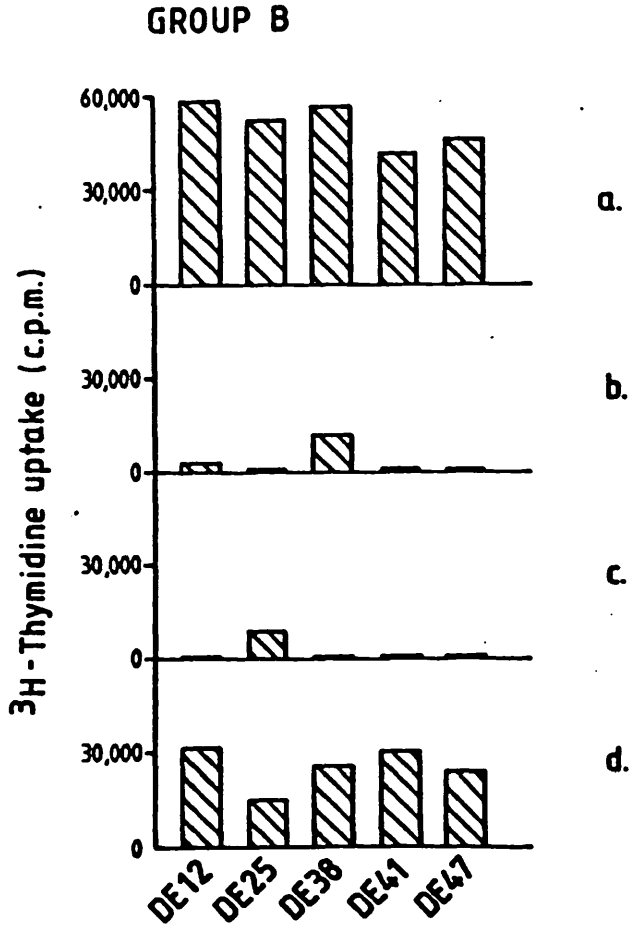

Figure 3.4b: Primary screen of the reactivity of a panel of CD4$^+$ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr. Group B: proliferative responses to D. farinae of 40-60,000 c.p.m.

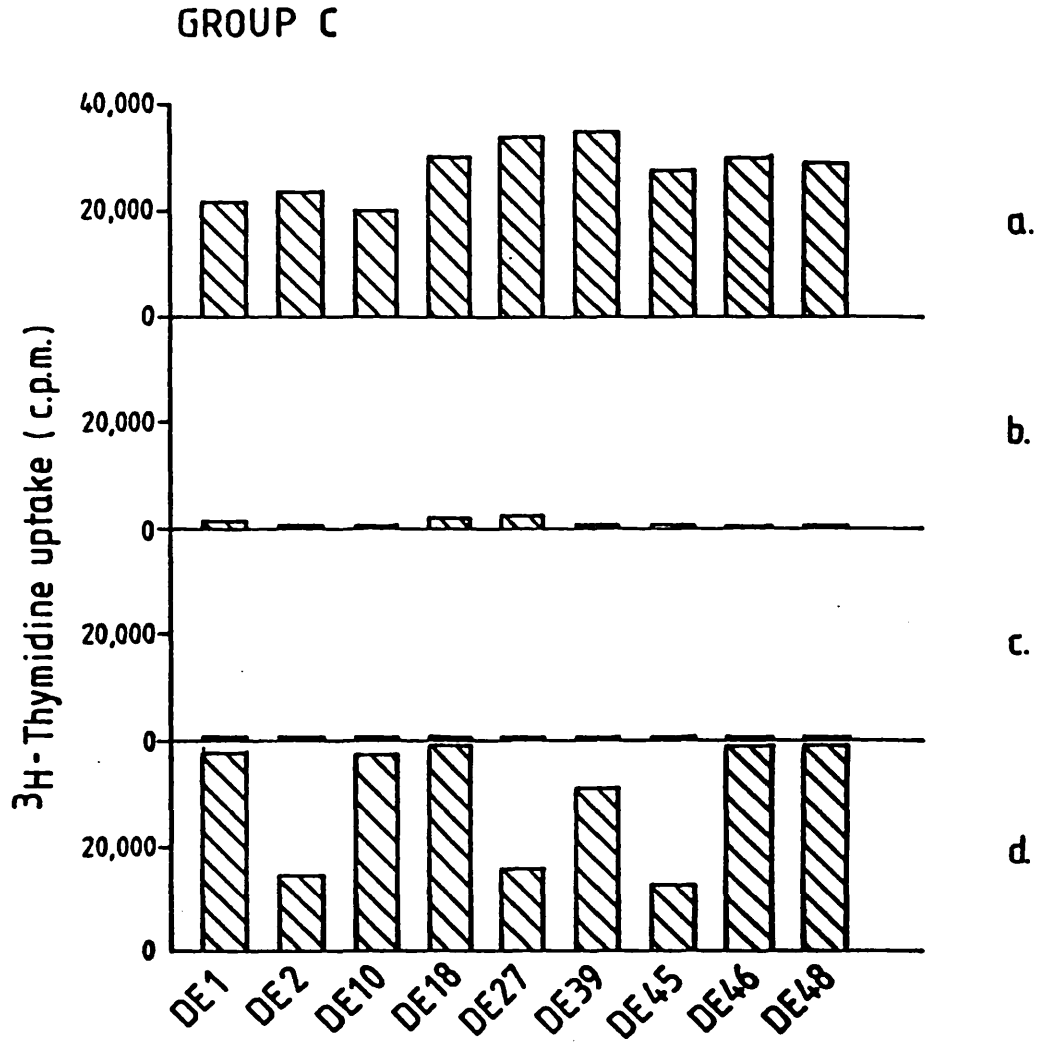

Figure 3.4c: Primary screen of the reactivity of a panel of CD4$^+$ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5×10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr. Group C: proliferative responses to D. farinae of 20-40,000 c.p.m.

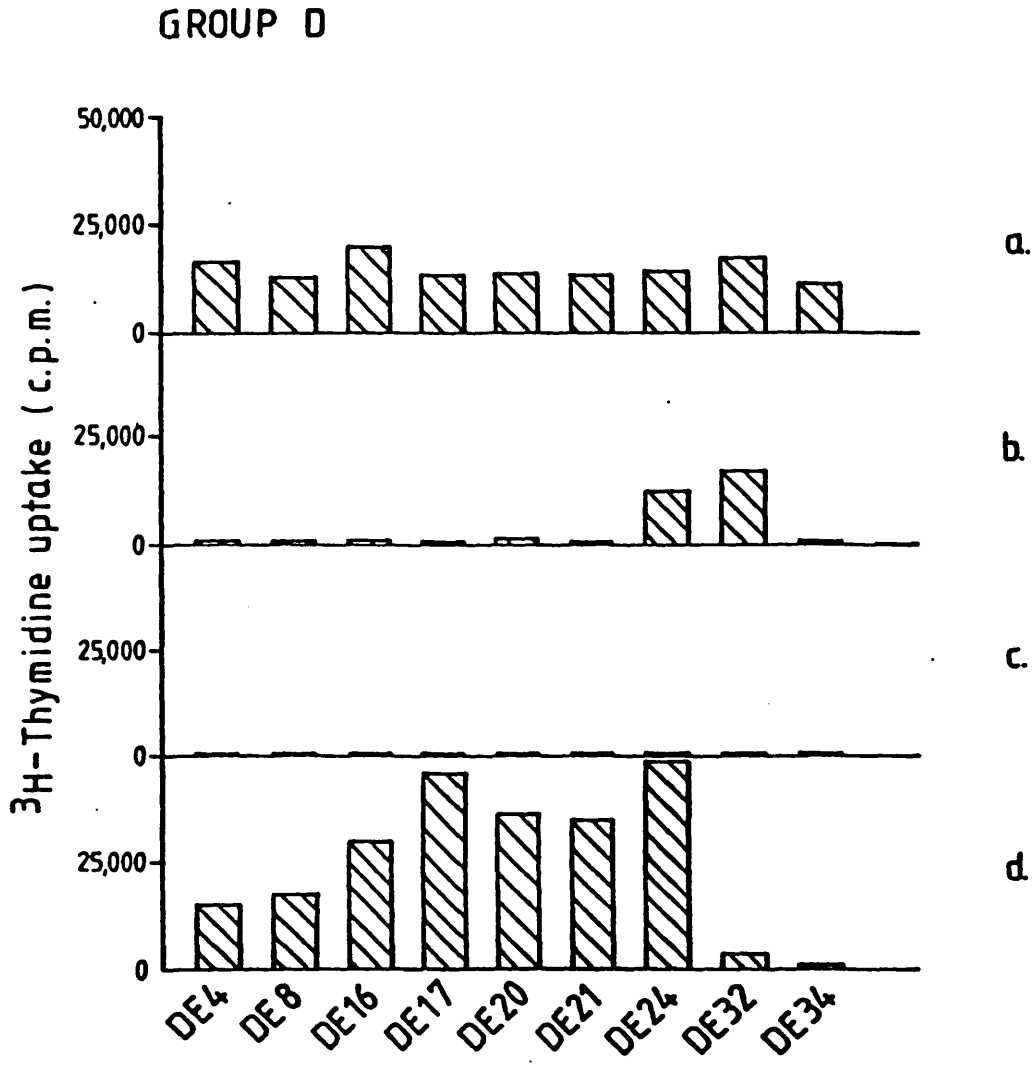

Figure 3.4d: Primary screen of the reactivity of a panel of CD4$^+$ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr. Group D: proliferative responses to D. farinae of 10-20,000 c.p.m.

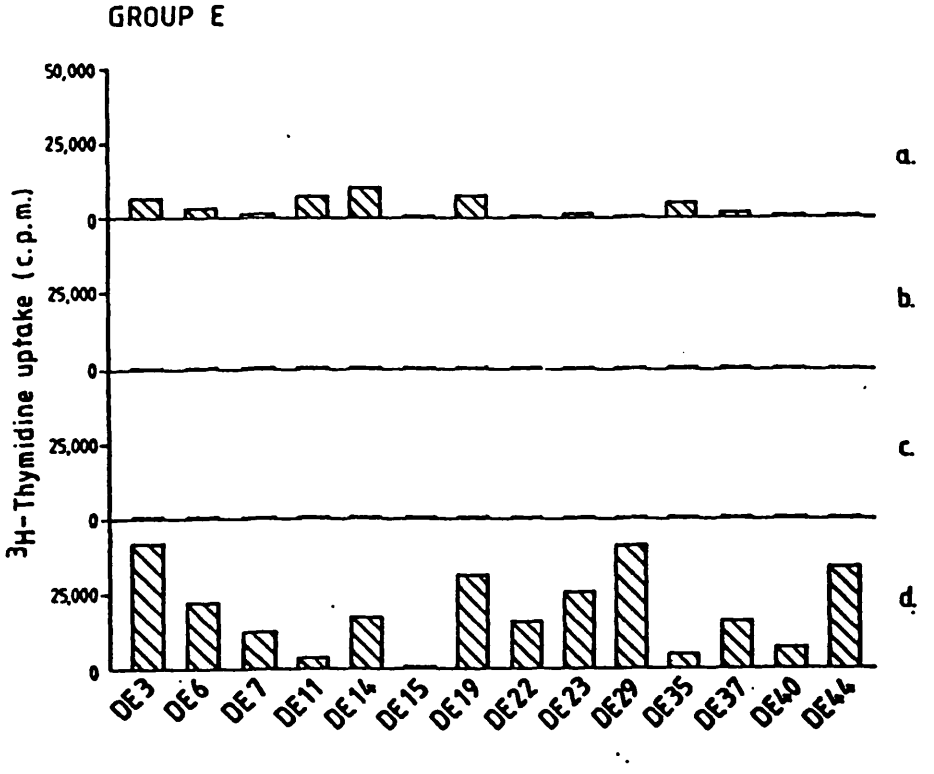

Figure 3.4e: Primary screen of the reactivity of a panel of CD4$^+$ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr. Group E: proliferative responses to D. farinae of 0-10,000 c.p.m.

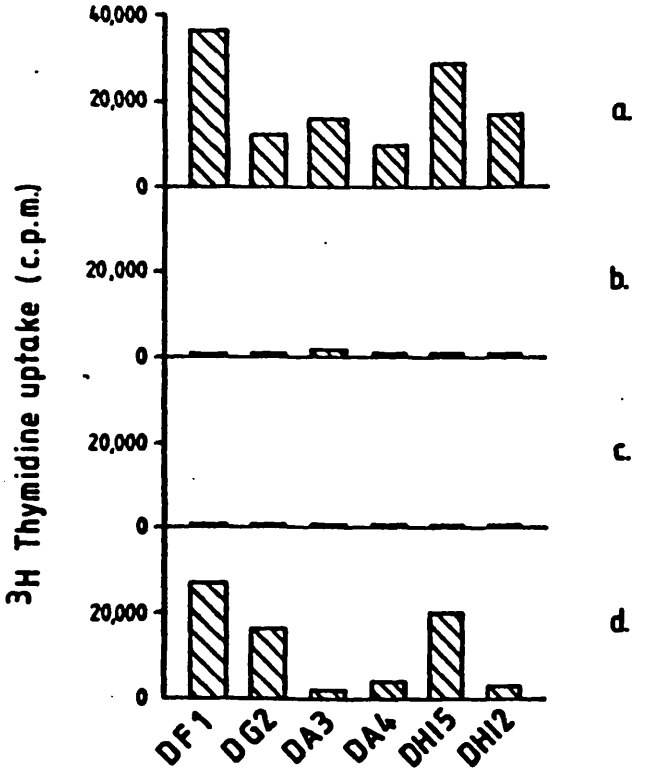

Figure 3.4f: Primary screen of the reactivity of a panel of CD4$^+$ T cell clones. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5×10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr.

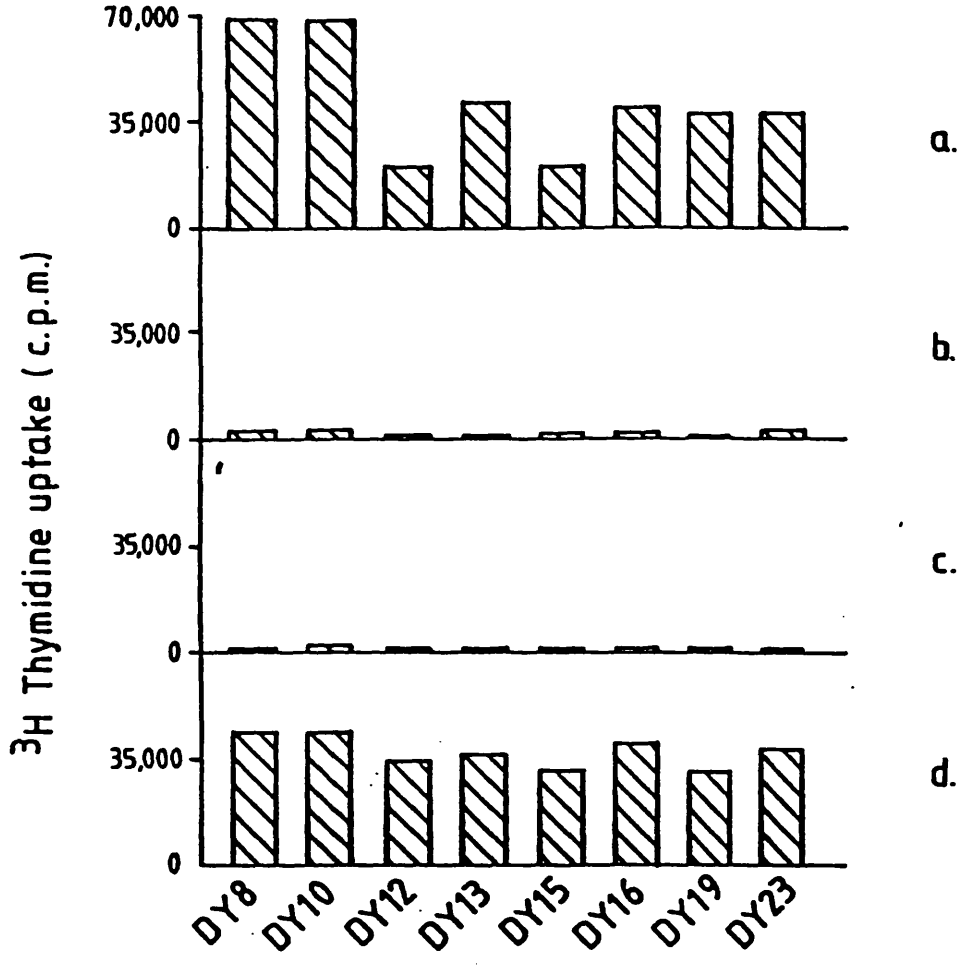

Figure 3.5a: Primary screen of the reactivity of a panel of T cell lines. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr.

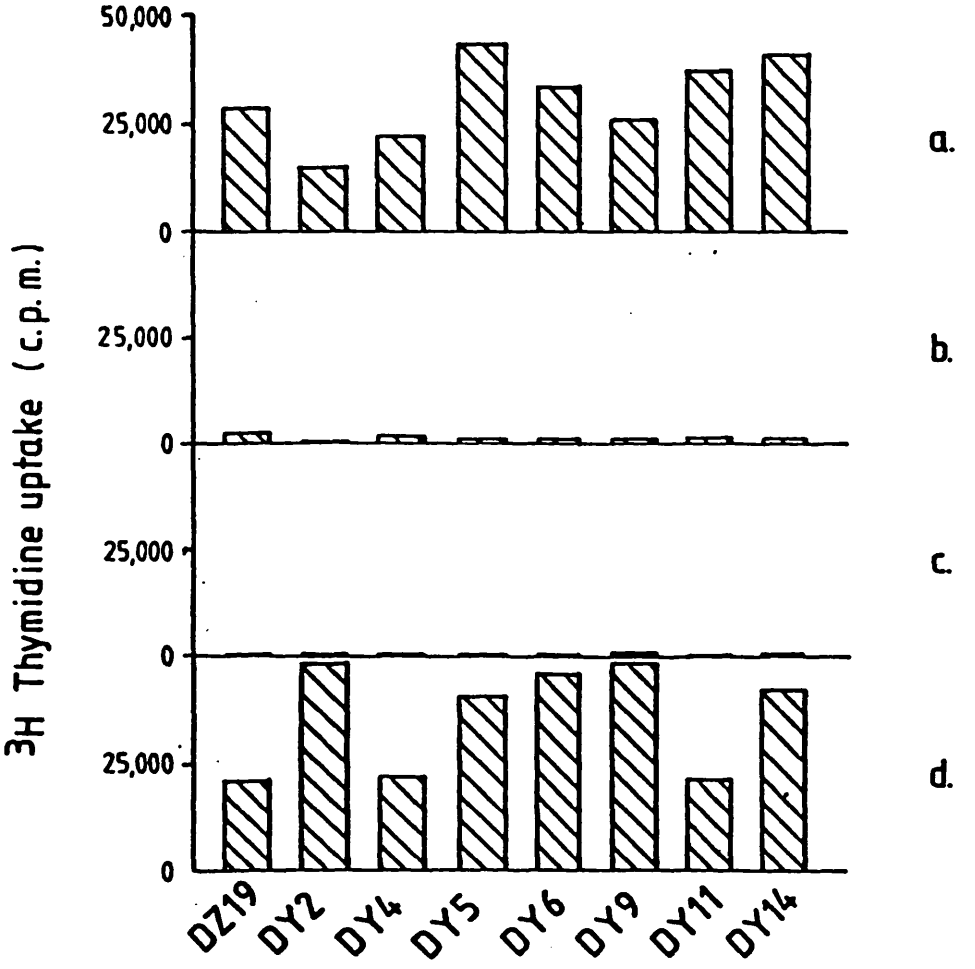

Figure 3.5b: Primary screen of the reactivity of a panel of T cell lines. Cloned T cells (10^4/well) were screened for responsiveness to D. farinae antigen (10^4 B.U./ml) in the presence of irradiated autologous PBMC (2.5 x 10^4/well) as accessory cells (a), responsiveness to accessory cells in the absence of antigen (b), responsiveness to antigen in the absence of accessory cells (c) and responsiveness to 10% IL-2 (d). Proliferation as correlated with [^{3}H]TdR uptake was determined at 72 hr.

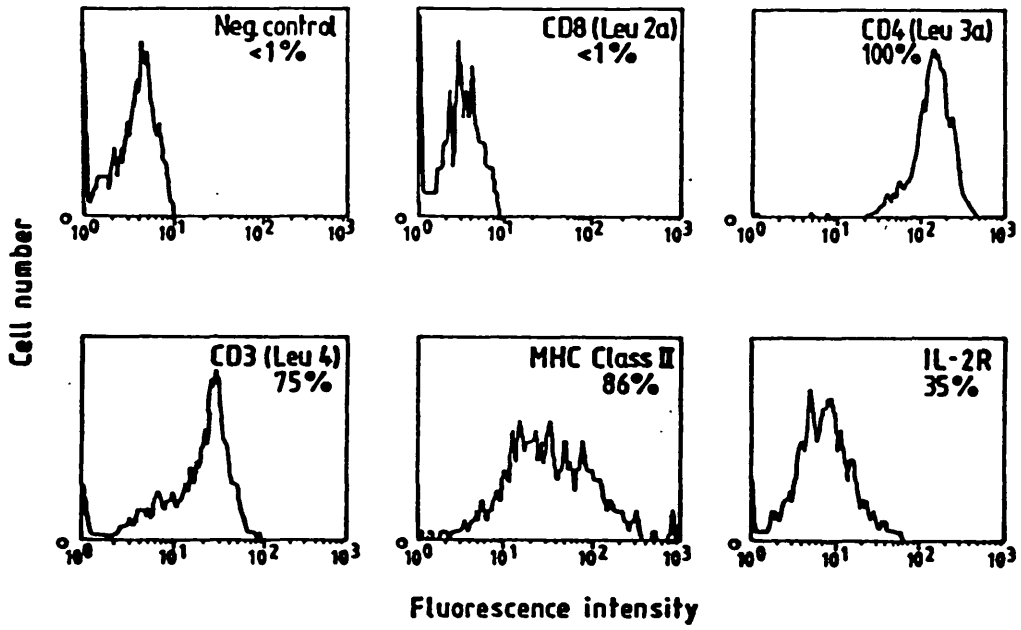

Figure 3.6: Indirect immunofluorescent cytofluorimetric analysis of clone DD11. Samples were analysed with monoclonal antibodies as indicated above the histograms. Percentage positivity is shown.

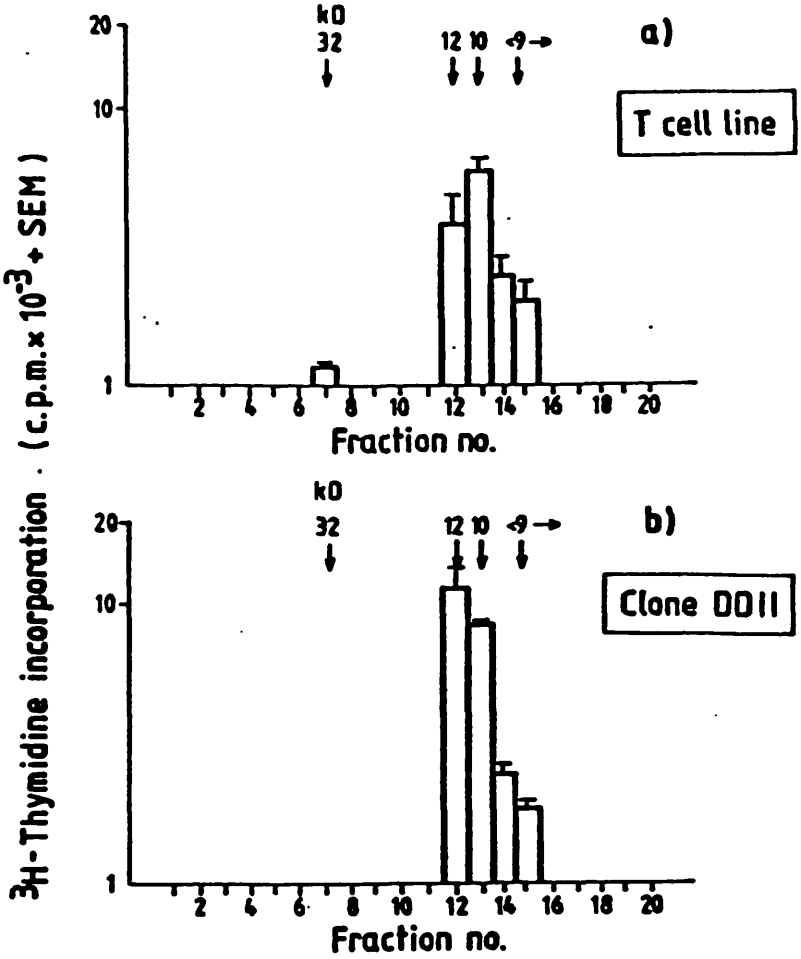

Figure 3.7: Differential pattern of reactivity of T cell line (a) and clone (b) to SDS-PAGE-separated antigens solubilised from immunoblots of D. farinae. T cells of the long term line DX and clone DD11 (5 x 10^4/ml) were cultured with SDS-PAGE-separated immunoblots (20 fractions) after solubilisation with DMSO, together with irradiated PBMC (2.5 x 10^5/ml) and assayed by [^{3}H]TdR incorporation after 72 hr. Background responses of DX and DD11 to accessory cells in the absence of antigen were 973 ± 20 and 66 ± 16 c.p.m., respectively.

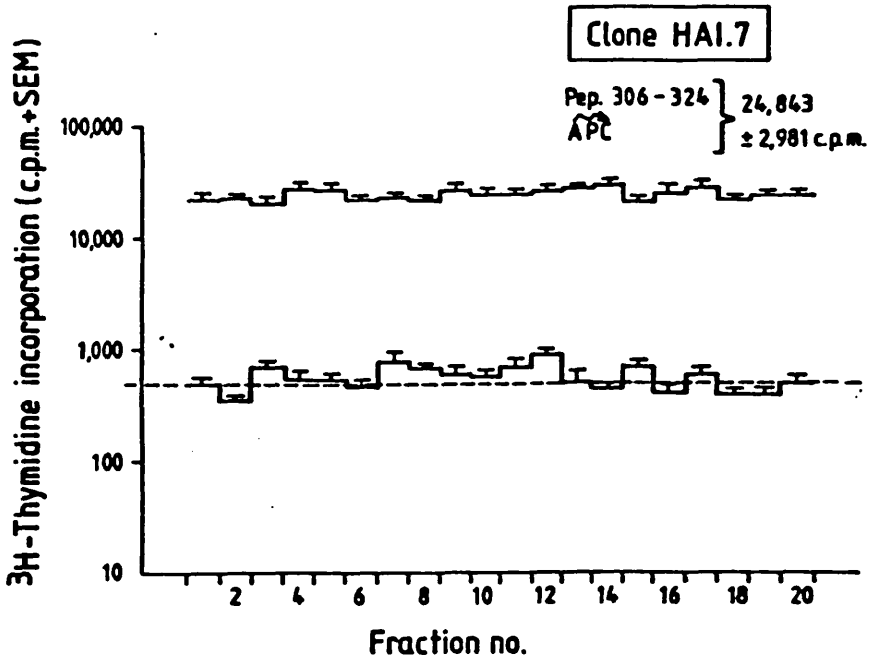

Fig 3.8: Exclusion of non-specific mitogenic and inhibitory effects of D. farinae immunoblots on influenza haemagglutinin specific T cell clone (HA1.7). Cloned T lymphocytes (10^4/well) were cultured with D. farinae immunoblots alone (lower graph) or together with irradiated histocompatible PBMC (2.5 x 10^5/ml) and HA peptide 306-324 (0.3 µg/ml; upper graph). Background controls of accessory cells and T cell clone (---).

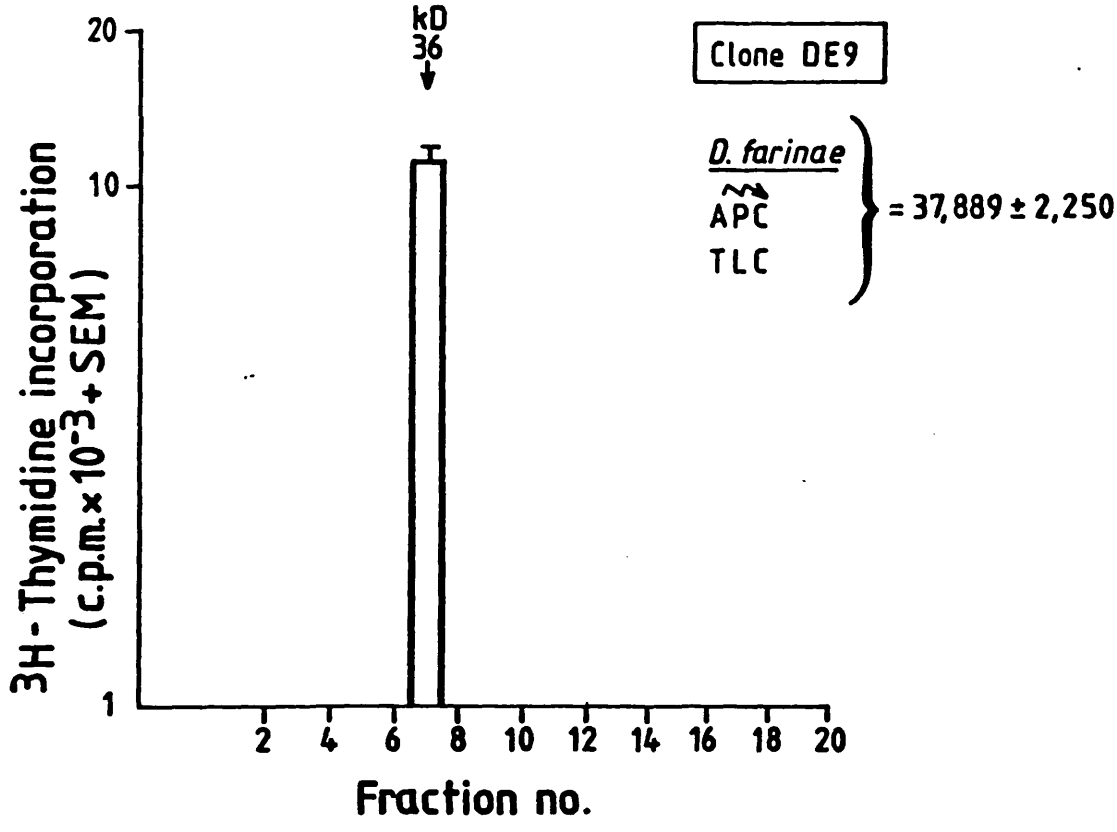

Figure 3.9: Pattern of reactivity of T cell clone DE9 to SDS-PAGE immunoblots of D. farinae. Cloned T cells (5 x 10^4/ml) were cultured with SDS-PAGE-separated immunoblots and assayed as described in legend to Fig. 3.7. Background responses of DE9 to accessory cells in the absence of antigen were 520 ± 16 c.p.m.

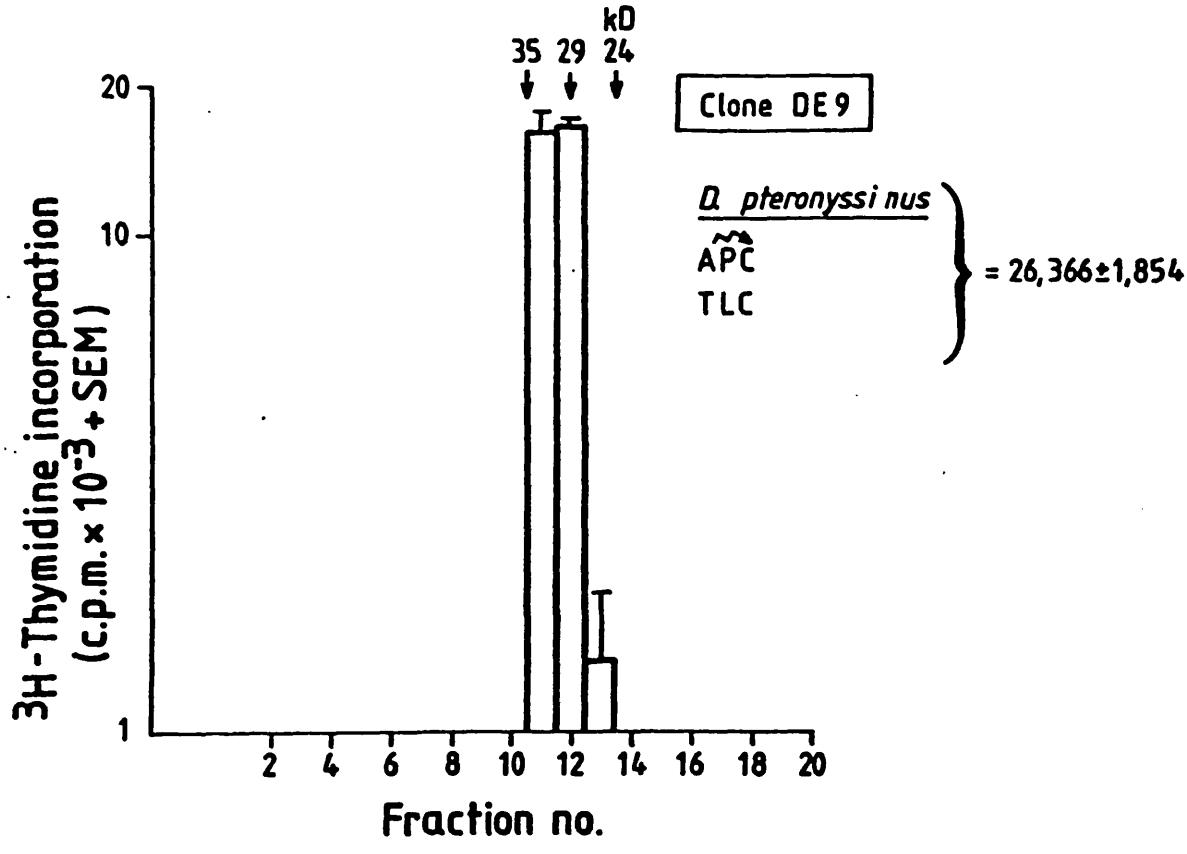

Figure 3.10: Response of cloned T cells to immunoblots of D. pteronyssinus. Cloned T cells (10^4/well) were stimulated with SDS-PAGE-separated immunoblots (20 fractions) after solubilisation with DMSO and reprecipitation, in the presence of irradiated autologous PBMC as a source of accessory cells. Proliferation was determined at 96 hr as previously. Background responses of DE9 to accessory cells in the absence of antigen were 480 ± 30 c.p.m.

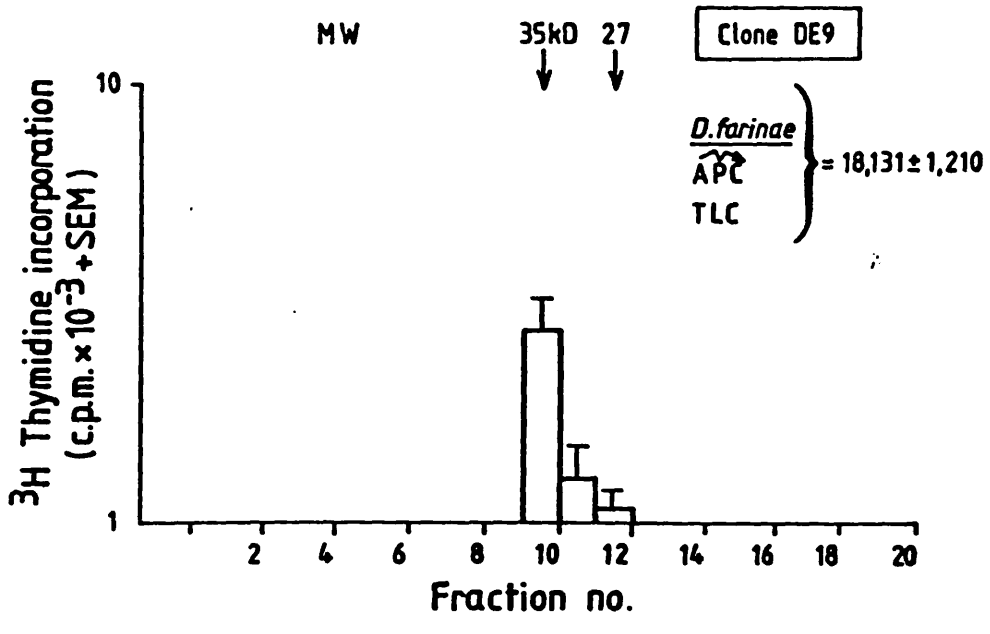

Figure 3.11: Response of cloned T cells to immunoblots of D. farinae. The response of T cell clone DE9 to immunoblots from a second SDS-PAGE-fractionated preparation of D. farinae was determined as previously. Background responses of DE9 to accessory cells in the absence of antigen were 407 ± 20 c.p.m.

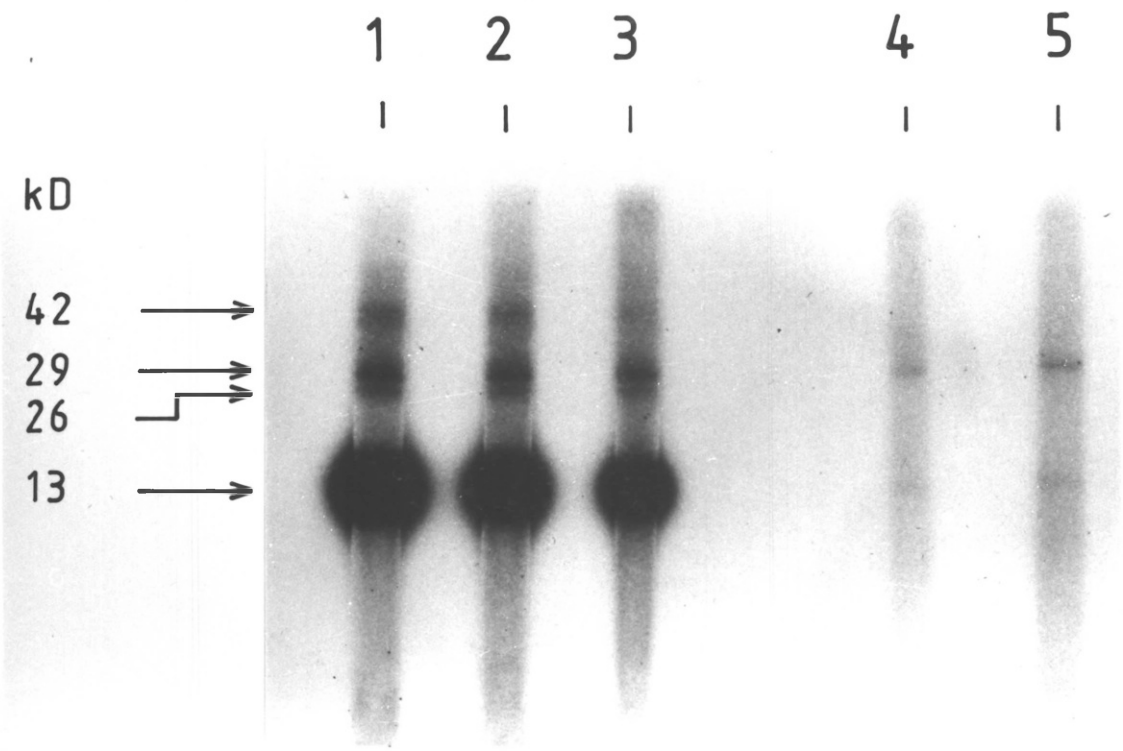

Plate 3: Autoradiograph of Western blots showing the IgE response of human sera to D. farinae mite body extract separated by SDS-PAGE and transferred to nitrocellulose. The strips were probed with sera from the atopic donor RM (undiluted, lane 1; 1:2, lane 2 and 1:5, lane 3) and a non-atopic donor (1:2, lane 4; 1:5, lane 5) and autoradiographed to detect binding of IgE.

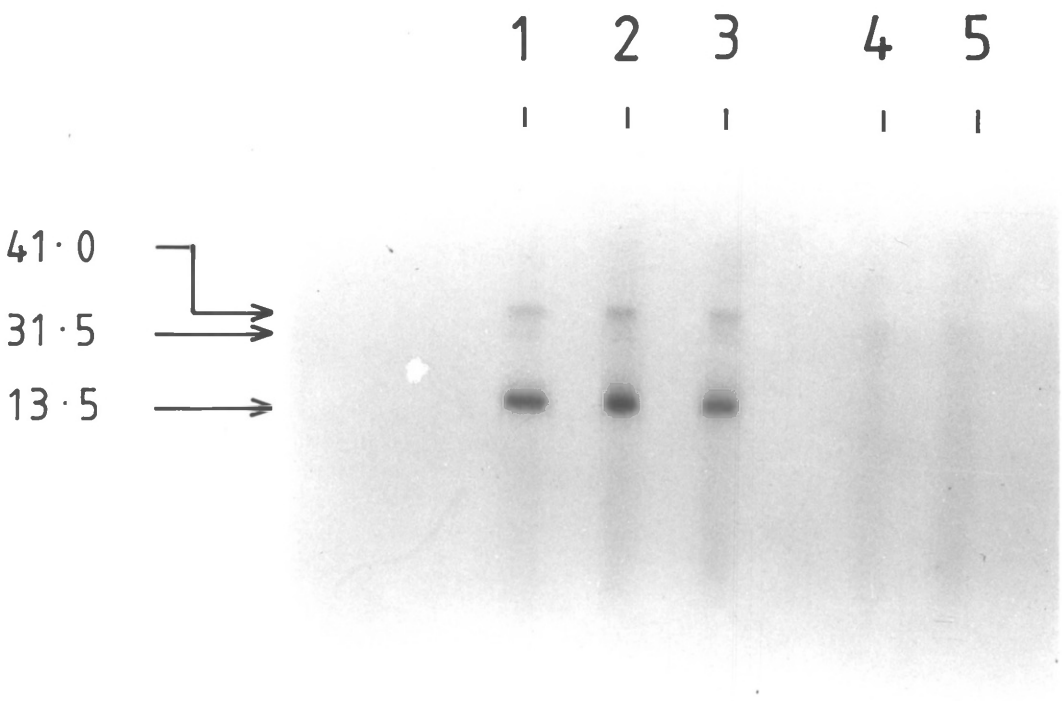

Plate 4: Autoradiograph of Western blots showing the IgE response of human sera to D. pteronyssinus mite body extract separated by SDS-PAGE and transferred to nitrocellulose. Immunoblots were probed with sera from the atopic donor RM and a non-atopic donor as described for Plate 2.

CHAPTER 4 MHC RESTRICTION SPECIFICITY OF T CELL ANTIGEN RECOGNITION

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4.1 INTRODUCTION

Major histocompatibility complex (MHC) class II proteins restrict antigen recognition of CD4+ T cells by forming distinct allele-specific molecular complexes with both "processed" fragments and the antigen receptor of T cells (Schwartz, 1985; Shevach and Rosenthal, 1973). The formation of these molecular interactions is necessary for T cell activation and the induction of immune responses. With respect to human CD4+ T cells it has been demonstrated that the HLA-D region gene products (DR, DQ and DP) can all function as restriction elements in the presentation of extrinsic antigen (Eckels and Lamb, 1985; Bergholtz, Thoresen and Thorsby, 1980; Qvigstad, Moen and Thorsby, 1984). The evidence for this has been largely derived from panel studies using allogeneic accessory cells (Eckels et al., 1982) and the inhibition of antigen-dependent activation of cloned T cells with anti-HLA class II antibodies (Lanzavecchia et al., 1983). However, the recent reports that murine L cells expressing HLA class II proteins are able to present antigen has provided an additional approach for analysing T cell restriction specificity (Austin et al., 1985; Lechler et al., in press).

In this chapter, the restriction specificity of a panel of cross-reactive and D.farinae specific T cell clones (described in chapter 3) derived from the peripheral blood of an atopic individual with house dust mite allergy of MHC haplotype HLA A2,24; B27,50; Bw6; C2; DR5(12),7; DRw52; DQ2,3; DP4 is investigated.

4.2 RESULTS

I The inhibitory effects of monoclonal antibodies reactive with HLA-DR, DQ and DP proteins on the proliferative response of D.farinae specific T cell clones.

To initially define the subsets of MHC class II molecules restricting antigen recognition, monoclonal antibodies directed against framework determinants of HLA-D-region molecules were added at the initiation of cultures to inhibit antigen-dependent proliferation of the T cell clones (Fig. 4.1). Both cross-reactive and species-specific T cell clones were selected for analysis (Table IV.1). Although a small degree of inhibition was observed with the Leu 10 (HLA-DQw1,w3) and B7/21 (HLA-DP) antibodies, maximal blocking was obtained with L243, which recognises a monomorphic DR determinant (alpha beta I; alpha beta III). At concentrations giving maximal staining of cells, the anti-DR antibody (0.025 µg/ml) inhibited the antigen-dependent response of all the T cell clones by 60% or greater, although the sensitivity of the clones to inhibition was variable with DE5 being inhibited by 100%. The minimal inhibition observed with anti-DP and -DQ antibodies at saturating concentrations may be due to the weak cross-reactivity of these antibodies for DR molecules.

II Analysis of MHC restriction using panels of allogeneic accessory cells.

Based on the serological inhibition analysis, T cell clones were further tested for responsiveness in their ability to recognise antigen presented by an accessory cell panel consisting of DR-matched and DR-

mismatched PBMC from 30 individuals, including RM as the DR5(12), 7, (52,-) autologous control (Table IV.2). Full haplotype of the accessory cell panel is shown (Table IV.3). The results of control cultures when the T cell clones and accessory cells were incubated together in the absence of antigen ranged from 47 to 540 c.p.m. (mean = 144 c.p.m.) (Table IV.4). In all cases the percentage SEM for triplicate cultures was less than 25%. Analysis of restriction specificities was performed after objectively assigning positive and negative responses using the T max programme suggested by Mendell (Mendell et al., 1977). For each clone in each experiment c.p.m. data were first normalised by $\log_{10}$ transformation, ranked and partitioned into high (positive) and low (negative) groups. This approach was used successfully to analyse the response patterns of alloreactive T cell clones (Rosen-Bronson et al., 1986).

Clone DE41 recognised antigen presented by 12/15 DR5+ accessory cells, failing to respond when the accessory cells 557G (DR5, w10), 589G (DR1,5[11]) and 814D (DR4,5[12]) were used. This pattern of recognition suggests that DE41 recognises antigen in association with the serologically-defined HLA-DR specificity DR5. A subtype of DR5 could account for the lack of presentation by the 3/15 accessory cells (Tieber et al., 1986).

Clone DE5 recognised 6/8 DR7+ accessory cells and 4/6 DR4+ accessory cells, failing to respond with 189P (DR3,7), 005G (DR1,7), 855E (DR2,4) and 727E (DR4,6). From the known Dw-subtype heterogeneity of both DR4 and DR7, DE5 may recognise a DRw53-associated restriction element. As all the DRw53 panel cells did not show functional

presentation the implication would be that subtypes of DRw53 exist, as seen with DRw52.

T cell clone DE9 recognised 9/15 DR5+ accessory cells, 1/3 DR2+ accessory cells 001C (DR2,-) and the DR8+ accessory cells 1009 (DR3,8). Examination of conserved residues in the beta chain of these alleles (DR2, DR5 and DR8) suggests antigen may bind to the sequence FLEDR (67-71) in the third variable region of the carboxyl terminal of the beta I domain.

Clone DD11 recognised 13/15 DR5+ accessory cells (failing to respond to 557G [DR10,5] and 589G, DR1,5[11]) and 1/4 informative DR3+ accessory cells (189, DR3, 7). It is noteworthy that 189P (DR3, 7) is a DR3 variant associated with HLA-B18 rather than the usual HLA-B8.

Clone DE12 appears similar to DD11 in its restriction specificity, however the response pattern is slightly different, primarily with respect to the recognition of accessory cells 239E (DR7,w9), 305G (DR2,7) and 847E (DR4,-).

Clone DE26 shows a restriction pattern that is not dissimilar to DD11 and DE12, with primary differences being in the recognition of accessory cells 239E (DR7,w9), 903E (DR4,7), 305G (DR2,7) and 557G (DR5,w10).

The patterns of restriction observed with these three clones suggest that they may recognise antigen in association with allelic forms of DRw52.

III Antigen presentation using murine fibroblasts transfected with HLA-D region genes - confirmation of the DRw52.

Results from both the serological inhibition and panel studies implied that antigen recognition by T cell clones DD11, DE12 and DE26 was restricted by proteins encoded by the DRw52 beta III gene. In order to investigate this further, murine L cells transfected with DRw52 subtype genes were used to present antigen to clone DE26. Control transfectants (Ltk-, the untransfected parental cell line and DR1+ L cells generously provided by Dr. R. Lechler) and autologous PBMC were used in parallel as accessory cells. The TR22.9 (LR6.1/52b) L cell line was transfected with the genomic DR beta III gene DRw52b of DR4/6, the DR alpha gene and the invariant gene. TR167-B2 (LR6.3/52c)L cell line was transfected with the cDNA DR beta III gene DRw52c of WT46, the genomic alpha gene and the invariant gene. (DRw52 transfectants were the kind gift of Dr. B. Mach).

Clone DE26 proliferated strongly only when the DRw52b+ transfectants were used to present antigen (Fig. 4.2) with the highest response being 50% of that observed with the autologous PBMC. Interestingly this response was seen at the highest antigen concentration tested (2×10^4 B.U./ml) whereas the highest response seen with clone DE26 and the autologous PBMC was at 5×10^3 B.U./mL No response was observed with any of the control transfectant cell lines or with the DRw52c+ transfectants in the presence or absence of antigens. Background responses of the cloned T lymphocytes cultured with the accessory cells alone were in all cases less than 713 c.p.m. Long term T cell line DX was also tested in this manner to assess at a polyclonal level the importance of the DRw52b restriction element in house dust mite

antigen recognition by this individual. A strong proliferative response was observed with the DRw52b+ transfectants, again the greatest proliferation was seen with the highest antigen concentration tested (2 x 10^4 B.U./ml) with the autologous PBMC giving the highest response at 5 x 10^3 B.U./ml (Fig. 4.3). The greatest response observed with the DRw52b+ transfectants was 62% of the response induced by the autologous PBMC. Background responses of the line cultured with the accessory cells alone were less than 726 c.p.m.

Initial analysis of the serological inhibition and panel studies suggested that the T cell clone DE5 may be restricted by the serologically-defined DR7 specificity. Subsequent analysis of the amino acid sequences of panel accessory cells capable of presenting to this clone suggested that DE5 may recognise a DRw53-associated restriction element. In order to investigate this further, murine L cells transfected with the human DR7 gene and L cells transfected with the DRw53 gene were used to present antigen. Responses of the clone using autologous irradiated PBMC as accessory cells and murine L cells transfected with DR4 and DR1 were used as controls. Proliferative responses of clone DE5 are shown in Fig.4.4. No proliferative response was inducible with any of the transfected L cells as accessory cells although they were each known to be capable of antigen presentation (DR1, Rothbard et al., 1988; DR4, Lamb et al., 1988; D. Wilkinson personal communication). In contrast, a strong proliferative response was observed using the autologous PBMC as accessory cells which showed a clear dose response, highest at 10^4 B.U./ml (the antigen concentration used in the serological inhibition and panel studies). Background responses of accessory cells and clone DE5 in the absence of antigen were less than 791 c.p.m.

4.3 DISCUSSION

The initial analysis of the restriction specificity of the T cell clones suggested that antigen recognition was regulated by immune response genes of the HLA-DR subregion, since only anti-HLA-DR and not HLA-DQ or HLA-DP specific antibodies markedly inhibited antigen dependent proliferation. Others have demonstrated the ability of murine monoclonal anti-HLA-DR antibodies and a rabbit anti-human "Ia" antiserum to inhibit antigen-dependent proliferation of a D. pteronyssinus reactive T cell line and suggested that HLA-DR molecules are able to function as restriction elements for the presentation of Dermatophagoides spp. antigens (Lanzavecchia et al., 1983). In order to determine the MHC class II alleles restricting T cell recognition of D. farinae a panel consisting of DR-matched and mis-matched PBMC were used as accessory cells. From the results obtained with large panels of presenting cells definition of the restriction elements appeared to be more complex than suggested by the serological analysis. In this context it should be remembered that the use of a small panel could suggest falsely simple restriction patterns and would thereby lead to an underestimation of the usage of MHC class II molecules in the presentation of antigen and the diversity of the T cell repertoire. Thus, in these experiments, if only three or four DR5+ presenting cells had been used, it is possible that the differences between DE41, for example, and other clones such as DE9 that also respond to antigen in association with some of the DR5+ presenting cells, would have been overlooked.

Only one clone (DE41) appeared to be restricted by MHC class II molecules with parallel serological definition. Even then this appeared to be a subtype of DR5 since not all DR5+ accessory cells were able to present antigen. Indeed it appears that there are at least two allelic forms of DR5 which have identical sequences in the first and second variable regions (residues 9-13 and 26-38 respectively) that differ at positions 67 and 71 in the third variable region of the beta I domain (Tieber et al., 1986; Bell et al., 1987; Todd, Bell and McDevitt, 1987). It has been suggested that these polymorphic regions and the generation of subtypes arise from gene conversion.

Population studies have demonstrated that HLA-DR5 is associated with specific immune responsiveness to the purified pollen antigen Amb a VI in atopic individuals as determined by the presence of serum IgE (Marsh et al., 1987). In contrast, responsiveness to Amb a V in the allergic population appeared to be restricted by the DR2/Dw2 haplotype (Marsh et al., 1982a and b).

Although the remaining T cell clones discussed here are restricted they show no consistent patterns that correspond to serologically defined HLA-DR specificities. However, this is not entirely unexpected since the regions of MHC class II determinants recognised by antibodies do not necessarily overlap with those involved in antigen binding or those that form contacts with T cell receptor molecules. From nucleic acid sequencing of cDNA clones obtained from lymphoblastoid cell lines homozygous by consanguinity, allelic variation of the serological DR types (1-9) have been analysed. Thereby, a set of the DR-specific sequences that determine the serological and functional polymorphisms

known to map to the HLA-DR subregion has been generated (Bell et al., 1987). Furthermore, two expressed loci were identified in the haplotypes DR2, -3, -4, -7 and -9, one locus product being present at a much lower frequency (4-7%) than the other. The low-frequency allele was highly conserved between each of the DRw53 (DR4, -7, -9) and the DRw52 (DR3, -5, -6) haplotypes. These are termed the supertypic specificities that are encoded by the beta III genes. Thus, the complex pattern of restrictions that were observed for the T cell clones other than DE41 may be related to DRw52 and DRw53 specificities. Until recently, little was known about antigen specific responses that were restricted by these molecules, perhaps because of the complexity of the system, which has now been examined at the molecular level (Gorski and Mach, 1986; Irle et al., 1988; Didier et al., 1986; Owerbach, Rich and Taneja, 1986). Gorski and Mach mapped the DR beta chain loci of the DR3, -5 and -6 haplotypes and were able to compare the allelic variation between the two expressed DR beta I and DR beta III gene products. In accordance with Bell and colleagues, they felt that gene conversion events over very short distances on the more polymorphic locus of DR beta I could explain the findings. Subsequently, the loci DRw52a and DRw52b could be defined with the generation of HLA-DR polymorphism within the DRw52 supertypic group being explained by a succession of gene duplication, divergence and conversion. The recently developed technique of oligonucleotide DNA typing has demonstrated that the supertypic specificity of HLA-DRw52 includes at least three alleles of beta III, named 52a, 52b and 52c (Fig. 4.5; Irle et al., 1988). Studies with alloreactive and antigen-specific T cell lines confirmed the existence of polymorphism within the DRw52 haplotypes (Irle et al., 1988).

In view of the serological inhibition data and molecular sequence analysis

of the haplotypes of the recognised accessory cells in the panel studies antigen recognition of clone DE26, when presented with house dust mite antigen by murine L cells transfected with the DRw52b gene, was examined. Marked antigen dependent proliferation was observed which was not seen with the control transfectants (DR1, DRw52c) or the Ltk- cells alone. Antigen recognition by the clone DE26 was, however, less than for the autologous PBMC. Possible reasons for this discrepancy include a decreased level of membrane HLA class II expression on the transfectant as compared with the autologous PBMC. Alternatively, this may reflect microheterogeneity of the transfected gene product or a deficiency of appropriate accessory molecules of antigen recognition. It is also possible that the irradiated autologous PBMC in the presence of antigen may exert a bystander effect (Lamb et al., 1982a). From the clonal panel studies it would appear that the DRw52b is a major restriction element in the recognition of house dust mite antigen by this subject. Assessment of antigen recognition by the long term line (DX) when presented with D. farinae by the DRw52b transfectant would support this hypothesis. To elicit a response of 62% of that seen with the autologous PBMC as accessory cells would suggest that a large proportion of the T cells of this subject use this restriction element. The fact that DRw52 is a haplotype common to a large percentage of the population raises interesting possibilities with respect to the recognition of house dust mite antigen and the potential development of a subunit vaccine.

In the same manner, antigen recognition by the T cell clone DE5 was examined using transfected murine L cells as the accessory cells. As DE5 recognised 6/8 DR7+ accessory cells from the panel studies and 4/6 DR4+ accessory cells, L cells transfected with DR7 and DR4 were used

with DR1 L cells and autologous PBMC as controls. Although all three transfectants were known to be capable of effective antigen presentation, clone DE5 failed to respond to house dust mite presented by any of these accessory cells but gave strong proliferative responses to the autologous cells. The failure of the L cell lines transfected with either DR7 or DR4 to present may be due to the absence of appropriate accessory molecules. Alternatively, given the known DW-subtype heterogeneity of both DR4 and DR7, DE5 may recognise a DRw53-associated restriction element, which is also known to encode molecular subtypes (Owerbach et al., 1986). However, the failure of the clone to respond to all DR4 and DR7 panel accessory cells raised the possibility that subtypes of DRw53 might exist, in an analagous manner to DRw52. Recent evidence suggests this is indeed the case (Gorski J, personal communication; Fig 4.6). Failure of the DRw53 transfectants to present to this clone would suggest that DE5 may recognise the DRw53.2 restriction element. Another possibility would be that DE5 is restricted by a class II molecule generated by gene transcomplementation. This is supported by the observation of Lotteau that DR alpha is able to pair with DQ beta and be expressed on the cell surface (Lotteau et al., 1987).

Although some human T cells are known to be restricted by HLA-DQ (Qvigstad et al., 1984) and HLA-DP (Eckels et al., 1983), the predominant restriction elements appear to be those encoded by HLA-DR (Eckels et al., 1984). Of particular interest is the observation that some DR2+, DR5+ and DR8+ presenting cells can activate the T cell clone DE9, suggesting that antigen may bind to conserved residues in the beta-chain of these alleles such as the sequence (FLEDR) corresponding to residues 67-71 (Fig. 4.7). This is speculative, although such conservation of residues in the third variable domain may allow for the

appropriate contact between MHC class II, antigen and the T cell receptor (Ronchese, Brown and Germain, 1987; Lamb et al., 1988). Based on sequence homologies (Bell et al., 1987), functional studies using mutated class II genes (Ronchese, Brown and Germain, 1987) and the crystallographic structure of HLA-A2 class I proteins (Bjorkman et al., 1987a and b) class II molecules may have a similar structure to MHC class I proteins (Brown et al., 1988). If so, then from modelling the structure of class II in class I the proposed combining site would consist of two parallel alpha helices overlaying a beta-sheet-platform that is held away from the cell membrane on two immunoglobulin-like domains. This would position residues 67-71 on the helical wall of the combining site. The critical residues of this region would appear to be 67 and 71 predicted to point into the combining site while 70 would point upwards potentially forming contact with the T cell receptor (Fig 4.8). Studies using site-directed mutagenesis of the alpha $beta^{bm12}$ gene and functional analysis of antigen and restriction specificity of the T cell response in wild type and the bm12 mutant have shown that differences in residues 67, 70 and 71 influence the T cell repertoire (Ronchese, Brown and Germain, 1987). Thus, conservation of the residues at positions 67, 70 and 71 in these alleles would allow class II proteins both to bind antigen and form the appropriate contacts with the T cell receptor. It is important to emphasise that the polymorphic residues in the beta sheet floor of the combining site formed by the NH_2-terminal half of the beta I domain also influence antigen binding.

Unfortunately, no correlation was observed between the antigen and restriction specificities of the T cell clones, although phenotypically they were identical ($CD4^+$, $CD3^+$, $UCHL1^+$, $CD8^-$) implying they are of the helper/inducer subset of T cells. Thus neither the cross-reactive nor

species-specific determinants of D. farinae appeared to be preferentially restricted by either of the haplotypes for this individual, such as has been reported for the cytotoxic response to the internal components of influenza virus (Gotch et al., 1987). Since allergic responses represent aberrant immunological recognition, an intriguing hypothesis would be that some T cells that recognise "allergenic epitopes" may interact with restriction elements encoded on the DR beta III chain. This is in contrast to the findings observed with other antigens (Ottenhoff et al., 1985). In order to identify MHC class II alleles determining high responder status to D. farinae, T cell epitopes within the major allergens (Der f I and II) need to be identified in atopic individuals and distinguished from those recognised by non-atopic subjects (Buckley et al., 1977; Rawle, Mitchell and Platts-Mills, 1984). Systematic study in allergic and non-allergic populations could elucidate this possibility and may prove of practical value in the design of hyposensitisation agents based on synthetic peptides.

Table IV.1 Antigen specificities of cloned T lymphocytes.

Clones	D. farinae	D. pteronyssinus	Grass-mix	Parietaria	No Ag
DE41	6,435 (7)	197 (40)	324 (8)	325 (9)	152 (44)
DE9	24,212 (6)	19,255 (2)	245 (39)	561 (41)	849 (38)
DD11	24,476 (5)	319 (81)	65 (49)	58 (16)	95 (32)
DE5	16,279 (9)	401 (28)	1,441 (21)	835 (12)	1,659 (10)
DE12	12,845 (6)	290 (19)	169 (27)	247 (6)	444 (24)
DE26	12,801 (3)	4,492 (6)	95 (13)	145 (47)	50 (13)

T lymphocytes (5 x 10^4/ml) were cultured with autologous irradiated PBMC (1.25 x 10^5/ml) in the presence of a panel of common aeroallergens including the inducing allergen, D. farinae. Proliferation as correlated with [^{3}H]TdR incorporation was measured at 72 hr. The results are expressed as mean c.p.m. (%SEM) for triplicate cultures. The data shown is the count of maximum proliferation over a dose-response curve for each antigen.

Table IV.2 Genetic restriction of T lymphocyte clones

Panel	DR	DRw	DE41	DE9	DD11	DE5	DE12	DE26
RM *	7, 5(12)	52, -	7732	28535	13130	15815	47541	16489
030G	7, 5(11)	52, 53	1178	9548	3864	4195	26873	101
857D	5, 7	52, 53	2040	11440	7447	3703	32439	8901
305G	2, 7	-, -	337	619	284	2547	3638	1875
903E	4, 7	-, 53	105	124	123	1989	2657	1464
239E	7, 9	-, 53	205	287	792	4111	8632	2844
189P	3, 7	52, 53	541	251	2367	902	14463	3551
005G	1, 7	-, 53	182	201	288	128	166	407
814D	4, 5(12)	52, 53	573	103	1895	1479	16401	3715
150G	4, 5(12)	52, 53	1606	15437	6300	3601	34711	9397
056G	5, -	52, 53	1243	7721	4302	279	26071	5453
129E	3, 5	52, -	1875	11084	4896	207	26719	7885
192E	1, 5	52, -	809	5208	3229	225	28740	6597
926D	5 (11), -	52, -	5529	17683	10045	317	45779	509
023C	8, 5(11)	52, -	1797	339	5237	654	38538	6200
826E	5, 6	52, -	1335	275	4223	245	23150	7049
088G	1, 5	52, -	853	220	3309	203	16527	4561
572G	10,5(12)	52, -	757	341	1802	96	13779	3809
557G	10, 5	52, -	461	9898	451	401	1853	2185
589G	1, 5(11)	52, -	311	268	381	102	1599	387

Table IV.2 Genetic restriction of T lymphocyte clones (continued)

Panel	DR	DRw	DE41	DE9	DD11	DE5	DE12	DE26
224G	1, -	-, -	206	277	470	405	845	933
161E	2, 9	-, 53	143	133	273	433	889	289
855E	2, 4	-, 53	122	453	159	772	1616	166
001C	2, -	-, -	315	5439	466	489	1295	1163
1009	3, 8	-, -	124	2000	571	234	1254	772
004C	3, -	52, -	201	289	335	268	697	421
804E	3, 6	52, -	225	1166	846	537	2902	1061
847E	4, -	-, 53	215	344	279	7189	9617	349
727E	4, 6	52, 53	213	142	455	120	339	206
013E	8, -	52, -	189	195	137	107	204	135

a) * Autologous cells

b) T-max analysis

c) Underlining indicates positive response

Table IV.3 HLA haplotype of accessory cell panel

	HLA loci								
Panel	A	B	Bw	C	DR	DRw	DQ	MC	DP
*RM	2, 24	27, 50	6	2	7, 12	52, -	2, 3		4,-
030G	2, 32	13, 45	4, 4	5, 6	7, 11	52, 53	2, 3	2, -	3, 4
857D	2, -	27, 57	4	2, -	5, 7	52, 53	2, 3	—	-, 4
305G	3, 29	18, 44	4, 6	-, -	2, 7	-, -	1, 2	-, -	4,
903E	30, 32	13, 47	4, 4	6, 7	4, 7	-, 53	2, 3	1, -	-, -
239E	2, 25	13, 37	4, 4	6, -	7, 9	-, 53	2, 3	-, -	-, -
189P	2, 29	18, 44	4, 6	3, 5	3, 7	52, 53	2, -	2, -	1, 4
005G	2, 24	7, 51	4, 6	7, -	1, 7	-, 53	1, 2	1, -	5, -
814D	2,24	35, 62	6, 6	3, 4	4, 12	52, 53	3, -	2, -	4,
150G	24, 31	27, 60	4, 6	2, 3	4, 12	52, 53	3, -	1, 2	3, 4
056G	2, 3	44, 62	4, 6	3, -	5, -	52, 53	2, 3	2, -	4, -
129E	1, -	8, -	6, 6	7, -	3, 5	52, -	2, 3	2, 3	2, -
192E	30, 32	27, 39	4, 6	1, 7	1, 5	52, -	1, 3	1, -	4, -
926D	25, 32	18, 44	4, 6	5, -	11, -	52, -	3, -	2, -	4, -
023C	2, -	18, 60	6, 6	3, 7	8, 11	52, -	3, -	2, -	-, -
826E	1, 2	37, 51	4	2, 6	5, 6	52, -	1, -	2, -	4, -
088G	2, 3	44, 60	4, 6	3, 7	1, 5	52, -	1, 3	-, -	1, -
572G	2, 11	37, 44	4, 4	5, -	10, 12	52, -	1, 3	-, -	-, -
557G	2, 11	35, 44	4, 6	4, 5	10, 5	52, -	1, 3	1, -	-, -
589G	11, 33	14, 35	6, 6	4, 8	1, 11	52, -	1, 3	1, 2	-, -

Table IV.3 HLA haplotype of accessory cell panel (continued)

	HLA loci								
Panel	A	B	Bw	C	DR	DRw	DQ	MC	DP
224G	2, -	7, 27	4, 6	2, 7	1, -	-, -	1, -	1, -	2, 4
161E	29, 32	44, 60	4, 6	3, -	2, 9	-, 53	1, 3	-, -	4, -
855E	2, 3	7, -	6	7, -	2, 4	-, 53	1, 3	1, -	3, 5
001C	3, -	7, -	6, 6	7, -	2, -	-, -	1, -	-, -	4, 5
1009	1, 2	8, 49	4, 6	7, -	3, 8	-, -	1, 2	-, -	1, 4
004C	1, -	8, -	6, 6	7, -	3, -	52, -	2, -	2, -	2, -
804E	2, 32	60, -	6, 6	2, 3	3, 6	52, -	1, 2	-, -	3, 4
847E	2, 68	52, -	4	-, -	4, -	-, 53	3, -	1, -	-, 4
727E	2, 11	55, 62	6	3, -	4, 6	52, 53	2, 3	-, -	4, -
013E	2, 25	27, 56	4, 6	1, 2	8, -	52, -	3, -	2, -	3, -

* autologous PBMC

Table IV.4

Unresponsiveness of cloned T lymphocytes to accessory cells without antigen.

Panel	DE41	DE9	DD11	DE5	DE12	DE26
*RM	540	75	143	79	169	99
030G	131	135	69	129	90	181
857D	353	73	285	141	115	84
305G	137	185	203	177	183	132
903E	314	115	172	138	83	83
239E	76	76	135	123	151	114
189P	106	87	239	150	93	117
005G	81	75	148	141	193	50
814D	114	92	171	107	189	135
150G	247	165	176	165	283	75
056G	123	102	178	140	291	185
129E	112	104	136	111	125	123
192E	83	79	107	136	112	128
926D	337	123	211	112	119	99
023C	175	181	64	164	122	47
826E	86	116	273	230	131	102
088G	61	50	215	81	83	123
572G	195	189	137	127	161	223
557G	199	125	116	139	94	182
589G	84	121	93	133	194	79

Table IV.4

Unresponsiveness of cloned T lymphocytes to accessory cells without antigen (continued).

Panel	DE41	DE9	DE11	DE5	DE12	DE26
224G	111	131	212	144	205	61
161E	56	110	101	105	118	80
855E	293	120	81	191	83	272
001C	441	73	79	312	68	68
1009	143	147	279	240	123	53
004C	373	62	89	313	95	139
804E	158	157	188	92	210	110
847E	316	59	163	191	138	75
727E	103	85	126	67	109	79
013E	101	59	100	233	83	99

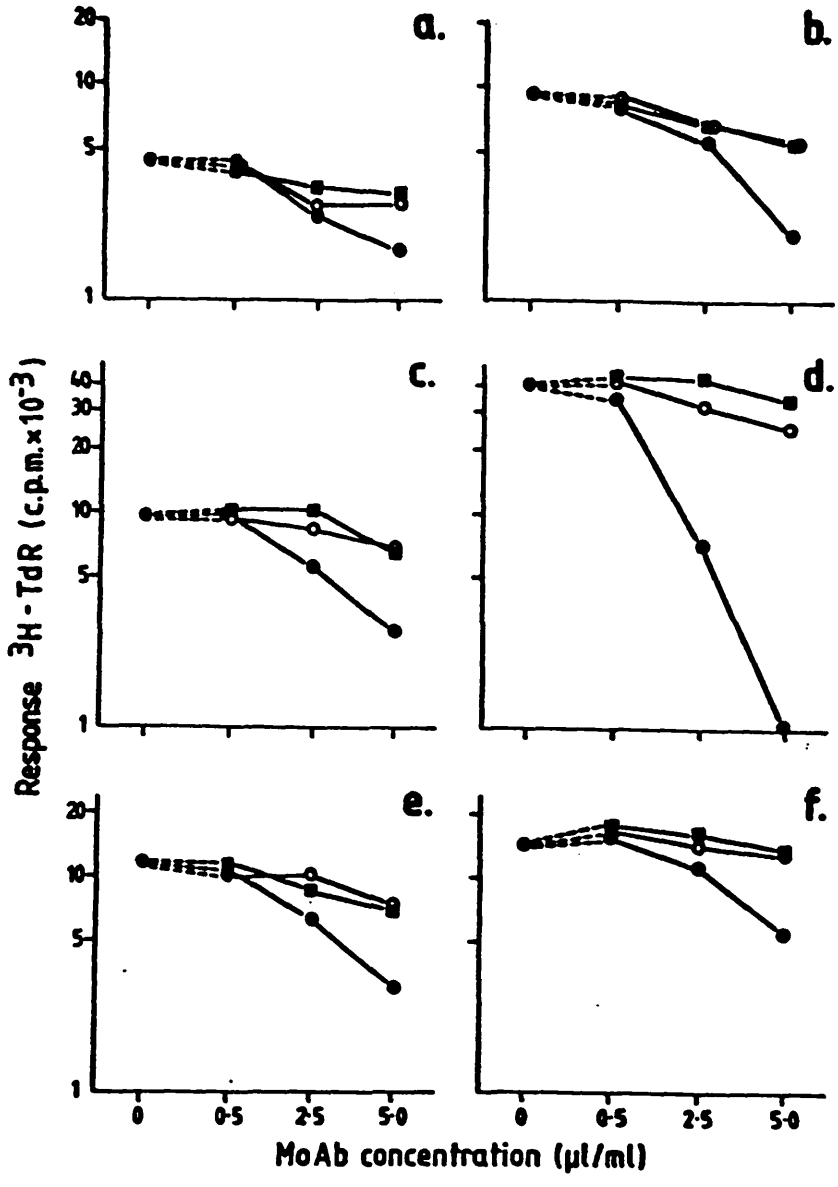

Figure 4.1: Panel of clones showing the dose response of the blocking effect of murine monoclonal antibodies against the human MHC class II determinants. T cell clones (10^4 cells/well) were cultured with D. farinae in the presence of autologous irradiated PBMC (2.5 x 10^4/well) as a source of accessory cell. Antibodies were added over a concentration range at the initiation of cultures. [^{3}H]TdR incorporation was determined at 72 hrs. Background responses of T cells and accessory cells in the absence of antigen were less than 200 c.p.m. Anti-HLA-DR (L243, 50 µg Ig/ml) ● ; anti-HLA-DP (B7/21, 25 µg Ig/ml)) O ; anti-HLA-DQ (Leu 10, 25 µg Ig/ml) ■. (a) DE41, (b) DE26, (c) DD11, (d) DE5, (e) DE9, (f) DE12.

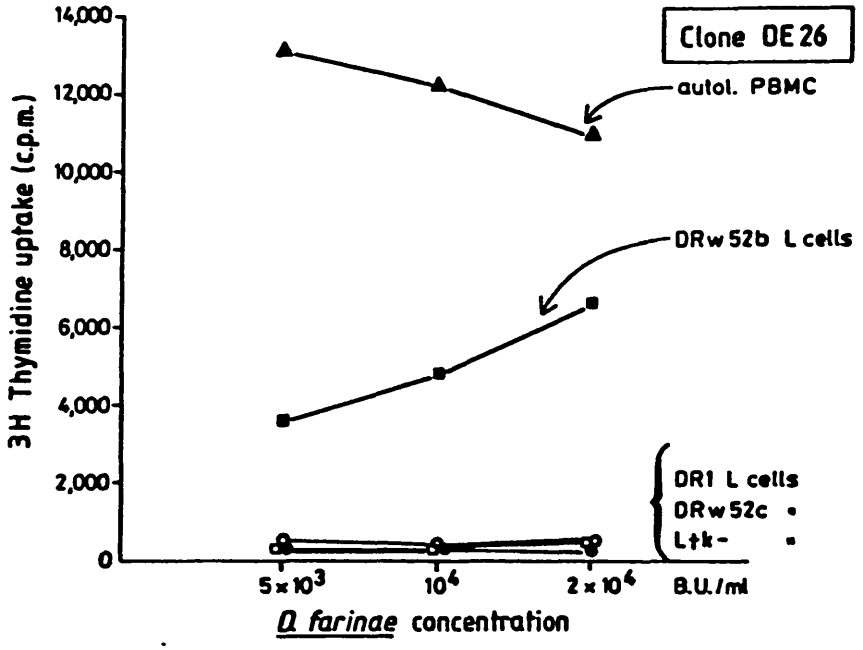

Figure 4.2: Response of clone DE26 to antigen presented by murine fibroblasts transfected with HLA-D region genes. T cells of clone DE26 (5 x 10^4/ml) were cultured with and without D. farinae antigen over a concentration range in the presence of accessory cells (10^5/ml), irradiated autologous PBMC (2,500 Rad) or mitomycin-C treated transfected murine L cells. Proliferation as correlated with [^{3}H]TdR incorporation was measured at 72 hr. The results are expressed as mean c.p.m. for triplicate cultures. Background responses of T cells and accessory cells in the absence of antigen or accessory cells alone were less than 713 c.p.m. Accessory cells used were: Ltk- cells ● ; DR1 L cells O ; TR22.9 (DRw52b) ■ ; TR167-B2 (DRw52c) □ ; autologous PBMC ▲.

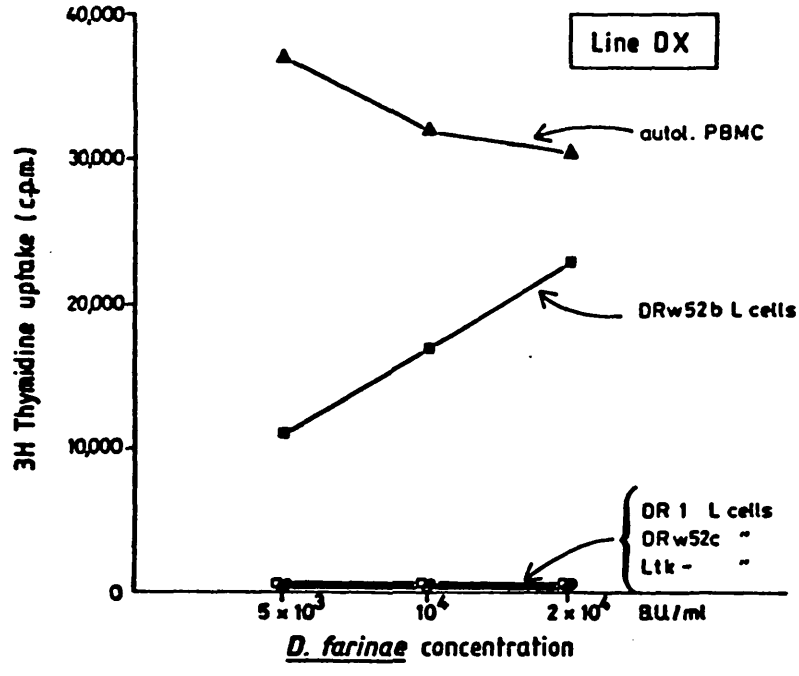

Figure 4.3: Response of line DX to antigen presented by murine fibroblasts transfected with HLA-D region genes. T cells of the long term line DX (5×10^4/ml) were cultured with and without D. farinae over a concentration range in the presence of accessory cells (10^5/ml) as for clone DE26 (Fig. 4.2). Background responses of T cells and accessory cells in the absence of antigen or accessory cells alone were less than 726 c.p.m.

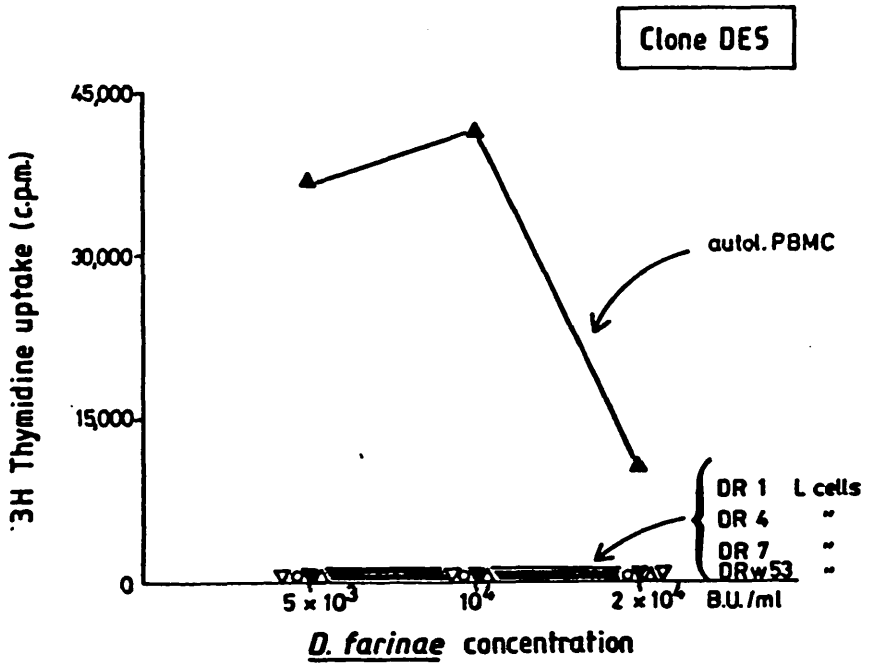

Figure 4.4: Response of clone DE5 to antigen presented by murine fibroblasts transfected with HLA-D region genes. T cells of clone DE5 (5 x 10^4/ml) were cultured with and without D. farinae antigen over a concentration range in the presence of accessory cells (10^5/ml), as described in Fig. 4.2. Proliferation responses were determined as previously. Background responses of T cells and accessory cells in the absence of antigen or accessory cells alone were less than 791 c.p.m. Accessory cells used were: autologous PBMC ▲ ; DR1 L cells O ; DR4 L cells ▲ ; DR7 L cells Δ ; DRw53 L cells ▽ .

Figure 4.5: Amino acid sequences of beta I domains of the allelic forms of DRw52

```
                          10        20        30        40        50
                          .         .         .         .         .
DR conserved         GDTRPRFLE----- CHFFNGTERV--L-R---NQEE--RFDSDVGE-RAV
DRw52a beta III      .........LRKSE ..........RY.D.YFH....FL.........Y...
DRw52b beta III      .........LLKSE ..........RF.E.HFH....YA.........Y...
DRw52c beta III      .........LLKSE ..........RF.E.YFH....FV.........Y...

                          60        70        80        90
                          .         .         .         .
DR conserved         -ELGRP-AE-WNSQKD-LE--R--VD-YCRHNYGV-ESFTVQRR
DRw52a beta III      T.....V..S......L..QK.GR..N........G........
DRw52b beta III      R.....D..Y......L..QK.GQ..N........V........
DRw52c beta III      T.....V..S......L..QK.GQ..N........V........
```

Figure 4.6: Amino acid sequences of beta I domains of DR4Dw15, DR7Dw7 and DRw53 alleles

```
                          10        20        30        40        50
                          .         .         .         .         .
DR conserved        GDTRPRFL-----ECHFFNGTERV--L-R---NQEE--RFDSDVGE-RAV
DR4Dw15 beta I      ........EQVKH............RF.D.YFYH...YV........Y...
DR7Dw7 beta I       ...Q....WQGKYK..........QF.E.LFY....FV........Y...
DRw53 beta III      ...Q....EQAKC....L......WN.I.YIY....YA.YN..L..YQ..
DRw53.2 beta III    ...Q....EQAKC....L......RN.I.YIY....YV.YN..L..YQ..

                          60        70        80        90
                          .         .         .         .
DR conserved        -ELGRP-AE-WNSQKD-LE--R--VD-YCRHNYGV-ESFTVQRR
DR4Dw15 beta I      T.....S..Y......L..QK.AA..T........G........
DR7Dw7 beta I       T.....V..S......I..DR.GQ..TV.......G........
DRw53 beta III      T.....D..Y......L..RR.AE..T...Y....V........
DRw53.2 beta III    T.....D..Y......L..RR.AE..T...Y....V........
```

Figure 4.7: Amino acid sequences of beta I domains of DR2Dw2, DR5Dw5 and DR8Dw8 alleles

```
                          10        20        30        40        50
                          .         .         .         .         .
DR conserved        GDTRPRFL-----ECHFFNGTERV--L-R---NQEE--RFDSDVGE-RAV
DR2Dw2 beta I       ........WQDKY...........RF.H.DFY....DL........Y...
DR5Dw5 beta I       ........EYSTS...........RF.D.YFY....YV........F...
DR8Dw8 beta I       ........EYSTG..Y........RF.D.YFY....YV........Y...

                          60        70        80        90
                          .         .         .         .
DR conserved        -ELGRP-AE-WNSQKD-LE--R--VD-YCRHNYGV-ESFTVQRR
DR2Dw2 beta I       T.....D..Y......F..DR.AA..T........G.........
DR5Dw5 beta I       T.....DE.Y......F..DR.AA..T........G.........
DR8Dw8 beta I       T.....S..Y......F..DR.AL..T........G.........
```

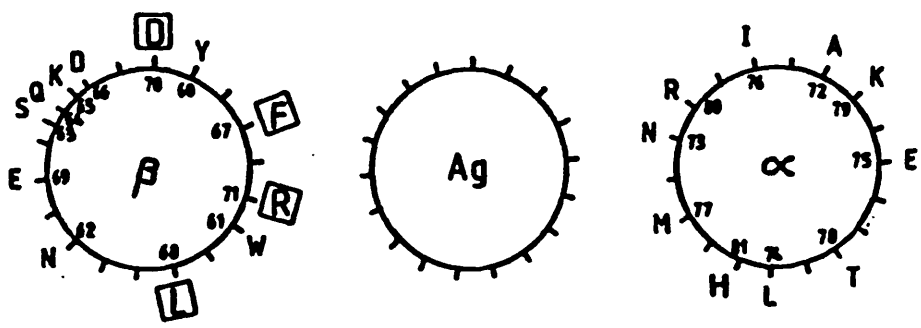

Figure 4.8: Model of the helical wall of the proposed binding site of DR2Dw2, DR5Dw5 and DR8Dw8 alleles.

CHAPTER 5 COMPARATIVE STUDY OF IgE and IgG REGULATION BY T CELLS FROM AN ATOPIC AND A NON-ATOPIC INDIVIDUAL

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5.1 INTRODUCTION

Immunoglobulin E (IgE) is responsible for allergic sensitivity in atopic subjects (Ishizaka, 1984). Advances in our understanding of the mechanisms of human IgE antibody responses in allergic individuals and the lack of such responses in non-atopic individuals have been hampered by the absence of suitable allergen-driven, reproducible systems for in vitro IgE synthesis by autologous B cells (Sampson and Buckley, 1981). Much of the available information has been derived from the analysis of pathological variants associated with hyper-IgE production (Geha et al., 1981; Buckley and Fiscus, 1975) or from the use of alloreactive (Lanzavecchia and Parodi, 1984) or autoreactive (Leung, Young and Geha, 1986) T cell clones. Polyclonal B cell activators such as pokeweed mitogen (PWM) or Epstein-Barr virus (EBV) have failed to induce IgE synthesis in peripheral blood B cells of both non-atopic and allergic donors and therefore have also failed to provide appropriate models for analysing IgE regulation (Saryan, Leung and Geha, 1983; Turner et al., 1983; Fiser and Buckley, 1979).

In this chapter, a Dermatophagoides spp. dependent in vitro model of IgE synthesis using autologous B cells is described. With this model, differences in the ability of allergen-reactive polyclonal T cells and clones from an atopic and a non-atopic individual to support IgE synthesis in such B cells are examined.

5.2 RESULTS

I Antigen specificity of polyclonal T cells and cloned T cells isolated from an atopic and a non-atopic individual

At the polyclonal level the response of peripheral T cells from both an atopic individual with perennial rhinitis and a non-atopic individual to D. farinae was comparable (Fig. 5.1a and b). In both cases PBMC proliferated in a dose-dependent manner when stimulated with soluble D. farinae extract, with the maximal response being seen at the highest antigen concentration tested (10^4 B.U./ml). In addition, proliferative responses were observed to the closely related member of the same genus, D. pteronyssinus and to Parietaria judaica as compared to the medium control. The maximal response to Parietaria judaica was also obtained at 10^4 B.U./ml in both subjects. Interestingly, to obtain the optimal response against D. pteronyssinus in the atopic subject a higher concentration of antigen (10^3 B.U./ml) was required as compared to the non-atopic subject (10^2 B.U./ml).

Cloned T cells derived from the same individuals (clones DE26 and DE9 from the atopic subject and clone DF1 from the non-atopic subject) proliferated strongly in a dose-dependent fashion in response to the inducing allergen, D. farinae, but failed to recognise the irrelevant aeroallergen mixed grass pollen (Fig 5.2) when tested over a concentration range. With all three clones the maximum response observed was obtained with the highest concentration of D. farinae tested (10^4 B.U./ml).

II Dot blot analysis of mRNA for IL-2 and IL-4 in T cell clones

The levels of IL-2 and IL-4 specific mRNA were compared for the T

cell clones DE9 and DE26 from the atopic and DF1 from the non-atopic individual six hours after activation of the cloned T lymphocytes on solid phase anti-CD3 antibody (OKT3). Dot blot analysis of clones DE9 and DE26 revealed the presence of comparable levels of IL-2 transcripts (positive at 1 x 10^5 cell-equivalents/spot; Plate 5; Fig. 5.3) whereas clone DF1 was positive at 5 x 10^5 cell-equivalents/spot. DE26 also gave positive hybridisation for IL-4 mRNA (1 x 10^5 cell-equivalents/spot) as opposed to DE9 which was only weakly positive when mRNA from five-fold the number of cell-equivalents/spot was probed and DF1, which had no detectable IL-4 specific mRNA. The clones, therefore, could not be ascribed to T_H1 or T_H2 subtypes as the presence of mRNA for the two lymphokines was not mutually exclusive. A summary of the lymphokine profile for the three clones is shown in Fig 5.3.

III Capacity of Dermatophagoides spp. specific T cell clones to support IgE and IgG synthesis

Investigation of the functional activity of the three Dermatophagoides spp. specific T cell clones (DE9 and DE26 from the allergic individual and DF1 from the non-allergic subject) revealed differences in their capacity to support in vitro immunoglobulin synthesis.

a. Atopic subject

Clone DE26 with easily detectable mRNA levels for IL-4 was able to collaborate with autologous E^- cells in the presence of the specific D. farinae allergen to support IgE synthesis and this was enhanced threefold by the addition of exogenous IL-4 (recombinant IL-4, 100 units/ml; Genzyme) at

the initiation of cultures (Table V.1, panel A). High levels of IgG were also synthesised in this system but in this experiment the total IgG synthesis was not enhanced by the addition of IL-4. Addition of the irrelevant allergen mixed grass pollen failed to elicit production of either IgE or IgG.

Clone DE9 with only barely detectable IL-4 transcripts helped IgE synthesis only when exogenous IL-4 was added to the cultures (Table V.1, panels B and C). The total IgG synthesis was also strongly enhanced in the presence of additional IL-4 (Table V.1, panels B and C; Table V.2). Again, the response observed was specific for the Dermatophagoides spp. allergen with the addition of mixed grass pollen to cultures failing to induce Ig synthesis. Furthermore, the addition of anti-interferon gamma (anti-IFN-gamma) antibody at the initiation of cultures and again 5 days later enhanced markedly IgE but not IgG synthesis induced by clone DE9 in the presence of exogenous IL-4 (Table V.1, panel C).

The failure of the autologous E^- cells cultured with antigen and IL-4 to stimulate marked IgE synthesis demonstrated the requirement for T cells (Table V.1, panel D). Similarly, IgG synthesis was low in the absence of T cells. Furthermore, peripheral T cells (E^+) from the atopic donor when cultured with autologous E^- cells and D. farinae induced the synthesis of IgE which was also enhanced by exogenous IL-4 (Table V.1, panel D). For polyclonal cultures, an increased number of cells were cultured (3×10^6 E^+/ml) because of the known paucity of sensitised T cells in the peripheral blood of an allergic individual (Halvorsen et al., 1986).

b. Non-atopic subject

Cloned CD4+ T cells (DF1) from the non-atopic donor which had no

detectable mRNA for IL-4 (Plate 5; Fig 5.3) were unable to induce IgE synthesis even in the presence of exogenous IL-4 (Table V.2, panel A) although they were able to support IgG secretion. This response was only observed with the specific D. farinae antigen and failed to occur with the control antigen, mixed grass pollen. Again, the total IgG synthesis was enhanced by the recombinant IL-4 added to the cultures. In general, although the D. farinae specific T cell clone from this subject could support IgG synthesis in vitro the levels of IgG produced were less than that observed using the clones from the atopic subject. The addition of anti-IFN-gamma antibody in the presence of exogenous IL-4 failed to induce IgE synthesis (Table V.2, panel A).

Stimulation of E^- cells from the non-atopic individual with allergen and peripheral T cells failed to induce IgE secretion although IgG was produced in low amounts (Table V.2, panel B). The addition of exogenous IL-4 to these cultures did not enhance IgE synthesis above that observed in the controls (increase of only 15 pg/ml; Table V.2, panel B).

The stimulation of B cell enriched populations from the non-atopic subject with cloned allogeneic T cells confirmed their capacity for the synthesis of IgE (Table V.2, panel C). This response was strongly enhanced in the presence of exogenous IL-4 for both IgE and IgG synthesis.

IV Analysis of sera from an atopic and a non-atopic individual for the presence of specific IgE using immunoblots of D. farinae

Autoradiographs using serum from the allergic donor of clones DE9 and DE26 showed strong IgE binding to the D. farinae immunoblots (Plate 6). A distinct band was evident at 13,000 molecular weight which corresponds to

the Der f II allergen of D. farinae which has recently been recognised as a major allergen. Fainter bands were also evident at 26,000 and 29,000 MW corresponding to the Der f I and Der f III allergens.

The autoradiographs using serum from the non-atopic clone donor showed no evidence of specific IgE directed at any of the determinants of D. farinae, consistent with the lack of allergic symptoms, negative skin prick tests and negative RAST testing (Table IL2).

5.3 DISCUSSION

The T cell dependence of IgE synthesis involved in atopic asthma, allergic rhinitis and eczema is well-established (Katz, 1980; Ishizaka, 1984). An understanding of why such responses only occur in "atopic" individuals has been hampered by the absence of a suitable allergen-driven, reproducible model for in vitro IgE synthesis. The limitations of currently available mitogen-stimulated models necessitated the development of an allergen-dependent system and the use of lymphocytes from both atopic and non-atopic individuals to understand the functional differences in their observed responses to Dermatophagoides spp.

The demonstration that the T cell repertoires of an atopic, house dust mite allergic individual and a normal, non-atopic individual both include cells capable of recognising Dermatophagoides spp. allergen has allowed the isolation of house dust mite specific cloned T lymphocytes in each case. The occurrence of such proliferative responses has proven controversial with some reports alleging that PBMC from non-atopic subjects can respond to aeroallergens (Halvorsen et al., 1986; Buckley et al., 1977) and others

describing a response only in allergic subjects (Rocklin et al., 1974; Eisenbrey et al., 1985). It is of interest, however, that in the majority of cases where a lack of response in non-atopic individuals was reported the entire aeroallergen was not used but rather a known antigenic determinant, selected as a major allergen on the basis of its capacity to evoke a vigorous IgE response in allergic subjects. Examples include the proliferative responses to Der p I, a major allergen of D. pteronyssinus, in house dust mite allergic individuals (Rawle, Mitchell and Platts-Mills, 1984) and responses to ragweed antigen E (Rocklin et al., 1974). Under normal circumstances, individuals allergic or non-allergic, are exposed to the entire allergen rather than a preselected antigenic determinant. When PBMC from both normal and atopic subjects are tested with the entire allergen of house dust mite (D. farinae or D. pteronyssinus), or ragweed, proliferation is seen with no obvious pattern of response being attributable to their underlying atopic status (Halvorsen et al., 1986). The subsequent ability to isolate allergen-specific T cell clones from allergic and non-allergic individuals allows the development of a model to examine differences between the two groups with respect to why one group develops an IgE response and manifestations of allergic diseases and the other group fails to do so.

Analysis of murine CD4+ T lymphocytes suggests that there are two non-overlapping subsets of CD4+ T lymphocytes (T_H1 and T_H2) that differ in their lymphokine production and functional activities (Mosmann and Coffman, 1987; Boom, Liano and Abbas, 1988). The T_H2 cells which produce IL-4 and IL-5 but not IL-2 or IFN-gamma are potent helpers for B cell IgE and IgG_1 synthesis (Howard and Paul, 1983; Coffman et al., 1986; Leung and Geha, 1987). Furthermore, relatively low concentrations of the lymphokine IFN-gamma are able to regulate this response by enhancing the synthesis of

IgG_{2a} and inhibiting IL-4 induced synthesis of IgG_1 and IgE through a direct action on B cells (Coffman and Carty, 1986; Snapper and Paul, 1987). Therefore, the allergen-specific T cell clones isolated from an atopic and a non-atopic individual were analysed for their ability to produce IL-4 using dot blot analysis for mRNA for IL-2 and IL-4 as parameters of lymphokine secretion (Mosmann and Coffman, 1987) and subsequently their capacity to provide help for IgE synthesis. Interestingly, analysis of the mRNA transcripts for IL-2 and IL-4 in these CD4+ clones suggests that there is no clear dichotomy in lymphokine production, as reported in the mouse. All three clones had detectable levels of IL-2 transcripts whereas both clones from the atopic subject (DE9 and DE26) also had evidence of IL-4 transcripts (Plate 5; Fig. 5.3). This is in agreement with the functional analysis of these lymphokines released by other human T cell clones stimulated with anti-CD3 antibody (Quint et al., 1988). No IL-4 mRNA was detectable in the cloned T lymphocytes from the non-allergic subject (DF1; Plate 5; Fig. 5.3). In all cases, clones were probed six hours after activation using solid phase anti-CD3 and actin probes were used as a control for the overall levels of mRNA in the cells.

Investigation of functional activity revealed that clone DE26 with high levels of mRNA for IL-4 was able to collaborate with autologous E^- cells in the presence of the specific D. farinae allergen to produce IgE and that this synthesis was augmented by the addition of exogenous IL-4 (Table V.1, panel A). The other clone isolated from the allergic subject, DE9, with barely detectable IL-4 transcripts, helped IgE synthesis only when exogenous IL-4 was added to the cultures (Table V.1, panels B and C). The requirement for T cells in this system was demonstrable by the failure of E^- cells cultured

with D. farinae and IL-4 alone to stimulate marked IgE synthesis (Table V.1, panel D). Similarly, the presence of antigen is required, for E^+ and E^- cells cocultured with IL-4 alone failed to induce IgE synthesis. These results suggest that antigen rather than IL-4 drives the T cells. The same pattern was observed at the polyclonal level, using peripheral T cells (E^+) from the atopic donor. When these E^+ cells were cocultured with autologous E^- cells and D. farinae IgE synthesis was also detected (Table V.1, panel D), implying that the clones were not unique cells but representative of those in the polyclonal population. IgE synthesis by the polyclonal population was much less, however, reflecting the low numbers of house dust mite immune T cells circulating in the peripheral blood (Halvorsen et al., 1986). The findings are consistent with the concept that B cells from atopic individuals are able to synthesise IgE when the appropriate T cell help is provided.

In contrast, the house dust mite specific $CD4^+$ T cell clone isolated from the non-allergic subject which had no detectable mRNA for IL-4 (Plate 5; Fig 5.3) was unable to support IgE synthesis even in the presence of exogenous IL-4 (Table V.2, panel A). Likewise, stimulation of E^- cells from this individual with allergen and peripheral T cells failed to induce IgE secretion (Table V.2, panel B) and the addition of exogenous IL-4 to these cultures did not enhance IgE synthesis above that observed in the controls (Table V.2, panel B). Thus the helper activity of the cloned T cells from the non-atopic subject was also similar to that displayed at the polyclonal level. Similarly, the absence of a D. farinae specific IgE response in the non-atopic individual was confirmed by immunoblotting and autoradiography (Plate 6) and supported the concept that the pattern observed in vitro reflected the in vivo status of this subject. A lack of clinical symptoms against the house dust mite and negative skin prick tests and RASTs also supported this

concept. Likewise the presence of all these features in the allergic subject supported the representative nature of the clonal response observed.

The stimulation of B cells from the non-atopic donor with cloned allogeneic T cells confirmed their capacity to synthesise IgE (Table V.2, panel C). It is well documented that non-atopic individuals are capable of mounting an IgE response following parasitic infection (Nutman et al., 1985) and allostimulation (Umetsu et al., 1985; Lanzavecchia and Parodi, 1984).

The failure of clone DF1, or of the peripheral E^+ cells from the non-atopic subject, to induce IgE synthesis cannot be explained solely by its inability to secrete IL-4 as the addition of exogenous IL-4 did not overcome the defect. Additional regulatory mechanisms may be required or there may be a defect in the IL-4 receptor. It has been suggested that the inability of T cells lacking IL-4 to support IgE synthesis is partly due to the production of IFN-gamma (Mosmann and Coffman, 1987; Coffman and Carty, 1986; Snapper and Paul, 1987). However, the clone DF1 was unable to provide appropriate help even when anti-IFN-gamma antibody and IL-4 were added (Table V.2, panel A) although marked enhancement of IgE synthesis was observed under similar culture conditions with the clone DE9 (Table V.1, panel C). Interestingly, these results are consistent with the recently reported finding, (Maggi et al., 1988), using PHA-driven T cell clones, which showed IL-4 dependent IgE synthesis in atopic but not non-atopic individuals. Extrapolating from these results, the observation that cloned CD4+ house dust mite specific T cells from the non-atopic individual were not able to support IgE synthesis while those from the atopic, house dust mite allergic subject were, failed to support the concept that the absence of IgE production in non-allergic subjects is due solely to the effects of CD8+ suppressor T cells or factors (Ishizaka, 1984).

If the T cell clones studied here recognise different epitopes within the allergen and the T cell recognition of a particular epitope within a protein can influence immune function as has been described for lysozyme (Adorini et al., 1979) then this would provide an additional explanation for the failure of DF1 to induce IgE synthesis. Nevertheless, from the phenotypic analysis it would appear that the clones DF1, DE26 and DE9 were derived from an overlapping functional subset of T cells. However, T cells from non-allergic subjects may respond to different components of Dermatophagoides spp. that are not associated with the induction of IgE but merely IgG as seen with the clone DF1 from the non-allergic subject studied here. As T cell antigen recognition depends upon the binding of peptide antigen to MHC proteins, the haplotype of the individuals will influence the epitopes recognised (Chapter 4). As information on the sequences of the major allergens of Dermatophagoides spp. becomes available it will be possible to test the hypothesis of the existence of "allergenic" versus "non-allergenic" epitopes. Alternatively, the specificities of the T cell repertoires of non-atopic and atopic subjects reactive with house dust mite may be overlapping, with the ability to induce IgE resulting from a regulatory difference in the specific T cells in each population rather than being a function of specificity. There is a precedent for this in that T cell clones able to confer protection and others able to induce arthritis in the rat model of adjuvant arthritis both recognised an identical sequence of the mycobacterial 65 kD protein, residues 180-188 (van Eden et al., 1988).

The absence of allergen-triggered IgE in the non-atopic system may have resulted from the absence of specific B cells which could act as highly efficient antigen presenting cells concentrating antigen via Ig receptors (Lanzavecchia, 1985) which were already clonally expanded in the atopic

individual. Alternatively, the failure to synthesise IgE may have been a result of a defect in isotype switching (Cebra, Komiser and Schweitzer, 1984; Radbruch et al., 1986; Gearhart, Sigal and Klinman, 1975) in the non-atopic subject. However, this explanation seems unlikely as IgE synthesis was inducible by allostimulation. Both subjects were able to synthesise IgG in response to D. farinae stimulation presented by autologous accessory cells and this was enhanced by the presence of exogenous IL-4.

With the experimental system described in this chapter it has been possible to analyse qualitative differences in helper activity for allergen-dependent in vitro IgE synthesis mediated by Dermatophagoides spp. specific T cell clones derived from an atopic and a non-atopic individual. A clear understanding of mechanisms involved in such IgE regulation is needed to facilitate the development of effective therapeutic regimens for allergic diseases, as discussed in the next chapter.

TABLE V.1: *In vitro* **immunoglobulin synthesis induced by polyclonal and cloned T cells isolated from an atopic subject.**

	T cells	E^-	antigen	IL-4	anti-IFN-gamma	IgE pg/ml	IgG ng/ml
A	DE26	+	-	-	-	0	48
	"	+	D.farinae	-	-	252	565
	"	+	grass pollen	-	-	0	30
	"	+	D. farinae	+	-	682	452
B	DE9	+	-	-	-	0	0
	"	+	D.farinae	-	-	0	73
	"	+	grass pollen	-	-	0	0
	"	+	D. farinae	+	-	595	2843
C	DE9	+	-	-	-	0	0
	"	+	D. farinae	-	-	0	145
	"	+	grass pollen	-	-	0	0
	"	+	D. farinae	+	-	150	330
	"	+	D. farinae	+	+	985	355
D	E^+	+	D. farinae	-	-	90	12
	-	+	D. farinae	-	-	0	0
	-	+	grass pollen	-	-	0	0
	-	+	D. farinae	+	-	41	11
	E^+	+	D. farinae	+	-	245	1335
	E^+	+	-	+	-	40	0

LEGEND TO TABLE V.1:

Polyclonal (E^+; 1.5 x 10^6/ml) or cloned T cells (2.5 x 10^5/ml) from an atopic subject were cocultured with antigen (D. farinae or mixed grass pollen; 5 x 10^3 B.U./ml) and autologous E^- cells (2.5 x 10^5/ml) in the presence or absence of recombinant IL-4. Supernatants were harvested after 10 days and total IgE (pg/ml) and total IgG (ng/ml) determined by ELISA. T cell clone DE26 (panel A) and DE9 (panels B and C) were isolated from the same atopic individual with perennial rhinitis. IgG and IgE synthesis by E^- cells from the atopic donor cultured with IL-4 and antigen without added T cells is shown (panel D). Polyclonal T cell help provided by E^+ cells was also determined (panel D). Background IgE and IgG synthesis by E^- cells cultured with antigen alone have been subtracted from the test groups. These were: for A, IgE $\leq$98 pg/ml, IgG $\leq$15 ng/ml; for B, IgE $\leq$90 pg/ml, IgG $\leq$15 ng/ml; for C, IgE $\leq$190 pg/ml, IgG $\leq$70 ng/ml; for D, IgE $\leq$47 pg/ml, IgG $\leq$14 ng/ml.

TABLE V.2: *In vitro* immunoglobulin synthesis induced by polyclonal and cloned T cells isolated from a non-atopic subject.

	T cells	E^-	antigen	IL-4	anti-IFN-gamma	IgE pg/ml	IgG ng/ml
A	DF1	+	-	-	-	0	0
	"	+	*D.farinae*	-	-	0	290
	"	+	grass pollen	-	-	0	0
	"	+	*D. farinae*	+	-	0	420
	"	+	*D. farinae*	+	+	0	120
B	E^+	+	-	-	-	0	0
	E^+	+	*D. farinae*	-	-	0	122
	-	+	*D. farinae*	+	-	20	190
	E^+	+	-	+	-	40	200
	E^+	+	*D. farinae*	+	-	55	360
C	E^+allo	+	-	-	-	93	26
	E^+allo	+	-	+	-	575	2385

Polyclonal (E^+) or cloned T cells were cultured as described for Table V.1. T cell clone DF1 (panel A) was isolated from a non-atopic subject. Total IgE and IgG synthesis by E^- cells from the non-atopic donor cultured with IL-4 and antigen without added T cells are shown (panel B). Polyclonal T cell help provided by E^+ cells (panel B) and cloned allogeneic E^+ cells (panel C) were determined. Background IgE and IgG synthesis by E^- cells cultured with antigen have been subtracted from the test groups. These were: for A, IgE $\leq$190 pg/ml, IgG $\leq$70ng/ml; for B and C, IgE $\leq$94 pg/ml, IgG $\leq$15ng/ml.

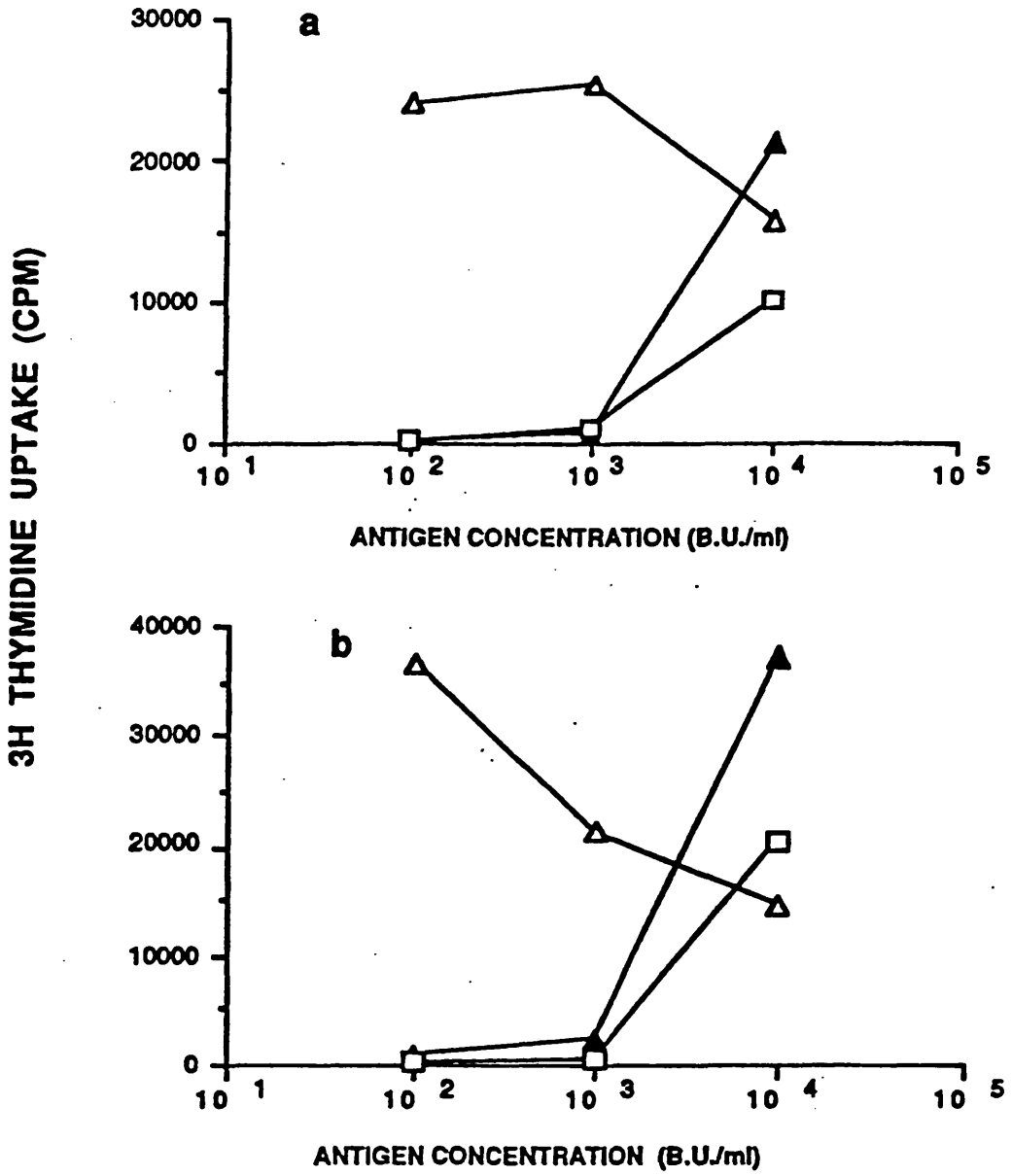

Figure 5.1: Antigen specificity of PBMC isolated from an atopic (a) and a non-atopic (b) individual. PBMC (2.5 x 10^5/ml) were cultured with D. pteronyssinus (△), D. farinae (▲) and Parietaria judaica (□) at different concentrations. Proliferation was determined by [^{3}H]TdR incorporation after six days. Background responses of PBMC in the absence of antigen were less than 115 c.p.m. The percentage SEM for triplicate cultures was less than 25% in all cases.

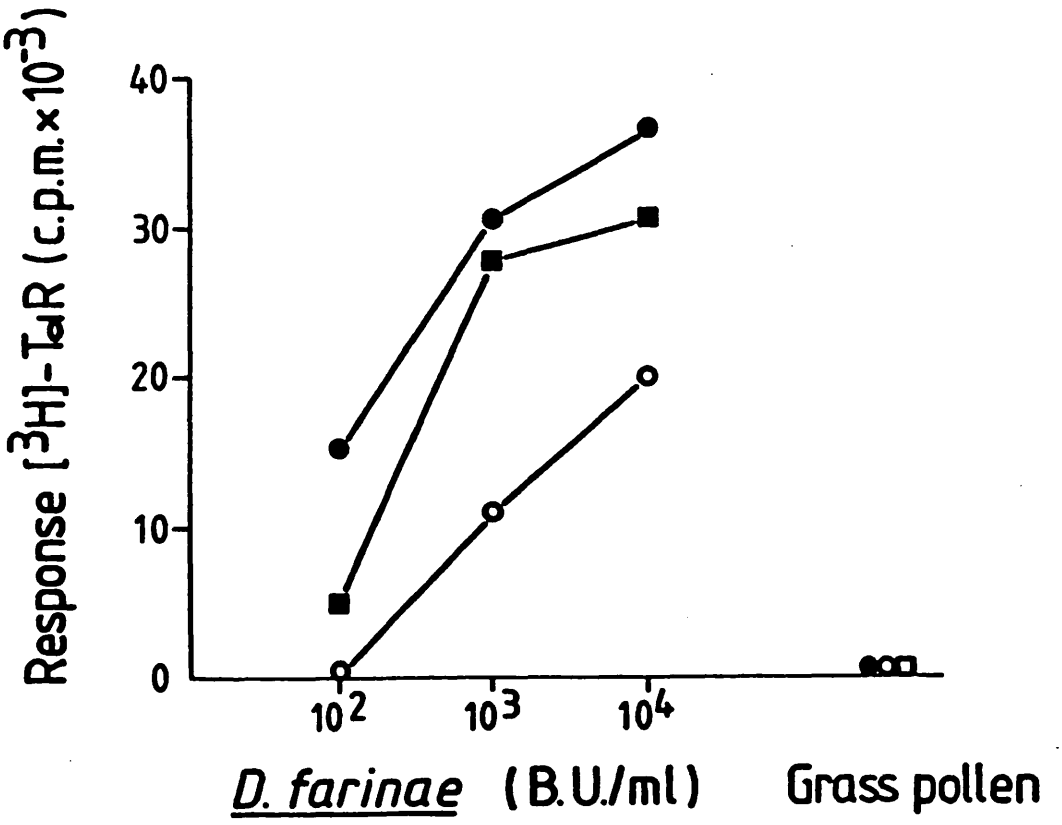

Figure 5.2: Antigen specificity of cloned T lymphocytes. Cloned T cells (5 x 10^4/ml) were cultured with D. farinae and mixed grass pollen over a concentration range in the presence of autologous, irradiated PBMC (1.25 x 10^5/ml). Proliferation was determined by [^{3}H]TdR incorporation at 72 hr. Dose response curves for D. farinae are shown; DE26 (○), DE9 (●) and DF1 (■). Only the maximum proliferative response to mixed grass pollen is plotted. Background responses to accessory cells in the absence of antigen were less than 275 c.p.m. and in all cases the percentage SEM was less than 25%.

	IL-4 mRNA	IL-2 mRNA	actin mRNA
DE9	+	+++	+++
DE26	+++	+++	+++
DF1	-	+	+++

Figure 5.3: Summary of the lymphokine profile of three <u>D. farinae</u> - reactive T cell clones.

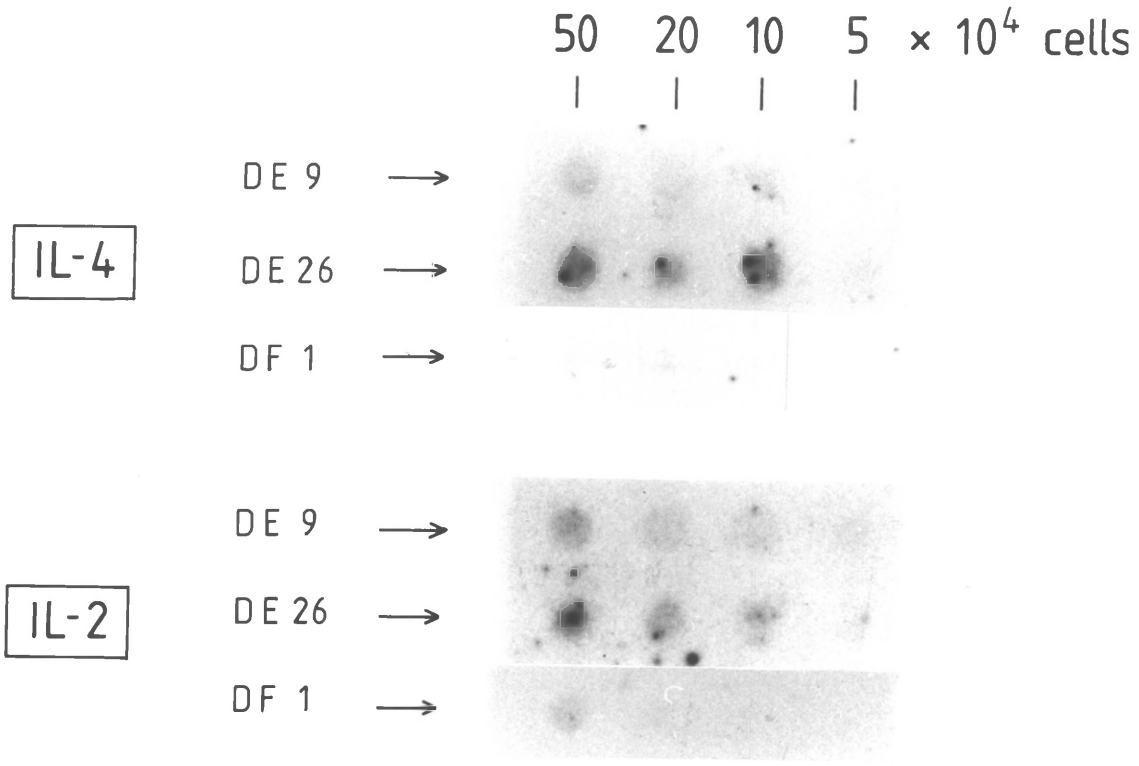

Plate 5: Dot blot analysis of mRNA for IL-2 and IL-4 in T cell clones. Cloned T lymphocytes, activated for six hours with solid phase anti-CD3, were probed using human IL-2 as cDNA and IL-4 oligonucleotides. Comparable mRNA for actin was present in all clones.

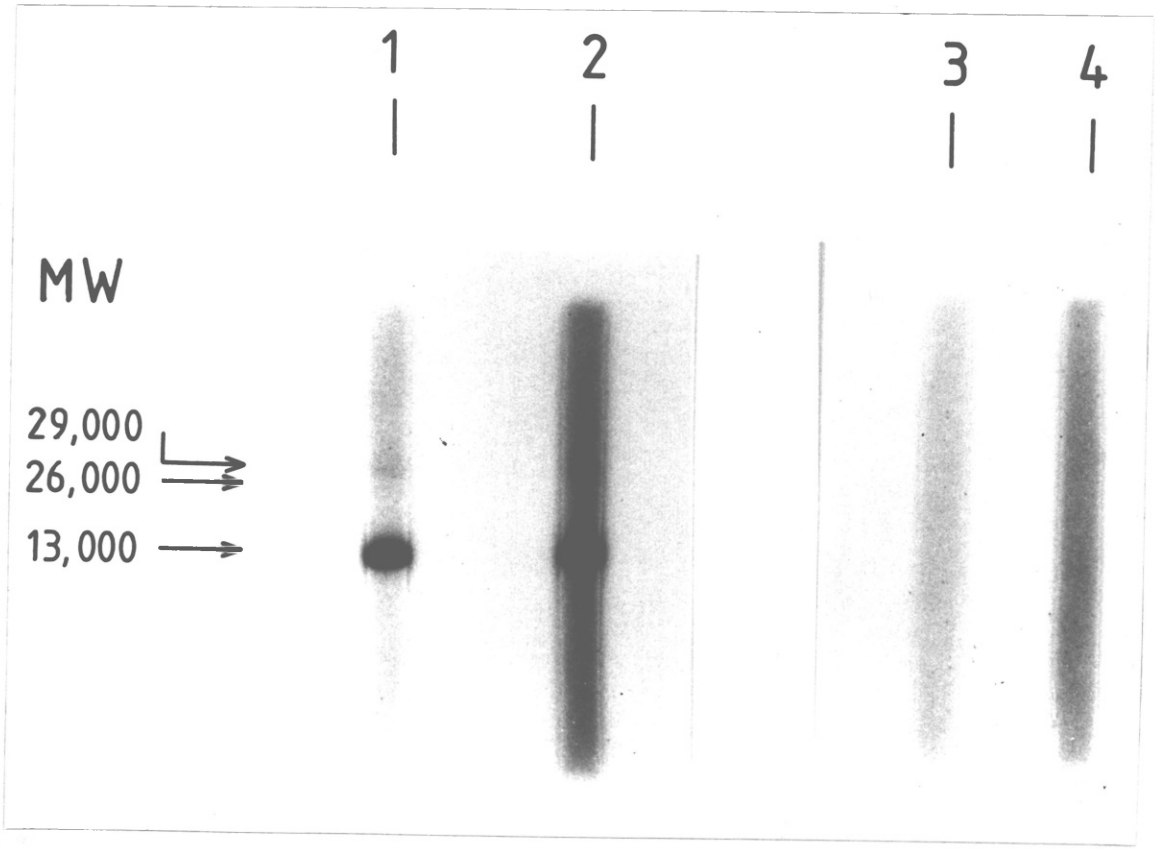

Plate 6: Autoradiographs of western blots showing the IgE response of human sera to D. farinae mite body extract separated by SDS-PAGE and transferred to nitrocellulose. Immunoblots were probed with sera from the mite allergic clone donor (1:2 dilution, lane 1; 1:5 dilution, lane 2) and from the non-allergic clone donor (1:2 dilution, lane 3; 1:5 dilution, lane 4) and autoradiographed to detect binding of IgE.

CHAPTER 6 IN VITRO MODELS FOR ANALYSING METHODS OF INDUCING HYPORESPONSIVENESS: PRELIMINARY STUDIES.

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6.1 INTRODUCTION

An area of clinical interest in house dust mite allergy is the development of protocols for hyposensitisation. The ability to develop models to investigate this depends upon not only knowledge of the antigen and restriction specificity of the T cell response but also a reproducible in vitro assay to determine IgE synthesis. Having established the necessary information (Chapters 3,4 and 5), in this chapter the possibility of developing models of hyposensitisation is examined.

The administration of crude extracts of allergen is widely used clinically for immunotherapy in a number of allergic responses but with variable effects (Ewan et al., 1988; Hunt et al., 1978). Although clinical protocols of hyposensitisation mimic certain of the characteristics of experimental models of T cell dependent low zone tolerance and are able to decrease IgE levels in certain instances the immunological mechanisms regulating this are ill-defined. The development of in vitro models has allowed the mechanisms of specific antigen-induced T cell tolerance using supraimmunogenic doses of synthetic peptide (Lamb et al., 1983) or the modification of class II restricted antigen presentation (Jenkins et al., 1987) to be analysed in detail. Therefore, the adaptation of these approaches may allow antigen-dependent T cell desensitisation to be examined as a potential means of clinical hyposensitisation. An alternative means of inhibiting the induction of an allergic response would be to block IgE synthesis at the level of the B cell itself. This could be achieved, for example, by blocking the IL-4 receptor as IgE synthesis appears to be highly IL-4 dependent (Howard and Paul, 1983; Coffman et al., 1986; Mosmann and Coffman, 1987).

In this chapter, preliminary experiments are described for an in vitro model

of low dose hyposensitisation using D. farinae. In addition, the ability to inhibit IL-4 secretion using the monoclonal antibody MR6 (Larche et al., 1988) that blocks the functional effects of the lymphokine IL-4 is examined, offering the potential of manipulating an allergic response. This focus on in vitro tolerance induction may yield information on which to develop protocols of hyposensitisation in vivo.

6.2 RESULTS

I Inhibition of IL-4 dependent IgE production by an antibody directed against the IL-4 receptor

Inhibition of IgE synthesis by manipulating B cells was investigated using serological inhibition by the antibody MR6 (putative anti-IL-4 receptor; Larche, Lamb and Ritter, 1988; Larche et al., in press). It had been demonstrated that MR6, a murine IgG_1 monoclonal antibody, detected a single polypeptide chain of approximately 145,000 MW when used in Western blotting on T cell lysates. This MW comigrated with the IL-4 receptor. FACS analyses on a variety of cell types had shown this molecule to be present at low density on the surface of T and B lymphocytes, myeloid lineage cells, some kidney epithelium and, at relatively higher concentrations, on the surface of cortical thymic epithelial cells (De Maagd et al., 1985; Larche, Lamb and Ritter, 1988). MR6 was also demonstrated to inhibit IL-4 induced T cell proliferation in a dose-dependent fashion (Larche et al., in press). More recently, FACS analysis of the modulation of cell surface molecules after T cell activation suggested that expression of the molecule detected by MR6 correlated inversely with that of the IL-2 receptor. Together, these data strongly suggested that MR6 binds to the human IL-4 receptor. The induction of IgE synthesis by polyclonal B cells in the presence of T helper clone DE9 or DE26, autologous E^- cells, D. farinae

allergen and exogenous IL-4 was abrogated by the addition of 2.5 μg/ml MR6 (Table VI.1, panels A and B) while the lower concentration of 1.25 μg/ml gave partial inhibition (71%; Table VI.1, panel C). Monoclonal antibodies MR6 and isotype-matched controls were added at the initiation of these cultures and again 5 days later.

The addition of MR6 also had a blocking effect on total IgG synthesis, giving either total (2.5 μg/ml, clone DE9, Table VI.1, panel B) or partial (75%, 1.25 μg/ml, clone DE9, Table VI.1, panel C; 61%, 2.5 μg/ml, clone DE26, Table VI.1, panel A) inhibition. In contrast, the isotype-matched controls gave markedly lower levels of inhibition for both IgE and IgG. Negative control antibody 1 (H17E2) gave 32% inhibition of the IL-4 enhanced IgE synthesis by clone DE26 and 45% inhibition of the maximal IgG synthesis (Table VI.1, panel A). Negative control antibody 2 (2118) gave 38% inhibition of maximal IgE synthesis by clone DE9 and 52% inhibition of the maximal IgG synthesis (Table VI.1, panel C).

II Antigen-induced decrease in T cell responsiveness to D. farinae

Low dose tolerance induction was investigated using Dermatophagoides spp. mite specific clone, DE26, cross-reactive for D. farinae and D. pteronyssinus (Chapter 3), restricted by DRw52b gene products (Chapter 4) and capable of supporting in vitro house dust mite dependent IgE synthesis from autologous B cells (Chapter 5).

In order to develop hyposensitisation protocols with potential functional relevance to the in vivo situation, it was necessary to commence the protocol with activated T cells, for both antigen presenting cells and inhaled aeroallergen are available to activate the allergen-specific T cells in vivo.

Therefore, after optimal activation with D. farinae (10^3 B.U./ml) in the presence of accessory cells and 10% IL-2, T cell cultures were pulsed with escalating concentrations of antigen, in the absence of accessory cells, starting at suboptimal antigen concentrations (10 B.U./ml) and increasing stepwise at daily intervals (10^2, 10^3, 10^3 B.U./ml) for a period of 5 days. The T cells were washed extensively and assayed for their ability to proliferate in response to the specific antigen, D. farinae and the irrelevant control antigen mixed grass pollen, in the presence of autologous irradiated PBMC as accessory cells or IL-2 alone. The proliferative response to D. farinae, the inducing antigen, was subsequently decreased to 60% of that obtained with the control T cells, although the response to IL-2 was maintained (Fig. 6.1). The lack of responsivenes to the control allergen mixed grass pollen was maintained in both cases.

6.3 DISCUSSION

Although there is limited understanding of the immunological mechanisms underlying allergen immunotherapy used in the treatment of allergic diseases, there is general agreement among allergists that hyposensitisation provides clinical benefit against some antigens. Insect venom hyposensitisation (wasp and bee allergy) is perhaps the most successful and best studied of the currently available treatment regimens, with controlled trials supporting the efficacy of this form of treatment (Hunt et al., 1978). Studies of the efficacy of the available house dust mite extracts, however, are less convincing (Gaddie, Skinner and Palmer, 1976; Newton, Maberley and Wilson, 1978).

The current approach to allergen hyposensitisation is to commence with minute doses of extract (.05μg) given by subcutaneous injection and increase

the strength of injection gradually at weekly intervals until the defined maximal dose is attained (100μg). Injection intervals are then slowly widened until the patient is receiving monthly or six weekly injections. Maximal strength, optimal injection interval and duration of treatment course have all been empirically determined with variation between treatment centres. House dust mite protocols are usually continued for 2-3 years and insect immunotherapy is carried out for a minimum of 3 years but indefinitely in some centres. Improved results with insect allergy have only been obtained since the availability of pure venom extracts rather than the previously used, and notoriously unsuccessful, whole body extracts. Extrapolating to the other allergens, it may be that immunotherapy results are generally disappointing because the preparations available are too crude, with the inability to obtain sufficiently high concentrations of relevant "allergenic" epitopes to render T cells unresponsive.

In order to study the current practice of allergen immunotherapy it was necessary to establish the antigen and restriction specificity of major components of the T cell repertoire reactive with house dust mite in addition to developing an allergen-dependent in vitro assay for the induction of IgE. Therefore, having established these using the aeroallergen house dust mite (Chapter 3-5), the more clinically directed goal of rendering the T cells unresponsive to this allergen could be approached.

Two main ways of immunological intervention are to block IgE synthesis at the B cell level using agents such as anti-IL-4 or anti-IL-4 receptor antibodies, or to functionally paralyse the allergen-immune T cells and so remove T cell help for specific IgE synthesis. The importance of IL-4 in the regulation of IgE production was reaffirmed by the observation that IL-4 dependent IgE production, induced by the clones DE9 and DE26, could be

abrogated by the addition of a monoclonal antibody putatively directed against the IL-4 receptor. Therefore, immunological modulation of the IL-4/IL-4 receptor interaction may have a role in the induction of hyporesponsiveness. However, there was also a clear decrease in the IgG response in contrast to the pattern reported by Finkelman and colleagues (Finkelman et al., 1986) in which an anti-IL-4 antibody inhibited IgE synthesis in an isotype-specific manner. The partial inhibition of IgG production was not inconsistent with MR6 recognising the IL4 receptor, as it has been demonstrated in the mouse that IL-4 is able to induce class switching to IgG_1 (but not to other IgG subclasses) in addition to IgE (Vitetta et al., 1985). The two isotype-matched control antibodies gave some inhibition of both IgE and IgG production. This pattern was not observed in other assays (IL-2, IL-4 and antigen-dependent proliferation; Larche et al., 1988) suggesting the non-specific blocking may be at the B cell level. It may result from steric hindrance of surface molecules required for B lymphocyte activation such as Fc binding. In the absence of MR6 inhibiting the binding of radiolabelled IL-4, evidence that MR6 recognises the IL-4 receptor must, however, remain circumstantial. From a clinical viewpoint, two major problems arise from attempts to block IgE synthesis using IL-4-related monoclonal antibodies. Firstly, IgE synthesis is required as a normal immune defence mechanism for both atopic and non-atopic individuals in parasitic infestation and therapeutic blocking of this response could have harmful repercussions. Secondly, the effect on IgG synthesis would also contraindicate the use of these agents as IgG is an important antibody involved in antibacterial defence. The anti-IL-4 antibody reported by Finkelman did not block IgG synthesis (Finkelman et al., 1986) but these studies were carried out in a mouse model and cannot be assumed to hold true for the human model.

The functional effects of hyposensitisation, whether operating via T or B cells or both, reveal some of the characteristics of experimental models of tolerance induction (Chiller and Weigle, 1972; Howard and Mitchison, 1975). The single exposure of cloned helper T cells to a supraoptimal concentration of the relevant peptide antigen in the absence of accessory cells has been shown to induce MHC class II restricted specific anergy to a subsequent immunogenic challenge with specific antigen (Lamb et al., 1983; Lamb and Feldmann, 1984) and was not due to cytolysis as the cells remained responsive to IL-2. Phenotypic changes in the T cells, most notably the downregulation of CD3-Ti concomitant with enhanced expression of IL-2 receptors accompanied unresponsiveness (Zanders et al, 1983; Lamb et al., 1987). Furthermore, the observed inactivation may also result from a lack of IL-2 expression as the addition of exogenous IL-2 resulted in proliferation. The failure to detect IL-2 mRNA after the preincubation of cloned T cells with peptide would support this explanation.

In the model described in this chapter, repeated stimulation with escalating doses of a crude polypeptide antigen mixture decreased specific responsiveness to the hyposensitising antigen, with an associated increase in the responsiveness to IL-2. The hyposensitising protocol, unlike that described by Lamb and colleagues, commenced with an activated T helper cell (being stimulated with autologous accessory cells and optimal concentrations of D. farinae antigen) as would be encountered in vivo. Subsequent identification of a peptide may allow a greater degree of inhibition using a low dose regimen as higher concentrations of the relevant determinant should be attainable. It is possible that a supraoptimal dose of the offending allergen would be an effective tolerising agent in vivo but

there is no safe way of determining the correct dosage and higher concentrations could result in fatal anaphylaxis so ethically such an approach is untenable.

Jenkins and Schwartz (1987) used a different approach to investigate the antigen specificity and presentation requirements for inactivation of T lymphocytes in vitro and in vivo. In murine studies, they used 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI)-treated splenocytes as modified antigen presenting cells. T cell clones exposed to these ECDI-treated splenocytes and antigen in vitro were rendered unresponsive for 8 days to subsequent antigenic stimulation by normal accessory cells. The model was then applied in vivo with the intravenous injection of antigen-coupled-ECDI-treated splenocytes resulting in the induction of in vivo unresponsiveness. Both the in vitro and in vivo methods displayed the same antigen and Ia molecule specificity as T cell activation. This, taken together with murine studies using planar membranes impregnated with MHC class II molecules (Quill and Schwartz, 1987) as antigen presenting cells, resulted in the consensus opinion that recognition of MHC class II and antigen in the absence of certain uncharacterised "second signals" may give an "off" signal to specific T cells. Again, the underlying mechanism of unresponsiveness was shown to be mediated by a decrease in m-IL-2 transcription (Jenkins et al., 1987). Of course, the use of chemically-modified antigen presenting cells does not present a therapeutic option.

In this chapter, the possibility that repeated in vitro exposure to low doses of specific antigen, D. farinae, simulating in vivo immunotherapy protocols, may have the same effect by inducing T cell tolerance is explored. It is necessary that such low dose regimens be developed as the risk of

anaphylaxis would render the potential use of supraimmunogenic stimuli, known to be capable of functional paralysis of T cells (Lamb et al., 1987), unacceptable in vivo.

Although CD8+ T "suppressor" cell regulation may be an important mechanism in vivo in non-atopic subjects, the data in this chapter taken together with the reports of Lamb and Jenkins, suggests that other mechanisms involving CD4+ T cells alone can downregulate the recognition of and response to antigen. In the presence of only cloned CD4+ T cells the proliferative response to D. farinae could be decreased by 42%, compared to the response of the control cloned T cells maintained with medium and IL-2 alone, when "hyposensitised" with specific D. farinae antigen. Furthermore, the ability of DE26 maintained in IL-2 in the absence of antigen to respond to an immunogenic pulse of D. farinae suggests that the mechanism of tolerance induction differs from that of Wilde and Fitch, (1984) who reported that IL-2 alone was able to induce specific anergy. The heightened response to a subsequent IL-2 stimulus of the "tolerised" T cells is consistent with an enhanced expression of the IL-2 receptor and decreased ability to transcribe the IL-2 gene (clone DE26 is known to possess easily detectable mRNA levels for both IL-2 and IL-4, Chapter 5) with a consequent relative increase in unoccupied surface IL-2 receptors. This result suggests that the induction of tolerance in DE26 may be by the same mechanism as that seen in the in vitro models described by Lamb and Jenkins.

In theory, a third possibility of preventing the induction of allergic responses to house dust mite would be to develop peptides able to occupy the combining site of the appropriate MHC class II molecules with sufficiently high affinity to displace allergen-derived peptides. From the analysis of the

restriction specificity of the house dust mite reactive T cell clones (Chapter 4) a competitive peptide capable of occupying the combining site of DRw52b class II proteins may be advantageous. There is a precedent for this approach to the regulation of immune responses in vivo. Adorini et al., (1988) demonstrated that a peptide derived from murine lysozyme (self peptide) differing by only 3 amino acids was able to compete and inhibit the presentation of hen egg lysozyme in vivo with the result that no immune response to hen egg lysozyme was generated.

These studies must be extended to examine different modes of antigen exposure which specifically diminish IL-4 production and support for IgE synthesis in response to antigen. In addition, further investigation of methods for inhibiting IL-4 secretion or its binding to T and B cells should be performed. Hence, mechanisms of hyposensitisation can be logically examined.

TABLE VL1 MR6 antibody inhibition of IL-4-dependent IgE production

	T cells	E^-	antigen	IL-4	MR6	IgE pg/ml	IgG ng/ml
A	DE26	+	D.farinae	-	-	355	356
	"	+	"	+	-	485	555
	"	+	"	+	+	≤46	220
	"	+	"	+	neg 1	329	305
	-	+	"	-	-	≤46	≤9
B	DE9	+	D. farinae	-	-	≤190	219
	"	+	"	+	-	340	400
	"	+	"	+	+	≤190	≤70
	"	+	Grass pollen	-	-	≤190	≤70
	-	+	D. farinae	-	-	≤190	≤70
	-	+	Grass pollen	-	-	≤190	≤70
C	DE9	+	D. farinae	-	-	≤90	88
	"	+	"	+	-	865	2858
	"	+	"	+	+	250	708
	"	+	"	+	neg 2	535	1358
	"	+	Grass pollen	-	-	≤90	58
	-	+	D. farinae	-	-	≤90	27
	-	+	Grass pollen	-	-	≤90	≤15

neg 1, negative control antibody (H17E2 anti-placental alkaline phosphatase)
neg 2, negative control antibody (2118 anti-lymphoma idiotype)

T cell clones DE9 and DE26 (2.5 x 10^5/ml), reactive with D. farinae, were cultured with autologous E^- PBMC (2.5 x 10^5/ml) and allergen (D. farinae or mixed grass pollen; 5 x 10^3 B.U./ml) in the presence or absence of recombinant IL-4. The anti-IL-4 receptor antibody (MR6) or isotype-matched negative control antibodies (final concentration of 2.5µg/ml in experiments A and B; 1.25 µg/ml in experiment C) were added to selected cultures at the initiation of cultures and again 5 days later. After 10 days IgE and IgG levels in the supernatant medium were measured by ELISA. Backgrounds for the assays were: for A, IgE ≤46 pg/ml, IgG ≤9 ng/ml; for B, IgE ≤190 pg/ml, IgG ≤70 ng/ml; for C, IgE ≤90 pg/ml, IgG ≤15 ng/ml.

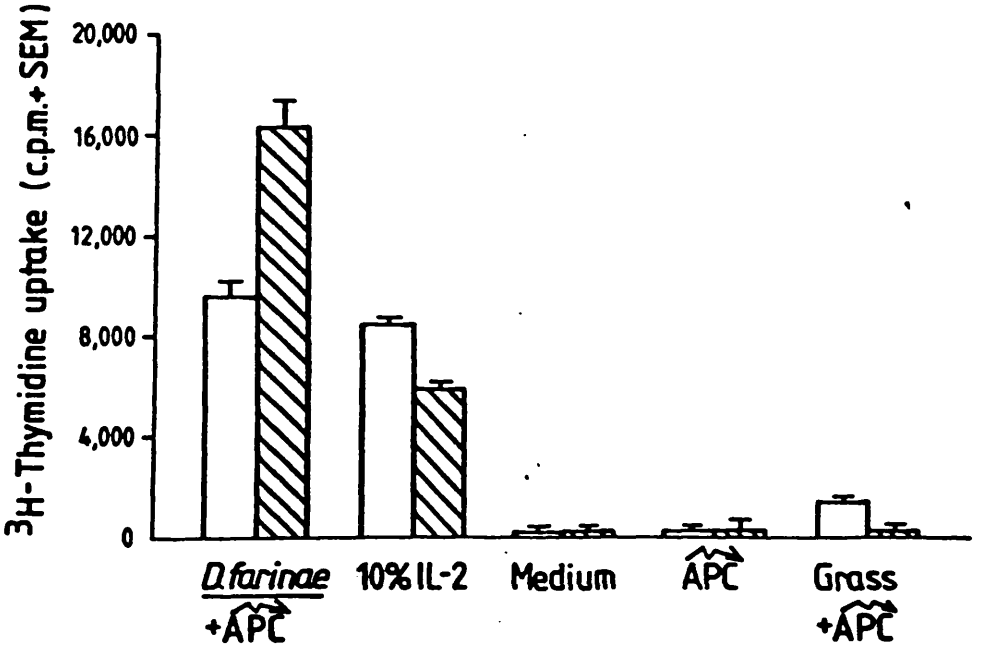

Figure 6.1: Antigen-induced hyporesponsiveness of clone DE26. T cells from clone DE26 (2.5 x 10^5/ml) were activated by stimulation with D. farinae (10^3 B.U./ml) in the presence of autologous accessory cells (2.5 x 10^5/ml) and 10% IL-2. After activation, T cell cultures were pulsed with escalating concentrations of D. farinae (10,10^2,10^3,10^3 B.U./ml) in the absence of accessory cells at daily intervals over a four day period. The T cells were then washed extensively and assayed for their ability to proliferate in response to D. farinae (10^4 B.U./ml) or the irrelevant mixed grass pollen in the presence of autologous accessory cells or IL-2 alone. Proliferation was determined at 72 hrs as previously. Control cultures were maintained in medium supplemented with IL-2 alone (hatched graph).

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In addition to the humoral immune response to aeroallergens (reviewed in Platts-Mills and Chapman, 1987), the role of effector cells such as mast cells, basophils and eosinophils together with their mediators have been extensively investigated (reviewed in: Kay, 1988; Cromwell, 1988; Kay et al., in press). Evidence is now being accumulated that lymphokines released by T cells are involved in the regulation of these effector cells. Eosinophilopoiesis and mast cell differentiation are regulated by IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 (reviewed in Kay et al., in press). Other factors have chemotactic activity for neutrophils, eosinophils, basophils and monocytes (Maestrelli et al., in press; Ming, Bersani and Mantovani, 1987).

It is now over two decades since IgE was identified as reaginic antibody and its importance in allergy established (Ishizaka, Ishizaka and Hornbrook, 1966). Furthermore, although IgE induced by allergen was reported to be T cell dependent (reviewed in Ishizaka, 1984), as was immunoglobulin in response to other protein antigens (reviewed in Abbas, 1988), it is surprising that neither the antigen specificity nor functional activity of T cells was examined more than superficially. The results of recent experiments have revealed that T cell-derived lymphokines such as IL-4, IL-5 and IFN-gamma are critical for the regulation of IgE production and therefore are potentially involved in inflammatory and allergic responses (reviewed in: Leung and Geha, 1987; Abbas, 1988). A detailed characterisation of the T cell repertoire reactive with aeroallergens is critical to the possible development of effective therapeutic regimens. Such regimens should ideally be directed at the pivotal cell, the helper T lymphocyte, rather than along the various effector pathways.

An initial limitation on the study of allergen-specific T lymphocytes stemmed from the lack of understanding of the growth requirements for the maintenance of such cells in long-term culture. However, with the vast improvement in technologies for the propagation of monoclonal populations of antigen-specific T cells over the last decade (reviewed in Fathman and Frelinger, 1983), it has become possible to analyse the specificity and functional activities of allergen-reactive T cells in detail and to attempt to modify these activities in vitro.

In this study, T cell responses to the most common mite species inducing allergy, Dermatophagoides spp., have been examined. It is unclear as to why 25-30% of the population mount a specific IgE response on exposure to common allergens. From serological analysis and the limited investigations of T cell responsiveness it was anticipated that the T cell repertoire of only atopic individuals would recognise house dust mite proteins (Rawle, Mitchell and Platts-Mills, 1984; Rocklin et al., 1974). However, being a complex mixture of foreign proteins it would seem unlikely that the T cell repertoire of non-atopic subjects would fail to contain cells capable of recognising house dust mite. If non-atopic T cells were to respond to house dust mite proteins both the specificity and quality of the T cell help may be different such that IgE is not induced. Therefore, it was important to study both atopic and non-atopic individuals and potentially identify differences between the two groups. Indeed, allergen-reactive T cell populations could be identified in both atopic and non-atopic individuals and in some instances the specificities overlapped at the protein level. However, more interestingly, the house dust mite-specific T cells could be expanded as monoclonal populations in both groups, providing an ideal system for the comparison of their functional activity (quality of help) with respect to the

induction of immunoglobulin synthesis. Although the cloned T cell populations from both atopic and non-atopic subjects could induce specific allergen-dependent IgG synthesis from autologous B cells, only those from the atopic house dust mite-allergic subject could support IgE synthesis. Clearly, the IgE synthesis was IL-4 dependent but this lymphokine was not the only requirement, for the addition of IL-4 to the non-atopic response was insufficient to "switch on" IgE synthesis. Furthermore, the presence of IFN-gamma could not solely explain the complexity of regulation in the non-atopic response. While IgE synthesis by the "atopic" B cells was augmented in the presence of anti-IFN-gamma antibodies, again this was insufficient to induce IgE synthesis in the non-atopic system. The demonstration that alloreactive cloned T cells could stimulate IgE synthesis from the "non-atopic" B cells suggested that they were functionally capable of IgE production. A model such as that developed in this study provides the means for further detailed analysis of the regulation of IgE synthesis in both atopic and non-atopic populations.

Historically, great importance has been attributed to the Der p I and Der f I (group I) allergens of Dermatophagoides spp., acknowledged as the "major" house dust mite allergens (Chapman and Platts-Mills, 1980; Heymann et al., 1986). This information was based on detailed serological studies of both atopic, house dust mite allergic individuals and non-atopic individuals. Therefore, it was surprising that the predominant allergen recognised by the T cells of the atopic donor characterised in detail in this study, was Der f II. Only recently have serological studies demonstrated that Der f II is an important allergen (Heymann et al., 1987). Perhaps this has resulted from difficulties in affinity-purification and the absence of the primary sequence. The question is raised as to how important the group II allergens are for T cell recognition of house dust mite. The significance of the Der f II allergen

may, however, merely reflect the Middle Eastern origin of the clone donor.

Unlike the reported cross-reactive serological responses to house dust mite allergens, the majority of the T cell response was directed towards species-specific determinants of D. farinae. Additionally, cross-reactive allergen-specific T cell populations could be identified. Both specificities of T cell clones were functionally important in that they could support IgE synthesis and, therefore, this raises a potential difficulty for the design of a vaccine for house dust mite hyposensitisation. Species-specific and cross-reactive determinants for the two major house dust mite species would need to be identified and included in such a subunit vaccine.

The analysis of the MHC restriction specificity of the Dermatophagoides spp.-reactive T cells was totally unexpected. From the literature, it would appear that most CD4+ T cells are restricted by HLA-DR molecules (Eckels et al., 1984). However, beta III in addition to beta I gene products were used as restriction elements in the responses to house dust mite, and overall the pattern of response was very heterogeneous. Significantly, T cell clones recognising antigen in association with either HLA-DR alpha beta I or alpha beta III molecules were capable of providing functional help for IgE synthesis in vitro. This is a further complication in the design of a vaccine. However, the predominant restriction element used appeared to be the supertypic specificity DRw52, accounting for approximately 50% of the specific T cell response as determined by allogeneic presenting cell panels and transfected L cells. Atopy and allergic diseases are very common in the population and the possibility of developing a useful vaccine for allergen hyposensitisation is increased if the allergen is recognised by a restriction element expressed at high frequency. The beta III gene products of DRw52 specificity are associated with HLA-DR3, 5 and 6 alleles which represent a

large proportion of the population. In order to demonstrate an association between T cell recognition of Der f II and DRw52, population studies will be required together with epitope mapping.

The induction of unresponsiveness to allergen can be attempted by modification of antigen recognition and activation at T cell or B cell levels. It seemed that both approaches could successfully decrease immune responsiveness to D. farinae. In preliminary studies using direct T cell inactivation with D. farinae, immune responsiveness to house dust mite could be decreased by 40% with a low dose hyposensitisation protocol. As there is much support for the concept that specific anergy in non-atopic subjects is mediated by CD8+ "suppressor" T lymphocytes (Rocklin et al., 1980), it was interesting that hyposensitisation could be induced in their absence. Other studies of direct inactivation of T cells by antigen have been performed both in vitro and in vivo (Jenkins and Schwartz, 1987). The observation that in vivo tolerance could be long-lasting (Jenkins and Schwartz, 1987) supports a possible role for functional clonal deletion as a therapeutic option. Although these models are informative and provide a foundation for the examination of the mechanisms regulating the induction of "tolerance" in functionally mature T cells, they used chemically-modified antigen presenting cells and as such are untenable for human use.

Modulation of the IgE response at the B cell level using the putative monoclonal antibody directed against the IL-4 receptor (MR6) was also possible. However, MR6 resulted in depression of both IgG synthesis and IgE synthesis. This presents obvious problems as IgG is critical in protective immunity to a number of pathogens. Similarly, IgE has an important protective role in parasitic infestation. Therefore, the effects of blocking

IL-4 either directly or at its receptor would seem too broad. Selective ablation of immune responsiveness to a specific aeroallergen which elicited only harmful consequences would be preferable.

The importance of inflammatory mediators in atopic allergy is well-established. Until quite recently, the role of the T cell in the generation of these mediators was not considered. However, it has now been demonstrated that activated CD4+ T lymphocytes are present in the peripheral blood of patients with acute severe asthma (Corrigan et al., 1988), in the skin of patients with late-phase allergen-induced wheal and flare responses as a model of late-phase asthmatic reactions (Frew and Kay, in press) and in the lungs of guinea pigs after allergen challenge (Frew et al., in press). Moreover, allergen-specific T lymphocytes have been shown to elaborate a 10kD neutrophil chemotactic factor (NCF) after specific antigen stimulation (Maestrelli et al., in press) and to release a leukotriene B_4 release enhancing factor (LREF) which augments LTB_4 production by human neutrophils in vitro (Tsai et al., 1987). The generation of an apparently identical NCF in vitro by influenza virus A-immune T cell clones using solid phase anti-CD3 stimulation has raised the possibility that it may be involved in the inflammatory events associated with both allergic and infectious diseases. Neutrophil chemotactic activity has been identified in the serum of asthmatic individuals during allergen-induced late-phase asthmatic reactions (Nagy, Lee and Kay, 1982). The asthma-associated neutrophil chemotactic activity has been found to be heterogeneous in terms of size and molecular weight and in some instances (for example, acute severe asthma) a low molecular weight (<20,000) peak of activity was observed (Buchanan, Cromwell and Kay, 1987). The importance of LTB4 as an inflammatory mediator is undisputed and it is known to be a powerful

mediator of bronchial mucosal oedema, increased vascular permeability and cellular infiltration (Cromwell, 1988). Studies examining the role of T cells in mediator generation, either directly or indirectly, are required with a comparison between allergen-specific T cells from both atopic and non-atopic individuals.

The analysis of the antigen and restriction specificity, combined with assays for determining the functional activity of the house dust mite-reactive T lymphocytes, will allow the modulation of immune responsiveness to house dust mite allergens to be examined further. The possibility of obtaining functional clonal deletion by direct antigen inactivation of T cells with house dust mite peptide(s) is intriguing. The study of Jenkins and Schwartz (1987) suggested that such inactivation might be long-lasting with the implication that this could be a potentially useful therapeutic approach. An alternative and intriguing possibility is to investigate the capacity of non-stimulatory peptides to competitively occupy the antigen combining site of those MHC class II proteins critical for the presentation of house dust mite allergens (Adorini et al., 1988). From the restriction specificity analysis presented in this study, DRw52 would be an appropriate allele for such an approach if it is demonstrated to have wider relevance on population screening.

BIBLIOGRAPHY OF REFERENCES

Abbas, A.K. (1988) A reassessment of the mechanisms of antigen-specific T cell-dependent B cell activation. Immunol. Today. 9, 89-94.

Abou-Zeid, C., Filley, E., Steele, J. and Rook, G.A.W. (1987) A simple new method for using antigens separated by polyacrylamide gel electrophoresis to stimulate lymphocytes in vitro by converting lines cut from western blots into antigen-bearing particles. J. Immunol. Meth. 98, 5-10.

Adorini, L., Harvey, M.A., Miller, A. and Sercarz, E.E. (1979) The fine specificity of regulatory T cells. II. Suppressor and helper T cells are induced by different regions of the hen egg-white lysozyme (HEL) in a genetically non-responder mouse strain. J. Exp. Med. 150, 293-306.

Adorini, L., Muller, S., Cardinaux, F., Lehmann, P.V., Falcioni, F. and Nagy, Z.A. (1988) In vivo competition between self peptides and foreign antigens in T cell activation. Nature. 334, 623-625.

Ajitkurmar, P., Geier, S.S., Kesari, K.V., Borriello, F., Nakagawa, M., Bluestone, J.A., Saper, M.A., Wiley, D.C. and Nathenson, S.G. (1988) Evidence that multiple residues on both the alpha helices of the class I MHC molecule are simultaneously recognised by the T cell receptor. Cell. 54, 47-56.

Allen, P.M. (1987) Antigen processing at the molecular level. Immunol. Today. 8, 270-273.

Allen, P.M., Matsueda, G.R., Evans, R.J., Dunbar, J.B., Marshall, G.R. and Unanue, E.R. (1987) Identification of the T cell and Ia contact residues of a T cell antigenic epitope. Nature. 327, 713-715.

Allen, P.M. and Unanue, E.R. (1984) Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. J. Immunol. 132, 1077-1079.

Allison, J.P. and Lanier, L.L. (1987) The structure, function and serology of the T cell antigen receptor complex. Ann. Rev. Immunol. 4, 503-540.

Anderson, J.A., Lane, S.R., Howard, W.A., Leiken, S. and Oppenheim, J.J. (1974) The effect of hyposensitisation on Alternaria-induced lymphocyte blastogenesis. Cell. Immunol. 10, 442-449.

Ansari, A.A., Shenbagamurthi, P., Kihara, T.K. and Marsh, D.G. (1987) Structural and immunological studies of Lolium perenne (rye) allergens Lol p I, II and III. Fed. Proc. 46, 1046 (Abs).

Austin, P., Trowsdale, J., Bodmer, W., Feldmann, M., Rudd, C. and Lamb, J.R. (1985) Functional expression of HLA-SB (HLA-DP) genes transfected into mouse fibroblasts. Nature. 313, 61-64.

Babbitt, B.P., Allen, P.M., Matsueda, G., Haber, E. and Unanue, E.R. (1985) Binding of immunogenic peptides to Ia histocompatibility molecules. Nature. 317, 359-360.

Beer, D.J., Osband, M.E., McCaffrey R.P., Soter, N.A. and Rocklin, R.E. (1982) Abnormal histamine-induced suppressor cell function in atopic subjects. N. Engl. J. Med. 306, 454-458.

Bell, J.I., Denney, D., Foster, L., Belt, T., Todd, J.A. and McDevitt, H.O. (1987) Allelic variation in the DR subregion of the human major histocompatibility complex. Proc. Natl. Acad. Sci. (U.S.A.) 84, 6234-6238.

Benacerraf, B. (1981) Role of MHC gene products in immune regulation. Science. 212, 1229-1238.

Benacerraf, B. and McDevitt, H.O. (1972) Histocompatibility-linked immune response genes. Science. 175, 273-279.

Benjamin, D.C., Berzofsky, J.A., East, I.J. et al., (1984) The antigenic structure of proteins: a reappraisal. Ann. Rev. Immunol. 2, 67-101.

Bergholtz, B.O., Thoresen, A.B. and Thorsby, E. (1980) HLA-D/DR restriction of macrophage-dependent antigen activation of immune T lymphocytes: cross-reacting allogeneic HLA-D/DR may partly substitute for self HLA-D/DR. Scand. J. Immunol. 11, 541-548.

Berzofsky, J.A. (1987) Structural features of protein antigenic sites recognised by helper T cells: what makes a site immunodominant? Year Immunol. 2, 28-38.

Biddison, W.E., Rao, P.E., Talle, M.A., Goldstein, G. and Shaw, S. (1983) Possible involvement of the T4 molecule in T cell recognition of class II HLA antigens: evidence from studies of proliferative responses to SB antigens. J. Immunol. 131, 152-157.

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. and Wiley D.C. (1987a) Structure of the human class I histocompatibility antigen, HLA-A2. Nature. 329, 506-512.

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. and Wiley, D.C. (1987b) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature. 329, 512-518.

Bluestein, H.G., Green, I. and Benacerraf, B, (1971) Specific immune response genes of the guinea pig. I. Dominant genetic control of immune responsiveness to copolymers of L-glutamic acid and L-alanine and L-glutamic acid and L-tyrosine. J. Exp. Med. 134, 458-470.

Blumenthal, M., Namboodiri, M., Mendell, N., Gleich, G., Elston, R. and Yunis, E. (1981) Genetic transmission of serum IgE levels. Am. J. Med. Genet. 10, 219-228.

Blumenthal, M., Awdeh, Z., Alper, C. and Yunis, E. (1985) Ra5 immune responses, HLA antigens and complotypes. J. Allergy Clin. Immunol. 75, 155 (Abs).

Blumenthal, M., Mendell, N. and Yunis, E. (1980) Immunogenetics of atopic diseases. J. Allergy Clin. Immunol. 65, 403-405.

Boom, W.H., Liano, D. and Abbas, A.K. (1988) Heterogeneity of helper/inducer T lymphocytes. II. Effects of interleukin 4- and interleukin 2-producing T cell clones on resting B lymphocytes. J. Exp. Med. 167, 1350-1363.

Bousquet, J., Calvayrac, P., Guerin, B., Hejjaoui, A., Dhivert, H., Hewiti, B. and Michel, F. (1985) Immunotherapy with a standardised Dermatophagoides pteronyssinus extract. I. In vivo and in vitro parameters after a short course of treatment. J. Allergy Clin. Immunol. 76, 734-744.

Bresser, J., Doering, J. and Gillespie, D. (1983) Laboratory methods. Quick blot: selective mRNA or DNA immobilisation from whole cells. DNA. 2, 243-254.

Brostoff, J., Greaves, M.F. and Roitt, I.M. (1969) IgE in lymphoid cells from pollen stimulated cultures. Lancet. i, 803-805.

Brostoff, J. and Roitt, I.M. (1969) Cell-mediated (delayed) hypersensitivity in patients with summer hayfever. Lancet. ii, 1269-1272.

Brown, J.H., Jardetzky, T., Saper, M.A., Samraoui, B., Bjorkman, P.J. and Wiley D.C. (1988) A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature. 332, 845-853.

Bruijnzeel, P.L.B., Hamelink, M.L., Prins, K. Remmert, G. and Meyling, F.H.J.G. (1987) Blood lymphocyte subpopulations in extrinsic and intrinsic asthmatics. Ann. Allergy. 58, 179-182.

Buchanan, D.R., Cromwell, O. and Kay, A.B. (1987) Neutrophil chemotactic activity in acute severe asthma ("status asthmaticus"). Am. Rev. Respir. Dis. 136, 1397-1402.

Buckley, R.H. and Becker, W.G. (1978) Abnormalities in the regulation of human IgE synthesis. Immunol. Rev. 41, 288-314.

Buckley, R.H. and Fiscus, S.A. (1975) Serum IgD and IgE concentration in immunodeficiency diseases. J. Clin. Invest. 55, 157-165.

Buckley, R.H. and Sampson, H.A. (1981) The hyperimmunoglobulinaemia E syndrome. In Franklin, E.C. Ed. Clinical Immunology Update, New York. Elsevier North-Holland, Inc.

Buckley, R.H., Seymour, F., Sanal, S.O., Ownby, D.R. and Becker, W.B. (1977) Lymphocyte responses to purified ragweed allergens in vitro. I. Proliferation responses in normal, newborn, aggammaglobulinaemic and atopic subjects. J. Allergy Clin. Immunol. 59, 70-78.

Bullock, J.D. and Frick, O.L. (1972) Mite sensitivity in house dust allergic children: evaluation by clinical and laboratory methods. Am. J. Dis. Child. 123, 222-226.

Butler, M., Atherton, D. and Levinsky, R.J. (1982) Quantitative and functional deficit of suppressor T cells in children with atopic eczema. Clin. Exp. Immunol. 50, 92-98.

Buus, S., Colon, S., Smith, C., Freed, J.H., Miles, C. and Grey, H.M. (1986) Interaction between a processed ovalbumin peptide and Ia molecules. Proc. Natl. Acad. Sci. (U.S.A.) 83, 4509-4513.

Buus, S., Sette, A., Colon, S.M., Miles, C. and Grey, H.M. (1987) The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. Science, 235, 1353-1358.

Buus, S. and Werdelin, O. (1986) Oligopeptide antigens of the angiotensin lineage compete for presentation by paraformaldehyde-treated accessory cells to T cells. J. Immunol. 136, 459-465.

Canonica, G.W., Mingari, M.C., Melioli G., Colombatti, M. and Moretta, L. (1979) Imbalances of T cell subpopulations in patients with atopic diseases and effect of specific immunotherapy. J. Immunol. 123, 2669-2672.

Cebra, J., Komisar, J. and Schweitzer, P. (1984) C_H isotype "switching" during normal B lymphocyte development. Ann. Rev. Immunol. 2, 493-548.

Chapman, M.D. (1988) Allergen-specific monoclonal antibodies: new tools for the management of allergic disease. Allergy. 43, (Suppl.) 7-14.

Chapman, M.D., Heymann, P.W. and Platts-Mills, T.A.E. (1987) Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus Dermatophagoides. J. Immunol. 139, 1479-1484.

Chapman, M.D., Heymann, P.W. and Platts-Mills, T.A.E. (1988) Epitope mapping of major dust mite (Dermatophagoides) allergens using monoclonal antibodies. In "Mite allergy - a world wide problem", The U.C.B. Institute of Allergy, pp. 27-29.

Chapman, M.D., Heymann, P.W., Wilkins, S.R., Brown, M.B. and Platts-Mills, T.A.E. (1987) Monoclonal assays for the major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. J. Allergy Clin. Immunol. 80, 184-194.

Chapman, M.D. and Platts-Mills, T.A.E. (1978) Measurement of IgG, IgA and IgE antibodies to Dermatophagoides pteronyssinus by antigen-binding assay, using a partially-purified fraction of mite extract (F_4P_1). Clin. Exp. Immunol. 34, 126-136.

Chapman, M.D. and Platts-Mills, T.A.E. (1980) Purification and characterisation of the major allergen from Dermatophagoides pteronyssinus - antigen P1. J. Immunol. 125, 587-592.

Chapman, M.D., Platts-Mills, T.A.E., Gabriel, M., Ng, H.K., Allan, W.G.L., Hill, L.E. and Nunn, A.G. (1980) Antibody responses following prolonged desensitisation with Dermatophagoides pteronyssinus extract. Int. Arch. Allergy Appl. Immunol. 61, 431-440.

Chapman, M.D., Rowntree, S., Mitchell, E.B., Di Prisco de Fuenmajor, M.C. and Platts-Mills, T.A.E. (1983) Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. J. Allergy Clin. Immunol. 72, 27-33.

Chapman, M.D., Sutherland, W.M. and Platts-Mills, T.A.E. (1984) Recognition of two Dermatophagoides pteronyssinus-specific epitopes on antigen P1 by using monoclonal antibodies: Binding to each epitope can be inhibited by serum from dust mite allergic patients. J. Immunol. 133, 2488-2495.

Chiller, J.M. and Weigle, W.O. (1972) Cellular basis of immunological unresponsiveness. Contemp. Top. Immunobiol. 1, 119-142.

Chua, K.Y., Stewart, G.A., Thomas, W.R., Simpson, R.J., Dilworth, R.J., Plozza, T.M. and Turner, K.J. (1988) Sequence analysis of cDNA coding for a major house dust mite allergen, Der p I. Homology with cysteine proteases. J. Exp. Med. 167, 175-182.

Clark, R.B. and Shevach, E.M. (1982) Generation of T cell colonies from responder strain 2 guinea pigs that recognise the copolymer L-glutamic acid L-lysine in association with nonresponder strain 13 Ia antigens. J. Exp. Med. 155, 635-640.

Coffman, R.L. and Carty, J. (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. J. Immunol. 136, 949-954.

Coffman, R.L., Ohara, J., Bond, M.W., Carty, J., Zlotnick, E. and Paul, W.E. (1986) B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. J. Immunol. 136, 4538-4541.

Cohn, M. and Epstein, R. (1978) T cell inhibition of humoral responsiveness. II. Theory on the role of restrictive recognition in immune regulation. Cell. Immunol. 39, 125-153.

Corrigan, C., Hartnell, A. and Kay, A.B. (1988) T lymphocyte activation in acute severe asthma. Lancet. i, 1129-1132.

Cromwell, O. (1988) Mediators in allergen-induced asthma. In "The allergic basis of asthma" Ed. A.B. Kay. Bailliere and Tindall, London. Pp. 197-216.

Dale, S. and Landmark, E. (1984) Characterisation of allergens in a crude extract of Dermatophagoides farinae and their identification in a new purified preparation. Allergy. 39, 572-585.

Dandeu, J.P., Le Mao, J., Lux, M., Rabillon, J. and David, B. (1982) Antigens and allergens in Dermatophagoides farinae mite. II. Purification of Ag 11 a major allergen from D. farinae. Immunology. 46, 679-687.

Decker, H. (1928) Asthma und milben. Munch. med. Wschr. 75, 515-516.

DeLisi, C. and Berzofsky, J.A. (1986) T cell antigenic sites tend to be amphipathic structures. Proc. Natl. Acad. Sci. (U.S.A.) 82, 7048-7052.

DeMaagd, R.A., Mackenzie W.A., Schuurman, H.J., Ritter, M.A., Price, K.M., Broekhuizen, R. and Kater, L. (1985) The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies. Immunology. 54, 745-754.

Didier, D.K., Schiffenbauer, J., Schuman, S., Abruzzini, L.F., Gorski, J., Watling, D.L., Tieber, V.L. and Schwartz, B.D. (1986) Characterisation of two distinct DR beta chain alleles at the beta III locus of the DR5 haplotype: beta III alleles are highly conserved. J. Immunol. 137, 2627-2631.

Dowse, G.K., Turner, K.J., Stewart, G.A., Alpers, M.P. and Woolcock, A.J. (1985) The association between Dermatophagoides mites and the increasing prevalence of asthma in village communities within the Papua New Guinea Highlands. J. Allergy Clin. Immunol. 75, 75-83.

Doyle, C. and Strominger, J. (1987) Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature. 330, 257.

Dreborg, S., Einarrson, R. and Longbottom, J. (1987) The chemistry and standardisation of allergens. In Weir and Hertzenberg (Eds): Handbk of Exp. Immunol. (Blackwell Scientific Publications) 1, 10.1-10.28.

Eckels, D.D., Lake, P., Lamb, J.R., Shaw, S., Woody J.N. and Hartzman R.J. (1983) SB-restricted presentation of influenza and herpes simplex virus antigens to human T lymphocyte clones. Nature. 301, 716-718.

Eckels, D.D. and Lamb, J.R. (1985) Human T lymphocyte clones; genetic control of specificity and function. In: T lymphocyte clones. (Ed. von Boehmer, H. and Haas, W.) Elsevier Biomedical Press. pp. 323-332.

Eckels, D.D., Lamb, J.R., Lake, P., Woody, J.N., Johnson, A.H. and Hartzman, R.J. (1982) Antigen-specific human T lymphocyte clones: Genetic restriction of influenza virus-specific responses to HLA-D region genes. Human-Immunol. 4, 313-324.

Eckels, D.D., Sell, T.W., Rosen-Bronson, S., Johnson, A.H., Hartzman, R.J. and Lamb, J.R. (1984) Human helper T cell clones that recognise different influenza haemagglutinin determinants are restricted by different HLA-D region epitopes. Immunogenetics. 19, 409-423.

Eisenbrey, A.B., Agarwal, M.K., Offord, K.P., Adolphson, C.R., Yunginger, J.W. and Gleich, G.J. (1985) Seasonal variation of in vitro lymphocyte proliferative response to ragweed antigen E. J. Allergy Clin. Immunol. 75, 84-90.

Erb, P. and Feldmann, M. (1975a) The role of macrophages in the generation of T helper cells. III. Influence of macrophage-derived factors in helper cell induction. Eur. J. Immunol. 5, 759-765.

Erb, P. and Feldmann, M. (1975b) The role of macrophages in the generation of T helper cells. The requirement for macrophages in helper cell induction and characteristics of macrophage-T cell interaction. Cell. Immunol. 19, 356-367.

Erb, P. and Feldmann, M. (1975c) Role of macrophages in in vitro induction of T helper cells. Nature. 254, 352-354.

Erb, P. and Feldmann, M. (1975d) The role of macrophages in the generation of T helper cells. II. The genetic control of the macrophage-T cell interaction for helper cell induction with soluble antigens. J. Exp. Med. 142, 460-472.

Evans, S.R., Pence, H., Kaplan, H. and Rocklin, R.E. (1976) The effect of immunotherapy on humoral and cellular responses in ragweed hayfever. J. Clin. Invest. 57, 1378-1385.

Ewan, P.W., Alexander, M.M., Snape, C., Ind, P.W., Agrell, B. and Dreborg, S. (1988) Effective hyposensitisation in allergic rhinitis using a potent partially purified extract of house dust mite. Clin. Allergy. 18, 501-508.

Falo, L.D., Benacerraf, B. and Rock, K.L. (1986) Phospholipase treatment of accessory cells that have been exposed to antigen selectively inhibits antigen-specific Ia-restricted, but not allospecific, stimulation of T lymphocytes. Proc. Natl. Acad. Sci. (U.S.A.) 83, 6994-6997.

Falo, L.D., Sullivan, K., Benacerraf, B., Mescher, M.F. and Rock, K.L. (1985) Analysis of antigen presentation by metabolically inactive accessory cells and their isolated membranes. Proc. Natl. Acad. Sci. (U.S.A.) 82, 6647-6651.

Fathman, C.G. and Frelinger, J.G. (1983) T lymphocyte clones. Ann. Rev. Immunol. 1, 633-655.

Feldmann, M. (1973) Induction of B cell tolerance by antigen specific T cell factor. Nature. 242, 82-84.

Fink, P.J., Matis, L.A., McElliot, D.L., Bookman, M. and Hedrick, S.M. (1986) Correlations between T cell specificity and the structure of the antigen receptor. Nature. 321, 219-226.

Finkelman, F.D., Katona, I., Urban, J.F., Snapper, C.M., Ohara, J. and Paul, W.E. (1986) Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine BSF-1. Proc. Natl. Acad. Sci. (U.S.A.). 83, 9675-9678.

Fiser, P.M. and Buckley, R.H. (1979) Human IgE biosynthesis in vitro: studies with atopic and normal blood mononuclear cells and subpopulations. J. Immunol. 123, 1788-1794.

Fox, P. and Siraganian, R.P. (1981) IgE antibody suppression following aerosol exposure to antigens. Immunology. 43, 227-234.

Freidhoff, L.R., Ehrlich-Kautzky, E., Grant, J.H., Meyers, D.A. and Marsh, D.G. (1986) A study of the human immune response to Lolium perenne (rye) pollen and its components, Lol p I and Lol p II (Rye I and Rye II). J. Allergy Clin. Immunol. 78, 1190-1201.

Frew, A.J. and Kay, A.B. The relationship between infiltrating CD4+ lymphocytes, activated eosinophils and the magnitude of the allergen-induced late-phase cutaneous reaction in man. J. Immunol. (in press)

Frew, A.J., Varley, J., Azzawi, M., Moqbel, R., Church, M.K., Holgate, S.T. and Kay, A.B. Bronchial T lymphocyte infiltration in the late asthmatic reaction in the guinea pig. Thorax (Abs) (in press)

Gaddie J., Skinner, C. and Palmer, K.N.V. (1976) Hyposensitisation with house dust mite vaccine in bronchial asthma. Br. Med. J. iii, 561-562.

Gatien, J.G., Merler, E. and Colten, H.R. (1975) Allergy to ragweed antigen E: effect of specific immunotherapy on the reactivity of human T lymphocytes in vitro. Clin. Immunol. Immunopathol. 4, 32-37.

Gaveriaux, C., Payne, T. and Loor, F. (1988) Factors regulating IgE synthesis. In: The Pharmacology of Lymphocytes. Handb. Exp. Pharm. 85, 275-290. Eds. Bray, M.A. and Morley, J. Springer-Verlag.

Gearhart, P.J., Sigal, N.H. and Klinman, N.R. (1975) Production of antibodies of identical idiotype but diverse immunoglobulin classes by cells derived from a single stimulated B cell. Proc. Natl. Acad. Sci. (U.S.A.) 72, 1707-1711.

Geha, R.S., Colten, H.R., Schneeberger, E. and Merler, E. (1975) Co-operation between human thymus-derived and bone marrow-derived lymphocytes in the antibody response to ragweed antigen E in vitro. J. Clin. Invest. 56, 386-390.

Geha, R.S. and Leung, D.Y.M. (1986) Cellular abnormalities in patients with elevated serum IgE levels. J. Allergy Clin. Immunol. 78, 995-999.

Geha, R.S., Reinherz, E.L., Leung, D., McKee, K.T., Schlossman, S.F. and Rosen, F.S. (1981) Deficiency of suppressor T cells in the hyper-immunoglobulinaemia E syndrome. J. Clin. Invest. 68, 783-791.

Geha, R.S. and Twarog, F., Rappaport, J., Parkman, R. and Rosen, F.S. (1980) Increased serum IgE levels following allogeneic bone marrow transplantation. J. Allergy Clin. Immunol. 66, 78-80.

Gerblich, A., Campbell, A.E. and Schuyler, M.R. (1984) Changes in T lymphocyte subpopulations after antigenic bronchial provocation in asthmatics. N. Engl. J. Med. 310, 1349-1352.

Germain, R.N. and Benacerraf, B. (1980) Helper and suppressor T cell factors. Springer Semin. Immunopathol. 3, 93-127.

Germain, R.N. and Malissen, B. (1986) Analysis of the expression and function of class II major histocompatibility complex-encoded molecules by DNA-mediated gene transfer. Ann. Rev. Immunol. 4, 281-316.

Gerrard, J.W., Rao , D.C. and Morton, N.E. (1978) A genetic study of immunoglobulin E. Am. J. Hum. Genet. 30, 46-58.

Gershon, R.K. (1974) T cell control of antibody production. Contemp. Top. Immunobiol. 3, 1-40.

Gershon, R.K. and Kondo, K.(1971) Infectious immunological tolerance. Immunology. 21, 903-914.

Gershon, R.K. and Kondo, K. (1972) Tolerance to sheep red cells: breakage with thymocytes and horse red cells. Science. 175, 996-997.

Girard, J.P., Rose, N.R., Kunz, M.I., Kobayashi, S. and Arbesman, C.E. (1967) In vitro lymphocyte transformation in atopic patients induced by antigens. J. Allergy. 39, 65-81.

Gleich, G.J., Jacob, G.L., Yunginger, J.W. and Henderson, L.L. (1977) Measurement of the absolute levels of IgE antibodies in patients with ragweed hayfever: effect of immunotherapy on seasonal changes and relationship to IgG antibodies. J. Allergy Clin. Immunol. 60, 188-198.

Gleich, G.J., Zimmerman, E.M., Henderson, L.L. and Yunginger, J.W. (1982) Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six year prospective study. J. Allergy Clin. Immunol. 70, 261-271.

Glimcher, L.H., Schroer, J.A., Chan, C. and Shevach, E.M. (1983) Fine specificity of cloned insulin specific T cell hybridomas: evidence supporting a role for tertiary conformation. J. Immunol. 131, 2868-2874.

Go, S., Michaels, D.L. and Ellis, E.F. (1970) Delayed hypersensitivity to pollen. Lancet i, 1115-1116.

Gonzalez, M.C., Diaz, P., Galleguillos, F.R., Ancic, P., Cromwell, O. and Kay, A.B. (1987) Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T cells in asthma. Relative increases in OKT8 cells in single early responders compared with those in late-phase responders. Am. Rev. Respir. Dis. 136, 600-604.

Goodfriend, L., Choudhury, A.M., Klapper, D.G., Coulter, K.M., Dorval, G., DelCarpio, J. and Osterland, C.K. (1985) Ra5G, a homologue of Ra5 in giant ragweed pollen: Isolation, HLA-DR associated activity and amino acid sequence. Mol. Immunol. 22, 899-906.

Gordon, J. and Guy, G.R. (1987) The molecules controlling B lymphocytes. Immunol. Today. 8, 339-344.

Gorski, J. and Mach, B. (1986) Polymorphism of human Ia antigens: gene conversion between two DR beta loci results in a new HLA-D/DR specificity. Nature. 322, 67-70.

Gotch, F.M., McMichael, A.J., Smith, G.L. and Moss, B. (1987) Identification of viral molecules recognised by influenza-specific human cytotoxic T lymphocytes. J. Exp. Med. 165, 408-416.

Green, I. and Benacerraf, B. (1971) Genetic control of immune responsiveness to limiting doses of proteins and hapten protein conjugates in guinea pigs. J. Immunol. 107, 374-381.

Groves, E.S. and Singer, A. (1983) Role of the H-2 complex in the induction of T cell tolerance to self minor histocompatibility antigens. J. Exp. Med. 158, 1483-1497.

Guillet, J.G., Lai, M.Z., Briner, T.J., Buus, S., Sette, A., Grey, H.M., Smith, J.A. and Gefter, M. (1987) Immunological self, nonself discrimination. Science. 235, 865-870.

Gupta, S., Frenkel, R., Rosenstein, M. and Grieco., M.H. (1975) Lymphocyte subpopulations, serum IgE and total eosinophil counts in patients with bronchial asthma. Clin. Exp. Immunol. 22, 438-445.

Gurka, G., Kalluri, A., McDonald, B., Ohman, J., Feigelson, P. and Rosenwasser, L.J. (1988) Allergen-specific T cell clones can be utilised to map allergenic epitopes for T cell activation. J. Allergy Clin. Immunol. 81, 195 (Abs).

Gurka, G., Ohman, J. and Rosenwasser, L.J. (1987) Human T cell clones specific for a mouse urinary protein allergen. J. Allergy Clin. Immunol. 79, 169 (Abs).

Halvorsen, R., Bosnes, V. and Thorsby, E. (1986) T cell responses to a Dermatophagoides farinae allergen preparation in allergics and healthy controls. Int. Archs. Allergy Appl. Immunol. 80, 62-69.

Hansburg, D., Heber-Katz, E., Fairwell, T. and Apella, E. (1983) Major histocompatibility complex-controlled antigen-presenting cell-expressed specificity of T cell antigen recognition. J. Exp. Med. 158, 25-39.

Harper, T.B., Gaumer, H.R., Waring, W., Brannon, R.B. and Salvaggio, J.E. (1980) A comparison of cell-mediated immunity and suppressor T cell function in asthmatic and normal children. Clin. Allergy. 10, 555-563.

Heber-Katz, E., Schwartz, R.H., Matis, L.A., Hannum, C., Fairwell, T., Appella,E. and Hansburg, D. (1982) Contribution of antigen presenting cell major histocompatibility complex gene products to the specificity of antigen-induced T cell activation. J. Exp. Med. 155, 1086-1099.

Hedrick, S.M., Nielsen, E.A., Kavaler, J., Cohen, D.J. and Davis, M.M. (1984) Sequence relationships between putative T cell receptor polypeptides and immunoglobulins. Nature. 308, 153-158.

Hemady, Z., Bloomberg, F., Gellis, S. and Rocklin, R.S. (1983) IgE production in vitro by human blood mononuclear cells: a comparison between atopic and non-atopic subjects. J. Allergy Clin. Immunol. 71, 324-330.

Heymann, P.W., Chapman, M.D., Aalberse, R. and Platts-Mills, T. (1987) Purification of two allergens, Der f II and Der f III, from the dust mite D. farinae. J. Allergy Clin. Immunol. 79, 192 (Abs.)

Heymann, P.W., Chapman, M.D. and Platts-Mills, T.A.E. (1986) Antigen Der f I from the dust mite Dermatophagoides farinae: structure comparison with Der p I from D. pteronyssinus and epitope specificity of murine IgG and human IgE antibodies. J. Immunol. 137, 2841-2847.

Hill, D.J., Hosking, C.S., Shelton, H.J. and Turner, M.W. (1981) Growing out of asthma: clinical and immunological changes over 5 years. Lancet. 2, 1359-1362.

Hirano, T., Yasukawa, K., Harada, H. etal. (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 324, 73-76.

Holck, A., Dale, S. and Sletten, K. (1986) Purification and characterisation of a major allergen from the house dust mite Dermatophagoides farinae. Allergy. 41, 408-417.

Holt, P.G., Batty, J.E. and Turner, K.J. (1981) Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. Immunology. 42, 409-417.

Holt, P.G. and Leivers, S. (1982) Tolerance induction via antigen inhalation: isotype specificity, stability and involvement of suppressor T cells. Int. Arch. Allergy Appl. Immunol. 67, 155-160.

Horn, N. and Lind, P. (1987) Selection and characterisation of monoclonal antibodies against a major allergen in D. pteronyssinus. Int. Archs. Allergy Appl. Immunol. 83, 404-409.

Howard, J.G. and Mitchison, N.A. (1975) Immunological tolerance. Prog. Allergy. 18, 43-96.

Howard, M. and Paul, W.E. (1983) Regulation of B cell growth and differentiation by soluble factors. Ann. Rev. Immunol. 1, 307-333.

Hsieh, K.H. (1982) Study of immunological changes after hyposensitisation in house dust sensitive asthmatic children. Ann. Allergy 48, 25-33.

Hsieh, K.H. (1984) Changes of lymphoproliferative responses of T cell subsets to allergen and mitogen after hyposensitisation in asthmatic children. J. Allergy Clin. Immunol. 74, 34-40.

Hsieh, K.H. (1985a) Enhancement of allergen-specific suppressor cell activity by T cell growth factor in house dust sensitive asthmatic children. Asian Pacific J. Allergy Immunol. 3, 37-42.

Hsieh, K.H. (1985b) Altered interleukin 2 (IL-2) production and responsiveness after hyposensitisation to house dust. J. Allergy Clin. Immunol. 76, 188-194.

Hsieh, K.H. (1987) Decreased expression of high affinity interleukin 2 receptors after hyposensitisation to house dust. Ann. Allergy. 59, 57-62.

Hsieh, K.H. (1988) Decreased production of interleukin 2 receptors after immunotherapy to house dust. J. Clin. Immunol. 8, 171-177.

Hunt, K.J., Valentine, M.D., Sobotka, A.K., Benton, A.W., Amodio, F.J. and Lichtenstein, L.M. (1978) A controlled trial of immunotherapy in insect hypersensitivity. N. Engl. J. Med. 299, 257-261.

Hurwitz, J.L., Heber-Katz, E., Hackett, C.J. and Gerhard, W. (1984) Characterisation of the murine T_H response to influenza virus haemagglutinin: evidence for three major specificities. J. Immunol. 133, 3371-3377.

Hwang, K.C., Fikrig, S.M., Friedman, H.M. and Gupta, S. (1985) Deficient concanavalin A-induced suppressor cell activity in patients with bronchial asthma, allergic rhinitis and atopic dermatitis. Clin. Allergy. 15, 67-72.

Ida, S., Hooks, J.J., Siragamian, R.P. et al. (1977) Enhancement of IgE-mediated histamine release from human basophils by virus: Role of interferon. J. Exp. Med. 145, 892-906.

Irle, C., Jaques, D. Tiercy, J.M., Fuggle, S.V., Gorski, J., Termijtelen, A., Jeannet, M. and Mach, B. (1988) Functional polymorphism of each of the two HLA-DR beta chain loci demonstrated with antigen-specific DR3- and DRw52-restricted T cell clones. J. Exp. Med. 167, 853-872.

Isakson, P.C., Pure, E., Vitetta, E.S. and Kramer, P.H. (1982) T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. J. Exp. Med. 155, 734-748.

Ishii, N., Baxevanis, C.N., Nagy, Z.A. and Klein, J. (1981) Responder T cells depleted of alloreactive cells react to antigen presented on allogeneic macrophages from non-responder strains. J. Exp. Med. 154, 978-982.

Ishizaka, K. (1983) Regulation of IgE response by IgE binding factors. Monogr. Allergy. 18, 52-80.

Ishizaka, K. (1984) Regulation of IgE synthesis. Ann. Rev. Immunol. 2, 159-182.

Ishizaka K., Ishizaka, T. and Hornbrook, M.M. (1966) Physicochemical properties of reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J. Immunol. 97, 75-85.

Ishizaka, K. and Sandberg, K. (1981) Formation of IgE binding factors by human T lymphocytes. J. Immunol. 126, 1692-1696.

Ishizaka, K., Yodoi, J., Suemura, M. and Hirashima, M. (1983) Isotype-specific regulation of the IgE response by IgE-binding factors. Immunol. Today. 4, 192-196.

Janeway, C.A. (1988) T cell development. Accessories or coreceptors? Nature. 335, 208-210.

Janeway, C.A., Carding, S., Jones, B., Murray, J., Portoles, P., Rasmussen, R., Rojo, J., Saizawa, K., West, J. and Bottomly, K. (1988) CD4 T cells: specificity and function. Immunol. Rev. 101, 39-80.

Jenkins, M.K., Pardoll, D.M., Mizuguchi, J., Quill, H. and Schwartz, R.H. (1987) T cell unresponsiveness in vivo and in vitro: Fine specificity of induction and molecular characterisation of the unresponsive state. Immunol. Rev. 95, 113-135.

Jenkins, M.K. and Schwartz, R.H. (1987) Antigen presentation by chemically-modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. J. Exp. Med. 165, 302-319.

Joffe, M.I., Kew, M. and Rabson, A.R. (1983) Lymphocyte subtypes in patients with atopic eczema, protein calorie malnutrition, SLE and liver disease. J. Clin. and Lab. Immunol. 10, 97-101.

Kaplan, M.E. and Clark, C. (1974) An improved rosetting assay for detection of human T lymphocytes. J. Immunol. Meth. 5, 131-135.

Kappler, J.W., Wade, T., White, J., Kushmir, E., Blackman, M., Bill, J., Roehm, N. and Marrack, P. (1987) A T cell receptor V beta segment that imparts reactivity to a class II major histocompatibility complex product. Cell, 49, 263-271.

Katz, D.H. (1980) Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. Immunology. 41, 1-24.

Katz, D.H. (1982) IgE antibody response in vitro: from rodents to man. Prog. Allergy. 32, 105-160.

Kay, A.B. (1988) Mechanisms in allergic and chronic asthma which involve eosinophils, neutrophils, lymphocytes and other inflammatory cells. In "The allergic basis of asthma" Ed. A.B. Kay. Bailliere and Tindall, London. Pp. 1-14.

Kay, A.B., Nadel, J.A., Henson, P.M., Irvin, C., Hunninghake, G. and Lichtenstein, L.M. (in press) The role of inflammatory cells and their mediators in bronchial hyperresponsiveness. In Proc. of Symposium on The Role of Inflammatory Processes in Airway Hyperresponsiveness, Florida, November, 1988.

Kern, R.A. (1921) Dust sensitisation in bronchial asthma. Med. Clin. North Am. 5, 751-758.

Kikkawa, Y., Kamimura, K., Hamajima, T. et al. (1973) Thymic alymphoplasia with hyper-IgEglobulinaemia. Paediatrics. 51, 690-696.

Kimoto, M., Krenz, T.J. and Fathman, C.G. (1981) Antigen-reactive T cell clones. III. Low responder antigen-presenting cells function effectively to present antigen to selected T cell clones derived from (high responder X low responder) F1 mice. J. Exp. Med. 154, 883-891.

Kinashi, T., Harada, N., Severinson, E. et al. (1986) Cloning of complementary DNA encoding T cell replacing factor and identity with B cell growth factor II. Nature. 324, 70-73.

King, T.P., Norman, P.S. and Connell, J.T. (1964) Isolation and characterisation of allergens from ragweed pollen. Biochemistry. 3, 458-468.

Kishimoto, T. (1982) IgE class specific suppressor T cells and regulation of the IgE response. Proc. Allergy. 32, 265-317.

Kontiainen, S. and Feldmann, M. (1977) Suppressor cell induction in vitro. III. Antigen specific suppression by supernatants of suppressor cells. Eur. J. Immunol. 7, 310-314.

Kontiainen, S. and Feldmann, M. (1978) Suppressor cell induction in vitro. IV. Target of antigen specific suppressor factor and its genetic relationship. J. Exp. Med. 147, 110-123.

Krilis, S., Baldo, B.A. and Basten, A. (1984) Antigens and allergens from the common house dust mite Dermatophagoides pteronyssinus. II. Identification of the major IgE binding antigens by crossed radioimmunoelectrophoresis. J. Allergy Clin. Immunol. 74, 142-146.

Kudo, K., Odudaira, H., Miyamoto, T. and Horiuchi, Y. (1978) IgE antibody response to mite antigen in the mouse. Suppression of an established IgE antibody response by chemically-modified antigen. J. Allergy Clin. Immunol. 61, 1-9.

Kuisaki, J., Atassi, H. and Atassi, M. (1986) T cell recognition of ragweed allergen Ra3: localisation of the full T cell recognition profile by synthetic overlapping peptides representing the entire protein chain. Eur. J. Immunol. 16, 236-240.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond). 227, 680-685.

Lake, P. and Mitchison, N.A. (1977) Regulatory mechanisms in the immune response to cell surface antigens. Cold Spring Harbor Symp. Quant. Biol. 41, 589-595.

Lamb, J.R., Eckels, D.D., Lake, P., Johnson, A.H., Hartzman, R.J. and Woody, J.N. (1982a) Antigen-specific human T lymphocyte clones: induction, antigen specificity, and MHC restriction of influenza virus-immune clones. J. Immunol. 128, 233-238.

Lamb, J.R., Eckels, D.D., Lake, P., Woody, J.N. and Green, N. (1982b) Human T cell clones recognise chemically-synthesised peptides of influenza haemagglutinin. Nature. 300, 66-69.

Lamb J.R. and Feldmann, M. (1984) Essential requirement for major histocompatibility complex recognition in T cell tolerance induction. Nature. 308, 72-74.

Lamb, J.R., O'Hehir, R.E. and Young, D.B. (1988) The use of nitrocellulose immunoblots for the analysis of antigen recognition by T lymphocytes. J. Immunol. Meth. 110, 1-10.

Lamb, J.R., Rees, A.D.M., Bal, V., Ikeda, H., Wilkinson, D., De Vries, R. and Rothbard, J.B. (1988) Prediction and identification of an HLA-DR-restricted T cell determinant in the 19kD protein of Mycobacterium tuberculosis. Eur. J. Immunol. 18, 973-976.

Lamb J.R., Skidmore, B.J., Green, N., Chiller, J.M. and Feldmann M. (1983) Induction of tolerance in influenza-virus immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. J. Exp. Med. 157, 1434-1447.

Lamb, J.R., Zanders, E.D., Eckels, D.D., Green, N., Webster, R.G., Woody, J.N., Lerner, R.A. and Feldmann, M. (1984) Inhibition of T cell proliferation by anti-antigen antibodies. Eur. J. Immunol. 14, 153-157.

Lamb, J.R., Zanders, E.D., Fische, A., Smith, S.H., Golay J.T., Green, N., Beverley, P.L.L. and Feldmann, M. (1985) Regulation of B lymphocytes by human T lymphocyte clones. Lymphokines. 10, 57-87.

Lamb, J.R., Zanders, E.D., Sewell, W., Crumpton, M.J., Feldmann, M. and Owen, M.J. (1987) Antigen-specific T cell unresponsiveness in cloned helper T cells mediated via the CD2 or CD3/Ti receptor pathways. Eur. J. Immunol. 17, 1641-1644.

Lancaster, F., Chui, Y.L. and Batchelor, J.R. (1985) Anti-idiotypic T cells suppress rejection of renal allografts in rats. Nature. 315, 336-337.

Lanzavecchia, A. (1983) One out of five peripheral blood B lymphocytes is activated to high rate Ig-production by human alloreactive T cell clones. Eur. J. Immunol. 13, 820-824.

Lanzavecchia, A. (1985) Antigen-specific interaction between T and B cells. Nature. 314, 537-539.

Lanzavecchia, A. and Parodi, B. (1984) In vitro stimulation of IgE production at a single precursor level by human alloreactive T helper clones. Clin. Exp. Immunol. 55, 197-203.

Lanzavecchia, A., Santini, P., Maggi E., Del Prete, G.F., Falagiani, P., Romagnani, S. and Ferrarini, M. (1983) In vitro selective expansion of allergen-specific T cells from atopic patients. Clin. Exp. Immunol. 52, 21-28.

Larche, M., Lamb, J.R., O'Hehir, R.E., Imami-Shita, N., Zanders, E.D., Quint, D.E., Moqbel, R. and Ritter, M.A. Functional evidence for a monoclonal antibody that binds to the human interleukin 4 receptor. Immunology. (in press)

Larche, M., Lamb, J.R. and Ritter M.A. (1988) A novel T lymphocyte molecule that may function in the induction of self-tolerance and MHC-restriction within the human thymic microenvironment. Immunology. 64, 101-105.

Lechler, R.I., Bal, V., Rothbard, J.B., Germain, R.N., Sekaly, R., Long, E.O. and Lamb, J. Structural and functional studies of HLA-DR restricted antigen recognition by human helper T lymphocyte clones using transfected murine cell lines. J. Immunol. (in press)

Lechler, R.I., Ronchese, F., Braunstein, N.S. and Germain, R.N. (1986) I-A restricted antigen recognition. Analysis of the roles of A alpha and A beta using DNA-mediated gene transfer. J. Exp. Med. 163, 678-696.

Lee, K.C., Wong, M. and Spitzer, D. (1982) Chloroquine as a probe for antigen processing by accessory cells. Transplantation. 34, 150-153.

Lee, F., Yokota, T., Otsuka, T. et al. (1986) Isolation and characterisation of a mouse interleukin cDNA clone that expresses B cell stimulatory factor 1 activities and T cell and mast cell stimulating activities. Proc. Natl. Acad. Sci. (U.S.A.) 83, 2061-2066.

Leung, D.Y.M., Bhan, A.K., Schneeberger, E.E. and Geha, R.S. (1983) Characterisation of the mononuclear cell infiltrate in atopic dermatitis

using monoclonal antibodies. J. Allergy Clin. Immunol. 71, 47-56.

Leung, D.Y.M., Brozek, C., Frankel, R. and Geha, R.S. (1984) IgE suppressor factors in normal human serum. Clin. Immunol. Immunopathol. 32, 339-350.

Leung, D.Y.M., Frankel, R., Brozek, C. and Geha, R.S. (1986) Potentiation of human IgE synthesis by plasma IgE binding factors from patients with the hyper IgE syndrome. J. Clin. Invest. 77, 952-957.

Leung, D.Y.M. and Geha, R.S. (1987) Regulation of the human IgE antibody response. Intern. Rev. Immunol. 2, 75-91.

Leung, D.Y.M., Rhodes, A.R. and Geha, R.S. (1981) Enumeration of T cell subsets in atopic dermatitis using monoclonal antibodies. J. Allergy Clin. Immunol. 67, 450-455.

Leung, D.Y.M., Young, M.C. and Geha, R.S. (1986) Induction of IgG_1 and IgE synthesis in normal B cells by autoreactive T cell clones. J. Immunol. 136, 2851-2855.

Levich, J.D., Parks, D.E. and Weigle, W.O. (1985) Tolerance induction in antigen specific T helper clones and lines in vitro. J. Immunol. 135, 873-878.

Levine, B.B., Ojeda, A. and Benacerraf, B. (1963) Studies on artificial antigens. III. The genetic control of the immune response to hapten poly-L-lysine conjugates in guinea pigs. J. Exp. Med. 118, 953-957.

Levine, B.B., Stember, R. and Fotino, M. (1972) Ragweed hayfever: genetic control and linkage to HLA haplotypes. Science. 178, 1201-1203.

Levy, D.A., Lichtenstein, L.M., Goldstein, E.O. and Ishizaka, K. (1971) Immunologic and cellular changes accompanying the therapy of pollen allergy. J. Clin. Invest. 50, 360-369.

Lieberman, R., Paul, W.E., Humphrey, W. and Stimpfling, J.H. (1972) H-2 linked immune response (Ir) genes. Independent loci for Ir-IgG and Ir-IgA genes. Science. 136, 1231-1240.

Lind, P. (1985) Purification and partial characterisation of two major allergens from the house dust mite Dermatophagoides pteronyssinus. J. Allergy Clin. Immunol. 76, 753-761.

Lind, P. (1986) Demonstration of close physiochemical similarity and partial immunochemical identity between the major allergen Dp 42, of the house dust mite, D. pteronyssinus and corresponding antigens of D. farinae (Df 6) and D. microceras (Dm 6). Int. Arch. Allergy Appl. Immunol. 79, 60-65.

Lind, P., Ingemann, L. and Brouvez, M. (1987) Demonstration of species-specific sensitisation to major allergens of Dermatophagoides species by solid phase absorption of human IgE antibodies. Scand. J. Immunol. 25, 1-10.

Lind, P. and Lowenstein, H. (1983) Identification of allergens in Dermatophagoides pteronyssinus mite body extract by crossed radioimmunoelectrophoresis with two different rabbit antibody pools. Scand. J. Immunol. 17, 263-273.

Livingstone, A.M. and Fathman, C.G. (1987) The structure of T cell epitopes. Ann. Rev. Immunol. 4, 477-502.

Lotteau, V., Teyton, L., Burroughs, D. and Charron, D. (1987) A novel HLA class II molecule (DRalpha-DQbeta) created by mismatched isotype pairing. Nature. 329, 339-341.

Lowenstein, H. (1978) Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. Prog. Allergy. 25, 1-62.

Maestrelli, P., O'Hehir, R.E., Lamb, J.R., Tsai, J-J., Cromwell, O. and Kay, A.B. Antigen-induced neutrophil chemotactic factor from cloned human T lymphocytes. Immunology. (in press)

Maggi, E., Del Prete, G.F., Tiri, A., Macchia, D., Parronchi P., Ricci, M. and Romagnani, S. (1988) T cell clones providing helper function for IgE synthesis release soluble factor(s) that induce IgE production in human B cells: possible role for interleukin 4 (IL-4). Clin. Exp. Immunol. 73, 57-62.

Maini, R.N., Dumonde, D.C., Faux, J.A., Hargreave, F.E. and Pepys, J. (1971) The production of lymphocyte mitogenic factor and migration-inhibition factor by antigen-stimulated lymphocytes of subjects with grass pollen allergy. Clin. Exp. Immunol. 9, 449-465.

Malissen, B., Peele-Price, M., Goverman, J.M., McMillan, M., White, J., Kappler, J., Marrack, P., Pierres, F., Pierres, M. and Hood, L. (1984) Gene transfer of H-2 class II genes: Antigen presentation by mouse fibroblast and hamster B cell lines. Cell 36, 319-327.

Malkovsky, M., Medawar, P.B., Thatcher, D.R. and Dore, C. (1985) Acquired immunological tolerance of foreign cells is impaired by recombinant rat interleukin 2 or vitamin A acetate. Proc. Natl. Acad. Sci. U.S.A. 82, 536-538.

Marsh, D.G., Hsu, S.H., Roebber, M., Ehrlich-Kautzky, E., Freidhoff, L.R., Meyers, D.A., Pollard, M.K. and Bias, W.B. (1982a) HLA-Dw2: A genetic marker for human immune response to short ragweed pollen allergen Ra5. I. Response resulting primarily from natural antigenic exposure. J. Exp. Med. 155, 1439-1463.

Marsh, D.G., Hsu, S.H., Roebber, M., Ehrlich-Kautzky, E., Freidhoff, L.R., Meyers, D.A. Pollard, M.K. and Bias, W.B. (1982b) HLA-Dw2: A genetic marker for human immune response to short ragweed pollen allergen Ra5. II. Response after ragweed immunotherapy. J. Exp. Med. 155, 1452-1463.

Marsh, D.G., Meyers, D.A. and Bias, W.B. (1981) The epidemiology and genetics of atopic allergy. N. Engl. J. Med. 305, 1551-1559.

Marsh, D.G., Friedhoff, L.R., Ehrlich-Kautzky, E., Bias, W.B. and Roebber, M. (1987) Immune responsiveness to Artemisifolia (short ragweed) antigen Amb a VI (Ra 6) is associated with HLA-DR5 in allergic humans. Immunogenetics. 26, 230-236.

Martin, W.J., Maurer, P.H. and Benacerraf, B. (1971) Genetic control of immune responsiveness to a glutamic acid, alanine, tyrosine copolymer in mice. I. Linkage of responsiveness to H-2 genotypes. J. Immunol. 107, 715-718.

Martinez, J.D., Santos, J., Stechschulte, D.J. and Abdou, N.I. (1979) Nonspecific suppressor cell function in atopic subjects. J. Allergy Clin. Immunol. 64, 485-490.

Martz, E. (1987) LFA-1 and other accessory molecules functioning in adhesions of T and B lymphocytes. Hum. Immunol. 18, 3-37.

Maryanski, J.L., Pala, P., Corradin, G., Jordan, B.R. and Cerottini, J.C. (1986) H-2-restricted cytolytic cells specific for HLA can recognise a synthetic HLA peptide. Nature. 324, 578-579.

Massie, F.S. and Wutanasupta, R. (1972) Detection of delayed hypersensitivity in ragweed pollinosis by an in vitro method. Ann. Allergy. 30, 676-684.

Matis, L.A., Glimcher, L.H., Paul, W.E. and Schwartz, R.H. (1983) Magnitude of response of histocompatibility-restricted T cell clones is a function of the product of the concentrations of antigen and Ia molecules. Proc. Natl. Acad. Sci. (U.S.A.) 80, 6019-6023.

Matzinger, P., Zamoyska, R. and Waldmann, H. (1984) Self tolerance is H-2 restricted. Nature. 308, 738-741.

McDevitt, H.O. and Benacerraf, B. (1969) Genetic control of specific immune responses. Adv. Immunol. 11, 31-74.

McDevitt, H.O. and Sela, (1965) Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. J. Exp. Med. 122, 517-531.

McDonald, S.M., Lichtenstein, L.M., Proud, D., Plant, M., Naclerio, R.M. and Kagey-Sobotka, A. (1987) Studies of IgE-dependent histamine releasing factors: Heterogeneity of IgE. J. Immunol. 139, 506-512.

McMichael, A.J., Gotch, F.M. and Rothbard, J. (1986) HLA B37 determines an influenza A virus nucleoprotein epitope recognised by cytotoxic T lymphocytes. J. Exp. Med. 164, 1397-1406.

Mendell, N.R., Lee, K.L., Reinsmoen, N., Yunis, E., Amos, B. and Emmel, L. (1977) Statistical methods for evaluating responses in HLA-D typing. Transpl. Proc. 9, 99-106.

Mengle-Gaw, L. and McDevitt, H.O. (1983) Isolation and characterisation of a cDNA clone for the murine I-E beta polypeptide chain. Proc. Natl. Acad. Sci. (U.S.A.) 80, 7621-7625.

Metzger, W.J., Zavala D., Richerson, H.B., Moseley, P., Iwamota, P., Monick, M., Sjoerdsma, K. and Hunninghake, G.W. (1987) Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs.

Description of the model and local airway inflammation. Am. Rev. Respir. Dis. 135, 433-440.

Meuer, S.C., Cooper, D.A., Hodgdon, J.C., Hussey, R.E., Fitzgerald, K.A., Schlossman, S.F. and Reinherz, E.L. (1983) Identification of the receptor for antigen and major histocompatibility complex on human inducer T lymphocytes. Science 222, 1239-1242.

Meyers, D. and Marsh, D. (1981) Report on a National Institute of Allergy and Infectious Diseases sponsored workshop on the genetics of total immunoglobulin E levels in humans. J. Allergy Clin. Immunol. 67, 167-170.

Miller, G. and Lipman, M. (1973) Release of infectious Epstein-Barr virus by transformed marmoset leucocytes. Proc. Natl. Acad. Sci. (U.S.A.) 69, 383-387.

Ming, W.J., Bersani, L. and Mantovani, A. (1987) Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leucocytes. J. Immunol. 138, 1469-1474.

Mitchell, E.B., Crow, J., Chapman, M.D., Jouhal, S.S., Pope, F.M. and Platts-Mills, T.A.E. (1982) Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet. 1, 127-130.

Mitchell, E.B., Crow, J., Rowntree, S., Webster, A.D.B. and Platts-Mills, T.A.E. (1984) Cutaneous basophil hypersensitivity to inhalant allergens: local transfer of basophil accumulation with immune serum but not IgE antibody. J. Invest. Dermatol. 83, 290-295

Mitchell, E.B., Crow, J., Williams, G. and Platts-Mills, T.A.E. (1986) Increase in skin mast cells following chronic house dust mite exposure. Br. J. Dermatol. 114, 65-73.

Mitchison, N.A. (1971) In: Cell Interaction in Immune Responses. (Makela, O., Cross, A. and Kosunen, R. Eds.) Pp. 249-260. Academic Press, N.Y.

Miyamoto, T., Oshima, S., Ishizaka, T. and Sano, S. (1968) Allergenic identity between the common floor mite (Dermatophagoides farinae Hughes 1961) and house dust as a causative agent in bronchial asthma. J. Allergy. 42, 14-28.

Moller, G. (Ed) (1987) Antigen requirements for activation of MHC restricted responses. Immunol. Rev. 98, 1-187.

Moorhead, J.W. (1981) Antigen receptors on murine T lymphocytes in contact sensitivity. I. Functional inhibition of effector T cells by monovalent 2,4 dinitrophenol: implication for a two receptor model. J. Exp. Med. 154, 1811-1826.

Mosmann, T.R. and Coffman, R.L. (1987) Two types of mouse helper T cell clone; implications for immune regulation. Immunol. To-day. 8, 223-227.

Murray A.B., Ferguson, A.C. and Morrison, B. (1980) The seasonal variation of allergic respiratory symptoms induced by house dust mites. Ann. Allergy. 45, 347-350.

Nagaya, H. (1985) Induction of antigen-specific suppressor cells in patients with hayfever receiving immunotherapy. J. Allergy Clin. Immunol. 75, 388-394.

Nagy, L., Lee, T.H. and Kay, A.B. (1982) Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. New Engl. J. Med. 306, 497-501.

Newton, D.A.G., Maberley, D.J. and Wilson, R. (1978) House dust mite hyposensitisation. Br. J. Dis. Chest. 72, 21-28.

Noma, Y., Sideras, T., Naito, T., Bergstedt-Lindqvist, S., Azuma, C., Severinson, E., Tanabe, T., Kinashi, T., Matsuda, F., Yaoita, Y. and Honjo, T. (1986) Cloning of cDNA encoding the murine IgG_1 induction factor by a novel strategy using SP6 promoter. Nature. 319, 640-646.

Nossal, G.J.V. (1983) Cellular mechanisms of immunological tolerance. Ann. Rev. Immunol. 1, 33-62.

Nutman, T.B., Volkman D.J., Hussain, R., Fauci, A.S. and Ottesen, E.A. (1985) Filarial parasite-specific T cell lines: induction of IgE synthesis. J. Immunol. 134, 1178-1184.

O'Garra, A., Warren, D.J., Holman, M., Popham, A.M., Sanderson, C.J. and Klaus, G.G. (1986) Interleukin 4 (B-cell growth factor II / eosinophil differentiation factor) is a mitogen and differentiation factor for preactivated murine B lymphocytes. Proc. Natl. Acad. Sci. (U.S.A.) 83, 5228-5232.

Ogasawara, K., Maloy, W.L. and Schwartz, R.H. (1987) Failure to find holes in the T cell repertoire. Nature. 325, 450-452.

O'Hehir, R.E., Eckels, D.D., Frew, A.J., Kay, A.B. and Lamb, J.R. (1988) MHC-class II restriction specificity of cloned human T lymphocytes reactive with Dermatophagoides farinae (house dust mite). Immunology. 64, 627-631.

Otten, G., Herold, K.C. and Fitch, F.W. (1987) Interleukin 2 inhibits antigen-stimulated lymphokine synthesis in helper T cells by inhibiting calcium-dependent signalling. J. Immunol. 139, 1348-1353.

Ottenhoff, T.H.M., Elferink, D.G., Hermans, J. and De Vries, R. (1985) HLA class II restriction repertoire of antigen-specific T cells. I. The main restriction determinants for antigen presentation are associated with HLA-D/DR and not with DP and DQ. Human-Immunol. 13, 105-116.

Owerbach, D., Rich, C. and Taneja, K. (1986) Characterisation of three HLA-DR beta genes isolated from an HLA-DR 3/4 insulin-dependent diabetic patient. Immunogenetics. 24, 41-46.

Ownby, D.R. and Buckley, R.H. (1979) Lymphocyte responses to ragweed antigens from different sources. J. Allergy. Clin. Immunol. 63, 65-66.

Paul, W.E. and Ohara, J. (1987) B cell stimulatory factor-1/interleukin 4. Ann. Rev. Immunol. 5, 429-459.

Phillips, L., Chambers, C., Underdown, B.J. and Zimmerman, B. (1987) Lymphocyte proliferation to antigen E: Demonstration of the restriction of antigen E-specific T cells to ragweed-allergic donors. J. Allergy Clin. Immunol. 79, 933-940.

Platts-Mills, T.A.E. and Chapman, M.D. (1987) Dust mites: Immunology, allergic disease, and environmental control. J. Allergy Clin. Immunol. 80, 755-775.

Polmar, S.H., Waldmann, T.A. and Terry, W.D. (1972) IgE in immunodeficiency. Am. J. Pathol. 69, 499-512.

Quill, H. and Schwartz, R.H. (1987) Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. J. Immunol. 138, 3704-3712.

Quint, D.J., Bolton, E.J., Mackenzie, A.R. and Zanders, E.D. (1988) Regulation of human IgE synthesis: lymphokine production by T lymphocyte clones from a patient with the hyper IgE syndrome. Submitted.

Qvigstad, E., Moen, T. and Thorsby, E. (1984) T cell clones with similar antigen specificity may be restricted by DR, MT (DC) or SB class II HLA molecules. Immunogenetics. 19, 455-460.

Radbruch, A., Burger, C., Klein, S. and Muller, W. (1986) Control of immunoglobulin class switch recombination. Immunol. Rev. 89, 69-83.

Rammensee, H-G. and Bevan, M.J. (1984) Evidence from in vitro studies that tolerance to self antigen is MHC-restricted. Nature. 308, 741-744.

Ratcliffe, M.J.H. and Lamb, J.R. (1988) Lymphocyte purification, growth cloning and functional assays. In: Handbk. Exp. Pharmacol. 85, 319-343. (Eds Bray, M.A. and Morley, J.) Springer-Verlag.

Rawle, F.C., Burr, M.L. and Platts-Mills, T.A.E. (1983) Long-term falls in antibodies to dust mite and pollen allergens in patients with asthma or hay fever. Clin. Allergy. 13, 409-417.

Rawle, F.C., Mitchell, E.B. and Platts-Mills, T.A.E. (1984) T cell responses to the major allergen from the house dust mite, Dermatophagoides pteronyssinus, antigen P1: comparison of patients with asthma, atopic dermatitis and perennial rhinitis. J. Immunol. 133, 195-201.

Reinherz, E.L., Kung, P.C., Goldstein, G. and Schlossman, S.F. (1980) A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a heteroantiserum termed TH_2. J. Immunol. 124, 1301-1307.

Ricci, M., Del Prete, G.F., Maggi, E., Lanzavecchia, A., Sala, P.G. and Romagnani, S. (1985) The in vitro synthesis of human IgE: reappraisal of a 5-year study. Int. Archs. Appl. Immunol. 77, 32-37.

Richter, M. and Naspitz, C.K. (1968) The in vitro blastogenic response of lymphocytes of ragweed-sensitive individuals. J. Allergy. 41, 140-151.

Rock, K.L. and Benacerraf, B. (1983) Inhibition of antigen-specific T lymphocyte activation by structurally related Ir gene-controlled polymers. J. Exp. Med. 157, 1618-1634.

Rocklin, R.E. (1976) Modulation of cellular-immune responses in vivo and in vitro by histamine-bearing lymphocytes. J. Clin. Invest. 57, 1051-1058.

Rocklin, R.E., Pence, H., Kaplan, H. and Evan, R. (1974) Cell-mediated immune responses of ragweed antigen E. J. Clin. Invest. 53, 735-744.

Rocklin, R.E., Sheffer, A.L., Greineder, D.K. and Melmon, K.L. (1980) Generation of antigen-specific suppressor cells during allergy desensitisation. N. Engl. J. Med. 302, 1213-1219.

Roebber, M., Klapper, D.G., Goodfriend, L., Bias, W.B., Hsu, S.H. and Marsh, D.G. (1985) Immunochemical and genetic studies of Amb t V (Ra5G), an Ra5 homologue from giant ragweed pollen. J. Immunol. 134, 3062-3069.

Roehm, N.W., Marrack, P. and Kappler, J.W. (1983) Helper signals in the plaque-forming cell response to protein-bound haptens. J. Exp. Med. 158, 317-333.

Rola-Pleszczynski, M. and Blanchard, R. (1981) Abnormal suppressor cell function in atopic dermatitis. J. Invest. Dermatol. 76, 279-283.

Romagnani, S., Biliotti, G., Passaleva, A. and Ricci, M. (1972) Mites and house dust allergy. II. Relationship between house dust and mite (Dermatophagoides pteronyssinus and Dermatophagoides farinae) allergens by fractionation methods. Clin. Allergy. 2, 115-123.

Romagnani, S., Maggi, E., Del Prete, G.F. and Ricci, M. (1983) IgE synthesis in vitro induced by T cell factors from patients with elevated serum IgE levels. Clin. Exp. Immunol. 52, 85-88.

Ronchese, F., Brown, M.A. and Germain, R.N. (1987) Structure-function analysis of the Ab^{bm12} mutation using site-directed mutagenesis and DNA-mediated gene transfer. J. Immunol. 139, 629-638.

Rosen-Bronson, S., Johnson, A.H., Hartzman, R.J. and Eckels, D.D. (1986) Human allospecific TLCs generated against HLA antigens associated with DR1 through DRw8. Immunogenetics. 23, 368-378.

Rosenthal, A.S. and Shevach, E.M. (1973) The function of macrophages in antigen recognition by guinea pig T lymphocytes. II. Role of the macrophages in regulation of the genetic control of the immune response. J. Exp. Med. 138, 1194-1212.

Rothbard, J.B. (1986) Peptides and the cellular immune response. Ann. Inst. Pasteur. 137, 518-526.

Rothbard, J.B., Lechler, R.I., Howland, K., Bal, V., Eckels, D.D., Sekaly, R.P., Long E.O., Taylor, W.R. and Lamb, J.R. (1988) Structural model of HLA-DR1 restricted T cell antigen recognition. Cell. 52, 515-523.

Rothbard, J.B. and Taylor, W.R. (1988) A sequence pattern common to T cell epitopes. EMBO J. 7, 93-100.

Rowntree, S., Cogswell, J.J., Platts-Mills, T.A.E. and Mitchell, E.B. (1985) Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. Arch. Dis. Child. 60, 727-737.

Saizawa, K., Rojo, J. and Janeway, C.A. (1987) Physical association of CD4 and the CD3: alpha: beta T cell receptor. Nature. 328, 260-263.

Sampson, H.A. and Buckley, R.H. (1981) Human IgE synthesis in vitro: a reassessment. J. Immunol. 127, 829-834.

Saryan, J.A., Leung, D.Y.M. and Geha, R.S. (1983) Induction of human IgE synthesis by a factor derived from T cells of patients with hyper IgE states. J. Immunol. 130, 242-247.

Saryan, J.A., Rappaport, J., Leung, D.Y.M., Parkman, R. and Geha, R.S. (1983) Regulation of human IgE synthesis in acute graft versus host disease. J. Clin. Invest. 71, 556-564.

Saxon, A., Morrow, C. and Stevens, R. (1980) Subpopulations of circulating B cells and regulatory T cells involved in in vitro immunoglobulin E production in atopic patients with elevated serum immunoglobulin E. J. Clin. Invest. 65, 1457-1468.

Schrader, J.W. (1986) The panspecific hemopoietin of activated T lymphocytes. Ann. Rev. Immunol. 4, 205-230.

Schuster, D., Pierson, D., Bongiovanni, B. and Levinson, A. (1979) Suppressor cell function in atopic dermatitis associated with elevated immunoglobulin E. J. Allergy Clin. Immunol. 64, 139-143.

Schuyler, M., Gerblich, A. and Urda, G. (1985) Atopic asthma: T lymphocyte subpopulations. Clin. Allergy. 15, 131-138.

Schwartz, R.H. (1985) T lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Ann. Rev. Immunol. 3, 237-261.

Schwarz, Y., Kivity S., Schlezinger M., Ilfeld, D., Grief, J., Topilsky, M. and Garti, Y. (1988) The in vivo effect of colchicine on clinical parameters and suppressor T cell function in patients with allergic bronchial asthma. J. Allergy Clin. Immunol. 81, 311 (Abs).

Sedgwick, J.D. and Holt, P.G. (1983) Induction of IgE-isotype specific tolerance by passive antigenic stimulation of the respiratory mucosa. Immunology. 50, 625-630.

Sedgwick, J.D. and Holt, P.G. (1984) Suppression of IgE response in inbred rats by repeated respiratory tract exposure to antigen: responder phenotype influences isotype specificity of induced tolerance. Eur. J. Immunol. 14, 893-897.

Sedgwick, J.D. and Holt, P.G. (1985) Induction of IgE-secreting cells and IgE isotype-specific suppressor T cells in the respiratory lymph nodes of rats in response to antigen inhalation. Cell. Immunol. 94, 182-194.

Sedgwick, J.D., Holt, P.G. and Turner, K.J. (1981) Production of a histamine releasing lymphokine by antigen or mitogen-stimulated human peripheral T cells. Clin. Exp. Immunol. 45, 409-413.

Sette, A., Buus, S., Colon, S., Smith, J.A., Miles, C. and Grey, H.M. (1987) Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. Nature 328, 395-399.

Shevach, E.M., Paul, W.E. and Green, I. (1972) Histocompatibility-linked immune response gene function in guinea pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. J. Exp. Med. 136, 1209-1214.

Shevach, E.M. and Rosenthal, A.S. (1973) Function of macrophages in antigen recognition by guinea pig T lymphocytes. II. Role of the macrophage in the regulation of genetic control of the immune response. J. Exp. Med. 138, 1213-1229.

Shimonkevitz, R., Kappler, J., Marrack, P. and Grey, H. (1983) Antigen recognition by H-2-restricted T cells. I. Cell free antigen processing. J. Exp. Med. 158, 303-316.

Sibbald, B. (1986) Genetic basis of asthma. Seminars Resp. Med. 7, 307-315.

Sillevis Smitt, J.H., Box, J.D., Hulsebosch, H.J. and Krieg, S.R. (1986) In situ immunophenotyping of antigen presenting cells and T cell subsets in atopic dermatitis. Clin. Exp. Dermatol. 11, 159-168.

Slavin, R.G., Tennenbaum, J.I., Becker, R.J., Feinberg, A.R. and Feinberg, S.M. (1963) Cell transfer of delayed hypersensitivity to ragweed from atopic subjects treated with emulsified ragweed extracts. J. Allergy. 34, 368-373.

Smith, J.M., Disney, M.E., Williams, J.P. and Goel, Z.A. (1969) Clinical significance of skin reactions to mite extracts in children with asthma. Br. Med. J. 2, 723-726.

Smith, H.R. and Steinberg, A.D. (1983) Autoimmunity - a perspective. Ann. Rev. Immunol. 1, 175-210.

Smith, S., Brown, M.H., Rowe, D., Callard, R.E. and Beverley, P.C.L. (1986) Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology. 58, 63-70.

Snapper, C.M., Finkelman, F.D. and Paul, W.E. (1988) Differential regulation of IgG_1 and IgE synthesis by interleukin 4. J. Exp. Med. 167, 183-196.

Snapper, C.M. and Paul, W.E. (1987) B cell stimulatory factor-1 (interleukin-4) prepares resting murine B cells to secrete IgG_1 upon subsequent stimulation with bacterial lipopolysaccharide. Science. 236, 944-947.

Stewart, G.A., Butcher, A., Lees, K. and Ackland, J. (1986) Immunochemical and enzymatic analyses of Dermatophagoides pteronyssinus. J. Allergy Clin. Immunol. 77, 14-24.

Stewart, G.A. and Holt, P. G. (1987) Immunogenicity and tolerogenicity of a major house dust mite allergen, Der p I from Dermatophagoides pteronyssinus in mice and rats. Int. Archs. Allergy Appl. Immunol. 83, 44-51.

Stewart, G.A. and Turner, K.J. (1980) Physicochemical and immunochemical characterisation of the allergens from the mite Dermatophagoides pteronyssinus. Aust. J. Exp. Biol. Med. Sci. 58, 259-274.

Strannegard, O. and Strannegard, L.L. (1978) T lymphocyte numbers and function in human IgE mediated allergy. Immunol. Rev. 41, 149-170.

Streicher, H.Z., Berkower, I.J., Busch, M., Gurd, F.R.N. and Berzofsky J.A. (1984) Antigen conformation determines processing requirements for T cell activation. Proc. Natl. Acad. Sci. (U.S.A.) 81, 6831-6835.

Takemori, T. and Tada, T. (1975) Properties of antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. I. In vivo activity and immunochemical characterisation. J. Exp. Med. 142, 1241-1253.

Taniguchi, M., Hayakawa, K. and Tada, T. (1976) Properties of antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. II. In vitro activity and evidence for the I region gene product. J. Immunol. 116, 542-548.

Taussig, M.J., Corvalen, J.R.F., Binns, R.M. and Holliman, A. (1979) Production of an H-2-related suppressor factor by a hybrid T cell line. Nature. 277, 305-308.

Tamir, R., Castracane, J.M. and Rocklin, R.E. (1987) Generation of suppressor cells in atopic patients during immunotherapy that modulate IgE synthesis. J. Allergy Clin. Immunol. 79, 591-598.

Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. and Hamuro, J. (1983) Structure and expression of a cloned cDNA for human interleukin-2. Nature. 302, 305-310.

Tee, R.D., Gordon, D.J., Hawkins, E.R., Nunn, A.J., Lacey, J., Venables, K.M., Cooter, R.J., McCaffery, A.R. and Newman-Taylor, A.J. (1988) Occupational allergy to locusts. An investigation of the sources of the allergen. J. Allergy Clin. Immunol. 81, 517-525.

Teh, H.S., Kisielow, P., Scott, B., Kishi, H., Umetsu, Y., Bluthmann and von Boehmer, H. (1988) Thymic major histocompatibility complex antigens and the alpha beta T cell receptor determine the CD4/CD8 phenotype of T cells. Nature. 335, 229-233.

Theuson, D., Speck, L.S., Lett-Brown, M.A. and Grant, J.A. (1979) Histamine releasing activity (HRA). I. Production by mitogen- or antigen-stimulated human mononuclear cells. J. Immunol. 123, 626-632.

Tieber, V.L., Abruzzini, L.F., Didier, D.K., Schwartz, B.D. and Rotwein, P. (1986) Complete characterisation and sequence of an HLA class II DR-beta chain cDNA from the DR5 haplotype. J. Biol. Chem. 261, 2738-2742.

Tjio, A.H., Hull, W.M. and Gleich, G.J. (1979) Production of human immunoglobulin E antibody in vitro. J. Immunol. 122, 2131-2133.

Todd, J.A., Bell, J.I. and McDevitt, H.O. (1987) HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature. 329, 599-604.

Tovey, E.R. and Baldo, B.A. (1987) Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. J. Allergy Clin. Immunol. 79, 93-102.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc. Natl. Acad. Sci. (U.S.A.) 76, 4350-4354.

Townsend, A.R.M., Gotch, F.M. and Davey, J. (1985) Cytotoxic T cells recognise fragments of the influenza nucleoprotein. Cell. 42, 457-465.

Townsend, A.R.M., Rothbard, J., Gotch, F.M., Bahadur, G., Wraith, D. and McMichael, A.J. (1986) The epitopes of influenza nucleoprotein recognised by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell. 44, 959-968.

Travers, P. and Bodmer, W.F. (1984) Preparation and characterisation of monoclonal antibodies against placental alkaline phosphatase and other human trophoblast associated determinants. Int. J. Cancer. 33, 633-641.

Tsai, J-J., Maestrelli, P., O'Hehir, R.E., Cromwell, O., Moqbel, R. and Kay, A.B. (1987) A T cell derived product which enhances neutrophil leukotriene B4 (LTB4) generation. Fed. Proc. 2, Abs 6696, A1449.

Turner, K.J., Holt, P.G., Holt, B.J. and Cameron, K.J. (1983) In vitro synthesis of IgE by human peripheral blood leucocytes. III. Release of preformed antibody. Clin. Exp. Immunol. 51, 387-394.

Umetsu, D.T., Leung, D.Y.M., Siraganian, R., Jabara, H.H. and Geha, R.S. (1985) Differential requirements of B cells from normal and allergic subjects for the induction of IgE synthesis by an alloreactive T cell clone. J. Exp. Med. 162, 202-214.

Unanue, E.R. (1984) Antigen-presenting function of the macrophage. Ann. Rev. Immunol. 2, 395-428.

Valverde, E., Huguet, J., Engel, P. and Garcia-Calderon, A. (1984) Cell-mediated immunity in perennial allergic rhinitis. Ann. Allergy. 52, 187-193.

van Bronswijk, J.E.M.H. and Sinha, R.N. (1971) Pyroglyphid mites (Acari) and house dust allergy: a review. J. Allergy. 47, 31-52.

van Eden, W., Thole, J.E.R., van der Zee, R., Noordzij, A., van Embden, J.D.A., Hensen, E.J. and Cohen, I.R. (1988) Cloning of the mycobacterial epitope recognised by T lymphocytes in adjuvant arthritis. Nature. 331, 171-173.

Vitetta, E.S., Ohara, J., Myers, C., Layton, J., Krammer, P.H. and Paul, W.E. (1985) Serological, biochemical and functional identity of B cell stimulatory factor-1 and B cell differentiation factor for IgG_1. J. Exp. Med. 162, 1726-1731.

Voorhorst, R., Spieksma, F.Th.M. and Varekamp, N. (1969) House dust atopy and the house dust mite Dermatophagoides pteronyssinus (Troussart, 1877). Leiden: Stafleu's Scientific Pub. Co.

Voorhorst, R., Spieksma, F.Th.M., Varekamp, H., Leupen, M.J. and Lyklema, A.W. (1967) The house dust mite (Dermatophagoides pteronyssinus) and the allergens it produces: identity with the house dust allergen. J. Allergy. 39, 325-337.

Watts, T.H., Gaub, H.E. and McConnell, H.M. (1986) T cell mediated association of peptide antigen and major histocompatibility complex protein detected by energy transfer in an evanescent wave-field. Nature. 320, 179-181.

Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. and Stobo, J. (1986) The role of the T3/antigen receptor complex in T-cell activation. Ann. Rev. Immunol. 4, 593-620.

Werdelin, O. (1982) Chemically related antigens compete for presentation by accessory cells to T cells. J. Immunol. 129, 1883-1891.

Widera, G. and Flavell, R.A. (1984) The nucleotide sequence of the murine $I\text{-}E^b$ beta immune-response gene: evidence for gene conversion events in class II genes of the major histocompatibility complex. EMBO J. 3, 1221-1225.

Wilcox, H.N.A., and Marsh, D.G. (1978) Genetic regulation of antibody heterogeneity: its possible significance in human allergy. Immunogenetics. 6, 209-225.

Wilde, D.B. and Fitch, F.W. (1984) Antigen-reactive cloned helper T cells. II. Exposure of murine cloned helper T cells to IL-2 containing supernatant induces unresponsiveness to antigenic restimulation and inhibits lymphokine production after antigenic stimulation. J. Immunol. 133, 636-641.

Witt, C., Stuckey, M.S., Woolcock, A.J. and Dawkins, R.L. (1986) Positive allergy prick tests associated with bronchial histamine responsiveness in an unselected population. J. Allergy Clin. Immunol. 77, 698-702.

Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S., Aleksander, I. and Mak, T. (1984) A human T cell specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature. 308, 145-149.

Yasueda, H., Mita, H., Yui, Y. and Shida, T. (1986) Isolation and characterisation of two allergens from Dermatophagoides farinae. Int. Arch. Allergy. Appl. Immunol. 81, 214-223.

Yokota R., Otsuka, T., Mosmann, T., Banchereau, J., DeFrance, T., Blanchard, D., De Vries, J.E., Lee, F. and Arai, K. (1986) Isolation and characterisation of a human interleukin c DNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell stimulating activities. Proc. Natl. Acad. Sci. (U.S.A.) 83, 5894-5898.

Young, D.B. and Lamb, J.R. (1986) T lymphocytes respond to solid-phase antigen: a novel approach to the molecular analysis of cellular immunity. Immunology. 59, 167-171.

Young, M.C., Geha, R.S., Maksad, K.N. and Leung, D.Y.M. (1986) Characterisation of human T cell-derived IgE potentiating factor. Eur. J. Immunol. 16, 985-991.

Young, M.C., Leung, D.Y.M. and Geha, R.S. (1984) Production of IgE potentiating factor in man by T cell lines bearing Fc receptors for IgE. Eur. J. Immunol. 14, 871-878.

Young, S.H., Zimmerman, R.E. and Smithwick, E.M. (1968) The in vitro response of human lymphocytes challenged by ragweed antigen. Paediatrics. 42, 976-979.

Zachary, C.B. and MacDonald D.M. (1983) Quantitative analysis of T lymphocyte subsets in atopic eczema, using monoclonal antibodies and flow cytofluorimetry. Br. J. Dermatol. 108, 411-422.

Zanders, E.D., Lamb, J.R., Feldmann, M., Green, N. and Beverley, P.C.L. (1983) Tolerance of T cell clones is associated with membrane antigen changes. Nature. 303, 625-627.

Zanders, E.D., Feldmann, M., Green, N. and Lamb, J.R. (1984) Direct evaluation of antigen binding to human T lymphocyte clones; involvement of MHC products in antigen binding. Eur. J. Immunol. 14, 1101-1105.

Zeitz, S.J., van Arsdel, P.P. and McClure, D.K. (1966) Specific response of human lymphocytes to pollen antigen in tissue culture. J. Allergy. 38, 321-329.

Ziegler, K. and Unanue, E.R. (1981) Identification of a macrophage antigen processing event required for I region restricted antigen presentation to T lymphocytes. J. Immunol. 127, 1869-1875.

Ziegler, K. and Unanue, E. (1982) Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. Proc. Natl. Acad. Sci. (U.S.A.) 79, 175-178.

Zinkernagel, R.M. and Doherty, P.C. (1974) Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature. 251, 547-548.

APPENDIX
Publications resulting from this study:

1. O'Hehir RE, Young DB, Kay AB and Lamb JR. (1987) Cloned human T lymphocytes reactive with Dermatophagoides farinae (house dust mite): a comparison of T - and B - cell antigen recognition. Immunology 62, 635-640.

2. O'Hehir RE, Eckels DD, Frew AJ, Kay AB and Lamb JR. (1988) MHC class II restriction specificity of cloned human T lymphocytes reactive with Dermatophagoides farinae (house dust mite). Immunology 64, 627-631.

3. Lamb JR, O'Hehir RE and Young DB. (1988). The use of nitrocellulose immunoblots for the analysis of antigen recognition by T lymphocytes. J. Imm. Meth. 110, 1-10.

4. Maestrelli P, O'Hehir RE, Lamb JR, Tsai J-J, Cromwell O and Kay AB (1988) Antigen-induced neutrophil chemotactic factor from cloned human T lymphocytes. Immunology (in press).

5. Larche M, Lamb JR, O'Hehir RE, Imami-Shita N, Zanders ED, Quint DE, Moqbel R and Ritter MA. (1988) Functional evidence for a monoclonal antibody that binds to the human interleukin-4 receptor. Immunology (in press).