Antigen-Presenting OKM5$^+$ Melanophages Appear in Human Epidermis After Ultraviolet Radiation

Kevin D. Cooper, M.D., Gabrielle R. Neises, B.S., and Stephen I. Katz, M.D., Ph.D.
Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

Ultraviolet radiation of murine skin in vivo or epidermal cells (EC) in vitro dramatically inhibits the antigen-presenting capacity of EC in vitro and results in the inhibition of immune responses to antigen challenge. In humans, UV exposure in vivo markedly inhibits alloantigen presentation by EC in the EC-lymphocyte reaction (ELR) when EC are harvested immediately after the administration of 4 times the minimal erythema dose (4 MED), whereas EC harvested 72 h after 4 MED (UV-EC) exhibit enhanced allostimulatory capacity in the ELR. This enhanced ELR reactivity is due to the appearance, in the epidermis, of bone marrow-derived OKT6$^-$ DR$^+$ cells which are distinct from Langerhans cells (LC) in their lack of surface OKT6 and in their ultrastructural morphology.

This report focuses on the phenotype and function of T6$^-$ Dr$^+$ UV-EC and on their relationship to known human antigen presenting cell (APC) subsets. Approximately 60% of T6$^-$ Dr$^+$ UV-EC bore the monocyte marker defined by monoclonal antibody OKM5, but lacked determinants recognized by OKM1, LeuM1, LeuM3, LeuM4, LeuM5, and Mac1. All T6$^-$ Dr$^+$ UV-EC bore the class II MHC antigen HLA-DQ (DC/DS), which is associated with a specialized subset of antigen-presenting monocytes capable of stimulation in the autologous mixed leukocyte reaction (AMLR). Panning of OKM5$^+$ UV-EC resulted in a population of cells which was markedly enriched in melanophages and which exhibited potent alloantigen-presenting capacity in the ELR. Since OKM5$^+$ T6$^-$ Dr$^+$ UV-EC were similar to the specialized APC minor subset of OKM1$^-$ OKM5$^+$ blood monocytes both in phenotype and in apparent phagocytic function, we examined other APC functions of UV-EC to assess the extent of this analogy. Relative to control EC (containing only LC as APC), UV-EC (containing functionally inactivated LC but many T6$^-$ Dr$^+$ APC) induced significantly greater degrees of T-cell proliferation in the presence of either tetanus toxoid antigen or the mitogen concanavalin A. UV-EC, as well as panning-purified OKM5$^+$ UV-EC, were also able to induce autologous T-cell proliferation in the absence of added antigen (autologous ELR), in contrast to control EC which were poor stimulators of an autologous ELR. Thus, although human EC 72 h after UV exposure are numerically and functionally depleted of LC, at least 2 additional subsets of T6$^-$ Dr$^+$ APC appear in the epidermis. One of these subsets is OKM1$^-$ OKM5$^-$ DQ$^+$. The major subset is OKM1$^-$ OKM5$^+$ DQ$^+$ and appears phenotypically and functionally analogous to the highly specialized OKM1$^-$ OKM5$^+$ subset of blood monocytes capable of phagocytosis, soluble antigen presentation, and stimulation in the AMLR. These non-LC APC subsets may be involved in altered APC-T cell activation mechanisms when skin is irradiated with UV in vivo. *J Invest Dermatol 86:363–370, 1986*

Manuscript received July 16, 1985; accepted for publication November 6, 1985.

Reprint requests to: Stephen I. Katz, M.D., Dermatology Branch, Building 10, Room 12N238, National Institutes of Health, Bethesda, Maryland 20892.

Abbreviations:
AMLR: mixed leukocyte reaction
APC: antigen-presenting cell(s)
ConA: concanavalin A
DTH: delayed type hypersensitivity
EC: epidermal cells
ELR: epidermal cell-lymphocyte reaction
FBS: fetal bovine serum
LC: Langerhans cell(s)
MED: minimal erythema dose
MHC: major histocompatibility complex
PBMNC: peripheral blood mononuclear cells
PBS: phosphate-buffered saline
R: responder (cells)
S: antigenic or mitogenic stimulus (tetanus toxoid or ConA)
T6: antigen on cells binding OKT6 antibody
UV-EC: epidermal cells harvested 3 days after 4 MED UV exposure

Antigen specific T-cell activation and proliferation is dependent upon recognition of antigen in the context of class II major histocompatibility complex (MHC) antigens which are expressed on several diverse cell types. These include macrophages [1], dendritic cells [2,3], Langerhans cells [4], B cells [5], and endothelial cells [6]. There is a growing body of evidence to support the contention that T cells discriminate between different antigen-presenting cell (APC) types [7,8] and that antigen presented by different APCs may activate functionally distinct T-cell subsets [9–17].

Normal epidermis contains a single population of APCs, Langerhans cells (LC) [4]. Human LC are dendritic, bear class II MHC antigens (Dr$^+$) and OKT6 antigen (T6$^+$) [18]. Presentation of antigen by LC is the likely mechanism for epicutaneous activation of T cells in the induction of antigen-specific delayed type hypersensitivity (DTH) [19–21]. An effective method of altering both epidermal APC function and the subsequent induction of T-cell immunity is by exposure of skin to UV radiation. This inactivates LC antigen-presenting function [22–25] and tends to lead to the induction of T cells which suppress DTH responses [26–29]. In mice, these suppressor mechanisms active in the modulation of contact sensitivity and in the development of tumors in UV-treated animals may be induced because of selective antigen

0022-202X/86/$03.50

presentation by a population of UV-resistant I-J$^+$ cells which induce T-cell suppression [29,30].

In addition to inactivation of the antigen presenting capacity of LC residing in the epidermis, UV exposure of intact animals activates a series of dynamic alterations in immune cell trafficking. These alterations result in shifts of murine APC from the spleen to the draining lymph nodes, dermis, and epidermis of UV-irradiated sites [31,32].

Few data are available on the functional effects of UV radiation in humans [24,33]. We have found that in vivo exposure of human skin to 4 times the minimal erythema dose (MED) of UV resulted in abrogation of epidermal LC alloantigen-presentation capacity in the epidermal cell-lymphocyte reaction (ELR) when the epidermal cells (EC) were harvested immediately after UV exposure [25]. In contrast, when the harvest of UV-irradiated EC was delayed, beginning at 24 h and peaking 72 h after UV exposure (UV-EC), a marked increase in alloantigen-presenting capacity was observed. This enhancement was due to the appearance of T6$^-$ Dr$^+$ bone marrow-derived cells containing phagocytosed melanosomes. These cells had neither the ultrastructural nor phenotypic criteria of LC.

In this study we addressed whether T6$^-$ Dr$^+$ EC were phenotypically and functionally analogous to any previously described subset of human APC, and whether activation of T cells by T6$^-$ Dr$^+$ EC differed from activation by LC. We have previously shown that, among potential human class II MHC antigen-bearing APC, T6$^-$ Dr$^+$ UV-EC were factor VIII-related antigen negative, surface immunoglobulin negative, and T-cell marker negative, demonstrating that T6$^-$ Dr$^+$ EC were not analogous to B cells, activated T cells, or antigen-presenting endothelial cells [25]. The phagocytic capacity of T6$^-$ Dr$^+$ EC for melanin, however, suggested that they were likely to be of monocyte/macrophage origin.

In this report, we describe T6$^-$ Dr$^+$ UV-EC as a heterogeneous population comprised of at least 2 populations of T6$^-$ Dr$^+$ non-LC: OKM5$^+$ cells, and OKM5$^-$ cells. We report that the OKM5$^+$ UV-EC are melanophagocytic and retain their functional capacity to stimulate allogeneic T cells. Their function as well as phenotype appears analogous to the highly specialized OKM1$^-$ OKM5$^+$ subset of blood monocytes, in that they have phagocytic capacity and can also stimulate autologous T cells in both the presence and absence of added antigens or mitogens.

MATERIALS AND METHODS

UV Exposure Normal human volunteers were irradiated with 16–80 mJ/cm^2 UV from a bank of FS 20 bulbs (Sylvania) to 2.4-cm^2 areas on the volar forearm to determine the MED. The bulbs emit 0.007 mJ/cm^2/s at 254 nm, 0.29 mJ/cm^2/s at 297 nm, 0.31 mJ/cm^2/s at 310 nm, and 0.005 mJ/cm^2/s at 350 nm at a 5-cm distance. Single doses of 4 MED were then given to multiple sites on the volar forearm 72 h prior to induction of suction blisters. Blisters were raised using suction chambers with a chamber temperature of 49–50°C produced by electrical heating coils and equipped with a telethermometer [34]. Blister tops (epidermis), which were free of dermal constituents, were removed under aseptic conditions and the wounds dressed with Opsite (Smith and Nephew, Welwyn Garden City, England) dressings.

Antibodies The specificity and source of the murine monoclonal antibodies directed toward cell surface markers are summarized in Table I. Polyclonal antibodies include: antihuman factor VIII-related antigen (Calbiochem-Behring, La Jolla, California), and fluoresceinated or biotinylated goat antimouse IgG (H + L chain specific, affinity purified, human serum absorbed) (Tago, Burlingame, California). Streptavidin conjugated with Texas Red was purchased from Bethesda Research Laboratories, Bethesda, Maryland. MOPC 21 IgG purified myeloma protein was obtained from Bionetics, Kensington, Maryland.

Direct and Indirect Immunofluorescence on Frozen Sections Volar forearm skin exposed to UV 72 h earlier or nonexposed skin was biopsied and frozen in OCT compound (LabTek Products, Naperville, Illinois). Six-micron sections were stained with fluorochrome-conjugated monoclonal antibodies at 1:10 or 1:20 in phosphate-buffered saline (PBS) containing 0.5% fetal bovine serum (FBS). Alternatively, sections were stained with unconjugated monoclonal antibodies followed by fluorescein-conjugated goat antimouse IgG. For double staining on the same section, one of 3 protocols was used: (1) fluoresceinated monoclonal antibody was used in conjunction with a rhodamine- or phycoerythrin-conjugated monoclonal antibody; or (2) an unconjugated monoclonal antibody, followed by fluoresceinated goat antimouse IgG, followed by a combination of murine myeloma IgG (in excess to occupy residual anti-IgG sites) and phycoerythrin- or rhodamine-conjugated murine monoclonal antibody; or

Table I. Murine Monoclonal Antibodies Directed Toward Human Cell Surface Markers

Antibody	Specificity	Source
OKT6	*M*, 49,000 T6 antigen distributed on LC and indeterminate cells of the epidermis, cortical thymocytes, and MOLT4 cells	Ortho Immunodiagnostics, Raritan, NJ
Leu 6	Non-complement fixing, used for staining Same as above except complement fixing Used for panning and complement lysis	Becton Dickinson, Sunnyvale, CA
Anti HLA-DR/Leu 10	Class II alloantigens on Langerhans cells, B cells, monocytes, antigen-presenting cells, activated T cells	Becton Dickinson
OKM1	Monocyte antigen (C3bi receptor)	Ortho
OKM5	Monocyte and platelet antigen	Ortho
Mac 1	Antihuman and murine monocyte antigen (C3bi receptor)	Hybritech San Diego, CA
Leu M1	Myelomonocytic antigen	Becton Dickinson
Leu M2 (Mac-120)	Human monocyte antigen	Becton Dickinson
Leu M3	Monocyte and macrophage antigen	Becton Dickinson
Leu M4	Neutrophilic granulocyte antigen	Becton Dickinson
Leu M5	Monocyte/macrophage antigen; shares *M*, 95,000 chain with LFA-1 and MAC-1	Becton Dickinson
anti CRII	B-cell antigen (C3d receptor)	Becton Dickinson
Mo-1	Myeloid antigen on monocytes, granulocytes, and null cells	Coulter Immunology Hialeah, FL
Mo-2	Myeloid antigen on monocytes and platelets	Coulter Immunology Westbury, NY

(3) an unconjugated monoclonal antibody, followed by a biotinylated goat antimouse IgG, followed by a combination of myeloma IgG, streptavidin-conjugated with Texas Red, and a fluorescein-conjugated IgG monoclonal antibody. Monoclonal murine antibodies of the appropriate class and subtype having irrelevant specificities were used as controls.

Preparation of Cell Suspensions In order to prepare single cell suspensions of epidermis, suction blisters were induced and the blister-top epidermal sheets were floated in 0.5% trypsin in Dulbecco's PBS for 40 min at 37°C and processed as previously described [25].

Peripheral blood mononuclear cells (PBMNC), which were used as responder cells in the allogeneic ELR, were obtained by centrifugation of heparinized venous blood over Ficoll-Hypaque density gradients (Bionetics). Autologous accessory cell-depleted T cells, which were used as responders in antigen presentation and autologous ELR assays, were prepared by sequential removal of APC from PBMNC by plastic adherence, exposure to 5 mM leucine methyl ester [35] and treatment with anti-HLA-DR, OKM1, and Leu M2 monoclonal antibodies plus complement (contained in fresh frozen young rabbit sera).

Two-Color Cell Surface Immunofluorescence Staining Cell suspensions were stained simultaneously with fluoresceinated OKT6 and phycoerythrin (or rhodamine)-conjugated anti-DR or fluorescein-conjugated OKM5 and phycoerythrin-conjugated anti-DR for 45 min at 4°C in PBS containing 0.5% FBS and 0.01% sodium azide, washed, and observed under a Leitz Orthoplan UV microscope using fluorescein and rhodamine filters and phase microscopy. Individual cells were scored as demonstrating both markers (i.e., $OKM5^+$, Dr^+), one marker ($OKM5^-$, Dr^+ or $OKM5^+$, Dr^-), or neither marker ($OKM5^-$, Dr^-). In some situations EC were stained in a 3-step method, first with unconjugated monoclonal antibodies followed by fluoresceinated goat antimouse IgG in the second step. The third step consisted of myeloma IgG (in excess to occupy residual anti-IgG sites) and phycoerythrin-conjugated anti-DR. Ethidium bromide was added in the last wash to counterstain dead cell nuclei in order to assess the viability. Controls included conjugated and unconjugated murine monoclonal antibodies of the appropriate class and subtype with irrelevant specificities.

Transmission Electron Microscopy Cell suspensions were pelleted at 400 *g* and incubated in phosphate buffer containing 6% glutaraldehyde for 3 h, washed, and left in PBS overnight at 4°C. The cells were postfixed in 1% OsO_4 1.5% potassium ferrocyanide, dehydrated in a graded series of methanol, and embedded in Epon. Thin sections were cut, stained with lead citrate and uranyl acetate, and viewed a Philips 400 electron microscope.

Depletion and Enrichment of Epidermal Cell Subpopulations Epidermal cells were enriched for $OKM5^+$ cells by a modified panning method. Epidermal cells suspensions were incubated with OKM5 antibody for 45 min at 4°C, washed, and added to 100-mm polystryrene Petri dishes previously coated with affinity-purified goat antimouse IgG. The dishes were placed on a level surface at 4°C for 40 min, swirled, and incubated another 40 min. Nonadherent $OKM5^-$ cells were harvested by collecting the first 2 aspirates from the plate. After 5 washes to remove nonadherent cells, adherent $OKM5^+$ cells were harvested by repeated vigorous pipetting. OKM5-depleted UV-EC were obtained by collecting nonadherent UV-EC. Any residual $OKM5^+$ cells in this nonadherent population were removed using indirect complement lysis by incubating with goat antimouse IgG for 30 min at 4°C, followed by complement lysis with young rabbit complement at 37°C for 45 min.

Functional Studies

Allogeneic Mixed ELR: Stimulator epidermal cell suspensions were x-irradiated with 2000 r from a ^{137}Cs source and added, in varying doses from 5,000 to 80,000 cells per well, to 50,000 allogeneic responder PBMNC in ELR medium [25] and incubated in 96-well round-bottom Linbro culture plates (Flow Laboratories, McLean, Virginia). In some experiments graded numbers of panning-purified $OKM5^+$ UV-EC or OKM5-depleted UV-EC were used as stimulators. One microcurie of tritiated thymidine (Amersham, Arlington Heights, Illinois) was added on day 6 to each well and the cultures were harvested 18 h later on a MASH harvester (MA Bioproducts, Maryland). Cell-associated radioactivity was measured by scintillation counting, and expressed as the mean ± SEM of triplicate determinations. Statistical comparisons between observations were considered significantly different when $p < 0.05$ using Student's *t*-test.

Presentation of Tetanus Toxoid and Concanavalin A (ConA) to T Cells: Varying concentrations of tetanus toxoid (Department of Public Health, Division of Biologic Laboratories, Boston, Massachusetts) or ConA (Calbiochem/Behring) and x-irradiated EC from control epidermis or UV-EC were mixed with 50,000 accessory cell-depleted autologous responder T cells and cultured for 7 days. Tritiated thymidine uptake was determined over the last 18 h of culture. As 10 μg/ml tetanus toxoid was determined to be the optimal concentration of antigen for use with EC, this dosage was used in all studies. ConA studies were performed at both suboptimal (2 μg/ml) and optimal concentrations (16 μg/ml).

In order to account for any autologous ELR or for any residual APC in the antigen- or mitogen-specific proliferation assays, the following formula was used:

$$\text{Net cpm} = \text{total cpm} - \{\text{cpm(EC + R)} + \text{cpm(S + R)}\}$$

where:

net cpm = antigen- or mitogen-specific proliferation,
total cpm = cpm in culture containing antigen or mitogen plus responder T cells plus EC,
cpm (EC + R) = Δ cpm in culture containing EC plus responder T cells (R) without added antigen or mitogen,
cpm (S + R) = Δ cpm in culture containing stimulus S (tetanus toxoid or ConA) plus responder cells but without EC.

Autologous ELR: Varying numbers of x-irradiated EC, UV-EC, or panning-enriched $OKM5^+$ UV-EC were mixed with 150,000 autologous accessory cell-depleted responders and incubated for 7 days. Tritiated thymidine incorporation was measured over the last 18 h of culture.

RESULTS

$T6^-$ Dr^+ Cells in UV-Exposed Epidermis Are Comprised of $OKM5^+$ and $OKM5^-$ Cell Subsets Volar forearm skin of normal volunteers was exposed to 4 MED UV radiation and suction blisters were induced at control sites and at UV-exposed sites 72 h later. The epidermis was removed, trypsinized into an epidermal cell suspension, and stained as described for single-step double fluorescent staining.

As compared with control EC which contained 1.8 ± 0.2% DR^+ cells (n = 13 individuals), EC harvested 72 h following UV exposure contained a higher percentage of DR^+ cells, 6.0 ± 0.7 (n = 13) (Fig 1). Virtually all control EC that expressed DR also expressed T6 (LC phenotype) whereas, by contrast, EC harvested 72 h after UV exposure contained a heterogeneous population of DR^+ cells (Fig 1). UV-EC contained a reduced percentage of $T6^+$ DR^+ LC (0.6 ± 0.1%, n = 12), a population of $OKM5^-$ $T6^-$ DR^+ cells (1.6 ± 0.3%, n = 7), and $OKM5^+$ $T6^-$ DR^+ cells. Viable $OKM5^+$ DR^+ cells comprised 3.8 ± 0.8% of viable EC (n = 7) (Fig 1) in UV-exposed epidermal cells as compared with nonexposed epidermal cells which rarely contained any $OKM5^+$ cells (<0.3%). All $OKM5^+$ EC expressed DR. Using phase microscopy, a variable proportion of viable Dr^+ cells (Fig 2*b*) appeared to contain a dark cytoplasm suggestive of ingested melanin (Fig 2*a*). Although virtually all $OKM5^+$ UV-EC were dark, $T6^+$ cells did not appear dark under phase microscopy. Thus $T6^+$ DR^+ UV-EC did not overlap with the $OKM5^+$ DR^+ cells by phase microscopy or by double fluorescence microscopy.

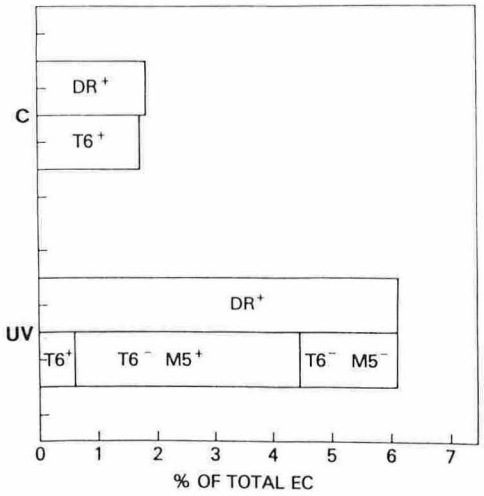

Figure 1. Histogram depicting Dr^+ EC subset phenotypes from control EC suspensions and EC suspensions obtained 72 h following in vivo UV exposure. Results expressed as percent of total EC specifically binding monoclonal antibody on viable EC as determined by double fluorescence microscopy. At least 1000 EC were counted per sample.

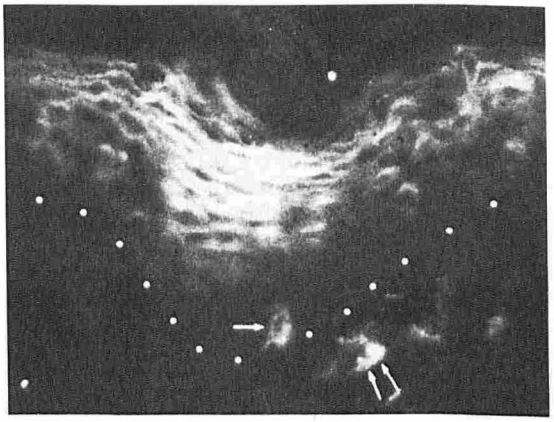

Figure 3. Localization of $OKM5^+$ cells to basal layer of epidermis. Skin biopsy was taken from the volar forearm of a normal volunteer 72 h following 4 MED UV exposure. Staining was performed on 6 μm- thick sections with OKM5 followed by fluoresceinated goat antimouse IgG. An $OKM5^+$ cells is noted in the basal layer of the epidermis (*single arrow*) as well as perivascularly in the dermis (*double arrows*). *Dots* denote dermal-epidermal junction. Stratum corneum staining is nonspecific. × 208.

Other monoclonal antibodies screened were OKM1, Leu M1, Leu M2, Leu M3, Leu M4, Leu M5, Mac 1, Mo-1, and Mo-2. All were uniformly negative except for OKM1, which had questionable staining on a minority of cells in 2 of 9 individuals. Staining of these DR^+ cells with Leu M2 was difficult to assess since keratinocytes bound substantial amounts of Leu M2. Only OKM5 was positive in all individuals tested. All DR^+ cells in UV-EC, as well as in control EC (C-EC), were HLA-DQ^+ (DC/DS^+).

In Situ Localization of $OKM5^+$ Cells to Basal Layer of Epidermis and to the Dermis Skin biopsies were taken from volar forearm skin 72 h after exposure to 4 MED UV. Frozen sections from 3 individuals were cut and stained with monoclonal antibodies as described. Staining with the OKM5 antibody demonstrated specifically stained cells in a basilar location in the epidermis (Fig 3) (confirming that these cells were within the epidermis), as well as in dermal perivascular and interstitial locations. A significant amount of nonspecific staining of the homogenized, UV-damaged upper epidermis/stratum corneum was observed not only when staining with OKM5 but also in sections stained with irrelevant monoclonal antibodies followed by fluoresceinated goat antimouse IgG. Control biopsies of normal human skin contained no detectable epidermal $OKM5^+$ cells and rare superficial dermal $OKM5^+$ cells. OKM1, Leu M1, Leu M3, Leu M4, Leu M5, and Mac-1 did not bind to epidermis of skin biopsied 72 h after UV. Double fluorescence microscopy of UV-irradiated skin after 72 h revealed that all $OKM5^+$ epidermal cells were DR^+, that, as in cell suspensions, approximately half of DR^+ cells in the spinous layer of the epidermis were $OKM5^-$, and that some DR^+ cells were $T6^+$.

$OKM5^+$ Cells Are Melanophages In an earlier study we demonstrated that DR^+ cells which were purified by panning and processed for electron microscopy contained a substantial percentage of melanophagic cells [25]. In this study we purified $OKM5^+$ cells from UV-EC using panning techniques and found that their purity was 41% as determined by fluorescence microscopy. This preparation was markedly enriched to 43% in cells containing melanosomes within phagolysosomes when ex-

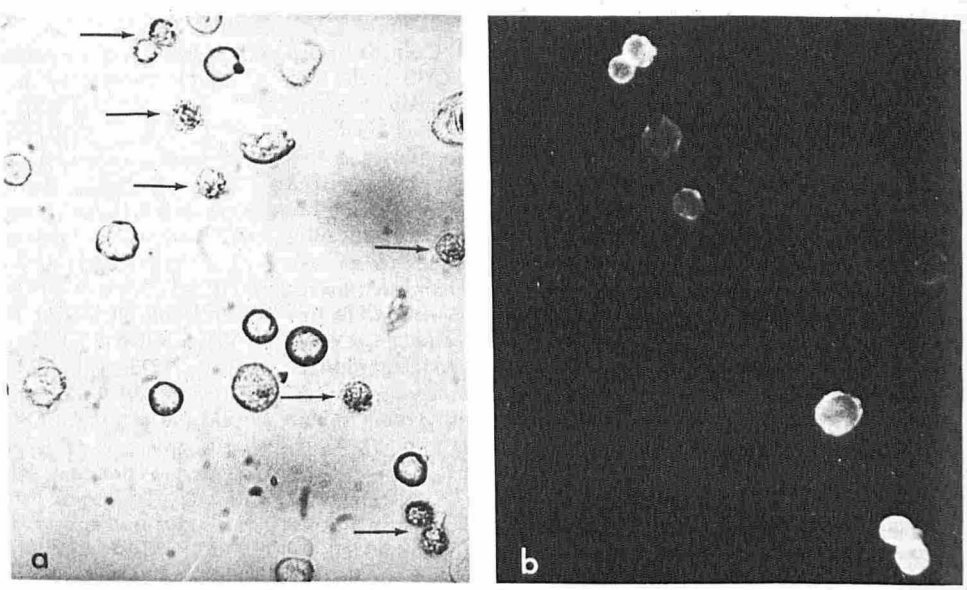

Figure 2. UV-EC with dark granular cytoplasm on phase microscopy (*a*) are Dr^+ by fluorescence microscopy (*b*) using phycoerythrin-conjugated anti-Dr (× 300).

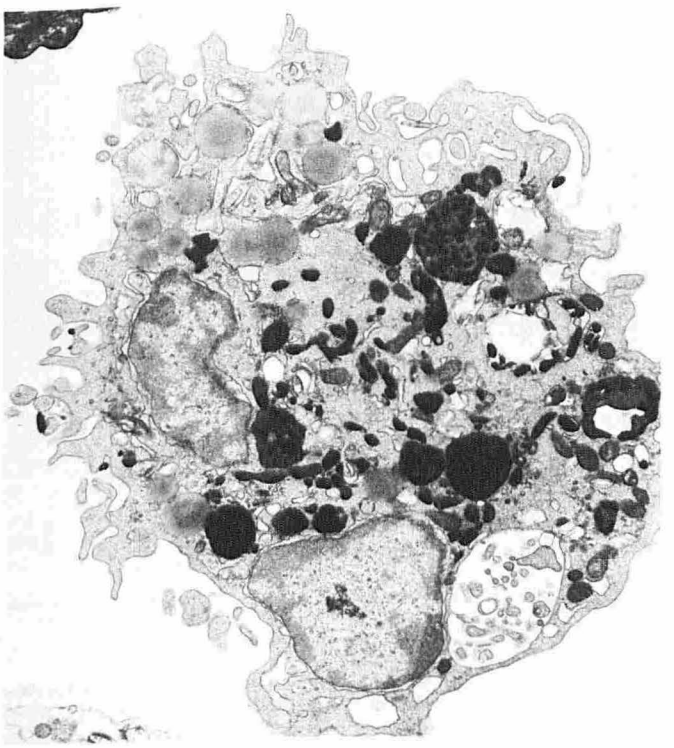

Figure 4. Transmission electron microscopic appearance of panning-enriched OKM5+ UV-EC. Cell shows numerous melanosomes within phagolysosomes, lipid bodies, lysosomal-rich cytoplasm, and lacks keratin filaments, premelanosomes, or Birbeck granules × 7660.

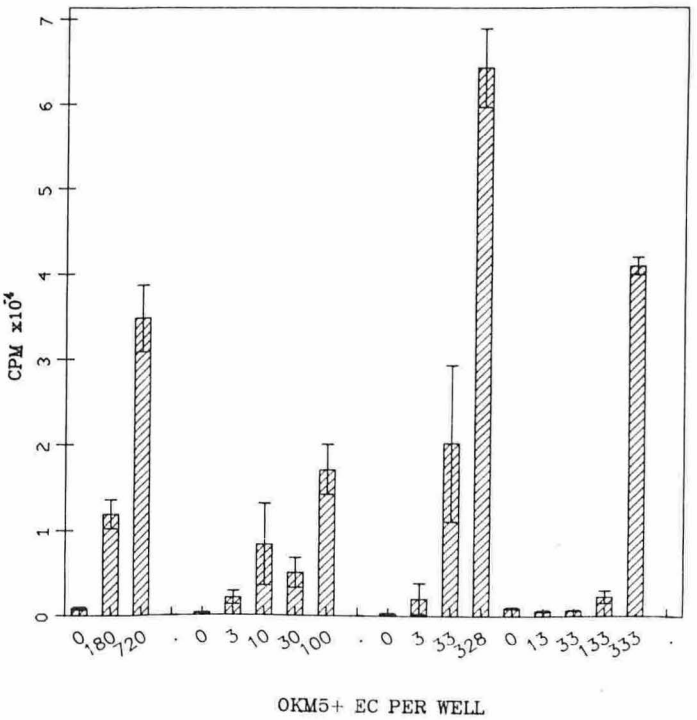

Figure 5. OKM5+ UV-EC present alloantigen in the ELR. Panning-enriched OKM5+ UV-EC were prepared from 4 individuals and added in graded numbers to 50,000 allogeneic responders. Results from each individual are expressed as cpm ± SEM tritiated thymidine uptake over last 18 h of a 7-day culture.

amined by transmission electron microscopy (Fig 4). The cells contained lysosomal-rich cytoplasm and indented nuclei, and appeared identical to melanophagic epidermal cells we had previously identified within the DR+ UV-EC population [25].

UV-EC were panning enriched for DR or OKM5 marker-bearing cells and their ultrastructural characteristics determined. Enumeration of relative proportions of LC, indeterminant cells (cells lacking Birbeck granules but otherwise identical to LC), and melanophages was performed at the ultrastructural level and the percentage of each cell type expressed as the percent of cells lacking features of keratinocytes or melanocytes. Among UV-EC enriched for DR+ cells, 6% were LC, 59% were melanophages, and 35% were indeterminant cells [25]. Enrichment for OKM5+ UV-EC, however, resulted in a DR+ population that was >80% melanophagocytic, only 19% indeterminant cells, and lacking LC. Therefore, these findings suggest that mainly OKM5+ cells comprise the melanophagocytic portion of DR+ UV-EC.

OKM5+ UV-EC Allostimulatory Capacity Since phagocytic function and antigen-presentation functions may be mediated by separate cell populations [2], we wished to determine whether the melanophagic OKM5+ cells which appear in epidermis 72 h after UV could be responsible for the enhanced allostimulatory capacity of UV-EC which we had previously observed [25]. OKM5+ cells were enriched by the panning technique as described and were tested in the ELR. OKM5+ UV-EC from all 4 individuals tested demonstrated that as few as 30–350 OKM5+ UV-EC per well were required to elicit significant proliferation in the allogeneic ELR (Fig 5).

Enhanced Soluble Antigen- and Mitogen-Presentation Capacity of EC Harvested 72 h after UV Exposure To determine the extent of similarities in function between blood OKM5+, OKM1− APC and EC obtained 72 h after UV irradiation, we determined whether UV-EC were capable of presenting soluble antigen and mitogen to autologous T cells. Optimal concentrations of tetanus toxoid (10 μg/ml) were added to cultures containing varying numbers of EC and autologous T cell-enriched responders. The autologous T cell-enriched responders themselves were depleted of antigen-presenting cells by adherence, leucine methyl ester exposure, and complement lysis of remaining Dr+, Leu M2+, OKM1+ cells. Background proliferation by APC-depleted, T cell-enriched responders plus tetanus toxoid antigen alone was <200 cpm [cpm (S + R)]. Specific proliferation (net cpm) induced by UV-EC (as APC) plus tetanus toxoid was significantly increased as compared with control EC (as APC) plus tetanus toxoid at both the 5,000 and 20,000 EC concentrations (Fig 6) (n = 3 individuals).

ConA at both suboptimal (2 μg/ml) and optimal (16 μg/ml) concentrations was added to cultures containing varying numbers of control EC or UV-EC (as APC) and 50,000 APC-depleted, T cell-enriched responders. Background proliferation by APC-depleted, T cell-enriched responders alone plus ConA was <1600 cpm [cpm (S + R)]. Marked increases in T-cell proliferation were observed when UV-EC were used as APC for ConA as compared with control EC (Table II).

UV-EC Induce Enhanced Autologous T-Cell Stimulation Since among blood OKM5+ cells, OKM1− OKM5+ cells are uniquely capable of stimulating autologous T cells to divide in the absence of antigen [11], we examined UV-EC containing OKM1− OKM5+ cells in this system. Forty thousand UV-EC mixed with 150,000 autologous APC-depleted T-cell responders were indeed capable of inducing marked proliferation whereas equal numbers of control EC from these individuals did not stimulate (Fig 7). Furthermore, 80,000 control EC also were not stimulatory in this system (data not shown). In most experiments, 5,000 UV-EC were not sufficient to stimulate substantial proliferation in the autologous ELR. The autologous ELR contributed little in antigen- and mitogen-containing cultures [cpm (EC + R)] since only 50,000 responders were used in those cultures, and

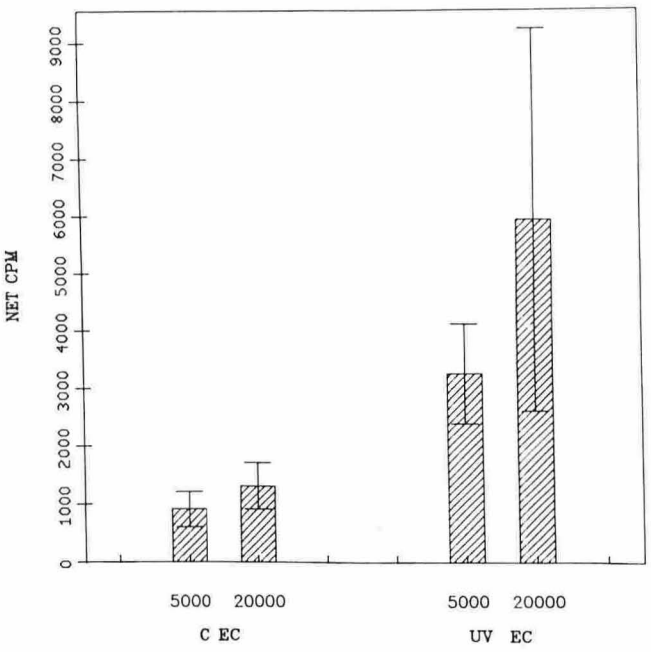

Figure 6. Enhanced presentation of tentanus toxoid to autologous T cells by UV-EC. Control (C) EC and UV-EC at 5,000 and 20,000 cells per well were mixed with 10 μg/ml tetanus toxoid and 50,000 autologous T cell-enriched, APCc-depleted responders for 7 days. Results expressed as specific proliferation due only to antigen presentation by EC in net cpm ± SEM tritiated thymidine uptake. Mean of 3 experiments from 3 individuals.

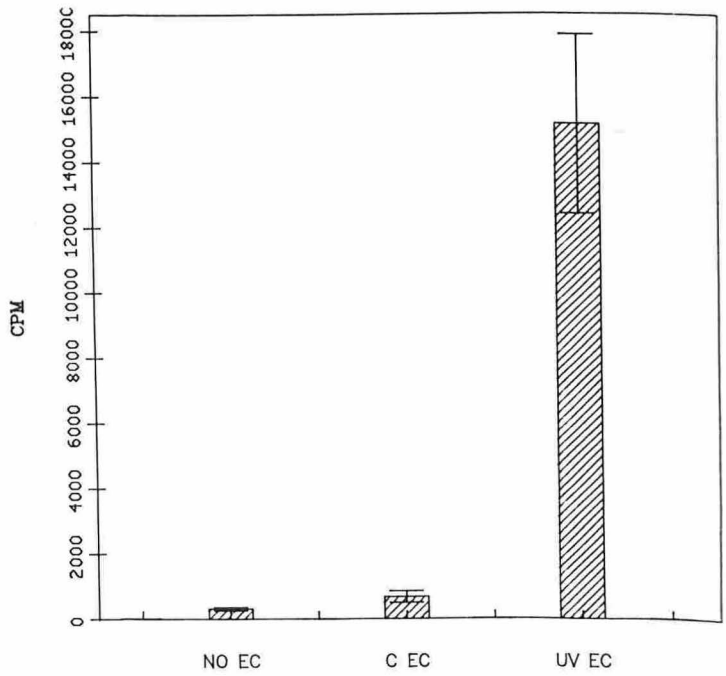

Figure 7. UV-EC induce autologous T-cell proliferation in the absence of added antigen. APC-depleted T cells (150,000) were cultured for 7 days either alone (no EC) or in the presence of 40,000 autologous control EC (C EC) or 40,000 UV-EC. Results expressed as cpm of tritiated thymidine uptake over the last 18 h of culture. Mean of 4 experiments from 4 individuals.

little autologous ELR proliferation occurred under these conditions.

Enriched OKM5$^+$ Cells Can Stimulate the Autologous ELR We next determined whether, among UV-EC, OKM5$^+$ EC could stimulate autologous T cells to proliferate in the absence of antigen. UV-EC were harvested, panning-enriched OKM5$^+$ UV-EC were prepared, mixed with 150,000 autologous APC-depleted T-cell responders, and cultured as above. Significant T-cell proliferation was induced by OKM5$^+$ UV-EC (Fig 8), whereas, as shown above, C-EC failed to induce T-cell proliferation.

DISCUSSION

In this report we provide phenotypic and functional evidence for the characterization of a second type of epidermal APC, distinct from LC, which appears in human epidermis following UV exposure. These UV-EC APC lack Birbeck granules, are OKM1$^-$, OKM5$^+$, OKT6$^-$, HLA-DQ$^+$ (DC/DS), HLA-DR$^+$, and differ functionally from LC in their phagocytic capacity and in their potent ability to activate autologous T cells in the absence of added antigen (autologous ELR). Thus, although within normal human epidermis APC are represented solely by a relatively homogeneous population of T6$^+$ LC, we have identified a type of APC which appears after UV irradiation and has a T-cell activation repertoire distinct from LC.

How do these T6$^-$ DR$^+$ melanophages in UV-irradiated epidermis differ from LC and how do both T6$^-$ Dr$^+$ UV-EC and LC relate phenotypically and functionally to human blood APC? Identification of monocyte APC subsets in human peripheral blood has been possible using monoclonal antibodies OKM1 and OKM5. OKM1 recognizes cell surface determinants of 160,000–170,000 daltons on the majority of monocytes and granulocytes as well as on null cells [36]. OKM5 recognizes an 88,000-dalton determinant which is more restricted on bone marrow-derived cells in that it is expressed only by monocytes and platelets [36]. Although OKM5 has been reported to bind to vascular cells of small vessels in many organs [37] it binds weakly at best to cutaneous vessels. The small fraction (5–10%) of non-T cells which are OKM1$^-$ OKM5$^+$ and surface membrane immunoglobulin negative have an enhanced capacity to function as stimulator cells in the autologous MLR [11], whereas the major monocyte population of OKM1$^+$ OKM5$^+$ cells does not exhibit this capacity. In contrast, both OKM1$^-$ OKM5$^+$ and OKM1$^+$ OKM5$^+$ cells have the ability to present soluble antigens, mitogens, and alloantigens to T cells [11]. T6$^-$ DR$^+$ UV-EC which appear in the epidermis 3 days after UV irradiation seem to be analogous to

Table II. Concanavalin A Presentation by Epidermal Cells to Autologous T Cells

	C-EC[a]			UV-EC[b]		
	5,000[c]	20,000	80,000	5,000	20,000	80,000
2 μg	<100	902 ± 236	4,784 ± 596	1,523 ± 207	5,353 ± 593	30,304 ± 625
16 μg	5,935[d] ± 3,524	20,968 ± 4,735	45,383 ± 8,610	18,639 ± 2,846	47,359 ± 3,478	54,805 ± 5,133

[a]C EC = EC harvested without any prior UV exposure.
[b]UV EC = EC harvested 72 h following UV exposure.
[c]Varying numbers of EC (5,000, 20,000, or 80,000) were mixed with 50,000 autologous T cells (depleted of accessory cells) in the presence of 2 μg/ml (2 individuals) or 16 μg/ml (3 individuals) ConA and the ensuing proliferation quantitated by [^{3}H]thymidine incorporation.
[d]Mean specific proliferation in net cpm ± SE.

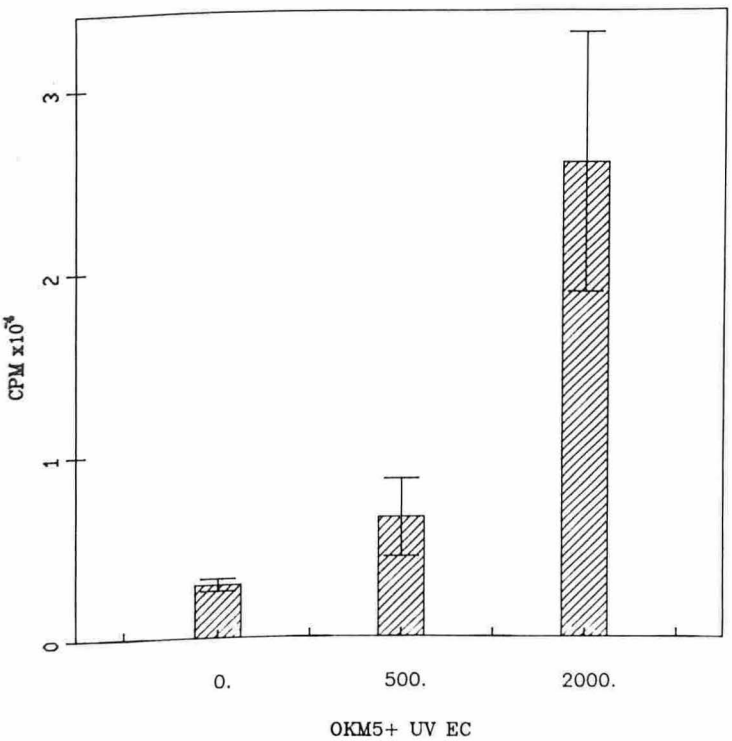

Figure 8. OKM5$^+$ UV-EC are stimulatory in the autologous ELR. Five hundred and 2,000 UV-EC panning enriched for OKM5$^+$ cells (36% purity) stimulate autologous T-cell proliferation in the absence of added antigen. Culture period = 7 days.

this specialized minor subpopulation of OKM1$^-$ OKM5$^+$ peripheral blood cells, both phenotypically and functionally. Both cell types present soluble antigens and mitogens, alloantigen, and, most importantly, self class II MHC antigens to T cells. In addition, both blood and UV-EC OKM1$^-$ OKM5$^+$ cells appear to be phagocytic.

Although both the OKM1$^+$ OKM5$^+$ APC population and LC have been proposed to be analogous to dendritic cells of the blood [11], OKM1$^-$ OKM5$^+$ cells differ from dendritic cells in their peroxidase reactivity, phagocytic capacity, and presence of lysosomes ultrastructurally [2,11,12] and LC differ in that they bear OKT6, FcIgG receptors, C3b receptors, and contain distinctive cytoplasmic Birbeck granules [2,4,12]. In this study, both OKM1$^-$ OKM5$^+$ DR$^+$ UV-EC and OKM1$^-$ OKM5$^-$ DR$^+$ UV-EC were clearly distinguishable from OKT6$^+$ LC in their melanophagic capacity or surface marker expression (Fig 1). Electron microscopy of panning-purified OKM5$^+$ EC demonstrated that OKM5$^+$ EC were identical to melanophages contained within DR$^+$ UV-EC which we have previously described [25].

An additional characteristic of monocytes which stimulate autologous T cells is the expression of HLA-DQ (DC/DS) [16]. The OKM5$^+$ cells which appeared in the UV-EC were also HLA-DQ positive and were the cells responsible for stimulation of autologous T cells in the absence of antigen.

In these studies and in numerous other experiments, unfractionated control EC demonstrated little stimulatory capacity for autologous T cells in the absence of added antigen (generally less than 1000 total cpm). This is consistent with other reports using human EC in culture with autologous T-cell responders [38]. By contrast, in the murine system, Aberer et al [39] demonstrated that BALB/c EC can function as stimulators of syngeneic T cells when serum from NZB mice was utilized in the culture, and that UV irradiation abrogated this capacity.

How might the T-cell response differ as a consequence of T-cell activation by autologous ELR stimulators rather than by LC which, in humans, are relatively poor stimulators in the autologous ELR? T cells that are activated in the autologous mixed leukocyte reaction (AMLR) are thought to be important in the T-cell regulation of other immune responses. This regulation appears to be part of a nonspecific suppressive immunoregulatory network involving the selectively greater proliferation of T cells that induce the development of suppressor-effector T cells [40]. Correspondingly, the predominant functional consequence of activation of T cells in an AMLR is the generation of noncytotoxic suppressor T cells which down-regulate B cells [13], cytotoxic T cells [14,15], and T-cell proliferation in response to mitogens and alloantigens [15,41]. Such regulatory T-cell activation by UV-EC may have relevance to the development of suppressor T-cell mechanisms after UV irradiation of skin.

The ability of EC harvested 72 h after UV irradiation to stimulate the autologous ELR, in contrast to control EC, suggests that UV induces the appearance in the epidermis of a select subpopulation of antigen-presenting cells which can activate T-cell populations distinct from those activated by LC. That melanophagic panning-purified OKM5$^+$ EC could stimulate in the autologous ELR shows that a cell population other than dendritic cells (which have poor phagocytic capacity) [2] can mediate this function. Substantial evidence for an analogy between epidermal OKM1$^-$ OKM5$^+$ T6$^-$ DR$^+$ UV-EC cells and the OKM1$^-$ OKM5$^+$ specialized subset of peripheral blood monocytes [11] is provided by the ability of OKM5$^+$ UV-EC to stimulate in the autologous ELR, their phagocytic ability, and their ability to present alloantigens, soluble antigens, and mitogens. It also raises the possibility that melanophages in certain inflammatory cutaneous disease states may function to activate T cells and regulate or otherwise participate actively in ongoing inflammatory processes.

REFERENCES

1. Rosenthal A, Shevach E: Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J Exp Med 138:1194–1212, 1973
2. Steinman R, Nussenzweig M: Dendritic cells: features and function. Immunol Rev 53:127–147, 1980
3. Van Voorheis W, Hain L, Steinman K, Kaplan G: Human dendritic cells. Enrichment and characterization from peripheral blood. J Exp Med 155:1172–1187, 1982
4. Stingl G, Tamaki K, Katz S: Origin and function of epidermal Langerhans cells. Immunol Rev 53:149–174, 1980
5. Ashwell J, DeFranco A, Paul W, Schwartz R: Antigen presentation by resting B cells. Radiosensitivity of antigen presentation function and two distinct pathways of T cell activation. J Exp Med 159:881–905, 1984
6. Hirschberg H, Evenson S, Henriksen T, Thorsby E: The human mixed lymphocyte-endothelial culture interaction. Transplantation 19:495–504, 1975
7. Ratcliffe M, Julius M, Kim K: Heterogeneity in the response of T cells to antigens presented by B lymphoma cells. Immunology 88:49–60, 1984
8. Shimada S, Katz S: TNP specific Ly-2$^+$ cytolytic T cell clones preferentially respond to TNP conjugated epidermal cells. J Immunol 135:1558–1563, 1985
9. Ramilla G, Erb P: Accessory cell dependent selection of specific T cell functions. Nature 304:442–445, 1983
10. Gershon R: Specialized antigen presenting cells. Splenic dendritic cells and peritoneal exudate cells induced by mycobacteria activate effector T cells that are resistant to suppression. J Exp Med 155:1344–1358, 1982
11. Shen H, Talle M, Goldstein G, Chess L: Functional subsets of human monocytes defined by monoclonal antibodies: a distinct subset of monocytes contains the cells capable of inducing the autologous mixed lymphocyte culture. J Immunol 130:698–705, 1983
12. Crow M, Kunkel H: Human dendritic cells. Major stimulators of the autologous and allogeneic mixed lymphocyte reactions. Clin Exp Immunol 49:338–346, 1982

13. James S, Yenokida G, Graeff A, Elson C, Strober W: Immunoregulatory function of T cells activated in the autologous mixed lymphocyte reaction. J Immunol 127:2605–2609, 1981
14. Sakane T, Green I: Specificity and suppressor function of human T cells responsive to autologous non T cells. J Immunol 123:584–589, 1979
15. Pazderka F, Angeles A, Kovithavongs T, Dossetor J: Induction of suppressor cells in autologous mixed lymphocyte culture (AMLC) in humans. Cell Immunol 75:122–133, 1983
16. Gonwa T, Picker L, Raff H, Gogert S, Silver J, Stobo J: Antigen presenting capacities of human monocytes correlate with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. J Immunol 130:706–711, 1983
17. Abramson S, Brown M, Puck J, Rich R: Antigen presentation to human T lymphocytes. II. Requirements for Mac-120$^+$ macrophages and responsiveness to IL-2. Cell Immunol 76:379–389, 1983
18. Fithian E, Kung P, Goldstein G, Rubenfeld M, Fenoglio C, Edelson R: Reactivity of Langerhans cells with hybridoma antibody. Proc Natl Acad Sci USA 78:2541–2544, 1981
19. Ptak W, Rozycha D, Askenase P, Gershon R: Role of antigen presenting cells in the development and persistence of contact hypersensitivity. J Exp Med 151:362–375, 1980
20. Tamaki K, Fujiwara H, Katz SI: The role of epidermal cells in the induction and suppression of contact sensitivity. J Invest Dermatol 76:275–278, 1981
21. Sullivan S, Bergstresser PR, Streilein JW: Intravenously injected, TNP-derivatized, Langerhans cell-enriched epidermal cells induce contact hypersensitivity in Syrian hamsters. J Invest Dermatol 84:249–252, 1985
22. Toews G, Bergstresser P, Streilein J, Sullivan S: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
23. Stingl G, Gazze-Stingl L, Aberer W, Wolff K: Antigen presentation by murine epidermal Langerhans cells and its alteration by UVB light. J Immunol 127:1707–1713, 1981
24. Sauder DN, Noonan FP, DeFabo EC, Katz SI: Ultraviolet radiation inhibits alloantigen presentation by epidermal cells: partial reversal by the soluble epidermal cell product, epidermal cell-derived thymocyte-activating factor (ETAF). J Invest Dermatol 80:485–489, 1983
25. Cooper K, Fox P, Neises G, Katz S: Effect of ultraviolet radiation on human epidermal cell alloantigen presentation: initial depression of Langerhans cell dependent function is followed by the appearance of T6$^-$ Dr$^+$ cells which enhance epidermal alloantigen presentation. J Immunol 134:129–137, 1984
26. Elmets C: *In vivo* low dose ultraviolet B (UV-B) irradiation induced T suppressor cells to contact sensitizing agents, The Effect of Ultraviolet Light on the Immune System. Johnson & Johnson, Inc., 1983, pp 317–333
27. Sauder D, Tamaki K, Moshell A, Fujiwara H, Katz S: Induction of tolerance to topically applied TNCB using TNP-conjugated ultraviolet light-irradiated epidermal cells. J Immunol 127:261–265, 1981
28. Fisher M, Kripke M: Further studies on the tumor-specific suppressor cells induced by ultraviolet radiation. J Immunol 121:1139–1144, 1978
29. Granstein R, Lowy A, Green M: Epidermal antigen-presenting cells in activation of suppression: identification of a new functional type of ultraviolet radiation resistant epidermal cell. J Immunol 132:563–565, 1984
30. Granstein R, Parrish J, McAuliffe D, Waltenbaugh C, Green M: Immunologic inhibition of ultraviolet radiation-induced tumor suppressor cell activity. Science 224:615–617, 1984
31. Lynch DH, Gurish MF, Daynes RA: The effects of high-dose UV exposure on murine Langerhans cell function at exposed and unexposed sites as assessed using in vivo and in vitro assays. J Invest Dermatol 81:336–341, 1983
32. Spangrude G, Banhard E, Ajioka R, Daynes R: Alterations in lymphocyte homing patterns within mice exposed to ultraviolet radiation. J Immunol 130:2974–2981, 1983
33. Czernielewski J, Vaigot P, Asselineau D, Prunières M: In vitro effect of UV radiation on immune function and membrane markers of human Langerhans cells. J Invest Dermatol 83:62–65, 1984
34. Kaplan A, Horakova Z, Katz S: Assessment of tissue fluid histamine in patients with urticaria. J Allergy Clin Immunol 61:350–354, 1978
35. Thiele D, Kurosaka M, Lipsky P: Phenotype of the accessory cell necessary for mitogen stimulated T and B cell responses in human peripheral blood: delineation by its sensitivity to the lysosomotropic agent, L-leucine methyl ester. J Immunol 131:2282–2290, 1983
36. Talle M, Rao P, Westberg E, Allegan N, Makowski M, Mittler R, Goldstein G: Patterns of antigenic expression on human monocytes as defined by monoclonal antibodies. Cell Immunol 78:83–99, 1983
37. Knowles DM II, Tolidjian B, Marboe C, D'Agati V, Grimes M, Chess L: Monoclonal anti-human monocyte antibodies OKM1 and OKM5 possess distinctive tissue distributions including differential reactivity with vascular endothelium. J Immunol 132:2170–2173, 1984
38. Braathen L, Kaamen T: Human epidermal Langerhans cells induce cellular immune response to trichophytin in dermatophytosis. Br J Dermatol 104:295–300, 1983
39. Aberer W, Stingl G, Stingl-Gazze L, Wolff K: Langerhans cells as stimulator cells in the murine primary epidermal cell-lymphocyte reaction: alteration by UV-B irradiation. J Invest Dermatol 79:129–135, 1982
40. Romain P, Morimoto C, Daley J, Palley L, Reinherz E, Schlossman S: Reactivity of inducer cell subsets and T8 cell activation during the human autologous mixed lymphocyte reaction. Clin Immunol Immunopathol 30:117–128, 1984
41. Innes J, Kuntz M, Kim Y, Weksler M: Induction of suppressor activity in the autologous mixed lymphocyte culture. J Clin Invest 64:1608–1613, 1979