Antigen-Presenting Activity of Non-Langerhans Epidermal Cells in Contact Hypersensitivity Reactions

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Baadsgaard, O., Lisby, S., Avnstorp, C., Clemmensen, O. & Lange-Vejlsgaard, G. Antigen-Presenting Activity of Non-Langerhans Epidermal Cells in Contact Hypersensitivity Reactions. *Scand. J. Immunol.* **32,** 217–224, 1990

Despite the critical role of the Langerhans cells in the induction of contact hypersensitivity reactions, non-Langerhans antigen-presenting cells in already sensitized individuals may play a role in the elicitation phase of a contact hypersensitivity reaction. Following epicutaneous challenge with antigens, the number of $CD1^+DR^+$ epidermal Langerhans cells increased in a time-dependent way and, concomitantly, $CD1^-OKM5^+DR^+$ epidermal non-Langerhans cells appeared. In parallel with this, the capacity of epidermal cells to present both alloantigens and auto/nominal antigens increased, and 4 days after initiation of the contact hypersensitivity reactions 33–53% of the epidermal antigen-presenting capacity was due to $CD1^-$ non-Langerhans antigen-presenting cells. Thus, contact hypersensitivity skin reactions are accompanied by the appearance of non-Langerhans antigen-presenting cells capable of presenting both alloantigens and auto/nominal antigens.

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In normal human epidermis the Langerhans cell is the only cell type capable of presenting antigens to T lymphocytes, thus eliciting T-cell activation [7, 8, 29]. Human epidermal Langerhans cells express CD1 [12] and major histocompatibility complex (MHC) class II molecules [16, 22]. The Langerhans cells are critical for the induction of delayed-type hypersensitivity reactions in mice [21, 31, 32]. Thus, sensitization through murine skin depleted of Langerhans cells results in the induction of antigen-specific tolerance rather than a contact hypersensitivity reaction [24, 30, 31, 32]. While the Langerhans cells are critical for the induction of the immune response in contact hypersensitivity reactions, non-Langerhans antigen-presenting cells may play a role in the effector phase of the immune response. This is supported by the findings in sensitized humans which demonstrate that irradiation of the skin with up to four minimal erythema doses (MED) of UVB,

a dose that normally efficiently abrogates the function of epidermal Langerhans cells, had no effect on subsequent challenge with contact allergens [27]. This could be the result of rapid recovery of functional Langerhans cell activity. However, since UVB irradiation induces the appearance of $CD1^-DR^+$ epidermal non-Langerhans cells [1–3, 5, 10, 11], this cell type may, in addition to the Langerhans cells, be capable of presenting antigens to primed T cells, eliciting T-cell activation. This observation prompted us to study the function and type of epidermal antigen-presenting cells present in the skin at various time points following initiation of allergic skin reactions. It was found that $CD1^-OKM5^+DR^+$ epidermal non-Langerhans antigen-presenting cells appear during hypersensitivity reactions and were in two experiments responsible for up to 33 and 53% of the total epidermal antigen-presenting capacity. Whether these cells function to up-

or down-regulate the immune function response is unknown.

MATERIALS AND METHODS

Patients. Nine patients with contact allergy verified by patch test participated in the study after giving informed consent. Each patient was challenged with serial dilutions of relevant hapten in order to determine a concentration of hapten which induced erthema and infiltration but not vesiculation (i.e. + to ++ reactions). The haptens used were primin, nickel sulphate, sodium dichromate, ethylene diaminedihydrochloride, yarrow, and clioquinol. Twelve-millimetre Finn chambers were used for patch testing. Four patches with haptens in petrolatum were applied on the volar forearm at least 5 cm apart, at 1, 3, 4, and 7 days prior to investigation. In addition, a chamber filled with petrolatum was used as vehicle control and applied for 48 h 4 days prior to the study. All patches were removed after 24 or 48 h.

Preparation of epidermal cell suspensions. Suction blister-derived epidermal cells were obtained from unchallenged areas and from sites challenged with vehicle (petrolatum) or hapten in petrolatum. Epidermal sheets were floated dermal side down on 0.25% trypsin (U.S. Biochemical Corp., Cleveland, Ohio, USA) in Dulbecco's phosphate-buffered saline (PBS) for 40 min at 37°C. Trypsinized epidermal sheets were transferred to 0.025% DNAse (Sigma, St Louis, Mo., USA) in Dulbecco's PBS, teased into a cell suspension, and were filtered through nylon gauze to achieve a single-cell suspension. Fetal bovine serum (FBS) was added to inactivate the trypsin, and the cells were washed three times in PBS containing 10% FBS. Epidermal cells were then resuspended in RPMI 1640 with added antibiotics, glutamine, and 10% human AB serum from non-transfused male donors. Cell viability (trypan blue exclusion) was monitored in haemocytometers and was more than 85%.

Epidermal cell-surface membrane staining. Cell suspensions were stained simultaneously with biotin-conjugated anti-HLA-DR (Becton Dickinson, Sunnyvale, Calif., USA) and either fluorescein isothiocyanate (FITC)-conjugated anti-HLe1 (CD45) (Becton Dickinson), OKT6 (CD1), OKM5 (Ortho Immunodiagnostics, Raritan, NJ, USA), or the appropriate isotype controls for 45 min at 4°C. The cells were then washed three times in Dulbecco's PBS containing NaN_3 and bovine serum albumin (BSA), and incubated with streptavidin-conjugated Texas red (Bethesda Research Labs, Bethesda, Md, USA) for 30 min at 4°C. The cells were washed, and propidium iodide was added to monitor viability. A total of 1000 cells was counted using a Zeiss microscope equipped with epifluorescence. The result was expressed as the percentage ± SEM of labelled viable cells (negative for propidium iodide) of total cell count. A three-step staining procedure was then applied, as previously described [21]. The cell suspensions were briefly incubated with unconjugated monoclonal antibody for 45 min at 4°C. A second incubation was performed using human serum-absorbed rhodamine-conjugated rabbit anti-mouse IgG (Dacopatts, Copenhagen, Denmark) diluted in PBS containing 10% vol/vol human AB serum. Finally, a third incubation was performed using normal mouse sera (in excess to occupy residual anti-IgG sites) and FITC-conjugated monoclonal antibody.

Depletion of leucocytes contained within the epidermal cell suspension. Leucocytes were depleted from the epidermal cell suspensions using magnetic beads coated with anti-mouse IgG (Dynal As, Skøyen, Norway). Epidermal cells pretreated with anti-HLA-DR, anti-Leu6 (Becton Dickinson), or a combination of the two antibodies were incubated for 30 min at 4°C, on a rotating rack; cells bound to beads were removed by exposure to a magnet, and the cell subsets remaining in suspension were harvested.

Preparation of peripheral blood mononuclear cells. Allogeneic or autologous peripheral blood mononuclear cells (MNC) were isolated from heparinized venous blood by Ficoll–Hypaque density-gradient centrifugation. MNC at the interface were collected, washed three times, and resuspended in RPMI 1640 with added antibiotics, glutamine, and 10% human AB serum.

Isolation of T cells. Human peripheral blood MNC were incubated on plastic for 1 h at 37°C. The macrophage-depleted non-plastic adherent MNC were then rosetted with 1% 2-aminoethyl-isothiouronium bromide hydrobromide-treated sheep red blood cells. A second Ficoll–Hypaque density-gradient centrifugation was performed to obtain non-rosetting B and null cells at the interface (E⁻) and rosetted T lymphocytes from the pellet (E⁺). The E⁺-cell fraction contained more than 95% T lymphocytes as determined by staining with fluoresceinated anti-Leu1 (CD5) (Becton Dickinson, Mountain View, Calif., USA). The T cells were further depleted of antigen-presenting cells by complement lysis of HLA-DR⁺ leucocytes.

Proliferative response of T lymphocytes to allogeneic or autologous epidermal cells. Allogeneic or autologous epidermal cell–lymphocyte in vitro reactions were performed in round-bottomed microtitre wells in 0.2 ml RPMI 1640 supplemented with 10% AB serum, glutamine, penicillin, and streptomycin. Various numbers of gamma-irradiated (2000 rad) epidermal cells were added to 50,000 or 100,000 responder allogeneic MNC or autologous T cells. The cultures were pulsed after 6 days with 1 μCi of [³H]TdR per well, harvested 18 h later on a Nunc cell harvester (Tecknunc, Roskilde, Denmark), and [³H] TdR incorporation was measured using a liquid scintillation counter.

Statistical analysis. Repeated-measures analyses of variance was used for statistical evaluation of the number of antigen-presenting cells. Student's *t*-test was used for evaluation of the [³H]TdR uptake by stimulated T cells.

RESULTS

Epidermal cells from contact hypersensitivity reactions contain increased numbers of CD1⁺DR⁺ non-Langerhans cells and CD1⁻OKM5⁺DR⁺ non-Langerhans cells

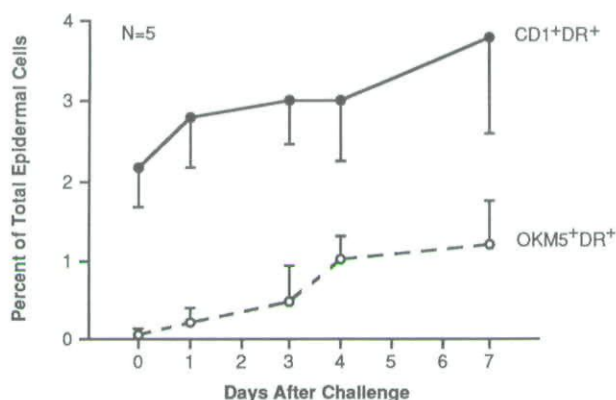Occlusion of the skin with petrolatum for 48 h induced the appearance of an increased number

FIG. 1. Five patients with verified allergic contact hypersensitivity were challenged with hapten 1, 3, 4, and 7 days prior to investigation. The number of $CD1^+DR^+$ epidermal Langerhans cells and the number of $OKM5^+DR^+$ epidermal non-Langerhans cells were determined from hapten-challenged sites and from a vehicle-(petrolatum) challenged site (0). In all five patients tested, the number of Langerhans cells increased in a time-dependent fashion, and concomitantly non-Langerhans $OKM5^+DR^+$ epidermal cells appeared. Results are expressed as mean values of five individuals $\pm$ SEM.

of $CD1^+DR^+$ Langerhans cells in the absence of detectable numbers of $CD1^-DR^+$ epidermal cells. Because of this immunostimulatory effect of the vehicle (petrolatum occlusion), all test results were compared with vehicle controls and not with unchallenged skin. Challenged skin from previously sensitized persons demonstrated an increased number of potential epidermal antigen-presenting cells in 5 out of 5 individuals tested ($n=5$) ($P \le 0.02$). Statistical significance was seen already at day 3. The number of $CD1^+DR^+$ Langerhans cells and $CD1^-DR^+/OKM5^+DR^+$ non-Langerhans epidermal cells was assessed in each individual following vehicle control and 1, 3, 4, and 7 days after challenge. The number of $CD1^+DR^+$ Langerhans cells increased in a time-dependent fashion in all individuals tested from day 1 (mean $2.2 \pm 0.06\%$, $n=5$) to a maximum at day 7 (mean $3.7 \pm 1.4\%$, $n=5$). Concomitantly, the number of $CD1^-DR^+/OKM5^+DR^+$ non-Langerhans epidermal cells also increased from the vehicle control baseline (mean $0\% + 0$, $n=5$) to a maximum at day 7 (mean $1.3 \pm 0.6\%$, $n=5$) (Fig. 1). The number of $CD1^-DR^+$ epidermal cells was equal to the number of $OKM5^+DR^+$ epidermal cells, and both the $OKM5^+$ epidermal cells and the DR^+ epidermal cells coexpressed HLe1, indicating that all $CD1^-DR^+/$

$OKM5^+DR^+$ epidermal cells were bone marrow-derived leucocytes and that no DR^+ or $OKM5^+$ keratinocytes were present. These data also indicate that $CD1^-DR^+$ epidermal cells coexpress OKM5. Furthermore, only a minority ($35 \pm 2\%$, $n=2$) of the $OKM5^+$ epidermal cells co-expressed OKM1, indicating that the majority of the $OKM5^+DR^+$ epidermal cells are $OKM1^-OKM5^+$.

Epidermal cells from contact hypersensitivity reactions demonstrate increased capacity to stimulate autologous T cells

In order to determine whether the increased number of Langerhans cells and non-Langerhans $CD1^-OKM5^+DR^+$ epidermal cells resulted in an enhanced capacity of epidermal cells to activate autologous T cells, epidermal cell lymphocyte reactions were performed. Increasing numbers of epidermal cells from a petrolatum-challenged area and from sites challenged with hapten 1, 3, 4, and 7 days before were added to antigen-presenting cell-depleted autologous T cells. At all epidermal cell concentrations tested from 10,000 to 160,000, primin- (0.01% in petrolatum) challenged epidermal cells demonstrated a time-dependent increase in the capacity to activate
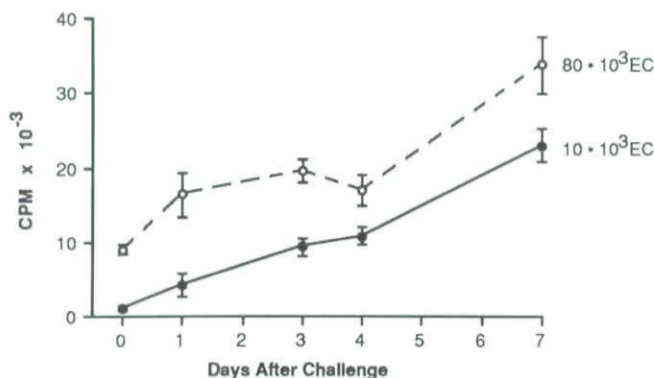
FIG. 2. Epidermal cells in suspension were obtained from petrolatum-challenged skin (0) and from skin challenged with hapten 1, 3, 4, and 7 days prior to investigation. One hundred thousand autologous T cells were stimulated with 10,000 (suboptimal number) and 80,000 (optimal number) gamma-irradiated epidermal cells, and the resulting T-cell proliferation was measured as [³H]TdR incorporation. Results are expressed as mean cpm ± SEM of triplicate wells from a representative experiment.

autologous T cells (Fig. 2.). Similar results were obtained in another patient using nickel sulphate. Results from the patient challenged with nickel sulphate on day 4 are shown in Fig. 5.

Contact hypersensitivity reactions result in an increased capacity of epidermal cells to activate allogeneic T cells

The increased capacity of hapten-challenged epidermal cells to activate autologous T cells could be the result of either an increased number of antigen-presenting cells or increasing presentation of processed hapten to the primed autologous T cells. To determine whether the challenged skin actually demonstrated an increased functional antigen-presenting capacity, allogeneic epidermal cell lymphocyte reactions were performed since this reaction is not affected by the presence of nominal antigen.

Hapten-challenged epidermal cells in 5 out of 5 individuals tested demonstrated enhanced capacity to activate allogeneic T cells relative to vehicle-challenged epidermal cells (Table I and Fig. 3). The mean percentage increases compared with vehicle-challenged control values at an epidermal cell concentration of 40,000 is shown in Table I ($n = 5$, $P \leq 0.05$, Student's t test). The increase was seen in all patients tested indepen-

TABLE I. The percentage increase in [³H]TdR uptake by allogeneic MNC stimulated with hapten-challenged skin relative to MNC stimulated with vehicle-challenged control skin 4 and 7 days after challenge in five different patients

Patient	Hapten	Day 4* (%)	Day 7 (%)
1	Yarrow	190 ± 8	168 ± 7
2	Ethylene diaminedihydrochloride	159 ± 8	172 ± 11
3	Clioquinol	121 ± 4	108 ± 8
4	Nickel sulphate	161 ± 20	ND
5	Sodium dichromate	113 ± 10	ND

* $P \leq 0.05$.
ND, not determined.

dent of the hapten used. However, the increase was less than when autologous T cells were used as responder cells. Furthermore, the allogeneic reaction peaked at day 3, whereas the autologous reaction was still increasing 7 days after hapten challenge.

Irrespective of these differences, hapten-challenged skin demonstrated increased capacity to present not only nominal and/or autoantigens but also alloantigens, and thus an increased function of antigen-presenting cells.
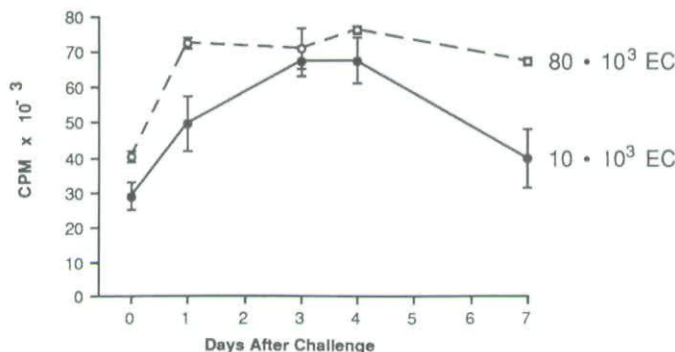
FIG. 3. Epidermal cells in suspension were obtained from petrolatum-challenged skin (0) and from skin challenged with hapten 1, 3, 4, and 7 days prior to investigation. Ten thousand (suboptimal number) and 80,000 (optimal number) gamma-irradiated epidermal cells were added to 50,000 allogeneic MNC, and the resulting T-cell proliferation was measured as [^{3}H]TdR incorporation. Results are expressed as mean cpm ± SEM of triplicate wells from a representative experiment.
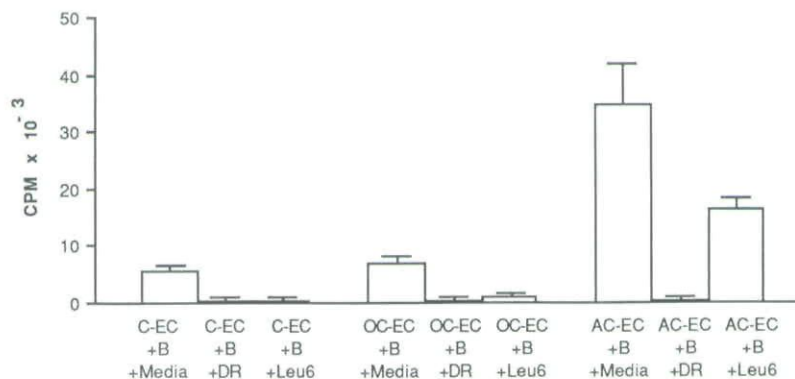

FIG. 4. Stimulation of 50,000 allogeneic MNC by 40,000 unchallenged epidermal cells (C-EC), vehicle-challenged epidermal cells (OC-EC), or hapten-challenged epidermal cells (AC-EC). The epidermal cells were treated with (1) magnetic beads + media alone (+ B + media); (2) anti-HLA-DR and magnetic beads (+ B + DR); and (3) Leu6 and magnetic beads (+ B + Leu6). Data are expressed as mean cpm ± SEM of triplicate wells from a representative experiment.

The capacity of allergen-challenged skin to activate T cells is dependent on both CD1$^+$DR$^+$ Langerhans cells and CD1$^-$DR$^+$ antigen-presenting cells

The discovery of the presence of non-Langerhans DR$^+$ epidermal cells in hypersensitivity reactions raises the possibility that non-Langerhans antigen-presenting cells may participate in the epidermal antigen presentation during an allergic skin reaction. To assess the functional antigen-presenting capacity of the non-Langerhans epidermal cells at day 4, we removed cells expressing CD1 (CD1$^+$DR$^+$ Langerhans cells) by incubation with Leu6 antibody plus Dynabeads, or cells expressing DR (CD1$^+$DR$^+$ Langerhans cells and CD1$^-$DR$^+$ non-Langerhans epidermal cells) by incubation with anti-HLA-DR plus Dynabeads. This decreased the number of CD1$^+$ or DR$^+$ epidermal cells to undetectable levels. Depletion of CD1$^+$(CD1$^+$DR$^+$ Langerhans cells) resulted in only partial abrogation of
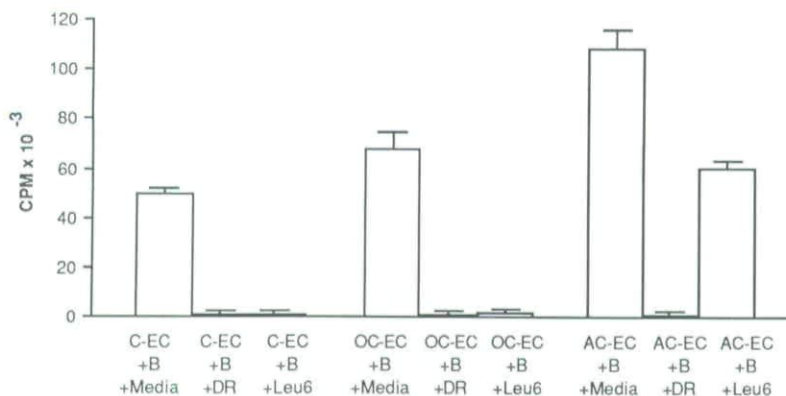
FIG. 5. Stimulation of 100,000 autologous T cells by 40,000 unchallenged epidermal cells (C-EC), vehicle-challenged epidermal cells (OC-EC), or hapten-challenged epidermal cells (AC-EC). The epidermal cells were treated with (1) magnetic beads (+B+media); (2) anti-HLA-DR and magnetic beads (+B+DR); and (3) Leu6 and magnetic beads (+B+Leu6). Data are expressed as mean cpm±SEM of triplicate wells from a representative experiment.

the epidermal antigen presentation capacity. In contrast, depletion of all DR$^+$ epidermal cells resulted in total abrogation of the epidermal alloantigen presentation capacity (AC-EC) (Fig. 4). This was not the result of incomplete removal of CD1$^+$ cells since depletion of CD1$^+$ cells contained within unchallenged (C-EC) or vehicle-challenged (OC-EC) epidermal cells totally abrogated the epidermal antigen-presenting function (Fig. 4). Similar results were obtained when autologous responder T cells were used, indicating that CD1$^-$DR$^+$ non-Langerhans antigen-presenting cells can present both alloantigens and autologous/nominal antigens (Fig. 5). In two experiments 33 and 53% of the total antigen-presenting cell activity of 40,000 epidermal cells was provided by non-Langerhans antigen-presenting cells (Fig. 4).

DISCUSSION

The Langerhans cell is the only known antigen-presenting cell in normal human epidermis, and its role as an antigen-processing cell in the induction of positive immune responses to antigen introduced through the skin is well established [14, 21, 31, 32]. However, the epidermal cellular sources for antigen presentation in the induction phase of allergic contact dermatitis reactions are less clear. Irradiation of sensitized individuals in vivo with a single dose of UVB

radiation sufficient to abrograte the function of Langerhans cells did not change the immune reactivity of the skin to subsequent challenge with antigen [27]. This could be explained by rapid recovery of epidermal Langerhans cells following the irradiation. However, normally more than 1 week is needed for recovery of epidermal Langerhans cells following in vivo UVB exposure [10]. Therefore, although the Langerhans cell is the critical antigen-presenting cell in the sensitization phase of the contact hypersensitivity response, epidermal non-Langerhans antigen-presenting cells could, in addition to Langerhans cells, play a role in the induction phase. In accordance with others we found an increased number of epidermal Langerhans cells following the contact hypersensitivity reactions [9, 13]. However, in addition to the increased number of Langerhans cells, a non-Langerhans antigen-presenting cell subtype appeared. These cells were distinct from the Langerhans cells in that they were smaller, non-dendritic, and they lacked the CD1 determinant and expressed the blood monocyte antigen defined by the monoclonal antibody OKM5. The majority of the OKM5$^+$ epidermal cells lacked expression of OKM1 and therefore are analogous to a minor subpopulation (<5%) of OKM1$^-$OKM5$^+$ blood monocytes which are uniquely capable of activating autoreactive immunoregulatory T cells [25] which may be important for down-regulation of immune responses [15, 20, 23, 28]. All CD1$^-$OKM5$^+$DR$^+$/

OKM5+DR+ epidermal cells expressed the common leucocyte antigen, CD45, demonstrating their bone marrow origin. Keratinocytes in allergic skin reactions can express both HLA-DR and OKM5 [19, 26, 33]. These molecules are probably induced by gamma interferon and other lymphokines released from local activated T cells [6]. We did not detect any HLe1⁻DR+ or HLe1⁻OKM5+ epidermal cells. This is probably because of the weak hypersensitivity reaction induced and the consequently minimal T-cell infiltration and lymphokine release.

Concomitantly with the increase in number of CD1+DR+ Langerhans cells and CD1⁻DR+ epidermal cells there was an increase in the antigen-presenting capacity of hapten-challenged epidermal cells. This enhanced capacity could result from either the increased number of Langerhans cells, the increased number of non-Langerhans cell DR+ epidermal cells, or both. In order to determine the relative contribution of the two different types of DR+ epidermal cells, CD1+ or DR+ epidermal cells were depleted from the stimulator population prior to culture with T cells. Depletion of CD1+ cells (CD1+DR+ Langerhans cells) from unchallenged or petrolatum-challenged epidermal cells totally abrogated the antigen-presenting cell function. In contrast, depletion of autologous CD1+ epidermal cells obtained 4 days after challenge with hapten only decreased the antigen-presenting cell function by 77 and 47% in two different patients. Depletion of DR+ epidermal cells (CD1+DR+ Langerhans cells and CD1⁻DR+ epidermal cells) totally abrogated T-cell activation. Thus, at day 4, 33 or 53% of the capacity of epidermal cells to activate T cells may be provided by non-Langerhans antigen-presenting cells.

The function of the heterogeneous population of CD1⁻DR+ non-Langerhans antigen-presenting cells in contact hypersensitivity skin reactions and irritant reactions [18] is unknown. They may function to enhance the ongoing immune response by presenting nominal antigen to primed T cells. However, they may also be analogous to the CD1⁻OKM5+DR+ antigen-presenting cell subtype that appears in human skin in response to certain wavelengths of UV irradiation [1, 2, 3, 5, 10, 11]. This UV-induced antigen-presenting cell population activates suppressor T-cell pathways and may function to down-regulate immune responses [2, 4]. Thus, the non-Langerhans antigen-presenting cells may represent a negative feedback mechanism down-regulating the inflammatory reaction in contact dermatitis.

Whether these non-Langerhans cells belong to the monocyte or Langerhans cell lineage is unknown. In cutaneous T-cell lymphoma CD1+OKM5+DR+ non-Langerhans cells are present in the epidermis. However, these cells lack Birbeck granules [17]. Thus, the CD1⁻OKM5+ epidermal cells may be precursors to Langerhans cells and in the epidermal environment they may lose their OKM5 expression and gain CD1 and Birbeck granules. However, they may also be distinct from the Langerhans cell lineage.

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