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Phenotype, Ultrastructure, and Function of $CD1^+DR^+$ Epidermal Cells that Express CD36 (OKM5) in Cutaneous T-Cell Lymphoma

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This study investigated the phenotype and function of different antigen-presenting cells (APC) present within the epidermis of patients with cutaneous T-cell lymphoma (CTCL). Involved epidermis of CTCL compared with uninvolved was found to contain increased numbers of $CD1^+DR^+$ APC. This population was heterogeneous and comprised both leucocytes of a novel $CD1^+DR^+CD36$ $(OKM5)^+$ phenotype and $CD1^+DR^+CD36^-$ indeterminate/Langerhans cells. The $CD1^+DR^+CD36^+$ leucocytes did not express TcR-1, CD5, CD15, or CD22, and only a minor population expressed CD11, demonstrating that they were neither T nor B cells, and did not belong to the major $CD11^+$ ($OKM1^+$) blood monocyte population. Electron microscopy of purified $CD36^+$ lesional epidermal cells (EC) demonstrated that they lacked Birbeck granules found on $CD1^+$-selected Langerhans cells, and most cells exhibited features of indeterminate cells or macrophages.

The capacity of EC from involved epidermis to present alloantigens was found to be increased relative to uninvolved epidermis in all patients tested, and this capacity was critically dependent upon the presence of $CD45^+DR^+$ bone marrow-derived cells but not on the presence of $CD45^-DR^+$ keratinocytes. Positive selection using MoAb against CD1 and CD36 demonstrated that both cell populations exhibited the capacity to stimulate T cells. The results indicate that a novel antigen-presenting cell population with a unique phenotype is present within involved skin of patients with mycosis fungoides. These cells express CD36 in addition to CD1 and have an ultrastructural appearance consistent with a dendritic antigen-presenting cell derivation.

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Mycosis fungoides (MF) is an uncommon disease contained within the broad spectrum of cutaneous T-cell lymphoma (CTCL). In CTCL, the malignant cells comprise mainly mature, activated T cells characterized by a high affinity for the skin, particularly the epidermis [13, 14]. The infiltrating T cells usually express the CD4 determinant on their surface and function as helper T cells [7, 24].

Antigen-specific activation of T cells is dependent upon the presence of antigen-presenting cells (APC) that express class II MHC antigens such as HLA-DR (DR). Within normal skin, this cell population is almost entirely composed of indeterminate cells and Langerhans cells (LC) [10, 20, 21, 34].

However, in the epidermis of CTCL patients the presence of an increased number of DR^+ cells has been reported. In these patients, the DR^+ epidermal cell (EC) population is heterogeneous and is comprised of both an increased number of $CD1^+DR^+$ LC or indeterminate cells and DR^+ keratinocytes [1, 2, 25, 35, 36].

The presence of DR^+ keratinocytes is also reported both in vitro and in other lymphoproliferative skin diseases associated with local infil-

tration of activated T cells [6, 18], and, although DR^+ keratinocytes have been speculated to be involved in the development and maintenance of the lymphotropic infiltrates, their actual function is still unknown [30]. Furthermore, OKM5 expression in skin has been reported. The increased number of epidermal LC has been proposed to play a role in the initial activation of the malignant T cells in CTCL, but additional populations of epidermal APC may also be involved [8].

Recently, we have demonstrated the presence of $CD36^+DR^+$ epidermal leucocytes within involved skin of CTCL patients. The $CD36^+DR^+$ leucocytes found in CTCL, if analogous to the $CD36^+DR^+$ leucocytes induced by UV irradiation, could represent a population of specialized APC [3, 11, 12, 31]. The aim of this study was phenotypically and ultrastructurally to characterize the potential APC populations contained within involved epidermal cell (IN-EC) suspensions from CTCL patients. In addition we asked which of the various IN-EC subpopulations could function as antigen-presenting cells, and we compared this with EC from clinically uninvolved skin (UN-EC) from the same patients.

MATERIALS AND METHODS

Patients. Nine patients with clinical CTCL (either histologically non-diagnostic or histologically diagnostic mycosis fungoides plaque stage) participated in the study. The histological diagnosis of CTCL was based on the presence of a pleomorphic dermal cellular infiltrate with epidermotropism as either single-cell exocytosis or Pautrier microabscesses. The cellular infiltrate comprised atypical mononuclear cells (MNC) with more or less pronounced indented nuclei. All patients were untreated for at least 2 months prior to investigation.

Epidermal cell suspensions. Suction blisters were raised from both involved and uninvolved skin of five patients and from involved skin of another four patients. In each patient, the suction blisters were obtained from sun-protected skin areas. Both involved and uninvolved skin samples were obtained from the same body region, symmetrically or as close as possible. Suction blister tops were trypsinized as previously described [25], and teased into a cell suspension. Following filtering through a nylon gauze, the cells were washed and resuspended in RPMI 1640 with added antibiotics, glutamine, and 10% heat-inactivated human AB serum (Sigma, St Louis, Mo., USA).

Isolation of T lymphocytes. Peripheral blood mononuclear cells were obtained by Ficoll–Hypaque density gradients as previously described [26] and were divided into adherent monocytes and non-adherent MNC by adherence to plastic. The monocyte-depleted MNC were then rosetted with 1% 2-(aminoethyl)-isothiouronium bromide hydrobromide-treated sheep red blood cells (SRBC). A second Ficoll–Hypaque density gradient centrifugation was performed to obtain non-rosetting B and null cells at the interface and rosetted T cells from the pellet. The T cells were then treated with anti-HLA-DR plus complement (C) for further purification and to eliminate activated T cells.

Antibodies. The specificity and source of the monoclonal antibodies (MoAb) used in the study are listed in Table I. As isotype controls, we used human glial fibrillary acidic protein (GFAP) (IgG1), amyloid A (IgG2a, Dakopatts, Copenhagen, Denmark), and anti-CD22 (IgG2b), Becton Dickinson, Sunnyvale, Calif., USA).

Polyclonal antibodies included rhodamine-conjugate goat anti-mouse IgG (human serum absorbed, Dakopatts) and rhodamine-conjugated sheep anti-mouse IgG1, IgG2a, and IgG2b (Serotec, Oxford, UK). Streptavidin conjugated with Texas red was obtained from Bethesda Research Laboratories, Bethesda, Md, USA.

TABLE I. Monoclonal antibodies used in this study

MoAb	Isotype	Source	Specificity
OKT6 anti-Leu6 (CD1)	IgG1 Ig2a	Ortho BD	Mv 49,000, distributed on LC/indeterminate cells in the epidermis
OKM1 (CD11)	IgG2b	Ortho	Monocyte antigen (C3bi receptor)
OKM5 (CD36)	IgG1	Ortho	Monocyte and platelet antigen, found on keratinocytes in diseased skin
Anti-Leu1 (CD5)	IgG2a	BD	Pan-T-cell marker
Anti-TcR1	IgG1	BD	Human T-cell antigen receptor α/β heterodimer
T4 (CD4)	IgG1	Coulter‡	T helper/inducer cell marker
Anti-Leu14 (CD22)	IgG2b	BD	Pan-B-cell marker
Anti-HLA-DR	IgG2a	BD	Class II MHC antigens
Anti-HLe1 (CD45)	IgG1	BD	Antigen expressed on cells originating from the bone marrow

* Ortho Immunodiagnostics, Raritan, NJ, USA.
† Becton Dickinson, Sunnyvale, Calif., USA.
‡ Coulter Immunology, Hialeah, Fla, USA.

Staining procedures. Double staining was performed using two methods, as previously described [26]. Briefly, the cells were incubated simultaneously with biotin-conjugated anti-HLA-DR or anti-CD4 (T4) and fluorescein isothyocyanate (FITC)-conjugated anti-CD1, anti-CD36 or anti-CD45 for 45 min at 4°C. Finally, the cells were washed and incubated with streptavidin-conjugated Texas red for 30 min at 4°C.

Another series of stainings was performed by incubating the cell populations with unconjugated MoAb of either IgG1, IgG2a, or IgG2b isotype for 45 min at 4°C, washing, and incubating with rhodamine-conjugated sheep anti-mouse IgG1, IgG2a, or IgG2b for 45 min at 4°C. Following extensive washing, a third incubation was performed using normal mouse sera (in excess to occupy residual anti-Ig sites) and FITC-conjugated MoAb for 45 min at 4°C. The following double stainings were performed: anti-CD1 (Leu 6), anti-CD36; anti-CD1(Leu 6), anti-CD45; anti-CD36, anti-CD45; anti-CD36, anti-CD5; anti-CD36, anti-TcR1; anti-CD22, anti-CD36; and anti-CD11, anti-CD36. Controls included omitting the primary MoAb or using MoAb of the appropriate isotype but with irrelevant specificities. Furthermore, the efficiency of the blocking procedure was measured using FITC-conjugated mouse IgG or FITC-conjugated MoAb of the appropriate isotype but with irrelevant specificity.

Electron microscopy of immunomagnetically separated EC subpopulations. The cells were resuspended in Hanks' balanced salt solution (HBSS) containing 5% normal goat serum (NGS), incubated with either anti-CD36 or anti-CD1 (Leu6) antibody at 4°C for 45 min. After incubation, the cells were washed twice in HBSS containing 1% NGS and mixed with Dynabeads (Dynabeads M-450, Dynal AS, Oslo, Norway) at a concentration of 4×10^6 Dynabeads per 10^6 cells. After incubation, the beads were drawn to the side of the tube using a magnet, and rinsed twice with HBSS containing 1% NGS. After removing the rinsing solutions, the beads were drawn to the bottom of the microfuge tube and centrifuged to obtain a workable pellet.

The pellets consisting of immunomagnetic beads and cells were fixed with 4% glutaraldehyde (Polysciences EM grade) in 1 M cacodylate buffer (pH 7.4–7.6). The pellets were rinsed in 1 M cacodylate and post-fixed in 1% OsO_4 in 1 M cacodylate buffer with 5% sucrose added. The pellets were then dehydrated in a series of ethanol, infiltrated in propylene oxide and Epon, and embedded in Epon. Ultrathin sections were cut and stained with lead citrate and uranyl acetate and examined under a Hitachi HU-11C electron microscope.

Allogeneic epidermal cell lymphocyte reaction (ALLO-ELR). Stimulator EC suspensions were X-irradiated with 2000 rad using a ^{137}Cs source, and the EC were added in varying numbers per well to 50,000 allogeneic responder MNC. The cultures were incubated in 96-well round-bottomed culture plates for 7 days in humidified air containing 5% CO_2. One μCi of tritiated marked thymidine ([^{3}H]TdR, Amersham International, UK) was added on day 6, and the cultures were terminated 18 h later. Following cell harvesting, the [^{3}H]TdR uptake was measured using a scintillation counter. The results are expressed as mean cpm ± SEM.

Rabbit complement-dependent lysis of epidermal cell subpopulations and T cells. EC suspensions or T cells were resuspended in phosphate-buffered saline (PBS) containing 2% fetal calf serum (FCS) and incubated with anti-DR MoAb for 45 min at 4°C. After washing, the cells were incubated with C (Pel-Freez, WI, UQ) in a non-toxic concentration for 35 min at room temperature (EC) or for 45 min at 37°C (T cells). Following extensive washing, the cells were resuspended in RPMI containing 10% AB serum. Controls included incubation with MoAb of the appropriate isotype but with irrelevant specificity followed by C lysis. These methods led to less than 0.1% residual positive EC and a purity of the T cells of >95%.

Magnetic separation of EC subpopulations. EC suspensions were resuspended in PBS containing 2% FCS and incubated with either anti-DR, anti-CD45, anti-CD1, or anti-CD36 MoAb for 45 min at 4°C. After washing, the cells were incubated with Dynabeads M-450 sheep anti-mouse IgG at a concentration of 0.1×10^6 beads per 1×10^6 EC at 4°C. Positive marked cell populations were then separated from unmarked cell populations by applying a magnet on the outer wall of the test tubes. The positively selected EC were washed and added in various numbers from 50 to 800 cells per well to 50,000 accessory cell-depleted allogeneic T cells. The purity of the positively selected cells was scored as follows. Using isotype control MoAb or omitting the MoAb, no binding of the beads to cell surfaces was observed, and the cells obtained this way did not exhibit any function in our assays. Using light microscopy, >90% of the positively selected cells were bound to immunomagnetic beads. The negatively selected cells were obtained as follows. The supernatants were obtained from the test tubes and a second incubation with Dynabeads was performed, using 1×10^6 beads per 1×10^6 EC. The supernatants were obtained, washed, and used as the negatively selected EC populations. This led to a purity of less than 0.1% residual positive cells.

RESULTS

CD36 (OKM5)⁺ EC in cutaneous T-cell lymphoma skin co-express the LC marker CD1

As we have previously demonstrated [25], IN-EC from CTCL patients, in contrast to UN-EC, contained CD36⁺ cells. Using double-fluorescence staining, the CD36⁺ EC population was found to be heterogeneous and contained CD36⁺CD45 (HLe1)⁻ keratinocytes, although the majority (68–80%) was comprised of CD36⁺CD45⁺ cells, indicating a bone marrow origin (Table II). In addition, co-expression of CD36 and DR was almost total, but CD36⁺DR⁻ cells, with the light microscopic appearance of medium-sized keratinocytes, were present. The majority of CD36⁺ EC lacked receptors recognized by the MoAb OKM1 (CD11), and no co-

TABLE II. Phenotypic markers on $CD36^+$ epidermal cells*

Phenotype		Staining	Range (%)
Anti-HLA-DR		+	
OKM5	(CD36)	+	
OKT6/anti-Leu6	(CD1)	+/−	31–78
Anti-TCR1†		−‡	
Anti-Leu1	(CD5)	−	
T4†	(CD4)	−	
Anti-Leu14	(CD22)	−	
OKM1	(CD11)	+/−	13–56
Anti-HLe1	(CD45)	+/−	68–80

* Data obtained from patients with MF plaque stage ($n=5$).

† ($n=2$).

‡ Less than 5% of the $CD36^+$ E C co-expressed this marker.

expression was found using MoAb against TcR-1, CD4, CD5, or CD22 (Table II). Using the MoAb anti-Leu6 (CD1), which in normal skin binds only to indeterminate cells and Langerhans cells [16, 27], we found co-expression of CD1 on 31–78% of the $CD36^+$ cells (Table II).

$CD36^+$ epidermal cells from clinically involved skin lack Birbeck granules of Langerhans cells as determined by electron microscopy

We next determined the ultrastructural appearance of $CD36^+$ potential antigen-presenting leucocytes within IN-EC of CTCL. EC were incubated with anti-CD36 or anti-CD1 and mixed with magnetic beads coated with goat anti-mouse IgG and the positively selected palette was fixed and examined under a transmission electron microscope. No cells were observed to contain Birbeck granules characteristic of LC. Forty-four per cent of the $CD36^+$ cells were classified as

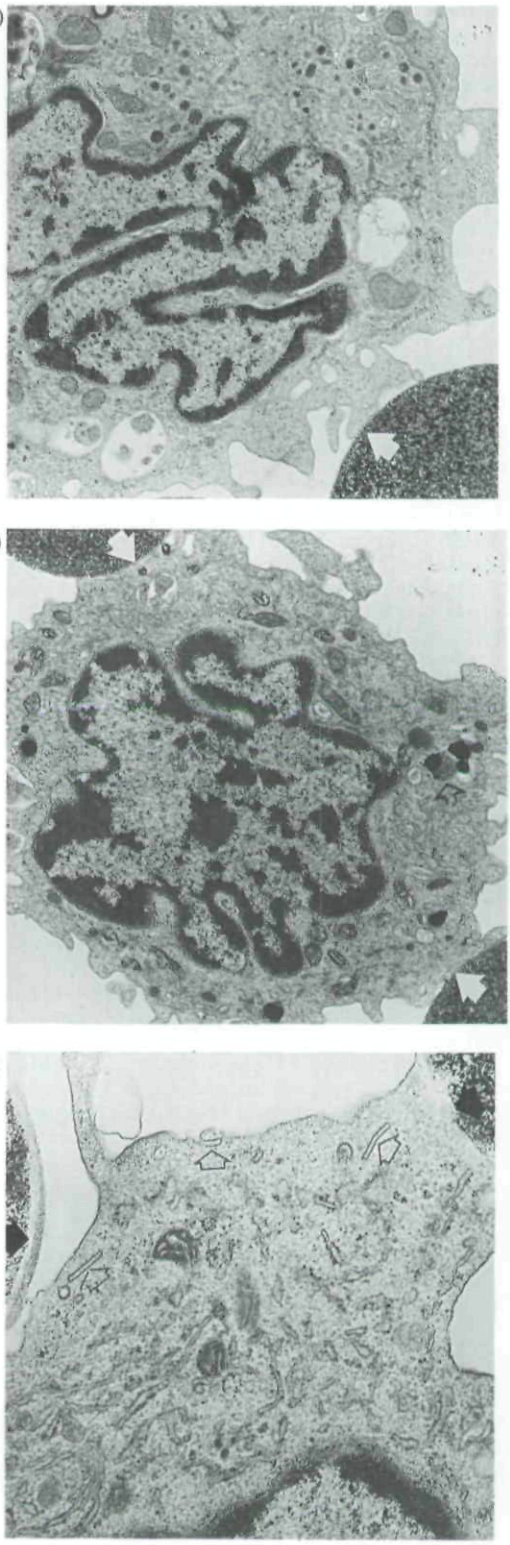

FIG. 1. (a) Epidermal cell in suspension from CTCL-involved epidermis coated with anti-CD36 (OKM5) and selected onto immunomagnetic beads coated with goat anti-mouse IgG. Closed arrow indicates immunomagnetic beads (×13,340). (b) Epidermal cell in suspension from CTCL-involved epidermis coated with anti-CD36 (OKM5) and selected via adherence to immunomagnetic beads (closed arrows) coated with goat anti-mouse IgG. Open arrow indicates melanophagocytic phagolysosome (×11,020). (c) Epidermal cell in suspension from CTCL-involved epidermis incubated with anti-Leu6 (CD1) followed by absorption onto immunomagnetic beads (closed arrows) coated with goat anti-mouse IgG. Birbeck's granules are denoted by open arrows (×11,020).

indeterminate cells with abundant cytoplasm, numerous mitochondria and microsomes, and relatively dense intermediate filaments (Fig. 1a). Fifty per cent of the CD36⁺ cells appeared to have some macrophage characteristics, mainly the presence of phagolysosomes. These cells generally had a less dense intermediate filament pattern and fewer microsomes (Fig. 1b). Interestingly, CD36⁺ cells with abundant cytoplasm were also observed to contain indented nuclei (Fig. 1a and b). In addition, 6% of the CD36⁺ cells were identified as keratinocytes, as identified by keratin tonofilaments. In contrast, no labelling was seen when using the isotype MoAb. Furthermore, only 21% of cells selected on the basis of anti-Leu6 (CD1) expression contained Birbeck's granules in their cytoplasm (Fig. 1c). Fifty seven per cent of the CD1⁺ cells were classified as indeterminate cells, and 21% contained phagolysosomes in their cytoplasm, with a similar appearance to the cell pictured in Fig. 1(b). Thus, even at the ultrastructural level, many CD1⁺ cells in CTCL epidermis have acquired the characteristics of macrophages.

Involved epidermal cells demonstrate enhanced allostimulatory capacity compared with uninvolved epidermal cells

Because of an increase in number of DR⁺ EC contained within CTCL plaques, we investigated whether the functional antigen-presenting capacity of IN-EC was increased compared with UN-EC. For this purpose we prepared ALLO-ELR, in which varying numbers of EC were incubated with 50,000 allogeneic responder MNC and the resulting T-cell proliferation was measured as [³H]TdR incorporation. Indeed, in all patients ($n=5$) the IN-EC were more potent stimulators than UN-EC (Fig. 2, $P<0.05$ by paired-sample Student t-test).

TABLE III. Depletion of MHC class II-positive EC using anti-HLA-DR followed by rabbit complement*

Undepleted EC†	DR-depleted EC	
IN-EC		
80000	77016 ± 1299	350 ± 51
20000	58336 ± 5537	423 ± 193
5000	40589 ± 789	133 ± 8
1250	3324 ± 413	155 ± 22
UN-EC		
80000	45015 ± 1845	331 ± 20
20000	42560 ± 144	158 ± 17
5000	15143 ± 2065	112 ± 20
1250	2781 ± 386	151 ± 27

* Data expressed as mean cpm ± SEM from one representative experiment.

† Cells treated with anti-GFAP followed by C lysis.

Depletion of DR⁺ epidermal cells abolishes the allogeneic epidermal cell lymphocyte reaction using epidermal cells from both clinically uninvolved and involved skin

We next wished to determine whether the

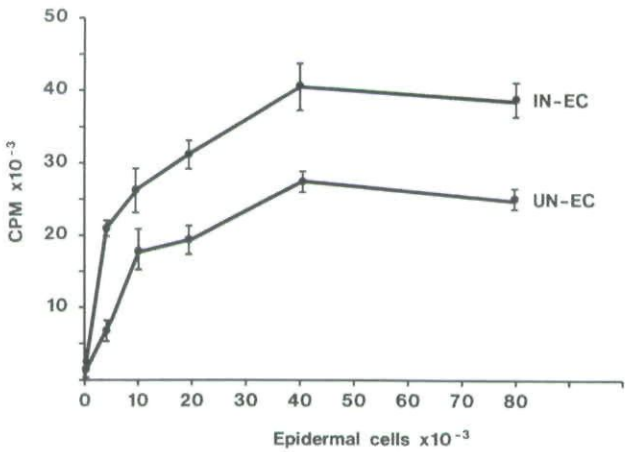

FIG. 2. Stimulation of 50,000 allogeneic MNC by varying numbers of involved (IN) EC or uninvolved (UN) EC. Data are expressed as mean cpm ± SEM from a representative experiment.

increased APC capacity of IN-EC was confined to DR$^+$ EC. In order to obtain this objective, both UN- and IN-EC suspensions were treated using anti-DR MoAb or a MoAb of the appropriate isotype but with irrelevant specificity, followed by C lysis. The EC were then incubated in increasing numbers from 1250 to 80,000 cells with 50,000 allogeneic MNC. The APC capacity of both IN-EC and UN-EC was totally abrogated using anti-DR MoAb (Table III).

The allostimulatory capacity of involved epidermal cells is dependent on CD45$^+$ DR$^+$ leucocytes and not DR$^+$ keratinocytes

We next addressed whether the CD36$^+$DR$^+$ EC or the CD45$^-$DR$^+$ keratinocytes contributed to the increased ALLO-ELR using IN-EC from MF patients. IN-EC were incubated either with MoAb against DR, CD1, CD45, or CD36 or with MoAb of the same isotype but with irrelevant specificities, followed by incubation with Dynabeads and magnetic depletion. EC populations magnetically depleted of DR$^+$, CD1$^+$, or CD45$^+$ cells were not able to induce any T-cell proliferation. In contrast, depletion of CD36$^+$ EC only slightly decreased the ALLO-ELR (Fig. 3).

Isolated CD36$^+$ EC do exhibit antigen-presenting capacity

To verify the functional capacity of different potential APC subsets in the skin, EC were positively selected using either anti-CD36 or anti-CD1 followed by incubation with Dynabeads. These cells were used as stimulators of accessory cell-depleted allogeneic T cells. Unselected EC suspensions (or cells obtained by incubation with isotype control MoAb) at doses from 50 to 800 cells produced only background levels of prolifer-

TABLE IV. Capacity of positively selected epidermal cell populations to stimulate allogeneic T cells*

Number of epidermal cells	EC†	CD1$^+$ EC‡	CD36$^+$ EC‡
800	1075 ± 1005	111,993 ± 7700	27,190 ± 8107
200	52 ± 12	67,993 ± 2148	20,733 ± 1115
100	72 ± 22	26,144 ± 2810	3,048 ± 1898
50	50 ± 5	16,628 ± 6684	2,220 ± 886
0	88 ± 2§		

* Undepleted EC.
† Positively selected CD1$^+$ or CD36$^+$ EC.
‡ Data obtained by culturing T cells alone.
§ Results expressed as mean cpm ± SEM of triplicate cultures.

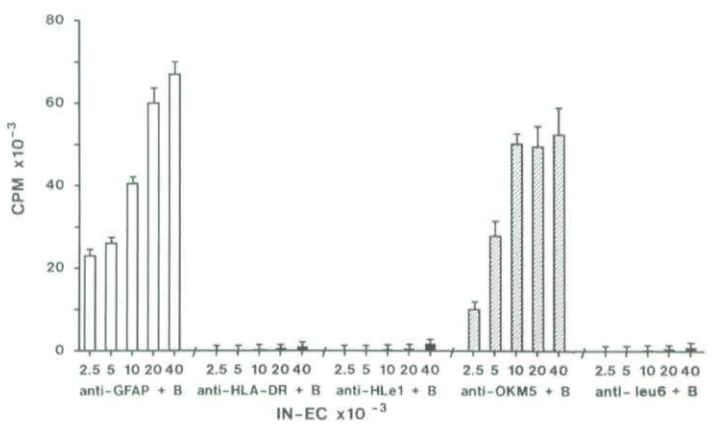

FIG. 3. Depletion of specific epidermal cell subsets. Open bars, IN-EC incubated with isotype MoAb; solid bars, IN-EC incubated with anti-HLA-DR, anti-Leu6 (CD1), or anti-HLe1 (CD45); striped bars, IN-EC incubated with OKM5 (CD36). Following incubation with the MoAb, the positively stained cell populations were removed by incubation with Dynabeads (B) and magnetic depletion. Data reveal the capacity of various numbers of EC populations to stimulate 50,000 allogeneic MNC, and are expressed as mean cpm ± SEM from a representative experiment.

ation. In contrast, both CD1⁺ LC and CD36⁺ EC potently stimulated allogeneic T cells (Table IV), demonstrating that, similar to CD1⁺ LC, the CD36⁺ potential APC were capable of presenting alloantigens.

DISCUSSION

Activation of T cells via a T-cell receptor mechanism normally required class II-positive APC, either blood monocytes or APC within the epidermis [15, 19]. Normal human epidermis contains only CD1⁺DR⁺ LC as the APC population, but under pathological conditions the epidermis may contain different potential APC populations [25]. For instance following UV irradiation, DR⁺ leucocytes are demonstrated within the epidermis [3, 4, 5, 11, 12], and those cells which lack CD1 but express CD36 could be analogous to a minor subset of blood monocytes expressing the CD11⁻ CD36⁺ phenotype [32]. Both epidermal and peripheral blood CD11⁻CD36⁺ leucocytes have the capacity to present alloantigens and to stimulate autologous T cells in both the presence and absence of added mitogens or antigens [4, 12, 32].

We recently described the presence of CD36⁺DR⁺ leucocytes in lesional epidermis of patients with MF, suggesting that alternative APC may exist in MF lesions [25]. We now demonstrate that, similar to UV-induced CD36⁺DR⁺ leucocytes, MF epidermal CD36⁺DR⁺ cells express a CD45⁺, TcR1⁻, CD4⁻, CD5⁻, and CD22⁻ phenotype. However, in contrast to UV-exposed skin, 31–78% of the CD36⁺ EC co-express CD1 molecules, suggesting that these cells are closely related to LC. The electron microscopic appearance of the CD36⁺ EC subset reveals that they lack Birbeck granules, and most of the cells exhibit the ultrastructural feature of cells intermediate between monocytes and LC, and can be termed indeterminate cells of the epidermis. Interestingly, following a bone marrow transplantation, the repopulating epidermal dendritic cells were also found to be CD1⁺DR⁺ and to co-express markers for monocytes/macrophages [28, 29].

CD36⁺CD1⁺ EC may represent a unique cell population or immature LC migrating to the skin in response to an ongoing inflammatory neoplastic process within the involved epidermis, or these cells may represent mature LC that have been modified by malignant T-cell lymphokines to express CD36 and lose Birbeck granules.

Because of the increased number of class II-positive cells in IN-EC from CTCL patients [25, 35], we investigated the antigen-presenting capacity of the EC. In all patients tested, IN-EC were more potent than UN-EC in stimulating allogeneic T-cell proliferation (Fig. 2).

We next investigated whether the increased ALLO-ELR using IN-EC was due to the increased number of DR⁺ APC or due to other cell populations contained within involved skin. Using the C-fixing MoAb anti-HLA-DR followed by C lysis, both IN- and UN-EC suspensions were depleted of DR⁺ cells, which encompasses indeterminate cells, LC and in involved skin also DR⁺ keratinocytes and virtually all the CD36⁺ EC. Both the IN- and UN-EC were unable to stimulate allogeneic MNC (Table III), indicating that the MHC class II-positive cells are essential in eliciting immune responses in skin, which is in agreement with previous reports using normal skin [11, 23].

It is likely, however, that although the CD1⁺DR⁺ EC exhibit strong APC activity other DR⁺ APC populations may contribute to the increased ALLO-ELR using IN-EC. We therefore determined whether the CD36⁺ EC or the DR⁺ keratinocytes contained within the skin of CTCL plaques were critical for the observed ALLO-APC function. The IN-EC populations were treated with anti-CD36, anti-CD1, anti-CD45, or anti-DR followed by depletion of positively stained EC subpopulations using immunomagnetic beads. The EC were then used as stimulators in ALLO-ELR. DR⁻ EC populations were again unable to stimulate T cells. Interestingly, removal of CD45⁺ epidermal leucocytes also abrogated the ALLO-ELR, indicating that the increased ALLO-ELR using IN-EC is totally dependent upon CD45⁺DR⁺ leucocytes and that the CD45⁻DR⁺ keratinocytes could not activate T cells directly (Fig. 3). In fact, DR⁺ kereatinocytes may actually provide a down-regulating signal if contact occurs with unprimed T cells [17]. Removal of CD1⁺ EC totally abrogated the APC activity of the skin, demonstrating the important role of the LC in T- cell activation.

Finally, removal of the CD36⁺ cells from the EC suspensions did not abrogate the ALLO-ELR. This could be due to the fact that OKM5 (anti-CD36) is a weak antigen, and thus the positive cells become more difficult to remove

from the cell suspension. Furthermore, in contrast to mixed lymphocyte reactions which express a linear correlation between cell numbers and T-cell proliferation, ALLO-ELR result in a plauteau range in which virtually no changes occur in the T-cell response to increased or decreased numbers of antigen-presenting cells (Fig. 2) [3, 11]. Thus, even after removal of the majority of $CD36^+$ EC the remaining $CD1^+CD36^-$ LC still produce high T-cell proliferation.

Because of this, we obtained positively selected $CD36^+$ EC, and demonstrated that these cells indeed exhibit APC activity (Table IV).

It could be argued that the $CD36^+$ EC population was contaminated by $CD1^+CD36^-$ LC, which in part could explain the APC activity seen in this cell population. However, this is highly unlikely because the majority (>90%) of the positively selected cells were bound to beads, the absence of MoAb or the use of isotype control MoAb did not result in specific binding of cells, and the very few cells obtained this way did not exhibit any functional capacity in our systems. Furthermore, the findings that no cells in the $CD36^+$ EC population exhibited Birbeck granules, as revealed by electron microscopy, supports these arguments. Taken together, the findings of APC activity of $CD36^+$ cells and no APC activity using $CD1^-$ EC populations indicate that the $CD36^+$ cells containing the APC activity are confined to the $CD1^+CD36^+$ subset. That $CD36^+$ cells in CTCL epidermis contain indented nuclei analogous to the nuclei of CTCL T cells suggest overlapping features of APC and T cells in CTCL, as previously suggested [9]. However, $CD36^+$ cells did not co-express CD4, CD5, or TcR-1, ruling out the possibility that these are indeed T cells. In conclusion, a novel $CD1^+CD36^+$ antigen-presenting cell which is both phenotypically and ultrastructurally distinct from the LC is present in the epidermis of patients with CTCL.

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REFERENCES

1 Aiba, S. & Tagami, H. HLA-DR antigen expression on the keratinocyte surface in dermatoses characterized by lymphocytic exocytosis (e.g. pityriasis rosea). *Br. J. Dermatol.* **111,** 285, 1984.

2 Aubock J., Romani, N., Grubauer, G. & Fritsh, P. HLA-DR expression on keratinocytes is a common feature of diseased skin. *Br. J. Dermatol.* **114,** 465, 1986.

3 Baadsgaard, O., Fox, D.A. & Cooper, K.D. Human epidermal cells from ultraviolet light-exposed skin preferentially activate autoreactive $CD4^+2H^+$ suppressor-inducer lymphocytes and $CD8^+$ suppressor–cytotoxic T lymphocytes. *J. Immunol.* **140,** 1738, 1988.

4 Baadsgaard, O., Lisby, S., Wantzin, G.L., Wulf, H.C. & Cooper, K.D. Rapid recovery of Langerhans cell alloreactivity, without induction of autoreactivity, after in vivo ultraviolet A, but not ultraviolet B exposure of human skin. *J. Immunol.* **142,** 4213, 1989.

5 Baadsgaard, O., Wulf, H.C., Wantzin, G.L. & Cooper, K.D. UVB and UVC, but not UVA, potently induce the appearance of $T6^-DR^+$ antigen-presenting cells in human epidermis. *J. Invest. Dermatol.* **89,** 113, 1987.

6 Basham, T.Y., Nickoloff, B.J., Merigan, T.C. & Morhenn, V.B. Recombinant gamma interferon induces HLA-DR expression on cultured human keratinocytes. *J. Invest. Dermatol.* **83,** 88, 1984.

7 Berger, C.L., Warburton, D., Raafaat, J., LoGerfo, P. & Edelson, R.L. Cutaneous T cell lymphoma: Neoplasm of T cells with helper activity. *Blood* **53,** 642, 1979.

8 Berti, E., Cavicchini, S., Cusini, M., Monti, M., Parolini, F. & Caputo, R. Heterogeneity of dermal $OKT6^+$ cells in inflammatory and neoplastic skin diseases. *J. Am. Acad. Dermatol.* **12,** 507, 1985.

9 Braverman, I.M. & Kupper, T.S. Both the epidermal dendritic population and the majority of epidermotropic cells in cutaneous T cell lymphoma (CTCL) bear both CD1 and T cell markers. *J. Invest. Dermatol.* **90,** 549, 1988.

10 Cooper, K.D., Breathnach, S.M., Caughman, S.W., Palini, A.G., Waxdal, M.L. & Katz, S.I. Fluorescence microscopic and flow cytometric analysis of bone marrow-derived cells in human epidermis. *J. Invest. Dermatol.* **85,** 546, 1985.

11 Cooper, K.D., Fox, P., Neises, G. & Katz, S.I. 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 that enhance epidermal alloantigen presentation. *J. Immunol.* **134,** 129, 1985.

12 Cooper, K.D., Neises, G.R. & Katz, S.I. Antigen-presenting $OKM5^+$ melanophages appear in human epidermis after ultraviolet radiation. *J. Invest. Dermatol.* **86,** 363, 1986.

13 Edelson, R.L. Cutaneous T cell lymphoma. P. 195 in Moschella (ed) *Dermatology Update New York.* Elsevier, Amsterdam, 1979.

14 Edelson, R.L. Cutaneous T-cell lymphoma: Myco-

sis fungoides, Sezary syndrome, and other variants. *J. Am. Acad. Dermatol.* **2,** 89, 1980.

15 Faure, M., Dezutter-Dambuyant, C., Schmitt, D., Gaucherand, M. & Thivolet, J. Human epidermal cell-induced generation of alloreactive cytotoxic T-lymphocyte responses against epidermal cells. *Scand. J. Immunol.* **21,** 441, 1985.

16 Fithian, E., Kung, P., Goldstein, G., Rubenfeld, M., Fenoglio, C. & Edelson, R.L. Reactivity of Langerhans cells with hybridoma antibody. *Proc. Natl. Acad. Sci. USA* **78,** 2541, 1981.

17 Gaspari, A.A., Jenkins, M.K. & Katz, S.I. Class II MHC-bearing keratinocytes induce antigen-specific unresponsiveness in hapten-specific TH1 clones. *J. Immunol.* **141,** 2216, 1988.

18 Gawkrodger, D.J., Carr, M.M., McVittie, E., Guy, K. & Hunter, J.A. Keratinocyte expression of MHC class II antigens in allergic sensitization and challenge reactions and in irritant contact dermatitis. *J. Invest. Dermatol.* **88,** 11, 1987.

19 Germain, R.N. Accessory cell stimulation of T-cell proliferation requires active antigen processing, Ia restricted antigen presentation, and a 2nd signal. *J. Immunol.* **127,** 1964, 1981.

20 Gothelf, Y., Hanau, D., Tsur, H., Sharon, N., Sahar, E, Cazenave, J.P. & Gazit, E. T6 positive cells in the peripheral blood of burn patients: Are they Langerhans cells precursors? *J. Invest. Dermatol.* **90,** 142, 1988.

21 Harrist, T.J., Muhlbauer, J.E., Murphy, G.F., Mihm Jr, M.C. & Bahn, A.K. T6 is superior to Ia (HLA-DR) as a marker for Langerhans cells and indeterminate cells in normal epidermis. *J. Invest. Dermatol.* **80,** 100, 1983.

22 Hunyadi, J. & Simon Jr M. Expression of OKM5 antigen on human keratinocytes in vitro upon stimulation with gamma-interferon. *Acta. Derm. Venereol. (Stockh.)* **66,** 527, 1986.

23 Katz, S.I., Cooper, K.D., Iijima, M. & Tsuchida, T. The role of Langerhans cells in antigen presentation. *J. Invest. Dermatol.* **85,** 96, 1985.

24 Kung, P.C., Berger, C.L., Goldstein, G., LoGerfo, P. & Edelson, R.L. Cutaneous T cell lymphoma: Characterization by monoclonal antibodies. *Blood* **57,** 261, 1981.

25 Lisby, S., Baadsgaard, O., Cooper, K.D., Thomsen, K. & Wantzin, G.L. Expression of OKM5 antigen on epidermal cells in mycosis fungoides plaque stage. *J. Invest. Dermatol.* **90,** 716, 1988.

26 Lisby, S., Baadsgaard, O., Cooper, K.D. & Vejlsgaard, G.L. Decreased number and function of antigen-presenting cells in the skin following application of irritant agents. *J. Invest. Dermatol.* **92,** 842, 1989.

27 Murphy, G.F., Bahn, A.K., Harrist, T.J. & Mihm Jr, M.C. In situ identification of T6 positive cells in normal human dermis by immunoelectron microscopy. *Br. J. Dermatol.* **108,** 423, 1983.

28 Murphy, G.F., Merot, Y.M., Tong, A.K.F., Smith, B. & Mihm, M.C. Depletion and repopulation of epidermal dendritic cells after allogeneic bone marrow transplantation in humans. *J. Invest. Dermatol.* **84,** 210, 1985.

29 Murphy, G.F., Messadi, D., Fonferko, E. & Hancock, W.W. Phenotypic transformation of macrophages to Langerhans cells in the skin. *Am. J. Pathol.* **123,** 401, 1986.

30 Nickoloff, B.J., Basham, T.Y., Merigan, T.C. & Morhenn, V.B. Keratinocyte class II histocompatibility antigen expression. *Br. J. Dermatol.* **112,** 373, 1985.

31 Phipps, R.P., Pillai, P.S. & Scott, D.W. Conversion of a tolerogenic to an immunogenic signal by P388AD.2 cells, a lymphoid dendritic cell-like tumor line. *J. Immunol.* **132,** 2273, 1984.

32 Shen, H.H., Talle, M.A., 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, 1983.

33 Simon Jr, M. & Hunyadi, J. Expression of OKM5 antigen on human keratinocytes in positive intracutaneous tests for delayed-type hypersensitivity. *Dermatologica* **175,** 121, 1987.

34 Tamaki, K., Stingl, G., Gullino, M., Sachs, D.H. & Katz, S.I. Ia antigens in mouse skin are predominantly expressed on Langerhans cells. *J. Immunol.* **123,** 784, 1979.

35 Tjernlund, U.M. Epidermal expression of HLA-DR antigens in mycosis fungoides. *Arch. Dermatol. Res.* **261,** 81, 1978.

36 Volc-Platzer, B., Groh, V. & Wolff, K. Differential expression of class II alloantigens by keratinocytes in disease. *J. Invest. Dermatol.* **89,** 64, 1987.

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