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HLA-DR-positive leucocyte subpopulations in human skin include dendritic cells, macrophages, and CD7-negative T cells

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SUMMARY

The immunophenotypes of the HLA-DR-positive leucocyte populations in normal human skin were studied using an extensive panel of monoclonal antibodies, which included antibodies from the Third International Leucocyte Differentiation Antigen Workshop (3rd LDAW). Langerhans' cells (LC) in the epidermis stained with antibodies from CD15c, Groups 10, 12a, 12b and 15, of the myeloid panel and from CD39 of the B-cell panel. However, LC did not react with CD14 antibodies or 63D3, which are frequently used to stain tissue macrophages. In addition to epidermal LC (26 cells/linear mm) a significant population of $CD1_a$-positive cells was identified in the papillary dermis (7 cells/linear mm of overlying epidermis). The dermal HLA-DR-positive leucocytes consisted of three cell populations. The most numerous cell type stained with antibodies to monocytes/macrophages. There were fewer, though substantial, numbers of T lymphocytes (mainly CD7-negative) and the least numerous was the population of $CD1_a$-positive cells. The $CD1_a$-positive cells and the population of dermal cells that stain with monocyte/macrophage markers are both potential antigen-presenting cells for the skin-associated immune system.

INTRODUCTION

Immune reactions are initiated by cells which present antigen in association with class II MHC products to appropriate recognition structures on T lymphocytes. Lymphoid dendritic cells (Steinman & Inaba, 1986), Langerhans' cells (LC) (Silberberg-Sinakin, Baer & Thorbecke 1978) and some cells of the monocyte–macrophage series (Werdelin, 1986) have all been shown to have antigen-presenting capacity. The main antigen-presenting cells studied in human skin have been identified as the $CD1_a$, HLA-DR- and ATPase-positive LC within the epidermis. The phenotypes of the leucocyte populations in the dermis have not been studied in detail and the relationship of the epidermal LC to the HLA-DR-positive leucocytes in the dermis (Daar *et al.*, 1983; Hogg *et al.*, 1986) is not clear. In this report we describe an extensive immunohistological analysis of the leucocytes present in human skin with particular emphasis on the cell membrane phenotype of the HLA-DR-positive cell populations.

MATERIALS AND METHODS

Tissue samples

Specimens of skin from the trunks of 10 human cadavers were obtained at autopsy. The individuals were aged 26–79 years old and the cause of death was either trauma or ischaemic heart disease. Five samples of skin were obtained from abdominal lipectomies or reduction mastectomies. The procedures were approved by the Christchurch Hospital Ethical Committee. The tissue was immediately snap-frozen in liquid nitrogen and stored at $-80°$ until required.

Antibodies

The monoclonal antibodies used and their sources are listed in Tables 1–4. The myeloid panel was obtained as a result of the laboratory's participation in the 3rd Leucocyte Differentiation Antigen Workshop (3rd LDAW) at which a preliminary report of some of these results was presented (McKenzie *et al.*, 1987). The clusters of differentiation (CD groups) and unclustered myeloid groups are as detailed in the workshop proceedings (McMichael *et al.*, 1987) and summarized by Boyd (1987). The antibodies were used at optimal dilutions established on appropriate control cell populations. CMRF-23 is an additional CD45 reagent that detects a non-restricted epitope on the leucocyte common molecule distinct from that recognized by CMRF-12 and 26 (Starling *et al.*, 1987).

Immunoperoxidase staining

Cryostat sections of 6 μm were placed on gelatin-coated slides, fixed for 10 min in cold acetone and kept at 4° for 1 hr to ensure complete evaporation of acetone prior to incubating the section with monoclonal antibody for 30 min. The sections were washed three times with PBS and incubated with affinity column-

Abbreviations: CD, cluster of differentiation; LC, Langerhans' cell; 3rd LDAW, Third Leucocyte Differentiation Antigen Workshop.

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Table 1. Reactivity of non-lineage specific antibodies with LC and dermal HLA-DR-positive cells

Cluster of differentiation specificity	Antibody name	Reactivity LC	Reactivity dermal DR-positive-cells	Reacting with other structures	Source/reference
HLA-DR+DQ	CMRF-20	+	Many cells	End*	This laboratory
HLA-DP	—	+	Many cells	End	BD‡
HLA-DQ	—	+	Many cells	End, weak	BD
HLA-DR	—	+	Many cells	End, weak	BD
HLA-DQ-associated	RFD1	+	Many cells	—	Poulter *et al.* (1986)
HLA-ABC	CMRF-6	+	Many cells	Ep†	This laboratory
CD11a	MHM24	—	Subset, mainly lymphocytes	—	3rd LDAW
CD11b	44,MO1	—	Few macrophages, weak	—	3rd LDAW
CD11c	3·9	—	Subset macrophages	—	3rd LDAW
CD18	MHM23	—	Lymphocytes and macrophages	—	3rd LDAW
CD45	CMRF-12, CMRF-23	+	Many cells	—	This laboratory
CD45R	CMRF-11	—	—	—	This laboratory
Factor VIII-related antigen	21-43-B7-07	—	—	End	Dr D. E. Joshua (Royal Prince Alfred Hospital, Sydney)

* End, endothelium
† Ep, epidermis
‡ BD, Becton-Dickinson.

purified $F(ab')_2$ rabbit anti-mouse $F(ab')_2$ (RAM) (prepared in this laboratory) at 25 μg/ml, blocked with 10% human $F(ab')_2$ and then 10% AB serum in PBS each for 15 min. After washing three times with PBS, the sections were incubated with peroxidase-conjugated goat anti-rabbit IgG (Tago Inc., Burlingame, CA) for 30 min, washed and developed with 0·08% 3′-3′ diaminobenzidine, 0·02% H_2O_2 in Tris-HCl, pH 7·4, for 8 min before counterstaining with haematoxylin or toluidine blue.

Immunofluorescence

Sections were not fixed but were treated as above except that, after the RAM incubation, FITC-conjugated affinity column-purified $F(ab')_2$ sheep anti-rabbit $F(ab')_2$ prepared in this laboratory was used. In some cases FITC-conjugated goat anti-mouse IgG (Tago) was used followed by FITC-conjugated swine anti-goat IgG (Tago). Sections were mounted in PPD-glycerol to preserve the fluorochrome. Double-labelling immunofluorescence involved additional incubations with phycoerythrin (PE)-conjugated anti-HLA-DR (Becton-Dickinson, Mountain View, CA) or biotin-conjugated monoclonal antibodies in the presence of an excess irrelevant monoclonal antibody followed by appropriate washing in PBS. In the latter instance a further incubation with PE-conjugated streptavidin (Becton-Dickinson) was used. Control sections indicated no cross-reactivity of the second reagent (PE) with the primary antibody sandwich (FITC). In some instances double labelling was performed by photographing before and after sequential complementary fluorescence staining. A Leitz Ortholux microscope with a L3 filter block was used with a Leitz Vario Orthomat photographic system (Wetzlar, FRG). $Cd1_a$-positive cell counts were performed on 15 sections using the photographic grid to define sequential transverse fields. Numbers of Leu 1-, OKT8- and 63D3-positive cells were counted in four samples of skin in a manner similar to that described by Bos *et al.* (1987), except that single 3·5–6·5-μm sections were used instead of two serial sections. Staining reactions were classified as positive or negative (Tables 1–4) and comments on intensity of staining are included in the text.

RESULTS

Bone marrow-derived cells in the dermis and epidermis were identified by a positive reaction with the CD45 antibodies, CMRF-12 and 23, which react with different unrestricted epitopes of the leucocyte common molecule. LC were identified in the epidermis, and dermal leucocytes were found mainly in a perivascular and periadnexal distribution, as well as scattered throughout the dermis, being more numerous in the papillary dermis and decreasing in numbers progressively in the reticular dermis. Most of the leucocytes were shown to be HLA-DR-positive on double labelling (Fig. 1). Only a few HLA-DR-negative dermal leucocytes were identified. The majority of these appeared to be mast cells as defined by toluidine blue staining. The anti-class II reagents (Table 1) stained the endothelium of blood vessels in addition to the bone marrow-derived cells (Fig. 1) and the identification of endothelial cells by anti-factor VIII staining aided interpretation of the class II MHC-positive structures (Fig. 1).

Epidermal $CD1_a$-positive cells

LC in the epidermis were identified readily by staining with the $CD1_a$ reagent, NA1/34. Virtually all of these cells in the epidermis were HLA-DR-positive but some of the LC were HLA-DP- and HLA-DQ-negative on double labelling with anti-HLA-DR. Some LC stained weakly with RFD1, an

Table 2. Reactivity of T- and B-cell antibodies with LC and dermal HLA-DR-positive cells

Cluster of differentiation specificity	Antibody name	Reactivity LC	Reactivity dermal HLA-DR-positive-cells	Reacting with other structures	Source/reference
$CD1_a$	NA1/34	+	Subset upper dermis	—	McMichael *et al.* (1979)
CD2	OKT 11	—	T lymphocytes	—	ATCC* (Rockville, MD)
CD3	OKT 3	—	T lymphocytes		ATCC
	Leu 4	—	T lymphocytes	Ep† Ecc‡, N§	BD¶
CD4	Leu 3a+3b	+	T lymphocytes and macrophages	—	BD
CD5	Leu 1	—	T lymphocytes	—	BD
CD7	4H9/Leu 9	—	Few T lymphocytes, weak	—	3rd LDAW
CD8	OKT 8	—	Subset T lymphocytes	—	ATCC
T-cell subset	Leu 8	—	—	Ep, Ecc	BD
CD19	HD37, 93-1B3	—	—	—	3rd LDAW
CD21	B2,THB5	—	—	—	3rd LDAW
CD37	BL14	—	—	—	3rd LDAW
CD39	G28-10,	+	Many cells	End**	3rd LDAW
	G28-8	+	Many cells	End	3rd LDAW
CDw40	G28-5	—	—	—	3rd LDAW
Restricted B+IDC††	2-7	—	Some cells mainly perivascular	Ep, End	3rd LDAW
Restricted B+IDC	7F7	—	Some cells mainly perivascular	End	3rd LDAW
NK cells	HNK1	—	Occasional/rare cells	N	ATCC
NK cells	NKH1	—	Occasional/rare cells	N	Coulter (Hialeah, FL)

* ACC, American Type Culture Collection.
† Ep, epidermis.
‡ Ecc, Eccrine duct.
§ N, nerve.
¶ BD, Becton-Dickinson.
** End, endothelium.
†† IDC, interdigitating cell.

antibody to an HLA-DQ-associated antigen. The epidermis contained 26 ± 6 (SD) LC/mm (range = 16–39 cells/mm, $n = 14$) and a significant population, 7 ± 4 (SD) cells/mm (range = 2–15 cells/mm, $n = 14$), of $CD1_a$-positive cells was noted in the upper dermis (Fig. 2).

Several of the monoclonal antibodies to leucocytes listed in Tables 1, 2 and 3 stained the $CD1_a$-positive LC. In each case results were confirmed by double-labelling studies using anti-HLA-DR. Most antibodies from the T-cell panel (Table 2) were non-reactive except for Leu 3a+3b (CD4), which stained some of the LC weakly (Fig. 2). Antibodies from the established B-cell clusters CD19, CD21 and CD37 did not stain LC; however, the CD39 antibodies, which have a wider specificity for B cells, some macrophages and interdigitating cells gave weak positive staining for LC (Table 2). Monoclonal antibodies which react with B cells and interdigitating cells of lymph nodes, namely G28-5 (CDw40), 2-7 and 7F7, did not react with LC.

Several antibodies from the myeloid panel stained LC (Table 3). The CD15c antibody 8-27-F6 stained LC in a granular pattern. The Group 10 antibody TE-3A-2C4 and antibodies from Groups 12a (Fig. 2) and 12b and Group 15 also stained LC. Not all LC were positive and the staining was weak to moderate in intensity. Group 10 and Group 15 antibodies stained additional cells in the basal layer of the epidermis, in a distribution typical for melanocytes.

The antibody DRC1 to follicular dendritic cells did not stain LC.

Dermal HLA-DR-positive leucocytes

Endothelial cells were distinguished from HLA-DR-positive leucocytes by positive staining with anti-factor VIII RAg. The remaining HLA-DR-positive dermal cells consisted of three identifiable cell types: $CD1_a$-positive dermal cells, cells which stained with antibodies to monocytes/macrophages and T lymphocytes. The relative numbers of $CD1_a$ (NA1/34)+: CD5 (Leu 1)+: 63D3+ cells in the dermis were 1:2·2:7·6 based on total counts of cells in four additional skin samples.

The majority of the HLA-DR-positive dermal cells stained with antibodies which react with monocytes (Table 3), such as

Table 3. Reactivity of myeloid cell antibodies with LC and dermal HLA-DR-positive cells

Cluster of differentiation specificity	Antibody name	Reactivity LC	Reactivity dermal DR-positive-cells	Reactivity with other structures	Source/reference
CD13	MCS2	—	Many cells. subepidermal	—	3rd LDAW
CD14A	FMC-32, UCHM1	—	Many cells	—	3rd LDAW
CD14B	My4, FMC17	—	Many cells	—	3rd LDAW
CD15a	CMRF-7	—	—	Basal layer Ep*	This laboratory
	CMRF-27	—	—	—	This laboratory
CD15c	8-27-F6	+ Granular	Many cells	—	3rd LDAW
CD16	Leu 11b	—	Few cells, weak	Variable Ep stain dermo-epidermal junction	BD†
CD17	T5A7	—	Many cells subepidermal, lacy pattern	—	3rd LDAW
CD31	SG134	—	—	End‡	3rd LDAW
CD32	CIKM5	—	Many cells	—	3rd LDAW
	2E1	—	Many cells	Ep	3rd LDAW
CD33	LIB2	—	—	—	3rd LDAW
CD34	My10	—	—	—	3rd LDAW
CD35	J3B11	—	—	Ep	3rd LDAW
CD36	5F1	—	—	Ep	3rd LDAW
W/S Group 10	SG133	—	Many cells	Ep, End	3rd LDAW
	TE-3A-2C4	+	Many cells	End, Mel§	3rd LDAW
W/S Group 12a	Y2/131,KiM6	+	Many cells	—	3rd LDAW
	Y-1/82A,EMB11	+	Many cells	—	3rd LDAW
W/S Group 12b	KiM7, RFD 7	+	Many cells	—	3rd LDAW
W/S Group 14	8F5	—	—	—	3rd LDAW
W/S Group 15	82H3, MOF11	+	Many cells	End, Mel	3rd LDAW
Follicular dendritic cells	DRC1	—	—	—	Dako
Monocytes	63D3	—	Many cells	—	Ugolini *et al.* (1980)
	CMRF31	—	Many cells	—	This laboratory
FcR1	32	—	—	—	Anderson & Looney (1986)

* Ep, endothelium.
† BD, Becton-Dickinson.
‡ End, epidermal.
§ Mel, melanocytes.

63D3 and the serologically identical antibody CMRF-31 (our unpublished data). A similar staining pattern was obtained with the CD14a and CD14b antibodies which react with monocytes, macrophages and dendritic reticulum cells. CD14b antibodies also react with tonsil LC and basophils. The CD13 antibodies and a CD17 antibody (T5A7) stained numerous subepidermal cells in a lacy or fibrillary pattern, as well as other cells in the papillary and reticular dermis, as has been reported previously (Hogg & Horton, 1987). Antibodies from Group 10, Group 12a and 12b and Group 15 also stained the HLA-DR-positive dermal cells but antibodies from Group 10 and Group 15 appeared to be less specific macrophages markers as they stained some dermal cells which were HLA-DR negative as well as endothelium.

The T lymphocytes in the dermis were $CD2^+$ (OKT 11), $CD3^+$ (Leu 4, OKT 3), and $CD5^+$ (Leu 1) and the majority of these were HLA-DR-positive on double labelling (Table 2). No Leu 1-positive B cells were identified. Many of the T cells occurred in a perivascular location (Fig. 3), where it was sometimes difficult to assess the true HLA-DR status of the cells because of the presence of HLA-DR-positive endothelium and the intimate association of the T cells with the HLA-DR-positive macrophages. Some T cells expressed IL-2R, and the CD25 antibody 2A3 stained more T cells than the other reagent used (BD IL-2R). Leu 8 did not stain the dermal T cells but stained epidermis and eccrine duct. The CD3 antibody Leu 4, as well as staining the dermal T cells, also stained nerve fibres strongly and epidermis to a variable degree. CD7 reagents (4H9/

Table 4. Reactivity of activation antigens with LC and dermal HLA-DR-positive cells

Cluster of differentiation specificity	Antibody name	Reactivity LC	Reactivity dermal DR-positive-cells	Reactivity with other structures	Source/reference
Activated T cells and macrophages	4F2	—	—	Basal layer Ep*	ATCC†
CD30	Kil	—	—	—	Dako
CD25	IL-2R	—	Few T cells, weak	—	BD
	2A3	—	Few T cells, weak	—	Prof. J D. Watson (Auckland University)
CD38	OKT10	—	—	—	ATCC
Transferrin receptor	CMRF2	—	Many cells positive	Ep, End‡, N§	This laboratory

* Ep, epidermis.
† ATCC, American Type Culture Collection.
‡ End, endothelium.
§ N, nerve.

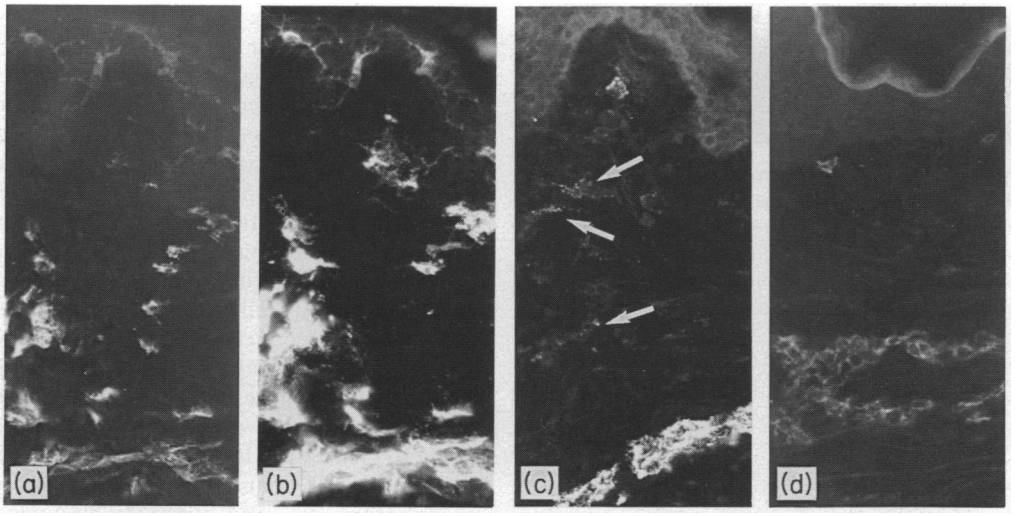

Figure 1. Human skin leucocyte populations stained using the immunofluorescence technique. In (a) staining with CMRF-12 and 23 antibodies to the leucocyte common antigen (FITC, green) reveals leucocytes in both the epidermis and dermis. Simultaneous staining with anti-HLA-DR (PE, red) in (b) indicates that most of these are HLA-DR-positive. Note the additional endothelial staining in (b). A serial section (c) stained with anti-FVIII RAg demonstrates endothelial staining. Some capillaries show weak granular staining (arrows). In (d) a collection of T lymphocytes adjacent to a dermal blood vessel is shown staining with OKT11 (CD2).

Leu 9) stained only a few T cells weakly. Approximately equal numbers of CD4- and CD8-positive T cells were present as judged by the number of CD8 (OKT8)-positive: CD5 (Leu 1)-positive T cells. Direct estimation of CD4-positive T cells was complicated by CD4-positive macrophage staining. An occasional $CD8^+$ cell was seen in the basal layer of the epidermis.

No B cells were detected in the skin (Table 2). Only an occasional cell reacted with the NK cell antibodies HNK1 and NKH1. Both of these antibodies stained nerve fibres.

The CD11 antigens (Table 1), each consisting of a different heavy chain non-covalently linked to a common light chain (CD18), are members of the LFA family (Leucocyte Function Antigens). Double-labelling studies and staining of serial sections were used to identify CD11a-positive cells, which corresponded in distribution and numbers with that of the T lymphocytes in the dermis; however, an occasional non-lymphocyte also stained. More dermal cells were CD18-positive, suggesting that this antigen was more widely expressed probably on macrophages as well as lymphocytes. A few dermal cells that on serial sections corresponded with macrophages were also positive for CD11b antibodies (CR3). CD11c-positive cells were found mainly in the papillary dermis. There were few CD11c-positive cells compared to the numbers of cells staining with the CD14a and CD14b monocyte/macrophage markers and 63D3.

Many of the dermal HLA-DR-positive cells also stained with CD32 antibodies to FcRII; fewer cells stained with CD16(FcR_{Lo}) and none with Ab32 to FcRI. Apart from the few

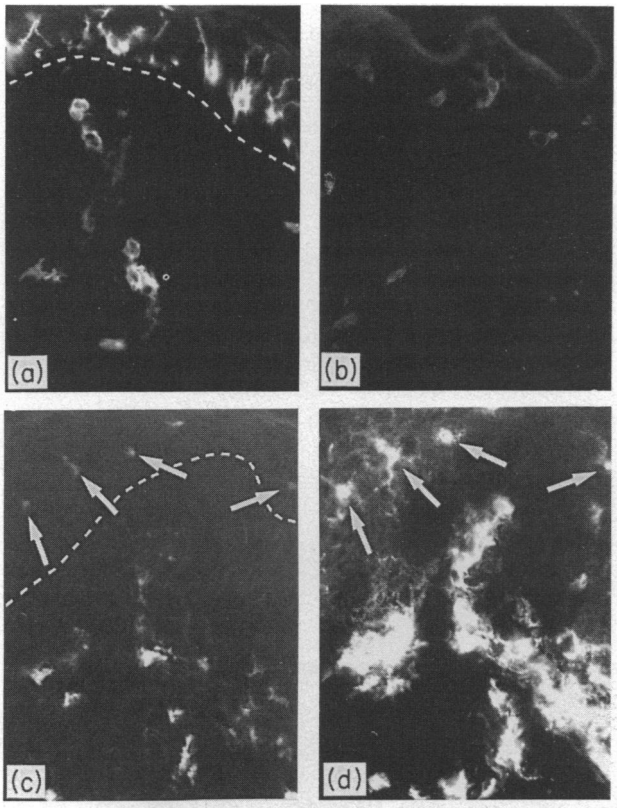

Figure 2. LC stained using the immunofluorescence technique. Staining with NA1/34 (CD1) (a) identifies LC in the epidermis and papillary dermis. LC also stained weakly with Leu 3a+b (CD4) (b). One of the Group 12 antibodies, EMB 11 stains LCs in the epidermis (arrows) as well as many of the HLA-DR-positive cells in the dermis (c). LC in (c) double label with anti-HLA-DR (d).

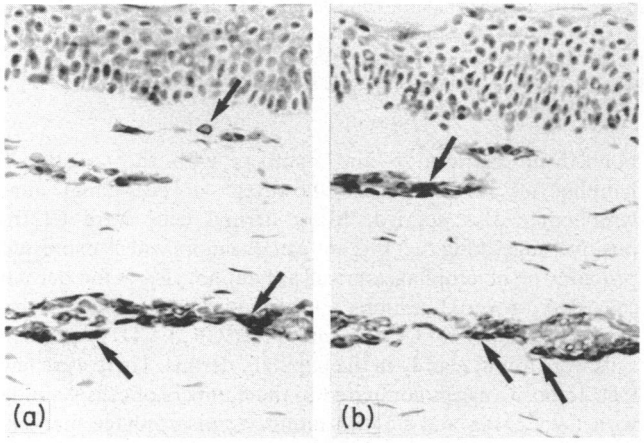

Figure 3. Immunoperoxidase staining of serial skin sections. (a) CD5 (Leu 1)-positive T lymphocytes (arrows) are clustered around blood vessels in the dermis. (b) CMRF-31-positive macrophages (arrows) are found in close association.

dermal macrophages positive for CD11b antibodies (CR3) no other complement receptor-bearing cells were detected in the dermis using CR2 (CD21) and CR1 (CD35) reagents.

Other staining patterns

The antibody CMRF-7 (CD15a) stained the basal layer of the epidermis strongly while 4F2, an antibody to activated T cells and monocytes, reacted in a similar pattern although only weakly.

DISCUSSION

LC within the epidermis are bone marrow-derived dendritic cells (Katz, Tamaki & Sachs, 1979), which are distinguished ultrastructurally by a unique organelle, the Birbeck granule (Birbeck, Breathnach & Everall 1961). In common with macrophages, LC possess ATPase activity, receptors for Ig and complement (Stingl, Wolff-Schreiner & Pichler 1977) and express class II MHC antigens (Rowden, Lewis & Sullivan, 1979). In addition LC have high-density expression of the $CD1_a$ antigen, a molecule with a similar structure to class I MHC antigens. The $CD1_a$ antigen, which is also found on immature thymocytes (McMichael *et al.*, 1979) has been widely exploited as a useful marker for staining LC (Murphy *et al.*, 1981), but apart from these antigens few other leucocyte differentiation antigens have been identified on LC. In this study, which used both immunofluorescence and immunoperoxidase techniques, LC were found to stain with monoclonal antibodies from Groups 10, 12a, 12b and 15 of the myeloid panel of the 3rd Leucocyte Differentiation Antigen Workshop, all of which also stain tissue macrophages. The CD39 antibodies, which react with macrophages, Kupffer cells, B cells, interdigitating cells and isolated tonsil dendritic cells (Hart & McKenzie, 1988), also stained LC. The presence of these antigens on LC might be interpreted as indicating some relationship between LC and the monocyte/macrophage series; however, it should be emphasized that LC did not stain with 63D3, CMRF 31 or with antibodies from CD14a and b, which stained macrophages in the dermis. Although the CD14 antibodies may not stain all tissue macrophages (Hogg *et al.*, 1984), it was also clear that antibodies from Groups 10, 15 and CD39 are not specific for tissue macrophages, as they stained some HLA-DR-negative dermal cells, endothelium and, in some cases, melanocytes or epithelium. Some LC were weakly CD4-positive, particularly with Leu 3, confirming previous reports that this antigen is found in LC (Wood, Warner & Warnke, 1983; Schmitt *et al.*, 1984; Groh *et al.*, 1986) and that our immunofluorescence technique was sufficiently sensitive to detect membrane antigen densities less than 5% that of the $CD1_a$ antigen (Schmitt *et al.*, 1984). Although our immunohistological techniques were optimized it must still be appreciated that cells expressing antigens in low density may not be detected by these techniques.

All LC appeared to be HLA-DR-positive on double labelling and the majority of these, although not all, were also HLA-DP- and HLA-DQ-positive. There have been conflicting reports as to whether all LC in the epidermis are HLA-DR-positive (Liu, Schroeter & Muller, 1986; Sontheimer, Stastny & Nunez, 1986) or not (Harrist *et al.*, 1983) and it has been suggested that the use of epidermal sheets for double-labelling immunofluorescence is a more sensitive technique than immunofluorescence on

frozen sections (Sontheimer *et al.*, 1986). Some LC and dermal cells were found to be RFD1-positive in contrast to previous reports that no RFD1-positive cells are present in normal skin (Poulter *et al.*, 1986). This staining pattern is consistent with the recent realization that RFD1 recognizes an HLA-DQ-associated antigen (Poulter *et al.*, 1986).

In this study we were unable to demonstrate consistent staining of LC in skin sections with antibodies to Fc or complement receptors, or with antibodies to dendritic reticulum cells, despite positive control staining of appropriate cells. For example the CD32 (anti-FcRII) antibodies stained dermal leucocytes, probably macrophages. Receptors for immunoglobulin and complement on LC were demonstrated originally by functional binding studies (Stingl *et al.*, 1977). The failure to detect these receptors using monoclonal antibodies implies either that these receptors on LC are different antigenically or, more probably, that they are present only in low density on LC compared to macrophages. The staining with CD32 antibodies on myeloid cells in tissue sections has been reported to be weak (Hogg & Horton, 1987), suggesting that even resting macrophages have a relatively low density of FcRII. These probable differences in receptor density perhaps distinguish LC from macrophages, instead of indicating a relationship as is commonly accepted, and further studies on isolated LC (stimulated and unstimulated) with these antibodies are indicated.

A substantial $CD1_a$-positive population of leucocytes was identified in the dermis. Classical LC with Birbeck granules have been considered to be rare in the dermis of normal skin (Zelickson, 1979). Breathnach (1975) originally used the term indeterminate cell to describe cells in the epidermis which lacked Birbeck granules, melanosomes and Merkel cell granules but had dendritic morphology. Using immunoelectronmicroscopy, Murphy *et al.* (1983) described a population of $CD1_a$-positive cells in the papillary dermis which they called indeterminate cells. Since the ultrastructural features of these cells are similar to those described for other cells of the dendritic cell family, they have also been referred to simply as dendritic cells. The function of the Birbeck granule is not understood but it appears probable that it is the result of endocytosis of the cell membrane consistent with the antigen-presenting role proposed for these cells.

The presence of the $CD1_a$ antigen on both epidermal and dermal dendritic cells suggests a close relationship, if not identity, for these cells despite the rarity of Birbeck granules in the dermal $CD1_a$-positive cells (Murphy *et al.*, 1983). Whether these dermal dendritic cells are in transit to the epidermis (Kaplan *et al.*, 1987) or are exiting the epidermis en route to draining lymphatics has yet to be answered. Certainly it has been proposed that LC traffic via the lymph, where they are identified as veiled cells (Drexhage *et al.*, 1979), to lymph nodes (Silberberg-Sinakin *et al.*, 1976) where they are considered to form part of the interdigitating cell population. Thus, the fact that LC were found to stain with CD39 antibodies, which also stain interdigitating cells and tonsil dendritic cells, is of some interest and may indicate a relationship between these cells. The possibility should also be considered, in light of recent data (Kaplan *et al.*, 1987), that epidermal LC may be shed, their turnover being linked to the turnover of the keratinocyte populations. Indeed, dermal LC may be predominantly recruited from the circulation rather than the epidermis in the case of dermal inflammatory reactions. These dermal LC may either enter the epidermis or leave via dermal lymphatics having at no stage entered the epidermis. Hence the dermal LC may be the more immunologically relevant cell as far as the central lymphoid system is concerned.

The identification of several different cell types in skin which express class II MHC antigens means that these cells must be considered, along with both the epidermal and dermal LC, as potential antigen-presenting cells. Vascular endothelial cells have been shown to be capable of antigen presentation (Hirschberg, Braathen & Thornsby, 1982) but, perhaps more relevant, there is clearly a substantial dermal macrophage population and these macrophages are often closely associated with T cells. The macrophages stained with the monocyte/macrophage markers 63D3 and the serologically identical antibody, CMRF-31, as well as with antibodies from CD14a and CD14b. Although initial studies reported that 63D3 reacted with a 200,000 MW single polypeptide chain (Ugolini *et al.*, 1980) it has been suggested recently, on the basis of DNA-mediated gene transfer experiments, that 63D3 and the CD14 antibodies react with different epitopes of a single, 55,000 MW cell membrane glycoprotein (Ashmun *et al.*, 1987). Other antibodies from the myeloid panel, Group 10, Group 12a and 12b and Group 15 antibodies, which stain macrophages, also stained many of these HLA-DR-positive cells in the dermis. Antibodies from Groups 10 and 15 appeared to be less specific macrophages markers as they also stained endothelium and HLA-DR-negative dermal cells.

In addition to macrophages, substantial numbers of T lymphocytes were identified in the dermis, and the majority of these were HLA-DR-positive on double labelling. The expression of HLA-DR and IL-2 receptors on these cells agrees with similar findings reported recently by Bos *et al.* (1987) and strongly suggests that these are activated T cells. The reason that activated T cells are present in normal skin clearly requires investigation. Although Bos *et al.* (1987) suggested that the dermal T cells might be activated by antigen-presenting endothelial cells, the physical association of these T cells with macrophages emphasizes the need to consider the latter cell population as possible antigen-presenting cells. Cloning of the dermal activated T cells might allow their specificity to be determined, but at present it can only be postulated that these T cells have either reacted to antigen (presented by LC, dermal macrophages or endothelial cells) or alternatively 'normal skin T cells' have an activated phenotype induced by lymphokines of epidermal origin. It is also of considerable interest that there were no Leu 8-positive T cells and only a few weakly CD7 (4H9/Leu 9)-positive T cells in normal skin. Some 75–95% of CD4 (Leu 3)+ cells and 50–80% of CD8 (Leu 2)+ cells in peripheral blood are Leu 8+ (Lanier *et al.*, 1983) and 85% of peripheral blood T cells are CD7+ (Link *et al.*, 1983). These results suggest that the T cells present in normal skin are a unique subset, possibly derived from the Leu 8-negative CD7-negative peripheral blood subset. In this regard it is of interest that a low percentage of Leu 8- and Leu 9 (CD7)-positive cells has been reported to be a feature of the T-cell infiltrate in mycosis fungoides as opposed to benign inflammatory dermal infiltrates (Turbitt & Mackie, 1986; Wood *et al.*, 1986). The former may be of dermal T-cell origin whilst the latter may be of peripheral blood origin.

This detailed immunohistochemical study makes several points relevant to understanding the function of the skin

immune system. There is clearly a significant dermal population of CD1-positive cells (Langerhans' cells/indeterminate cells), as well as a large number of HLA-DR-positive macrophages and these may play a much greater role in antigen presentation than hitherto considered. Furthermore we have identified a substantial population of dermal T lymphocytes which are found predominantly in a perivascular and periadnexal distribution in association with the HLA-DR-positive macrophages. These T cells have an activated phenotype and may reflect a unique Leu 8- and CD7-negative T-cell subset.

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