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Epidermal DR+T6− Dendritic Cells in Inflammatory Skin Diseases

BARBARA S. BAKER,[1] S. LAMBERT,[1] A. V. POWLES,[2] H. VALDIMARSSON[3] and LIONEL FRY[2]

[1]*Department of Immunology, St. Mary's Hospital Medical School and* [2]*Department of Dermatology, St. Mary's Hospital, London, England, and* [3]*Department of Immunology, Landspitalinn, Reykjavik, Iceland*

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T lymphocyte and dendritic cell subpopulations were counted in three biopsies each of endogenous eczema and pityriasis rosea and two of lichen planus and compared with previous findings in psoriatic lesions. In common with psoriasis, proportionately more CD4 T cells than CD8 T cells were DR+ in both epidermis and dermis of all lesions. In addition, total numbers of epidermal dendritic cells were significantly increased in endogenous eczema and pityriasis rosea, and variably in lichen planus lesions. Interestingly, a DR+T6− subpopulation of dendritic cells was present in varying proportions in all three skin lesion types. Electron microscopy of DR+T6− dendritic cells from psoriatic lesions, using an immunogold staining technique, showed the cells to be of the Langerhans' cell lineage. DR+T6− dendritic cells are a subpopulation of Langerhans' cells which are not specific to psoriasis, but present in the lesions of other benign, inflammatory skin conditions in which CD4 T cells are preferentially activated. *Key words: Immunopathology; DR+T6− Langerhans cells; T lymphocytes.* (Received October 5, 1987.)

B. Baker, Dermatology Research, St. Mary's Hospital, Praed St., London W2 1NY., England.

We have recently identified a subpopulation of dendritic cells, which express HLA-DR antigen but lack the T6 antigen characteristically expressed by Langerhans' cells (DR+T6−), in the epidermis of psoriatic lesions (1, 2). These cells are rarely observed in normal or uninvolved psoriatic epidermis, although Cooper et al. (3) have reported the appearance of DR+T6− cells in normal human epidermis after UV irradiation. We have postulated that DR+T6− cells are immature Langerhans' cells which have been recruited at an abnormal rate into the epidermis as part of the psoriatic process (2).

Cyclosporin A treatment of psoriasis resulted in a rapid and selective depletion of these cells from psoriatic epidermis before clinical improvement was apparent and at rate which correlated with clearance of psoriasis (2). Cyclosporin A is known to be an immunosuppressive drug which selectively inhibits T helper (CD4) cell function by impairing the production and effects of Interleukin−2 (4). The treatment-induced disappearance of DR+T6− cells may, therefore, be secondary to the effects of cyclosporin on mediator production by CD4 T cells.

The aim of this study was therefore to determine whether

1) DR+T6− dendritic cells are unique to psoriatic lesions, or present in the lesional skin of other inflammatory diseases. Eczema, pityriasis rosea and lichen planus were chosen because these diseases are associated with infiltration of T lymphocytes which are predominantly of the T helper/inducer subset (5–7). A double-staining immunofluorescent technique previously employed in the study of psoriatic lesions (1) was used to count T lymphocyte and dendritic cell subpopulations.

2) DR+T6− dendritic cells in psoriatic epidermis are of the Langerhans' cell lineage, using an immunogold staining technique and electron microscopy.

MATERIAL AND METHODS

Samples

For electron microscopy, keratome or punch biopsies of lesional skin were obtained from 3 patients with chronic plaque psoriasis. Foreskin samples constituted the normal skin controls.

Punch biopsies were taken from lesional skin of 3 patients with endogenous eczema of <1-several years standing (2 discoid and 1 seborrheic), 3 patients with pityriasis rosea of <3 weeks duration and from 2 patients with lichen planus, one of short (less than a year) and the other of long (few years) duration.

None of the patients had been receiving topical or systemic treatment for at least 2 weeks or 1 month, respectively, before the biopsies were taken. The 2-mm punch biopsies were immediately frozen in liquid nitrogen, embedded in Tissue Tek II OCT compound (Lamb, London) and stored at −80°C for up to 3 months. Five-micrometre sections were cut on a cryostat (Slee, London) air-dried for at least 30 min, and either stained immediately or stored at −80°C.

Electron microscopy

Dendritic cells in either epidermal sheets or suspensions of epidermal cells were double-stained for DR and T6 antigens using an immunogold technique.

(a) *Preparation of Epidermal sheets/suspensions:* Biopsies were cut into small pieces and floated dermal side down in 0.3% trypsin plus 0.1% EDTA in phosphate-buffered saline (PBS) at 37°C for either 25 min for epidermal sheets or 1 h for epidermal suspensions. Epidermal sheets were carefully peeled off from the dermis and washed in PBS. Suspensions were prepared by gently scraping off the epidermal cells with forceps; the cells were washed twice with complete MEM (Minimal Essential Medium supplemented with 0.1 mg/ml streptomycin, 100 U/ml Crystapen and 10% heat-inactivated fetal calf serum (FCS)) and passed through gauze to remove the stratum corneum.

Epidermal sheets were replaced in later experiments by suspensions of epidermal cells because it was difficult to obtain an intact epidermal layer using trypsin which split the epidermis above the basal layer of epidermis. Keratome biopsies, which consisted of epidermis and upper dermis, also proved unsuitable because of the lack of penetration of the antibodies due to the thickness of the skin.

(b) *Immunogold Double-Staining:* Epidermal sheets were fixed in freshly prepared PLP (periodate–lysine–paraformaldehyde) at 4°C for 2 h and then washed twice in PBS. The labelling procedure was as follows: 1) OKT6 antibody diluted 1:10, 18 h at 4°C; 2) Goat anti-mouse antibody conjugated to gold granules of 40 nm size, diluted 1:10, 2 h at 37°C; 3) Anti-HLA-DR antibody, undiluted, 18 h at 4°C; 4) Goat anti-rat antibody conjugated to gold granules of 5 nm size, diluted 1:10, 2 h at 37°C.

Epidermal suspensions, which were first pelleted and the medium removed, were similarly treated except that there was a shorter fixation time (5 min), the incubations were for 1 h at 37°C and the gold antibodies were used at a 1:2 dilution.

Both epidermal preparations were post-fixed in 2.5% glutaraldehyde in cacodylate buffer for 1 (sheets) or 2 h (suspensions), then in 1% osmium tetroxide at 4°C for 1 h, and embedded in Araldite resin. Ultrathin sections were examined, after post-staining with uranyl acetate and lead citrate, with a Philips 300 electron microscope using a voltage of 80 kV.

Double Immunofluorescence Labelling

T lymphocyte and dendritic cell subpopulations in skin sections were counted by a double-staining technique as previously described (1). Briefly, chloroform/acetone-fixed sections were given 30 min incubations with biotin-conjugated Leu 2a (suppressor/cytotoxic T cells), Leu 3a (helper/inducer T cells) or (unconjugated) OKT6 (Langerhans cells) monoclonal antibodies, followed by biotin-conjugated goat anti-mouse antibody (Tago, Burlingame, Calif., USA), and finally rhodamine-labelled avidin (Vector Laboratories, Burlingame). The sections were then immediately stained for HLA-DR antigen by incubation for 30 min with monoclonal anti-HLA-DR (YE2/36 HLK) antibody (8) followed by fluorescein-labelled rabbit anti-rat antibody. After mounting in 10% PBS in glycerol the sections were examined under a Leitz fluorescent microscope equipped with selective filters for fluorescein and rhodamine.

Epidermal dendritic cells expressing DR molecules and/or OKT6 determinants were counted in 50 high-power fields (×50 objective) and were designated as DR+T6+, DR+T6− or DR−T6+ dendritic cell subpopulations. Total and DR+ T cell numbers were also counted in 50 high-power fields of the

epidermis, but dermal T cell counts were expressed as numbers per papillary and reticular dermis of one 2-mm section. It should be noted that DR expression by T and dendritic cells could be distinguished from that of keratinocytes on the basis of both morphology and double-staining.

RESULTS

Immunofluorescence

Epidermis. T lymphocytes were present in the epidermis of all biopsies from lesional skin of endogenous eczema, pityriasis rosea and lichen planus examined (Table I). Although there was variability as to which T cell subpopulation predominated, the percentage of DR+CD4 T cells generally exceeded that of DR+CD8 T cells in these diseases, as previously observed in psoriatic lesions (Table I). However, there was some difficulty in assessing DR expression by T cells in the epidermis of eczema and lichen planus lesions due to the marked expression of DR antigen by keratinocytes. DR+ keratinocyte staining was also observed in 2/3 pityriasis rosea biopsies. In contrast, DR expression by keratinocytes was infrequently observed in psoriatic lesions.

DR+T6− dendritic cells, which are very rarely found in normal epidermis but have previously been observed in psoriatic lesions, were present in the lesional epidermis of each skin condition studied (Fig. 1 *a, b*). However the proportion of these cells, in relation to the total dendritic cell numbers, varied both within and between diseases (Table I). Furthermore, as in psoriatic lesions, total dendritic cell numbers were significantly increased compared with normal skin in both eczema and pityriasis rosea, and in a late lesion of LP (Patient 2, Table I). Moreover, the highest numbers were observed in the epidermis of eczema lesions.

Dermis. A greater proportion of CD4 than CD8 T cells was generally observed in the dermis and, in all cases, the percentage of DR+CD4 T cells exceeded that of DR+CD8 T cells (Table II).

Table I. *T lymphocyte and DR+T6− dendritic cell subpopulations in the epidermis of endogenous eczema, pityriasis rosea and lichen planus lesions*

Skin disease	Patient	T lymphocytes			Dendritic cells	
		Total CD4	Total CD8	CD4/CD8	DR+T6−	Total[b]
Endogenous eczema	1	142 (46)[a]	70 (41)[a]	2.03	211 (13)[c]	1656
	2	137 (50)	50 (44)	2.74	10 (1)	1200
	3	144 (51)	283 (21)	0.51	184 (15)	1262
Pityriasis rosea	1	106 (32)	162 (33)	0.65	287 (28)	1014
	2	156 (55)	29 (24)	2.97	9 (1)	818
	3	86 (43)	163 (20)	0.53	22 (3)	835
Lichen planus	1	90 (86)	50 (28)	1.80	209 (38)	547
	2	54 (30)	149 (24)	0.36	370 (42)	879
Psoriasis	Mean (n=17)	199 (16) ±21	365 (9) ±44	0.66 ±0.10	128 (18) ±20	775 ±49

[a] % DR^+ of total CD4 or CD8 T cells.
[b] Normal range = 304–621 total dendritic cells/50 high-power fields of epidermis.
[c] % of total dendritic cell numbers.

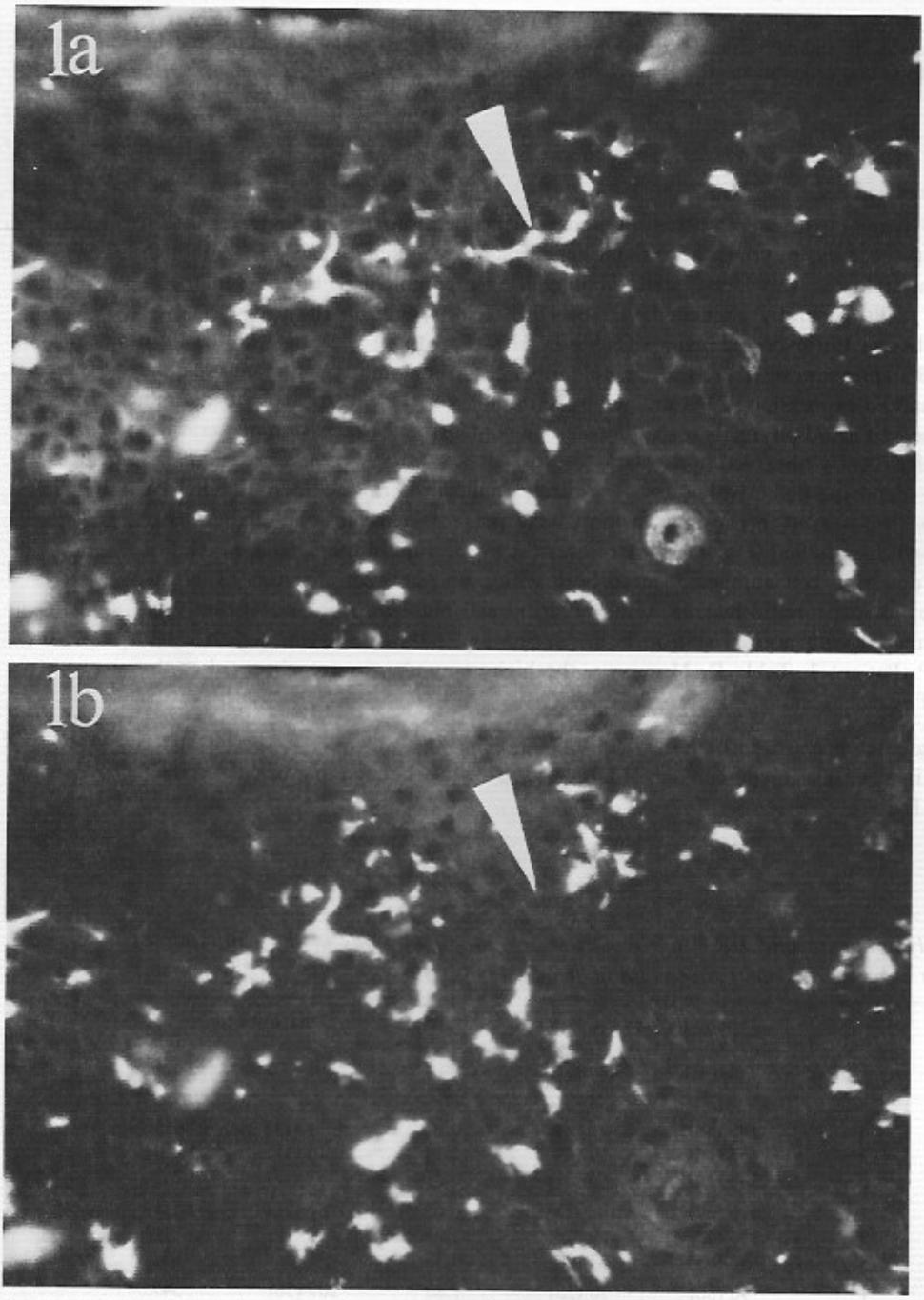


Fig. 1 a, b. The epidermis from an endogenous eczema lesion double-stained with (*a*) Anti-DR, and (*b*) OKT 6 antibody combination. Arrowhead indicates a DR+ dendritic cell which does not express T6 antigen.

Absolute numbers are not given for the lichen planus lesions as the extent of the infiltrates made accurate counting impossible.

Electron Microscopy

Keratinocytes were not labelled by the immunogold staining method, with the exception of a single DR+ keratinocyte observed in a psoriatic epidermal suspension, and relative numbers of Langerhans' cells (LC) were small. However, typical LCs which contained LC granules in the cytoplasm and expressed both DR and T6 antigens were observed in both normal and psoriatic epidermis (Fig. 2*a–c*). There was heavier labelling for DR than for T6 antigen in each case (Fig. 2*a*). In addition, in psoriatic (but not normal) epidermis, there were cells labelled with numerous small gold granules (DR antigens) but very sparse large gold granules (T6 antigens) scattered along the cell membrane (Fig. 3*a, b*). Such cells almost certainly correspond to the DR+ dendritic cells which do not stain for T6 antigen by immunofluorescence (Fig. 1*a, b*), and are therefore designated as DR+T6− cells. LC granules were present in 2 DR+T6− cells (Fig. 3*b*) but not in 4 other such cells studied. Judging by the ultrastructural characteristics, i.e. lack of desmosomes, tonofilaments and melanosomes, these DR+T6− cells appeared to be of the LC lineage (Fig. 3*a*).

DISCUSSION

We have previously reported the presence of a subpopulation of epidermal dendritic cells, HLA-DR positive but lacking the T6 antigen characteristically expressed by Langerhans' cells, in the lesional skin of psoriatic patients (1, 2).

This study has established that these epidermal DR+T6− dendritic cells are not unique to psoriasis but are present in other benign inflammatory skin diseases such as endogenous eczema, pityriasis rosea and lichen planus. Although we (unpublished observations), and others (9), have observed interdigitating dendritic (RFD1+) cells in the epidermis of psoriatic lesions, it is unlikely that the DR+T6− cells are of this phenotype because RFD1+ cells are present in smaller numbers, and are almost exclusively T6+ (unpublished

Table II. *T lymphocyte subpopulations and HLA-DR expression in the dermis of lesions of endogenous eczema, pityriasis rosea and lichen planus*

Skin disease	Patient	Total CD4	DR+CD4	Total CD8	DR+CD8	CD4/CD8
Endogenous eczema	1	639	374 (58)[a]	179	56 (31)[a]	3.57
	2	586	359 (61)	213	54 (25)	2.75
	3	270	172 (64)	52	18 (35)	5.19
Pityriasis rosea	1	450	336 (75)	212	84 (40)	2.12
	2	216	164 (76)	ND	ND	ND
	3	346	224 (65)	406	169 (42)	0.85
Lichen planus	1		Total CD4>Total CD8			ND
	2		DR+CD4>DR+CD8			ND
Psoriasis	Mean	204	107 (52)	119	40 (32)	2.05
	(n=17)	±24	±15.2	±18	±8.8	±0.26

[a] % of total CD4 or CD8 T cells.
ND = Not done.

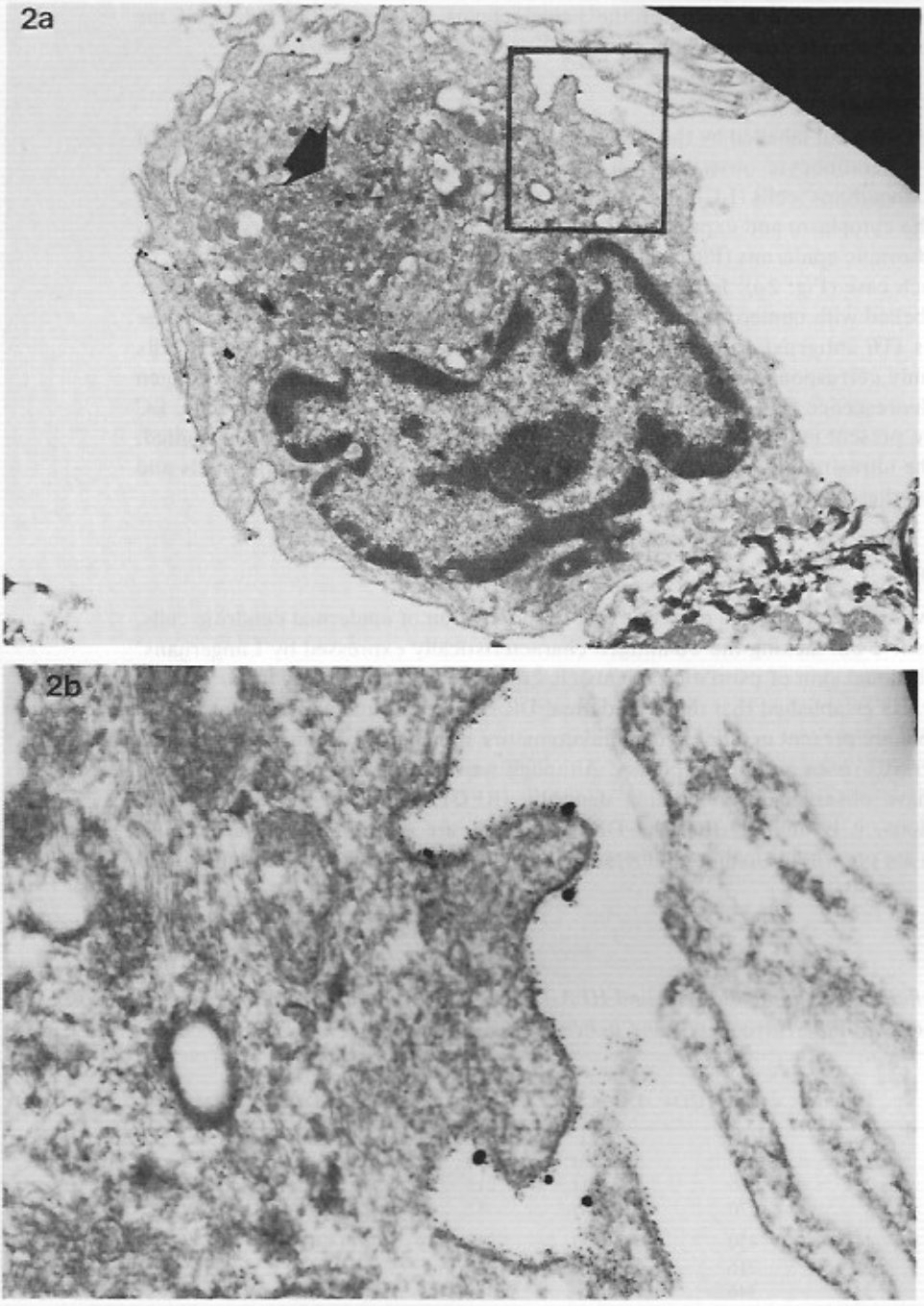
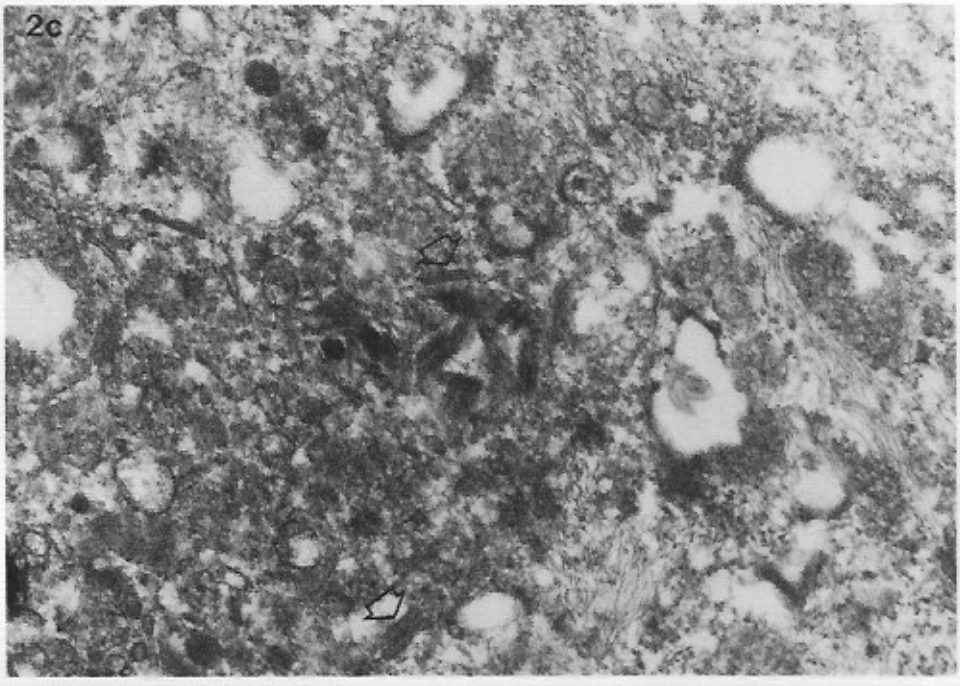


Fig. 2 a, b, c. (*a*) A Langerhans' cell observed in the epidermal sheet from a psoriatic lesion showing labelling with large (T6) and small (DR) gold granules. Arrow indicates area considered in (*c*). (*b*) Higher magnification of the boxed area in (*a*) showing gold granules in close apposition with the cell membrane. (*c*) Higher magnification of Langerhans' cell granules (arrows) in the cytoplasm.



observations). We have therefore postulated that this subpopulation of dendritic cells are immature Langerhans' cells which have been recruited at an abnormally rapid rate into the epidermis (2). The electron microscopic findings reported here support the view that DR+T6− dendritic cells are of the LC lineage although LC granules have not been observed in all the DR+T6− cells studied. This may be due to technical reasons, or alternatively may indicate that the cells are at different stages of maturity (10). It has been proposed that the formation of LC granules takes place within the environment of the epidermis (10, 11). As T6 antigen expression is probably subsequent to LC granule formation, as suggested by a recent study of DR and T6 antigen expression by LCs in embryonic and fetal skin (12), and by the findings reported here, the intact epidermal milieu may also be necessary for the induction of this characteristic marker of mature LCs. Indeed, keratinocytes have been shown to induce the expression of T6 antigen in cultured neoplastic T lymphocytes (13). The lack of T6 antigen on a subpopulation of epidermal LCs in inflammatory skin lesions reported here may be due to the very rapid influx of these cells into the epidermis allowing insufficient time for induction of T6 expression to take place.

As previously reported (6, 7) the majority of T lymphocytes in the dermis of endogenous eczema, pityriasis rosea and lichen planus skin lesions are of the CD4 phenotype. In the epidermis of these lesions CD4 and CD8 T cells are present in varying numbers and proportions. However, in both epidermis and dermis, a larger proportion of CD4 than CD8 T cells are activated (as defined by HLA-DR expression). Thus there is a similar degree of T lymphocyte infiltration in these diseases to that previously reported in psoriatic lesions (2).

This study suggests that the recruitment of DR+T6− Langerhans' cells into the epidermis may be part of a pathogenic pathway common to a variety of T cell-mediated skin diseases.

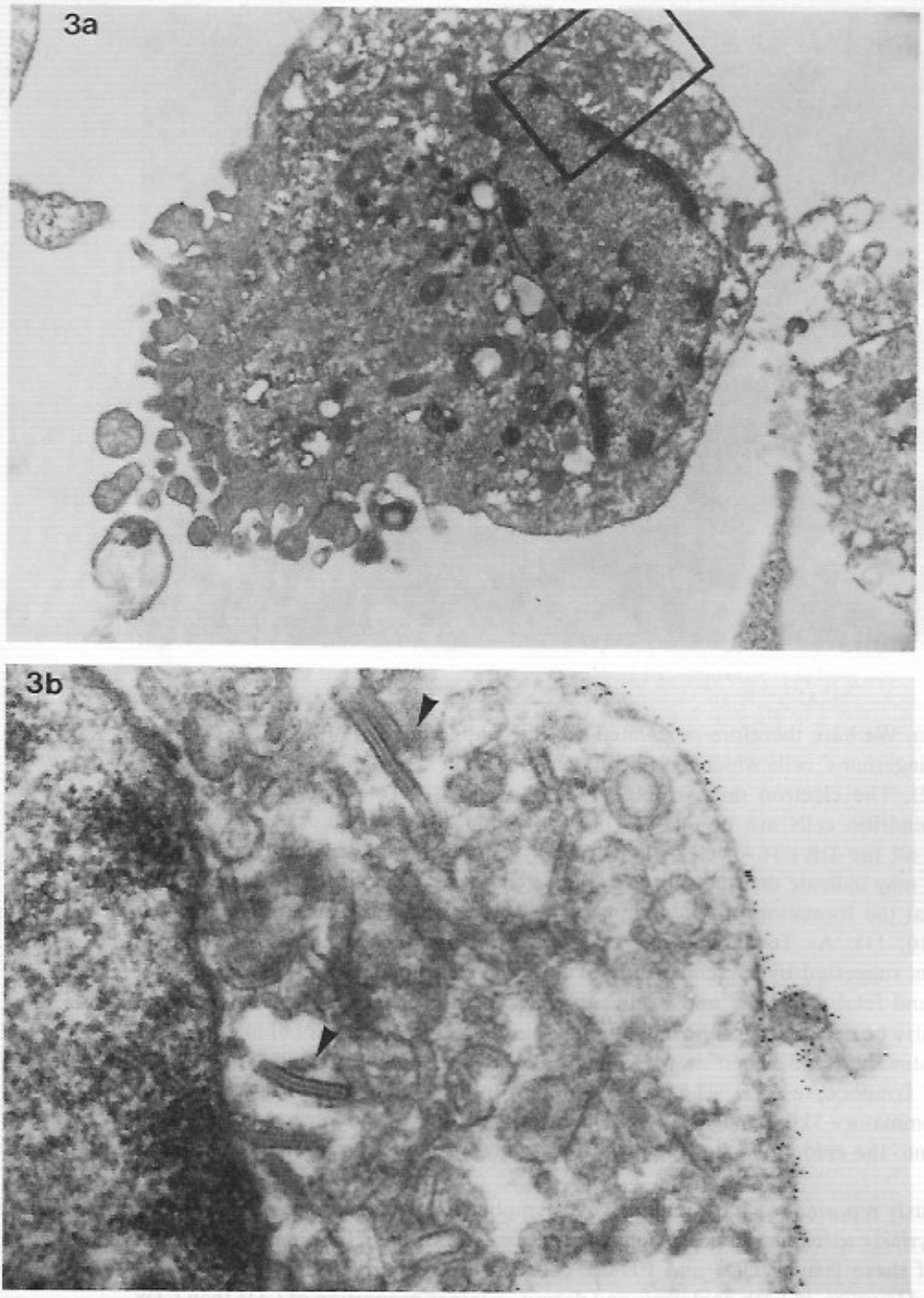


Fig. 3 a, b. (*a*) A Langerhans' cell observed in the epidermal sheet from a psoriatic lesion showing heavy labelling with small gold granules (DR antigen) and very few large gold granules (T6 antigen). (*b*) Higher magnification of boxed area in (*a*) showing Langerhans' cell granules (arrowheads).

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