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Phenotypic characterization of the early cellular responses in allergic and irritant contact dermatitis

D. J. GAWKRODGER, EVA McVITTIE, MARY M. CARR, J. A. ROSS & J. A. A. HUNTER *Department of Dermatology, University of Edinburgh, Royal Infirmary, Edinburgh*

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SUMMARY

Despite qualitative similarities there were subtle differences between the nickel allergic and dithranol irritant dermatitis reactions. In both responses, dermal and epidermal cellular infiltrates developed, which were predominantly of Leu 3a phenotype with lesser numbers of Leu 2a positive cells. Dermal infiltrates were larger in the allergic response, but epidermal invasion was greater in the irritant reaction. In the allergic challenge response, Leu 3a reactive cells appeared in the dermis and epidermis by 4 h. At 48 h, both reactions showed skin infiltration by Leu M3 positive macrophages, and had increased numbers of cells in the epidermis expressing class II antigens. The number of Leu 6 reactive Langerhans cells in the epidermis was almost halved at 48 h in the irritant reaction, but Langerhans cell counts were increased by a third between 24 and 48 h of the allergic response. Ultrastructural studies showed disruption of the Langerhans cell mitochondrial cristae at 8 h in the irritant reaction, with few identifiable epidermal Langerhans cells at 48 h. At 1 h in the allergic response, electron microscopy identified two populations of Langerhans cells; the majority showed an electron-dense cytoplasm with vacuoles, and the rest appeared normal. Peripolesis was noted in both types of reaction.

Keywords allergic contact dermatitis nickel sensitivity immunophenotyping dithranol irritant dermatitis

INTRODUCTION

The Langerhans cell and the T lymphocyte play a central role in allergic contact dermatitis (Wolff & Stingl, 1983) but few studies have followed the temporal development of the elicitation reaction. Allergic contact dermatitis is an example of delayed hypersensitivity, and antigen presentation by Langerhans cells is thought to occur in the skin (Silberberg *et al.*, 1975) with subsequent immunological processing in regional lymph nodes (Lens *et al.*, 1983).

Pathological changes in experimental irritant dermatitis show some qualitative similarities to those in allergic reactions (Kanerva, Ranki & Lauharanta, 1984). However, the dermal lymphocytic infiltrates may be larger in the allergic response (Scheynius *et al.*, 1984) and, in the irritant model, the numbers of epidermal Langerhans cells may be reduced (Ferguson, Gibbs & Beck, 1985). We aimed, therefore, to quantify the early cellular changes in the hypersensitivity response to nickel, and to compare it to an irritant reaction. Dithranol was chosen as the irritant because it produces a delayed type of irritancy (Malten, den Arend & Wiggers, 1979), is widely used in the treatment of psoriasis, and rarely causes sensitivity (Lawlor & Hindson, 1982).

Correspondence: Dr D. J. Gawkrodger, Department of Dermatology, Royal Infirmary, Edinburgh EH3 9YW, UK.

MATERIALS AND METHODS

Subjects and challenge. Fifteen women and one man who were positive to nickel on patch testing (mean age 45 years, range 24–71) were challenged on forearm skin with 5% nickel sulphate in 60 mg of petrolatum (Trolab Hermal-Chemie Kurt Hermann, Hamburg, West Germany) in a 12 mm Finn Chamber (Epitest Ltd Oy, Helsinki, Finland). Punch biopsies were taken using 2% lignocaine local anaesthesia at 1, 4, 6, 8, 24 and 48 h after applying the patch, and a sample of non-challenged skin was taken in each case. Biopsies were immediately divided into samples for cryosectioning (snap frozen in liquid nitrogen), electron microscopy (fixed in 2% glutaraldehyde) and routine haematoxylin and eosin (H & E) histology (fixed in Bouin's solution). Five males and one female (mean age 55 years, range 30 to 76), who were not allergic to nickel, served as controls for the assessment of the effect of 60 mg petrolatum alone or 60 mg petrolatum containing 5% nickel sulphate, biopsies being taken at 8 h in each case.

In the irritant study, six male and three female volunteers (mean age 54 years, range 23 to 76), were patch tested on normal looking forearm skin with 60 mg petrolatum containing 0·1% dithranol (approximately 60 μg dithranol) with 0·5% salicylic acid, in a 12 mm Finn Chamber. Six of the subjects had chronic plaque psoriasis but none had received recent local or systemic corticosteroids, ultraviolet therapy or dithranol. Salicylic acid 0·5% in 60 mg petrolatum was used as a control. Punch biopsies were taken using local anaesthesia at 8 h and 48 h for dithranol (the patch having been removed at 24 h in the latter case), and at 8 h for the control. A biopsy of normal skin was taken in each case. The biopsies were divided up as for the nickel study.

Immunoperoxidase technique. Cryocut 5 μm thick sections were placed on poly-L-lysine coated slides, fixed in acetone at room temperature for 20 min, and preincubated with 20% normal rabbit serum in Tris-buffered saline (0·001 M, pH 7·6) for 10 min. They were then incubated for 1 h at room temperature with the monoclonal antibody made up at the appropriate dilution in 20% normal rabbit serum in Tris-buffered saline (Table 1). After washing they were incubated with rabbit anti-mouse immunoglobulin conjugated with horse radish peroxidase (Dakopatts a/s, Glostrup, Denmark) for 1 h. Development of the enzyme reaction was achieved using 0·03% diaminobenzidine (Sigma Chemical Company Ltd, Poole, England) with 0·068% imidazole (BDH Chemicals Ltd, Poole, England) and 0·02% hydrogen peroxide in Tris-buffered saline applied for 5 min. Sections were counterstained with Mayer's haemalum.

Quantification of cellular infiltrates. Epidermal counts were performed by noting the number of reactive cells overlying 200 consecutive basal keratinocytes; the mean of at least three readings was taken. Cells staining with Leu 6 were counted only if a cell body with two or more dendritic processes was seen. When Leu 6 and DA6.147 were studied, the number of cells within the basal

Table 1. The monoclonal antibodies used

Monoclonal antibody	Origin	Specificity	Working dilution
anti-Leu 2a*	Becton-Dickinson‡	Cytotoxic/suppressor T lymphocytes	1:40
anti-Leu 3a*	Becton-Dickinson‡	Delayed hypersensitivity/helper T lymphocytes	1:40
anti-Leu 6*	Becton-Dickinson‡	Langerhans cell/common thymocyte	1:20
anti-Leu M3*	Becton-Dickinson‡	Monocyte/macrophage	1:20
DA6.147†	K. Guy§	DR (α chain) + DQ	neat

* Purified ascites.

† Culture supernatent.

‡ Becton-Dickinson Monoclonal Center Inc, Mountain View, California, USA.

§ MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh.

layer and above it were recorded separately. Quantification of the upper dermal infiltrate required an eye-piece graticule with an objective optical system giving × 500 magnification. The number of reactive cell bodies within 10 consecutive adjacent graticule areas immediately below the dermo-epidermal junction was noted and a mean figure obtained. All counting was done by one observer using coded slides. The mean for each time point came from an analysis of data from between three and six different patients. The results were analysed statistically using a two-sample *t*-test comparing mean cell counts at a particular time point with the value in non-challenged skin.

Ultrastructural examination. Samples for electron microscopy were post-fixed in osmium, dehydrated and embedded in Epon. Thin sections were cut on an LKB Ultratome III, stained with uranyl acetate and lead citrate, and examined in a Philips 301 electron microscope.

RESULTS

Macroscopic changes. At 24 h after the application of a nickel sulphate patch test, nearly all nickel sensitive subjects showed eczematous changes, but before this time, biopsied sites did not show eczema clinically. By 48 h, vesiculation was seen in some individuals. Dithranol occlusion for 8 h did not produce any overt skin changes, but by 48 h each patch test showed a uniform erythema with oedema although no vesiculation.

H & E histology. Routine H & E examination of the nickel allergic sites revealed a slight mononuclear cell infiltrate in the upper dermis and epidermis of many subjects between 4 and 8 h. At 24 h, intercellular oedema in the epidermis was often observed. The mononuclear infiltrate, which showed a periappendageal emphasis, had increased by 48 h, and in some subjects, vesicle formation had developed in the epidermis. In the dithranol sites, a few perivascular mononuclear cells were demonstrated in the upper dermis at 8 h, but by 48 h, all biopsies showed upper dermal oedema and intercellular oedema in the epidermis, with necrosis of keratinocytes at several places. A moderate perivascular mononuclear infiltrate was evident in the mid and upper dermis, with epidermal invasion at certain points.

Leu 2a and Leu 3a reactivity. Although irritant and allergic reactions both show increases in Leu 2a and Leu 3a positive cells, the rates of change differ. In the irritant response, Leu 2a and Leu 3a reactive cells in the epidermis have trebled in number at 8 h and increased 7-fold at 48 h (Table 2). The increase is initially less in the allergic reaction, especially for Leu 2a and is still less for Leu 3a at 48 h, but the latter epidermal count for Leu 2a is similar to the irritant one. Contrarywise, the allergic dermal infiltrates tend to be bigger, with increases of about 2-fold at 8 h and 4-fold at 48 h for both Leu 2a and Leu 3a (Figs 1 & 2). Leu 3a reactive cells outnumber Leu 2a positive ones at most time points, the exact ratio varying between 6:1 to 1·4:1 but often being about 3:1. The dermal and epidermal changes at 8, 24 and 48 h were all statistically significant ($P < 0{\cdot}05$ or less) for both reaction types except for the irritant 8 h dermal Leu 3a count and the allergic 8 h epidermal Leu 2a count. Some of the epidermal cells reacting with Leu 3a may have been Langerhans cells (Groh *et al.*, 1986), and this may explain our findings of a few Leu 3a positive cells in unchallenged, clinically normal skin.

Leu 6 reactivity. Different changes in epidermal Leu 6 reactivity were seen in the irritant and allergic responses. In the allergic model, epidermal Leu 6 cell counts were raised by 30% at 8 h, and 50% at 24 to 48 h, but in the irritant reaction, numbers had fallen to half normal at 48 h and the Langerhans cells looked abnormal, with loss of dendrites (Table 2; Fig. 2). In both reactions, the dermal counts, which were usually small, were nearly twice normal at most time points, except for at 1 h in the allergic response (Fig. 3). Several of these changes are statistically significant. In the allergic dermatitis reaction, some subjects showed an increase in the proportion of Langerhans cells occupying a basal position between 4 and 24 h (data not shown), and in one subject with a distinct spongiotic reaction, Langerhans cells with long dendrites were found in the upper Malpighian layer and in microvesicles (Fig. 2).

Leu M3 and DA6.147 reactivity. No Leu M3 positive macrophages are present in the normal epidermis, and few are found in the normal dermis. In both allergic and irritant reactions, the dermal counts rose about 3-fold at 48 h, with Leu M3 reactive cells entering the epidermis (Table 2;

Table 2. Epidermal cell counts: positive cells per 200 basal keratinocytes (mean ± s.e.m.)

Biopsies analysed	Leu 2a	Leu 3a	Leu 6	DA6.147	Leu M3
Nickel sensitive					
Non-challenged	0·69 ± 0·22	2·94 ± 0·48	20·00 ± 0·61	13·81 ± 0·58	0
1 h	1·00 ± 0·58	1·33 ± 0·33	23·33 ± 0·88*	12·66 ± 1·86	0
4 h	1·00 ± 0·74	4·60 ± 0·93	22·20 ± 1·59	15·60 ± 2·16	0
6 h	1·33 ± 0·88	3·33 ± 0·88	28·33 ± 2·19*	17·00 ± 1·53*	0
8 h	1·00 ± 0·26	6·33 ± 1·85*	25·83 ± 1·38*	13·50 ± 1·73	0
24 h	2·83 ± 0·95*	10·40 ± 2·36*	29·83 ± 1·82*	15·16 ± 1·21	0·50
48 h	10·00 ± 3·82*	13·80 ± 3·71*	28·60 ± 3·06*	39·80 ± 9·77*	3·50 ± 0·87*
Non-nickel sensitive					
Non-challenged	1·00 ± 0·37	3·67 ± 0·72	19·00 ± 0·52	11·50 ± 0·22	0
5% $NiSO_4$ 8 h	0·67 ± 0·67	5·00 ± 1·73	20·50 ± 1·53	12·50 ± 1·50	0
Petrolatum 8 h	2·00 ± 1·15	4·67 ± 1·33	18·50 ± 0·29	12·00 ± 1·53	0
Dithranol irritant					
Non-challenged	1·22 ± 0·32	4·37 ± 0·60	19·44 ± 0·60	12·78 ± 0·68	0
0·5% salicylic acid 8 h	1·50 ± 0·50	5·50 ± 0·50	20·00 ± 0·58	14·00 ± 1·73	0
8 h dithranol	3·75 ± 1·18*	12·75 ± 3·28*	21·00 ± 1·58	17·00 ± 1·22*	0
48 h dithranol	8·00 ± 1·38*	30·50 ± 2·72*	12·50 ± 1·85*	31·60 ± 4·43*	5·00 ± 1·34*

*Statistical analysis using a two-sample *t*-test, with counts compared to the non-challenged results ($P < 0·05$ or less).

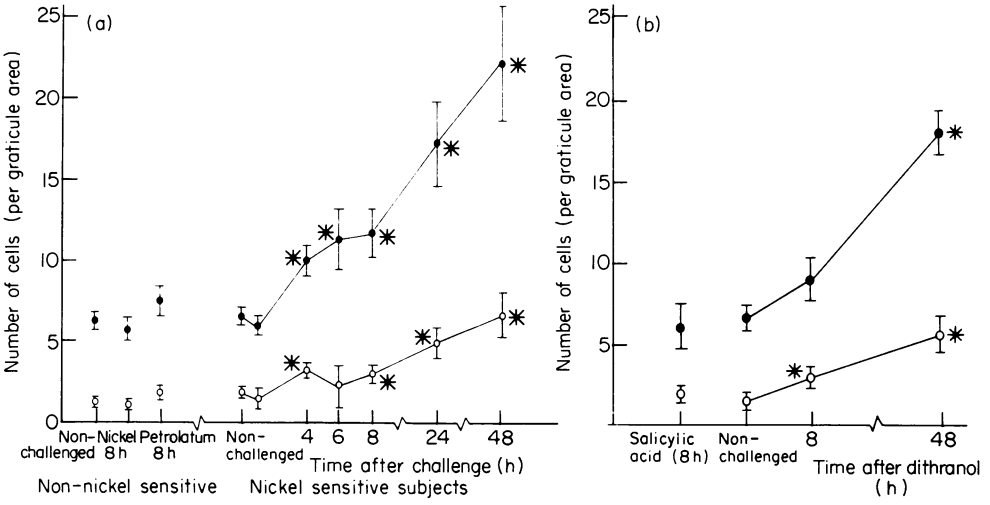

Fig. 1. Upper dermal counts (positive cells per graticule area) for Leu 3a (●) and Leu 2a (○), at various time points for (a) the nickel allergic and (b) the dithranol irritant reactions: mean ± s.e.m. *$P < 0·05$.

Fig. 3). Most DA6.147 positive cells in the normal epidermis are Langerhans cells, and cells reactive with this monoclonal antibody make up 60 to 70% of those which stain with Leu 6. The 3-fold increment in DA6.147 reactive cells at 48 h in both reaction types (Table 2) probably represents activity on Langerhans cells, and activated lymphocytes and macrophages in the epidermis. The dermal pattern for DA6.147 showed enhancement at 48 h for irritant and allergic responses,

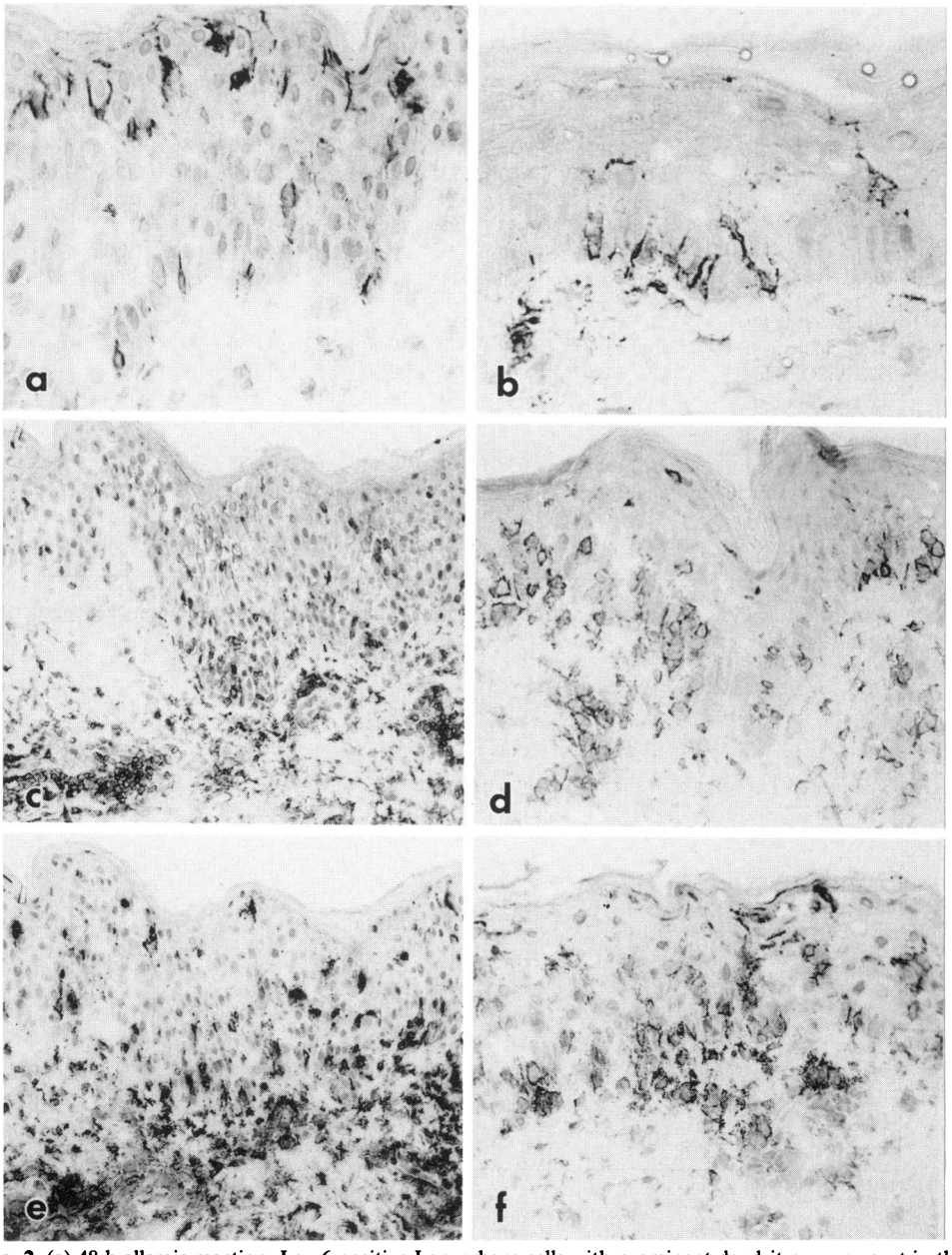

Fig. 2. (a) 48 h allergic reaction: Leu 6 positive Langerhans cells with prominent dendrites are present in the upper malpighian layer; a few cells occupy a basal position (× 320). (b) 48 h irritant reaction: Leu 6 positive Langerhans cells are reduced in number in the epidermis and have few dendrites. Some occupy a basal position, a few are present in the upper dermis (× 500). (c) 48 h allergic reaction: large numbers of Leu 3a positive T cells have invaded the dermis and epidermis (× 160). (d) 48 h irritant reaction: Leu 3a reactive cells have invaded the dermis and epidermis (× 320). (e) 48 h allergic reaction: the DA6.147 reactive epidermal cells include Langerhans cells, activated lymphocytes and macrophages; the dermal infiltrate is prominent in this section (× 160). (f) 48 h irritant reaction: increased numbers of DA6.147 reactive cells are present in the epidermis (× 320).

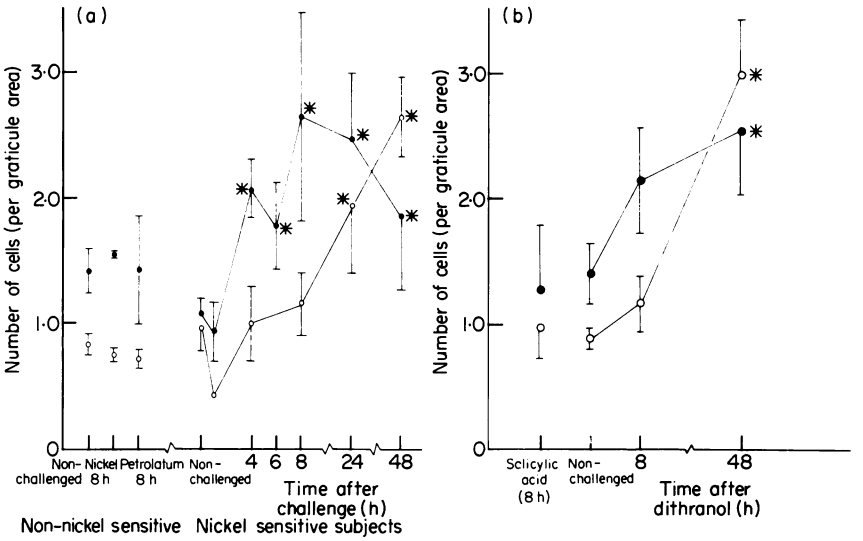

Fig. 3. Upper dermal counts (positive cells per graticule area) for Leu M3 (○) and Leu 6 (●) reactive cells at various time points in the (a) nickel allergic and (b) dithranol irritant reactions: mean ± s.e.m. $^*P < 0{\cdot}05$.

probably due to the infiltration of activated T cells and macrophages (Fig. 2). No keratinocyte reactivity with DA6.147 was observed.

Changes in controls. Minor increases in Leu 3a positive cells in the epidermis were noted after 8 h occlusion with petrolatum (one subject), petrolatum with salicylic acid (two subjects) and with 5% nickel sulphate in petrolatum (two non-nickel sensitive subjects); in addition, two subjects showed slight increases in Leu 2a and 3a reactive cells in the dermis with petrolatum. These changes were numerically small and not statistically significant. No changes with other monoclonal antibodies were found in the controls.

Inter-personal variability. Detailed analysis showed variation between individuals in the rate and intensity at which the allergic and irritant contact reactions developed. For example, in the nickel hypersensitivity reaction, not all subjects showed an epidermal influx of Leu 3a reactive cells at 4 h, but all did by 48 h, although it varied between five and 24 cells per 200 basal keratinocytes. Other such variations occurred and the standard errors give an indication of this. In the irritant study, the six psoriatics were compared to the three non-psoriatics, although valid comparisons were difficult due to the small numbers in each group. In non-involved, non-patch tested skin, the psoriatics had marginally more Leu 2a positive cells in the dermis than in the normals' skin, but no more Leu 3a reactive cells. Non-involved psoriatic epidermis on average also had more DA6.147 reactive cells, 13·3 per 200 basal keratinocytes, compared to 12·2 for non-psoriatic epidermis. Forty-eight hours after dithranol application, psoriatic subjects had slightly more Leu 2a positive cells, and fewer Leu 3a reactive cells in the dermis, than the non-psoriatic individuals did, although all subjects showed substantive increases over baseline values. The lower Leu 3a dermal count at 48 h was the only statistically significant difference between the psoriatic and non-psoriatic subjects (two sample *t*-test $P < 0{\cdot}05$).

Electron-microscopic examination. One hour after nickel allergic challenge, Langerhans cells with electron dense cytoplasm and vacuoles occupied a basal position (Fig. 4) and outnumbered normal looking suprabasal Langerhans cells by 2 to 1. Peripolesis in the epidermis was found at 4 h and Langerhans cells containing Birbeck granules were noted in the dermis. Lymphocytes and macrophages infiltrated the dermis and epidermis at 48 h.

Changes in the irritant reaction were dramatic: the mitochondrial cristae of Langerhans cells were disrupted at 8 h (Fig. 4) though the outer membranes were not affected. Mitochondria within keratinocytes showed similar but less severe changes. No intact Langerhans cells containing

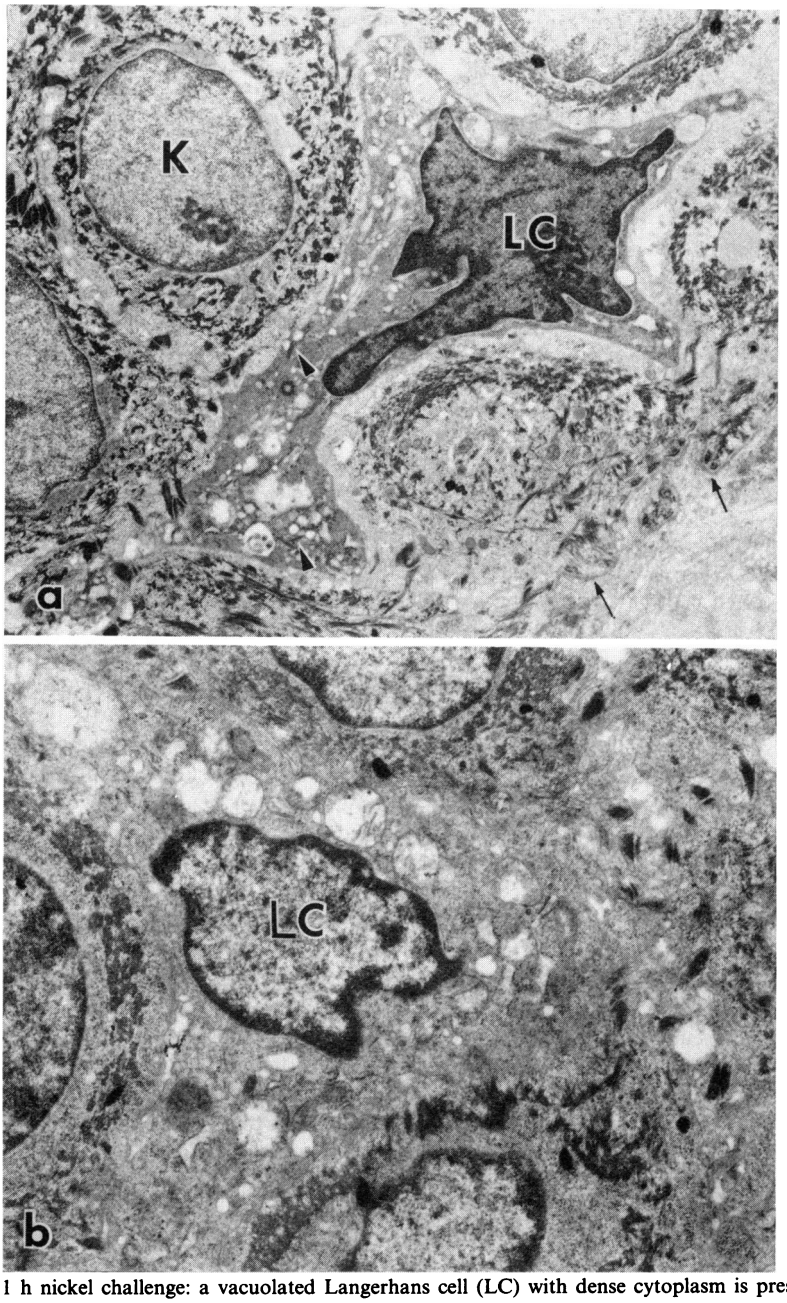

Fig. 4. (a) 1 h nickel challenge: a vacuolated Langerhans cell (LC) with dense cytoplasm is present in the suprabasal layer of the epidermis. The surrounding keratinocytes (K) appear normal. Birbeck granules are shown by arrowheads and the basal lamina is demonstrated by arrows (electron micrograph, × 11800). (b) 8 h dithranol reaction: disrupted cristae are seen within the swollen mitochondria of a Langerhans cell (LC). Surrounding keratinocytes appear normal (electron micrograph, × 15000).

Birbeck granules were found in the epidermis at 48 h, but acantholysis, debris from damaged cells, active lymphocytes and macrophages containing phagosomes were present. In one subject, a Birbeck-granule-positive Langerhans cell was apposed to an active lymphocyte in the upper dermis. Mast cells and basophils were not prominent in the infiltrate of either irritant or allergic reactions.

DISCUSSION

Qualitative similarities in the cellular infiltrates of experimentally induced allergic and irritant contact dermatitis have been noted (Kanerva *et al.*, 1984; Scheynius *et al.*, 1984). We confirm that the infiltrating T cell and macrophage types are similar, but the dithranol irritant model which we studied was distinguished from the nickel allergic reaction by a fall in epidermal Langerhans cell numbers and a quantitatively greater epidermal influx of Leu 3a reactive cells at 48 h. Reduced epidermal Langerhans cell numbers were found with the irritant sodium lauryl sulphate (Ferguson *et al.*, 1985) but were not seen in a previous dithranol study (Kanerva *et al.*, 1984), possibly due to different experimental conditions. Although the epidermal lymphocytic infiltrate may be greater in the irritant reaction, we observed, as others have, that dermal T cell numbers are larger in the allergic situation (Scheynius *et al.*, 1984; Ferguson *et al.*, 1985).

Leu M3 reactive macrophages were slightly more numerous in the epidermis of the 48 h irritant reaction than in the 48 h allergic response. Ultrastructurally, these macrophages contained phagocytosed melanin granules and resembled those seen in ultraviolet damaged epidermis (Cooper *et al.*, 1985). Their presence probably reflects the degree of tissue damage induced by dithranol and they may non-specifically augment the local cellular infiltrate. Peripolesis was noted in both irritant and allergic reactions, emphasizing that it is not an exclusive feature of the latter (Kanerva *et al.*, 1984). Basophils and mast cells were not prominent ultrastructurally in the infiltrates of either reaction. They may be important in the early stages of the contact hypersensitivity response (Dvorak *et al.*, 1974; Askenase & Van Loveren, 1983), and we have not excluded their participation as we did not look specifically for them. However, our findings of an early rise in epidermal Leu 6 reactive cell numbers in the allergic reaction, with some cells occupying a basal position, supports the suggestion that the Langerhans cell is instrumental in the initiation of allergic contact dermatitis (Carr *et al.*, 1984).

The rate and magnitude of cellular responses varied between individuals within both the allergic and irritant groups. This presumably reflects differences in the degree of hypersensitivity, and the susceptibility to irritants. The only significant difference between the psoriatic and non-psoriatic subjects in the irritant study was a reduced Leu 3a dermal response in the psoriatic group at 48 h. Baker *et al.* (1984) reported increased Leu 2a and Leu 3a reactive cells in the uninvolved dermis of subjects with guttate psoriasis. We found slightly but not significantly raised dermal Leu 2a counts, although the Leu 3a numbers were similar to normal. However, we did not examine as many subjects as Baker *et al.* (1984), and our individuals had chronic plaque, not guttate, psoriasis.

Subtle differences existed between allergic and irritant reactions. The main disparity was in the fate of the Langerhans cells which are damaged by irritants but increased in number during an allergic challenge. Since Leu 2a and Leu 3a reactive cells each represent heterogeneous groups, future study may reveal other differences between allergic and irritant responses by defining more precisely the T cell subtypes involved in these reactions.

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