# T and B Lymphocytes, Macrophages and Langerhans' Cells during the Course of Contact Allergic and Irritant Skin Reactions in Man

## An Immunohistochemical and Electron Microscopic Analysis

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The *in situ* distribution of lymphocyte subclasses, macrophages and Langerhans' cells was analysed with histochemical (ANAE staining), immunohistochemical (immunoperoxidase) and electron microscopic methods during the course of epicutaneous allergic and irritant skin reactions. Serial biopsies were taken during the first 5–72 hours following the allergen (nickel and chromium) or irritant (non-anoic acid and dithranol) application. Empty and petrolatum-filled Finn chambers were used as controls. No significant differences in the number of mononuclear inflammatory cells or their subclasses were found in the early recognition and elicitation phases of the allergic or irritant-induced reactions. Nor was there any difference in the frequency of lymphocyte–Langerhans' cell contacts, although exocytic cells were most frequent in dithranol reactions. *Key words: Contact dermatitis; T and B lymphocytes; Langerhans' cell.* (Received November 9, 1982.)

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The immunopathological differences between allergic and irritant skin reactions are still unresolved. In a recent study by Reitamo et al. (17) the inflammatory cell subtypes were shown to be similar in fully developed allergic and irritant skin reactions elicited by the epicutaneous test in human skin. On all occasions, T lymphocytes were described as the dominant cell type. The aim of this study was to further characterize the inflammatory cell subclasses with histochemical, immunohistochemical and electron microscopic methods *in situ*, and to compare the early antigen recognition phase of allergic skin reaction to irritant and control reactions in an epicutaneous test system. Particular attention was paid to the distribution of lymphocytes subclasses and Langerhans' cells during the course of the reaction. Serial dilutions of the allergen and irritant substance were made on each occasion, to allow the comparison of reactions with clinically equal intensity.

## MATERIALS AND METHODS

### Patients

15 patients previously tested and known to have delayed-type hypersensitivity to either nickel (Ni) or chromium (Cr) volunteered for the study. The patients were otherwise healthy.

### Epicutaneous tests

Finn-chambers were used for the epicutaneous tests (13). The test chambers were removed after 5–24 hours. Empty 8-mm aluminium chambers (C) and white petrolatum-filled chambers (P) were used as

controls. Each patient was tested simultaneously for one allergen, for two irritants, non-anoic acid (NA) (28) and dithranol (D), and for the controls. Nickel (Ni) and chromium (Cr) were first tested in three concentrations (0.03 %, 0.10 % and 0.32 % in petrolatum), and the lowest concentration giving a clinically positive test reaction during 4 days of observation was chosen. Non-anoic acid and dithranol were similarly tested in serial dilutions (NA 20 % and 40 %, D 0.025 mMkg$^{-1}$, 0.05 mMkg$^{-1}$ and 1 mMkg$^{-1}$) to provoke a clinically analogous reaction to that given by the allergic compound.

*Biopsies*

A skin biopsy of the allergen, irritant, and control reactions was taken simultaneously on 3 or 4 occasions during 5–72 hours. The results of the biopsies taken at 5, 6, 7 or 8 hours have been combined and given as mean values for 6 or 7 hours. There were altogether 7 biopsies of empty chamber, 7 of petrolatum, 8 of Ni, 8 of Cr, 14 of NA and 11 of D test sites.

*Histochemical and immunohistochemical studies*

One-half of the biopsy was fixed in Baker's for ANAE staining and processed as previously described (16). Briefly, the tissues were fixed for 4—8 hours, washed in Holt's solution, dehydrated in acetone and embedded in paraffinwax. For the ANAE staining the paraffin sections were deparaffinized, washed, and incubated in the presence of $\alpha$-naphthylacetate and hexazotized pararosaniline in Sørensen's phosphate buffer in conditions described elsewhere (15), for 4 h at room temperature. Thereafter the slides were counterstained with toluidine blue, cleared in methanol and mounted.

The other half of the tissue was fixed in Bouin's for 4–8 hours, dehydrated and embedded in paraffinwax for the demonstration of intracellular immunoglobulin with the PAP-immunoperoxidase technique (22). The sections were first deparaffinized, incubated in methanol – $H_2O_2$ to abolish the endogenous peroxidase, washed, and incubated with normal swine serum and then with either rabbit-antihuman IgG, -IgM, -IgA (diluted 1 : 1000) or -IgE (diluted 1 : 10) (Dakopatts, Copenhagen, Denmark). Thereafter the sections were washed in PBS, incubated with swine anti-rabbit Ig (Dakopatts, diluted 1 : 100) for 30 min, washed, and incubated with PAP (peroxidase–anti–peroxidase(raised in rabbit, Dakopatts, diluted 1 : 500) for 30 min. The peroxidase was then visualized with 3-amino-9-ethylcarbazole (Sigman Chemical Comp., St. Louis, Mo, USA) and dimethylformamide. The slides were counterstained with Mayer hematoxylin and mounted. To detect non-specific staining the primary antibody was omitted. Routine hematoxylin-eosin staining of each biopsy was also done.

*Quantitation of inflammatory cells*

The infiltrating cells were counted with a Zeiss microscope equipped with an ocular supplied with a grid (8836 $\mu m^2$) by using 10×100 magnification and oil immersion. In each section, five squares were counted form the perivascular infiltrates. Lymphocytes expressing the dot-like ANAE reaction (T-ANAE), cells expressing the diffuse type ANAE reaction (M-ANAE), and ANAE-negative cells were counted separately. T-ANAE cells were considered as T-lymphocytes, M-ANAE cells were considered to represent cells of monocyte–macrophage lineage and ANAE-negative mononuclear cells to represent mainly cells of B lymphocyte lineage (14). The peroxidase-positive plasma cells and the cells infiltrating the epidermis (exocytic cells) were counted in the same way. All samples were counted blind.

*Electron microscopic studies*

Part of the biopsies were fixed for electron microscopy in cacodylate or phosphate buffered (0.1 M; pH 7.3) 2.5 % glutaraldehyde for 2 hours or longer at 4°C and post-fixed with 1 % osmium tetroxidase, dehydrated in graded series of alcohol and embedded in Epon 812. Ultrathin sections were cut with a diamond knife on a LKB ultramicrotome, stained with lead citrate and examined with a Jeol 100S or a Jeol 100CX electron microscope operated at 80 kV. Samples from 24 biopsies were examined electronmicroscopically.

# RESULTS

## I. *Light microscopic studies*

*Histology of the reactions.* On all occasions, an inflammatory cell infiltrate was seen perivascularly and near the follicles in upper dermis. Exocytic cells were seen in the epidermis, being most numerous in dithranol reactions. Some intercellular oedema (spongiosis) was apparent, but a fully developed eczema with blistering was never seen histologically, probably because the allergen/irritant concentrations were initially chosen
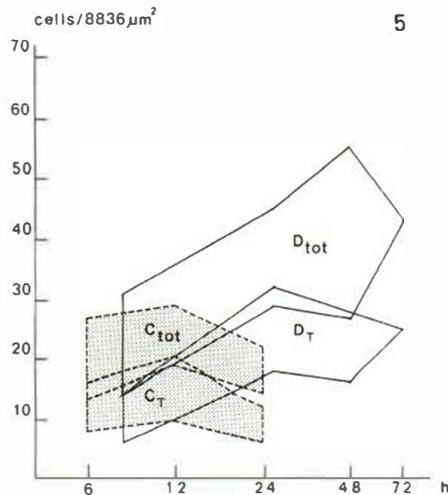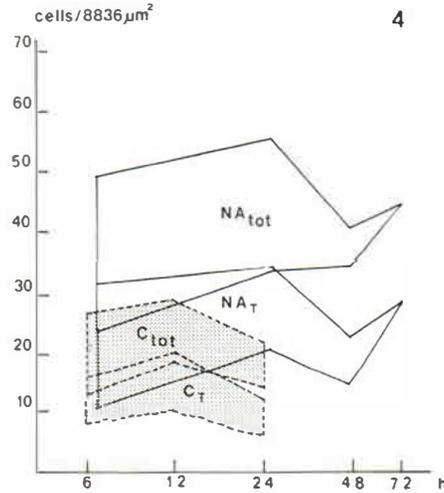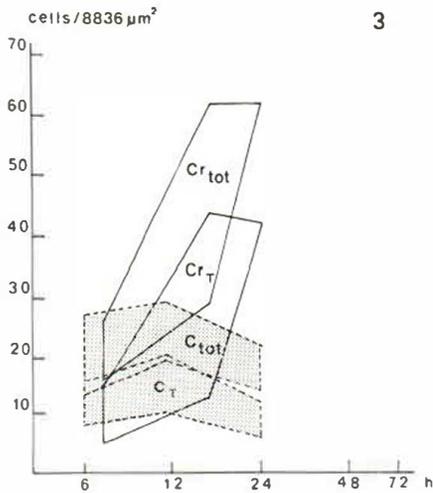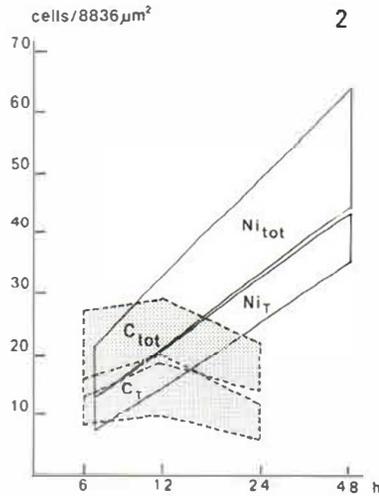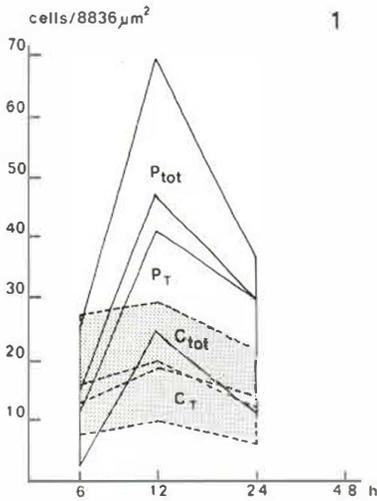
Fig. 1. The numbers (mean ± SD) of inflammatory mononuclear cells in upper dermis elicited by empty Finn-chamber ($C_{tot}$) and white petrolatum- filled Finn-chamber ($P_{tot}$) epicutaneous test, and the number of T-ANAE lymphocytes in the respective reactions ($C_T$ and $P_T$).

Fig. 2. The numbers (mean ± SD) of inflammatory mononuclear cells ($Ni_{tot}$) and T-ANAE lymphocytes ($Ni_T$) in upper dermis in epicutaneous nickel reactions.

Fig. 3. The numbers (mean ± SD) of inflammatory mononuclear cells ($Cr_{tot}$) and T-ANAE lymphocytes $Cr_T$ in upper dermis in epicutaneous chromium reactions.

Fig. 4. The numbers (mean ± SD) of inflammatory mononuclear cells ($NA_{tot}$) and T-ANAE lymphocytes ($NA_T$) in upper dermis in epicutaneous non-anoic acid reactions.

Fig. 5. The numbers (mean ± SD) of inflammatory mononuclear cells ($D_{tot}$) and T-ANAE lymphocytes ($D_T$) in upper dermis in epicutaneous dithranol reactions.

to give the weakest clinically detectable reaction. Neutrophilic granulocytes were very seldom seen, but occasionally appeared in reactions biopsied more than 24 hours after the challenge.

*Lymphocyte subclasses and macrophages.* The total numbers of mononuclear inflammatory cells infiltrating the dermis in the various allergic, irritant and control reactions are shown in Figs. 1–5. Empty or petrolatum-filled chambers gave no erythema or other sign of inflammation at the time of biopsy. However, a slight increase in the number of perivascular cells infiltrating the dermis was usually seen when compared with uninvolved skin where only about 10–15 infiltrating cells were recorded in a similar area. There was also a clear cellular response to plain petrolatum stimulus over that of empty chamber background (Fig. 1). This 'control response', however, had subsided by 24 h. Instead, the cellular response provoked by nickel and chromium steadily increased towards the end of observation period, i.e. 48 h (Figs. 2 and 3). A very similar initial reaction pattern was provoked by the irritant compound non-anoic acid, but the number of infiltrating cells then decreased (Fig. 4). The reaction pattern of dithranol was similar to that of NA, but slower (Fig. 5).

The distribution of lymphocytes showing dot-like ANAE activity (T-ANAE, T-lymphocytes) closely followed the degree of inflammation in all reaction types (Figs. 1–5).

Table I. *The ratio of T-ANAE/M-ANAE cells in the inflammatory infiltrates and the number of exocytic ANAE-positive cells in allergic, irritant, and control epicutaneous test reactions*

| Test compund[a] | Time of biopsy[b] (h) | n | T-ANAE/M-ANAE cells in the inflammatory infiltrates | ANAE + cells/0.04 mm$^2$ epidermis |
|---|---|---|---|---|
| Empty chamber | 6 | 3 | 1.9±0.8[c] | 1±1 |
|  | 12 | 2 | 2.8±1.2 | 1±1 |
|  | 24 | 2 | 1.3±0.6 | 1±1 |
| P | 6 | 3 | 1.4±1.8 | 3±2 |
|  | 12 | 2 | 2.9±0.3 | 2±1 |
|  | 24 | 2 | 4.4±4.1 | 2±1 |
| Ni | 7 | 4 | 1.9±0.9 | 3±0 |
|  | 24 | 1 | 1.1[d] | 6 |
|  | 48 | 2 | 5.0±1.1 | 2 |
| Cr | 6 | 3 | 3.1±3.1 | 1±5 |
|  | 16 | 2 | 5.1±4.6 | 2±2 |
|  | 48 | 1 | 11.0 | 5 |
|  | 72 | 1 | 2.2 | 7 |
| NA | 7 | 5 | 1.3±0.8 | 4±3 |
|  | 24 | 5 | 5.0±1.4 | 3±1 |
|  | 48 | 3 | 0.7±0 | 5±4 |
|  | 72 | 1 | 5.8 | 0 |
| D | 8 | 3 | 1.8±1.0 | 3±0 |
|  | 24 | 5 | 3.2±1.6 | 6±2 |
|  | 48 | 3 | 1.4±0.3 | 4±4 |
|  | 72 | 1 | 1.9 | 2 |

[a] P = white petrolatum-filled chamber. Ni = nickel, Cr = chromium, Na = non-anoic acid, D = dithranol.
[b] Hours after allergen/irritant application, chambers were removed latest at 24 h.
[c] Mean ± SD.
[d] One determination only.

Table II. *Quantitation of the electron microscopy analysis*
*n* = number of patients studied

| Type of skin test | Total number of Langerhans' cells counted | Number of Langerhans' cells showing apposition to mononuclear cells |
|---|---|---|
| Positive contact allergic test sites (*n*=6) | 40 | 3 |
| Positive contact irritant test sites (*n*=9) | 54 | 4 |
| Controls (petrolatum or empty chambers) (*n*=4) | 26 | 2 |

The number of cells expressing diffuse ANAE activity (M-ANAE, monocytes, macrophages) did not increase in reactions provoked by empty chambers. In the response to petrolatum, nickel, chromium, and non-anoic acid, they had increased in number by 24 h whereafter their number decreased to the initial values—except in Cr reactions, where the number of M-ANAE cells remained high. The number of M-ANAE cells in dithranol reactions peaked at 48 h. The ratio of T-ANAE cells to M-ANAE cells in the above reactions is given in Table I.

The distribution of ANAE-negative mononuclear cells (not shown) was as follows: in empty chamber and NA reactions the number of ANAE-negative cells did not increase, but with petrolatum the number of these cells peaked at 12 h, with Ni at 24 h, with Cr at 16 h and D at 48 h, and then decreased to the initial levels.

*Immunoglobulin-containing (plasma) cells.* Empty chamber, petrolatum, NA, D and Ni did not induce any significant plasma cell response. Chromium induced IgA- and IgG-containing plasma cells to appear at perivascular locations between 24 h and 48 h (twice the number of plasma cells seen in control reactions, i.e. about 20 cells/0.04 mm$^2$).

*Cells infiltrating the epidermis (exocytic cells).* The predominant exocytic cell (Table I) was an ANAE-positive cell. ANAE-negative exocytic cells were occasionally seen in three biopsies. The number of exocytic cells in the different reactions was approximately the same. The only significant difference was the low number of ANAE-positive exocytic cells in empty chamber reactions compared with the high numbers recovered in dithranol reactions at 24 h (p=0.03 in Student's *t*-test).

## II. *Ultrastructural studies*

Chief attention was paid to the Langerhans' cells and their apposition to mononuclear cells. It was noted (Table II) that Langerhans' cell–mononuclear cell contacts are seen not only at contact allergic patch test sites, but also at irritant test sites (Fig. 6 AC) as well as after Finn chamber occlusion with (Fig. 6 B) or without white petrolatum. A morphological distinction in the Langerhans' cells could not be made in the different groups. Thus,

*Fig. 6 A.* Small lymphocyte (*Ly*) juxtaposed (*thick arrow*) to Langerhans' cell (*L*). The Langerhans' cell shows widened endoplasmic reticulum (*er*) and Golgi areas (*G*), numerous Birbeck granules (*thin arrow*) and disrupted mitochondriae (*m*). Irritant (dithranol) patch test. 30 h. ×14 800.

*Fig. 6 B.* Lymphocyte (*Ly*)–Langerhans cell (*L*) contact after 5 hours of petrolatum-filled chamber occlusion. *I*, intercellular space; *c* centriole. ×22 000.

*Fig. 6 C.* Active-looking Langerhans cell. Irritant (non-anoic acid) patch test. 24 h. *Er*, widened endoplasmic reticulum containing fine granular substance; *ly*, lysosome; *li*, lipid droplet; *arrow*, Birbeck granule. ×22 000.
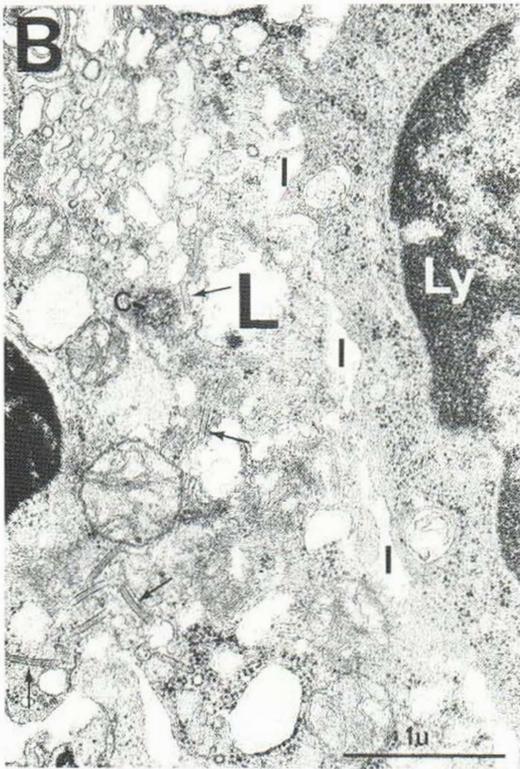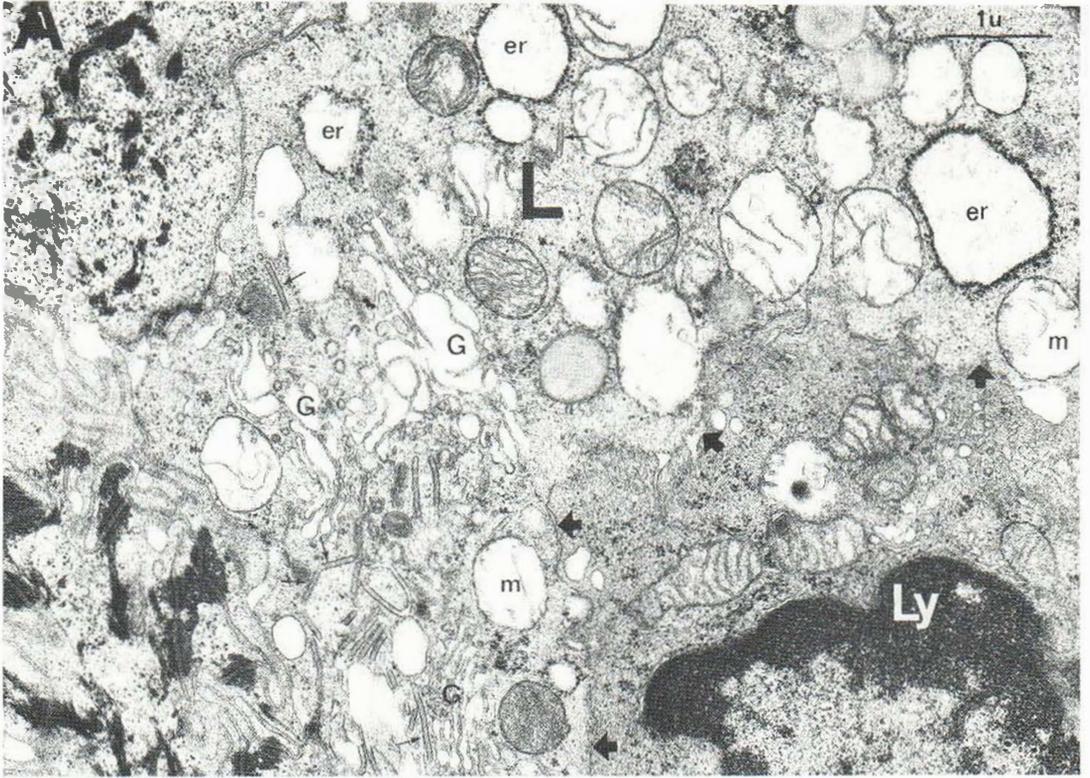
'active'-looking Langerhans' cells with widened rough endoplasmic reticulum, suggesting protein synthesis, were seen also after an irritant reaction (Fig. 6).

## DISCUSSION

Differentiation between allergic and irritant skin reactions is a common problem in clinical dermatology. The histology of allergic and irritant skin reactions is the same, usually that of any eczema (7, 10, 12). The immuno-pathology of the reaction is not completely known. It has been shown that the cell involved in the antigen presentation is the Langerhans' cell (1, 2, 21). This cell has many properties common to macrophages, such as Fc- and $C_3$-receptors (25) and surface Ia (DR) antigens (6, 19). Ia antigen expression has been postulated to be associated with the capacity to present antigens to T lymphocytes (24). The delayed hypersensitivity reactions are considered to be T lymphocyte-mediated and a specific 'delayed hypersensitivity T cell' has been suggested to exist—at least in mice (11).

The histochemical ANAE staining was chosen because in ANAE-stained sections one can simultaneously recognize the lymphocyte subclasses (T-ANAE or negative, i.e. T or B lymphocytes) and cells of macrophage origin (M-ANAE). One must remember, however, that stimulated lymphocytes can change their 'original' pattern of ANAE-staining, and possibly activated macrophages, too (26). Skin fibroblasts did not express ANAE activity as demonstrated in dermatofibromas and cultured skin fibroblasts (Niemi & Ranki, unpublished). The ANAE-positive exocytic cells in epidermis represented both T-ANAE and M-ANAE pattern, the latter probably including Langerhans' cells too (4, 9).

The fine-structure analysis showed that Langerhans' cell—lymphocyte contacts are not specific for allergic reactions as was originally suggested by Silberberg (20). Our observation is in accordance with the recent report of Lindberg and Forslind (8), that occlusion, *per se,* results in apposition of Langerhans' cells to mononuclear cells. Lymphocyte–Langerhans' cell contacts have also been reported in malignant epidermotrophic lymphomas (18), lamellar ichthyosis (5), atopic dermatitis (27) and in normal skin (3). Thus, the significance of Langerhans' cell–lymphocyte contacts needs further clarification, although firm evidence of its significance in allergic contact reactions has been published (23).

Concerning the number and distribution of lymphocyte subclasses (T and B cells), our results confirm those of Reitamo et al. (17) that there is no significant difference between the major lymphocyte subclasses in allergic and irritant skin reactions. In this study we have traced the initially appearing inflammatory cells after antigen recognition, by studying the site of antigen application as early as after 5 hours' exposure. The slight increase in the perivascular cell population seen in the control reactions was probably due to a non-specific stimulus, possibly occlusion. As there was no difference in the number of T and B lymphocytes or other immunocompetent cells in the early phases of the allergic/irritant reactions, it may be assumed that the recognition and early elicitation phases are performed by a heterogeneous, pluripotent group of cells, the real differences being possibly due to the lymphokines secreted by these cells.

Further investigations with monoclonal antibodies and immunoperoxidase techniques to detect T inducer/helper and -suppressor/cytotoxic cells in these reactions will possibly also help us to understand the role of the Langerhans' cell in T lymphocyte subset–B lymphocyte interactions in allergic skin reaction.

## ACKNOWLEDGEMENTS

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