

Langerhans cells in human allergic contact dermatitis contain varying numbers of Birbeck granules

Double staining immunohistochemistry with OKT6 and Lag antibody *

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Summary. Dynamic changes in human Langerhans cells (LCs) were studied with OKT6, anti-HLA-DR antibody, and Lag antibody in allergic contact dermatitis (ACD). Both T6-positive (T6⁺) cells and Lag-positive (Lag⁺) cells in the epidermis decreased in number from 0 to 48 h, but then gradually increased after day 7 of ACD. Lag⁺ cells after day 7 manifested a variety of staining intensities from weak to strong. It was also shown, after day 7, that some T6⁺ cells were Lag negative whereas all Lag⁺ cells were T6 positive. Flow cytometric analysis suggested that Lag-strongly-positive cells and Lag-weakly-positive cells belonged to the same population, and that the relative amount of Lag antigens in T6⁺ LCs gradually increased after day 7. Immunoelectron microscopy revealed that the Lag-strongly-positive cells contained numerous Lag-reactive Birbeck granules (BGs) whereas the Lag-weakly-positive cells contained fewer BGs in the cytoplasm. In some Lag-weakly-positive cells, no BGs were detected.

Introduction

In allergic contact dermatitis (ACD), Langerhans cells (LCs) present the contact allergens to lymphocytes, which then proliferate and release lymphokines that in turn cause damage to LCs (Silberberg et al. 1974, 1975, 1984). From 0 to 72 h, after lymphokine injury, epidermal LCs decrease in number in ACD. On the other hand, a few investigators have observed recovery in the number of epidermal LCs after day 3 of ACD (Aiba et al. 1984; Bressler et al. 1984; Bian and Bing-He 1985; Marks et al. 1987).

OKT6 and anti-HLA-DR antibody have been used in immunohistochemical studies of LCs (Harrist et al.

1983; Mackie and Turbitt 1983; Dezutter-Dambuyant et al. 1985; De Jong et al. 1986). These monoclonal antibodies (mAbs) react to the surface antigens of LCs but not to the Birbeck granules (BGs) which are the most characteristic marker of LCs (Birbeck et al. 1961). Thus, reactivity to these mAbs, especially to HLA-DR, was not absolutely specific for LCs (Harrist et al. 1983), since other HLA-DR-positive cells can infiltrate to the skin under the inflammatory conditions and keratinocytes can also express HLA-DR antigens in several dermatoses (Aiba and Tagami 1984; Lampert 1984; Gawkrödger et al. 1987). It is also possible that the cells in the skin reactive to these mAbs belong to a population different from the BG-containing LCs.

Recently, we established a mAb designated "Lag", that specifically reacts to BGs and the related structures of human LCs, and showed that this antibody is useful in identifying LCs in normal and diseased human skin such as Letterer-Siwe disease (Kashiwara et al. 1986; Kashiwara-Sawami et al. 1988). In this report, epidermal LCs in ACD were studied with Lag and other mAbs to clarify the dynamics of BG-containing LCs in allergic reactions.

Materials and methods

Tissue preparation. Three healthy volunteers, 2 females and 1 male, aged 28–57 years, were sensitized on their forearms with 0.05 ml of 1% DNCB (dinitrochlorobenzene) dissolved in acetone and were subsequently challenged, 14 days post-sensitization, with 0.01 ml of 0.1% DNCB on multiple sites. Skin specimens were obtained from challenged backs at 0 (non-challenged site), 3, 6, 12, 24, and 48 h after the DNCB challenge and were used for immunohistochemical analysis. The remaining sites on their backs were covered for 48 h, biopsied on days 7, 9, and 11 after the challenge, and used for immunohistochemical analysis as well. Epidermal cells were obtained from suction blisters which were raised from the challenged forearms of one volunteer (Furukawa et al. 1987) and were isolated for flow cytometric analysis. Normal human epidermal cells obtained from a resected breast were used as controls for flow cytometric analysis.

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Monoclonal antibodies. The mAbs used were as follows: OKT6 (anti-CD1a antibody, Ortho Diagnostic Systems, Raritan, NJ, USA) (Harrist et al. 1983), anti-HLA-DR antibody (Becton Dickinson, FACS Systems, Sunnyvale, CA, USA) and Lag antibody (prepared in our laboratory and specifically reactive to BGs and related structures of human LCs) (Kashihara et al. 1986).

Immunoperoxidase staining. Biopsy specimens were cut into small pieces, fixed immediately in periodate-lysine-paraformaldehyde solution at 4° C, and embedded in optimal cutting temperature (OCT) compound Tissue-Tek II (Miles Laboratories, Naperville, IL, USA). Three serially-cut cryosections, 6- μ m-thick, were sequentially placed on glass slides. Each section was stained using avidin-biotin-peroxidase complex procedures (Hsu et al. 1981). Briefly, the primary mAb was added; OKT6 (5 μ g/ml) in the first section, Lag antibody (5 μ g/ml) in the second section, and anti-HLA-DR antibody (0.25 μ g/ml) in the third section. All sections were treated with biotinylated anti-mouse IgG (4 μ g/ml, Vector Laboratories, Burlingame, CA, USA), and avidin-biotin-peroxidase complex (Vector). The sections were then incubated in a solution of diaminobenzidine (0.2 mg/ml) and hydrogen peroxide (0.1 μ g/ml) in PBS, and counterstained with methylgreen. Positive cells were counted in a "blind" fashion by three dermatopathologists under direct observation at a magnification of 400 $\times$ in 20 unit lengths (each 0.25 mm) using a calibrated eyepiece micrometer. Only cells with green stained nuclei surrounded by brown granular precipitates were counted and the density of LCs in the epidermis was expressed as the number of positive cells per mm of epidermis. Results were statistically analyzed using Student's *t*-test.

Double immunofluorescence staining. Purified anti-T6 antibody and Lag antibody was conjugated with tetramethylrhodamine isothiocyanate (RITC) (Smith et al. 1962) and fluorescein isothiocyanate (FITC) (Riggs et al. 1958), respectively. Cryostat sections were incubated for 1 h at room temperature with a mixture of RITC-conjugated anti-T6 antibody (10 μ g antibody/ml) and FITC-conjugated Lag antibody (5 μ g antibody/ml). Each section was examined under an epifluorescence microscope (Nikon, Tokyo, Japan) through a G filter system for RITC-conjugated anti-T6 antibody or through a B filter system for FITC-conjugated Lag antibody (Kashihara-Sawami et al. 1988).

Immunoelectron microscopy. Cryostat sections (8 μ m thick) were treated sequentially with Lag antibody, biotinylated anti-mouse IgG, and avidin-biotin-peroxidase complex. They were fixed with 1% glutaraldehyde in PBS for 5 min, and treated with diaminobenzidine and hydrogen peroxide in PBS. The sections were then post-fixed for 1 h in 1% osmium tetroxide in water, dehydrated in a graded ethanol series, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and analyzed under an H-300 electron microscope (Hitachi, Tokyo, Japan) (Kashihara et al. 1986).

Immunoelectron microscopy was intensely done on the areas where LCs manifested a variety of staining intensities with lag antibody in the same field.

Preparation of epidermal cell suspensions. The specimens of resected breast were cut into small pieces and incubated overnight at 4° C in 0.5% dispase (Boehringer, Mannheim, W. Germany) in PBS. The epidermis was stripped from the dermis using forceps. The epidermis was also obtained from the tops of suction blisters on challenged forearms. These sheets were treated for 20 min at 37° C with 0.25% trypsin (Difco Laboratories, Detroit, MI, USA) in PBS and single cell suspensions were then obtained by pipetting and filtration. The viability of epidermal cells was more than 80% when measured by the trypan blue dye exclusion test.

Flow cytometric analysis. Epidermal cell suspensions were double stained with OKT6 and Lag antibody. The sequence of the staining procedures was as follows: OKT6, biotinylated anti-mouse IgG,

phycoerythrin (PE)-conjugated streptavidin, cold acetone for fixation and permeabilization, mouse IgG to quench residual anti-mouse IgG sites, and FITC-conjugated Lag antibody. Immunolabeling was performed at 4° C. Flow cytometric analysis was performed on epidermal cell suspensions. Fifty thousand cells were analyzed using a Becton-Dickinson FACS 440 equipped with laser excitation at 488.8 nm. Electronic compensation was employed using LCs stained with PE-conjugated OKT6, LCs stained with FITC-conjugated Lag, and LCs double stained with both antibodies. Cells were enumerated relative to log integrated green fluorescence, log integrated red fluorescence, forward angle light scatter, and right angle light scatter. The specificity of the staining was confirmed by immunofluorescence and flow cytometric analysis.

Results

Macroscopic observation of the challenged skin showed a mild inflammatory reaction from 3 h after the challenge. The reaction peaked at 48 h and then regressed. Scales and pigmentation were evident after day 7. Immunohistochemical observation showed no marked differences in the dynamics of LCs among three subjects.

LCs before elicitation

LCs in the skin were identified by immunoperoxidase staining with each mAb. Lag-positive (Lag⁺) cells were distributed evenly in the suprabasal layer as reported previously (Kashihara et al. 1986). T6-positive (T6⁺) cells and HLA-DR-positive (HLA-DR⁺) cells were ob-

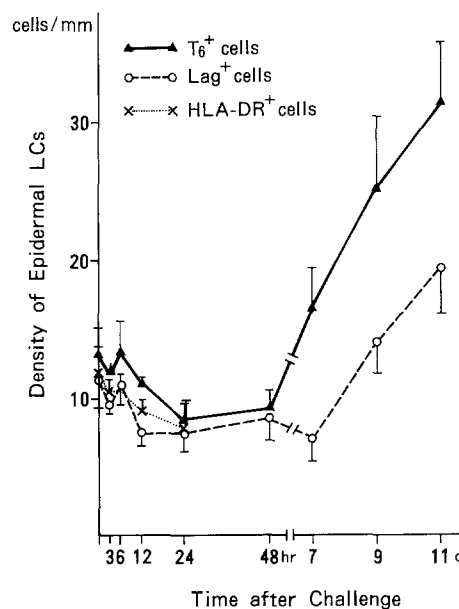


Fig. 1. The change in densities of epidermal LCs during ACD. Three volunteers were sensitized with 1% DNCB, and 14 days later their backs were challenged with 0.1% DNCB. Punch biopsies were taken at various times after the challenge and stained with OKT6, anti-HLA-DR, or Lag antibody. Positive cells were counted under direct observation and the density of LCs was expressed as the number of positive cells per mm of epidermis. Because of strong HLA-DR expression of keratinocytes, individual HLA-DR⁺ LCs were hardly identified after 48 h. Vertical bars indicate one standard deviation

Table 1. Immunohistochemical analysis of DNCB-challenged skin; location of T6⁺ cells and Lag⁺ cells

	Number of positive cells					
	0 h	3–12 h	24–48 h	7 days	9 days	11 days
<i>T6⁺ Cells</i>						
Epidermis						
Upper			+			±
Mid		+	+	+	+	++
Lower	+	+		+	+	++
Dermis						
Papillary		+		+	+	+
Reticular		+		+	++	+
<i>Lag⁺ Cells</i>						
Epidermis						
Upper			+			
Mid		+	+	±(S)	+(S)	++(S)
Lower	+	+		+(W)	+(SW)	+(SW)
Dermis						
Papillary					+(W)	+(SW)
Reticular					±(SW)	±(SW)

The number of positive cells is graded: ± slight to mild, + moderate, ++ marked. Blank means that almost no positive cells were observed.

(S)= The infiltrating cells were mainly Lag-strongly-positive.

(W)= The infiltrating cells were mainly lag-weakly-positive.

(SW)= The infiltrating cells consisted of both Lag-strongly-positive cells and Lag-weakly-positive cells

served in the same manner. Although T6⁺Lag⁺ cells were occasionally found (data not shown), no significant differences were noted in the total numbers of Lag⁺ cells, T6⁺ cells, and HLA-DR⁺ cells in the epidermis (Fig. 1). Lag⁺ cells, T6⁺ cells, and HLA-DR⁺ cells were only rarely seen in the dermis.

LCs from 3 to 48 h after the challenge

The challenge with DNCB resulted in a slight decrease in the number of Lag⁺ cells, T6⁺ cells, or HLA-DR⁺ cells in the epidermis during the first 24 h (Fig. 1). Because of the strong expression of HLA-DR antigens by keratinocytes, individual HLA-DR⁺ LCs could not be clearly differentiated from HLA-DR⁺ keratinocytes at 48 h. The morphology and location of Lag⁺ cells in the epidermis changed gradually. From 3 to 12 h, they were present in the lower and mid-epidermis with prominent extending dendrites. From 24 to 48 h, they were generally located in the mid- and upper epidermis (Table 1). The dendrites of Lag⁺ cells were not prominent in this stage. T6⁺ cells and HLA-DR⁺ cells in the epidermis showed the same pattern of distribution and morphology as Lag⁺ cells.

T6⁺ cells appeared in the dermis at 3 h after the challenge with DNCB, and increased in number until 12 h in two of three subjects (Table 1). On the other hand, very few Lag⁺ cells were seen in the dermis in all three subjects. Most of the perivascular infiltrating cells in the reticular dermis exhibited HLA-DR antigens on their surfaces.

LCs after 7 days after the challenge

Throughout this phase, we could not accurately estimate the number of HLA-DR⁺ LCs because of the presence of HLA-DR⁺ keratinocytes and HLA-DR⁺ infiltrating cells.

The number of epidermal T6⁺ cells on day 7 was larger than at 48 h, and it greatly increased on days 9 and 11 (Fig. 1). Most of the T6⁺ cells in the epidermis were located in the mid- and lower epidermis (Table 1). No significant variety in staining intensity was noted in the T6⁺ cells. A considerable number of T6⁺ cells were observed in the dermis throughout the course (Fig. 2).

LCs, on the other hand, manifested a variety of staining intensities with Lag antibody after day 7. Some cells were stained as strongly as those before elicitation and others were stained only weakly and to varying degrees. Therefore, we classified Lag⁺ cells into Lag-strongly-positive cells (with intensities similar to LCs before elicitation) and Lag-weakly-positive cells (with intensities less than LCs before elicitation). Lag-strongly-positive cells were observed mainly in the mid-epidermis on day 7, and also in the mid- and lower epidermis on days 9 and 11, while Lag-weakly-positive cells were mainly observed in the lower epidermis throughout the course (Table 1). The number of epidermal Lag⁺ cells on day 7 was slightly smaller than that before elicitation, and it gradually increased on days 9 and 11. The number of Lag⁺ cells was always smaller than that of T6⁺ cells throughout the course. No clusters of more than two LCs or mitotic figures were observed either with OKT6

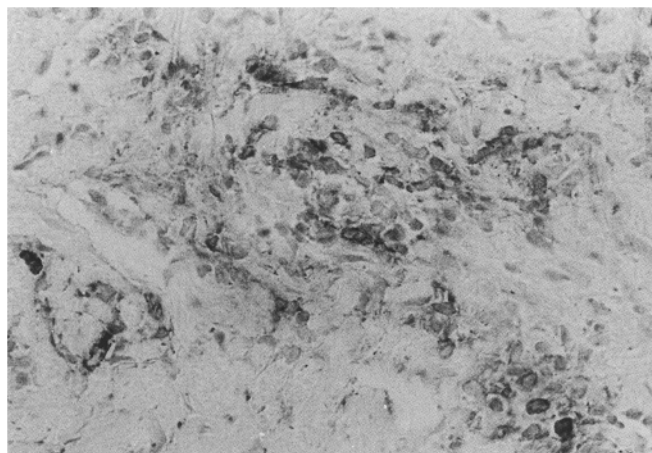


Fig. 2. Immunoperoxidase staining of human skin on day 7 of ACD. Cryosections were stained with OKT6. A considerable number of T6⁺ cells were seen in the perivascular dermis. They were mainly Lag negative. $\times 125$

or Lag antibody. Most of Lag⁺ cells in the papillary dermis were stained weakly, while only a small number of them were stained strongly on days 9 and 11. Lag⁺ cells were scarcely seen in the reticular dermis.

The double immunofluorescence staining study with RITC-labeled T6 antibody and FITC-labeled Lag antibody after day 7 revealed that most of the T6⁺ cells in the mid epidermis were stained strongly with Lag antibody, while those in the lower epidermis were stained weakly to varying degrees (Fig. 3). Some T6⁺ cells in the epidermis were not reactive to Lag antibody though all Lag⁺ cells were reactive to OKT6. It was also revealed that most of T6⁺ cells in the papillary dermis were stained weakly with Lag antibody while those in the reticular dermis had no reactivity to it.

To clarify the differences between Lag-strongly-positive cells and Lag-weakly-positive cells, immunoelectron microscopy was done with Lag antibody. It revealed that in Lag-strongly-positive cells, numerous BGs including typical racket shaped ones were surrounded by dense reaction products in the cytoplasm (Figs. 4 and 5). On the contrary, less BGs associated with the reaction products were detected in Lag-weakly-positive cells (Figs. 4 and 5). In some Lag-weakly-positive cells, reaction products seemed to associate only with indeterminate tiny vesicular structures, and obvious BGs were not noted (Fig. 6).

Epidermal LCs on days 0, 7, 14, and 21 were analyzed by two-color flow cytometry with OKT6 and Lag antibody to clarify the relationship between Lag-strongly-positive cells and Lag-weakly-positive cells. The stainability and specificity of these mAbs were confirmed by the fact that double stained cells showed green fluorescence (Lag antibody) in their cytoplasm and red fluorescence (OKT6) on their cell surfaces (Fig. 7). After 7 days of ACD, there was a broad distribution of fluorescence intensities of Lag antibodies in T6⁺ cells (Fig. 8B, C, D), with the mean fluorescence intensity gradually increasing during the course (Fig. 8, Table 2).

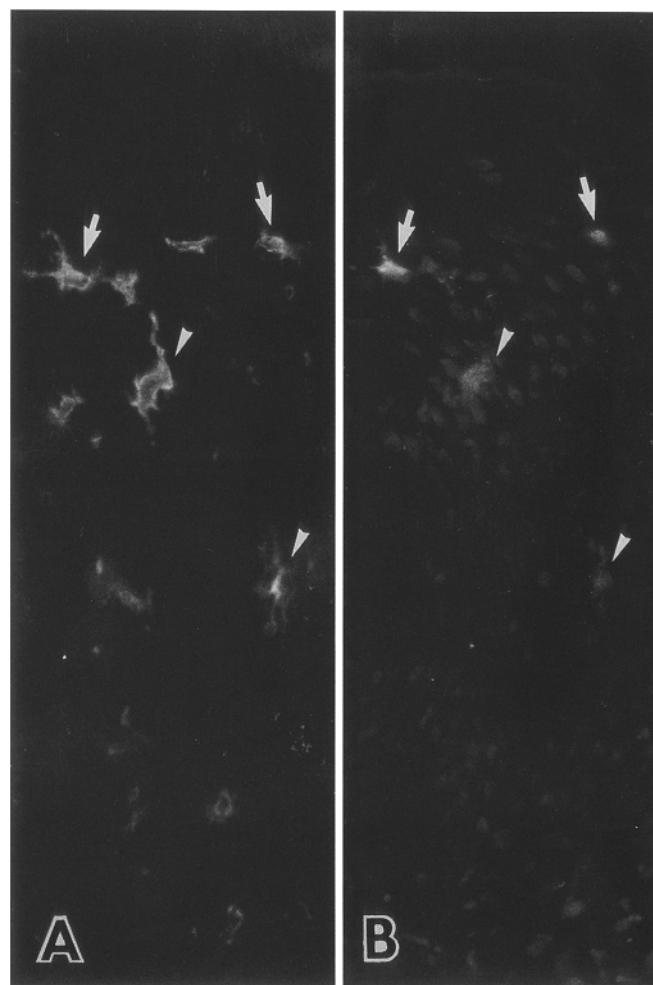


Fig. 3 A and B. Double immunofluorescence micrographs of human skin on day 7 of ACD. Cryosections were stained with RITC-conjugated anti-T6 antibody and FITC-conjugated Lag antibody. The fluorescence of RITC is seen in A, and the fluorescence of FITC in B. A lot of T6⁺ cells were seen in the acanthotic epidermis whereas fewer Lag⁺ cells were seen in the same area. Some T6⁺ cells in the mid-epidermis stained strongly with Lag (arrows) while others stained only weakly (arrowheads). Other T6⁺ cells were Lag negative. $\times 1000$

Table 2. Flow cytometric analysis of epidermal cell suspensions from normal human skin and DNCB-challenged skin

	No. of samples	Mean values of fluorescence intensity of T6 ⁺ Lag ⁺ cells with	
		OKT6	Lag
Normal human skin	7	109.1 $\pm$ 34.7	91.9 $\pm$ 39.7
Challenged skin on day 7	1	54.7	6.5
Challenged skin on day 14	1	121.1	47.4
Challenged skin on day 21	1	103.3	63.9

Discussion

The results of this study concerning epidermal LCs before 48 h of ACD were generally consistent with pre-

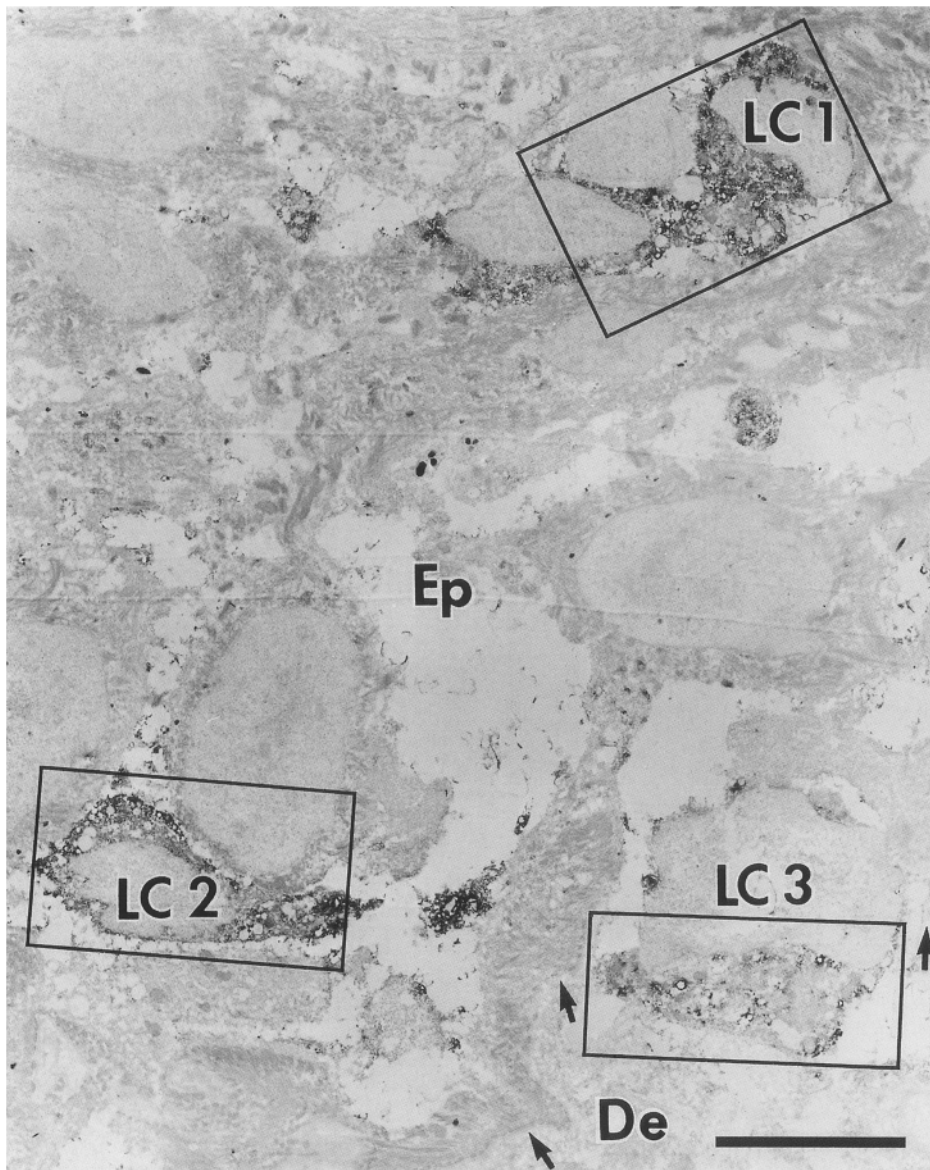


Fig. 4. Low magnification of an immunoelectron microscopy study on day 9 of ACD. LCs with reaction products to Lag antibody were observed in the spongiotic epidermis (*Ep*). The LC (*LC1*) in the mid-epidermis with lobulated nucleus contains more Lag-reaction products than LC (*LC2*) in the lower epidermis. The LC (*LC3*) which is just passing basal lamina (*arrows*) from the dermis (*De*) into the epidermis contains the least amount of Lag-reaction products. Bar = 5 μ m

vious reports (Silberberg et al. 1974, 1975, 1984; Aiba et al. 1984; Bressler et al. 1984; Bian and Bing-He 1985; Marks et al. 1987). There were no marked differences in the dynamic changes of epidermal $T6^+$ cells, Lag^+ cells, and $HLA-DR^+$ cells, all of which decreased in number gradually in this phase of ACD. They seemed to move slowly from the lower to the upper epidermis as if they were removed from the epidermis (Hunziker and Winkelmann 1978; Emtestam and Lindberg 1985; Cole et al. 1987).

After the decrease, the number of both $T6^+$ cells and Lag^+ cells in the epidermis subsequently increased after day 7. Lag^+ cells showed a variety of staining intensities with Lag antibody: Most of the $T6^+Lag^+$ cells in the mid-epidermis stained strongly with Lag antibody, while those in the lower epidermis stained weakly and with varying intensities. In addition, some $T6^+$ cells were Lag negative whereas all Lag^+ cells were $T6$ positive.

Although Lag-strongly-positive cells were observed to have many cytoplasmic BGs, only a few BGs were detected in Lag-weakly-positive cells which had not been observed in normal human skin (Kashihara et al. 1986) or in the skin before 48 h of ACD.

To clarify the relationship between Lag-weakly-positive cells and Lag-strongly-positive cells, epidermal cells after day 7 of ACD were analyzed by two-color flow cytometry. Quantification of fluorescence intensity with both OKT6 and Lag antibody showed that $T6^+$ cells consisted of one continuous population with respect to staining intensities with Lag antibody. This suggests that Lag-weakly-positive cells and Lag-strongly-positive cells result from the same cell lineage. Flow cytometric analysis also reveals that, during this phase of ACD, the mean fluorescence intensity in the $T6^+$ cells detected with Lag antibody increases gradually. These two results strongly suggest that, although $T6^+$ LCs have only a small

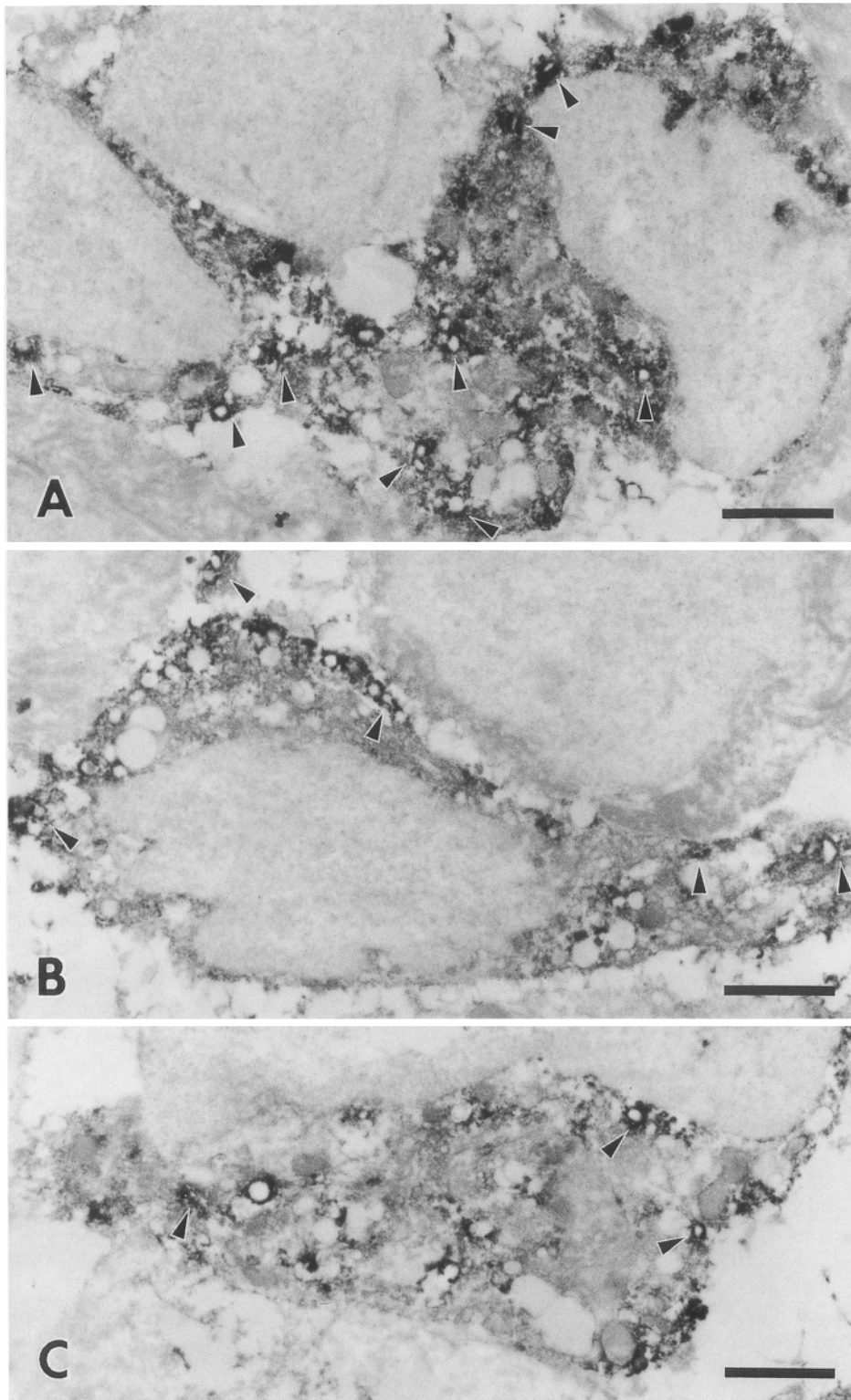


Fig. 5. **A** Enlarged photomicrograph of the uppermost rectangular area in Fig. 4 (*LC1*). The peroxidase-reaction products are localized around numerous BGs (arrowheads) in the cytoplasm. **B** Enlarged photomicrograph of the left rectangular area in Fig. 4 (*LC2*). Fewer BGs with Lag-reaction products (arrowheads) are distributed in the periphery of the cytoplasm. **C** Enlarged photomicrograph of the lower right rectangular area in Fig. 4 (*LC3*). BGs reacting with Lag antibody (arrowheads) are rare. All bars are 1 μ m

number of Lag antigens per individual cell on day 7, the number of Lag antigens gradually increased thereafter.

It is not clear whether these Lag-weakly-positive cells are on their way to form BGs or to be damaged to lose BGs. It is possible that $T6^+$ LCs may be damaged

by residual inflammatory cells and lose BGs around the dermo-epidermal junction. However, in our study, $T6^+$ LCs were gradually increased in number. Although the direction of the migration is not proved, this increase suggests more numbers of $T6^+$ LCs migrating from the dermis to the epidermis than those migrating vice versa.

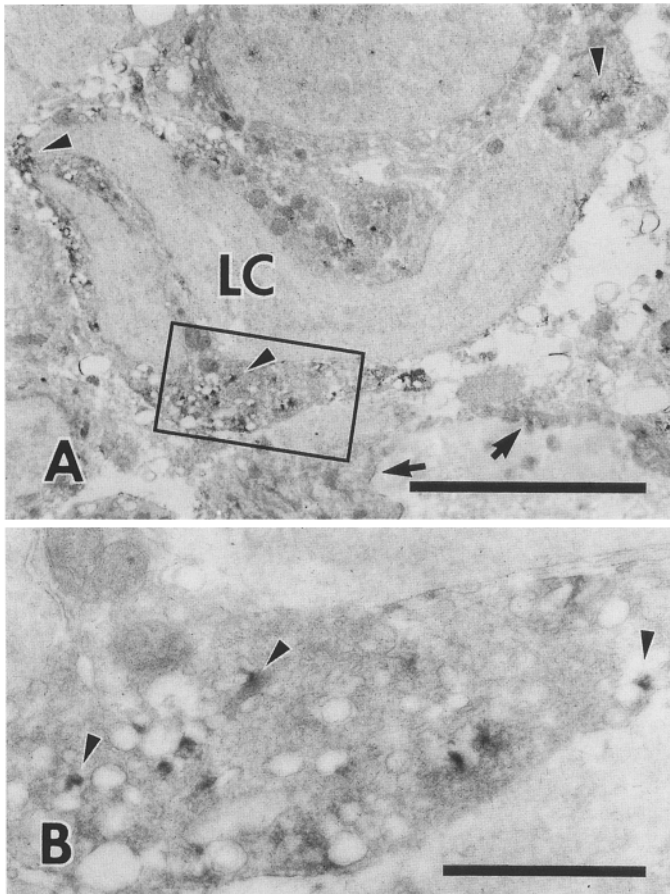


Fig. 6. **A** Low magnification of an immunoelectron microscopy of an LC just above the dermo-epidermal junction (arrows) shows few Lag-reaction products (arrowheads) in the cytoplasm. Bar = 5 μ m. **B** Enlarged photomicrograph of the rectangular area of **A** reveals that the reaction products are associated only with indeterminate tiny vesicular structures (arrowheads). Bar = 1 μ m

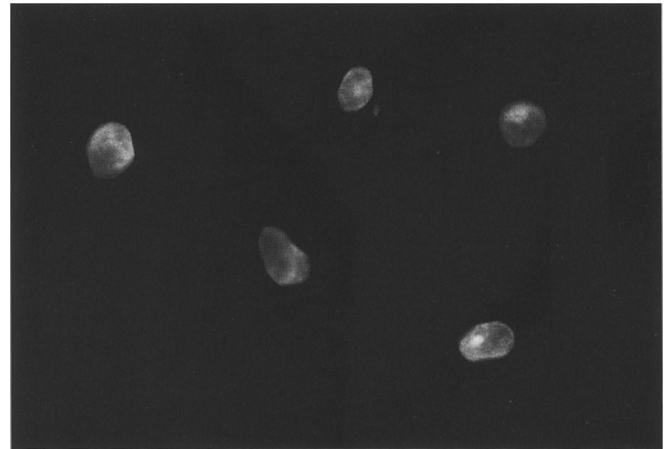


Fig. 7. Double immunofluorescence staining of epidermal cells from normal human skin. Epidermal cells were double stained with OKT6 and Lag antibody. Note the green fluorescence (Lag antibody) in their cytoplasm and red fluorescence (OKT6) on their cell surfaces. $\times 500$

$T6^+$ LCs were distributed in a manner such that they were mainly Lag-negative in the reticular dermis, Lag-weakly-positive in the papillary dermis and lower epidermis, and Lag-strongly-positive in the mid-epidermis. These data about the number and the distribution strongly suggest that at least considerable numbers of $T6^+$ LCs migrate from the dermis into the epidermis to form BGs around the dermo-epidermal junction.

LCs are classically identified with electron microscopy by detecting BGs (Birbeck et al. 1961). However, the nature of BGs is not clear. There have been several reports suggesting that BGs are lost whereas immunologic accessory functions in primary responses increase during in vitro cultivation (Schuler and Steinman 1985; Inaba

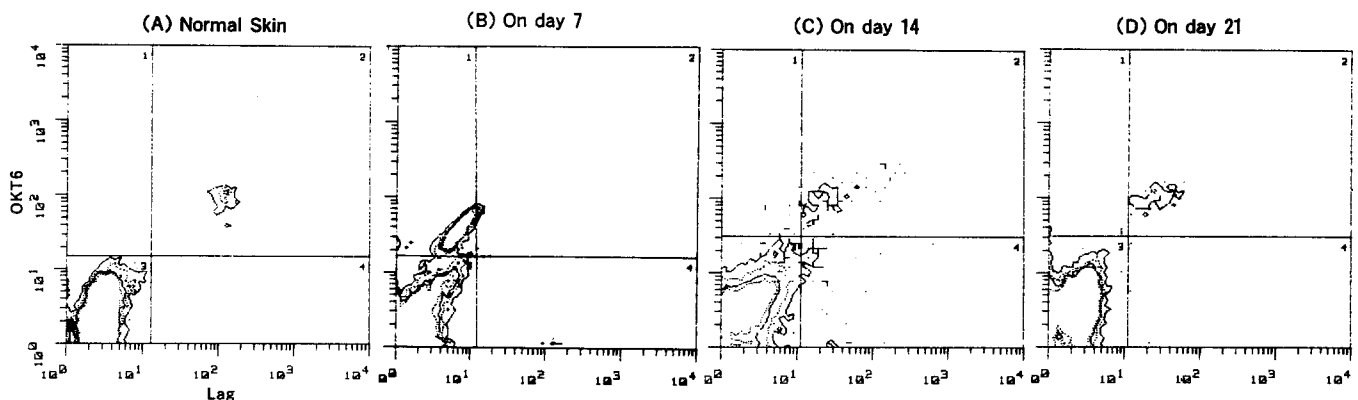


Fig. 8A-D. Contour plot showing two-color flow cytometric analysis of epidermal cells from normal human skin and DNCB-challenged skin. Epidermal cells of normal human skin were obtained from a resected breast, and epidermal cells of the challenged skin were taken from one volunteer. Epidermal cells were double stained with OKT6 (PE) and Lag antibody (FITC). The vertical and hori-

zontal axes denote log integrated red and green fluorescence intensity, respectively. **A** Epidermal cells from normal human skin. **B** Epidermal cells from DNCB-challenged skin on day 7. **C** Epidermal cells from DNCB-challenged skin on day 14. **D** Epidermal cells from DNCB-challenged skin on day 21. Note that $T6^+$ cells were stained continuously with Lag antibody from weak to strong

et al. 1986; Shimada et al. 1987). In the present study, we showed that LCs in human ACD contain varying numbers of BGs and that some LCs have no detectable BGs. This strongly suggests that all of T6⁺ cells in the skin, regardless of the presence of BGs, are categorized into LCs. The fate and the function of LCs and BGs remains to be elucidated, and Lag antibody may be helpful in further investigations.

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References

- Aiba S, Tagami H (1984) HLA-DR antigen expression on the keratinocyte surface in dermatosis characterized by lymphocytic exocytosis (e.g. pityriasis rosea). *Br J Dermatol* 111:285–294
- Aiba S, Sizawa H, Obata M, Tagami H (1984) Dynamic changes in epidermal Ia-positive cells in allergic contact sensitivity reactions in mice. *Br J Dermatol* 111:507–516
- Bian Z, Bing-He W (1984) Cytochemical and ultrastructural studies of the Langerhans' cells: sequential observations in experimental contact allergic reaction. *Int J Dermatol* 24:653–659
- Birbeck MS, Breathnach AS, Everall JD (1961) An electron microscopic study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. *J Invest Dermatol* 37:51–64
- Bressler MF, Zaino RJ, Marks JG (1985) Changes in lymphocytes and Langerhans cell population in human contact dermatitis. *J Invest Dermatol* 82:419
- Cole S, Faiweather JM, Townsend KMS (1987) Quantitative studies of the fate of epidermal Langerhans cells after X-irradiation of guinea-pig and mouse footpad skin. *Br J Dermatol* 116:55–65
- De Jong MCJM, Blanken R, Nanninga J, Van Voorst Vader PC, Poppema S (1986) Defined in situ enumeration in T6 and HLA-DR expressing epidermal Langerhans cells: morphologic and methodologic aspects. *J Invest Dermatol* 87:698–702
- Dezutter-Dambuyant C, Schmitt D, Faure M, Horisberger M, Thivolet J (1985) Immunogold technique applied to simultaneous identification of T6 and HLA-DR antigens on Langerhans cells by electron microscopy. *J Invest Dermatol* 84:465–468
- Emtestam L, Lindberg M (1985) Langerhans cell and mild irritant reactions. *J Invest Dermatol* 84:437
- Furukawa F, Huff JC, Weston WL, Norris DA (1987) Serum-free serial culture of adult human keratinocytes from suction-blister roof epidermis. *J Invest Dermatol* 89:460–463
- Gawkrodger DJ, Carr MM, McVittie E, Guy K, Hunter JAA (1987) Keratinocyte expression of MHC class II antigens in allergic sensitization and challenge reactions and in irritant contact dermatitis. *J Invest Dermatol* 88:11–16
- Harrist TJ, Muhlbauer JE, Murphy GF, Mihm MC, Bhan AK (1983) T6 is superior to Ia (HLA-DR) as a marker for Langerhans cells and indeterminate cells in normal epidermis: A monoclonal antibody study. *J Invest Dermatol* 80:100–103
- Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29:577–580
- Hunziker N, Winkelmann RK (1978) Langerhans cells in contact dermatitis of the guinea pigs. *Arch Dermatol* 114:1309–1313
- Inaba K, Schuler G, Witmer M, Valinsky J, Atassi B, Steinmann RM (1986) The immunologic properties of purified Langerhans cells: distinct requirements for the stimulation of unprimed and sensitized T lymphocytes. *J Exp Med* 164:605–613
- Kashihara M, Ueda M, Horiguchi Y, Furukawa F, Hanaoka M, Imamura S (1986) A monoclonal antibody specifically reactive to human Langerhans cells. *J Invest Dermatol* 87:602–607
- Kashihara-Sawami M, Horiguchi Y, Ikai K, Takigawa M, Ueda M, Hanaoka M, Imamura S (1988) Letterer-Siwe disease: immunopathologic study with a new monoclonal antibody. *J Am Acad Dermatol* 18:646–654
- Lampert IA (1984) Expression of HLA-DR (Ia like) antigen on epidermal keratinocytes in human dermatoses. *Clin Exp Immunol* 57:93–100
- Mackie RM, Turbitt ML (1983) Quantitation of dendritic cells in normal and abnormal human epidermis using monoclonal antibodies directed against Ia and HTA antigens. *J Invest Dermatol* 81:216–220
- Marks JG, Zaino RJ, Bressler MF, Williams JV (1987) Changes in lymphocyte and Langerhans cell populations in allergic and irritant contact dermatitis. *Int J Dermatol* 26:354–357
- Riggs JL, Seiwald RJ, Burckhalter JH, Downs CM, Metcalf TG (1958) Isothiocyanate compounds as fluorescent labeling agents for immune serum. *Am J Pathol* 34:1081–1097
- Schuler G, Steinman RM (1985) Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. *J Exp Med* 161:526–546
- Shimada S, Caughman W, Sharrow SO, Stephany D, Katz SI (1987) Enhanced antigen-presenting capacity of cultured Langerhans cells is associated with markedly increased expression of Ia antigen. *J Immunol* 139:2551–2555
- Silberberg I, Baer RL, Rosenthal SA (1974) The role of Langerhans cells in contact allergy: an ultrastructural study in actively induced contact dermatitis in guinea pigs. *Acta Derm Venereol* 54:321–331
- Silberberg I, Baer RL, Rosenthal SA, Thorbecke GJ, Berezowsky V (1975) Dermal and intravascular Langerhans cells at sites of passively induced allergic contact sensitivity. *Cell Immunol* 18:435–453
- Silberberg I, Baer RL, Rosenthal SA (1984) Role of Langerhans cells in allergic contact hypersensitivity: review of findings in man and guinea pigs. *J Invest Dermatol* 66:507–516
- Smith ML, Carski TR, Griffin CW (1962) Modification of fluorescent-antibody procedures employing crystalline tetramethylrhodamine isothiocyanate. *J Bacteriol* 83:1358–1364