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Immunological Activation of Dermal Langerhans Cells in Contact with Lymphocytes in a Model of Human Inflamed Skin

Fuminori Katou,* Haruo Ohtani,† Anne Saaristo,‡ Hiroshi Nagura,† and Katsutoshi Motegi*

From the Departments of Oral and Maxillofacial Surgery I and Pathology,† Tohoku University, Sendai, Japan; and the Molecular/Cancer Biology Laboratory and Department of Pathology,‡ Haartman Institute, University of Helsinki, Helsinki, Finland*

Langerhans cells play an important role in the skin's immune system. Little is known, however, about the antigen-presenting capacity of Langerhans cells in the context of skin inflammation. By immunohistochemistry we investigated the phenotypic characteristics of epidermal and dermal Langerhans cells and their spatial relationship with infiltrating lymphocytes. We studied skin flaps autotransplanted to the oral cavity to fill a defect after maxillofacial cancer surgery. In 15 of 21 cases sampled for the present study, the skin flaps were severely inflamed by *Candida albicans* infection. In contrast to the normal skin, such inflamed skin showed a marked increase in CD1a$^+$ dermal Langerhans cells. Double immunohistochemistry revealed that dermal Langerhans cells abundantly expressed B7–2 (CD86), a representative costimulatory molecule, and CD83, a marker of mature dendritic cells. Furthermore, these dermal Langerhans cells were in close contact with CD4$^+$/CD45RO$^+$ lymphocytes. This cell-to-cell contact was further visualized by immunoelectron microscopy. Langerhans cells were also observed within lymphatic vessels that were identified by the expression of vascular endothelial growth factor receptor-3. Ki-67 labeling indices were 4.2% in CD4$^+$ T cells and 0.8% in CD8$^+$ T cells within the dermis. Factor XIIIa$^+$ dermal dendrocytes were distributed outside the clusters of lymphocytes and were not in contact with them. Our observations indicate that dermal Langerhans cells in the inflamed skin are activated to express common phenotypes to mature dendritic cells so that they could stimulate neighboring memory CD4$^+$ T cells. *(Am J Pathol 2000, 156:519–527)*

Transplantation of autologous vascularized skin flaps to the oral cavity after the radical resection of advanced oral cancer has held a solid position for the past three decades as the treatment of choice, because of its usefulness in the reconstruction of huge defects.[1,2] Skin is a highly differentiated organ, and its principal function is as a barrier that protects our living body against harmful exogenous pathogens as well as against desiccation to maintain homeostasis.[3] It remains unknown how the skin flaps in the oral cavity adapt themselves to the wet microenvironment to maintain homeostasis. Recently inflammatory changes in skin flaps transplanted to the oral cavity have been noticed.[4,5] The histopathological features of this inflammation resembled those of psoriatic lesions, characterized by a dense infiltration of papillary dermis by CD4$^+$ T cells, epidermal hyperplasia, and loss of the granular layer with thin parakeratotic stratum corneum.[6] This inflammation was mainly caused by *Candida albicans* infection.[6] During this study, the authors noticed that CD1a$^+$ Langerhans cells increased in the dermis and decreased in the epidermis. The presence of clustered Langerhans cells within the dermis has already been demonstrated in diverse inflammatory skin diseases, including psoriasis,[7,8] atopic dermatitis,[8] lichen planus,[9] allergic contact dermatitis,[10] and delayed-type hypersensitivity.[11] However, the significance of the dermal Langerhans cells in such inflammatory lesions is still obscure, and it has not been referred to in recent reviews concerning dendritic cells (DCs).[12,13]

DCs are the most capable antigen-presenting cells (APCs) required to initiate the immune responses. Langerhans cells are paradigmatic DCs that are present in the skin and oral and bronchial mucosa. Precursor Langerhans cells migrate to the epidermis via the hematogenous route. After an antigen capture, Langerhans cells migrate to the regional lymph nodes and convert into mature DCs that are capable of priming naive T cells.[13] During the process of maturation, Langerhans cells show increased expression of MHC class II molecules and costimulatory molecules B7–1 (CD80) and B7–2 (CD86).[14,15] This maturing process is also associated with a high level of expression of CD83 and CD40.[13,16] These phenotypical changes in Langerhans cells are commonly observed in the maturation process of dendritic cells in general.[12,13] This maturation process is

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Address reprint requests to Fuminori Katou, M.D., D.D.S., Department of Oral and Maxillofacial Surgery I, Tohoku University School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. E-mail: fkatou@mail.cc.tohoku.ac.jp.

Table 1. List of Primary Antibodies

Antibody	Clones	Working dilution	Sources
CD1a	NA1/34	1:100 (1.5 μg/ml)	Dako (Copenhagen, Denmark)
CD1a-PE	BL6	1:1	Coulter (Miami, FL)
Birbeck granules	Lag	1:1000 (0.2 μg/ml)	Immuno-biological Lab. (Fujioka, Japan)
CD80 (B7-1)	Mab104	1:10 (20 μg/ml)	Immunotech (Marseille, France)
CD86 (B7-2)	BU63	1:100 (10 μg/ml)	Ancell (Bayport, MN)
CD83	HB15a	1:1000 (1 μg/ml)	Immunotech
Factor XIIIa	(polyclonal)	1:2000	Calbiochem (La Jolla, CA)
CD4 (Leu3a)	SK3	1:50	Becton Dickinson (San Jose, CA)
CD4-PE	SK3	1:1	Becton Dickinson
CD8	DK25	1:100 (1.5 μg/ml)	Dako
CD28	L293	1:100 (10 μg/ml)	Becton Dickinson
CD45RA	2H4	1:100 (2.5 μg/ml)	Coulter
CD45RO	UCHL-1	1:100 (4 μg/ml)	Dako
CD68	EBM11	1:500 (0.8 μg/ml)	Dako
VEGFR-3	909F9	1:1000 (2 μg/ml)	Prof. Kari Alitalo[27]
Blood endothelia	PAL-E	1:10	Sanbio (Uden, Netherlands)
Ki-67	MIB-1	1:50	Immunotech

PE, phycoerythrin.

assumed to be a prerequisite for priming naive T cells in the secondary lymphoid organs.[12,13] There are two counterreceptors of B7 expressed on T cells: CD28 and CTLA-4. The CD28 ligation induces a positive signal for T cell proliferation and cytokine production.[17] The role of CTLA-4 has been less evident, and is suggested to be involved in a peripheral T cell tolerance by transmitting negative signals.[18] Another population of DCs has been identified in the human dermis that is characterized by the expression of factor XIIIa.[19–21] These DCs, termed dermal dendrocytes, are as potent as Langerhans cells in their antigen presentation capacity *in vitro*[21] and are involved in the pathogenesis of psoriasis.[22]

In this study we immunohistochemically investigated the possible involvement of Langerhans cells or factor XIIIa$^+$ dermal dendrocytes in antigen presentation during the local immune responses in inflamed skin tissue autotransplanted to the oral cavity. We used CD1a and Lag as representative markers of Langerhans cells. Lag reacts specifically with a 40-kd glycoprotein present in human Birbeck granule, helping to identify Langerhans cells at the light microscopic level.[23] We reveal here 1) phenotypical characterization of dermal Langerhans cells, suggesting their immunological activation and 2) identification of lymphatic vessels that contained these activated Langerhans cells. We discuss the roles of Langerhans cells, which are more active in the context of inflammatory skin tissue than is generally conceived.

Materials and Methods

Surgery and Tissue

Autotransplantation of a forearm skin flap was performed for the reconstruction of an extensive defect in the oral cavity caused by curative resection of advanced oral squamous cell carcinoma. The skin flap included the epidermis, dermis, subcutaneous adipose tissue, underlying muscle fascia, and vascular pedicle. Twenty-one biopsy specimens were obtained during the second surgery, performed approximately 2 years after the primary surgery to revise the contour of skin flaps. Of these 21 cases of skin flaps, 15 were analyzed that were severely inflamed by *Candida albicans* infection. The inflamed dermis was extensively infiltrated by macrophages and neutrophils, and the fungal pathogens were confirmed by culture study.[6] As a control, normal forearm skin (five cases) was sampled from the remnant tissue trimmed off at the time of the initial surgery.

Fixation

For immunohistochemistry and immunoelectron microscopy, biopsy specimens were fixed in periodate-lysin-4% paraformaldehyde (PLP) for 6–12 hours at 4°C.[24] After they were washed in phosphate-buffered saline (PBS) containing sucrose, the fixed specimens were rapidly frozen in dry ice/acetone after embedding in OCT compound (Miles, Elkhart, IN). For immunofluorescence staining, parts of the biopsy specimens were immediately frozen without prefixation.

Single Immunohistochemistry (Enzyme-Linked)

PLP-prefixed frozen sections were used with the biotin-streptavidin-peroxidase method (Histofine staining kit; Nichirei, Tokyo, Japan). The chromogen was 3′,3-diaminobenzidine tetrahydrochloride (DAB) (Dojin, Kumamoto, Japan). The primary antibodies used are listed in Table 1. The specificity was confirmed by replacing the primary antibodies with PBS. The sections were counterstained with methyl green or hematoxylin.

Quantitative Assessment of CD1a$^+$, Lag$^+$, CD83$^+$, CD80$^+$, and CD86$^+$ Cells

Positive cells were quantified by light microscopy (40 objective), using an ocular grid (the area of the grid was 0.0625 mm^2). Ten noncontiguous and nonoverlapping

Table 2. Quantitative Assessment of CD1a-, Lag-, CD83-, CD80-, and CD86-Positive Cells per 0.0625 mm^2

Antibody	Inflamed skin		Normal skin	
	Epidermis	Dermis	Epidermis	Dermis
CD1a	2.6 ± 1.3	13 ± 8.0	5.1 ± 1.2	0.1 ± 0.3
Lag	0.4 ± 0.5	1.9 ± 1.3	1.4 ± 0.6	0
CD83	0	11 ± 8.4	0	0
CD80 (B7-1)	0	0.8 ± 1.5	0	0
CD86 (B7-2)	0.6 ± 1.1	14 ± 5.0	0	0.1 ± 0.2

Results are mean ± SD.

squares were randomly chosen for the cell counting in the epidermis or dermis. We judged a cell with a nucleus and clear immunoreactivity to be a positive cell. Positive cells obviously in the vessel lumens were excluded in this assessment.

Immunoelectron Microscopy for CD86, CD83, and Lag

The same staining method as in light microscopic immunohistochemistry was used.[25] The specimens were fixed in 0.5% glutaraldehyde for 30 seconds before DAB reaction and in 1% osmium tetroxide for 20 minutes after DAB reaction. After dehydration in ethanol, the specimens were embedded in Epon. Ultrathin sections were observed with a Hitachi H-600 electron microscope.

Double Immunofluorescence Staining

Frozen sections mounted on glass slides were fixed for 10 minutes in chilled acetone before use. The following combinations of double immunofluorescence staining were performed in five representative cases: 1) combinations of CD1a and B7–1, CD1a and B7–2, CD1a and CD83, or CD1a and Lag; 2) combinations of CD4 and CD45RA, or CD4 and CD45RO; and 3) combinations of factor XIIIa and CD4, factor XIIIa and CD68, factor XIIIa and CD1a, factor XIIIa and CD83, or factor XIIIa and Lag. In combinations 1 and 2, primary unlabeled mouse monoclonal antibody (B7–1, B7–2, CD83, Lag, CD45RA, or CD45RO) was applied overnight at 4°C. The sections were then washed and incubated for 2 hours at 4°C with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Fab fragments (Jackson ImmunoResearch, West Grove, PA). The second-step immunostaining was carried out with phycoerythrin (PE)-conjugated anti-CD1a or anti-CD4. In combination 3, rabbit primary antibody (factor XIIIa) was detected by FITC-conjugated goat anti-rabbit antibody (Biosource International, Camarillo, CA), and mouse antibody (CD4, CD68, CD1a, CD83, or Lag) was detected by tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse antibody (Biosource International).[26] The sections were observed and photographed with an Olympus AX-80 microscope.

Double Immunohistochemistry (Enzyme-Linked)

The following combinations of double immunohistochemistry were performed with PLP-prefixed frozen sections of eight representative cases: 1) combinations of Lag and B7–2, or factor XIIIa and B7–2; 2) combinations of CD4 and CD1a, CD4 and B7–2, CD4 and CD83, or CD4 and Lag; 3) combinations of CD1a and vascular endothelial growth factor receptor-3 (VEGFR-3),[27] CD83 and VEGFR-3, Lag and VEGFR-3, B7–1 and VEGFR-3, B7–2 and VEGFR-3, factor XIIIa and VEGFR-3, or CD68 and VEGFR-3; 4) a combination of VEGFR-3 and PAL-E.[28] The first-step immunohistochemistry was performed by the same method as for the single enzyme-linked immunohistochemistry, using 4-chloro-1-naphthol (Sigma, St. Louis, MO) or 3-animo-9-ethylcarbazole (AEC) (Vector, Burlingame, CA). After washing in 0.2 mol/L glycine buffer (pH 2.2) for 10 minutes, the second-step immunohistochemistry was carried out with the indirect method, using anti-mouse rabbit antibody conjugated with alkaline phosphatase as the secondary antibody (Dako, Glostrup, Denmark). The chromogen was Fast Red substrate (Sigma) or Vector Blue (Vector). For a combination of mouse and rabbit (factor XIIIa) antibodies, the treatment of the glycine buffer was omitted.

Semiquantitative Assessment of the Ratios of Double-Positive Cells to Dermal $CD1a^+$ Cells in the Inflamed Skin

We semiquantitatively graded the ratios of cells positive for Lag, CD83, CD80, or CD86 to the dermal $CD1a^+$ cells in the inflamed skin by double immunofluorescence staining.

Table 3. Semiquantitative Assessment of the Proportions of Double-Positive Cells to Dermal $CD1a^+$ Cells in Inflamed Skin

Cell	Lag	CD83	CD80 (B7-1)	CD86 (B7-2)
Dermal $CD1a^+$ cells	+	++++	+	++++

+, 0–25%; ++, 25–50%; +++, 50–75%; ++++, 75–100%.

Assessment of Proliferation Activity of CD4$^+$ and CD8$^+$ T Cells in the Dermis of Inflamed Skin

Double immunohistochemistry for CD4 or CD8 and Ki-67 was performed as follows in 15 cases (modified from Ref. 29). First, sections were stained with anti-CD4 or CD8 with DAB as a chromogen, using an indirect immunoperoxidase method. Then sections were left in an autoclave for 5 minutes at 120°C to retrieve Ki-67 antigen. The sections were stained with anti-Ki-67, using a Histofine kit; reacted with nickel-cobalt DAB; and counterstained with hematoxylin. The labeling index was measured as the number of Ki67$^+$/CD4$^+$ or Ki67$^+$/CD8$^+$ cells among 100 CD4$^+$ or CD8$^+$ cells.

Results

Quantitative and semiquantitative results are shown in Tables 2 and 3.

Normal Control Skin

CD1a$^+$ cells formed a network in the epidermis, whereas dermal CD1a$^+$ cells were only sparsely distributed. Epidermal Lag$^+$ cells made up approximately a quarter of epidermal CD1a$^+$ cells. There were no Lag$^+$ cells in the dermis. Virtually no cells were found to be positive for B7–1, B7–2, or CD83, indicating an absence of activated Langerhans cells in the normal skin. CD4$^+$ and CD8$^+$ T cells were sporadically present only in the dermis. Factor XIIIa$^+$ dermal dendrocytes were present adjacent to the vessels in the dermis (not shown).

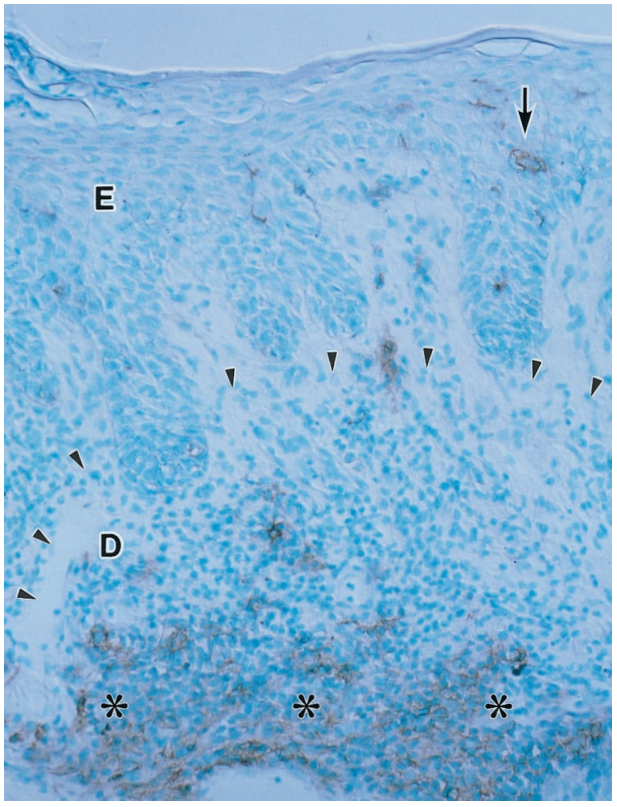

Figure 1. Immunohistochemistry for CD1a (brown) in inflamed skin autotransplanted to the oral cavity (methyl green counterstaining). Note an abundance of dermal CD1a$^+$ cells (**asterisks**) in the area of lymphocytic infiltrate (**arrowheads**), contrasted by a decrease in epidermal CD1a$^+$ cells (**arrow**). E, epidermis; D, dermis. Original magnification, ×50.

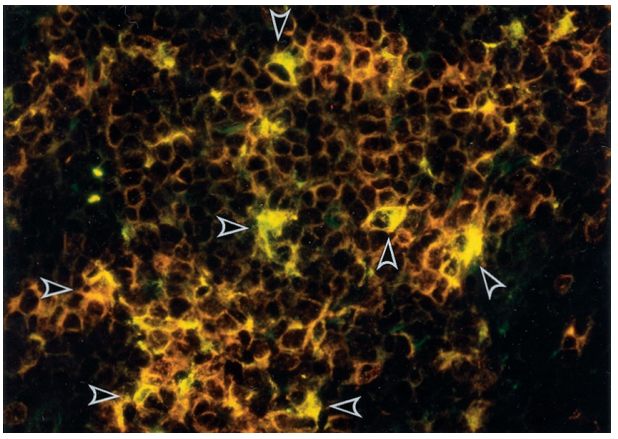

Figure 2. Double immunofluorescence for Lag (green) and CD1a (red) in inflamed dermis. Lag$^+$/CD1a$^+$ cells (yellow, **arrowheads**) constitute a part of CD1a$^+$ cells (red) in the inflamed dermis. Note an absence of Lag$^+$ cells without CD1a expression (green). Original magnification, ×100.

Inflamed Skin (Autotransplanted Skin Flap)

Compared with the normal counterparts, the number of CD1a$^+$ cells decreased by half in the epidermis, whereas dermal CD1a$^+$ cells increased markedly. Dermal CD1a$^+$ cells were frequently located in the clusters of lymphocytes (Figure 1). Lag$^+$ cells occurred in the dermis, whereas there was a marked reduction of them in the epidermis. Dermal Lag$^+$ cells always coexpressed CD1a, constituting a part of CD1a$^+$ cells (about one-sixth) (Figure 2). B7–2$^+$ or CD83$^+$ cells abounded in the dermis, showing a typical dendritic shape, but they were quite scarce in the epidermis (Figure 3). Double staining revealed that the vast majority of dermal CD1a$^+$ cells

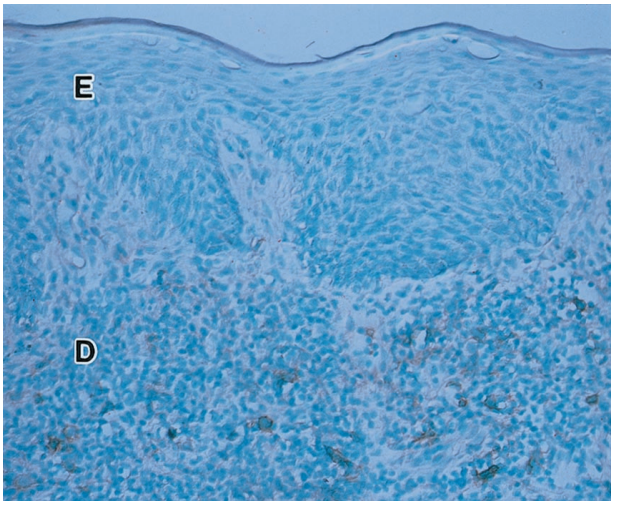

Figure 3. B7–2 expression (brown) in inflamed skin (methyl green counterstaining). Note an abundance of B7–2$^+$ cells in the inflamed dermis and an absence of B7–2$^+$ cells in the epidermis. E, epidermis; D, dermis. Original magnification, ×50.

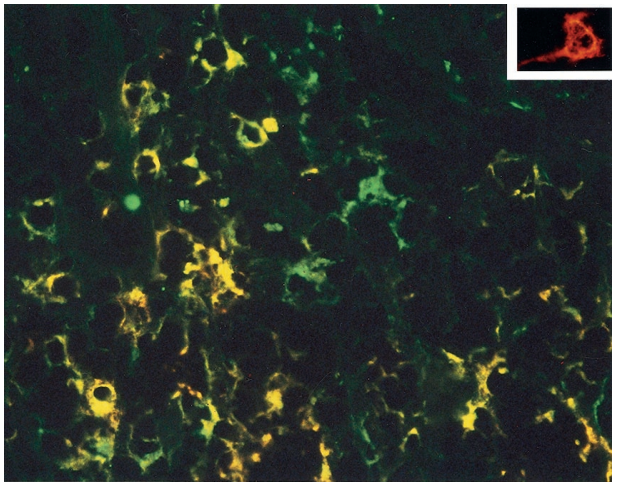

Figure 4. Double immunofluorescence for B7–2 (green) and CD1a (red) in inflamed skin. Yellow cells are double positive for B7–2/CD1a. Most $CD1a^+$ cells in the dermis coexpress B7–2, whereas $CD1a^+$ cells in the epidermis do not express B7–2 (**inset**). Original magnification, ×100.

coexpressed B7–2 and CD83, in contrast to a lack of B7–2 or CD83 in epidermal $CD1a^+$ cells (Figure 4). $B7–1^+$ cells were only observed in the dermis sporadically, amounting to 1/18th of $B7–2^+$ cells. $B7–1^+$ cells were particularly evident within the lumen of thin-walled vessels (identified as lymphatic vessels; *vide infra*) (Figure 5). Factor $XIIIa^+$ cells were located outside the cluster of lymphocytes, virtually lacking contact with lymphocytes (Figure 6). They are partly positive for B7–2 or CD68, but negative for CD1a, CD83, or Lag (not shown). $B7–2^+$/$CD83^+$ mononuclear cells abundantly located within the cluster of lymphocytes in the dermis were judged to be activated Langerhans cells because of positivity for CD1a and Lag (partly) and negativity for factor XIIIa. These activated, dermal Langerhans cells projected their cytoplasmic processes to the neighboring lymphocytes (Figure 7). Most lymphocytes aggregated in the dermis were $CD4^+$/$CD45RO^+$/$CD45RA^-$, contrasting with a sparse distribution of $CD8^+$ T cells (Figures 8 and 9). Almost all T cells expressed CD28 (not shown). Some

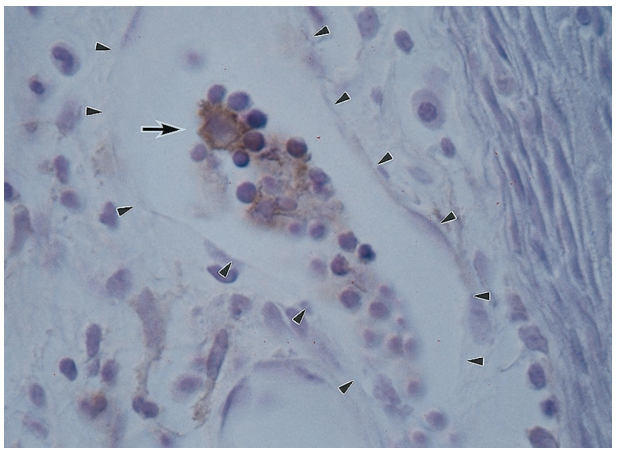

Figure 5. B7–1 expression (brown) in the dermis of inflamed skin (hematoxylin counterstaining). $B7–1^+$ cells (**arrow**) were selectively observed in the lumen of thin-walled vessels (**arrowheads**). Original magnification, ×100.

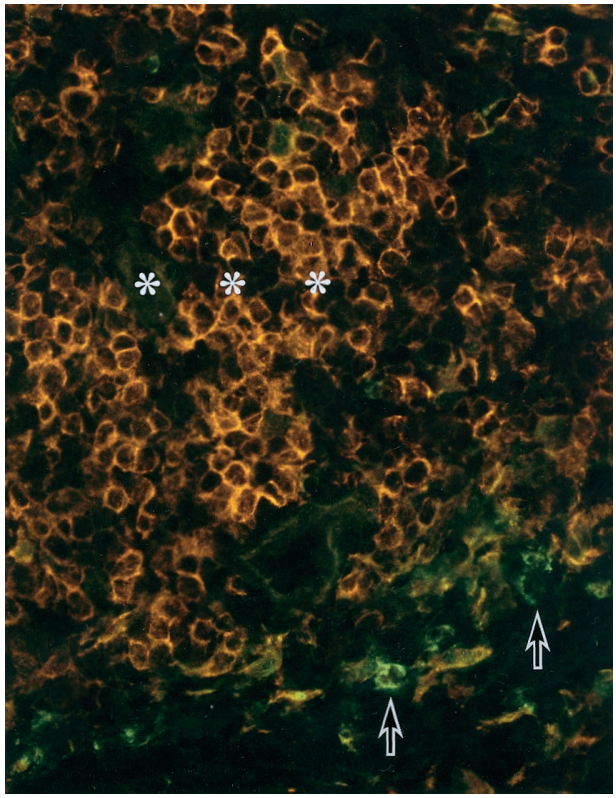

Figure 6. Double immunofluorescence for factor XIIIa (green) and CD4 (red) in the dermis of inflamed skin. Factor $XIIIa^+$ dermal dendrocytes (**arrows**) are located at the periphery of the cluster of $CD4^+$ T cells (**asterisks**) and are not in contact with them. Original magnification, ×50.

$VEGFR\text{-}3^+$ lymphatic vessels in the dermis,[27] but not $PAL\text{-}E^+$ blood vessels,[28] contained mononuclear cells with a phenotype of $CD1a^+$/$B7–1^+$/$B7–2^+$/$CD83^+$ and Lag^+ (partly) (Figure 10). These mononuclear cells were also judged to be activated Langerhans cells. Lymphocytes were admixed within the lumen of the same lymphatic vessels. Neither factor $XIIIa^+$ dermal dendrocytes nor $CD68^+$ cells were found in the lumen of $VEGFR\text{-}3^+$ lymphatic vessels (not shown).

Immunoelectron microscopy confirmed direct cell-to-cell contact between $B7–2^+$ or $CD83^+$ dermal Langerhans cells and lymphocytes (Figure 11). These dermal Langerhans cells had numerous tangled cytoplasmic processes labeled for B7–2 or CD83. Lag^+ dermal Langerhans cells also attached to lymphocytes (not shown).

Ki-67 labeling indices were 4.2% in $CD4^+$ T cells and 0.8% in $CD8^+$ T cells in the dermis of inflamed skin (Figure 12).

Our findings for the inflamed skin tissue are summarized in Figure 13.

Discussion

The present immunohistochemical study is the first to show that $CD1a^+$ dermal Langerhans cells in inflamed skin were activated to express B7–2 and CD83 in close contact with $CD4^+$/$CD45RO^+$ T cells (memory phenotype). Factor $XIIIa^+$ dermal dendrocytes did not express

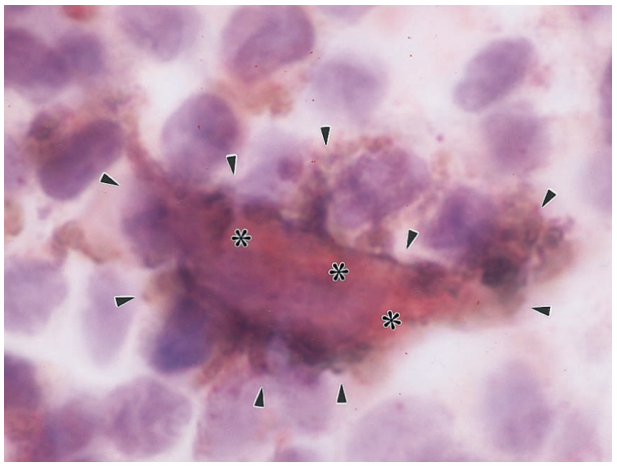

Figure 7. Double staining for Lag (red, **asterisks**) and B7–2 (black, **arrowheads**) in the dermis of inflamed skin (hematoxylin counterstaining). The $Lag^+/B7–2^+$ dermal Langerhans cell is in close contact with lymphocytes. Note the presence of $B7–2^+$ cell processes holding lymphocytes. Original magnification, ×500.

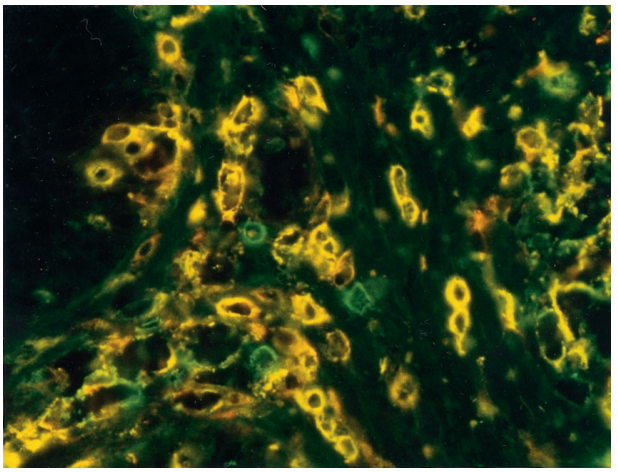

Figure 9. Double immunofluorescence for CD45RO (green) and CD4 (red) in the dermis of inflamed skin. An abundance of yellow cells indicates that almost all $CD4^+$ T cells infiltrating the dermis are $CD45RO^+$, a memory phenotype. Original magnification, ×100.

CD83, being out of contact with lymphocytes. These $CD4^+$ T cells had a higher labeling index of Ki-67 (4.2%) than did $CD8^+$ T cells (0.8%). We further confirmed that activated Langerhans cells were present in lymphatic vessels, suggesting a process of migration to the regional lymph nodes.

We used CD1a and Lag as representative markers of Langerhans cells. Lag^+ cells constituted a part of $CD1a^+$ cells in both the epidermis and dermis. This could be explained by the facts that precursor Langerhans cells lack Lag^+ Birbeck granules and Langerhans cells lose Birbeck granules during the migration from the epidermis.[30] This suggested that the presence of Lag^+ Birbeck granules is sufficient but not a prerequisite for the identification of Langerhans cells.[31] Factor $XIIIa^+$ dermal dendrocytes, another subpopulation of DCs in human dermis, do not express CD1a or Lag *in situ*.[19,20] Langerhans cells were originally defined as epidermal dendritic-shaped cells of neural origin by Paul Langerhans in 1868,[32] and their functions as APCs were identified later.[12,13] With all of this taken into account, we designate abundantly distributed dermal $CD1a^+$ cells in our materials as dermal Langerhans cells.

Dermal Langerhans cells expressed B7–2 abundantly but B7–1 sporadically in the inflamed skin. This predominant expression of B7–2 over B7–1 has already been demonstrated on $CD68^+$ cells in human intestines.[33,34] *In vitro* study of Langerhans cells suggested a crucial role of B7–2 in the induction of T cell proliferation with little dependence on B7–1.[35] B7–2, therefore, could be an important molecule for costimulation by Langerhans cells. We observed the expression of CD83 on dermal Langerhans cells and a lack of CD83 on factor $XIIIa^+$ dermal dendrocytes in the inflamed skin. This observation further supports the conclusion that dermal Langerhans cells in the inflamed skin are of mature DC pheno-

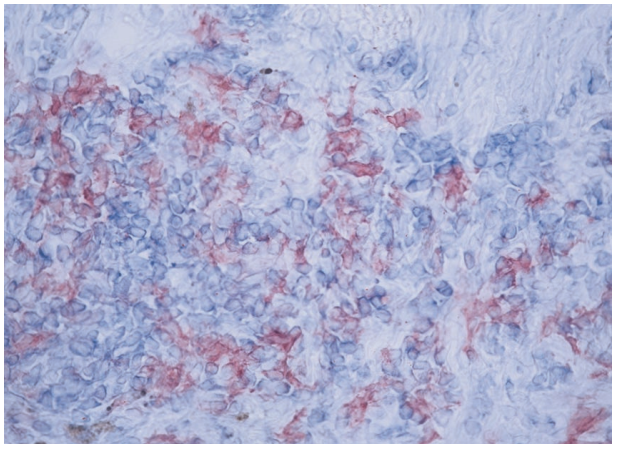

Figure 8. Double staining for CD1a (red) and CD4 (blue) in the dermis of inflamed skin (no counterstaining). Dermal $CD1a^+$ cells are admixed with $CD4^+$ T cells. Original magnification, ×132.

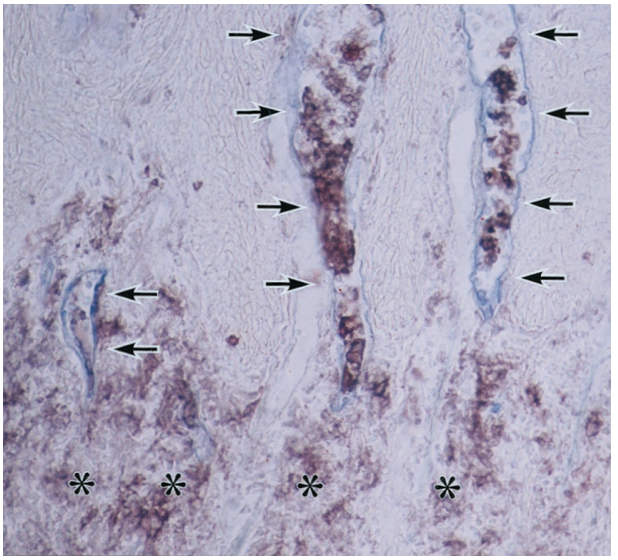

Figure 10. Double staining for B7–2 (black) and VEGFR-3 (blue) in inflamed skin (no counterstaining). $B7–2^+$ Langerhans cells are aggregated within $VEGFR\text{-}3^+$ lymphatic vessels (**arrows**). Note $B7–2^+$ dermal Langerhans cells (**asterisks**) as well as Langerhans cells within the lymphatic vessels. Original magnification, ×50.

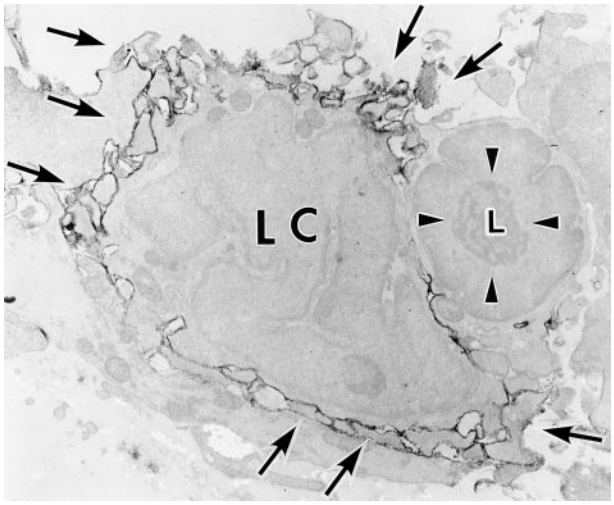

Figure 11. Immunoelectron microscopy for B7–2 in the dermis of inflamed skin. Black represents the immunoreactivity for B7–2. The B7–2$^+$ dermal Langerhans cell (LC) is in cell-to-cell contact with a lymphocyte (L). Note numerous cytoplasmic processes positive for B7–2 (**arrows**). The lymphocyte possesses a large nucleolus (**arrowheads**), another finding of activation. CD83$^+$ dermal Langerhans cells showed the same features (not shown). Original magnification, ×5000.

type.[36] We also confirmed direct cell-to-cell contact between CD83$^+$/B7–2$^+$ dermal Langerhans cells and lymphocytes, suggesting that these Langerhans cells could function as APCs. These findings are consistent with *in vitro* data suggesting that the majority of antigen-

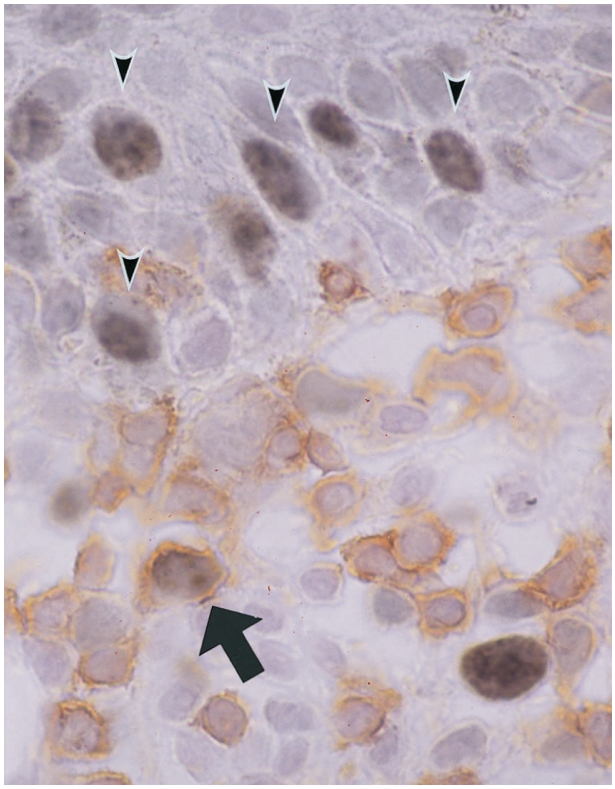

Figure 12. Double staining for CD4 (brown) and Ki-67 (black) in the dermis of inflamed skin (hematoxylin counterstaining). The **arrow** indicates a Ki-67$^+$/CD4$^+$ T cell. Note Ki-67 expression on keratinocytes as a control (**arrowheads**). Original magnification, ×330.

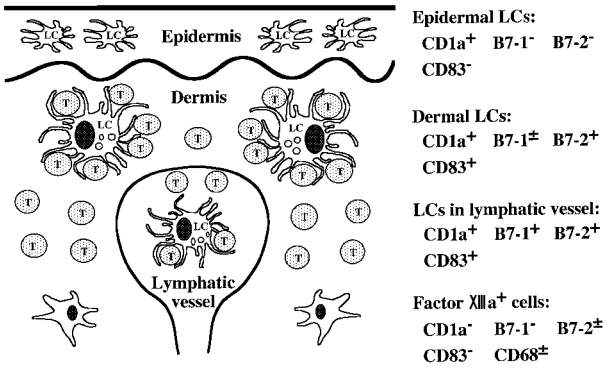

Figure 13. A schema of the results. LC, Langerhans cell; T, T cell.

presenting capacity resides in the CD1a$^+$ Langerhans cell-like dendritic antigen-presenting cell population.[37] The lack of CD83 on factor XIIIa$^+$ dermal dendrocytes and the limited contact with lymphocytes suggest that they are not principal APCs in our specimens.

Our present data showed that CD4$^+$ and CD8$^+$ T cells had a proliferation activity with the labeling indices of Ki-67 antigen of 4.2% and 0.8%, respectively. These figures may be lower than the Ki-67 labeling index of CD8$^+$ T cells in Epstein-Barr virus-associated gastric cancer (13%).[29] Ki-67 expression in circulating blood T cells has been reported to be 1.1% in CD4$^+$ T cells and 1% in CD8$^+$ T cells in healthy individuals, and 6.5 and 4.3% in HIV-1-infected individuals, respectively.[38] We speculate that CD4$^+$ T cells were significantly proliferating in the locale, demonstrating ongoing responses between APCs and T cells. Our morphological method, therefore, could be an *in vivo* counterpart of mixed lymphocyte reaction *in vitro*. CD4$^+$ T cells in inflamed skin tissue mostly showed a memory phenotype, suggesting that activated, dermal Langerhans cells would restimulate primed CD4$^+$ T cells. Activated dermal Langerhans cells could also be stimulated by T cells via the CD40/CD40 ligand pathway.[16] The communication between activated dermal Langerhans cells and primed T cells is possibly reciprocal.

There appears to be a variation among the patterns of costimulation by B7 depending on the types of T cells. For example, the cytokine production of Th1-type (interleukin-2 (IL-2) and interferon-γ (IFN-γ)), but not that of Th2-type (IL-4), requires the costimulation of primed helper T cells by B7.[39,40] And this costimulation by B7 is more effective for the production of IL-2 than that of IFN-γ.[41] These *in vitro* data are consistent with the present *in situ* observations on the possible activation of memory T cells by APCs.

We have also observed that Langerhans cells were located in lymphatic vessels, which probably represents a process of migration to the regional lymph nodes.[42] It is of particular interest that Langerhans cells in lymphatic vessels are fully matured and thus able to express B7–1 as well as B7–2 and CD83.

In the present study, inflammation was chiefly caused by *Candida albicans* infection. The main defense against fungi is provided by the T-cell-mediated acquired immunity and the innate immunity by macrophages or neutro-

phils.[43] In the experimental model of candidiasis, protective immunity by phagocytes is well correlated with the Th1 response.[44] The production of IFN-γ and IL-2 by Th1 cells is dependent on the costimulatory signal by B7.[39–41] IFN-γ enhances the activities of macrophages and neutrophils in the killing process of *Candida albicans*,[45] and IL-2 protects neutrophils from apoptotic death.[46] These further support our notion that B7–2$^+$ activated dermal Langerhans cells could play a defensive role against fungi through restimulating memory CD4$^+$ T cells in our study.

In conclusion, we have morphologically demonstrated the occurrence of activated dermal Langerhans cells in inflamed skin, which express common phenotypes to mature dendritic cells. These cells could play important roles in the immune mechanisms against pathogens. It remains to be clarified how dermal Langerhans cells or factor XIIIa$^+$ dermal dendrocytes are involved in the local antigen presentation in other inflammatory dermatoses.

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