Identification of dendritic cell subsets in psoriasis vulgaris: Immunohistochemical study

Sang Gun Park

Department of Medicine
The Graduate School, Yonsei University

Identification of dendritic cell subsets in psoriasis vulgaris: Immunohistochemical study

Sang Gun Park

Department of Medicine
The Graduate School, Yonsei University

Identification of dendritic cell subsets in psoriasis vulgaris: Immunohistochemical study

Directed by Professor Min-Geol Lee

The Master's Thesis
Submitted to the Department of Medicine
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Master of Medical Science

Sang Gun Park

June 2005

This certifies that the Master's Thesis
of **Sang Gun Park** is approved.

Thesis Supervisor: ***Prof. Min-Geol Lee***

Thesis Committee Member: ***Prof. In-Hong Choi***

Thesis Committee Member: ***Prof. Wook Lew***

The Graduate School
Yonsei University

June 2005

Acknowledgements

I appreciate professor Min-Geol Lee, M.D., Ph.D., Department of Dermatology, Yonsei University Medical College, Seoul, Korea. He supervised and encouraged me to study this subject.

I also appreciate professor In-Hong Choi, M.D., Ph.D., and Wook Lew, M.D., Ph.D.. They gave me experienced advice and warm support.

I could not forget the warm support of You Chan Kim, M.D., Ph.D., who is a Professor of the Department of Dermatology, Ajou University. I am very grateful to Nam Soo Jang for technical assistance.

I am especially grateful to my family members, especially my parents, who have tolerated the years of my study with patience. I give my love and admiration to them.

Table of Contents

List of Tables

List of Figures

ABSTRACT

Identification of dendritic cell subsets in psoriasis vulgaris: Immunohistochemical study

Sang Gun Park

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

In the past, the view of the pathogenesis of psoriasis was focused on the primary dysfunction of keratinocytes. In recent years, however, it has been viewed as a T cell-mediated autoimmune disease. Moreover, newly defined dendritic cell subsets such as inflammatory dendritic epidermal cells and plasmacytoid dendritic cells have been identified in various inflammatory skin diseases, including psoriasis.

In this study, we attempted to stain and compare the dendritic cell subsets including Langerhans cells, inflammatory dendritic epidermal cells, and plasmacytoid dendritic cells in the involved and uninvolved skin of five psoriasis patients and in normal skin of two healthy volunteers. Immunohistochemical analysis confirmed that Langerhans cells were present in the involved and uninvolved skin of psoriasis patients and in the skin of healthy volunteers. However, Langerhans cells expressed CD83 only in the involved skin. Inflammatory dendritic epidermal cells and plasmacytoid dendritic cells were also found in the epidermis and dermis of the involved skin, respectively, but not in the uninvolved skin and in normal skin. Unlike Langerhans cells, they did not express CD83.

In conclusion, we have found that most of the CD83+ dendritic cells in psoriatic lesions are Langerhans cells. Although inflammatory dendritic epidermal cells and plasmacytoid dendritic cells did not express CD83, they may play a role in the pathogenesis of psoriasis.

Key words: dendritic cell, Langerhans cell, inflammatory dendritic epidermal cell, plasmacytoid dendritic cell, psoriasis

Identification of dendritic cell subsets in psoriasis vulgaris: Immunohistochemical study

Sang Gun Park

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

I. INTRODUCTION

Psoriasis is a chronic relapsing inflammatory disease of the skin that affects about 2% of the world's population.[1-3] Physical trauma, infection, stress, several drugs (particularly β-blockers, lithium, chloroquine, and indomethacin) have been known to be triggering factors, however, the etiology of psoriasis remains enigmatic.[1,2] In the past, increased keratinocyte proliferative activity was regarded as the primary event. However, in the last decade, the view of psoriasis as an autoimmune-mediated disease has

gradually replaced its previous view as a primary dysfunction of keratinocytes. Several studies have argued that activated T lymphocytes may play the role of central regulators of inflammatory reactions in the skin and may induce keratinocyte hyperproliferation.

Dendritic cells (DC) are considered to be the most potent antigen-presenting cells, and are thought to be one of the essential cells for the activation of T cells. In the psoriatic plaque, the total number of T cells and DC greatly increase, and this increase in both subsets represents an activated status.[3,4] Recent studies have focused on the changes of DC in inflammatory skin diseases such as psoriasis.[5-7] Langerhans cells (LC; $CD1a^{+++}$, $Langerin^{++}$, $CD11b^{-}$, $CD11c^{+/-}$) containing Birbeck granules are found in the normal epidermis and the involved skin of psoriasis, and do not express CD206. CD206 (mannose receptor) has been thought as a differentiation hallmark of immature DC. Newly defined DC in the inflamed epidermis, which are termed inflammatory dendritic epidermal cells (IDEC), lack Birbeck granules and Langerin (CD207). In contrast to LC, IDEC ($CD1a^{+}$, $CD11b^{+++}$, $CD11c^{+++}$) which express

CD206 are found in the inflammatory epidermis in conditions such as psoriasis, contact dermatitis, and atopic dermatitis, but not in the normal epidermis.[4-6] Another subset of DC in humans is the plasmacytoid dendritic cells (pDC), which seem to be specialized for the detection of viral infection due to their ability to secrete large amounts of anti-viral type I interferon (IFN: IFN-α and IFN-β).[4,6,7] Blood dendritic cell antigen-2 (BDCA-2) has been considered to be selectively expressed in pDC.[6,8] In normal skin, pDC ($CD4^+$, $CD11c^-$, $CD123^+$) are known to be low or absent, like IDEC. Wollenberg et al.[6] reported that lesional skin samples from atopic dermatitis contained high numbers of IDEC, but had low numbers of pDC. This may explain the predisposition of patients with atopic dermatitis to viral infections such as herpes simplex virus, which leads to eczema herpeticum.[3,6,7] In contrast, lupus erythematosus showed high numbers of pDC, but had low numbers of IDEC. In the involved skin of psoriasis and contact dermatitis, both subsets increased in number, and pDC were found in the basal layer of epidermis and papillary dermis of psoriatic plaques.[6] Therefore, it seems likely that various

inflammatory skin diseases show distinct disease-specific compositions of DC.

Among diverse techniques in DC research, visualization *in situ* is thought to be fundamental, e.g. immunohistochemical staining. In this study, we initially tried to find optimal conditions for staining DC in the frozen tissue of human skin samples. Then, in normal skin, the involved and uninvolved skin of psoriatic patients, we investigated and compared the presence and the surface antigen expression of these human DC subsets (LC, IDEC, pDC) using immunohistochemical methods.

II. MATERIALS AND METHODS

1. Patients and tissue materials

The subjects of this study were five patients with psoriasis vulgaris (aged 12 to 69, mean age 28.6; four males and one female) and two healthy volunteers (aged 7 and 69, both males). The diagnosis of psoriasis in each case was based on a tissue biopsy of the involved skin. The patients either had no history of treatment, or not been treated for at least four weeks. After informed consent was obtained, each patient received a 4-mm punch biopsy under local anesthesia. In the patients with psoriasis, in addition to the involved skin sample, another tissue sample was obtained from uninvolved skin located more than 10 cm apart from the involved skin.

The biopsies were embedded in Tissue-Tek OCT compound (Sakura Finetek U.S.A., Inc., Torrance, CA, USA) and stored at -70℃ until further processing.

2. Immunohistochemical staining

Frozen tissue was cut into 6-㎛-thick sections and mounted on silane coated microscope slides (Muto Pure Chemicals, Tokyo, Japan). After the cryostat sections were air-dried, they were fixed in 4℃ acetone for 10 min and washed with phosphate-buffered saline.

The tissue sections were stained using a Histostatin-DS kit (Zymed Lab., South San Francisco, CA, USA) according to the manufacturer's recommendations. A series of single-color immunostaining procedures was performed to find the optimal conditions for staining DC in frozen tissues. During this procedure, primary antibodies (Ab) were applied at various dilutions, as listed in Table 1, overnight at 4℃ or for 90 min at room temperature. Then, we analyzed and compared each slide. The monoclonal Ab, their sources, and the range of dilution used in this study as primary Ab are listed in Table 1. In this step, CD1a, Langerin, CD11c and CD83 Ab were applied to the skin sections of the healthy volunteers, and CD206, BDCA-2 and CD83 Ab to the skin sections of the psoriatic patients. Based on these results, single- and two-color immunostaining were performed on the skin sections from

the psoriatic patients. Because there was no significant difference between the previous results for the different conditions of primary Ab incubation time and temperature, the first primary monoclonal Ab were applied overnight at 4℃, and the second primary monoclonal Ab were applied for 90 min at room temperature in the two-color staining procedure. With this procedure, the cells reacted with the first primary Ab to stain dark purple to black, and reacted with the second primary Ab to stain light brown to red. The control isotype-matched mouse IgG1 and IgG2b Ab always showed negative staining.

Table 1. Panel of monoclonal antibodies used in this study

Antibody	Manufacturer	Antibody type	Recommended dilution	Dilution used in this study
CD1a	BD Biosciences	Mouse IgG2b	1:1	1:1,2,4
Langerin	Immunotech	Mouse IgG1	1:40	1:50,100,200
CD83	Serotech	Mouse IgG2b	1:2~10	1:20,40,80, 160,320,640
CD11c	BD Biosciences	Mouse IgG1	1:10~50	1:50,100,200
CD206	BD Biosciences	Mouse IgG1	-	1:25,50,100
BDCA-2	Miltenyi Biotec	Mouse IgG1	-	1:10,20,40
CD31	BD Biosciences	Mouse IgG1	1:10~50	1:100

3. Immunohistochemical evaluation and scoring

Two independent investigators independently reviewed all of the sections without knowledge of which antibody they were scoring. Discrepancies in estimations were reconciled by a concurrent review using a multi-headed microscope.

Immunostained cells were quantified separately in the epidermis and dermis. The quantity of staining was evaluated using a semiquantitative scale from 0 to 3, where 0 = none, 1 = few cells (less than 10 cells in the tissue section), 2 = moderate density, and 3 = many positive cells. The scale was adjusted for each part of the skin, the epidermis and dermis respectively, so that score 3 referred to the maximum number of positive cells within all specimens.

III. RESULTS

1. Determining optimal immunostaining conditions of primary antibodies in the skin tissue samples of healthy volunteers and patients with psoriasis vulgaris.

We performed a series of single-color immunostaining experiments with the skin tissue samples of healthy volunteers to determine the optimal dilution of primary antibodies such as CD1a, Langerin, CD11c, and CD83 Ab.

For the Ab against CD1a, Langerin, or CD11c, there were no significant differences among the given range of dilution or between the different conditions of incubation temperature and time. However, when incubated overnight at 4℃ with CD1a Ab at 1:4 dilution and Langerin Ab at 1:200 dilution, the tissue sections were stained slightly in some areas. Since the CD83 Ab, at a range of 1:20-80 dilution, caused nearly the entire epidermis to be stained darkly in a non-specific pattern, it was applied at a more diluted concentration of 1:160-640. We observed less diffuse epidermal staining pattern at 1:160 and

1:320 dilutions, and little staining in the epidermis at a 1:640 dilution. We found little difference between the conditions of incubation time and temperature in CD83 Ab. Because CD83 is expressed in activated DC, normal skin could serve as a negative control for CD83 expression, and the same procedure was repeated in the tissue sections of the psoriatic patients at a dilution range of 1:160-640.

We performed the previous procedure using different primary Ab, such as CD83, CD206, and BDCA-2 Ab, in the tissue sections of the involved skin of psoriatic patients, because activated DC (CD83+), IDEC (CD206+), and pDC (BDCA-2+) are known to be present in psoriatic skin, but not in normal skin.

As in the previous results, there was no significant difference at the given range of dilution or between the different conditions of incubation temperature and time in CD206 and BDCA-2 Ab. Immunolabelling with CD83 Ab showed a pattern of dark staining at lower dilutions similar to that found with the normal samples. Like the previous immunostaining, the epidermis was stained little diffusely at a 1:640 dilution.

In conclusion, the results were not influenced by the different conditions of incubation time and temperature, and we decided to set the optimal dilution of primary Ab used in this study as follows:

CD1a	1:2;	Langerin	1:100;
CD11c	1:200;	CD83	1:640;
CD206	1:100;	BDCA-2	1:40.

2. DC antigens in the involved and uninvolved skin from psoriatic patients and the normal skin from healthy volunteers detected by single-color immunostaining

Single-color immunostaining was done at the above dilutions in the involved and uninvolved skin from the patients with psoriasis vulgaris, and in the normal skin from the healthy volunteers. More than five sections were evaluated for each of these DC antigens (Table 2). The number of stained cells in all specimens was not significantly different from the number of stained cells in each individual for the same primary Ab.

CD1a+ cells were observed in the epidermis and dermis of nearly all specimens including normal skin. In the epidermis,

these cells were more frequently found in the normal skin, and were less evident in the psoriatic skin. In the dermis, on the contrary, the psoriatic plaque showed more CD1a+ cells than the normal skin. Langerin immunostaining showed a similar staining pattern to that of CD1a, except for its absence in the dermis of the normal skin. When comparing the immunolabelling obtained from these two markers, we observed that the CD1a immunolabelling presented with more background staining but better visualized dendrites, whereas the Langerin immunolabelling was more specific and typically located at the cell body.

CD83+ cells were rarely present in the normal skin, but were considerably increased in the skin of the psoriatic patients, especially in the involved skin. The greatest portion of the cells was found primarily in the upper dermis. We observed more conspicuous differences in the CD83 staining than the CD1a and Langerin staining.

CD11c immunolabelling did not show significant differences among the dermis of all tissue sections. However, CD11c+ cells were found in the epidermis of the involved and uninvolved

Table 2. Single-color immunostaining in the involved and uninvolved skin from the patients with psoriasis vulgaris and normal skin from healthy volunteers

Antibody	Epidermis			Dermis		
	Normal skin	Patients		Normal skin	Patients	
		Uninvolved skin	Involved skin		Uninvolved skin	Involved skin
CD1a	3 (3)	2.2 (2-3)	1.4 (1-2)	1 (1)	1 (0-2)	2 (2)
Langerin	2 (2)	2.2 (1-3)	1.8 (1-2)	0 (0)	0.8 (0-1)	2 (2)
CD83	0 (0)	0.2 (0-1)	1.8 (1-2)	0.5 (0-1)	1.4 (1-2)	2.2 (2-3)
CD11c	0 (0)	0.4 (0-1)	0.6 (0-1)	2 (2)	1.4 (1-2)	1.8 (1-2)
CD206	0 (0)	0.2 (0-1)	1.2 (1-2)	2.5 (2-3)	2.4 (2-3)	2.8 (2-3)
BDCA-2	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0-1)	2.6 (2-3)

Numbers indicate median and range (described in parentheses) of a semiquantitative scale scored in five patients with psoriasis (involved and uninvolved skin) and two healthy volunteers (normal skin).

skin of some patients, but not in the normal skin samples.

In the CD206 immunolabelling, there were no significant differences among the dermis samples, but scattered stained cells were present in the epidermis of the involved skin of the psoriatic patients, and were not present in the normal skin. The skin sample of one patient showed a few stained cells in the epidermis of uninvolved skin. These cells may correspond to IDEC.

BDCA-2 immunolabelling showed characteristic findings. In the epidermis, we did not find any cells stained with BDCA-2 Ab in any tissue specimens. There were no BDCA-2+ cells in the dermis of the normal skin or the uninvolved skin, whereas they were present only in the dermis of the psoriatic plaque except for the skin sample of one patient, which contained a few positively stained cells in the uninvolved skin. These cells may correspond to pDC.

3. Identification of DC subsets in the involved and uninvolved skin from psoriatic patients by two-color immunostaining

We performed two-color immunostaining to visualize each DC subset in the tissue sections. Double-positive cells were counted, and the results are shown in Table 3.

Immunolabelling with CD1a and Langerin Ab showed double-positive cells in the epidermis, and less frequently in the dermis. These cells may correspond to LC. In the epidermis of the involved skin, there were also a small number of CD1a+ Langerin- cells, but CD1a- Langerin+ cells were rarely found. In the epidermis of the uninvolved skin, almost all CD1a+ cells were also stained with Langerin, and CD1a+ Langerin- cells were rarely found.

We performed immunostaining with CD1a and CD206 Ab to identify IDEC and to distinguish epidermal DC, i.e., LC and IDEC. Double-positive cells were found only in the epidermis, not in the dermis, and except for the skin sample of one patient, they were present only in the involved skin (Figure 1). Like single-color staining with CD1a and Langerin Ab, double-positive cells in the epidermis of the involved skin seemed to be lower in number than those of the uninvolved skin.

Table 3. Two-color immunostaining with Ab of markers for each DC subset in the involved and uninvolved skin from the patients with psoriasis vulgaris

Antibodies	Epidermis		Dermis	
	Uninvolved skin	Involved skin	Uninvolved skin	Involved skin
CD1a / Langerin	1.8 (1-2)	1.6 (1-2)	0.2 (0-1)	0.4 (0-1)
CD1a / CD206	0.2 (1)	1.2 (1-2)	0 (0)	0 (0)
Langerin / CD206	0 (0)	0 (0)	0 (0)	0 (0)
CD206 / BDCA-2	0 (0)	0 (0)	0 (0)	2.6 (2-3)

Numbers indicate median and range (described in parentheses) of a semiquantitative scale scored in five patients with psoriasis (involved and uninvolved skin). Only double-positive cells were counted.

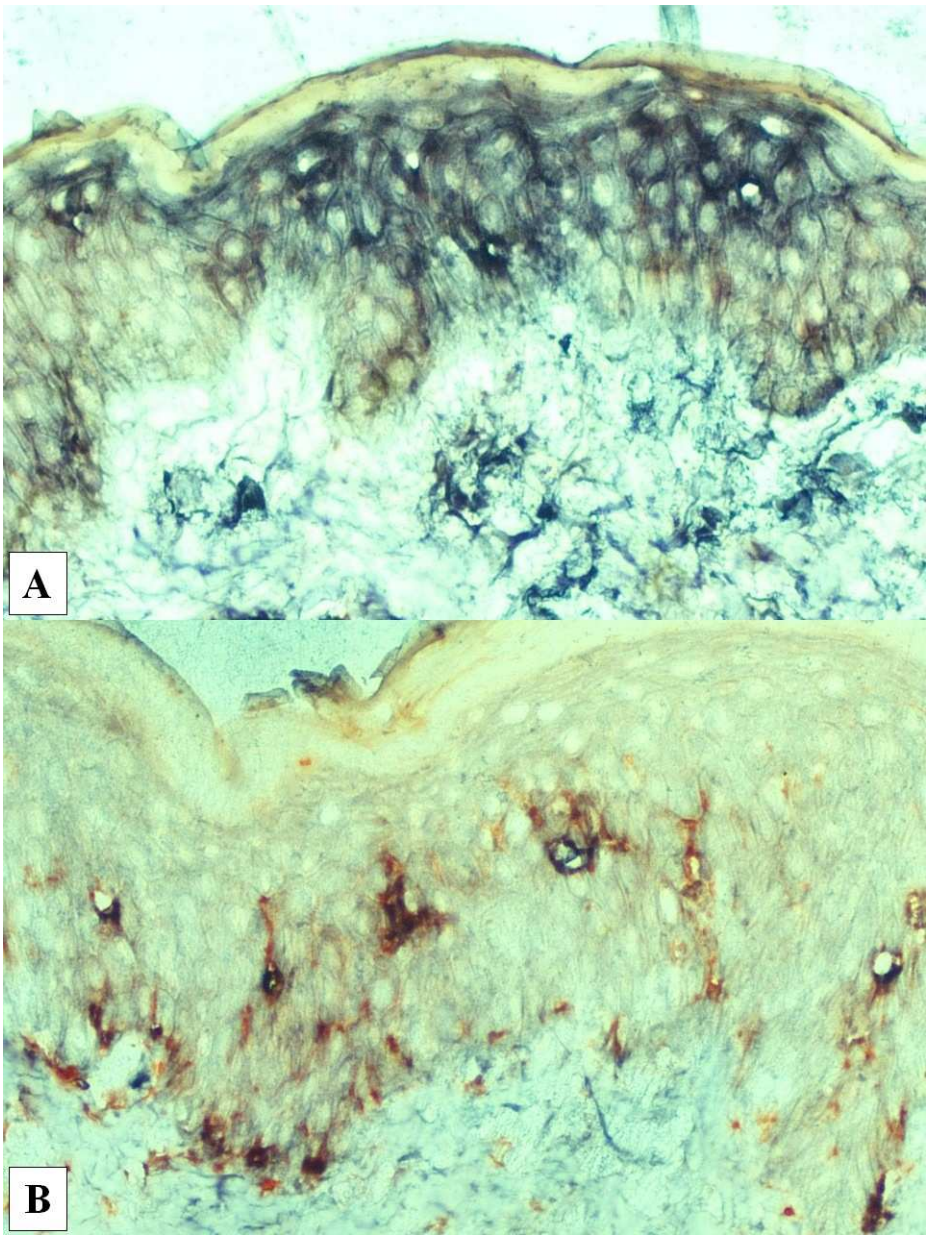

Figure 1. Two-color immunostaining with CD1a (black) and Langerin Ab (light brown) in the uninvolved skin (A) and the involved skin (B) of psoriatic patients. CD1a+ CD206+ cells (IDEC) were present in the epidermis of the involved skin, but not in the uninvolved skin.
(Original magnification, ×200)

Because Langerin is known to be expressed exclusively by LC, immunolabelling with Langerin and CD206 Ab was performed to evaluate the CD206 expression of Langerin+ cells. As expected, there were no double stained cells.

Double staining with CD206 and BDCA-2 Ab was performed, and numerous double-positive cells were found in the dermis of the involved skin of the psoriatic patients (Figure 2). Both CD206+ BDCA-2- cells and CD206- BDCA-2+ cells were occasionally found. Because BDCA-2+ cells were characteristically found only in the dermis of the psoriatic plaque, we did not observe CD206+ BDCA-2+ cells in the specimens of uninvolved skin or in the epidermis of the involved skin. These findings suggested that most pDC in the psoriatic plaque express CD206.

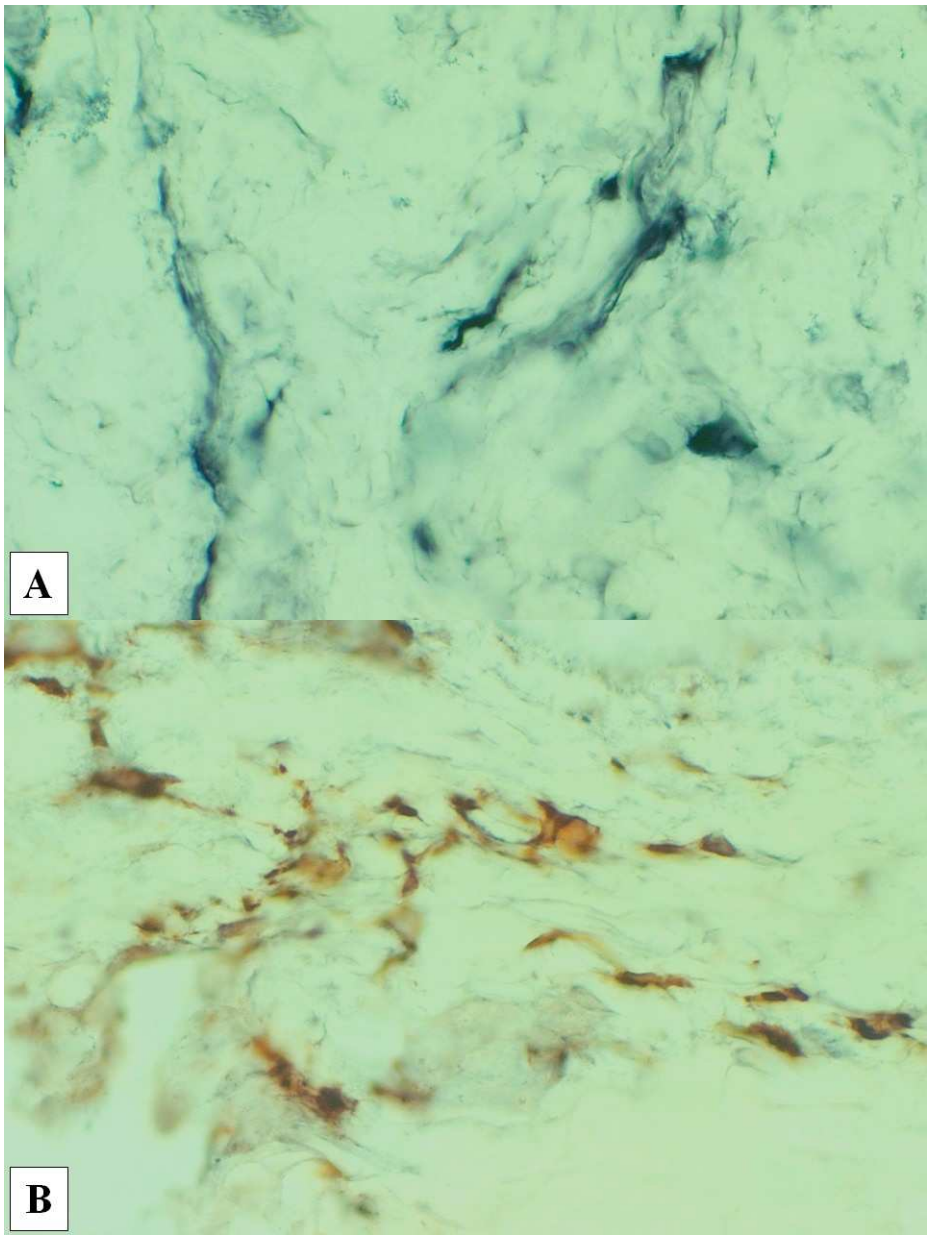

Figure 2. Two-color immunostaining with CD206 (black) and BDCA-2 Ab (light brown) in the uninvolved skin (A) and the involved skin (B) of psoriatic patients. CD206+ BDCA-2+ cells were present only in the dermis of involved skin, not in uninvolved skin.
(Original magnification, ×400)

4. DC subsets showing the activated phenotype (CD83+) in the psoriatic skin

To evaluate which subset of DC expresses CD83, we performed two-color immunostaining with Ab of CD83 and markers of each DC subset: the results are shown in Table 4.

Two-color immunolabelling with CD83 and BDCA-2 Ab showed that BDCA-2+ cells (pDC) did not express CD83 (Figure 3). In CD83 and CD206 immunostaining, a few CD206+ cells showed weak CD83 expression in the epidermis of one psoriatic patient. Apart from this tissue section, we did not observe any cells expressing both CD83 and CD206 (Figure 4).

In double staining with CD1a and CD83 Ab, CD1a+ CD83+ cells were present in the epidermis of all involved skin samples and uninvolved skin sample from one patient (Figure 5). They were also found in the dermis of some psoriatic plaques in small numbers. Double-color immunolabelling with Langerin and CD83 Ab showed similar results to those for the CD1a and CD83 immunolabelling (Figure 6).

Table 4. Two-color immunostaining with Ab of CD83 and markers for each DC subset in the involved and uninvolved skin from the patients with psoriasis vulgaris

Antibodies	Epidermis		Dermis	
	Uninvolved skin	Involved skin	Uninvolved skin	Involved skin
CD83 / BDCA-2	0 (0)	0 (0)	0 (0)	0 (0)
CD83 / CD206	0 (0)	0.2 (0-1)	0 (0)	0 (0)
CD1a / CD83	0.2 (0-1)	1.4 (1-2)	0 (0)	0.6 (0-1)
Langerin / CD83	0.2 (0-1)	1.8 (1-2)	0 (0)	0.6 (0-1)

Numbers indicate median and range (described in parentheses) of a semiquantitative scale scored in five patients with psoriasis (involved and uninvolved skin). Only double-positive cells were counted.

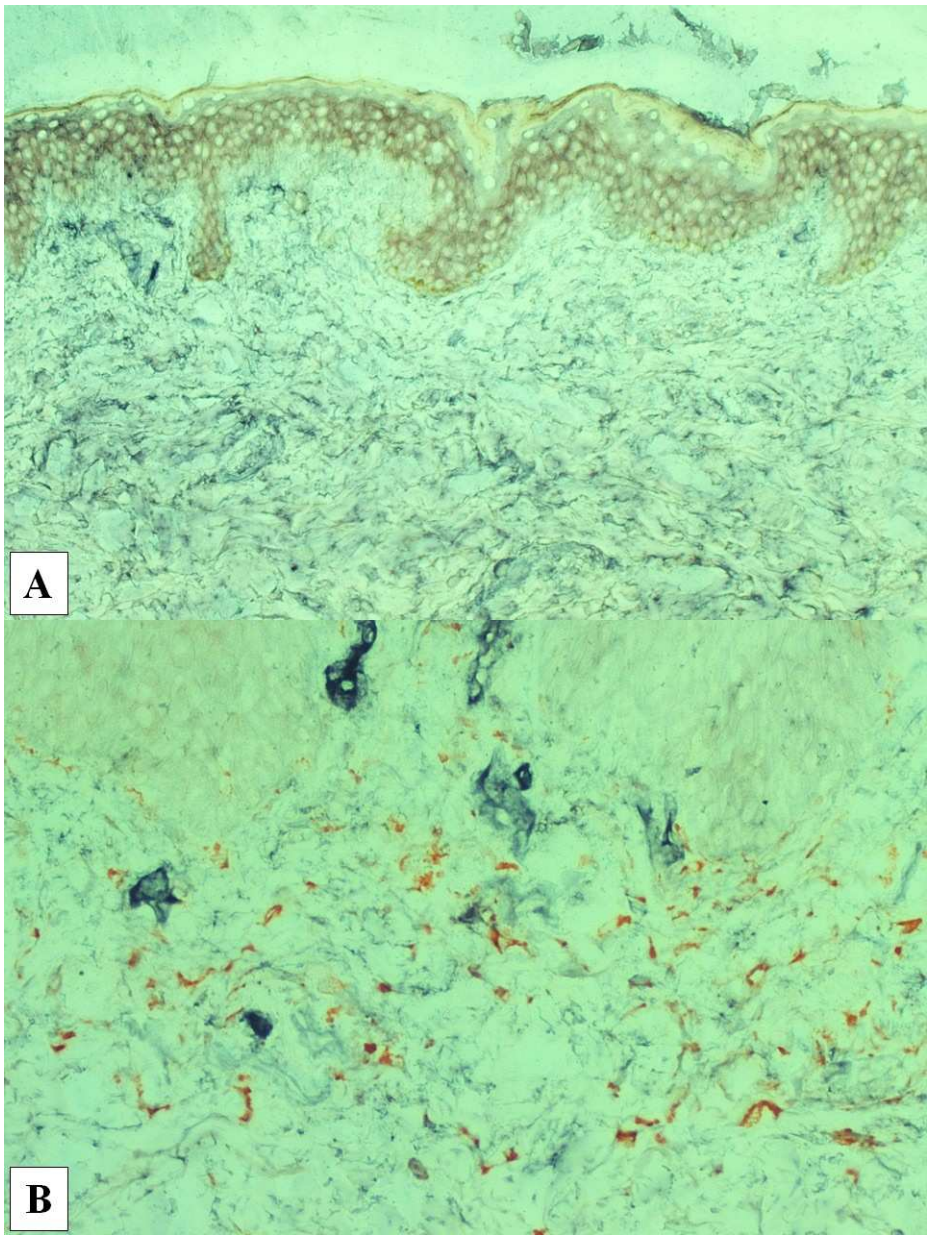

Figure 3. Two-color immunostaining with CD83 (black) and BDCA-2 Ab (light brown) in the uninvolved skin (A) and the involved skin (B) of psoriatic patients. BDCA-2+ cells (pDC) in the dermis of psoriatic plaques did not express CD83.
(Original magnification, A: ×100; B: ×200)

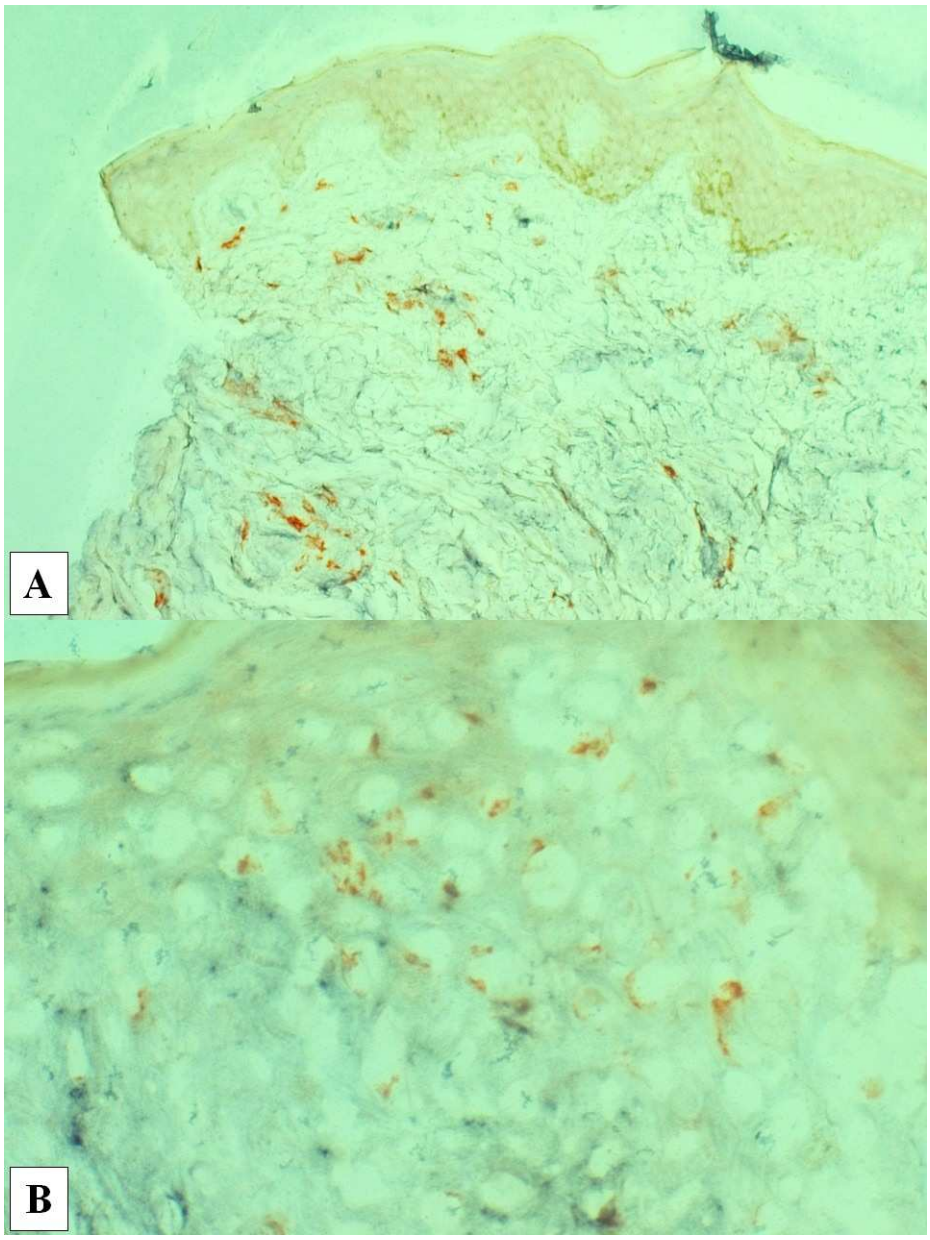

Figure 4. Two-color immunostaining with CD83 (black) and CD206 Ab (light brown) in the uninvolved skin (A) and the involved skin (B) of psoriatic patients. CD206+ cells in the epidermis of psoriatic plaques (IDEC) did not express CD83.
(Original magnification, A: ×100; B: ×400)

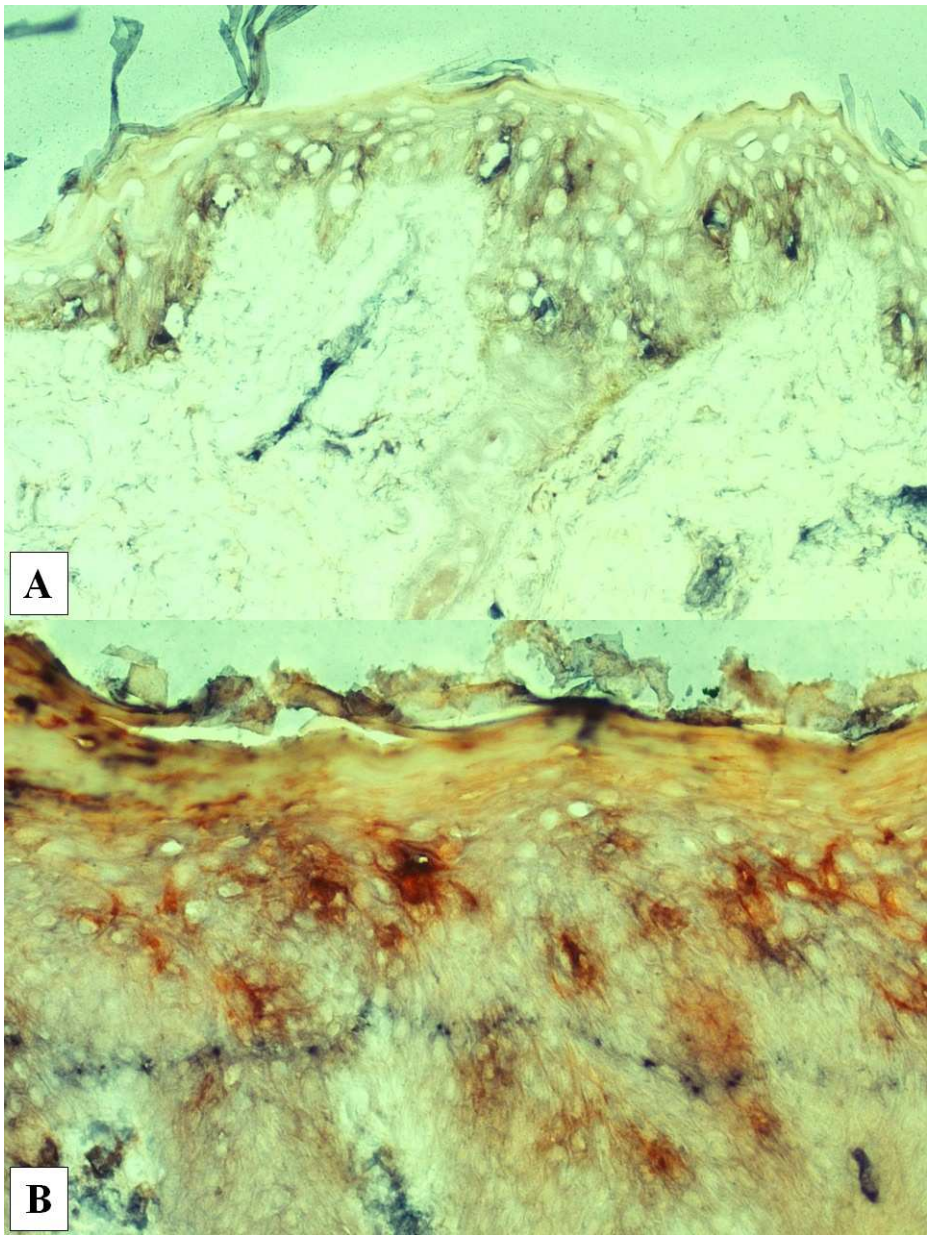

Figure 5. Two-color immunostaining with CD1a (black) and CD83 Ab (light brown) in the uninvolved skin (A) and the involved skin (B) of psoriatic patients. CD1a+ cells in the epidermis of psoriatic plaques expressed CD83.
(Original magnification, A: ×100; B: ×200)

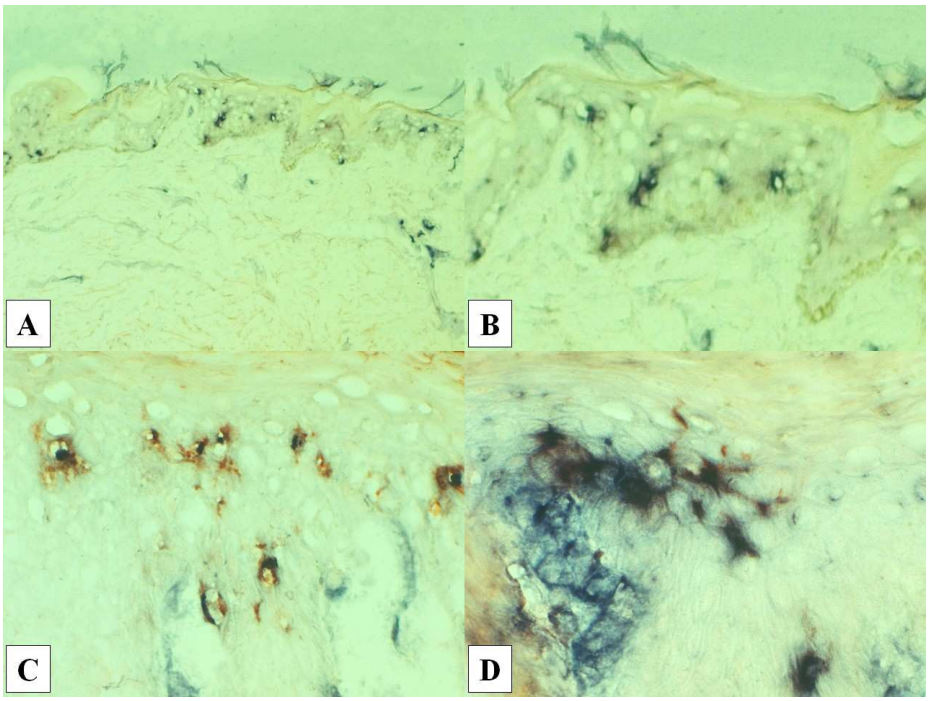

Figure 6. Two-color immunostaining with Langerin (black) and CD83 Ab (light brown) in the uninvolved skin (A,B) and the involved skin (C,D) of psoriatic patients. Langerin+ cells (LC) in the epidermis of psoriatic plaques expressed CD83. (Original magnification, A: ×200; B: ×400; C,D: ×400)

5. Two-color immunostaining with Ab of CD31 and DC markers

Since a part of the staining pattern in the dermis resembled capillaries when we performed immunostaining with CD11c, CD83, CD1a and Langerin Ab, we did two-color immunostaining with CD31 and each of the antibodies.

When stained with CD31 and Langerin Ab, most of the Langerin+ dermal structures resembling vessels expressed CD31, and these double-stained structures were present in the dermis of both the involved and the uninvolved skin (Figure 7). Double-color immunolabelling with CD31 and other DC Ab (CD1a, CD11c, and CD83 Ab) showed the same results.

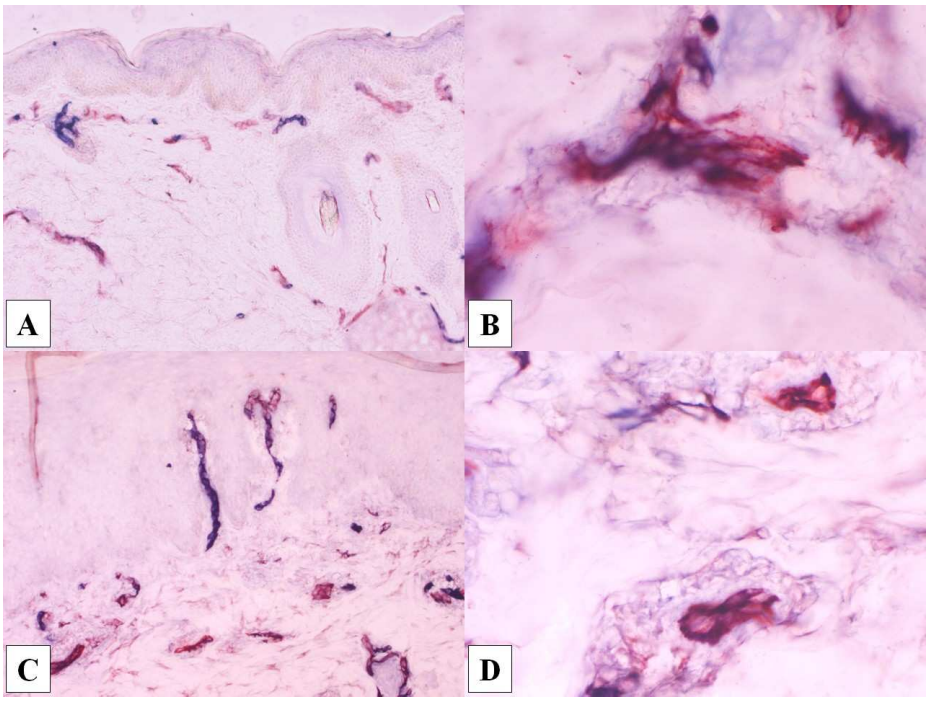

Figure 7. Two-color immunostaining with CD31 (black) and Langerin Ab (red) in the uninvolved skin (A,B) and the involved skin (C,D) of psoriatic patients. Vessel-like Langerin+ dermal structure expressed CD31.
(Original magnification, A,C: ×100; B,D: ×400)

IV. DISCUSSION

Recently, newly identified subsets of DC (IDEC and pDC), which were low in number or absent in normal skin, were reported to be involved in various inflammatory skin diseases. In lupus erythematosus, high numbers of pDC and low numbers of IDEC were observed. In contrast, skin lesions from atopic dermatitis contained many IDEC, but only very few pDC. Lesional skin samples from patients with psoriasis and contact dermatitis showed high numbers of both subsets.[3,5-7] Therefore, various inflammatory skin diseases might show distinct disease-specific compositions of DC, which reflect the etiology of the condition.

Psoriasis is a chronic inflammatory and T cell-mediated cutaneous disease. DC are considered to be involved in the pathogenesis, because DC are known to be the most potent antigen-presenting cells that play a crucial role in initiating immune responses.[3,4,7] In this study, we tried to identify LC, IDEC, and pDC in the skin samples from psoriatic

patients, and to evaluate their surface antigen expression through immunohistochemistry.

The results of immunostaining were hardly influenced by the range of antibody dilution or the different conditions of incubation time and temperature used in this study. Therefore, it seems unlikely that immunolabelling requires strictly accurate incubation conditions and concentrations of primary Ab. It is possible that staining with more diluted Ab than those of our study could produce similar results. Since double staining involves many steps and requires much time, in order to obtain the results that afternoon, we tried to apply the first primary antibodies for overnight incubation at 4℃ the previous evening.

Among CD1 isoforms, only CD1a is closely related to Langerin. Langerin is localized not only on the cell surface, but also intracellularly in close association with Birbeck granules. CD1a is transported from the cell surface to the Birbeck granules, where it colocalizes with Langerin. It is possible that Langerin serves to internalize microbial glycolipids to the Birbeck granules, where the glycolipids are loaded into

CD1a for presentation to T cells. Therefore, the coupling of CD1a and Langerin in Birbeck granules could enable the uptake and presentation of specific glycolipid antigens to T cells by LC.[9,10] In addition to these observations, since CD1a and Langerin are considered to be specific markers for LC, we expected that CD1a and Langerin immunostaining might be much alike in morphology. In our study, the distribution of CD1a+ cells and Langerin+ cells was very similar. However, CD1a immunolabelling showed more background staining but better visualized dendrites than Langerin immunolabelling. Several immunohistochemical studies using CD1a and Langerin Ab have pointed out the different staining patterns of these Ab.[11,12] Based on these studies and our observations, Langerin seems to be the most specific marker for the detection of LC.

Previous studies have reported increases in the overall number of CD11c+ cells in psoriasis plaques.[3,4] However, this increase was not observed in our study. This may be due to the semiquantitative scale, as the '2' scale in this study contained a wide range of cell numbers.

Recent experiments have identified a new DC subset in the epidermis of inflamed skin, which is termed IDEC. These cells differ from LC in that they express CD206 and lack Birbeck granules and Langerin, and are rarely found in normal skin.[3-6,13,14] CD206, the mannose receptor, has been regarded as a differentiation hallmark of immature DC that is not expressed in the monocytes, mature DC and LC. CD206 is expressed on human macrophages and various cells in visceral organs in addition to DC.[15] Therefore, CD206+ cells could be detected in the dermis of all specimens in our study. Previous experiments also showed similar results.[5] CD206 may be involved in the phagocytosis of mannose-coated particles, in the endocytosis of mannosylated glycoproteins, or in receptor-mediated facilitated antigen presentation, as many glycoproteins, particularly those from bacteria and fungi, are mannosylated.[5] IDEC express CD11c strongly, and it seems most likely that they are the epidermal component of CD11c+ DC found in both the epidermis and dermis of psoriasis plaques.[3,4] However, their role in psoriasis remains unresolved.

pDC, another new DC subset, exclusively express the specific marker BDCA-2.[6,16-18] BDCA-4 is also expressed in pDC, but is also up-regulated on CD11c+ myeloid DC after maturation.[18] Therefore, it seems that the most specific marker for the detection of pDC is BDCA-2.

Psoriatic plaques contain high numbers of mature or activated CD83+ DC.[4,7] Koga et al.[7] morphologically identified CD83+ DC in psoriatic skin lesions. They have found that greater numbers of CD83+ DC were present in the upper dermis than in the epidermis, and CD83+ DC were in close contact with lymphocytes. From these results, they suggested that mature CD83+ DC activate neighboring T cells in psoriasis. However, since they did not conduct double-color immunostaining, it was unclear whether the CD83+ cells in the psoriasis skin were LC or other DC subset. Using flow cytometry, Abrams et al.[19] found that LC express various kinds of costimulatory molecules, including CD83, in lesional skin samples from psoriasis patients. In our study, we have identified three subsets of DC in psoriatic plaques, none of which were present in normal and uninvolved skin, except LC. Among these DC subsets, only LC

expressed CD83, which is currently considered to be the most specific marker of activated and mature DC. Moreover, CD1a+ or Langerin+ cells showed a tendency to decrease in the epidermis and a tendency to increase in the dermis of skin samples from psoriasis patients, especially in the involved skin samples. These observations lead to the conclusion that CD83+ cells in psoriasis plaques are mature LC, which migrated to the dermis from epidermis. Langerhans cells stay in close contact with T cells and stimulate T cells by the classical T-cell activation pathway.

Although IDEC and pDC did not express CD83, we could observe these cells only in the psoriatic plaques, not in the uninvolved and normal skin. Therefore, they might engage in the pathogenesis of psoriasis by a different manner. Besides T-cell activation by unknown Ag presentation, alternative immune activation pathways in psoriasis seemed to be innate immune mechanisms, which might play a crucial role of chronic cellular activation in psoriasis plaques.[3,4] For example, through the interleukin-12 (IL-12)-IL-18 pathway, T-cell activation and release of IFN-γ could be attained without T

cell receptor engagement. Keratinocytes in psoriatic skin lesions express a high level of CD1d, which enables the presentation of glycolipids to natural killer T-cells. CD91, the heat-shock protein (HSP) receptor, is highly expressed on dermal fibroblasts and DC in psoriasis skin lesions, and several HSPs have shown the ability to induce both NF-κB activation in fibroblasts and the up-regulation of costimulatory molecules on DC.[3,4,20,21]

IDEC and pDC are known to express CD83 in their active status.[22] However, they did not express CD83 in our study except for one patient who showed weak expression in IDEC. Differential Toll-like receptor (TLR) expression on DC subsets has been described. For example, myeloid DC express TLR4, TLR7, and not TLR9, whereas pDC express TLR7, TLR9 and not TLR4.[23] Lee et al.[24] have found that strong expression of IL-23, which plays a role in type 1-polarized T cell immune reactions, was detected in monocytes and CD11c+ DC in skin samples from psoriasis patients. Since IDEC are considered to be the epidermal component of myeloid (CD11c+) DC, both IDEC and pDC seem to be involved in innate immunity through somewhat

different mechanisms. As T cells are rarely present in the epidermis of psoriasis plaques, IDEC might not directly activate T cells like LC. It should also be acknowledged that pDC, which were found only on the dermis of psoriatic plaques in our study, secrete large amounts of anti-viral type I IFN, which can up-regulate the synthesis of most IFN-regulated genes such as STAT1, p48, ISF3γ, IRF1, IRF4, IRF7, etc.

Vessel-like staining patterns of some primary Ab (CD1a, Langerin, CD11c, and CD83 Ab) were observed in our study. These were reacted with Ab of CD31, the endothelial marker. Even in uninvolved skin, these double-stained structures were also present. It seemed very unlikely that CD31+ endothelial cells expressed DC markers such as CD1a, Langerin, CD11c and CD83. Therefore, we have concluded that DC might migrate to vessels, and DC migration could occur even in the grossly normal appearing, uninvolved skin from psoriatic patients.

In this study, we identified three subsets of DC in psoriatic plaques using double-color immunohistochemistry. We also found that CD83+ DC are LC, but that IDEC and pDC do not express CD83. Although IDEC and pDC did not express CD83, the

activation marker of DC, it is possible that these three subsets of DC may be involved in the pathogenesis of psoriasis though somewhat different mechanisms. However, further experiments are needed to clarify their exact roles.

V. CONCLUSION

In this study, we performed single- and double-color immunolabelling in the frozen tissue of five psoriatic patients and two healthy volunteers. The results are summarized as follows:

1. Compared with CD1a immunolabelling, Langerin immunolabelling was more specific for LC staining, and presented with less background staining.
2. CD1a+ Langerin+ cells (LC) were identified in the involved and uninvolved skin of the psoriatic patients and in the skin of the healthy volunteers.
3. CD1a+ CD206+ cells (IDEC) were found only in the epidermis of the involved skin form the psoriatic patients.
4. BDCA-2+ cells (pDC) were present only in the dermis of the involved skin from the psoriatic patients, except in one patient, who also had BDCA-2+ cells in the uninvolved skin. pDC expressed CD206.
5. CD83+ cells were considerably increased in the skin of the psoriatic patients, especially in the involved skin.

These cells expressed CD1a and Langerin. However, CD206+ or BDCA-2+ cells did not express CD83. These findings suggest that CD83+ cells correspond to LC.

6. Dermal structures resembling vessels in this study expressed CD1a, Langerin, CD11c, CD83, and CD31. These results suggest that DC in the skin of psoriatic patients may migrate around vessels, even if they are in the uninvolved skin.

Based on this study, we have concluded that various subsets of DC are involved in the pathogenesis of psoriasis, and that LC show the activated phenotype (CD83+). Although IDEC and pDC did not express the activation marker (CD83), they may play an important role, because they were present in the psoriatic plaques, and not in the normal skin.

REFERENCES

1. Van de Kerkhof P. Psoriasis, In: Bolognia JL, Jorizzo JL, Rapini RP, editors. Dermatology. London: Mosby, 2003:125-149.
2. Christophers E, Mrowietz U. Psoriasis, In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, editors. Dermatology in general medicine. 6th ed. New York: McGraw-Hill, 2003:407-427.
3. Lew W, Bowcock AM, Krueger JG. Psoriasis vulgaris: cutaneous lymphoid tissue supports T-cell activation and 'Type 1' inflammatory gene expression. Trends Immunol 2004;25:295-305.
4. Chamian F, Krueger JG. Psoriasis vulgaris: an interplay of T lymphocytes, dendritic cells, and inflammatory cytokines in pathogenesis. Curr Opin Rheumatol 2004;16:331-337.
5. Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Gunther S, Moderer M. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. J Invest Dermatol 2002;118:327-334.
6. Wollenberg A, Wagner M, Gunther S, Towarowski A, Tuma E, Moderer M et al. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in

inflammatory skin diseases. J Invest Dermatol 2002;119:1096-1102.

7. Koga T, Duan H, Urabe K, Furue M. In situ localization of CD83-positive dendritic cells in psoriatic lesions. Dermatology 2002;204:100-103.

8. Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. J Exp Med 2001;194:1823-1834.

9. Mizumoto N, Takashima A. CD1a and langerin: acting as more than Langerhans cell markers. J Clin Invest 2004;113:658-660.

10. Hunger RE, Sieling PA, Ochoa MT, Sugaya M, Burdick AE, Rea TH, et al. Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. J Clin Invest 2004;113:701-708.

11. Seguier S, Bodineau A, Godeau G, Pellat B, Brousse N. Langerin+ versus CD1a+ Langerhans cells in human gingival tissue: a comparative and quantitative immunohistochemical study. Arch Oral Biol 2003;48(4):255-262.

12. Chikwava K, Jaffe R. Langerin (CD207) staining in normal pediatric tissues, reactive lymph nodes, and childhood histiocytic disorders. Pediatr Dev Pathol 2004;7:607-614.
13. Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. J Invest Dermatol 1996;106:446-453.
14. Wollenberg A, Wen S, Bieber T. Phenotyping of epidermal dendritic cells: clinical applications of a flow cytometric micromethod. Cytometry 1999;37:147-155.
15. Pontow SE, Kery V, Stahl PD. Mannose receptor. Int Rev Cytol 1992;137B:221-244.
16. Bangert C, Friedl J, Stary G, Stingl G, Kopp T. Immunopathologic features of allergic contact dermatitis in humans: participation of plasmacytoid dendritic cells in the pathogenesis of the disease? J Invest Dermatol 2003;121:1409-1418.
17. Narbutt J, Lesiak A, Zak-Prelich M, Wozniacka A, Sysa-Jedrzejowska A, Tybura M, et al. The distribution of

peripheral blood dendritic cells assayed by a new panel of anti-BDCA monoclonal antibodies in healthy representatives of the polish population. Cell Mol Biol Lett 2004;9:497-509.

18. Chaperot L, Perrot I, Jacob MC, Blanchard D, Salaun V, Deneys V, et al. Leukemic plasmacytoid dendritic cells share phenotypic and functional features with their normal counterparts. Eur J Immunol 2004;34:418-426.

19. Abrams JR, Kelley SL, Hayes E, Kikuchi T, Brown MJ, Kang S, et al. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. J Exp Med 2000;192:681-694.

20. Bonish B, Jullien D, Dutronc Y, Huang BB, Modlin R, Spada FM, et al. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. J Immunol 2000;165:4076-4085.

21. Curry JL, Qin JZ, Bonish B, Carrick R, Bacon P, Panella J, et al. Innate immune-related receptors in normal and psoriatic skin. Arch Pathol Lab Med 2003;127:178-186.

22. Cravens PD, Melkus MW, Padgett-Thomas A, Islas-Ohlmayer M, Del P Martin M, Garcia JV. Development and activation of human dendritic cells in vivo in a xenograft model of human hematopoiesis. Stem Cells 2005;23:264-278.

23. Ito T, Amakawa R, Kaisho T, Hemmi H, Tajima K, Uehira K, et al. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. J Exp Med 2002;195:1507-1512.

24. Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, et al. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. J Exp Med 2004;199:125-130.

국 문 요 약

건선에서 면역화학적 염색을 통한 수지상 세포의 아형 규명

<지도교수 이민걸>

연세대학교 대학원 의학과

박상건

건선은 전세계적으로 발생하며 인구의 약 1-3%에서 발생하는 매우 흔한 피부질환으로 외상, 감염, 차고 건조한 기후 등의 악화 요인들은 알려져 있으나 그 원인에 대해서는 명확히 밝혀져 있지는 않다. 건선의 병인으로 전에는 표피세포의 과증식이 일차적인 원인으로 생각되었으나, 이후 T 세포 매개의 자가면역 질환으로 알려지고 있으며, 최근에는 수지상 세포 또한 중요한 역할을 하는 것으로 알려지고 있다.

수지상 세포에는 랑게르한스 세포, inflammatory dendritic epidermal cell, dermal dendritic cell, plasmacytoid dendritic cell 등이 알려져 있으며, 최근에는 건선 이외의 여러 염증성 피부질환에서 이러한 수지상 세포의 아형들이 다른 기전으로 관여하는 것으로 알려지고 있다.

본 연구에서는 랑게르한스 세포, inflammatory dendritic epidermal cell, plasmacytoid dendritic cell 등의 수지상 세포를

건선 환자의 병변 및 비병변 조직과 정상인의 조직에서 염색하여 비교해 보고자 하였다. 다섯명의 건선환자와 2 명의 정상인을 대상으로 면역화학적 염색을 시행하여 다음과 같은 결과를 관찰할 수 있었다. 건선환자의 병변 및 비병변 조직과 정상인의 조직에서 랑게르한스 세포를 발견할 수 있었고, 병변 부위에서는 CD83 을 표현하였다. Inflammatory dendritic epidermal cell 과 plasmacytoid dendritic cell 은 각각 병변 조직의 표피와 진피에서 발견되었으며, 비병변 부위와 정상조직에서는 발견되지 않았다. 이들은 병변 부위에서 증가되었지만, 랑게르한스 세포와는 달리 CD83 을 표현하지는 않았다.

본 연구를 통해, 저자들은 건선 병변 조직에서의 CD83 양성 수지상 세포는 대부분 랑게르한스 세포임을 알 수 있었다. Inflammatory dendritic epidermal cell 과 plasmacytoid dendritic cell 이 CD83 을 표현하지는 않았지만, 이들은 비병변 부위에서는 발견되지 않고 병변 부위에서만 발견되는 점으로 보아, CD83 을 발현하면서 성숙되지는 않았지만, 건선의 발병기전에 관여할 것으로 사료된다.

핵심 되는 말: 수지상 세포, 랑게르한스 세포, inflammatory dendritic epidermal cell, plasmacytoid dendritic cell, 건선