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Role of Adhesion Molecules in the Development of Pustular Lesions in Patients with Pustulosis Palmaris et Plantaris

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The expression of adhesion molecules and the ligands on endothelial cells and infiltrating inflammatory cells in lesional skin specimens from patients with pustulosis palmaris et plantaris was studied. Intercellular adhesion molecule-1 and E-selectin were expressed on endothelial cells of microvessels in the papillary dermis. Intercellular adhesion molecule-1 was also expressed focally on keratinocytes in the epidermis of the lesional skin. On the other hand, lymphocyte function-associated antigen-1, Mac-1 and sialyl Lewis^x were expressed on infiltrating inflammatory cells. Further, flow cytometric analysis demonstrated that circulating leukocytes in peripheral blood from patients with pustulosis palmaris et plantaris expressed the ligands of adhesion molecules. It is therefore suggested that the expression of adhesion molecules and the ligands on circulating leukocytes, endothelial cells, infiltrating inflammatory cells and keratinocytes might be closely related to the formation of pustular lesions in patients with pustulosis palmaris et plantaris. ***Key words: ICAM-1; LFA-1; keratinocyte.***

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The development of inflammation in the skin requires cell-to-cell communication between infiltrating inflammatory cells and resident cutaneous cells (1). This intercellular communication is mediated by various cytokines, chemotactic factors, and adhesion molecules. Recently, it has been reported that adhesion molecules play an important role in the binding of immune cells to endothelial cells on blood vessels, their movement to the epidermis, and their binding to keratinocytes (1–3). Of particular importance is the movement of inflammatory cells that express lymphocyte function-associated antigen-1 (LFA-1) into the epidermis and their subsequent binding to keratinocytes via the surface expression of intercellular adhesion molecule-1 (ICAM-1) (1–3). A study of the expression of adhesion molecules and the ligands on infiltrating inflammatory cells and cutaneous cells is essential to elucidate the complex cellular interactions in the development of cutaneous inflammatory lesions.

Pustulosis palmaris et plantaris (PPP) is a chronic, relapsing disorder occurring on either the palms or the soles or both (4). Crops of small, deep-seated pustules are seen within areas of erythema and scaling. Histologically, many neutrophils are present within the cavity of the pustule, and a number of inflammatory infiltrates can be seen beneath the pustule (4). Very early lesions may show a mononuclear infiltrate in the lower epidermis overlying the tips of papillae, followed by the formation of an intraepidermal vesicle containing mostly mononuclear cells. Subsequently, the vesicle expands and there is a massive invasion of the cavity by neutrophils, which penetrate the intracellular spaces of the vesicle wall. However, the formation of inflammatory skin lesions of PPP has not been fully identified. In the present study we investigated the expression of adhesion molecules and the ligands on endothelial cells, infiltrating inflammatory cells, keratinocytes, and circulating leukocytes in order to elucidate the development of pustular lesions of PPP.

MATERIALS AND METHODS

Patients

The subjects were 14 Japanese patients with PPP (7 women and 7 men, with an age range of 36–65 years), who were under treatment at Aichi Medical University Hospital. We also examined 9 normal healthy subjects who were roughly matched in age and sex to patients with PPP.

Antibodies

Monoclonal antibodies to ICAM-1 and E-selectin (ELAM-1) were purchased from R & D systems, Abingdon, England. Anti-sialyl-Le^x (SLe^x) monoclonal antibody were obtained from Becton Dickinson, San Jose, USA. Monoclonal antibodies to LFA-1 (CD11a) and Mac-1 (CD11b) were obtained from Immunotech, Marseille, France.

Tissue preparation

Punch biopsy specimens, 3 mm in diameter, were obtained from the pustular lesions and non-lesional skin of palms in the patients under local anesthesia. Punch biopsy specimens from the palms of healthy controls were also used. The biopsies in OCT compound were snap-frozen immediately in liquid nitrogen, and 4-μm thick sections were then cut from the frozen specimens and placed on each slide, to give the most representative assessment. They were air-dried for 24 h at room temperature.

Immunohistochemical staining technique

The sections were fixed in acetone for 10 min prior to staining. For the blocking of the endogenous peroxidase activity, the sections were treated with methanol containing 0.3% hydrogen peroxide for 15 min at room temperature and washed in 0.01 M phosphate-buffered saline (PBS), pH 7.2, containing 2% normal horse serum. They were incubated with appropriately diluted various monoclonal antibodies at 4°C for 1 h. The immunoreactivity was detected with avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, Calif., USA). Finally, they were stained in a solution of 3,3-diaminobenzidine (0.2 mg/ml) and 0.01% hydrogen peroxide in PBS. Sections were counterstained with methyl green. In negative control sections, an irrelevant monoclonal antibody was used.

Cell preparation

Heparinized peripheral blood was layered on Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) and centrifuged at 1,800 rpm for 20 min. Polymorphonuclear leukocytes (PMN) were prepared by hemolysis of erythrocytes in buffy coat with a lysis buffer (0.826% NH_4Cl, 0.1% $KHCO_3$, 0.0037% EDTA2Na, pH 7.4). Peripheral blood mononuclear

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cells (PBMC) were collected from the plasma-Ficoll interface. PMN and PBMC were washed with RPMI 1640 medium 3 times.

Laser flow cytometry

PMN and PBMC were incubated with appropriately diluted various antibodies against adhesion molecules for 30 min at 4°C. They were washed with RPMI 1640 containing 5% fetal calf serum 3 times and then stained with anti-mouse Ig antibody conjugated with fluorescein isothiacyanate (FITC) (Dako, Kyoto, Japan) by an indirect immunofluorescence staining method. Immunofluorescence-positive cells were analyzed with the aid of a laser flow cytometer (FACS 440, Becton Dickinson, Mountain View, USA). PMN and PBMC were inspected by gating with the forward and side scatter. The fluorescence intensity was expressed in a log scale.

RESULTS

Expression of ICAM-1 and E-selectin in lesional skin specimens from patients with PPP

The expression of ICAM-1 and E-selectin in lesional skin specimens from patients with PPP was studied by immunohistochemical staining. Typical experimental results are shown in Fig. 1. ICAM-1 was expressed on blood vessels in the papillary dermis (Fig. 1A), and ICAM-1-positive keratinocytes were grouped focally in the epidermis (Fig. 1A and B). There were few infiltrates around ICAM-1-positive areas (Fig. 1B). In pustular lesions ICAM-1 was found to be expressed on the wall of the pustule and the blood vessels in the papillary dermis (Fig. 1C). E-selectin was also expressed on blood vessels in the papillary and subpapillary dermis (Fig. 1D). In non-lesional palm skin specimens from patients with PPP, ICAM-1 was expressed weakly on blood vessels in the papillary dermis as compared with lesional skin specimens. But there were no ICAM-1-positive keratinocytes in the epidermis of non-lesional specimens (data not shown). No significant expression of the adhesion molecules was detectable in skin specimens from normal healthy controls.

Expression of SLe^x, LFA-1 and Mac-1 on infiltrating inflammatory cells in lesional skin specimens from patients with PPP

The expression of SLe^x, LFA-1 and Mac-1 on infiltrating inflammatory cells in lesional skin specimens from patients with PPP was studied by immunohistochemical staining. Typical experimental results are shown in Fig. 2. SLe^x, Mac-1 and LFA-1 were positively stained on inflammatory cells infiltrating beneath the pustule (Fig. 2). They were also detected on PMN infiltrating into the pustule.

Expression of SLe^x, LFA-1 and Mac-1 on PMN and PBMC in peripheral blood from patients with PPP

The expression of SLe^x, LFA-1 and Mac-1 on PMN and PBMC in peripheral blood of patients with PPP was studied by laser flow cytometry. Typical experimental results are shown in Figs. 3 and 4. In the histogram analysis a large number of

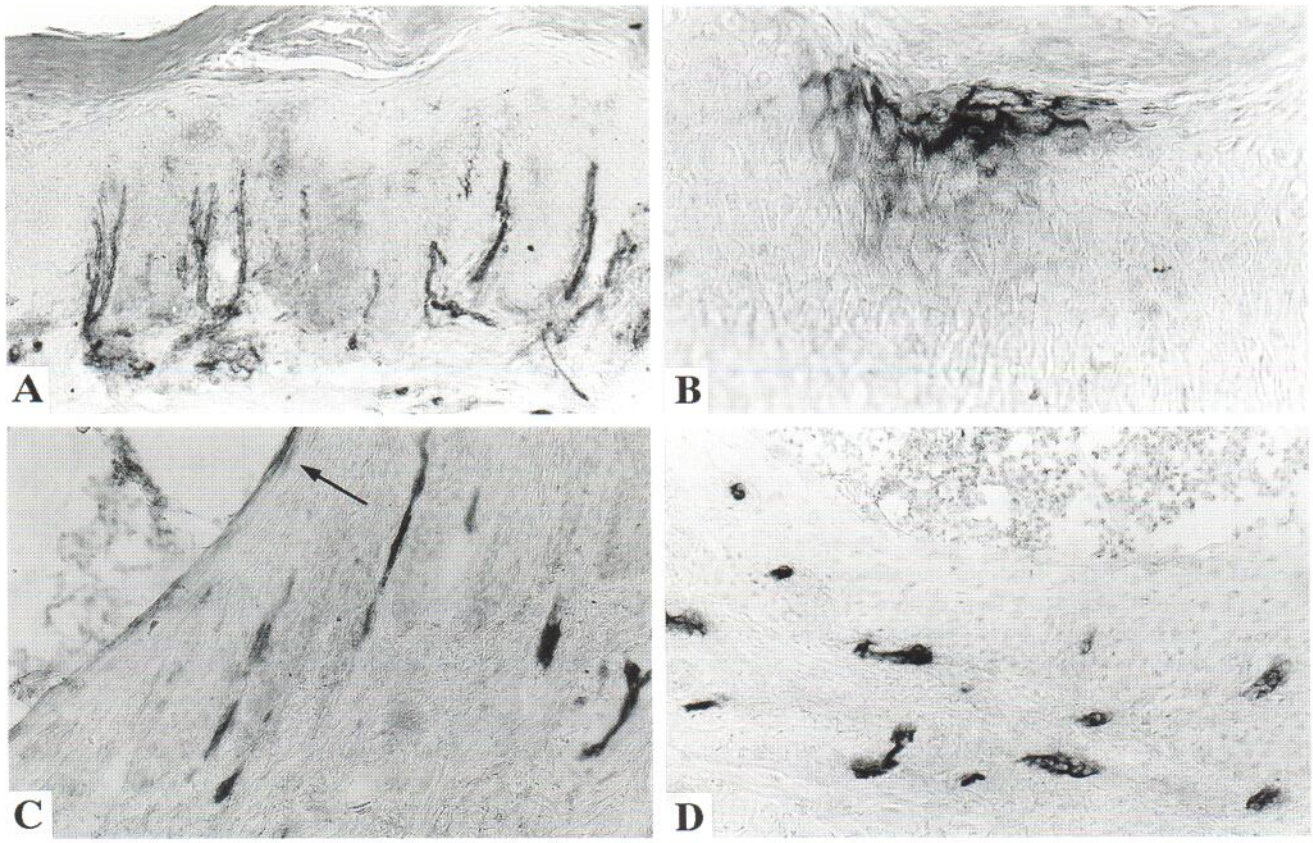

Fig. 1. Expression of ICAM-1 and E-selectin in lesional palm skin specimens from patients with PPP. The specimens were immunohistochemically stained by an antibody to ICAM-1 (A, B, C) or E-selectin (D). ×200. It should be noted that ICAM-1 was expressed on the wall of the pustule (*arrow*).

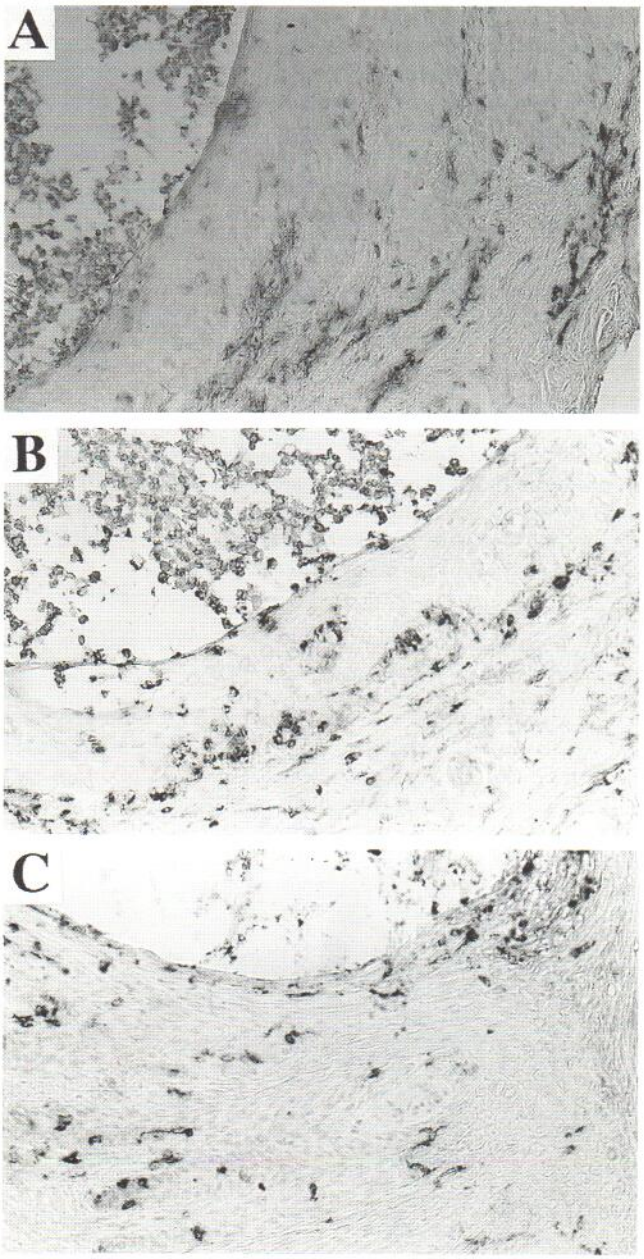

Fig. 2. Expression of SLe^x, LFA-1 and Mac-1 on infiltrating inflammatory cells in lesional palm skin specimens from patients with PPP. The specimens were immunohistochemically stained by an antibody to LFA-1 (A), Mac-1 (B) or SLe^x (C). ×200.

PMN shifted rightward, indicating augmented expression of SLe^x, LFA-1 and Mac-1 on their surface (Fig. 3). The frequency of the positive reactivity for each antigen in patients with PPP is shown in Table I. LFA-1, Mac-1 and SLe^x were expressed on 13, 8, and 5 out of 14 patients, respectively. LFA-1 antigen was expressed on most patients with PPP. PMN from healthy controls occasionally expressed those antigens weakly. Next, PBMC from a typical patient with PPP were examined (Fig. 4). The expression of LFA-1 was enhanced on a large part of PBMC from the patient with PPP, and the enhancement of Mac-1 expression was detected on a small part of those PBMC. However, SLe^x was undetectable on PBMC.

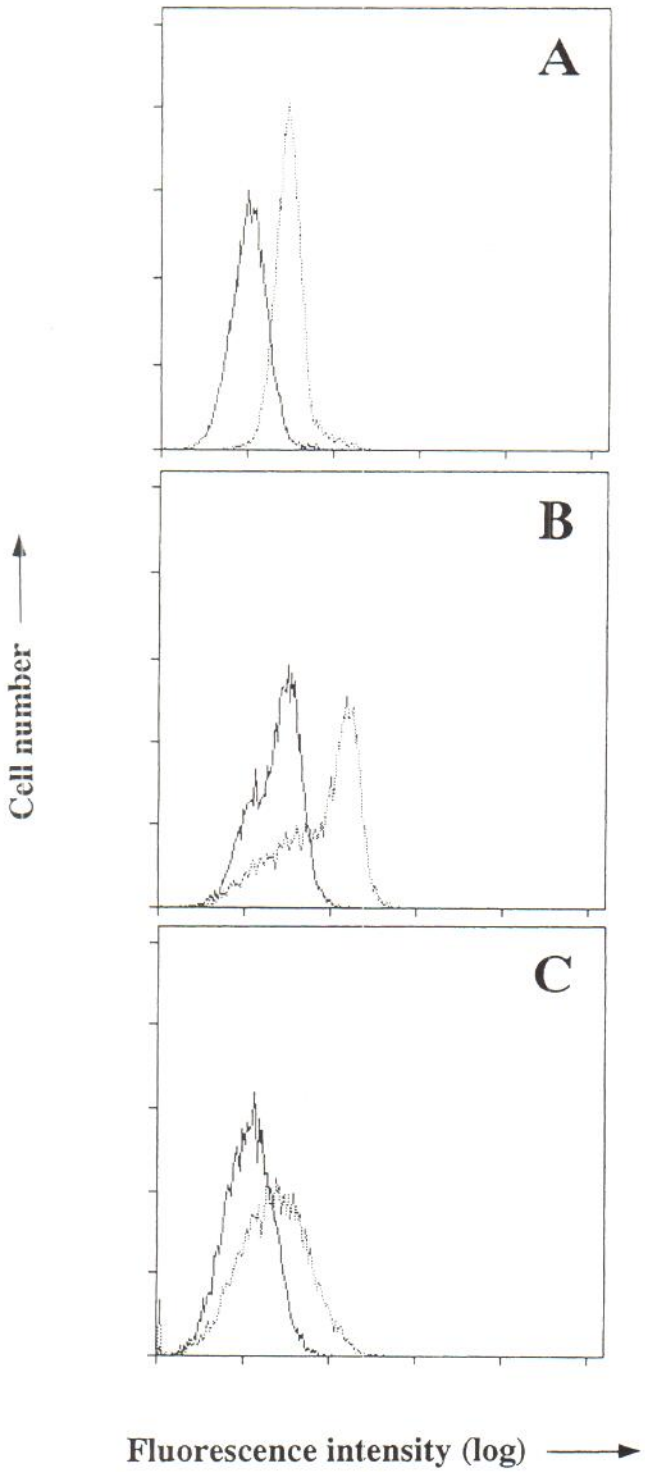

Fig. 3. Expression of SLe^x, LFA-1 and Mac-1 on PMN in peripheral blood from patient with PPP. PMN from patient with PPP (·····) and healthy controls (——) were stained by immunofluorescence using an antibody to LFA-1 (A), Mac-1 (B) or SLe^x (C). The fluorescence intensity is expressed on a log scale.

DISCUSSION

In the present study we have demonstrated that various adhesion molecules and ligands, such as ICAM-1, E-selectin, LFA-1, Mac-1 and SLe^x, were definitely expressed on pustular

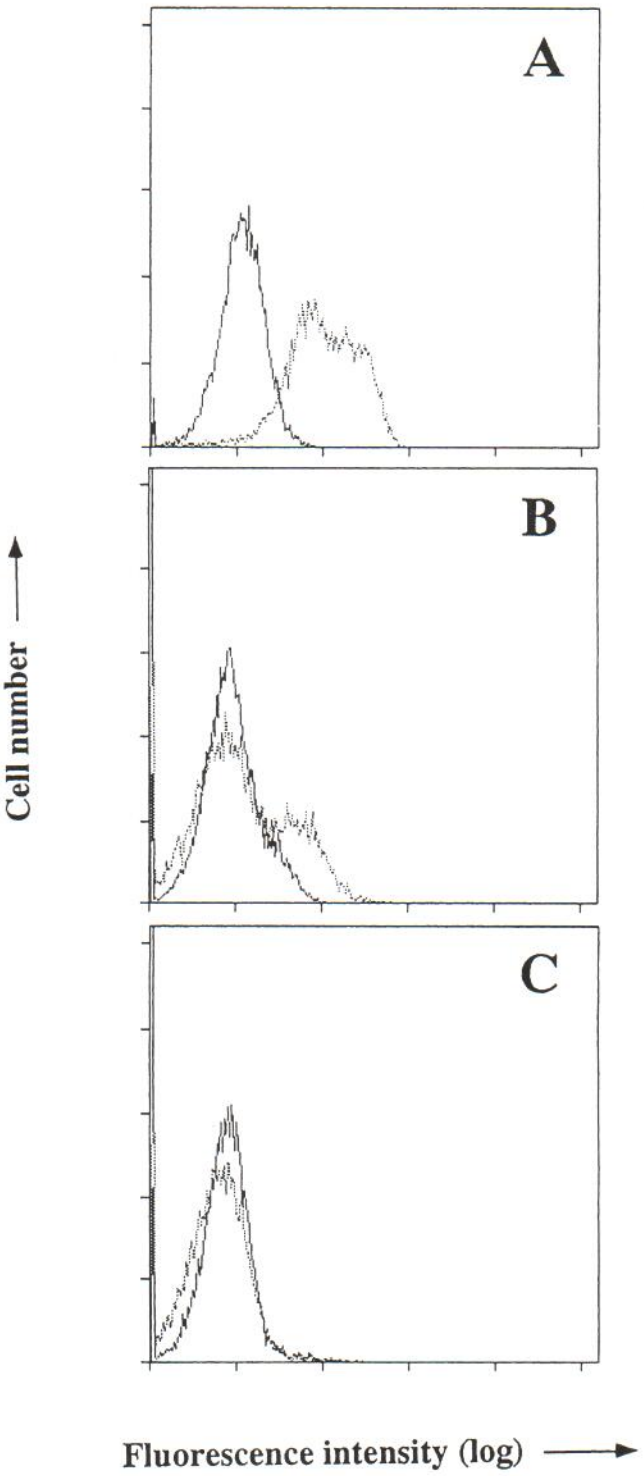

Fig. 4. Expression of SLe^x, LFA-1 and Mac-1 on PBMC in peripheral blood from patient with PPP. PMN from patient with PPP (·····) and healthy controls (——) were stained by immunofluorescence using an antibody to LFA-1 (A), Mac-1 (B) or SLe^x (C). The fluorescence intensity is expressed on a log scale.

lesions in patients with PPP. ICAM-1 and E-selectin were expressed on endothelial cells of microvessels in the papillary and subpapillary dermis, and ICAM-1 was also expressed on keratinocytes in the epidermis of the lesional skin. On the other hand, LFA-1, Mac-1 and SLe^x were expressed on infiltrating inflammatory cells. Our findings indicate that these adhesion molecules and ligands might play a crucial role in the initiation and maintenance of pustular lesions in patients with PPP. It has been reported that adhesion molecules are expressed on endothelial cells and infiltrating leukocytes in chronic inflammatory skin diseases, such as psoriasis, and are essential to the accumulation of inflammatory cells in those lesions (5–8). Our finding was essentially consistent with these studies of psoriasis. Furthermore, the present study has demonstrated that the ligand-expressing leukocytes circulate in peripheral blood of patients with PPP. To our knowledge, there are few studies on the expression of the ligands of adhesion molecules on circulating leukocytes in patients with chronic inflammatory diseases, such as PPP and psoriasis. It should be noted that the expression of the ligands on circulating leukocytes would be important in the process of recruitment of inflammatory cells, which leave the confines of the blood vessel lumen to perivascular tissue.

Table I. *Enhanced expression of ligands to adhesion molecules on PMN in peripheral blood from patients with PPP*

Ligands	Enhanced/total No. (positive %)
LFA-1	13/14 (93)
Mac-1	8/14 (57)
SLe^x	5/11 (45)

Nickoloff et al. (1) reviewed the role of adhesion molecules in inflammatory skin diseases. Their hypothetical idea in the molecular basis for the increased trafficking of leukocytes into the skin is that leukocytes expressing LFA-1 bind to ICAM-1-expressing endothelial cells, which are stimulated by cytokines, such as interferon-γ and interleukin-1. Their idea is supported by our present finding that ICAM-1 and LFA-1 were expressed on blood vessels in the lesional skin and circulating leukocytes, respectively. It was also demonstrated that Mac-1, another ligand of ICAM-1, seemed to be involved as well as LFA-1. Moreover, the expression of E-selectin and its ligand, SLe^x, on endothelial cells and circulating leukocytes provides new evidence that a combination of E-selectin and SLe^x also participates in the binding of leukocytes to endothelial cells. It has been reported that the expression of selectins on circulating leukocytes causes their rolling on endothelial cells (9, 10). The importance of these adhesion molecules in the binding of circulating leukocytes to endothelial cells is supported by the finding that inflammatory cells infiltrating into the lesional skin expressed them.

Of particular interest is that ICAM-1-positive keratinocytes were grouped focally in the epidermis of lesional skin, but not non-lesional skin. There was no significant infiltration of lymphocytes which could produce cytokines for induction of ICAM-1. It is likely, therefore, that ICAM-1 was expressed on keratinocytes through the antigen-non-specific mechanism. The pathogenesis of PPP might be closely related to the selective expression of ICAM-1 on keratinocytes in the palms or the soles. The focal expression of ICAM-1 on keratinocytes might be the targets in the chemotaxis of inflammatory cells towards the epidermis (3). Actually, a close spatial and temporal association between elevated ICAM-1 expression and the appearance of leukocytes in cutaneous inflammatory

lesions has been described (11–13). The accumulation of infiltrates to ICAM-1-positive areas might initiate the formation of the pustule. It is not unlikely that the focal expression of ICAM-1 on keratinocytes in early lesions may be related to its expression at the wall of the pustules in advanced lesions. However, it has been reported that epidermal expression of ICAM-1 is not a primary inducer of cutaneous inflammation in transgenic mice (14). Presumably, chemotactic factors for leukocytes, such as IL-8 (15) and C5a (16), might be another key element in production of PPP lesions. It was of interest that IL-8 was immunohistochemically stained at the neighbourhoods of ICAM-1-positive areas in the epidermis (data not shown).

The mechanism of the onset of PPP has been a subject of controversy. The metal allergy, smoking and focal infection theories have been suggested as possibilities (17, 18). Delayed type hypersensitivity to heavy metals and microbial infections at tonsils may cause the activation of peripheral blood leukocytes and endothelial cells in blood vessels via released cytokines and induce the expression of adhesion molecules on them (19). When keratinocytes would incidentally express ICAM-1, circulating leukocytes which expressed LFA-1 and Mac-1 could bind to endothelial cells, and move to the epidermis. It should be clarified how adhesion molecules are expressed selectively on epidermal keratinocytes in the palms and soles of patients with PPP.

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