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Expression of Beta-2 Integrin Molecules on Human Keratinocytes in Cytokine-mediated Skin Diseases

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Integrins are cell surface molecules of importance in a wide variety of cellular functions, including morphogenesis, cell migration and cell matrix interactions. The beta-2 (B2) integrin (leukocyte integrin, CD11/CD18) subfamily comprising three members, each consisting of a shared beta subunit (CD18) non-covalently associated with unique alpha subunits (CD11a, CD11b, CD11c). In the present study, we have analysed the expression pattern of B2 integrins on the surface of human keratinocytes (HKs) in biopsies obtained from healthy volunteers, from positive tuberculin skin tests and from patients with acute urticaria (AU), lichen planus (LP), psoriasis vulgaris (PV), mycosis fungoides (MF) or purpura pigmentosa chronica (PPC). In biopsies obtained from positive tuberculin tests and from the clinically involved skin of patients with LP, PV, MF or PPC, a multifocally occurring, suprabasal peroxidase-positive reaction was observed on the membranes of the HKs when the monoclonal antibodies (MABs) Dako CD11a, Dako-p150, 95 or Dako CD18 were used. In contrast, no specific staining of the HKs was observed with the same MABs in biopsies from healthy volunteers, from patients with AU and in the uninvolved skin specimens obtained from the other patients. The HKs from PV, LP, MF, PPC and AU patients and those from the healthy subjects failed to give a positive reaction when the MAB against CD11b (OKM1) was used. Our present findings provide further evidence that HKs may be actively involved in cell adhesion processes. ***Key words: CD11; CD18; Tuberculin-positive skin; Lichen planus; Psoriasis vulgaris; Mycosis fungoides; Purpura pigmentosa chronica.***

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Recent investigations have demonstrated that human keratinocytes (HKs) are able to express cell surface antigens characteristic of effector and/or accessory cells of the immune system. Thus, they react specifically with the monoclonal antibody (MAB) Leu-11b(CD16), which is a typical marker of natural killer cells, granulocytes and macrophages, and under certain circumstances (in different cytokine-mediated dermatoses such as lichen planus (LP), psoriasis vulgaris (PV), purpura pigmentosa chronica (PPC), etc.) they may also become HLA-DR, HLA-DQ, CD21, CD36, CD54, CD11a and CD18-positive cells (1–7).

To obtain further information regarding the surface characteristics of HKs, we have investigated the expression of beta-2 (B2) integrins on these cells in skin biopsies obtained from tuberculin-positive reactions, from healthy volunteers, and from patients with LP, PV, mycosis fungoides (MF), PPC, or acute urticaria (AU) in vivo. The present study provides evidence that, in the lesional skin of patients with LP, PV, MF or PPC, and also in the positive tuberculin skin reaction, HKs of the epidermal basal cell layers may exhibit a multifocally occurring specific peroxidase-positive reaction with the MABs against CD11a, CD11c and CD18.

MATERIAL AND METHODS

Patients

Investigatio s were carried out on surgical skin specimens from healthy volunteers ($n = 5$) who underwent plastic surgery, on punch biopsy specimens (3 mm) of involved and uninvolved skin from patients with LP ($n = 5$), PV ($n = 5$), MF stage II ($n = 2$), PPC ($n = 2$), AU ($n = 2$) and on biopsies obtained from positive tuberculin skin

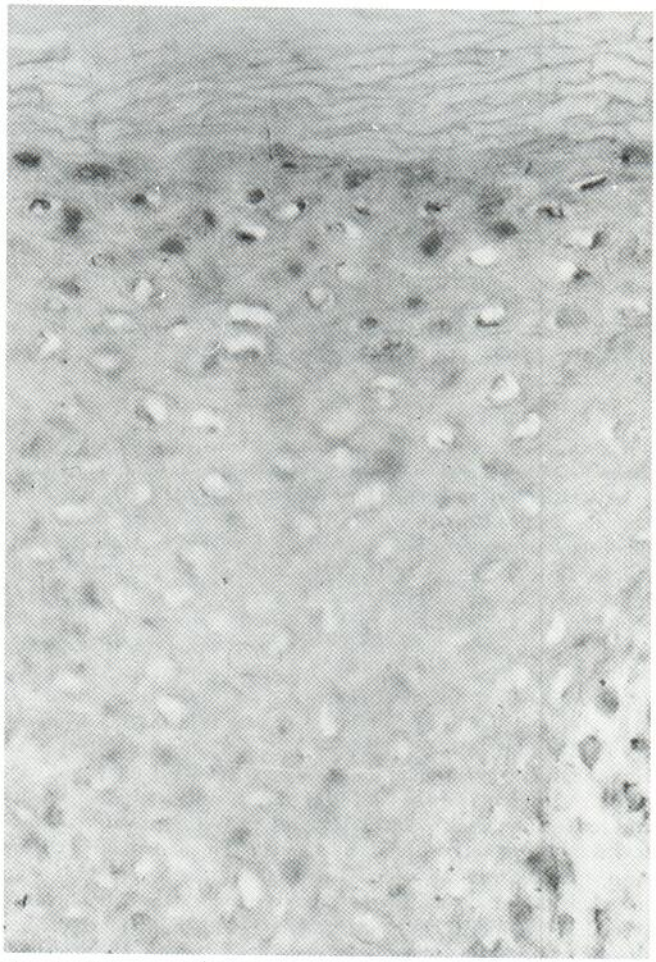

Fig. 1. Absence of CD11b expression on lesional keratinocytes in lichen planus (AEC, ×400).

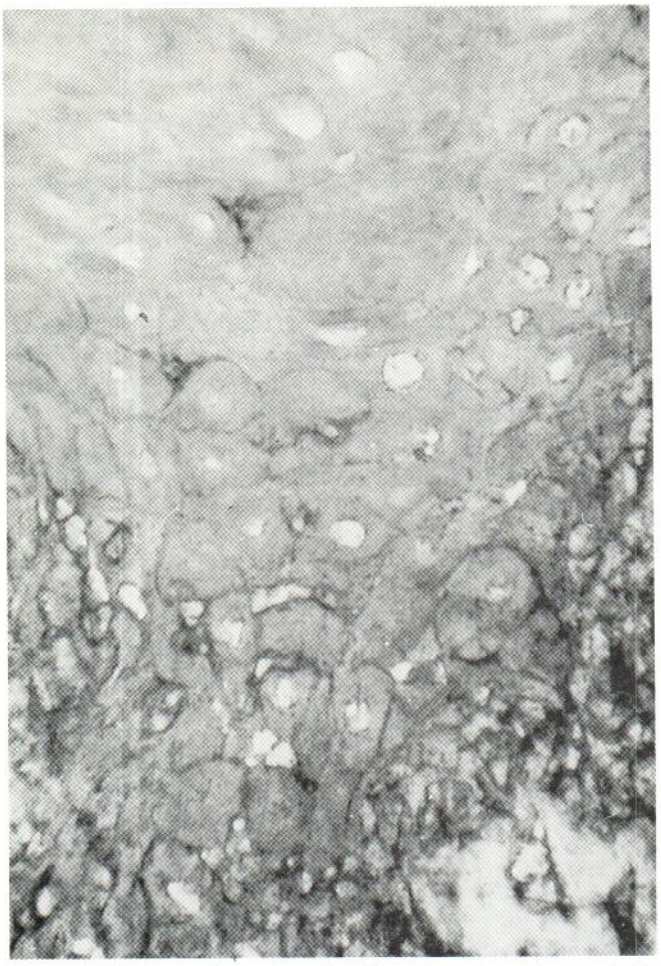

Fig. 2. CD11a expression on suprabasal keratinocytes and on dendritic cells in tuberculin-positive skin (AEC, ×400).

tests (72 h after injection of purified protein derivate; $n = 3$). None of the patients had been involved in any systemic treatment or had used topical corticosteroids during the last 3 weeks before the biopsy. Each specimen was immediately frozen in liquid nitrogen.

Immunohistochemistry

4-μm sections were cut on a cryostat and reactivity with the MABs Dako CD11a(CD11a), Dako-p150,95(CD11c), Dako-CD18(CD18) (Dako Diagnostika, Hamburg, FRG) and OKM1(CD11b) (Ortho Diagnostics, Raritan, N. J., USA) were visualized by means of a multistep immunoperoxidase method described by Poppema et al. (8). Briefly, cryostat sections were frozen at −70°C for at least 24–48 h. After thawing, they were immediately washed in phosphate-buffered saline (PBS), pH 7.2, and incubated with the monoclonal antibodies for 30 min at room temperature. The sections were subsequently washed three times in PBS and incubated with peroxidase-conjugated rabbit-antimouse antibody (Dakopatts, Glostrup, Denmark) diluted 1:10 with human AB serum diluted 1:1 with PBS (SPBS). This was followed by PBS washing and by a 30-min incubation with peroxidase-conjugated swine-antirabbit antibody (Dakopatts) diluted 1:10 with SPBS. After three washes with PBS, the specific peroxidase activity was revealed by using aminoethyl carbazole (AEC) as substrate, followed by a 10-min rinse in distilled water. Finally, counter-staining was performed with hemalum. Two kinds of negative controls were included: first, omission of the primary monoclonal antibody and, second, substitution of the primary antibody with irrelevant antibodies (mouse monoclonal antibodies IgG_1 and IgG_2; Becton Dickinson).

RESULTS

There was no specific peroxidase-positive staining concerning OKM1(CD11b) expression on the epidermal cells (neither HK nor Langerhans' cells) in the biopsy specimens from the investigated persons, but in all skin specimens from positive tuberculin reactions and from patients with LP, PV, MF or PPC, the dermal inflammatory infiltrate exhibited numerous OKM1-positive cells (Fig. 1).

On the other hand, in all biopsies from positive tuberculin skin reactions and from the lesional skin of patients with LP, PV, MF or PPC, a multifocal, suprabasal peroxidase-positive reaction was observed on the membranes of HKs when exposed to Dako CD11a(CD11a) (Fig. 2), Dako-p150,95(CD11c) or Dako CD18(CD18) (Fig. 3) MABs. With the same antibodies, no specific staining of HKs was observed in skin specimens from healthy volunteers, in those from patients with AU, or in the clinically uninvolved skin of patients with LP, PV, MF or PPC.

Dendritic cells in the skin of healthy subjects, in tuberculin-positive skin, and in skin specimens obtained from patients with LP, PV, MF or PPC gave specific peroxidase-positive reactions with the MABs against CD11a, CD11c and CD18.

DISCUSSION

The understanding of the skin immune system (SIS) has advanced remarkably in recent years. An increasing body of evidence proves that the HKs are active and important participants in the SIS. Thus, the HKs are able to produce a large number of cytokines with defined immunological activities (9), they are able to synthesize and express cell surface moieties characteristic of immune cells (1–7), and they react specifically

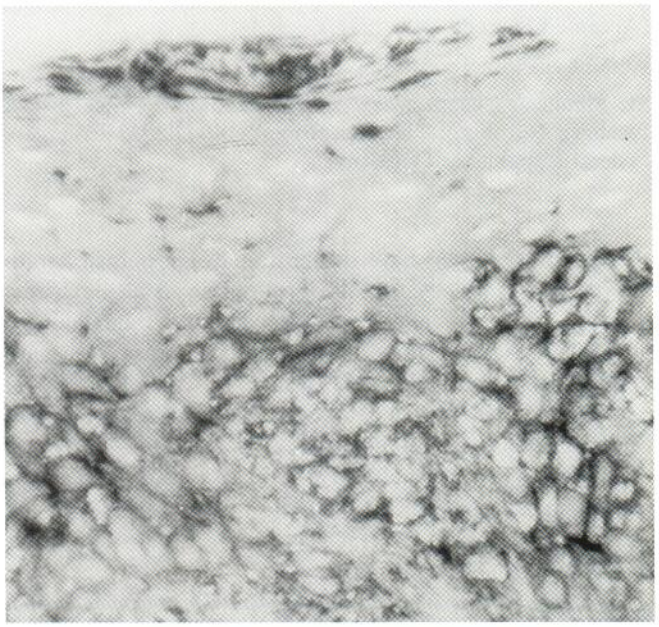

Fig. 3. CD18 expression on suprabasal keratinocytes in lesional skin of patients with psoriasis vulgaris. Note the specific positive staining of the Munro abscess as well (AEC, ×250).

with immune modulating cytokines such as interferon-gamma and tumour necrosis factor (10–11).

Adhesion molecules are rapidly growing groups of well-characterized cell surface moieties of physiological importance (12). The leukocyte integrin subfamily (CD11/CD18 integrin subfamily, B2 integrin molecules) is involved in mediating leukocyte adhesion processes in virtually all stages of the cellular immune response (13, 14). These molecules, each composed of a shared beta subunit (CD18) non-covalently associated with a unique alpha chain (CD11a, CD11b, CD11c), are involved in both cell-cell and cell-matrix interactions and are expressed on haematopoietic cells (12, 14).

The maintenance of the cutaneous homeostasis probably depends on cellular interactions between keratinocytes, Langerhans' cells, melanocytes, Merkel cells(?), fibroblasts, endothelial cells, T-lymphocytes and other lymphoid cells. It is well known that HKs are actively involved in wound healing processes and in cutaneous cell-cell interactions, and that under certain circumstances they are able to phagocytose and kill living microorganisms as well (2). All of these processes are presumed to be closely related with different kinds of cytokine signals and with surface molecules of the involved cells. B2 integrins belong among the key structures of these cell surface moieties (12, 14).

In healthy skin, the expression of CD11a, CD11b, CD11c and CD18 on Langerhans' cells is well documented, whereas the HKs of the healthy skin do not express these molecules (15, 16). Similarly, we have also seen some CD11a, CD11c and CD18-positive dendritic cells in the skin of healthy subjects, in tuberculin-positive skin, and in skin specimens obtained from patients with AU, LP, PV, MF or PPC.

Recently, we reported that, in the clinically involved skin of patients suffering from LP, the HKs of the basal cell layer exhibit focally membranous specific peroxidase-positive staining when exposed to MABs against CD11a and CD18 (5). Our present findings fit in well with these data. Moreover, the same phenomena were also detectable in other cytokine-mediated skin diseases, such as PV, MF or PPC. The lesional HKs in the aforementioned dermatoses may express CD11c as well. It remains to be elucidated whether the acquired, presumedly not disease-specific, B2 integrin (CD11a, CD11c, CD18) expression of HKs in cytokine-mediated skin diseases is due to the synthesis of these molecules by HKs themselves, and whether it fulfils an active function.

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