Human Epidermal Langerhans' Cells in Bullous Pemphigoid

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Through the epidermal analysis of 13 patients with bullous pemphigoid compared to controls, using OKT6 monoclonal antibodies on the light microscopic level and electron-microscopy, we found a redistribution of the Langerhans' cells towards the basal membrane in combination with an increased total number of Langerhans' cells. This redistribution was also noted in clinically normal skin from patients with bullous pemphigoid. The findings may be consistent with the theory of antigen presentation. (Received April 28, 1987.)

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It is considered today that the epidermal Langerhans' cells (LC) is a subpopulation of the mononuclear phagocyte system with the capacity of binding and presenting antigens. The Langerhans cells do also possess receptors for C_3 and the Fc-fragment of immunoglobulins at the cell surface. Both qualitative and quantitative alterations have been reported in the Langerhans cell population at various pathological conditions (4). The purpose of the present investigation was to study the epidermal Langerhans cell population in patients with bullous pemphigoid.

MATERIAL AND METHODS

To perform an analysis of the Langerhans' cells at the light microscopic level the monoclonal antibody OKT6 was used as marker for the cells (5, 6). This was complemented by an ultrastructural examination on a limited number of patients with bullous pemphigoid.

Studied population

Standard histological and immunohistochemical criteria were fulfilled in the 17 included BP-patients (subepidermal bullae, linear desposits of C_3 and/or IgG at the basal membrane). The median age was 82 (56–96) years. At the time of biopsy five patients received no treatment, four had 10 mg prednisolone a day, while one patient had 50 mg daily of the same drug, and seven patients were treated with a potent steroid ointment. Thirteen patients were included in the light microscopic group and four in the electron microscopic study.

The 18 sex and age matched controls were outpatients at the dermatological clinic with ulcus cruris, seborrhoic keratosis or controls after treatment for basaliomas (median age 76 (67–84) years).

Biopsies

Skin biopsies were taken with a 4 mm punch. The patients with BP were biopsied both from clinically involved erythematous or papular skin and from clinically uninvolved skin from the lower arm, while their controls were biopsied from the lower arm only.

Assessment of OKT6 positive cells

The biopsies were frozen and stored at −70°C. Sections of the skin biopsies, 8 μm thick, were cut on a crytostat and stored overnight at −70°C. After thawing, the sections were fixed in icecold acetone and stained with OKT6 monoclonal antibodies (Ortho Diagnostic System Inc., Raritan NJ, USA) in a dilution of 1/100. The antibodies were visualized with an avidin-biotin-immuno peroxidase assay (Vectastain ABC® kit, Vector Laboratories Inc., Burlingame, Calif., USA), as previously described (7). For the control of OKT6-specificity additional slides were stained with OKT4 and OKT8 antibodies or the primary antibody was omitted as a negative control. Each section was evaluated by,

at least, two investigators in a light microscope using the 400 power field. The number of OKT6 positive dendritic cells were counted per 4 mm length of interfollicular epidermis. Each OKT6 positive dendritic cell in which a nucleus could be seen was counted. Both the total number, and those situated adjacent to the basal membrane, of the OKT6 positive dendritic cells were counted.

Transmission electron microscopy

The biopsies were fixed with 2.5% glutaraldehyde in a 0.1 M phosphate buffer, postfixed in 2% osmiumtetroxide in water, dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 G electron microscope at 60 kV. The Langerhans' cells were identified by the presence of Birbeck's granulae.

Statistical procedure

The Wilcoxon test for two samples and the Wilcoxon test for pair differences were used in the statistical calculations.

RESULTS

The result of the quantitative analysis is given in Table I. In the biopsies from the BP patients there was a significantly increased number of epidermal OKT6 positive cells in the diseased skin compared to the macroscopically noninvolved skin ($p<0.05$) and also compared with the controls ($p<0.05$). In both macroscopically uninvolved and involved skin of the BP patients, a higher percentage of the OKT6 positive cells were found close to the basal membrane, compared with the controls ($p<0.05$) (Table I), which implies a redistribution of LC towards the basal layer of epidermis in bullous pemphigoid.

At the ultrastructural level a mild acanthosis was seen in the specimens obtained from the involved skin. In one patient the involved skin displayed marked changes with acantholysis and a prominent dermal cell infiltrate (including eosinophils) just beneath the basal lamina. In this biopsy the basal keratinocytes were affected, as showed by an accumulation of glycogen in the cells. Both in the involved and the non-involved skin, LC were found from basal layer up to the upper spinous layer and transversally sectioned cell processes were found throughout the epidermis. A few occasional LC appeared to be injured as reflected by the occurrence of dilated endoplasmatic reticulum and perinuclear space and a more electron dense (condensed) cytoplasm than normal. In total, thirty LC were identified in epidermis (8 cells in non-involved skin and 22 in involved skin). Of these cells 52% were located at the level of the basal cell layer (75% in non-involved and 36% in involved skin).

DISCUSSION

The etiology of bullous pemphigoid remains obscure. The BP antigen is found both in normal and diseased skin (1, 3). Complement, autoantibodies directed against the basal

Table I. *The total number of OKT6 positive cells per 4 mm epidermis and the percentage of OKT6 positive cells in the basal cell layer of epidermis in patients with bullous pemphigoid and in the controls*

Mean and (standard deviation) are given. *=$p<0.05$, compared with the controls

	Total number	Percentage of cells in the basal layer
Pemphigoid ($n=13$)		
Non-involved skin	42.5 (10.4)	40.9 (14.3)*
Involved skin	59.7 (13.6)*	29.6 (13.7)*
Controls ($n=18$)	42.6 (13.9)	17.5 (9.5)

membrane zone, degradative enzymes from neutrophils and eosinophils are other important factors involved (2). So far, not much interest in the research has been focused on the role of the Langerhans' cell in the disease. It has recently been proposed that the epidermis is an integrated part of the so-called SALT (Skin associated lymphoid tissue) and as such a unique location for immunological events (8). In this SALT hypothesis LC would supply the epidermis with its antigen presenting function. We have used OKT6 monoclonal antibodies and electron microscopy to study the epidermal LC population in biopsies from patients with BP. An increased number of LC in clinically involved skin in the BP patients was found, confirming recently reported results by others (9). We did also register a higher percentage of LC in the basal layer of epidermis in the biopsies from the BP patients. This distribution was confirmed at the ultrastructural level, and is possibly a phenomenon parallel to the reported presence of cells with an antigen presenting capacity at the dermal side of the basal lamina in diseases characterized by the deposition of immune complexes at the epidermal-dermal junction (10). Our results show that there are both quantitative and qualitative changes in the epidermal LC population in bullous pemphigoid. It has been demonstrated that non-specific stimuli might cause an increase in the number of epidermal Langerhans' cells (11). In the present study, a higher percentage of LC was found in the basal layer of epidermis, not only in involved skin, but also in macroscopically normal skin from BP patients. This may indicate that the redistribution of the LC in the epidermis of patients with BP is not caused by non-specific inflammation. The density of the T6 antigen-bearing LC in normal human skin does not significantly differ in various anatomical regions, except for low numbers on the soles (12). Biopsies of the uninvolved skin from BP-patients and controls in the present study were taken from the lower arm. Biopsies from lesional skin were obtained from the arms, the legs, the chest and the back. Thus, the findings in the present study cannot be explained by the normal anatomical variations of LC density.

The redistribution of LC may be explained by the presence of complement and immunoglobulins at the basal membrane zone in BP, since LC carry receptors for them (4). However, the possibility also exists, that this redistribution within the epidermis in BP is a primary event, and that LC might be involved in the detection and the processing of the antigen(s) localized at the basal membrane zone.

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REFERENCES

1. Diaz LA, Calvanico NJ, Tomasi TB, Jordon RE. Bullous pemphigoid antigen: Isolation from normal human skin. J Immunol 1977; 118: 455–460.
2. Gammon WR, Merritt CC, Lewis DM, Sams WM, Carlo JR, Wheeler CE. An in vitro model of immune complex mediated basement membrane zone separation caused by pemphigoid antibodies, leukocytes and complement. J Invest Dermatol 1982; 78: 285–290.
3. Jordon RE, Kawana S, Fritz KA. Immunopathologic mechanisms in pemphigus and bullous pemphigoid. J Invest Dermatol 1985; 85: 72–78.
4. Wolff K, Stingl G. The Langerhans' cell. J Invest Dermatol 1983; 80: 17–21.
5. Fithian E, Kung P, Goldstein G, Rubenfeld M, Fenoglio C, Edelson R. Reactivity of Langerhans' cells with hybridoma antibody. Proc Natl Acad Sci USA 1981; 78: 2541–2544.
6. Murphy G, Bhan A, Sato S, Mihm Jr M, Harrist T. A new immunologic marker for Langerhans' cells. N Engl J Med 1981; 304: 791–792.
7. Emtestam L, Kaaman T, Hovmark A, Åsbrink E. An immunohistochemical staining of epidermal Langerhans' cell in tinea cruris. Acta Derm Venereol (Sthlm) 1985; 65: 240–243.

8. Streilein JW, Tigelaar RE. SALT: Skin-associated lymphoid tissues. In: Parrisch J, Kripke ML, Morison L, eds. Photoimmunology. New York and London: Plenum Medical, 1983: 95–132.
9. Nestor MS, Cochran AJ, Ahmed AR. Mononuclear cell infiltrates in bullous disease. J Invest Dermatol 1987; 88: 172–175.
10. Drijkoningen M, De Vos R, De Wolf-Peeters C, Degreef H, Desmet V. Immunoelectron microscopic investigation of HLA-DR-positive dendritic cells at the dermo-epidermal junction in skin disorders associated with the deposition of immune complexes. Ultrastruct Pathol 1986; 10: 123–128.
11. Lindberg M, Emtestam L. Dynamic changes in the epidermal OKT6 positive cells at mild irritant reactions in human skin. Acta Derm Venereol (Stockh) 1986; 66: 117–120.
12. Berman B, Chen VL, France DS, Dotz WI, Petroni G. Anatomical mapping of epidermal Langerhans' cell densities in adults. Br J Dermatol 1983; 109: 553–558.

Effects of PUVA and Mechlorethamine Treatment of Psoriatic Patients on Epidermal Langerhans' Cells

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Using OKT6 monoclonal antibody, we investigated the number of epidermal Langerhans' cells (LCs) in involved skin from patients with psoriasis, before and after mechlorethamine (HN_2) or PUVA treatment. The number of LCs remained at about pretreatment number during three weeks of HN_2 treatment alone, though they were reduced after 10 systemic PUVA treatments. Therefore, in contrast to PUVA which influences LCs, HN_2 seems to have little effect on LCs. LCs in psoriatic plaques were, in number, 3–4 times less numerous than those in uninvolved, nontreated epidermis. *Key words: Langerhans' cells; Psoriasis; PUVA; Mechlorethamine.* (Received April 3, 1987.)

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Both PUVA therapy and topically applied mechlorethamine (nitrogen mustard; HN_2) (1), an alkylating agent among cytotoxic drugs, have been demonstrated to be effective in the treatment of psoriasis. The topical HN_2 can be easily applied by the patient himself, but most persons eventually develop contact hypersensitivity to the compound. In those patients, sensitization to HN_2 is prevented by PUVA (2, 3). Mechanisms possibly responsible for these results are the reduction of Langerhans' cells (LCs) in the skin after UV radiation and/or induction of antigen-specific suppressor cells (4).

In the present study, we investigated the first possibility. Quantitative distribution of LCs in involved psoriatic epidermis was studied, before and after treatment of PUVA or HN_2. LC enumeration was also performed in uninvolved skin of patients.

MATERIAL AND METHODS

Patients and treatment schedules

Twenty-six patients with stationary psoriasis were included in the study. They were arranged into two groups. Five patients (group A) were treated with HN_2 alone. Twenty-one patients (group B) were treated with systemic PUVA followed by HN_2.

Group A. A freshly prepared aqueous solution of 0.02 % HN_2 was applied daily to the skin lesions.

Group B. Ten systemic PUVA treatments (three times per week) were given before initiation of the daily applications of HN_2, as previously described (3). Subsequently, HN_2 alone was applied.