Acta Derm Venereol (Stockh) 1989; 69: 101–104

Immunopathological Investigations in Purpura Pigmentosa Chronica

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We studied the cell infiltrates and antigenic characteristics of keratinocytes in biopsies from purpura pigmentosa chronica (PPC), with eleven monoclonal antibodies against several cell surface markers of effector and/or accessory cells of the immune system and compared the reactivity patterns with those in biopsies from uninvolved skin. Immunohistochemical staining revealed a predominance of activated helper T lymphocytes in the cutaneous inflammatory infiltrate. In contrast to uninvolved skin, lesional keratinocytes were found to express HLA-DR, OKM5, Leu-8 and Leu-11b (CD16) antigens in all biopsies from the involved skin. We demonstrate here for the first time the in vivo expression of several effector and/or accessory cell markers on lesional keratinocytes and infiltrate cells in PPC. ***Key words: Pigmented purpuric dermatosis; Lymphocytes; Keratinocytes.***

(Accepted October 4, 1988.)

Acta Derm Venereol (Stockh) 1989; 69: 101–104.

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Use of different in vivo and in vitro experimental models for cutaneous delayed-type hypersensitivity in recent studies yielded evidence that human keratinocytes are able to express characteristic markers for cells possessing monocyte/macrophage (HLA-DR, OKM5) and natural killer cell (CD16) activities (1–7). To our knowledge, little has been published concerning the antigenic characteristics of lesional keratinocytes and the distribution of the phenotypes of immunocompetent cells in dermal infiltrate in purpura pigmentosa chronica (PPC) (8), in a group of dermatoses characterized by petechiae and brownish pigmentation without any associated hematologic disorders, probably initiated by a cell-mediated immunological response to antigenic changes in the skin (9–14). Consequently, we investigated the antigenic properties of lesional keratinocytes and infiltrate cells with a panel of monoclonal antibodies against several effector and/or accessory cells (Table I) in patients suffering from PPC.

MATERIAL AND METHODS

Eight patients (3 females, 5 males; median age 35 years, range 16–69 years) with clinically and histologically (8) typical PPC were included in the study. Mean duration of disease was 9 months (range 3–54). The clinical spectrum of PPC consisted of 6 patients with eczematid-like purpura (15) and 2 patients with Schamberg's disease (16). No patient had previously received any topical or systemic immunosuppressive treatment of PPC.

Punch biopsies were taken from the lesional and the clinically uninvolved skin (distance from PPC lesion > 50 mm), snap-frozen in liquid nitrogen and stored at −70°C. 4-µm sections were cut on a cryostat and reactivity with monoclonal antibodies OKM1, OKM5, OKT6 (Ortho Pharmaceutical Corp., Raritan, N. J., USA), Leu-2a, Leu-3a, Leu-4, Leu-7, Leu-8, Leu-11b, IL-2 and HLA-DR (Becton Dickinson, Mountain View, Calif., USA) (Table I) was visualized using a multistep immunoperoxidase method as previously described (2). Control sections using mouse monoclonal antibodies IgG_1 and IgG_{2a} (Becton Dickinson) were processed according to the same procedure.

RESULTS

A clear predominance of Leu-4 (CD3)- (Fig. 1 *a*), Leu-3a (CD4)- (Fig. 1 *b*), HLA-DR- and partially IL-2 (CD25)-positive mononuclear cells was seen in the dermal lesions of PPC, in contrast to the small numbers of Leu-2a (CD8)-, Leu-7-, Leu-8- and Leu-11b (CD16)-positive cells (Table II). In the same biopsies a dense perivascular infiltrate of OKM5-, OKM1 (CD11)- and OKT6 (CD1)-positive cells was also revealed. In the epidermis, infiltrating T-lymphocytes, mostly positive for IL-2 (CD25), were seen.

In all biopsies from lesional skin, keratinocytes exhibited a net-like specific positive staining above the dermal mononuclear infiltrate when exposed to HLA-DR, OKM5 (Fig. 2), Leu-8 and Leu-11b antibodies (Table III). OKM5+Leu-8+HLA-DR+ keratinocytes predominated in each case in the stratum spinosum and granulosum. In the same biopsies, Leu-11b+HLA-DR+ keratinocytes were seen (except in the stratum corneum) in the whole epidermis. OKT6 (CD1)-positive cells were mainly seen in the stratum Malpighi.

Table I. *Monoclonal antibodies used in the study*

CD = cluster designation

Antibody	Working dilution	Specificity
Leu-2a (CD8)	1:500	Suppressor/cytotoxic T lymphocytes, cortical thymocytes
Leu-3a (CD4)	1:800	Helper/inducer T lymphocytes, cortical thymocytes, Langerhans' cells
Leu-4 (CD3)	1:800	T lymphocytes (pan T, mitogenic)
Leu-7	1:200	Large granular lymphocytes (natural killer cells), neuro-ectodermal tissue
Leu-8	1:200	T lymphocytes, B lymphocytes, monocytes, granulocytes
Leu-11b (CD16)	1:25	Natural killer cells, granulocytes
HLA-DR	1:1 000	B lymphocytes, monocytes/macrophages, Langerhans' cells, activated T lymphocytes, activated keratinocytes
IL-2 (CD25)	1:50	Activated T lymphocytes, interleukin-2 receptor
OKM1 (CD11)	1:100	Monocytes (C3bi receptor), granulocytes, null cells
OKM5	1:400	Monocytes, platelets, ultraviolet irradiated melanophages
OKT6 (CD1)	1:800	Langerhans' cells and indeterminate cells of the epidermis, cortical thymocytes

Table II. *Predominant staining pattern of the dermal inflammatory cells in PPC*

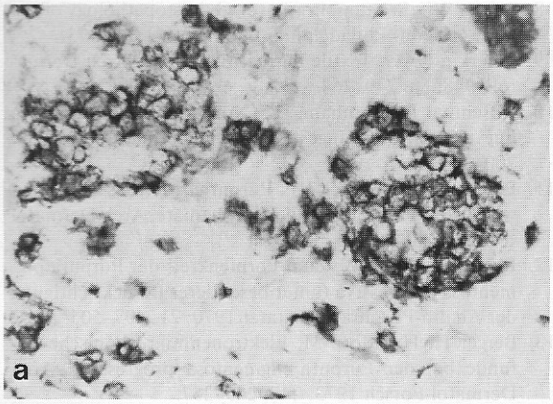
Monoclonal antibody						
Leu-4	Leu-3a	Leu-2a	HLA-DR	IL-2	OKT6	OKM5
+++[a]	+++	+	+++	+/++	+++	++

[a] +++: 70% of the cells or more stained; ++: 30–70% of cells stained; +: less than 30% of cells stained.

Table III. *Antigenic characteristics of keratinocytes in lesional and clinically uninvolved skin of PPC patients (n=8)*

Antibody	Lesional skin	Uninvolved skin
Leu-2a (CD8)	–	–
Leu-3a (CD4)	–	–
Leu-4 (CD3)	–	–
Leu-7	–	–
Leu-8	+	–
Leu-11b (CD16)	+	+[a]
HLA-DR	+	–
IL-2 (CD25)	–	–
OKM1 (CD11)	–	–
OKM5	+	–
OKT6 (CD1)	+	–

[a] Linear, fine granular staining at the dermo-epidermal interface (4).



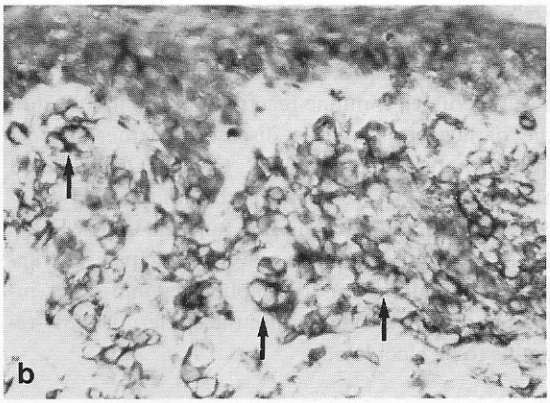


Fig. 1. (*a*) Dermal infiltrate consisting of Leu-4-positive T lymphocytes in PPC (amino ethyl-carbazole, ×130) (*b*) The majority of the inflammatory cells show a positive membranous staining pattern for Leu-3a (↑) (amino ethyl-carbazole, ×130).

DISCUSSION

Four closely related clinical patterns based on common histological (12, 17, 18) electron microscopical (13, 19) and immunological (20–22) features constitute PPC (8): purpura annularis teleangiectodes of Majocchi, progressive pigmentary dermatosis of Schamberg, pigmented purpuric lichenoid dermatitis of Gougerot & Blum and eczematid-like purpura of Doucas & Kapetanakis. On the basis of these features and in the light of certain encouraging therapeutic approaches (23) a cell-mediated immune reaction seems to be involved in the pathogenesis of this pigmented purpuric disease (12, 14, 24). However, to our knowledge, no study so far has dealt with immunohistochemical in vivo analysis of the dermal infiltrate and lesional keratinocytes in PPC.

Positive intracutaneous tuberculin tests of 72 h duration as classical representatives of delayed-type hypersensitivity reactions are useful in vivo models with which to investigate immunological changes in the epidermis. A recent study showed that human keratinocytes became HLA-DR-positive in one such an experimental model (1). The finding of HLA-DR expression by epidermal keratinocytes has been regarded as a marker of a cell-mediated immune reaction in the skin (25). In addition, further studies revealed the expression of OKM5 and Leu-11b antigens on human keratinocytes in positive delayed-type skin test areas as well (2, 4).

Our present data indicate that, as in positive delayed-type hypersensitivity reactions, PPC keratinocytes beside HLA-DR (26) do express OKM5, OKT6, Leu-8 and Leu-11b (CD16) antigens which are markers for effector and/or accessory cells of the immune system (Table III). The fact that lesional keratinocytes in PPC display the same markers as those of positive delayed-type hypersensitivity reactions—in

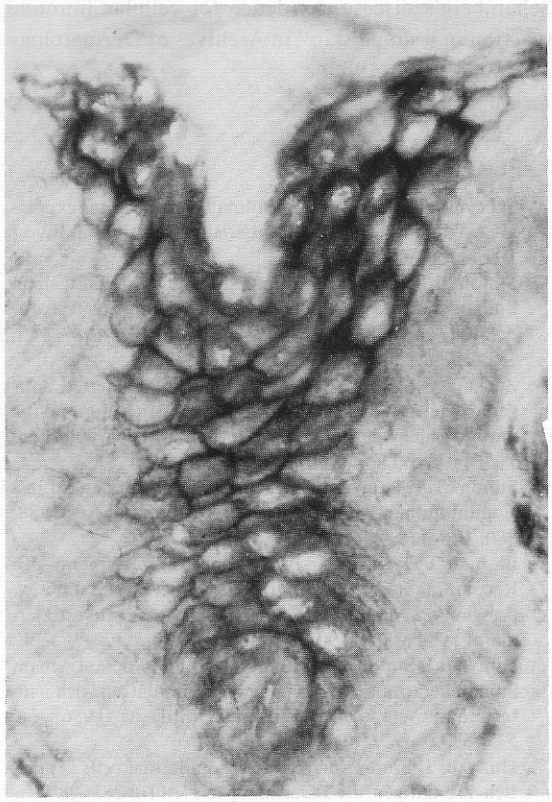


Fig. 2. OKM5 antigen expression on lesional keratinocytes in PPC (amino ethyl-carbazole, ×540).

view of the constitution of the dermal inflammatory infiltrate as well (Leu-4+, Leu-3a+, HLA-DR+, IL-2+ preponderance (27))—is a further suggestion that a cell-mediated immune reaction may be involved in the pathogenesis of this disease. Whether the acquired expression of HLA-DR, OKM5, OKT6, Leu-8 and Leu-11b (CD16) antigens by lesional keratinocytes in PPC fulfil an immune function—HLA-DR+ OKM5+ keratinocytes may well participate in trapping and maintaining T lymphocytes and other inflammatory leukocytes within the epidermis (28)—remains to be elucidated.

ACKNOWLEDGEMENTS

The expert technical assistance of Miss B. Husslein is gratefully acknowledged. This work was supported by a grant from Wilhelm Sander-Stiftung (no. 87.010.1).

ADDENDUM IN PROOF

Since submission of this article for publication, Aiba S & Tagami H reported "Immunohistologic studies in Schamberg's disease. Evidence for cellular immune reaction in lesional skin" in Archives of Dermatology 124: 1058–1062, 1988.

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