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Phenotypic Characterization *in situ* of Inflammatory Cells in Pityriasis (Tinea) versicolor

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The cellular response in pityriasis (tinea) versicolor lesions was analysed *in situ* with an immunohistochemical double staining technique combined with periodic acid-Schiff staining in frozen sections of skin biopsies from 9 patients. The proportions of B and T cells and subpopulations of T cells in the blood were normal as were the proliferative responses of blood mononuclear cells against various B- and T-cell mitogens and antigens. Fungi were observed in stratum corneum in all lesions, and there were moderate cell infiltrates in both epidermis and dermis as compared to biopsies from normal-looking skin. The majority of the infiltrating perivascular cells reacted with anti-Leu 1 antibodies (all mature peripheral T cells). Anti-Leu 2a reactive cells ('suppressor/cytotoxic' phenotype) were few and scattered, whereas anti-Leu 3a reactive cells ('helper/inducer' phenotype) dominated. This investigation demonstrates that pityriasis versicolor is not a simple overgrowth of the fungus in stratum corneum, but is accompanied by infiltrating immunocompetent cells in both epidermis and dermis. *Key words: Fungal disease; Skin biopsies; Monoclonal antibodies; Serum antibodies; Blood lymphocytes.* (Received March 6, 1984.)

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Pityriasis (tinea) versicolor is a chronic superficial fungal disease. The etiological agent *Pityrosporum orbiculare*, a dimorphic lipophilic yeast, is also found in the normal human cutaneous flora (1, 2). As patients with pityriasis versicolor have no increased susceptibility to infections, major abnormalities of the immune system are unlikely. Earlier studies have shown that blood mononuclear cells from patients with pityriasis versicolor have a decreased reactivity against extracts from *P. orbiculare* but react normally against other antigens and T-cell mitogens (3, 4). Circulating antibodies against *P. orbiculare* have been found in both patients with pityriasis versicolor and healthy adults (5).

In the light microscope pityriasis versicolor lesions usually exhibit only modest changes (6, 7). Hyperkeratosis and slight acanthosis are present in the epidermis and in the dermis there may be moderate and essentially perivascular infiltrates of lymphocytes, plasma cells and histiocytes (6, 7).

In the present study the patterns of inflammatory cells in the skin lesions were correlated to the presence of fungi, serum antibody titers, the proportions of B and T cells and subpopulations of T cells in the blood as well as to the proliferative capacity in vitro of blood mononuclear cells (BMN).

MATERIAL AND METHODS

Characteristics of patients

Nine patients, 6 females and 3 males, mean age 42 years (range 23–58) with pityriasis versicolor participated in the study. The criteria for including the patients were: clinical picture, fluorescence

under Wood's light, and positive microscopical identification. Eight patients had recurrent infections (mean 3.4 recurrences). The mean duration of the disease or current episode was 1.5 years (range 0.5–3 years). Associated diseases were seborrheic dermatitis in one and psoriasis in 2 patients.

Culture of P. orbiculare

Skin scales were taken, with a curette, from lesions and normal-looking skin on the back. The specimens were transferred to a glucose-neopeptone-yeast extract medium with the addition of olive oil (2%), Tween 80 (0.1%), and glycerol monostearate (2.5 g/l), earlier described (1), and incubated at 37°C for 4 days.

Indirect immunofluorescence (IIF) technique on sera

Antibodies against *P. orbiculare* were estimated as earlier described (5) using fluorescein isothiocyanate (FITC)-labeled antihuman IgG from Behringwerke AG (Marburg, W. Germany, Lot 40 E/OTKD G49 01179).

Preparation of blood cells

BMN were isolated from heparinized blood by centrifugation on Ficoll-Hypaque. Blood lymphocytes (BLC) were prepared by treatment of BMN with carbonylated iron powder (8).

Detection of surface markers on BLC

The percentage of BLC with surface immunoglobulin was estimated with indirect immunofluorescence (8). The percentages of total T cells and subpopulations of T cells in the BLC population was detected using the monoclonal antibodies anti-Leu 1, anti-Leu 2a and anti-Leu 3a (Becton-Dickinson Corp., Sunnyvale, Ca., USA) for staining with the ABC-technique (reagents from Vector Laboratories, Burlingame, Ca., USA) of cells fixed on microscope slides as described before (9).

Functional studies on BMN

The proliferative capacity in vitro of BMN was evaluated by measuring the incorporation of ^{3}H-Tdr after stimulation with various mitogens and antigens. *Branhamella catarrhalis* and anti-β_2-microglobulin were used as B-cell mitogens and phytohemagglutinin (PHA), concanavalin A (con A), soluble protein A, pooled allogeneic BMN and purified protein derivate (PPD) for stimulation of T cells. Cultures with *B. catarrhalis*, anti-β_2-microglobulin, PHA and con A were harvested on day 3, cultures with protein A on day 5 and cultures with allogeneic cells or PPD on day 6 at the peak of proliferation.

Skin biopsies

3 mm punch biopsies were taken from lesions and normal-looking skin on the back in all patients. The specimens were kept in Histocon® (Histolab, Bethlehem Trading Ltd, Gothenburg, Sweden) at 4°C for less than 24 hours. The specimens were then frozen and cut in a cryostat as previously described (10).

Immunohistochemical staining

Frozen skin sections, 6 μm thick, were investigated using a modified (10) sensitive double immunoenzymatic technique originally described by Mason & Sammons (11). This method permits simultaneous recognition of cells binding mouse monoclonal antibodies (peroxidase-catalysed brown staining) and those binding rabbit anti-HLA-DR antibodies (alkaline phosphatase-catalysed blue staining). The antibodies used, dilutions, combinations and the present knowledge about the specificities of the monoclonal antibodies are summarized in Table I. In control experiments staining was not observed when the primary antibodies were omitted or replaced by normal rabbit serum.

Periodic acid-Schiff (PAS) staining

After immunohistochemical staining the skin sections were further processed for PAS staining to visualize the presence of fungi. In control experiments PAS staining did not affect the previous immunohistochemical staining or vice versa.

RESULTS

Positive cultures of *P. orbiculare* were obtained from all lesions and from normal-looking skin in 8 of the 9 patients. The mean antibody titer in sera against *P. orbiculare* was 342.2±132.5 (± SEM).

Surface markers and functional reactivity of BLC

The percentages of BLC with various surface markers were similar in the patients and in normal blood donors. In the patient group (n=9) the percentages of BLC (mean ± SD) reacting with anti-Ig, anti-Leu 1, anti-Leu 2a and anti-Leu 3a antibodies, were 3.3±1.5, 69.4±10.9, 21.9±8.3 and 48.0±11.6, respectively. The corresponding figures for normal blood donors (5 females and 10 males, mean age 33 years; range 21–58) were 4.6±1.9, 75.2±7.3, 28.5±9.0, and 45.6±9.2. The proliferative responses of the patients BMN against *B. catarrhalis*, anti-β_2-microglobulin, PHA, con A, soluble protein A, allogeneic BMN and PPD were all normal. Thus no major abnormality could be found in the patients' BLC.

General characteristics of the skin biopsies

Round and short fungal hyphae were observed in stratum corneum in all biopsies from lesions (Fig. 1). Only one of the biopsies from normal-looking skin exhibited a few round fungal cells in the acroinfundibulum of a slightly dilated hair follicle.

In the lesions there were a moderate infiltration of inflammatory cells in dermis and epidermis as compared to normal-looking skin.

HLA-DR expression and T-cell patterns

In biopsies from normal-looking skin HLA-DR antigens were expressed on dendritic cells in the epidermis, on a few scattered cells in the dermis and on endothelial cells. Anti-Leu 1 reactive cells were found in low numbers perivascularly and occasionally at the level of the basal lamina.

The lesions were characterized by moderately increased numbers of anti-Leu 1 reactive cells in both epidermis and dermis, where they were mainly located perivascularly. The majority of the perivascular cells reacted with anti-Leu 3a antibodies (Fig. 1 *a*) whereas anti-Leu 2a expressing cells were few and scattered (Fig. 1 *b*). In all the biopsies the numbers of HLA-DR reactive cells in the dermis were increased. Close association of anti-Leu, mainly anti-Leu 3a reactive cells with HLA-DR reactive dendritic cells was seen in both epidermis and dermis (Fig. 1 *a*).

Table I. *Characteristics of antibodies, dilutions used and specificity of monoclonal antibodies*

Rabbit antibody (dilution)	Mouse monoclonal antibody (dilution)	Specificity of monoclonal antibodies
Anti-HLA-DR[a] (1/160)	Anti-Leu 1[b] (1/32–1/64)	All peripheral T-cells
	Anti-Leu 2a[b] (1/32–1/64)	'Suppressor/cytotoxic' T-cells
	Anti-Leu 3a[b] (1/32–1/64)	'Helper/inducer' T-cells
Anti-HLA-DR[a] or normal rabbit serum (1/160)	Anti-IgG[c] (1/1 280)	IgG heavy chains
	Anti-IgM[c] (1/1 280)	IgM heavy chains
	OKM 1[d] (1/20)	Monocytes/macrophages, granulocytes
	Anti-Leu 6[b] (1/32–1/64)	Langerhans cells, subpopulation of immature thymocytes
	Anti-HLA-DR[b] (1/64)	Framework determinant of HLA-DR molecules

[a] Klareskog et al. 1978 (25).
[b] Becton-Dickinson Corp., Sunnyvale, CA, USA.
[c] Seward Laboratory, London, England.
[d] Ortho Diagnostic Systems Inc., Raritan, NJ, USA.

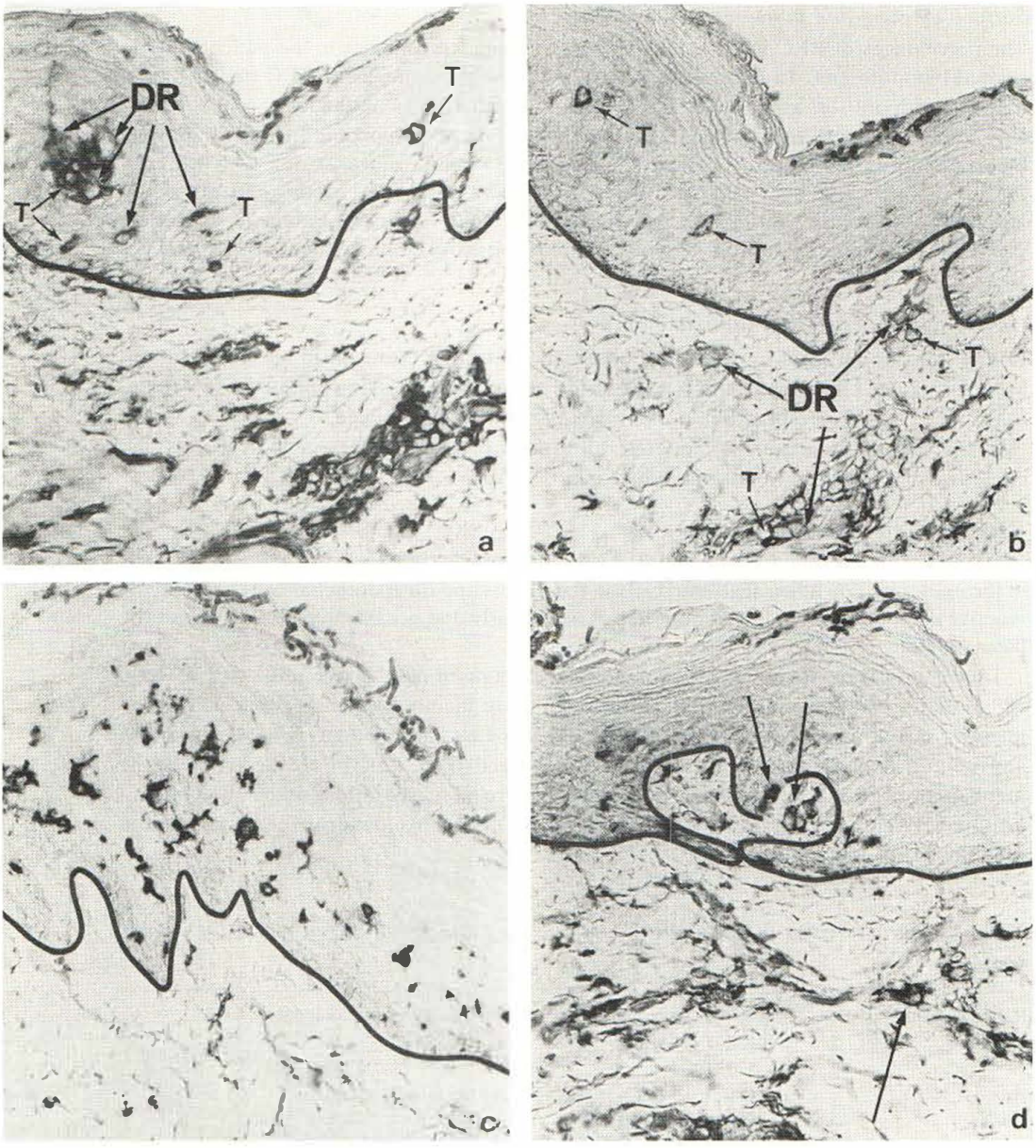

Fig. 1. Combined immunohistochemical and PAS staining of frozen sections of a human skin biopsy from a pityriasis versicolor lesion. The fungi are seen in the stratum corneum. Cells reacting with mouse monoclonal antibodies are visualized by peroxidase catalysed staining and those reacting with rabbit anti-HLA-DR antibodies by alkaline phosphatase catalysed reaction (arrows marked 'DR'). The solid lines indicate the epidermal basal lamina. (*a* and *b*) Rabbit anti-HLA-DR antibodies combined with in *a*) anti-Leu 3a antibodies reacting with most of the perivascular cells and with infiltrating cells in the epidermis (arrows marked 'T') and in *b*) anti-Leu 2a antibodies reacting with a few of the cells (arrows marked 'T'). (*c*) Anti-Leu 6 antibodies reacting with Langerhans cells in the epidermis. (*d*) OKM 1 reacting cells (three of the latter are indicated by arrows).

Anti-Leu 6 and OKM 1 reactive cells

In biopsies from normal-looking skin anti-Leu 6 reactive dendritic cells were found in similar numbers and locations as the HLA-DR reactive cells in epidermis. They were sparse in the dermis, where they occurred close to epidermis or vessels. OKM 1 reactive cells were only found in the dermis and in about the same frequency as the anti-Leu 6 reactive cells.

In the lesions the number of anti-Leu 6 reactive cells was increased in the dermis in 2 biopsies. Anti-Leu 6 reactive Langerhans cells were not observed in close spatial relation to fungi (Fig. 1 *c*). The number of OKM 1 reactive cells was slightly increased in the dermis in 4 biopsies (Fig. 1 *d*) and a few OKM 1 reactive cells were also observed in the epidermis in 2 biopsies.

Immunoglobulin-bearing cells

Only occasionally an immunoglobulin-bearing cell was observed in either the biopsies from normal-looking skin or lesions.

DISCUSSION

In agreement with earlier studies (3, 4) we found normal proliferative responses of BMN against various B- and T-cell mitogens and antigens in patients with pityriasis versicolor. The proportions of B and T cells and subpopulations of T cells in the blood were normal. We also confirmed that the mean antibody titer in sera against *P. orbiculare* was in the same range as described previously (5).

Light-microscopic studies have revealed slight (6) to moderate (7) changes in the epidermis and dermis in pityriasis versicolor. In an electron-microscopic study there was an increased number of Langerhans' cells in the epidermis and a perivascular infiltrate of lymphocytes in the dermis (13).

In the present study there were increased numbers of T cells in both epidermis and dermis. The majority of the infiltrating T cells expressed Leu 3a antigens ('helper/inducer' phenotype) whereas few and scattered cells were anti-Leu 2a reactive ('suppressor/cytotoxic' phenotype). Similar results have been described previously in delayed type of hypersensitivity reactions (10, 15), and in a number of skin disorders (16, 17). In contrast to this distribution of T-cell subsets, with a dominance of 'helper/inducer' T cells, lymphoid skin infiltrates in graft versus host disease consist of a virtually pure population of 'suppressor/cytotoxic' T cells (18). In cutaneous infiltrates of leprosy, differences between T-cell subsets were found in lepromatous and tuberculoid infiltrates (19). In the lepromatous form, where *Mycobacterium leprae* multiplies extensively in the skin macrophages, the T cells consist almost exclusively of 'suppressor/cytotoxic' cells whereas in the tuberculoid form, with few viable bacteria, the predominant T cell is of the 'helper/inducer' subclass. It is likely that these variations in proportions of T-cell subsets reflect differences in the pathogenetic mechanisms and immune responses involved. Immunomorphological studies on T-cell subsets may thus add valuable information about local immune responses.

A close association between HLA-DR expressing cells and T cells of mainly the 'helper/inducer' phenotype was observed in the lesions. The dendritic HLA-DR expressing cells in the epidermis were probably Langerhans' cells. Since epidermal Langerhans' cells have been suggested to induce a cellular immune response to trichophytin in dermatophytosis (20), the *in situ* observation may be compatible with an antigen-presenting situation (21, 22, 23). However, this is not necessarily the case since the mere occlusion of

the skin with Finn chambers or irritative contact reactions result in close association between Langerhans' cells and mononuclear cells/T cells (15, 24).

The present study demonstrates that even if *P. orbiculare*, in pityriasis versicolor, is only found in the stratum corneum, pityriasis versicolor is not only a simple overgrowth of the fungi, but induces more profound cellular changes in both epidermis and dermis.

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