

# Plasma Cells in the Dermal Infiltrate of Mycosis Fungoides are of Polyclonal Origin

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**There are several reports on the occurrence of immunoglobulin-producing B-cell lymphomas in Sézary's syndrome and mycosis fungoides, which may be the consequence of the helper activity of the neoplastic T-cells. Therefore we investigated skin biopsies of 50 patients with mycosis fungoides regarding the presence of plasma cells and their immunoglobulin profile. Nine of these patients had plasma cell nests, most frequently located at the lower edge of the infiltrate. IgE was detectable consistently, and IgG, IgM and IgA could also be demonstrated in the majority of these cases;  $\kappa$ - and  $\lambda$ -chains were present in equal amounts. Our results demonstrate polyclonal activation of plasma cells in a subgroup of mycosis fungoides patients. Key word: Ig subclass.**

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Mycosis fungoides (MF) is a helper T cell lymphoma of low-grade malignancy. It usually originates in the skin, showing eczematous patches which then transform into infiltrated plaques and finally become ulcerating tumours (1). The natural course of MF ranges from 10 to 15 years. Findings associated with a shortening of the mean survival time are increasing size of involved skin areas, palpable lymph nodes, presence of lymphocytes with a cerebriform nucleus (Sézary cells) in the peripheral blood and involvement of visceral organs (2-5).

Histologically, the plaque stage exhibits the most characteristic findings of MF: small lymphocytes with a cerebriform nucleus (Sézary cells) aggregate within the epidermis, thus forming Pautrier's microabscesses. A band-like infiltrate of cerebriform lymphocytes, histiocytes, and sometimes large lymphocytes with dark irregular nuclei (mycosis cells) is situated in the papillary body and the upper dermis. Eosinophils and plasma cells, the latter often forming nests at the

base of the infiltrate, are present in varying amounts from patient to patient (1).

There are several lines of evidence indicating that the neoplastic T-cells are functionally able to activate B-cells and to promote their differentiation into plasma cells: i) plasma cells are present in the dermal infiltrate of MF (1); ii) serum IgE and IgA levels are elevated in many patients with MF and seem to correlate roughly with the activity of the disease, since they return to normal during remission and may increase before the clinical manifestation of a relapse (6-8); iii) immunoglobulin producing B-cell lymphomas may develop in patients with MF (9). Moreover, the presence of plasma cells in the dermal infiltrate may be associated with an unfavorable prognosis (10).

As there exists no systematic investigation concerning this aspect of MF, we investigated biopsies of 50 MF patients regarding the presence of plasma cells and their immunoglobulin profile.

## MATERIAL AND METHODS

Biopsies were taken from 50 patients with MF with patches or plaques and stained by Giemsa's method. In each section, 200 cells were differentiated as small or large lymphocytes, plasma cells, mast cells, or eosinophils. Melanophages and siderophages were counted as 'others', whereas histiocytes were not included in the differentiation, since they are difficult to distinguish from endothelial cells.

Intracellular immunoglobulins were demonstrated by a three-stage immunoperoxidase technique. After incubation with the primary antibody (polyclonal rabbit antiserum) in optimal concentrations, the sections were washed in Tris buffer. Thereafter, they were incubated with swine anti-rabbit antiserum and finally soluble complexes of horseradish peroxidase and rabbit anti-horseradish peroxidase. Peroxidase activity was visualized using diaminobenzidine as substrate.

## RESULTS

In those skin infiltrates in eczematous and plaque stages of mycosis fungoides without plasma cell in-

Table I. Frequency of various infiltrating cells in mycosis fungoides with and without plasma cellular participation (50 cases; median and 95 % confidence interval)

|                   | With plasma cellular participation (n=9) | Without plasma cellular participation (n=41) |
|-------------------|--|--|
| Small lymphocytes | 61.5 (38.5-70.5)                         | 74 (66.5-81)                                 |
| Large lymphocytes | 1.5 (0-3)                                | 4.5 (2.5-8.5)                                |
| Mast cells        | 7.5 (0-17.5)                             | 7.75 (3.5-11)                                |
| Plasma cells      | 19.5 (1.5-31)                            | 0  |
| Eosinophils       | 10 (0-29)                                | 0 (0-2.5)                                    |
| Others            | 0 (0-1)                                  | 4 (0.5-7.5)                                  |

involvement, small lymphocytes with a cerebriform nucleus are the predominant type cell; their median count is 74. The median for mast cells was 7.75, for eosinophils 0, and 4.5 for large lymphocytes (Table I). Plasma cells were detected in 9 cases. These biopsies showed two striking differences compared with the infiltrates without plasma cells (Table I): the median of small lymphocytes decreased to 61.5, whereas the median of eosinophils is 10. The median of plasma cells is 20.

In all cases of plasma cellular participation we investigated the presence of light and heavy chains (except IgD) in these cells. All 9 biopsies proved positive for IgE. IgG was present 8 times, IgA 7 and IgM 5 times.  $\kappa$ - and  $\lambda$ -chains were equally distributed (Table II).

No correlation was detected between age and immunological or histological findings.

## DISCUSSION

Mycosis fungoides has already been established as a clinical entity by Alibert (11) and Bazin (12). Despite

its typical clinical picture and course, Hödl (13) demonstrated the histopathological heterogeneity of MF and proposed a classification which gives additional prognostic data. In this classification, a small-cell lymphoid type, a mixed-cell polymorphic type, a medium sized type and a large-cell type, are distinguished. Cases of plasma cell involvement would be classified as mixed-cell polymorphic type, which accounted for 40% of Hödl's cases. In such cases the malignant T cells may secrete a variety of lymphokines, thereby attracting eosinophils and B cells (see Table I). Consistent with this concept is the finding that B cells show enhanced immunoglobulin production in the presence of Sézary cells (14).

The association of MF and Sézary syndrome with B cell neoplasias such as monoclonal gammopathies (15, 16), multiple myeloma (9, 17) and lymphoplasmacytoid immunocytoma (18) is well established. It has sometimes been speculated about a possible causal relation of this association (17, 18). Recently, mouse B-cell hybridomas and plasmocytomas have been described (19, 20), whose in vitro growth is

Table II. Investigation of light and heavy chains of immunoglobulins in the plasma cellular infiltration of mycosis fungoides

| Pat.  | IgG | IgM | IgA | IgE | $\kappa$ | $\lambda$ |
|-------|-----|-----|-----|-----|----------|-----------|
| K. R. | ++  | +   | +   | ++  | ++       | +         |
| E. J. | ++  | ∅   | ∅   | ++  | +++      | +         |
| K. B. | +++ | +   | ++  | ++  | ++       | ++        |
| H. R. | ++  | ∅   | ∅   | ++  | ++       | ++        |
| M. M. | ++  | ∅   | +   | ++  | +        | +         |
| E. J. | ++  | +   | +   | +   | +        | +         |
| E. J. | ++  | +   | ++  | ++  | ++       | ++        |
| H. R. | ++  | +   | +   | ++  | +        | +         |
| H. K. | ∅   | ∅   | ++  | +   | ++       | ++        |

∅, not present; +, single cells; ++, formation of small nests; +++, formation of small and large nests.

strictly dependent on IL-6 (21). Since IL-6 is produced by T-cells and induces differentiation of activated B-cells into plasma cells, it might play a crucial role in the development of B-cell lymphomas in MF.

In such animal models, but also in the human system, development of B cell lymphomas is preceded by polyclonal B cell activation, which precedes the outgrowth of a malignant clone. If a similar pathogenesis holds true for B cell lymphomas associated with MF, early polyclonal B cell activation should be detectable.

In fact, many patients with MF show elevated levels of serum immunoglobulins, particularly of IgE and IgA (6-8). Moreover, the presence of cold agglutinins has been observed in MF and Sézary syndrome (22). Our finding of all classes of immunoglobulins and the equal distribution of  $\kappa$ - and  $\lambda$ -chains clearly proves the polyclonal nature also of the plasma cells within the dermal infiltrate.

Finally, the fact must be stressed that the polyclonal B cell activation in MF preferentially involves IgE and IgA (6-8); this contrasts with most other states with polyclonal B cell activation, as in systemic lupus erythematosus, Epstein Barr virus infection, or HIV infection, where IgG predominates (23). This remarkable selection for IgE and IgA is not yet explained, but might give a hint to possible antigens involved in the pathogenesis of MF. Moreover, as the immunoglobulin class switch from IgM to IgE is mediated by IL-4, one can speculate that the malignant T cells synthesize IL-4 too and because of this property may belong to the TH2 subgroup of helper T cells (24); this hypothesis can be tested using *in situ* hybridization with various cytokine probes.

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tion in liquid paraffin it was found to be partially effective against scabies, comparing 13 scabies with Lindane (5). In the present study a more cosmetically acceptable formulation, 2% permethrin in a cream base (6), was used to investigate the clinical efficacy and the transdermal absorption in the treatment of scabies patients.

MATERIAL AND METHODS

The study was conducted in the Dermatology Department of the Karolinska Hospital, Stockholm, Sweden. The study was approved by the local ethics committee. The study included 20 patients, 12 males and 8 females, with an age range of 23-63 years, in whom skin scrapings showed mixed and/or typical scabies. Exclusion criteria were: other skin diseases, pregnancy, lactation, severe skin conditions requiring special therapy with corticosteroids, absence of psychiatric or psychiatric disorders, chronic diseases or use of other drugs. Patients were treated for scabies within the past 4 weeks and were treated with 2% permethrin cream under medical supervision at the outpatient department. The cream was applied to the skin of the whole body, excluding head and neck, and the weight of the cream used was recorded. Each patient was instructed to have a thorough wash with 2% permethrin cream for the first 3 weeks after the treatment and thereafter wash with 2% permethrin cream daily. The cream could also be used.

The clinical effect was assessed by a visual score system prior to and 7, 14 and 28 days after treatment. A scoring system of lesions (0-4) (papules (1), and vesicles (2)) was applied to 2 sites and 4 sites (thorax, forearm, extensor, proximal and distal) in 6 sites (head/neck, axilla, forearm, extensor, buttock and leg/ankle). The total duration was kept possible for each individual patient. No side-effects were reported according to a simple scoring system.

Reduction in the haemoglobin level, erythrocyte sedimentation rate, serum creatinine and urea, AST, ALT, serum albumin, alkaline phosphatase, serum cholesterol, triglycerides and glucose, and urine protein and glucose were examined before and 7 days after the treatment.

A reflection of the degree of permethrin absorption was assessed indirectly by determination of conjugated and unconjugated 2-(2,6-dichlorophenyl)ethyl 2-(2,6-dichlorophenyl)ethylcarbamate (CVA 1342 dichlorophenyl-2,6-dichlorophenylcarbamate) as a metabolite of permethrin. Urine from 30 patients before the treatment (mean) patients received 10 ml of urine before the treatment.

The clinical efficacy and transdermal absorption of permethrin, a new synthetic insecticide was investigated in ten scabies patients. All patients were successfully treated with one application of a cream, containing 2% permethrin. Apart from mild postscabies dermatitis no side-effects were observed. The mean weight of cream used per patient was 25 g (range 21-32), mean content of permethrin 1.250 mg. The degree of permethrin absorption was assessed indirectly by determination of conjugated and unconjugated 2-(2,6-dichlorophenyl)ethyl 2-(2,6-dichlorophenyl)ethylcarbamate (CVA 1342 metabolite of permethrin) excretion in urine using two dimensional gas chromatography-mass spectrometry. It was found that during the first 48 hours (mean) the mean estimated absorption was 6 mg (range 3-11), which is approximately 0.2% of the total dose.

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Gamma benzene hexachloride (lindane) is widely used for the treatment of scabies, because of its demonstrated efficacy, ease of use and acceptability to patients. The safety of this drug, however, is the subject of considerable controversy (1). Several authors have suggested that its use should be abandoned in young children and pregnant women (2, 3). In its place gamma benzene hexachloride or precipitated sulfur have been recommended, but it is questionable whether these things are either as effective as or more safe than lindane (1). Recently (4) aqueous malathion (0.1% w/w) was described as an effective substitute compared with benzenehexachloride in the treatment of children with scabies. Data on absorption and on responses of clinical laboratory parameters after whole body application to man were not collected. However, the 39 malathion treated patients showed no sign of clinical toxicity.

Permethrin is a pyrethroid synthetic pyrethroid with broad spectrum insecticidal activity and low mammalian toxicity. It is a racemic mixture of six and diastereomers in the ratio 25:1:22. As a 10% solu-