Acta Dermatovener (Stockholm) 59: 125–128, 1979

OCCURRENCE OF IMMUNOGLOBULINS AND COMPLEMENT IN THE SKIN OF PATIENTS UNDERGOING TOPICAL TREATMENT OF MYCOSIS FUNGOIDES

Gunnar Volden and Gunnar Husby

Department of Dermatology and Institute of Immunology and Rheumatology, Rikshospitalet, Oslo, Norway

Abstract. Deposits of immunoglobulins (IgG, IgA and IgM) and complement were occasionally found in lesional skin of mycosis fungoides patients. However, after complete remission of the skin lesions, deposits of immunoglobulins and complement were found, corresponding to previous infiltrates or tumours, in about half of the patients. These deposits appeared as globular bodies in the upper part of the dermis closely connected with the basement membrane.

Key words: Mycosis fungoides; Immunoglobulins and Complement

Mycosis fungoides (MF) is a malignant T-cell lymphoma (2, 13) consistently localized in the skin before internal dissemination occurs. The etiology of MF is unknown, although some authors have suggested that an immune imbalance caused by persistent antigenic stimulation may lead to an autoimmune reaction and in some cases to the development of MF (11).

Lai A Fat & Cormane (7) demonstrated deposits of immunoglobulins and complement in lesional skin of MF patients. Such deposition coincided with the infiltrative stage, and was not found in the premycotic stage, while a small number were discovered in the tumour stage.

This report deals with the detection of immunoglobulin and complement deposits in apparently healed skin lesions following treatment of MF patients.

MATERIALS AND METHODS

Sixteen MF patients were studied, all having a clinical course and findings typical of MF (Table I). The diagnosis was histologically verified in 14 cases and was fairly compatible with MF in the remaining 2 cases (nos. 1 and 4). Eleven of the patients were classfied as being in the plaque stage, 4 in the tumour stage and one patient (no. 6) was diagnosed as a case of Sézary syndrome. Thirteen of the patients had previously received treatment for MF including topical corticosteroids (13 cases), superficial X-ray (4 cases, but not on the actual biopsy area in this study) and chemotherapy (1 case, no. 5). The actual treatment (Table I) was whole-body topical mechlorethamine (HN_2) for 10 patients. Complete resolution of all skin lesions occurred in 9 of them, while the skin in one patient (no. 6) was almost normalized. Three patients achieved complete remission after treatment with the psoralen 8-MOP and longwave ultraviolet light (PUVA) (10).

Biopsy specimens from apparently normal and lesional skin (plaques and tumours) were obtained from 10 MF patients before treatment, and, after remission, from normal skin and from skin where a previous lesion had been present but which appeared normal at the time the biopsy was taken—except for pigment changes (hypo- and hyperpigmentation) in 13 cases. Biopsies were taken from exactly the same spot before and after treatment in 7 cases (nos. 1, 4, 6, 8, 9, 12 and 15).

Antisera and fluorochrome conjugates. Antibodies against human IgG, IgA, IgM, $F(ab')_2$ and the complement factor C3 were produced in rabbits, correctly absorbed and thereafter conjugated with fluorescein-isothiocyanate (FITC) as described in detail by Husby et al. (1973). Normal rabbit IgG was also labelled with FITC and used as control conjugate.

Immunofluorescence microscopy. Skin tissue specimens obtained at biopsy were embedded in OCT compound (Ames Company, Indiana, USA) and immediately snap-frozen in dry ice–acetone. The sections were treated for 30 min in a moist chamber with the various FITC-conjugated antisera, washed twice for 15 min in phosphate-buffered saline, pH 7.4 (PBS), mounted in a 1 : 1 mixture of glycerol PBS (6). The stained sections were thereafter examined in a Leitz Orthoplan microscope with UV light source and filter combinations as described elsewhere (6). Micrographs were taken with an Orthomate camera.

Quantitation of serum immunoglobulins. Radial immunodiffusion (8) was employed for the determination of serum immunoglobulin (IgG, IgA, IgM) levels.

RESULTS

The results are presented in Table I. Before treatment with mechlorethamine or PUVA, deposits of

Fig. 1. Frozen section from an apparently healed skin lesion in a patient with mycosis fungoides stained with fluoresceinated anti-$F(ab')_2$ showing numerous globular bodies containing immunoglobulins (×200).

immunoglobulins were demonstrated i lesional skin in 2 out of 10 patients. The immunofluorescence staining was most intense in the Sézary patient (no. 6) and was much less pronounced in one MF patient (no. 4). Complement was found in only one patient (no. 16). The deposits of immunoglobulins and complement were seen as globular bodies in the upper part of the dermis in the Sézary patient and as granular deposits in the dermis closely apposed to the basement membrane in the MF patients. After healing of the cutaneous lesions, deposits of IgG and IgM and to a lesser extent IgA were detected in the skin of 7 out of 13 patients. Complement was found in the skin of 4 patients. The deposits of immunoglobulins and complement appeared as globular bodies of varying size located in the upper

Table 1. *Deposits of immunoglobulins and complement in lesional skin before treatment and in apparently normal skin on the same spot as a previous lesion after clearance*

CR=complete remission, PR=partial remission. Degree of immunofluorescent staining was recorded, from zero to +++ according to stength of fluorescence and amount of fluorescent material

Patient no.	Treatment		Stage before treatm.	Clinical result	Before treatment				After treatment			
	HN_2	PUVA			IgG	IgA	IgM	C_3	IgG	IgA	IgM	C_3
1		+	III	CR	−	−	−	−	−	−	−	−
2	+		II	CR					−	−	−	−
3	+		II	CR					−	−	−	−
4	+		II	CR	+	−	+	−	+	−	+	−
5	+		III	CR					++	−	−	−
6	+		Sézary syndr.	PR	++	+	++	−	−	−	−	−
7	+		II	CR					−	−	−	++
8		+	II	CR	−	−	−	−	+	+	+	−
9	+		III	CR	−	−	−	−	−	−	−	−
10	+		II	CR					+	+	+++	+
11			II		−	−	−	−				
12	+		III	CR	−	−	−	−	+++	+	++	−
13		+	II	CR					+	+	+	+
14			II		−	−	−	−				
15	+		II	CR	−	−	−	−	++	+	+++	+
16			II		−	−	−	+				

part of the dermis, closely connected with the basement membrane (Fig. 1). To check the specificity of immunofluorescence, it was shown that no similar staining was achieved with fluoresceinated normal rabbit IgG. In addition, specific immunofluorescence was completely abolished by pre-incubation of the various fluoresceinated antisera with the relevant antigens (IgG, IgA, IgM and C3). No immunoglobulins or complement were found in unaffected skin, either before or after treatment.

Normal immunoglobulin levels (IgG, IgA and IgM) were found in the serum of all 16 patients before and after clearance of the skin lesions.

DISCUSSION

This study showed that immunoglobulins and/or complement were occasionally present in biopsy specimens from lesional skin of MF patients, obtained before treatment. However, after healing of the skin lesions, considerable amounts of immunoglobulins and complement were found in apparently healed skin, corresponding to the previous infiltrates or tumours in many of the patients studied. The findings suggest that deposition of immunoglobulins and complement seems to take place during successful skin treatment of MF.

Our findings differ to a certain degree from those of Lai A Fat & Cormane (7), who demonstrated deposits of immunoglobulins and complement in lesional skin before treatment in most of their MF patients in the infiltrative stage. These authors found immunoglobulins and complement located in the blood vessel wall and scattered in the dermis.

In contrast to the findings in the MF patients, the Sézary patient included in this study showed deposition of immunoglobulins before treatment but not after partial remission of the skin lesions. This finding was unexpected, since it is impossible to discriminate between these diseases histologically, and since the same percentages of B- and T-lymphocytes from erythrodermic skin of Sézary patients and from plaques of MF patients have been found (1).

The mechanism of the deposition of immunoglobulins and complement in healed skin at sites of previous MF lesions is not clear. One possible answer may be that such deposition is a result of the treatment itself. The successful local treatment of MF resulting in an effective depletion of lymphocytes, mainly T-cells which have been shown to be present in the skin lesions (1), may lead to a change in the immunologic balance. Thus the T-cell depletion may include loss of regulatory (suppressor) lymphocytes and thereby disturb the ability of the antibody producing cells (B-cells) to discriminate between "self" and "not-self", or simply make the B-cells more active in antibody production. Antigens from decomposed cells exposed during treatment may then act as immunogens in vivo, resulting in the production and deposition of complement-fixing antibodies to such antigens. The globular bodies found in the upper part of the dermis resemble the so-called 'elastic globes' which Hashimoto (4) revealed by electron microscopy were derived from degeneration of basal epithelial cells.

It is interesting that we have seen internal dissemination of the disease in many patients studied by the Scandinavian Mycosis Fungoides Study Group a few weeks to months after complete healing of all cutaneous lesions (3, 9). It therefore seems that a certain protective mechanism connected with the process in the skin may be abolished during successful treatment of skin lesions. It is tempting to assume that this factor is related to T-lymphocytes. The skin lesions in MF may thus represent an immunologic surveillance system—possibly made up of T-cells—which prevents internal/systemic dissemination of the disease. A similar protective effect of T-cells has been suggested for other malignant tumors (6). As soon as these lesions heal as a result of local treatment, such dissemination will tend to occur. This would also explain the protracted course of the disease which is observed in many patients where clearance of skin lesions does not occur.

REFERENCES

1. Burg, G. & Braun-Falco, O.: Classification and differentiation of cutaneous lymphomas. Enzyme-cytochemical and immunocytological studies. Br J Dermatol *93:* 597, 1975.
2. Edelson, R. L., Lutzner, M. A., Kirkpatrick, Ch. J., Shewach, E. M. & Green, J.: Morphologic and functional properties of the atypical T-lymphocytes of Sézary syndrome. Mayo Clin Proc *49:* 558, 1974.
3. Fischer, T., van Vloten, W. A. & Volden, G.: Internal dissemination of mycosis fungoides despite successful local therapy. Acta Dermatovener (Stockholm) *58:* 88, 1978.
4. Hashimoto, K.: Apoptosis in lichen planus and several other dermatoses. Acta Dermatovener (Stockholm) *56:* 187, 1976.
5. Husby, G., Blomhoff, J., Skrede, S., Elgjo, K. &

Gjone, E.: Detection of immunoglobulins in paraffin-embedded liver biopsies. Scand J Gastroenterol *8:* 621, 1973.
6. Husby, G., Hoagland, P. M., Strickland, R. G. & Williams, R. C., Jr: Tissue T and B cell infiltration of primary and metastatic cancer. J Clin Invest *57:* 1471, 1976.
7. Lai A Fat, R. F. M. & Cormane, R. H.: Immunohistopathological observations in the skin of patients with mycosis fungoides. Arch Dermatol Forsch *249:* 349, 1974.
8. Mancini, G., Carbonara, A. O., Heremans, J. P.: Immuno-chemical quantitation of antigens by single radial immunodiffusion. Immunochemistry *2:* 235, 1965.
9. Molin, L., Skogh, M. & Volden, G.: Successful PUVA-treatment in the tumour stage of mycosis fungoides associated with the appearance of lesions in organs other than the skin. Acta Dermatovener (Stockholm) *58:* 189, 1978.
10. Roenigk, H. H.: Photochemotherapy for mycosis fungoides. Arch Dermatol *113:* 1047, 1977.
11. Tan, R. S.-H., Butterworth, C. M., McLaughlin, H., Malka, S. & Samman, P. D.: Mycosis fungoides—a disease of antigen persistence. Br J Dermatol *91:* 607, 1974.
12. Winkelmann, R. K.: Clinical studies of T-cell erythroderma in Sézary syndrome. Mayo Clin Proc *49:* 519, 1974.

Received April 24, 1978

Gunnar Volden, M.D.
Department of Dermatology
Rikshospitalet
Oslo
Norway