Sézary Syndrome: Relative Increase in T Helper Lymphocytes Demonstrated by Monoclonal Antibodies

J. Thivolet, R. Fulton, P. Souteyrand, M. Gaucherand and A. Claudy

U. 209 INSERM, Laboratoire de Recherche Dermatologique et Immunologie, Clinique Dermatologique, Hôpital Edouard Herriot, F-69374 Lyon Cédex 2, France

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Abstract. Helper and suppressor T lymphocytes were measured in 7 patients with the Sézary syndrome and 6 patients with mycosis fungoides, by indirect immunofluorescence using monoclonal antibodies. A large relative increase in helper cells was found in the Sézary syndrome, while the results in mycosis fungoides were normal.

The Sézary syndrome is a rare systemic disease which presents as erythroderma and peripheral lymphadenopathy. A characteristic finding is the presence of large mononuclear cells with cerebriform nuclei in the peripheral blood and skin—the so-called Sézary cell. These cells have been shown to be thymus-derived lymphocytes (4, 5, 21). Mycosis fungoides is a related condition in which morphologically and immunologically identical cells initially preferentially infiltrate the skin and lymph nodes but are occasionally seen in the peripheral blood. It has been suggested that the two conditions are part of a spectrum of cutaneous T cell lymphoma, where the Sézary syndrome represents a leukemic phase of mycosis fungoides (6). We have used monoclonal antibodies specific for T lymphocyte subsets to demonstrate that in the Sézary syndrome there is a large relative increase in T helper lymphocytes and that mycosis fungoides shows no similar imbalance of T lymphocyte subpopulations in peripheral blood.

PATIENTS

Seven patients with typical features of the Sézary syndrome and persistent circulating Sézary cells were studied. There were 4 women and 3 men with an age range of 25–75 years. Peripheral blood Sézary cell counts varied from 7% to 52% of total white cells. Most of the patients had received various systemic treatments such as systemic corticosteroids, chemotherapy, leukophoresis and PUVA therapy. Six patients with histologically proven mycosis fungoides were studied—4 men and 2 women with an age range of 49–71 years. Four patients had plaque lesions only and 2 had, in addition, early tumours. One had superficial lymphadenopathy, but none had detectable circulating Sézary cells. All had received a range of conventional systemic therapies similar to the Sézary syndrome group. 20 sex- and age-matched healthy controls were included in the study.

METHODS

Three monoclonal antibodies, produced by the Ortho Pharmaceutical Corporation, USA, named OKT3, OKT4 and OKT8, were kindly donated by Cilag-Chimie, France. These had been produced by the hybridoma technique in which myeloma cells are fused with spleen cells from mice immunized with human T lymphocytes. The myeloma cells subsequently produce highly specific IgG antibodies to surface antigens on human T lymphocytes and subsets (12). OKT3 identifies 100% of human peripheral T cells, OKT4 identifies the T helper/inducer subset and OKT8 identifies the suppressor/cytotoxic subset.

Mononuclear cells were separated from peripheral blood by “Ficoll-Paque” (Pharmacia) density centrifugation. After washing, the cells were suspended in RPMI-1640 supplemented with 5% foetal calf serum and 25 mM Hepes at a concentration of 5 × 10⁶ cells/ml. 10 microlitres of each reconstituted antibody (OKT3, OKT4, OKT8) was incubated with 200 μl of the cell suspension on ice for 30 min. After washing, the cell pellet was incubated on ice for 30 min with 100 μl of fluorescein-conjugated goat anti-mouse IgG (Nordic) which had been diluted 1 in 10 in RPMI-1640. Fluorescent cells were counted on a Leitz Orthoplan microscope and compared with the total number of cells.

Statistical analysis was carried out by Student’s t-test.

RESULTS

The mean percentage (± standard error of the mean) of OKT3+ cells was calculated. In the Sézary syn-
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There was an increased percentage \( p < 0.1 \) of OKT3+ cells (78±16) compared with controls (67±10). This finding is consistent with previous findings that the Sézary syndrome is a proliferation of thymus-dependent lymphocytes. In mycosis fungoides there was no difference in the percentage of OKT3+ cells (62±11) compared with controls (67±10). The percentage of OKT4 (helper) cells (75±15) was significantly \( p < 0.01 \) increased and the percentage of OKT8+ (suppressor) cells (17±11) was very significantly \( p < 0.001 \) decreased in the Sézary syndrome, compared with controls (55±10). There was no significant difference in the percentages of OKT4+ or OKT8+ cells in the mycosis fungoides group, compared with controls.

The balance between helper (OKT4+) and suppressor (OKT8+) T lymphocytes was expressed as the ratio OKT4+/OKT8+ (Fig. 1). This eliminates the contamination by non-T cells in the mononuclear suspension. This ratio was significantly increased \( p < 0.01 \) in the patients with Sézary syndrome, compared with controls. This indicates a significant excess of T helper cells over T suppressor cells in the Sézary syndrome. The OKT4+/OKT8+ ratio in patients with mycosis fungoides did not differ from controls, indicating a normal balance of helper and suppressor subsets.

DISCUSSION

On the basis of functional tests it has been suggested that the Sézary syndrome is a proliferation of T helper lymphocytes (1, 3, 13) though others have demonstrated a suppressor capacity (7, 9). The development of the hybridoma technique has allowed the selection and large-scale production of antibodies from a single clone directed against a chosen antigen (10). Murine antibodies have been produced against stable surface antigens of mature human peripheral T lymphocytes and T cell subsets. These can distinguish specifically and consistently each subpopulation of T cells without cross-reaction or reaction with B or null lymphocytes, granulocytes or monocytes. In this study 3 monoclonal antibodies—OKT3, OKT4 and OKT8—were used to study the peripheral T cell characteristics of the Sézary syndrome and mycosis fungoides. These antibodies distinguish all peripheral T cells (OKT3), the T helper cell subset (OKT4) and the T suppressor cell subset (OKT8) (17, 18, 19). The antibodies distinguish the phenotypic differences of lymphocytes rather than their functional capacity to help or suppress in immunological reactions.

Using these monoclonal antibodies, we have shown that the circulating lymphocytes in the Sézary syndrome are predominantly OKT3+, thus confirming the thymus-dependent character of this disease. In contrast, the circulating lymphocytes in early cases of mycosis fungoides showed a normal percentage of OKT3+ cells. Furthermore, we have demonstrated that there is a relative increase in circulating OKT4+ T helper cells, compared with OKT8+ T suppressor cells in the Sézary syndrome with a normal number in mycosis fungoides, as was recently ascertained by Kung et al. (11). Although most patients had received several forms of treatment, this elevation could not be related to a specific therapy and the levels in mycosis fungoides were consistently normal despite a similar range of treatment. This finding is in keeping with an immunomicroscopic study where peripheral blood Sézary cells were shown to express the OKT3 and OKT4 phenotypes but not the OKT8 phenotype (20).

The increase in helper/suppressor ratio appears only when Sézary cells are detectable in the peripheral blood, as in the classical Sézary syn...
There is no T-cell disturbance when the disease is purely cutaneous as in our group of patients with peripheral blood common to both diseases, since cells is therefore not a basic abnormality of the bone marrow (8, 15). It has been suggested that the T cell interfollicular areas of lymph nodes contain predominantly OKT4+ (helper) cells, while the suppressor phenotype was the predominant cell in the T cell population of the bone marrow (8, 15). In mycosis fungoides it has been shown that there is preferential infiltration by lymphoid cells in the OKT4+ lymph node zone (16). An overspill of these cells with the helper phenotype into the peripheral blood from the lymph nodes could explain our findings of an increased proportion of helper cells in the Sézary syndrome. The Sézary syndrome may thus be regarded as a leukemic phase of mycosis fungoides. In the future, it will be worthwhile to compare simultaneously the phenotype and the functional activity of the tumoral cells in the epidermotropic cutaneous T cell lymphomas (22).

REFERENCES


Deposits of Complement and Immunoglobulins in Vessel Walls in Pyoderma gangrenosum

Susanne Ullman, Poul Halberg and Jette Howitz

Department of Dermatology, Rigshospitalet and Department of Medicine, Division of Rheumatology, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark

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Abstract. Previous immunofluorescence studies on pyoderma gangrenosum (PG) proved negative. Biopsies from the ulcer edge of 8 patients with PG were examined by immunofluorescence microscopy. Deposits of complement C3 were seen in the vessel walls of all samples, IgM in three and IgA in one. Granular deposits of C3 were seen at the dermal-epidermal junction in 2 patients. Biopsies from clinically normal skin of 6 of the patients were negative. It is suggested that deposition of immune complexes in the dermal vessel walls may play a role in the pathogenesis of PG.

Key words: Pyoderma gangrenosum; Immunofluorescence; Vessel walls

Histopathological examination of pyoderma gangrenosum (PG) lesions has given conflicting results. Some authors have described vasculitis as an important feature (6, 7), whereas others found no inflammatory changes in the vessel walls (1, 2, 4). Several immunofluorescence studies did not find deposits of immunoreactants either in the vessel walls or at the dermal-epidermal junction (1-7).

MATERIAL AND METHODS

Eight patients (7 women and one man) were included in the study. Besides PG, 2 patients had ulcerative colitis, one had Crohn's disease and one had seropositive, erosive deformating, nodular rheumatoid arthritis without active synovitis. Four patients had no other diseases. Biopsy specimens were obtained from lesional skin at the edge of the ulcers. A preferred and the undermined destroyed part of the edge was avoided. Six patients had biopsies performed from clinically uninvolved skin of the buttock as well.

By direct immunofluorescence microscopy the biopsy specimens were examined for deposits of IgG, IgM, IgA and complement C3 (8). Material from lesional skin of 6 of the patients was also stained with hematoxylin-eosin.

RESULTS

Immunofluorescence microscopy

Deposits of complement C3 were found in the dermal vessel walls of all samples of involved skin (Fig. 1). In three samples, deposits of IgM and, in one, deposits of IgA were found in the vessel walls. Granular deposits of C3 were seen in the dermal-epidermal junction in two biopsies. No deposits were found in any of the biopsies from clinically uninvolved skin.

Light microscopy

Endothelial cell proliferation and perivascular and intramural infiltrates of neutrophils and mononuclear cells were found in the dermal vessels of all six biopsies from lesional skin. Neither fibrinoid deposits, necrosis, nor nuclear dust were seen in any of the biopsies.

COMMENT

Deposits of immunoreactants were found in the dermal vessels of all biopsies from lesional skin of patients with PG. C3 was found in all cases, and immunoglobulins were also found in some cases. According to one report on a patient with paraproteinaemia and PG, vasculitis was found in the ulcer as well as in vasculitis lesions elsewhere in the skin. Immunofluorescence microscopy revealed deposits of IgG and complement C3 in the vessels of the vasculitic lesions (7). However, the ulcer itself was not examined by this technique. Several authors were unable to find deposits of immunoglobulins or complement in vessels of lesional skin of patients with PG (1-6). In this study, the indurated zone at the edge of the ulcer was biopsied in order to avoid the necrotic central area. In the other studies quoted the exact areas biopsied were not mentioned. Variations in biopsy sites may account for the discrepancies in the findings.

Fibrinoid deposits, necrosis and nuclear dust were not found by histopathological examination of lesional skin of our patients. On the other hand,