PHENOTYPE OF CELLS INVOLVED IN MYCOSIS FUNGOIDES AND SÉZARY SYNDROME (BLOOD AND SKIN LESIONS): IMMUNOMORPHOLOGICAL STUDY WITH MONOCLONAL ANTIBODIES

D. Schmitt, P. Souteyrand, J. Brochier, J. Czernielewski, and J. Thivolet

Abstract. The phenotypic expression of immunocompetent cells involved in lesions of cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome) was studied by using monoclonal antibodies specific for T-cell subsets and HLA-DR antigens (OKT3, T4, T6, T8) (BL6, BL5, BL2). In Sézary syndrome, the phenotype of Sézary cell is homogeneous (T3+, T4+, T8−, T6−, BL2−) and corresponds to the phenotype expressed by the normal helper T-cell population. At the ultrastructural level, the discontinuous distribution of OKT4 and OKT3 antigenic sites on the cell surface is similar to the HTLA antigens. In skin lesions, the Langerhans cells expressed two specificities shared by normal thymocytes (T6 and BL6). We suggest that the expression of these antigens could be related to the epithelial microenvironment (epithelium-dependent differentiation antigens). The tumoral lymphoid cells infiltrating the skin showed the same phenotype (helper type) as the circulating Sézary cells (T3+, T4+, T8−, T6−). Moreover, the T3+ cells also expressed HLA-DR antigens and corresponded to activated T lymphocytes. The various subpopulations of immunocompetent cells seemed to be related to the MHC-derived antigens.

Among the malignant cutaneous lymphomas, the epidermotropic T-cell lymphoma (mycosis fungoides and Sézary syndrome) are characterized by dense dermal infiltrates and epidermal exocytosis of proliferating cells in skin lesions.

In mycosis fungoides the T-cell nature of the tumoral cells was demonstrated by using membrane markers (27). In the Sézary syndrome, direct evidence of a T-cell nature was obtained by studying E-rosette forming cells and immunolabelling of the HTLA antigens, specific for the T-cell lineage (19, 29). The functional capacity of Sézary cells to stimulate immunoglobulin synthesis in vitro has been appreciated in some cases of Sézary syndrome. The results are not uniform; in some reports, Sézary cells had a T-cell helper capacity (1, 3, 16), and in others, a T-cell suppressor activity (7, 10).

Recently, the use of the hybridoma technique has permitted the production of murine monoclonal antibodies specific for T lymphocytes and subsets (12, 13). Therefore, a way to investigate lymphoid populations is to analyse surface of cytoplasmic properties of cells in situ.

The identification of T-cell subsets in tissue sections by using monoclonal antibodies and immunohistochemical techniques has recently been demonstrated in normal lymphoid tissues (9), human intestinal mucosa (20) and tumoral lymph nodes (28).

Using indirect immunofluorescence on circulating lymphocytes, in the Sézary syndrome it has been demonstrated that the majority of the cells express the helper phenotype OKT4 (15, 7, 6, 2, 14). The aim of this study was to obtain direct and more precise information about the phenotype of cells involved in epidermotropic cutaneous T-cell lymphoma in blood and skin lesions.

MATERIAL AND METHODS

Five typical cases of the Sézary syndrome (SS) with persistently high levels of circulating Sézary cells and 5 typical cases of Mycosis fungoides (MF) were studied. In Sézary syndrome, the peripheral blood Sézary cell counts ranged between 7 and 52% of all white cells. Most of the patients had received various systemic treatments such as corticosteroids, chemotherapy and PUVA therapy.

Tissue specimens

Circulating Sézary cells: In the cases of Sézary syndrome, the peripheral blood lymphocytes were isolated using Ficoll-Paque (Pharmacia) density centrifugation. In each case, the helper/suppressor (OKT4/OKT8) ratio was greatly elevated as compared with controls (6) (mean ratios: 1.33±0.32 in controls, 6.84±4.96 in the Sézary syndrome).

Skin specimens: Punch biopsies of infiltrated plaques of MF and SS cases were removed under local anaestheisia.
Circulating Sézary cell labelled by OKT3 antibodies and contrasted by uranyl acetate and lead citrate (X8000).

Half was frozen in liquid nitrogen and half was fixed for routine histological and ultrastructural processing.

Monoclonal antibodies

Four monoclonal antibodies, produced by Ortho Pharmaceutical Corporation USA, called OKT3, OKT4, OKT8 and OKT6, were kindly donated by Cilag-Chimie France. Inducer/helper T lymphocytes have been shown to react with the monoclonal antibody termed OKT4. In contrast, the suppressor/cytotoxic T-cell subset was recognized by the monoclonal antibody OKT8. All the peripheral T lymphocytes express a common antigen, termed OKT3. OKT6 antibody was produced against thymocytes (13). Three monoclonal antibodies produced by J. Brochier using the same technique (INSERM U 80, Hôpital E. Herriot, Lyon, France) were also used in this study (BL2, BL5, BL6), specific for HLA-DR antigens, leukocytes, and thymocytes, respectively.

Techniques

Immunoelectron microscopy on circulating cells: Monoclonal antibodies OKT3, OKT4, OKT8 and HLA-DR (BL2) were used at the dilution 1:10 for 30 min at 37°C on cells fixed by 3% paraformaldehyde. The murine antibodies, fixed on the cell surface, were revealed by a peroxidase conjugate at the dilution 1:20 for 30 min at 37°C (rabbit anti-mouse Ig-peroxidase, Nordic). The peroxidase activity was revealed using 3,3'-diaminobenzidine. The cells were postfixed for 20 min with osmium tetroxide and included in epoxy medium. Ultrathin sections were examined without staining with an Hitachi HU12A electron microscope. Some sections were post-stained with lead citrate and uranyl acetate.

In each case of Sézary syndrome, 200 Sézary cells were observed under the electron microscope.

With each monoclonal antibody, the percentage of positive Sézary cells was estimated directly by ultrastructural observation in each case of Sézary syndrome.

Immunohistochemical procedure on skin sections:

Five-micron frozen sections were prepared, air-dried and fixed in acetone for 10 min at 4°C. Indirect immunofluorescence was performed. First-layer antibodies were added (monoclonal antibodies at the dilution 1:10) and the sections incubated at 30 min at 37°C. They were then washed in PBS (pH = 7.2–20 min). Fluorochrome-conjugated second-layer antiserum (Meloy goat anti-mouse Ig, labelled with FITC, dilution 1:10) was next added, incubated and washed as above. Slides were mounted in a glycerol PBS mixture. The sections were examined under a Leitz fluorescence microscope.

Control reactions: The same reactions were performed using a negative serum (non-immunized mouse serum) and a positive serum (anti-HTLA serum produced in rabbit; Institut Mérieux, France). The non-specific fixation of the conjugate was controlled by using PBS instead of the monoclonal antibodies.

Results

(a) Phentotypic expression of circulating Sézary cells

By electron microscopy, the majority of the circulating lymphocytes were found to express a T3 phenotype. In each case of Sézary syndrome all the circulating Sézary cells were specifically labelled by OKT3 and OKT4 antibodies (Fig. 1). The quantitative results obtained by immunoelectronmicroscopy with each monoclonal antibodies are summarized in Table 1. In each case of SS, a very small number of Sézary cells (<2%) expressed the OKT8

Table 1. Phenotype of circulating Sézary cells (immunoelectronmicroscopy)

<table>
<thead>
<tr>
<th>AG</th>
<th>%*</th>
<th>Specificity</th>
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<tr>
<td>T3+</td>
<td>100</td>
<td>T lymphocytes</td>
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<tr>
<td>T4+</td>
<td>&gt;98</td>
<td>Helper/inducer T lymphocytes</td>
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<td>T8−</td>
<td>&gt;98</td>
<td>Suppressor/cytotoxic</td>
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<tr>
<td>T6−</td>
<td>100</td>
<td>&quot;Common thymocytes”</td>
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<tr>
<td>BL2−</td>
<td>100</td>
<td>HLA-DR antigen</td>
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<td>HTLA**</td>
<td>100</td>
<td>T lymphocytes</td>
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</table>

* % obtained from 200 cells counted in the electronmicroscope.
** Polyclonal heteroantibodies.
No Sédary cells expressed OKT6 or HLA-DR specificities.

The surface distribution of T3 and T4 antigens was discontinuous and appeared as small electron-dense deposits all over the cell surface. The typical ultrastructural characteristics of the Sédary cells (the villous membrane and the cerebriform nucleus) were especially clear after post-staining.

(b) Phenotype of mononuclear cells in tumoral skin lesions

A summary of the results for the various antigens is presented in Table II.

Phenotypic expression of Langerhans cells

Fig. 2. Mycosis fungoides. Cutaneous infiltrated plaque. Specific labelling of dendritic Langerhans cells with OKT6 monoclonal antibodies (×500).

Table II. Phenotype of mononuclear cells in skin infiltrates of MF and SS

<table>
<thead>
<tr>
<th>Cases</th>
<th>MF</th>
<th>SS</th>
<th>Conclusions</th>
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<tr>
<td></td>
<td>T3</td>
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<td>Control</td>
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<td></td>
<td>HTLA</td>
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</table>

Table II. Phenotype of mononuclear cells in skin infiltrates of MF and SS

Moreover, in all specimens, these dendritic T6-positive cells expressed the HLA-DR antigens. The staining was strongly positive. In general, OKT6+ HLA-DR+ dendritic cells were distributed randomly within the epidermis. A large number of intra-epidermal and dermal infiltrating lymphocytes were intensely stained with anti-HLA-DR monoclonal antibodies (BL2 specificity). No obvious spatial relationships between dendritic HLA-DR+, T6+ cells and HLA-DR+ lymphocytes were observed (Fig. 3).

Similar results were obtained in MF and SS cutaneous lesions.

**Phenotype of lymphocytes in skin lesions** (Figs. 4, 5). Similar results were obtained in MF and SS. In all the samples, epidermal and dermal lymphocytes reacted with OKT3 and OKT4 antibodies, often intensely, sometimes weakly (Fig. 4). Some tumoral cells, especially the more blastic ones, remained unlabelled by OKT4 antibodies. No significant number of the lymphoid cells was labelled by OKT8 antibodies. Moreover, a large percentage of these cells expressed the HLA-DR antigens in the dermis and in the epidermis (BL2 specificity) (Fig. 5). No difference was found between the OKT3+ cells and the HTLA+ cells. The majority of HTLA+ cells expressed the helper/inducer phenotype. In the epidermis some of these cells appeared isolated or within aggregates contained in Pautrier micro-abscesses. The number and location of OKT4+ lymphocytes in the epidermis were irregular and lacked correlation with the location and number of T6+ BL6+ Langerhans cells.
DISCUSSION

(a) Phenotype of circulating Sézary cells

When using monoclonal antibodies, the circulating lymphocytes in the Sézary syndrome are predominantly OKT3+, thus confirming the thymus-dependent character of this disease. The quantitative results obtained under the electron microscope demonstrated the homogeneity of the phenotypes T3+, T4+, T8−, T6−, BL2−. The low percentage of OKT8+ Sézary cells could be explained by recent data reported by Van Der Loo and his colleagues (26) who observed a few cells with similar ultrastructural Sézary-like morphology in white blood cells of normal subjects. These new conditions make it impossible to distinguish by electron microscopy between the very small number of tumoral OKT8-positive Sézary cells and the normal OKT8-positive Sézary-like cells.

Using immunoperoxidase, our results are direct ultrastructural evidence of the helper/phenotype of the Sézary cell. They confirm the results obtained by indirect immunofluorescence using the same monoclonal antibodies on peripheral blood lymphocytes in the Sézary syndrome (2, 6, 7, 14, 15). The phenotypes T3+, T4+, T8−, T6−, HLA-DR− are usually correlated with a functional helper activity. This tallies with some studies (7). In others, Sézary cells seemed to have a suppressor capacity. In the future, it will be worthwhile to compare simultaneously the phenotype and the functional activity of tumoral cells in the epidermotropic cutaneous T-cell lymphomas (21).

The surface distribution of OKT3, OKT4 and OKT8 antigens on the lymphocytes is similar to that found with anti-HTLA antibodies and HLA antigens (19).

(b) Phenotypic expression of Langerhans cells

This paper describes the expression of the BL6 specificity by Langerhans cell.

The presence of T6 specificity on Langerhans cells was recently described by immunofluorescence (23) and immunoelectron microscopy (17).

In cutaneous lymphoma, dermal Langerhans cells, characterized by OKT6 specificity, seem to be a significant observation to distinguish T and B cell infiltrates (4).

This paper confirms the presence of the OKT6 specificity on Langerhans cells. Moreover, the existence of the BL6 specificity expressed by these dendritic cells provides valuable information on the relationship between thymus and epidermis. The presence of common antigens (T6, BL6) shared by thymocytes and Langerhans cells is not explained. If we consider the location of the T6+, BL6+ cells, it appears that the expression of this specificity seems to be related to the existence of an epithelial microenvironment. Out of this epithelial environment these cells do not express this specificity. If thymocytes are T6+, BL6+, circulating T lymphocytes, are T6−, BL6−. In the same way, if Langerhans cells are T6+, BL6+, the monocytes, from which those cells are derived, are T6−, BL6−. We propose the hypothesis of an expression of epitheliumentdependent antigens by these free cells in close microanatomical relationship with an epithelial environment.

Using monoclonal antibodies, this paper confirms...
the presence of HLA-DR Ag on epidermal Langerhans cells as was previously reported with polyclonal antibodies (11, 18). This observation supports the suggestion that they are related to cells from the monocyte–macrophage series and play a significant role in T-cell immune reactions (22).

(c) Phenotype of tumoral lymphocytes of cutaneous T-cell lymphoma

The expression of T3 specificity by the MF cells confirms the T-cell nature of these tumoral cells. In dermis and epidermis the proliferating lymphocytes expressed the phenotype (T4+, T8–) of the normal helper circulating lymphocyte subpopulation. In the Sézary syndrome, the same phenotype of tumoral cells is observed in skin and blood. These results led us to conclude that the epidermotropic cutaneous T-cell lymphoma represents a homogeneous expression of helper T cells and can be included among the helper cell neoplasms.

Some T3+ skin infiltrating cells also expressed HLA-DR antigens. Despite agreement that la antigens exist on cells other than lymphocytes (24), the expression of la antigens on peripheral T lymphocytes has been a matter of controversy. Recent reports indicated the presence of la antigens on a small proportion of T cells corresponding to activated T lymphocytes (5). The significance of the finding of la molecules on activated T cells is not known. We have observed the existence of a population of activated T cells in the tumoral infiltrates of epidermotropic T-cell lymphoma. Further in vitro studies are needed to determine the significance of HLA-DR antigens in cutaneous lesions (25).

The relationship between T-cell subsets and MHC-derived antigens is not clear. Recent studies have shown that the distribution of T cells with suppressor/cytotoxic or inducer phenotype in the intestinal epithelium and lamina propria may be related to the differential expression of la-like and HLA-A, B, C antigens in intestinal mucosa (20). This observation allowed us to consider the epidermis as a special microenvironment related to the T cell subset functions and other immunocompetent cells.

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