Evolution of a Lymphoma With Helper T Cell Characteristics in Sézary Syndrome

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A patient with Sézary syndrome presented with disease limited to the skin and circulating abnormal lymphocytes but subsequently developed a diffuse undifferentiated lymphoma. Malignant cells from a lymph node effaced with the morphologically undifferentiated lymphoma were characterized as being of thymus-derived (T cell) origin. We have previously shown that circulating malignant lymphocytes from some patients with Sézary syndrome are malignant cells that function as helper cells for Ig biosynthesis by normal B lymphocytes. In the present case, either peripheral blood lymphocytes, comprised mainly of Sézary cells, or cells from the malignant lymph node, containing morphologically undifferentiated lymphomatous cells, greatly augmented pokeweed mitogen-driven IgM synthesis by purified normal B lymphocytes. Thus both the peripheral blood lymphocytes and lymphoma cells from this patient could perform a helper function for Ig biosynthesis in vitro. The undifferentiated lymphoma in this case probably represented an extension of the original malignant clone of helper T cells rather than an unrelated lymphoma that developed de novo. This case illustrates that a T cell malignancy may be morphologically either relatively well differentiated, as in the circulating Sézary cells, or relatively undifferentiated, as in the lymphomatous node, and still express a residual helper T cell function. Furthermore, this case supports the view that it may be possible to classify certain morphologically undifferentiated lymphomas (or immunoblastic sarcomas) according to their immunoregulatory capacity in vitro.

SÉZARY SYNDROME is a primary cutaneous lymphoma characterized by an exfoliative erythroderma, generalized lymphadenopathy, and circulating malignant lymphocytes (Sézary cells) that have characteristic deeply folded or cerebriform nuclei. While the bone marrow is relatively spared, virtually all tissues may be involved, and most patients die within five years of diagnosis.

Mycosis fungoides is histologically indistinguishable from Sézary syndrome but lacks the leukemic phase. Many investigators, including ourselves, consider mycosis fungoides and Sézary syndrome to be varying clinical manifestations of a single malignant disease and draw no other distinction between them.

The non-Hodgkin lymphomas may be classified according to the histopathologic criteria of Rappaport as either nodular or diffuse and then subclassified further based on the apparent cell of origin and state of differentiation. Newer approaches to classification, as proposed by Lennert et al. and Lukes and Collins, attempt to define whether malignant cells have a thymic (T cell) origin, a bursa-equivalent (B cell) origin, or a macrophage (histiocytic) origin based on a combination of morphologic, cytochemical, and immunologic criteria.
Sézary syndrome and mycosis fungoides are now considered collectively as malignancies of T lymphocytes, since abnormal lymphocytes from these patients form rosettes with sheep red blood cells (E rosettes) and are killed by anti-T cell antisera. Our own studies have shown that circulating lymphocytes from certain Sézary patients with very high white blood cell counts serve as helper cells for immunoglobulin biosynthesis in vitro by highly purified normal B cells. Thus Sézary syndrome may represent a malignant proliferation of a helper T cell subclass of T lymphocytes in some cases.

We here report our studies on cells obtained from a malignant lymph node from a patient with Sézary syndrome. This patient presented with disease limited to the skin and circulating abnormal lymphocytes but later developed a large-cell lymphoma that was not morphologically characteristic of the Sézary syndrome. Cells from this malignant lymph node formed E rosettes, a feature of T cells. In addition, cells from the lymph node as well as cells from the patient's peripheral blood performed a helper function for immunoglobulin production in vitro by normal B cells. Thus despite the morphologically undifferentiated appearance malignant cells from a totally effaced lymph node retained T cell membrane markers and served as helper T cells.

The undifferentiated lymphoma in this case probably represented an extension of the original clone of malignant helper T cells rather than a de novo lymphoma. It may be feasible to classify other lymphomas not only according to surface marker features but also as to their functional properties.

CASE REPORT

First admission. H.K., a 65-yr-old white male, was admitted to the Metabolism Branch of the National Cancer Institute on 7/7/76. Over a 2-mo period he had developed a generalized pruritic erythroderma with diffuse lymphadenopathy. Physical examination showed diffuse erythema and dry
skin, but no plaques, and discrete 2 x 2 cm nodes in the neck, axilla, and groin. There was no hepatomegaly. Laboratory data included the following: Hb 14.6 g/dl; Hct 42.6%; WBC 9600/mm³ with 22% lymphocytes (most being characteristic of Sézary cells) (Fig. 1).

The skin biopsy (S76-2320), performed by his referring physicians, was reviewed and interpreted as consistent with mycosis fungoides or Sézary syndrome. Despite abnormal paraaortic nodes on lymphangiogram, lymph node biopsies from the left groin and right axilla showed only dermatopathic lymphadenitis (Fig. 2). Normal studies included liver-spleen scan, bone scan, intravenous urogram, chest x-ray, and whole lung tomograms. Biopsies obtained from the liver and bone marrow showed no evidence of neoplastic disease. The patient was then treated with topical steroids, and total body electron beam therapy was instituted.

Second admission. The patient was readmitted on 10/4/76 because of the sudden increase in size of all lymph nodes that occurred after 1000 rads of total body electron beam therapy. Physical examination showed scaling red skin, but no plaques, and striking cervical, axillary, and inguinal-femoral lymph nodes that were matted and firm. Laboratory data included the following: Hb 15.4 g/dl; Hct 44.4%; platelets 252,000/mm³; WBC 7600/mm³ with 8% lymphocytes (many lymphocytes had cerebriform nuclei typical of Sézary syndrome).

A lymph node biopsy from the right cervical chain showed an undifferentiated, pleomorphic lymphoma with a high mitotic index (Fig. 3). Negative studies included repeat liver-spleen scan, bone scan,

Fig. 2. Lymph node biopsy showing changes of reactive hyperplasia. (A) Low power, showing intact architecture with follicular and paracortical hyperplasia. x 100. (B) High power, showing morphologically normal lymphocytes in the paracortex. x 950.
Fig. 3. Lymph node biopsy showing involvement by malignant lymphoma. × 950.

liver biopsy, bone marrow biopsy, and lumbar puncture. The patient was then started on intensive systemic chemotherapy.

MATERIALS AND METHODS

Isolation of peripheral blood lymphocytes for surface marker studies was performed by buoyant density centrifugation through Ficoll-Hypaque. Peripheral blood lymphocytes for assays of immunoglobulin biosynthesis were obtained from the supernatant of heparinized blood sedimented at 37°C.

Preparation of normal T and B cells. Normal peripheral blood lymphocytes obtained by Ficoll-Hypaque centrifugation were separated into highly purified populations of B cells (the immediate precursors of immunoglobulin-secreting cells) and T cells by immunoabsorbent column separation as previously described.14 The B cell fraction was then subjected to an additional overnight depletion of E-rosette forming cells in order to remove any residual T cell contamination.

Preparation of lymph node cells for surface marker studies. A 3 x 3 cm lymph node was removed from the right anterior cervical chain under local anesthesia in the operating room and placed in normal saline. Part of the tissue was processed for histology; the remainder was gently minced and then passed through a fine stainless steel wire mesh to obtain a single-cell suspension. After lysis of erythrocytes by NH4Cl buffer pH 7.4, the cells were washed three times and resuspended in balanced salt solution.

Storage of cells in liquid nitrogen. The remainder of peripheral blood lymphocytes and lymph node cells not used in initial studies were suspended in RPMI-1640 containing 10% dimethylsulfoxide (DMSO) and 30% heat-inactivated fetal calf serum (FCS). Cells were then cooled at a rate of ~1°C in the vapor phase of liquid nitrogen. When needed for further assays, cells were thawed rapidly and washed free of DMSO, then checked for viability with trypan blue.

Immunologic marker studies. Mononuclear cell suspensions were evaluated for cells bearing receptors for complement (EAC), the Fc portion of IgG (EA), and sheep erythrocytes (E) as previously described.14 The IgM and IgG reagents used for EAC and EA immunotyping, respectively, were kindly provided by Dr. Michael M. Frank, NIAID, NIH. Rosetted cells were enumerated in the presence of trypan blue, and Wright-stained cytocentrifuge preparations were examined for cytologic identification of rosetted cells. Cells were studied for the presence of cell surface immunoglobulin using a polyvalent antiserum prepared against human γ, α, δ, ε, and λ chains as previously described.13

Assay of immunoglobulin biosynthesis in vitro. The details of this assay have been presented previously.11,17 Briefly, lymphocytes were cultured in vitro in the presence or absence of pokeweed mitogen (PWM), a known mitogen for B cells. The amount of immunoglobulin produced and secreted into the culture medium was then determined by a very sensitive double-antibody radioimmunoassay specific for IgM using a technique similar to that previously described for IgE.14
In the present study, freshly prepared normal B and T cell populations were employed. Previously frozen peripheral blood lymphocytes and lymph node cells from patient H.K. were rapidly thawed at 37°C, then washed free of DMSO. All cell populations were then washed four times through FCS to remove contaminating immunoglobulin and resuspended in culture medium at a concentration of 2 x 10^6 cells/ml. The culture medium consisted of RPMI-1640 (Grand Island Biological, Grand Island, N.Y.) containing 4 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% heat-inactivated FCS. Aliquots (1 ml) of cells were then cultured either with or without PWM, 10 μl/culture, in 1-dram vials for 12 days at 37°C in 5% CO₂. The amount of IgM secreted into the culture medium was then determined.

**Assay for helper function.** In the absence of T cells, PWM-stimulated B cells produce only small quantities of immunoglobulin. The ability of cocultured lymphocytes to enhance immunoglobulin production by normal B cells may then be used as an index of helper function. When the lymphocytes added to the normal B cells are a mixed population, it is necessary to selectively inactivate B cells and suppressor T cells while allowing helper T cells to express themselves. We previously showed that helper T cells are radioresistant while suppressor T cells and B cells are radiosensitive. In addition, helper T cell influence predominates at low T to B cell ratios. Thus to assess helper function irradiated (2000 rads) peripheral blood lymphocytes or lymph node cells were cultured with normal B cells at either the standard T cell/B cell ratio (two T cells per B cell) or a low T cell/B cell ratio (0.2 T cells per B cell). The amount of IgM secreted into the medium after 12 days in culture with PWM was then determined.

**RESULTS**

**Pathology.** Several biopsies and tissue examinations were performed during the course of this patient’s illness. The tissue histology generally paralleled the clinical course. Thus a skin biopsy (S76-2320, not shown) was performed on 6/21/76 when the patient presented with a clinical picture typical of Sézary syndrome. It showed irregular acanthosis and mild hyperkeratosis of the surface epithelium with a high dermal lymphocytic infiltrate composed of atypical cerebriform cells, without other inflammatory elements. There was exocytosis of individual cells into the epidermis as well as rare clusters of three to ten cells within the epidermis. Pathologic diagnosis was consistent with mycosis fungoides or Sézary syndrome. At this same time a lymph node biopsy (not shown) was obtained from the left inguinal region (S76-2320); it showed intact lymphoid architecture and features of dermatopathic lymphadenitis. Cerebriform lymphoid cells were identified within vessels and as occasional cells within the paracortex. These changes were not felt to be diagnostic of Sézary syndrome. Another lymph node biopsy was taken from the right axillary region 1 mo later (S76-2526) and had the identical histologic findings (Fig. 2) of dermatopathic lymphadenitis.

Four months later the patient’s clinical picture suddenly changed as peripheral lymph nodes began to massively enlarge. A cervical lymph node biopsy (S76-3513) was then obtained (Fig. 3), showing complete effacement of normal lymph node architecture by a diffuse infiltrate of abnormal cells that extended into surrounding adipose tissue. The walls of small vessels were frequently infiltrated. The cells ranged from 20 to 30 μm in diameter and had a moderate amount of cytoplasm that stained moderately with methyl green pyronin and indistinctly with eosin. The cells had round to oval nuclear contours, and cerebriform cells were very rarely seen. Nuclear chromatin was finely clumped, with one or two central eosinophilic nucleoli. Mitotic figures were numerous (>20/HPF). Admixed cytologically normal plasma cells were only rarely identified. Pathologic diagnosis was malignant lymphoma, undifferentiated, pleomorphic.
**Immunologic markers.** Because of the change in the patient's clinical disease to an aggressive lymphoma and because of the unusual histologic picture, attempts were made to determine whether this lymphoma represented the progression of the T cell malignancy previously confined to skin and peripheral blood or the presentation of a de novo lymphoma unrelated to Sézary syndrome. Accordingly, immunologic marker studies were performed on cells obtained from the malignant lymph node (Table 1); 44% of these cells formed rosettes with sheep red blood cells (E rosettes), a feature of human T cells. Both rosetting and nonrosetting cells were large, transformed tumor cells (Fig. 4). Studies of peripheral blood lymphocytes from this patient showed normal percentages and numbers of T and B cells both before and after development of the lymphoma (data not shown). Most of these circulating lymphocytes had the characteristic morphology of Sézary cells by both light- and electron-microscopic criteria (Figs. 1, 4). It thus appeared that the lymphoma did not arise de novo but was an extension of the T cell malignancy initially involving the skin and circulating lymphocytes.

**Immunoglobulin biosynthesis in vitro.** We first asked whether or not the patient's peripheral blood lymphocytes and lymph node cells could synthesize IgM in response to PWM. As can be seen in Table 2, peripheral blood lymphocytes from control subjects had geometric mean synthesis values for IgM of 3002 ng after 12 days in culture with PWM. By contrast, peripheral blood lymphocytes from patient H.K. synthesized only 634 ng IgM under the same conditions. Prior irradiation (2000 R) reduced the amount of IgM produced to negligible quantities. It is important to emphasize that the IgM-secreting capacity of this patient's circulating lymphocytes reflected the relatively low leukemic cell count. The patient's circulating lymphocyte population contained enough normal B cells to produce a limited, albeit subnormal, quantity of immunoglobulin in vitro.

There are several possible explanations for the low IgM production in response to PWM by peripheral blood lymphocytes despite normal numbers of circulating B cells in this patient. One possibility might be the presence of excessive suppressor cell influence analogous to our finding in common variable hypogammaglobulinemia. To date, however, we have not found excessive suppressor cell activity in any Sézary patient studied. Another possibility might be abnormal T to B cell interaction, a prerequisite for B cell responsiveness to PWM. Clearly, Sézary T cells can interact with normal B cells to induce immunoglobulin synthesis, but the appropriate cell separations and recombinations have not yet been done to address the question of autologous T to B cell interactions in Sézary patients. Yet another possibility might be defects in accessory cells, such as macrophages, essential for PWM responsiveness by B cells. Finally, B cells might be intrinsically defective in the ability to respond to PWM. Further studies are necessary to distinguish between these possibilities.

**Table 1. Lymph Node Histology and Surface Markers of Patient H.K.**

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<td>E rosettes</td>
<td>54%</td>
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<tr>
<td>EA rosettes</td>
<td>16%</td>
<td>3%</td>
</tr>
<tr>
<td>EAC rosettes</td>
<td>25%</td>
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<td>Surface Ig</td>
<td>18%</td>
<td>0.5%</td>
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Fig. 4. Photomicrograph of malignant cells forming spontaneous E rosettes. (A) Peripheral blood lymphocytes. × 950. (B) Lymph node cell. × 950.
Cells from the tumor-effaced lymph node were unable to synthesize IgM in response to PWM. This inability to secrete immunoglobulin corresponds to our previous experience with the circulating lymphocytes from patients with Sézary syndrome with very high leukemic counts. The circulating lymphocytes from such patients secrete essentially no immunoglobulin in response to PWM because their circulating normal B cells have been diluted by enormous numbers of malignant T cells. By analogy, the patient’s lymph node was totally effaced by malignant cells, many bearing T cell markers, leaving virtually no cells with B cell markers (Table 1). The failure of cells from the malignant lymph node to produce immunoglobulin in response to PWM can therefore be explained by the lack of B cells in the malignant cell population.

Assay for helper function. We next addressed the issue of whether or not lymphocytes from the patient’s peripheral blood could help IgM production by B cells from a normal individual. Figure 5 shows the results of two separate experiments. As can be seen, neither the purified normal B cells nor the patient’s ir-
radiated peripheral blood lymphocytes made significant amounts of IgM in the presence of PWM. When normal B cells were cocultured with irradiated peripheral blood lymphocytes from patient H.K. at either standard (Fig. 5A) or low (Fig. 5B) T cell/B cell ratios, the amount of IgM secreted was greatly augmented. Therefore circulating lymphocytes from this patient did perform a helper function in vitro.

Cells from the patient’s malignant lymph node were assayed for helper function in a similar fashion. Figure 6 shows one such experiment. The lymph node cells alone made no IgM in response to PWM, but at standard T cell/B cell ratios increased IgM production by normal B cells from 228 ng alone to 1905 ng in coculture. More importantly, when these lymph node cells were irradiated and then added in coculture with normal B cells at low T cell/B cell ratios, 1033 ng of IgM was still produced. Thus the malignant lymph node cells met our criteria for helper function in vitro by augmenting IgM production by normal B cells at low T cell/B cell ratios following irradiation.

**DISCUSSION**

The immune system can be conveniently divided into the bone marrow–derived (B cell) compartment, responsible for humoral antibody formation, and the thymus-derived (T cell) compartment, responsible for delayed hypersensitivity, graft rejection, and other manifestations of cell-mediated immunity. T cells also play a critical role in regulating normal B cell maturation into immunoglobulin-secreting cells. T cells are required for immunoglobulin production by B cells (a helper effect) but may also act to inhibit antibody production (a suppressor effect). Different classes of T cells mediate help or suppression. In NZB/W mice, loss of suppressor cell function with age seems to allow autoantibody formation and a clinical picture similar to that seen in systemic lupus erythematosus in man.
contrast, excessive suppressor function has been shown experimentally in bursectomized chickens\textsuperscript{22} and in many adult humans with common variable hypogammaglobulinemia.\textsuperscript{12} Thus there appears to be a critical T cell regulatory system involving a delicate balance between helper and suppressor T cell functions.

Sézary syndrome is an example of a T cell malignancy. This primary cutaneous lymphoma may progress to frank visceral involvement. When visceral or secondary lymph node involvement does occur, the infiltrating cells usually resemble the Sézary cells seen in the skin and peripheral blood.\textsuperscript{2,23} Less commonly, there is progression to an undifferentiated or pleomorphic lymphoma in which the neoplastic cells do not express the typical morphologic features of Sézary cells, as in the present case. These tumors have been variously classified as Hodgkin disease, histiocytic lymphoma, or reticulum cell sarcomas.\textsuperscript{24–26} More recently these tumors have been classified as immunoblastic sarcomas\textsuperscript{27} in recognition of their immunologic derivation. Most recently Lukes and Collins classified a large series of non-Hodgkin lymphomas as to their immunologic origin.\textsuperscript{8} While the majority of tumors in that series were of B cell origin, some immunoblastic sarcomas and all Sézary-mycosis fungoides tumors were morphologically characteristic of T lymphocytes. Lukes and Collins postulated that the large, transformed-appearing cells in the tumor phase of mycosis fungoides or Sézary syndrome might constitute an immunoblastic sarcoma of T cells. The present case afforded a unique opportunity to confirm or deny the relationship between these two processes. Since 41% of the cells from an effaced malignant node formed spontaneous E rosettes, a human T cell marker, the malignant cells are likely to have been of T cell origin. Morphologically, the malignant node in this case (Fig. 3) was similar to the immunoblastic sarcoma described by Schwarze and Ude.\textsuperscript{27}

In addition to categorizing this patient's lymphoma as one of T cell origin, we were able to study cells from an effaced malignant lymph node as well as circulating lymphocytes comprised mainly of Sézary cells in an assay in vitro for helper activity for immunoglobulin biosynthesis by normal peripheral blood B cells. We recently showed that leukemic lymphocytes from some patients with Sézary syndrome perform a helper function in this assay.\textsuperscript{11} Such circulating malignant cells from Sézary patients probably represent a malignant expansion of a helper T cell subset of normal T lymphocytes. Cells from the malignant lymph node and peripheral blood augmented IgM production by normal B cells when irradiated and added in coculture at low T cell/B cell ratios, thus meeting our criteria for helper function in vitro (Figs. 5, 6).

It might be argued that the observed helper activity of the lymph node cells is an artefact of contamination by circulating peripheral blood lymphocytes. This is unlikely, since the helper activity was present at low T cell/B cell ratios, conditions where any blood contamination would be diluted tenfold. Furthermore, the lymph node appeared to be effaced by a uniform population of undifferentiated cells with no morphologic evidence of contamination by peripheral blood cells (Fig. 3). Finally, if significant contamination of the lymph node cells by circulating lymphocytes had occurred, one might expect IgM production by the lymph node cells proportional to that contamination. As shown in Table 2, however, the patient's circulating lymphocytes made 634 ng IgM in response to pokeweed mitogen, while cells from the malignant lymph node made none. There is thus no
evidence of contamination by peripheral blood lymphocytes to explain the helper activity of the malignant lymph node.

An unresolved issue is the low IgM synthesis in response to pokeweed mitogen by the patient's peripheral blood lymphocytes despite the demonstration of helper T cell activity and normal numbers of circulating B cells. This phenomenon has been seen in several of our Sézary patients with relatively low numbers of circulating leukemic cells. Several of our patients have also shown a subnormal antibody response following immunization with certain antigens (Lawrence EC, et al: unpublished observations), and several died of infectious complications. Thus this question is of more than academic interest. Although it is possible that certain patients may have additional defects in B cell responsiveness, T to B cell interaction, or accessory cell function, we have not yet addressed these questions directly. Hopefully, the rigorous cell separations and reconstitution procedures needed to differentiate between these various possibilities will be forthcoming.

This case is unique in that cells from a morphologically undifferentiated T cell lymphoma, similar to the T cell immunoblastic sarcoma of Lukes and Collins, were seen to express a residual helper T cell function. There appears to have been an evolution from limited involvement of skin and blood to generalized lymph node destruction by cells having the same origin as but different morphologic appearance from the original circulating Sézary cells. This is analogous to the progression of chronic lymphocytic leukemia (CLL), a B cell leukemia, to a large-cell lymphoma with B cell markers (Richter syndrome). Brouet et al. postulated that progression to a lymphoma in CLL represents an expansion of the original clone of malignant B cells; however, the case presented here probably represents the lymphomatous expansion of the original clone of malignant helper T cells.

A number of critical insights into the nature of the humoral immune response were made possible by studying neoplasms of the B cell/plasma cell series. The recognition that so-called paraproteins derived from patients and certain animals with myeloma (a malignant proliferation of plasma cells) represent extremely homogeneous immunoglobulins was an indispensable step in understanding the structural, functional, metabolic, and genetic aspects of humoral immunity. Similarly, the study of malignant T cells and their products may prove to be as rewarding in resolving questions regarding cellular immunity and especially T cell regulation of humoral immune responses.

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REFERENCES

5. Rappaport H: Tumors of the hematopoietic system, in: Atlas of Tumor Pathology, sec. III,
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EC Lawrence, S Broder, ES Jaffe, RC Braylan, WO Dobbins, RC Young and TA Waldmann