Comparison of Histologic and Immunologic Heterogeneity of Non-Hodgkin's Lymphomas

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The lymphocyte surface marker phenotype in 11 selected cases of non-Hodgkin's lymphomas was determined with anti-immunoglobulin and mouse monoclonal antibodies against human lymphocyte antigens. A complement-mediated cell cytotoxicity assay on suspensions of the tumor cells was compared with an indirect immunoperoxidase technique on frozen tissue sections. Both methods gave good results in tumors with a uniform cell population, but the frozen section technique was superior in heterogeneous tumors. The six B-cell neoplasms were heterogeneous with respect to expression of surface immunoglobulin and Ia antigens. The five T-cell tumors were morphologically heterogeneous and also highly variable in their expression of different T-cell specific antigens. (Am J Pathol 1981, 105:97–106)

THE IMPORTANCE OF classifying non-Hodgkin's lymphomas according to immunologic phenotype has long been recognized.[1,2] Most early studies were performed on suspensions of cells from B-cell lymphomas, in part because these are common. However, since it has been recognized that most lymphoblastic lymphomas are of thymic origin,[3] and also that certain morphologic patterns can be associated with peripheral T-cell lymphomas,[4,5] recently more attention has been paid to T-cell neoplasms.

Although the techniques for the identification of lymphocyte surface markers in frozen sections by either immunofluorescence[6,7] or immunoperoxidase[2,8] are well known, they have mainly been used to identify B cells by means of anti-immunoglobulin antibodies. A few reports on the use of carefully absorbed anti-T-cell heteroantisera have been published.[8,9] However, the new monoclonal hybridoma antibodies are now readily available, and immunologic detection of specific lymphocyte subpopulations in frozen sections of thymus,[10] lymph node,[11] and other tissues[12] is possible. The reagents have not yet been extensively used in the study of lymphomas, although several recent reports exist.[13–15]

The present study of 11 cases of non-Hodgkin's lymphomas was undertaken first to compare the immunohistochemical and cell suspension techniques, and also to define the nature of the surface antigens on cells from some cases of T-cell non-Hodgkin's lymphomas. We found that the immunoperoxidase technique on frozen sections has considerable advantages over cell suspension assays and that it is an excellent technique for demonstrating the cell type and phenotypic heterogeneity of T-cell lymphomas.

Materials and Methods

Lymph node biopsy specimens were obtained in the fresh state from the operating room. (For Case 2, tissue for the immunohistochemical studies was obtained from the patient at autopsy, and a previous biopsy specimen from the same site was used for the original histopathologic diagnosis). A portion of tissue was teased into fresh Hanks' balanced salt solution supplemented with 20% fetal calf serum. Cells were washed and separated on a Ficoll-Hypaque gradient when necessary to remove dead cells and other debris. Lymphocyte surface antigens were identified by a complement-mediated cytotoxici-

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ty test with a battery of monoclonal antibodies and normal serum controls. In addition, aliquots of the suspension were incubated overnight at 4 C with sheep red blood cells to determine E rosette formation, and in some cases surface immunoglobulin (SIg) or other surface membrane antigens were identified by direct or indirect immunofluorescence techniques. These methods have been described previously in detail.[16]

For the immunoperoxidase procedure, an additional portion of tissue was embedded in gelatin and snap frozen in liquid nitrogen-cooled isopentane. Six-micron cryostat sections were cut, air dried, and fixed briefly in cold acetone. All material was stored at −70 C until use. Sections were stained using a modified indirect immunoperoxidase procedure.[17] Briefly, following a preincubation in 10% normal rabbit serum, the tissue was incubated with appropriately diluted (usually 1:5 to 1:100) mouse monoclonal antibody or normal mouse serum control for 1 hour, followed by horseradish peroxidase-conjugated rabbit anti-mouse IgG (light and heavy chain) diluted 1:50 in a mixture of normal rabbit and human sera. Surface immunoglobulin was detected with goat anti-human immunoglobulin followed by peroxidase-conjugated rabbit antibody to goat immunoglobulin. Enzyme activity was detected with a diaminobenzidine-H_2O_2 reaction and the sections were counterstained with hematoxylin.

The stained sections were then examined by light microscopy, but because these were frozen sections, the morphologic appearance was obviously not as clear as in routine paraffin-embedded material. Nonetheless, positive staining was easily recognized. In most cases, staining took the form of a distinct rim of brown precipitate on positive cells, indicating the presence of a specific surface antigen. In some cases, cytoplasmic immunoglobulin could also be detected as a more discrete mass of precipitate within the cell. It was also important to distinguish the surface pattern from an "interstitial" pattern that occurs due to the presence of antigen between the cells. This was of practical importance only when staining for gamma heavy chain or kappa light chain, and in any case the interstitial pattern was usually distinctive. In the case of the T-cell monoclonals, surface staining was the principal pattern seen, though in some cases there appeared to be a small amount of cytoplasmic staining of questionable significance. The Ia antigen, however, frequently demonstrated staining between cells, and in some cases in cytoplasm as well. This was only present in areas that were also surface Ia positive, suggesting that antigenic material had been leached out from around positive cells during processing.

T-cell phenotypes were defined with monoclonal antibodies to pan- or broad-spectrum T-cell antigens (Leu 1, OKT3, 3A1), thymic leukemia antigens (OKT6, 12E7), Ia antigens (HLADR), T helper-inducer antigen (OKT4, Leu 3A), and T-suppressor-cytotoxic antigen (OKT8, Leu 2A). The monoclonals OKT3, OKT4, OKT6, and OKT8 were purchased from Ortho Diagnostics (Boston, Mass), and Leu 1, Leu 2A, Leu 3A, and HLADR were purchased from Becton Dickinson (Sunnyvale, Calif). The antibodies 3A1 and 12E7 were gifts of Dr. Barton Haynes and Dr. Ronald Levy, respectively. Other xenoantisera were purchased from Cappel (Cochranville, Pa) or Meloy (Springfield, Va).

The 11 non-Hodgkin's lymphoma cases described here were classified by routine light-microscopic examination of either formalin- or Zenker's-fixed tissue sections according to a modified Rappaport classification. All were diffuse lymphomas. The cases were in part selected to illustrate the immunohistochemical phenotyping of T-cell lymphomas; thus, they do not reflect the prevalence of T-cell lymphomas in our patient population.

Results

The 11 non-Hodgkin's lymphomas studied included two well differentiated lymphocytic lymphomas, two lymphoblastic lymphomas, six diffuse mixed or large cell lymphomas, and one malignant lymphoma with a high content of epithelial histiocytes (Lennert's lymphoma). From their morphologic appearance alone, the tumors could be divided into six cases with a homogeneous cell population and five with a more heterogeneous cell population, either because of partial involvement of a node with tumor or because of a mixture of cell types within the tumor. In the homogeneous tumors, the interpretation of phenotypes of the frozen sections was straightforward, and, in the three cases in which both suspension data and section data were obtained, there was substantial agreement between cytotoxicity and immunoperoxidase results. In contrast, the frozen-section technique often provided more information on the heterogeneous tumors that was not discernible from the suspension data alone.

Homogeneous Tumors

The data from six homgeneous tumors are summarized in table 1. The first case was a mediastinal

Table 1—Phenotypic Characterization of Homogeneous Tumors

Case	Histologic diagnosis	Phenotype* E (%)	SIg	Kappa	Lambda	T-antigens†	Ia	Immunopathologic diagnosis
1	Lymphoblastic lymphoma	61	–	NT	NT	+	–	T-cell lymphoblastic lymphoma
2	Lymphoblastic lymphoma	NT	+(IgM)	+	–	–	+	B-cell lymphoblastic lymphoma
3	Well differentiated lymphocytic lymphoma (WDLL)	14	80%	–	+	–‡	+	B-cell WDLL
4	Well differentiated lymphocytic lymphoma	1	6%	NT	NT	+	–	T-cell WDLL
5	Large cell lymphoma	NT	+(IgM)	+	–	–	–	B-cell large cell lymphoma
6	Large cell lymphoma	NT	–	NT	NT	+	+	T-cell large cell lymphoma

* – or + denotes immunoperoxidase reactivity in frozen sections; where a number appears it refers to % positive cells by direct immunofluorescence technique with an anti-polyvalent immunoglobulin serum.

† Reactivity by both cytotoxic and immunohistochemical studies with any of several anti-T-cell-specific antibodies, as further elaborated in Table 3.

‡ Reacted weakly with Leu 1. See text.

NT = not tested.

mass from a young child, which morphologically contained a uniform population of medium-size cells with diffuse chromatin that were diagnostic of lymphoblastic lymphoma (Figure 1). The immunologic phenotype of the cells in suspension was E^+, and they reacted with antibodies directed against most T-cell and thymic antigens. They also lacked surface immunoglobulin (SIg) and were Ia^-. By immunoperoxidase technique, an identical phenotype was found. A positive reaction was indicated by distinct rim staining of the lymphoma cells. Figure 2A shows a positive stain with the monoclonal antibody 3A1, and Figure 2B shows a negative reaction for Ia. In contrast to this case, Case 2 represents another mediastinal mass that, although morphologically very similar to the first case, was serologically different. Figure 3 shows a section, stained with hematoxylin and eosin, of a tumor very similar to that of Case 1. However, this tumor failed to stain with any of the monoclonal anti-T-cell antibodies but instead was positive for SIgM (Figure 4), kappa (but not lambda) light chain, and Ia. Thus, this tumor is best considered a B-lymphoblastic lymphoma, since it contains Ia and monotypic surface immunoglobulin.

The next two examples of homogeneous tumors were lymph node biopsies from patients with chronic lymphocytic leukemia. The histopathologic features in both cases were similar and characteristic of well differentiated lymphocytic lymphomas. The surface marker data, obtained both by analysis of cell suspension and by tissue section, illustrated that these were two different tumors (Table 1). One case had surface immunoglobulin with only lambda light chain and was Ia^+, typical for a B-cell lymphoma. It did not react for most T-cell antigens, but was weakly positive with the antibody Leu 1. This antibody is one of several that have been shown to react with an antigen present on T cells and B-cell chronic lymphocytic leukemia (CLL), but not other B-cell lymphomas.[18] The second case, in contrast, was surface immunoglobulin negative and failed to form E rosettes, and thus might have been called a "null" cell lymphoma if analysis had been limited to this type of surface marker analysis. However, sections of the tumor reacted with several of the anti-T-cell monoclonal antibodies (Table 3). A similar phenotypic pattern was seen with the cell suspensions. Thus, this tumor is an example of a T-cell well differentiated lymphocytic lymphoma.

The remaining two cases of monomorphic tumors were both large cell lymphomas and were studied by

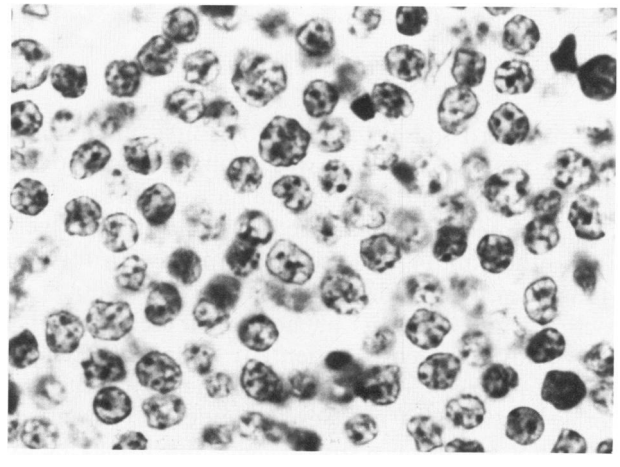

Figure 1—Case 1. Lymphoblastic lymphoma. Tumor cells are medium size, often irregular, and have finely dispersed chromatin. Mitotic figures are present. (H&E, ×1000)

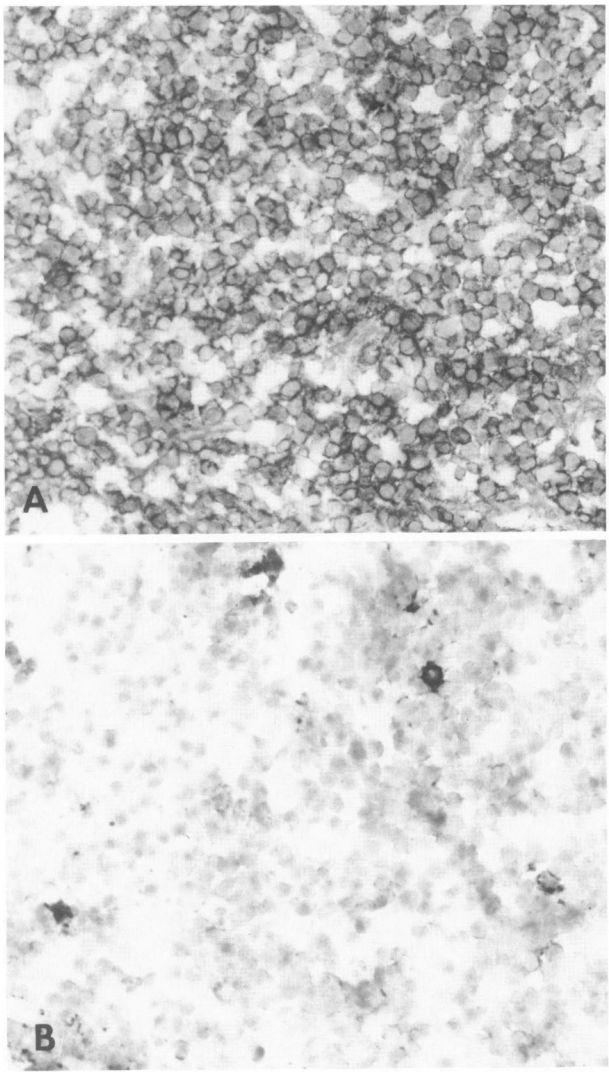

Figure 2—Case 1. **A**—Frozen section stained by immunoperoxidase technique with the monoclonal anti-T-cell antibody 3A1. Note uniform rim staining of nearly all cells. **B**—Immunoperoxidase stain for Ia antigen. Only a few positive cells, probably macrophages, are present. (Hematoxylin counterstain, × 325)

immunoperoxidase technique. The first tumor was a mediastinal mass which was found, in spite of its location, to be a B-cell lymphoma with surface IgM-kappa. Interestingly, it failed to stain for Ia antigen. The other tumor was from a patient with mycosis fungoides. Although this was a T-cell lymphoma reacting with OKT3, its phenotype differed from that of the usual mycosis patient. It lacked the Leu 3A (helper) antigen and was Ia^+ and thus different from other examples of mycosis fungoides.[18] The antigen Ia is not found on resting T cells, but it may be expressed when T cells undergo antigen activation or stimulation.[19] The significance of this discovery is discussed later.

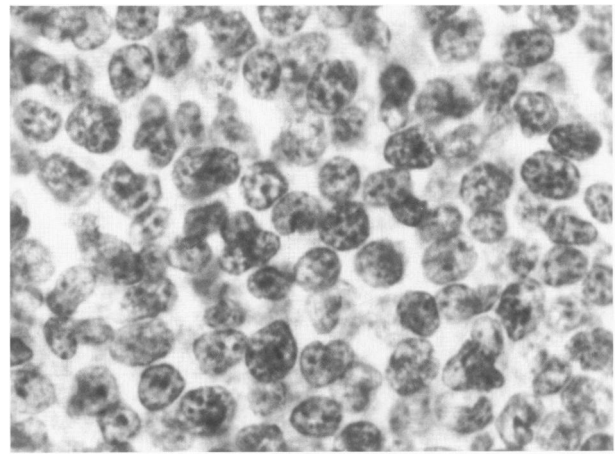

Figure 3—Case 2. Lymphoblastic lymphoma. Notice medium-size cells with finely distributed chromatin. Nuclear contours are fairly regular. (H&E × 1000)

Heterogeneous Tumors

The remaining cases were all diffuse large cell or diffuse mixed cell lymphomas that had a heterogeneous cellular composition. These were all studied by both the isolated cell suspension cytotoxicity and *in situ* frozen-section techniques. A comparison of these findings is summarized in Table 2. In general, when there was a mixture of normal and neoplastic cells, particularly with the B-cell lymphomas, the suspensions were often relatively enriched for the normal cells. Thus, in Cases 7 and 9, the section data showed a marked preponderance of B cells with either a few admixed T cells (Case 7) or an area of B-cell tumor clearly separate from residual normal node with many T cells (Case 9). Even Case 10, which

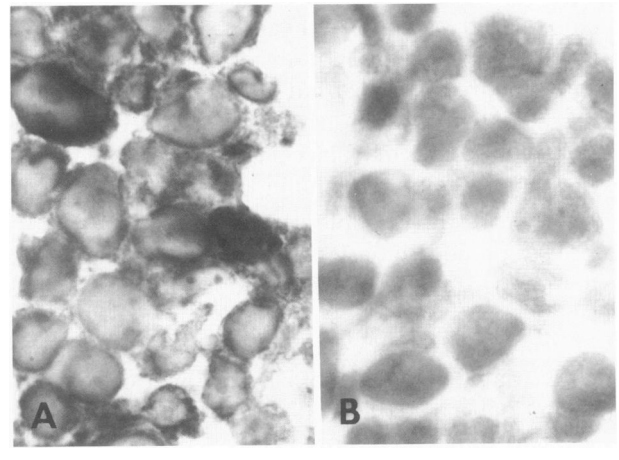

Figure 4—Case 2. Frozen section. **A**—Immunoperoxidase stain for IgM. **B**—Immunoperoxidase stain for the thymus leukemia antigen OKT6. Notice the granular rim staining for immunoglobulin and the lack of staining with OKT6. (Hematoxylin counterstain, × 1000)

Table 2—Phenotypic Characterization of Heterogeneous Tumors

Case	Histologic diagnosis	Suspension phenotype* E	SIg	T Antigens†	Ia	Section phenotype	Immunopathologic diagnosis
7	Mixed cell lymphoma	56	45	34	66	Tumor cells $SIg^+\lambda^+\kappa^-Ia^+$ Some T cells	B-cell mixed cell lymphoma
8	Mixed cell lymphoma	64	6	±	24	Tumor cells T^+ Some residual B cells	T-cell mixed cell lymphoma
9	Large cell lymphoma	52	24	NT	36	Tumor cells $Ia^+SIg^-T^-$ Only focal node involvement	B-cell large cell lymphoma
10	Large cell lymphoma	58	NT	±	+	Virtually all cells $Ia^+SIg^+\kappa^+\lambda^-$	B-cell large cell lymphoma
11	Lennert's lymphoma	50	58	±	±	Mixed SIg^+ and T^+ cells with histocytes	Probable T-cell lymphoma

* Where a number appears it refers to the percentage of positive cells by immunofluorescence technique with the appropriate antiserum. The designation + indicates more than 50% of cells were killed in the cytotoxicity test; the designation ± means fewer than 50% of the cells were killed. Background killing with normal serum or non-complement fixing antibodies varied from 10% to 20-30%.

† Reactivity with any of several anti-T-cell-specific antibodies, as further elaborated in Table 3.

NT = not tested.

had an overwhelming proportion of B cells in section, had 20% T cells in the suspension. Because of this result, the immunologic phenotype of the tumors was not always apparent from cytotoxic analysis of the cell suspensions. Other investigators have advocated examination of Wright-stained cytocentrifuge preparations of E (and EAC) rosettes in order to distinguish neoplastic from normal T (and B) cells.[1] When this technique was applied to Case 7, however, both small lymphocytes and some large atypical lymphoid cells formed E rosettes (Figure 5A). When combined with the suspension data, this result might have suggested a T-cell origin for this tumor. However, in section, at least two thirds of the cells stained for SIg, and virtually all stained for lambda rather than kappa light chain (Figure 6). These cells were also Ia^+. Thus, we interpret this case as a B-cell lymphoma with admixed and probably reactive T lymphocytes. To investigate further, we looked for the expression of Ia antigens on the surface of the T cells. We prepared an E-rosette preparation after staining for Ia antigen by indirect immunofluorescence technique and found that 40% of the rosetting cells had membrane fluorescence, which indicated that some but not all of the T cells were Ia positive as well.

In contrast to Case 7, Case 8 represents an example of a peripheral T-cell lymphoma. This classification was suggested strongly by the suspension data, in which there were few cells positive for SIg and larger numbers of rosetting cells. A Wright-stained cytocentrifuge preparation of the E rosettes is shown in Figure 5B, where it can be seen that, morphologically, the tumor cells are not much different from the reactive T cells of Case 7 (Figure 5A). Although there was a significant number of Ia-positive cells in the suspension, when a combined Ia-E-rosette preparation was made, we found that none of the Ia-positive cells formed rosettes. In section, the specimen contained large tumor cells infiltrating throughout the node, but there were some spared areas as well. As il-

Table 3—Phenotypic Comparison of T-Cell Lymphomas

Case	Diagnosis	E	OKT3	3A1	Leu 1	Leu 3A/ OKT4	Leu 2A/ OKT8	OKT6	12E7	Ia
1	Lymphoblastic lymphoma	+	+	+	+	+	+	+	+	–
4	Well differentiated lymphocytic lymphoma	–	+	–†	+	+	–†	–	+	–
6	Large cell lymphoma (mycosis fungoides)	NT	+	–	–	–	–	–	NT	+
8	Mixed cell lymphoma	+	+	–	+	+	–	–	NT	–
11	Lennert's lymphoma	±	±	–	±	–	±	–	NT	±‡

* The designation + indicates reactivity of the majority of tumor cells by immunoperoxidase technique in frozen tissue section; the designation ± indicates reactivity of about half the cells. E rosettes were determined on cell suspensions.

† Approximately 10-20% of the tumor cells were positive for these antigens.

‡ The Ia-positive cells in this case were probably B and not T cells (See Table 2).

NT = not tested.

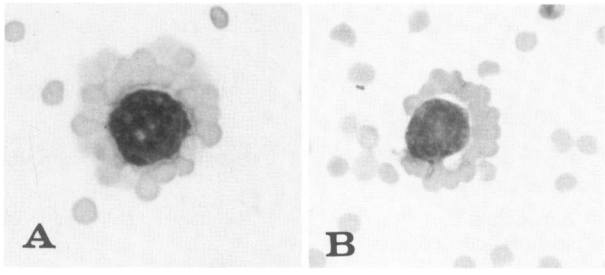

Figure 5—Cytocentrifuge smear of E-rosette preparation from Case 7(**A**) and Case 8(**B**). Large, atypical lymphoid cells are present in both cases. (Wright's, ×680)

lustrated in Figure 7, the tumor stained with some anti-T-cell antibodies, whereas the smaller cells did not stain. The complete T-cell phenotype of the tumor, as shown in Table 3, was $OKT3^+$ Leu 1^+ $3A1^-$ $OKT4^+$ $OKT8^-$ Ia^-. As mentioned above, this is the phenotype seen in most patients with mycosis fungoides. However, although this patient did have skin lymphoma and circulating atypical cells, his skin lesions were neither clinically nor histopathologically those of mycosis fungoides.

The next case is an example of large cell lymphoma, which on section only involved part of the lymph node (Figure 8A). In this case, the suspension data were ambiguous because of the relative enrichment of the suspension with normal B and T lymphocytes. Lymphocytes from the uninvolved portion of the node stained with all the T-cell antibodies, but the tumor did not (Figure 8B). Tumor staining for SIg for both heavy and light chains was equivocal, but when stained for Ia the tumor showed extensive diffuse staining on the surface and, to a lesser extent, between the cells (Figure 9). Because the tumor did not stain with any T-cell antibodies, and because the cells were nonspecific esterase negative (and thus not macrophages), we believe that this is a B-cell neoplasm. The SIg^-Ia^+ phenotype of some B-cell large cell lymphomas has been previously recognized.[15] Case 10 is a more typical example of a B-cell large cell lymphoma with the more common SIg^+Ia^+ phenotype. This diagnosis was apparent from both the suspension and the section, although, as mentioned, there was a relative enrichment by normal T cells in the suspension.

The final case, an example of Lennert's lymphoma, illustrates that in some cases of tumors with mixed cell populations even analysis of the frozen section may not provide a clear-cut immunologic diagnosis. In this case, weakly positive SIg^+ cells were admixed with cells that reacted with several anti-T-cell reagents, though neither cell type could be definitely recognized as the neoplastic cell. In suspension, the ratio of kappa-positive to lambda-positive cells was about 2:1, which suggests that this is not a B-cell tumor. Lennert's lymphoma is generally considered a T-cell tumor.[1,5]

Comparison of T-Cell Tumors

The data presented so far indicate that the immunoperoxidase staining of frozen sections is useful for distinguishing B- and T-cell tumors when they are morphologically similar. However, by using monoclonal antibodies to define T-cell subpopulations, more information can be gathered about differences among T-cell tumors. A summary of the phenotypic data on the four definite T-cell lymphomas and the Lennert's lymphoma is shown in Table 3. It can be seen that these five tumors are markedly heterogeneous with respect to expression of T-lymphocyte antigens. This is not surprising since some of them are morphologically and clinically dissimilar. Nonetheless, several significant points emerge. First, there was heterogeneity with respect to expression of the pan-T antigens. OKT3 was present on all of the tumors. 3A1 was present on the lymphoblastic lymphoma, but was weak or completely absent from the other four tumors. The lymphoblastic lymphoma was the only tumor to express the thymic antigen OKT6. The well-differentiated lymphocytic lymphoma, though lacking this marker, expressed the immature T lymphocyte antigen 12E7.[20] In addition, three of the five tumors expressed the helper antigen, while the phenotypic distribution of the suppressor antigen was similar to that seen for 3A1, although it was also present in the Lennert's lymphoma. Finally, as mentioned above, one of the T-cell tumors expressed Ia.

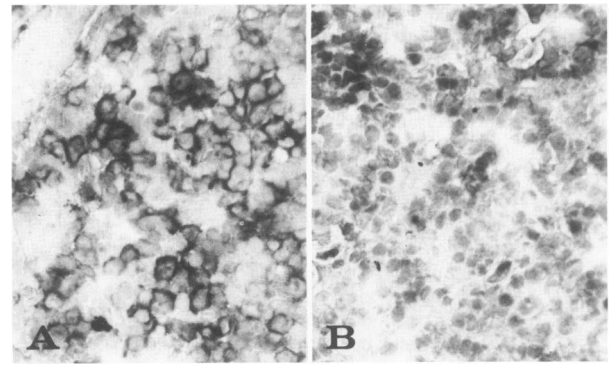

Figure 6—Case 7. **A**—Frozen section. Immunoperoxidase stain for lambda light chain. Notice distinct rim staining of the majority of the cells in the section. A few nonstaining cells are also present. **B**—Serial section stained for kappa light chain. Notice the lack of surface staining but the presence of weak interstitial staining. (Hematoxylin counterstain, ×250)

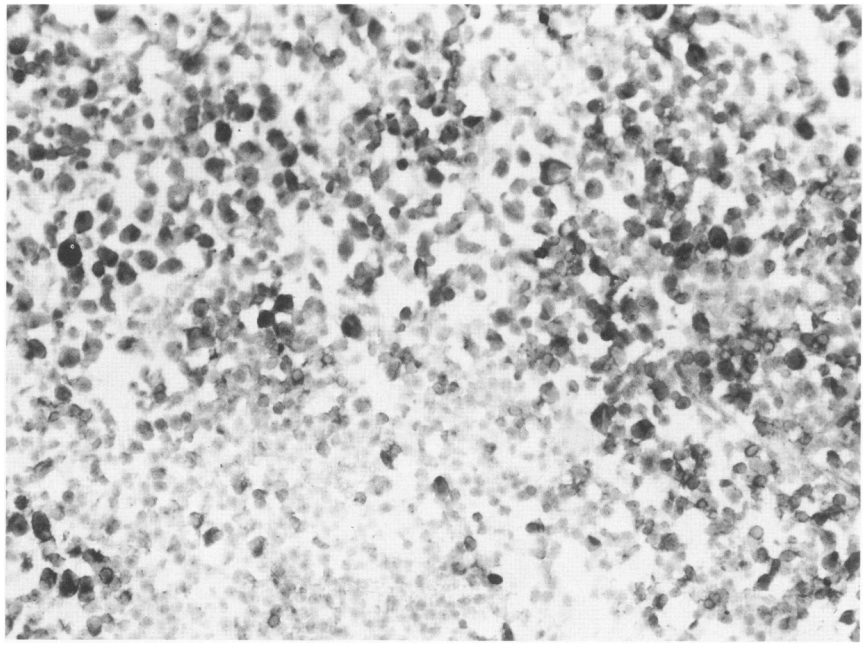

Figure 7—Case 8. Frozen section. Immunoperoxidase stain for the T-cell antigen OKT3. Notice intense staining of most of the large lymphoid cells with an area of nonstaining, smaller cells near the bottom of the photograph. The latter represent residual normal lymphoid cells in the tissue. (Hematoxylin counterstain, ×250)

Discussion

One purpose of the present study was to determine the immunologic phenotype of non-Hodgkin's lymphomas by immunocytochemistry, and to compare that to the one obtained by the more conventional analysis of cell suspensions. Our results show that, in cases in which tumor completely replaces normal tissue, interpretation of marker phenotype is straightforward, no matter which technique is used. However, in those cases in which there is a heterogeneous cell population, more information can be obtained with immunocytochemical analysis on frozen sections for several reasons. First, one has the opportunity to examine the tissue histologically and to determine the morphologic appearance and pattern of the cells that are staining. In this respect, immunoperoxidase technique is superior to immunofluorescence technique, since the tissue can also be counterstained and examined with a conventional light microscope. Thus, in Case 9 above, where the suspension contained a mixture of T and B cells, the tumor cells could be seen to stain for Ia, while adjacent uninvolved lymph node contained many T cells. The second reason that interpretation of frozen-section analysis is superior is that cell suspensions may not be representative samples of the node. Although quantitation on frozen section is often difficult, in many cases, an estimate of the percentage of cells staining differed greatly from the result obtained by suspension analysis. In one case, (Case 7), analysis of suspension might have led to an erroneous classification. One reason for this difference in cases in which there is low viability is that the suspension cells are usually subjected to gradient purification. Another advantage to the frozen-section procedure is that it is a technique that is readily adaptable to any existing pathology laboratory and requires no special procedures for preparation or analysis of cell suspensions.

The examples of lymphomas presented here are a mixed group with respect to histologic classification. Even among tumors with similar morphologic appearance, however, there was heterogeneity with respect to phenotype, as defined by reaction with anti-immunoglobulins, and monoclonal anti-Ia and anti-T-cell-specific antibodies. Six of the 11 cases were either diffuse mixed cell or diffuse large cell lymphomas. Of these, four were B-cell and two were T-cell tumors. Two of the B-cell tumors were SIg^+Ia^+, one was SIg^+Ia^-, and one was SIg^-Ia^+. Warnke et al[15] recently studied 30 cases of large cell lymphomas and found them all to be B-cell tumors, with all three combinations of SIg and Ia phenotypes noted. Their failure to find a single T-cell tumor in this group is unusual, since generally up to 15% of large cell lymphomas are thought to be of T-cell origin.[1,21] However, Warnke et al base their claim on their failure to find tumors that reacted with a single T-cell monoclonal antibody, 17F12 (equivalent to Leu 1). Our cases of peripheral T-cell lymphoma are heterogeneous with respect to expression of different T antigens, and at least one did not express the Leu 1 anti-

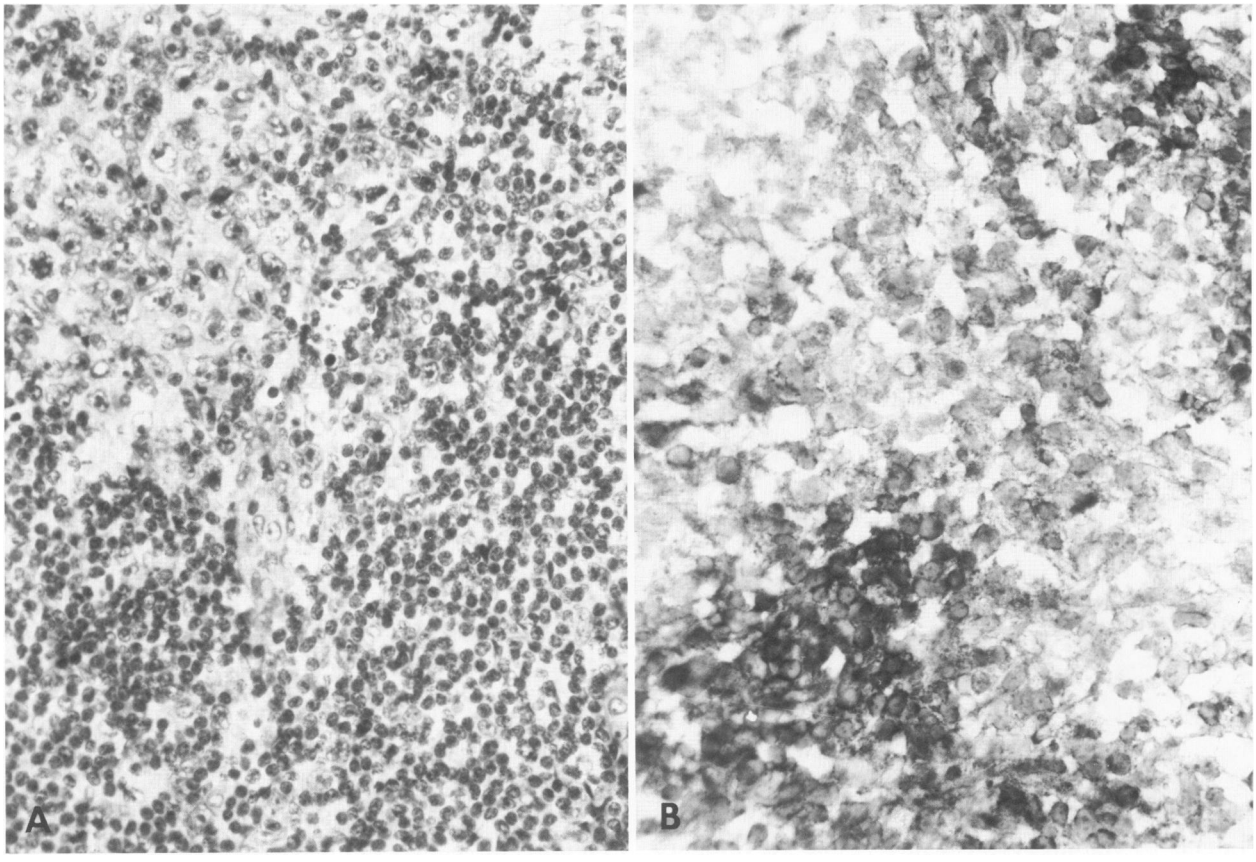

Figure 8—Case 9. **A**—Paraffin-embedded section showing large tumor cells with vesicular nuclei and prominent nucleoli infiltrating into a region of normal lymphocytes. (H&E, × 400) **B**—Frozen section from a similar region stained by immunoperoxidase for the T-cell antigen 3A1. The smaller lymphocytes are strongly positive, but the large tumor cells do not stain. (Hematoxylin counterstain, × 400)

gen (table 3). One of our T-cell tumors did, however, express Ia antigen. Thus, it is possible that, in the study of Warnke et al, some of the SIg^-Ia^+ $17F12^-$ tumors are of T-cell phenotype.

The expression of Ia by a T-cell lymphoma, in our case in a patient with mycosis fungoides, is of interest, since Ia antigens appear on T cells only after antigen or mitogen activation.[19,22] Ia has occasionally been found on peripheral T-cell lymphomas, particularly T-cell CLL.[23,24] However, Stein et al[8] described an Ia^+ T antigen$^+$ T-zone lymphoma analyzed with rabbit heteroantisera, and Halper et al,[25] in a study of Ia expression on malignant lymphomas, found one Ia^+T^+ unclassifiable lymphoid proliferation among 34 cases of malignant lymphomas. It would be of interest to know if the presence of Ia antigen in such patients could be correlated with some *in vivo* evidence of antigen activation of T cells. Recently, T-cell lymphomas associated with polyclonal hypergammaglobulinemia have been reported,[26] but in only one of the five cases were the T-cell tumors Ia positive. The present patient did not have hypergammaglobulinemia. Cells of patients with mycosis fungoides have the ability to carry out helper function but generally lack the Ia antigen, although cultured cell lines from these patients express Ia antigens when established with conditioned medium containing T-cell mitogens.[27] The phenotype of mycosis fungoides was reported by Haynes et al to be $3A1^-OKT3^+T_H^+T_S^-Ia^-$.[27] Our case differs in that the tumor cells have no detectable helper antigen and are Ia^+. However, one reason for this difference may be that Haynes et al studied only peripheral blood cells of patients with Sézary syndrome. The $3A1^-T_H^+$ phenotype was seen in another of our cases but in a patient who did not have mycosis fungoides. In addition, the T cells present in the Lennert's lymphoma were $3A1^-$. The fact that this antigen is present on 85% of normal peripheral blood T cells[28], and is readily demonstrable on non-neoplastic T lymphocytes in B-cell lymphomas (Figure 8B) further suggests the T-cell origin for this neoplasm. In this case, too, the T cells expressed the suppressor but not the helper antigen. The heterogeneity of expression of T-cell antigens by T-cell lymphomas parallels what has been seen in T-cell leukemias.[23,27,30-32]

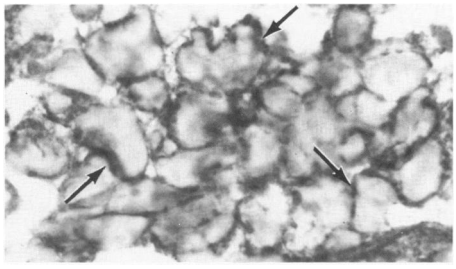

Figure 9—Case 9. Frozen section stained by immunoperoxidase for Ia antigen. There is intense granular rim staining around most large tumor cells, some of which is indicated by the arrows. A small amount of staining is seen between cells, possibly due to antigen leaching off the tumor cells. (Hematoxylin counterstain, ×680)

Among our other lymphomas were two cases that were diagnosed morphologically as lymphoblastic lymphomas. These tumors are considered the tissue phase of acute lymphocytic leukemia (ALL) and are generally of T-cell origin, but one of our two cases was of B-cell lineage. This is a very unusual finding. Although B-cell ALL can be associated with tissue lymphoma, the histologic analysis of these tumors invariably shows them to be Burkitt's lymphomas.[29] Our case had none of the features of Burkitt's tumor. Our T-cell lymphoblastic lymphoma had the phenotype $OKT_3^+T_H^+T_S^+$, which is not common, although some cortical thymocytes express all three of these antigens.[32] The T-cell ALLs have been extensively studied with heteroantisera and monoclonal antibodies and have been found an extremely heterogeneous group.[27,30-32]

In contrast to ALL, many fewer examples of T-CLL (or well differentiated lymphocytic lymphoma) have been extensively phenotyped. There is, however, evidence for some heterogeneity in this group of tumors as well, at least with respect to expression of Ia.[23,24] Most cases have lacked thymic antigens, although one example of Thy^+ T-CLL has been reported.[33] In our case, the phenotype $12E7^+$ $OKT6^-$ suggests a thymic, or immature, T-lymphocyte origin for the tumor, even though the 12E7 marker is not completely thymus specific.[20] However, the cell suspension from this tumor reacted with the thymocyte-specific monkey antiserums αTHY1/PC and αTHY2/PC,[32] which further suggests its thymic origin.

The present work demonstrates the phenotypic complexity of the T-cell non-Hodgkin's lymphomas. The epidemiologic and clinical significance of this finding is, at this time, uncertain. More cases need to be studied, and, more important, tumor phenotype and clinical behavior need to be correlated. The frozen-section immunoperoxidase technique will be very useful in such correlative studies.

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