Use of Monoclonal Antibodies for Analyzing the Distribution of the Intermediate Filament Protein Vimentin in Human Non-Hodgkin's Lymphomas

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A series of human non-Hodgkin's lymphomas was examined for immunoreactivity with monoclonal antibodies to the intermediate filament protein vimentin with the use of an avidin–biotin immunoperoxidase method. The lymphoid cell nature of each tumor was established with the use of a panel of monoclonal antibodies to lymphoid cell differentiation antigens. There were 28 B-cell and 2 T-cell lymphomas in the series; of the 30 tumors, 11 (37%) were immunoreactive for vimentin. There was no correlation between vimentin immunoreactivity and the histopathologic type of lymphoma. In some tumors, there was nonspecific stromal immunoreactivity for vimentin, but the neoplastic lymphocytes were not immunoreactive. The selective expression of vimentin in non-Hodgkin's lymphomas may be due to masking of the appropriate epitopes or to selective expression of the vimentin gene in certain tumors. On the basis of these results, monoclonal antibodies to vimentin appear to be of limited usefulness in establishing the diagnosis of non-Hodgkin's lymphoma (Am J Pathol 1985, 120:351–355)

THE IMMUNOHISTOCHEMICAL characterization of human malignant neoplasms has become a useful adjunct in tumor diagnosis. In particular, immunophenotyping of tumors with reagents specific for different types of intermediate filament proteins is helpful in classifying neoplasms with respect to their origin from epithelial or mesenchymal tissues.[1,2] Intermediate filaments are filamentous structures 7–11 nm in diameter which are intermediate in size between smaller microfilaments and larger microtubules.[3,4] These structural proteins represent families of related, though not identical, polypeptides whose expression in various cells and tissues appears to depend upon processes of cellular differentiation.

Epithelial cells contain intermediate filaments related to epidermal tonofilaments which are classified as cytokeratins.[4] Mesenchymal cells contain only one type of intermediate filament protein, vimentin.[5] Other intermediate filament proteins appear to be rather restricted in their distribution, such as desmin in muscle cells,[6] neurofilament in neurons,[7] and glial fibrillary acidic protein in astrocytes.[8]

These findings have suggested that intermediate filament proteins could serve as differentiation markers and aid in determining the histogenesis of cells of unknown origin, including those occurring in malignant tumors. Indeed, antibodies to the intermediate filament protein vimentin have been used to classify tumors as being of mesenchymal origin, including sarcomas, lymphomas, and melanomas.[9]

A recent study of normal human peripheral lymphoid tissue has shown that a monoclonal antibody to vimentin is reactive with a limited number of cell types, including lymphocytes in the periarteriolar lymphocyte sheath of the spleen, tingible body macrophages, fibroblastic reticulum cells in lymph nodes, marginal zone macrophages in the spleen, and endothelial cells in lymphoid tissues.[10] This limited distribution of vimentin in lymphocytes prompted us to extend our studies to malignant lymphomas. All of the tumors were established as lymphoid in origin by the use of a panel of monoclonal antibodies, and the results were compared

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with those obtained with the use of two monoclonal antibodies to vimentin.

Materials and Methods

Tissues

Biopsy specimens were obtained fresh. Adequate blocks of fresh tissue were embedded in cryoprotectant (OCT, Ames) in an aluminum foil vessel and rapidly frozen by immersion in a dry ice–isopentane bath.[11] Frozen blocks were stored at −70 C until ready for sectioning. Cryostat sections were cut at 6-μ, air-dried at room temperature for 1–2 hours, fixed in reagent grade acetone for 20 minutes at room temperature, and air-dried again.

Immunoperoxidase Staining

Sections were rehydrated briefly in modified phosphate-buffered saline (PBS)[12] and incubated for 20 minutes in each step at room temperature in the following avidin–biotin immunoperoxidase staining protocol[13,14]: 1) appropriately diluted unlabeled primary monoclonal antibody, 2) biotinylated antibody to primary antibody, and 3) avidin–peroxidase. Sections were washed with PBS between incubations. After the last incubation and PBS wash, sections were incubated with 3,3′-diaminobenzidine (DAB, 0.02%) in 0.01% H_2O_2 in PBS for 5 minutes and washed. The DAB staining was intensified by incubation for 5 minutes in 0.5% $CuSO_4$ in 0.9% NaCl.[15] After washing in water, sections were dehydrated through alcohol, cleared with xylene, and mounted with Permount. Due to very low background, blocking of endogenous peroxidase activity is not required in this system.[13]

To ensure proper staining patterns, frozen sections of human tonsil were stained with all monoclonal antibodies employed in this study. Negative controls on neoplastic tissue sections consisted of substitution of antibody to keratin in place of the other primary antibodies.

Photomicrographs were made with the use of an interference filter combination which enhances the contrast of the DAB reaction product.[16]

Antibody and Other Reagents

The antibodies used and their specificities and sources are listed in Table 1. Suppliers were Becton-Dickinson (Mountain View, Calif), Labsystems (Chicago, Ill), and Enzo Biochem Inc. (New York, NY). The antibodies were diluted in PBS supplemented with 0.2% bovine albumin[17] to a titer which gave specific staining with minimum nonspecific background. Biotinylated anti-mouse and anti-rabbit IgG and avidin–peroxidase were from Biomeda (Foster City, Calif).

Table 1—Monoclonal Antibodies Used in This Study

Antibody	Specificity	Source	Refernce
Anti-vimentin	Intermediate filament vimentin	Labsystems; Enzo	23
Anti-keratin	Cytokeratin	Enzo	23
HLe-1	Hematopoietic cells	Becton–Dickinson	24
Leu 14	B cells	Becton–Dickinson	25
Leu 4 + Leu 5	T cells	Becton–Dickinson	26
Anti-IgM	IgM heavy chain	Becton–Dickinson	27
Anti-κ	κ light chain	Becton–Dickinson	28
Anti-λ	λ light chain	Becton–Dickinson	28

Histopathologic Evaluation

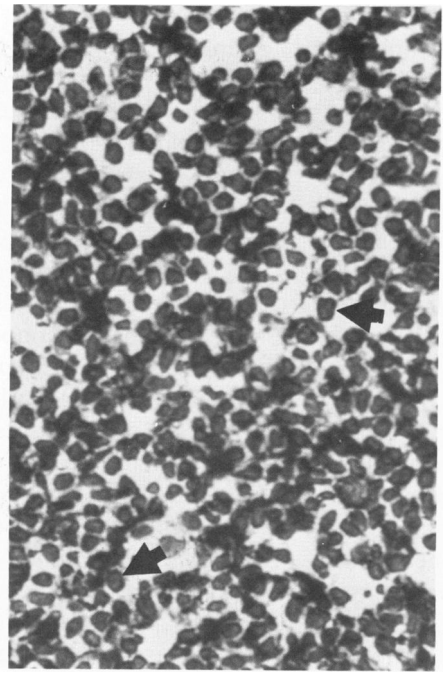
Sections of B5- and formalin-fixed tissue stained with hematoxylin and eosin (H&E) and embedded in paraffin were evaluated. Tumors were classified according to the National Cancer Institute Working Formulation[18] and the Rappaport classification.[19]

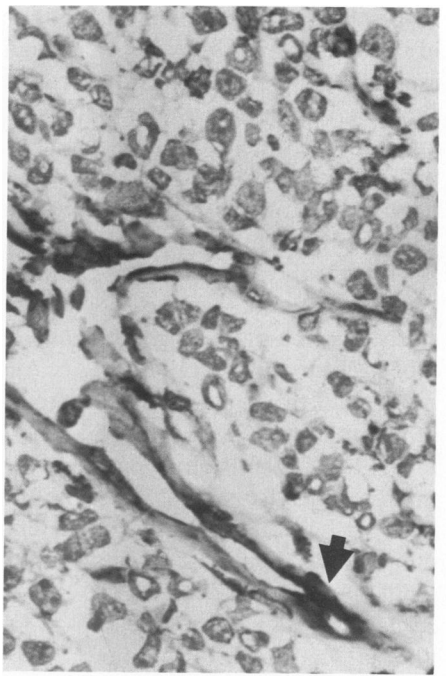
Results

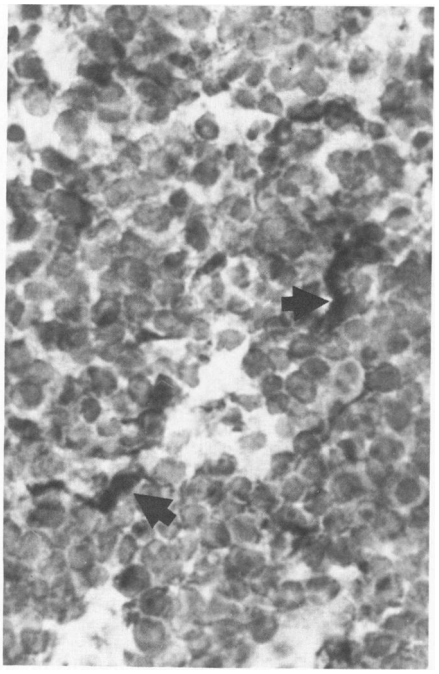
To ensure that the monoclonal antibodies exhibited proper immunoreactivity, frozen sections of control tonsil were stained with each batch of tumors studied. The staining patterns[11] obtained with the monoclonal antibodies were: pan-leukocyte (HLe-1)—all lymphoid cells; pan-T-cell (Leu 4 + Leu 5)—all T cells in paracortex and germinal center; pan-B-cell (Leu 14), IgM, kappa and lambda—B cells in follicles and paracortex; anti-vimentin—endothelium, reticulum cells, and

Table 2—Distribution of Vimentin Immunoreactivity in Histopathologic Categories of Lymphoma

Histopathology	Vimentin immunoreactivity (number)	
	Vimentin-positive	Vimentin-negative
Diffuse small lymphocytic (SL)	3	5
Follicular predominantly small cleaved-cell (FSC)	0	1
Follicular mixed small cleaved and large-cell (FM)	0	1
Follicular predominantly large-cell (FL)	2	0
Diffuse small cleaved-cell (DSC)	1	1
Diffuse mixed small cleaved- and large-cell (DM)	1	1
Diffuse large-cell (DL)	4	5
Large-cell immunoblastic (IBL)	0	2
Lymphoblastic (LBL)	0	2
Small non-cleaved-cell (SNC)	0	1







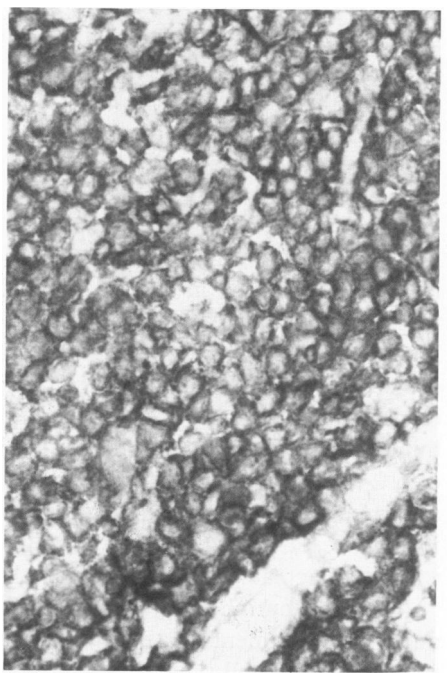


Figure 1—Malignant lymphoma showing immunoreactivity for vimentin in the neoplastic lymphocytes (*arrows*). (Immunoperoxidase, ×480) **Figure 2**—Malignant lymphoma lacking immunoreactivity for vimentin. Note the endothelial immunoreactivity in the blood vessel (*arrow*). (Immunoperoxidase, ×480) **Figure 3**—Malignant lymphoma with stromal immunoreactivity (*arrows*). Note that the neoplastic lymphocytes are not immunoreactive (Immunoperoxidase, ×480) **Figure 4**—Malignant lymphoma immunoreactive with the pan-leukocyte antibody HLe-1. (Immunoperoxidase, ×480)

some lymphocytes. A total of 30 malignant non-Hodgkin's lymphomas was studied. A variety of histopathologic categories was examined (Table 2). All cases of lymphoma were verified by immunophenotyping with a panel of monoclonal antibodies to lymphoid cell types. Twenty-eight of the lymphomas were B-cell in origin, and 2 were T-cell in origin. Eleven out of 30 (37%) of the lymphomas were immunoreactive with monoclonal antibodies to vimentin. Only cases bearing definite cytoplasmic or rim-like membrane staining were scored as vimentin-positive. Stromal immunoreactivity, which was frequent, was not considered as positive lymphoid cell immunoreactivity. An inspection of Table 2 indicates that there was no apparent correlation between histopathologic categories and the presence or absence of immunoreactivity for vimentin in the tumors studied.

Figures 1–4 show the various types of immunoreac-

tivity observed with the monoclonal antibodies used in this study. Figure 1 shows a malignant lymphoma immunoreactive for vimentin. In contrast, Figure 2 shows a lymphoma that is not immunoreactive for vimentin. Figure 3 shows a lymphoma with stromal immunoreactivity for vimentin but with neoplastic lymphocytes lacking immunoreactivity. In contrast, Figure 4 shows membrane immunoreactivity of a lymphoma for HLe-1, the pan-leukocyte monoclonal antibody.

In order to examine the cases in more detail, they were studied with a panel of monoclonal antibodies to establish the lymphoid cell of origin. All of the lymphomas exhibiting immunoreactivity to vimentin were B-cell lymphomas (11/28, or 39%). The 2 T-cell lymphomas (1 SL and 1 LBL histopathologically) were not immunoreactive for vimentin. Of the B-cell lymphomas showing immunoreactivity for vimentin, 8 contained monoclonal kappa light chains and 3 contained monoclonal lambda light chains. Seven of 11 tumors bore IgM heavy chain immunoreactivity. Of the B-cell lymphomas that were not immunoreactive for vimentin, 10 contained monoclonal kappa light chains and six contained monoclonal lambda light chains. One tumor was a lymphoblastic lymphoma containing only cytoplasmic IgM. Nine of 17 tumors were immunoreactive for IgM.

Discussion

These results show that less than 50% of non-Hodgkin's lymphomas were immunoreactive with two murine monoclonal antibodies directed against the intermediate filament protein vimentin. The histopathologic diagnosis of lymphoma was confirmed in every case by immunophenotyping with a panel of monoclonal antibodies. All of the lymphomas which were immunoreactive for vimentin were B-cell in origin, but only 2 of the cases studied were T-cell in origin. There appeared to be no preferential association of vimentin immunoreactivity with the immunophenotype or histopathologic category of malignant lymphomas.

In normal peripheral lymphoid tissues, monoclonal antibodies to vimentin reveal immunoreactivity limited to only certain cell types.[10] Some lymphocytes in the periarteriolar sheath of the spleen, a T-cell zone, were immunoreactive. In general, lymphocytes in other areas of spleen and in tonsil and lymph node were not immunoreactive. Vimentin immunoreactivity was present in some types of reticulum cells, namely, histiocytic reticulum cells (tingible-body macrophages) in germinal centers of secondary follicles and fibroblastic reticulum cells in the mantle zone of secondary follicles. In addition, marginal zone macrophages in the spleen were immunoreactive. Finally, endothelial cells in all peripheral lymphoid tissues were immunoreactive.

Previous studies suggested that all malignant lymphomas were immunoreactive for vimentin.[9,20,21] These studies employed polyclonal antisera to detect vimentin and immunofluorescence staining techniques. There is no mention of using immunophenotyping to confirm the histopathologic diagnosis of malignant lymphoma. The reason or reasons for the discrepancy between previous studies using polyclonal antisera and the present work, employing monoclonal antibodies, are open to speculation. First, it is possible that the polyclonal antiserum cross-reacts with a pan-leukocyte antigen, such as HLe-1, and that studies showing positive staining were detecting the cross-reaction, rather than vimentin. Second, background or stromal immunoreactivity may be interpreted as being present in neoplastic lymphocytes. The immunoperoxidase staining technique employed in the present work gives less background staining and is more sensitive than immunofluorescence techniques,[22] and so it should be less likely to lead to subjective misinterpretation. In particular, it is much easier to distinguish stromal staining with the immunoperoxidase method than it is with immunofluorescence methods. As shown in Figure 3, it is obvious that stromal immunoreactivity for vimentin is present in lymphomas in which the neoplastic cells fail to exhibit immunoreactivity. Third, it is possible that the two monoclonal antibodies employed to detect vimentin react with an epitope which is masked in some cells. This would seem unlikely based on the data showing no difference in vimentin immunoreactivity with respect to lymphoma immunophenotype or histopathologic features. A final possibility is that the gene for vimentin is expressed only in some neoplastic lymphocytes. If a gene probe for vimentin were available, it would be useful for studying the possibility of selective gene expression by detecting vimentin messenger RNA.

No discrepancies in immunoreactivity were noted with the two monoclonal antibodies to vimentin. It is not known whether they react with the same epitope, but the present study shows that this might be the case. Other investigators have noted that one of these anti-vimentin monoclonal antibodies fails to react with lymphomas.[23] Although monoclonal antibodies to vimentin appear to be reliable markers for some tumors, such as melanomas and sarcomas, their usefulness in identifying non-Hodgkin's lymphomas appears to be limited.

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