IMMUNOHISTOCHEMISTRY OF LYMPHOMAS IN FROZEN TISSUE SECTIONS

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This paper will briefly review the history and perspective of an immunohistologic approach to lymphomas, specimen handling and processing, methods that are utilized, the rationale for immunophenotyping, review normal lymphocyte development and maturation, and then outline typical phenotypes of lymphoproliferative disease.

Cell suspension studies have been used for two decades and continue to be one of the cornerstones for immunologic characterization of lymphomas (1). Tissue section immunostaining of lymphomas was initiated by Taylor who used the peroxidase-antiperoxidase technique on paraffin sections to refine the distribution of immunoglobulin phenotypes in Hodgkin's disease and in some non-Hodgkin's lymphomas (2). Warnke et al documented in 1978 the false negative immunoglobulin staining in paraffin sections and the utility of frozen tissue section immunohistology using immunofluorescence (3), and further demonstrated the denaturation of immunoglobulin or loss of antigenicity induced by a variety of fixatives (4). In 1980, immunoglobulin phenotypes defined by immunoperoxidase techniques were described using frozen tissue sections (5), and staining of T and B cell antigens was accomplished using monoclonal antibodies and frozen tissue sections with biotin-avidin and unlabeled immunoperoxidase techniques (6,7). In 1981, the concordance was verified between frozen section immunoperoxidase techniques and cell suspension studies (8). Epstein et al recently developed a series of monoclonal antibodies that detect differentiation antigens in paraffin sections of fixed
material, and that are specific in part for individual subgroups of B-cell tumors (9).

It should be emphasized that immunophenotyping is not at this time a primary mode of diagnosis, but is rather one of several tools providing information that should be integrated together to form a final pathologic diagnosis.

SPECIMEN PROCESSING

Key to processing lymphoid tissue is the development of a comprehensive accession protocol (10,11). Fresh tissue received should be divided into several different aliquots. Well-fixed, thinly sectioned material for light microscopy remains the cornerstone of pathologic classification. The few monoclonal antibodies active in paraffin sections seem to work best in Bouin’s or B5 solutions (9,11). Formalin fixed paraffin sections are helpful in recognition and subclassification of Hodgkin’s disease, since lacunar cells are best visualized this way. For optimal cellular detail, particularly nuclear detail, B5 is the preferred fixative. If the quantity of tissue is limited, B5 is preferred, if enough material for only one fixative is available. B5-fixed tissue is also used for morphometry in plastic sections. A small piece of tissue should be embedded and held for electron microscopy, but not routinely processed.

Touch preparations from the freshly cut surface of the node are helpful in terms of evaluating the Wright’s stain. In the pre-monoclonal antibody era, identification of T cells other than with sheep erythrocyte rosettes depended on cytochemical profiles and were very valuable at that time. Currently, the Wright’s stain can be used to make some decisions about the extent of immunophenotyping at the time of specimen accession and to recognize macrophages.

A fresh cell suspension should also be prepared from the tissue. A comparatively large volume of tissue (>1 cm$^3$) is usually required to extract enough cells to do all the markers. Generally, $1 \times 10^6$ cells per antigen desired are required for ease of flow cytometry analysis. The derived