Enzyme immunohistochemistry: review of technical aspects and diagnostic applications

Raymond R. Tubbs, D.O.
Department of Pathology

Khalil Sheibani, M.D.
Sharad D. Deodhar, M.D., Ph.D.
Department of Immunopathology

William A. Hawk, M.D.
Department of Pathology

The era of immunohistochemistry was introduced by Coons et al[1] in 1941 when antibodies were successfully labeled with a fluorochromatic compound. Shortly thereafter, localization of tissue antigens was successfully accomplished with the use of fluorochromatic labels.[2] Initially a research tool, immunofluorescence became an essential diagnostic technique for the evaluation of many disease states, particulary autoimmune diseases mediated by immune complexes or autoantibody deposition.

It soon became clear that certain limitations such as special instrumentation requirements and lack of permanency were accorded immunofluorescent procedures. Consequently, immunohistochemical systems were developed that permitted the visual localization of a tissue antigen as a permanent preparation with the potential for visualization of adjacent tissue morphology. The successful conjugation of antibodies with enzymes and unlabeled antibody methods made immunomicroscopy practical. Both enzyme-labeled antibody and unlabeled antibody (antienzyme) methods allowed identification of tissue antigens by formation of permanent color products in histologic sections with excellent morphologic detail.[3,4]

This paper reviews the rationale underlying enzymatic immunomicroscopic procedures, tech-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

niques currently available, characteristics of currently available chromogens, safety for personnel, quality control of immunohistochemical systems, and clinical diagnostic applications of the procedure.

Biochemistry of enzyme immunomicroscopy

Many different enzymes are potential antibody labels, including acid phosphatase, β-glucuronidase, 5′-nucleotidase, glucose oxidase, and horseradish peroxidase.[5–7] However, horseradish peroxidase has been the enzyme label used most frequently since it is readily available and relatively inexpensive; well-established conjugation methods have been developed for conjugation with antibody.[8]

The biochemical reaction that occurs at the histochemical level can be summarized in the equation below:

$$H_2O_2 \text{ (to be reduced)} + H_2R \text{ (Chromogen donor)} \xrightarrow{\text{Peroxidase}} R \text{ (Oxidized-chromogen donor)} + 2H_2O$$

The substrate, hydrogen peroxide, is important in the reaction only in that it accepts hydrogen from the chromogen. Ideally, the molecular change in the oxidized chromogen results in a reaction product insoluble in organic solvents which differs in color from its parent compound. The amount of substrate necessary to make the reaction proceed is very small, with usual useful working concentrations of hydrogen peroxide in most systems ranging from 0.0003% to 0.003%.

The proportion of conjugated antibody to enzyme is evaluated by the ratio of enzyme to antibody protein.[8] This is probably best expressed as a molar ratio implying the number of molecules of enzyme coupled to one molecule of antibody protein. At a ratio of three to four enzyme molecules per protein molecule there is loss of antibody binding, enzyme function, and penetration. For most enzyme-labeled antibody immunomicroscopy tests, a molar ratio of 1 enzyme molecule per protein molecule is adequate. This allows high function of both antibody and enzyme and good penetration (peroxidase + antibody = 40,000 + 160,000 = 200,000 molecular weight). For enzyme-linked immunosorb assay (ELISA) a molar ratio of between two- and three-enzyme molecules per molecule of antibody may be desirable. Currently, numerous commercial preparations of high-titer antibodies with optimum enzyme-protein conjugation ratios are available. However, lot-to-lot differences and variations between various companies' antisera exist and, therefore, the reactivity and specificity of every commercial reagent must be confirmed.

Tissue processing

The choice of type of tissue processing is largely dependent upon the individual microscopic system. Extracellular immune complexes and autoantibodies may be detected in paraffin-embedded tissue with the use of posttrypsinization techniques. Such preparations do provide superior morphology. However, frozen tissue is preferable for most studies since 10% to 25% of cases positive with cryostat frozen section immunofluorescence are negative even if dewaxed paraffin-embedded tissue is pretreated with trypsin.[9,10]

The effect of tissue fixation is especially important in the evaluation of lymphoproliferative disorders. It has been shown that fixation using any mordant solution markedly alters the immunoglobulin products associated with non-Hodgkin's and Hodgkin's lym-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

phoma cells and reactive lymphocytes.[11,12] Although paraffin-embedded tissue sections may be counterstained to give excellent cellular detail in immunomicroscopic sections,[13] spurious immunostaining of non-Hodgkin's lymphomas not infrequently occurs.[11,14] Furthermore, immunostained cryostat frozen sections are amenable to counterstaining with hematoxylin and eosin or other counterstains permitting some definition of cellular morphology.[15]

For paraffin-embedded systems, reports vary widely as to the superiority of different fixatives.[16–22] It has been suggested that 2% formaldehyde is superior to 4% formaldehyde.[21] Some investigators have found cacodylate-buffered paraformaldehyde superior to Bouin's solution for cytoplasmic immunoglobulins and Bouin's best to preserve antigenicity of hormones.[22] In our experience, the best approach is to evaluate each immunomicroscopic system independently with regard to optimum fixative solutions.

When submitting tissue for paraffin embedding, an important consideration is the thickness of the original tissue specimen when placed in fixative. To ensure complete tissue penetration, 1- to 2-mm thick sections should be placed in abundant volumes of appropriate fixative.

The alleged problems of immunoglobulin diffusion and spurious staining said to occur with cryostat frozen section immunohistochemistry have not proved serious under close scrutiny.[11,13] Study of frozen section material yields reproducible results and observations consistent with well-established concepts of monoclonality in non-Hodgkin's lymphomas in most cases.[11,12,15,23] Furthermore, small amounts of alcohol used in paraffin embedding markedly alter the immunoglobulin phenotypes of proliferating lymphoid cells.[11,12] Both direct and unlabeled frozen section immunohistochemistry readily detect appropriate immunoglobulin phenotypes.[23–25]

Chromogens

Table 1 summarizes data currently available for chromogens used in immunoperoxidase methods. Each chromogen offers certain advantages but has some disadvantages, and many ques-

Table 1. Properties of chromogens

Immunohistochemical label	Solubility in organic solvents	Color	Carcinogenicity (laboratory animals)	Federal regulations of use
Fluorochromatic				
Fluoroscein isothiocyanate	Not applicable	Green	?	–
Tetramethyl rhodamine	Not applicable	Red	?	–
Enzymatic				
Benzidine dihydrochloride	–	Blue	+	+
DAB (diaminobenzidine)	–	Brown-black	?*	–
TMB (tetramethyl benzidine)	–	Blue	?†	–
HYR (Hanker-Yates reagent, p-phenylenediamine & pyrocatechol)	–	Black	?†	–
AEC (aminoethylcarbazole)	+	Red-brown	+	–

* One study has reported that diaminobenzide (3,3′,4,4′-Tetraminodiphenylether·4 HCl or 4,4′-Oxybis-o-phenediamine) did not act as a carcinogen in experimental animals.[31]

† Commercial sources of these reagents specify that this chromogen is noncarcinogenic but, to our knowledge, studies of carcinogenesis of these compounds sponsored by the federal government have not been done.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

tions regarding the safety of these compounds (and fluorochrome markers) remain unanswered.[26] Benzidine dihydrochloride gives a stable blue color reaction product, which has been associated with greater sensitivity than other available chromogens.[27–29] Governmental regulations have made use of this compound impractical.[30]

The most widely used immunohistochemical chromogen has probably been 3,3-diaminobenzidine dihydrochloride monohydrate (DAB).[13] This reagent yields a brown to black color reaction product, which is not soluble in organic solvents and does not crystallize on the tissue sections. DAB is not currently regulated to our knowledge and in one study did not demonstrate carcinogenesis in experimental animals.[31] Tetramethylbenzidine (TMB) has been advocated as an alternative chromogen that has not been associated with carcinogenesis in laboratory animals, but crystallization on the tissue sections is a problem.[28,29,32]

The availability of multiple types of peroxidative chromogens yielding different color reaction products having different tinctorial properties allows the simultaneous visualization of more than one antigen in the same tissue section.[33–35] These results can be achieved without elaborate double incubation steps.[35] Similar double-labeling studies have also been done with the use of a combination of enzyme labels such as glucose oxidase and horseradish peroxidase.[36]

Aminoethylcarbazole (AEC) has also been advocated as a useful chromogen.[37,38] However, recent evidence suggests that carcinogenic potential in laboratory animals does exist, and this reagent may be regulated by the government in the future.[39]

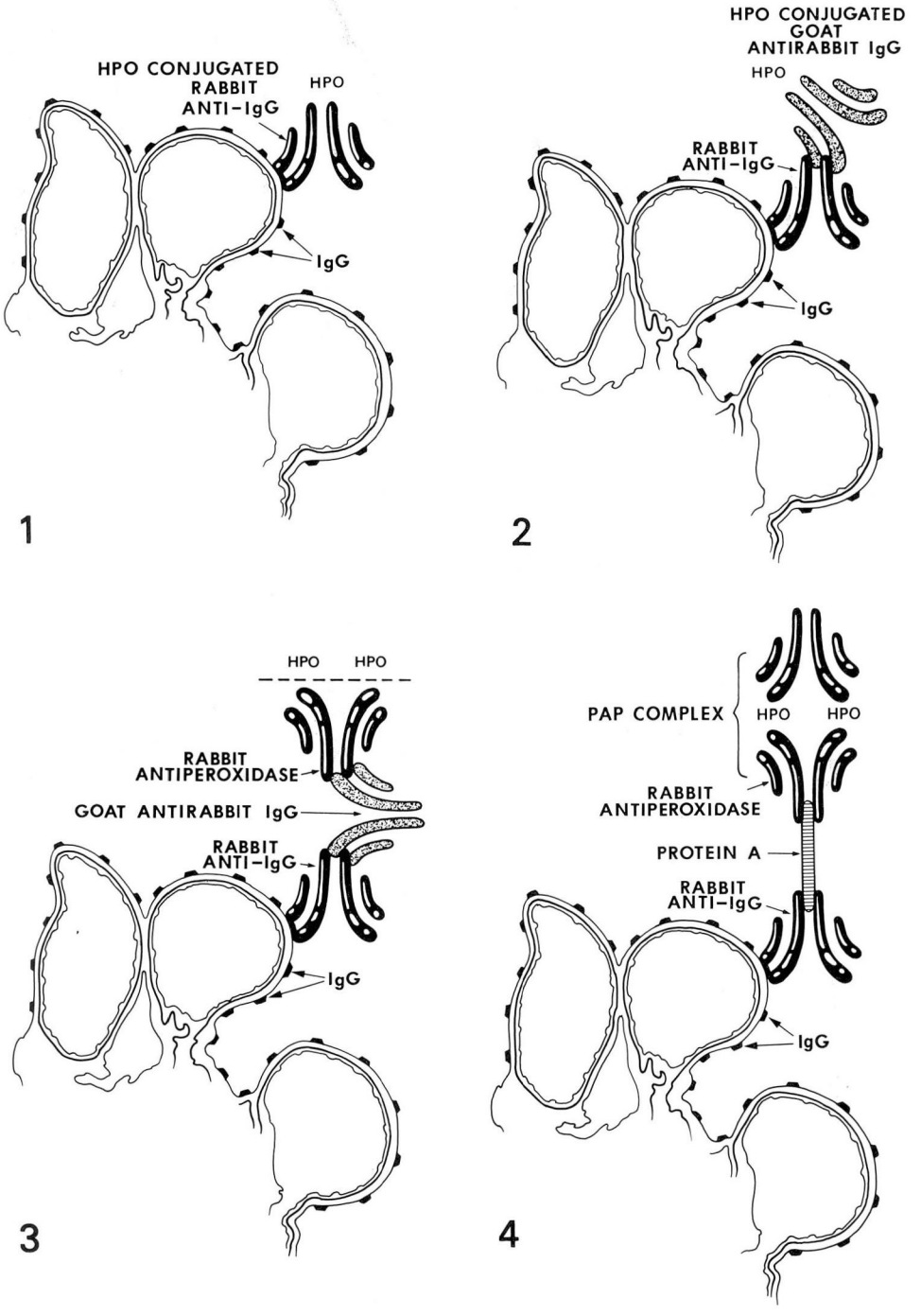
Hanker et al[40] have developed a chromogenic reagent (p-phenylenediamine and pyrocatechol) that incorporates the better qualities of benzidine derivatives and that has no currently identified carcinogenic properties.[41] The biochemistry of this chromogen depends upon the peroxidation of aromatic alcohols in the presence of phenolic compounds.[40]

From the results of a recent study comparing nine methods for immunohistochemical chromogen systems it was concluded that TMB provided the greatest sensitivity and specificity.[32] However, these conclusions have been challenged, and Hanker-Yates reagent (HYR) has been suggested as a superior immunohistochemical chromogen.[42] Published reports have described variable methodology for HYR procedures, and the differential sensitivity of TMB and HYR may be attributable to minor technical variations. HYR reagent works well when a sequence of fresh substrate-chromogen solutions are used with addition of the substrate just prior to placing the sections into the chromogen solution.[41] At present one of the more useful reagents would appear to be HYR since it has no known carcinogenic potential to our knowledge,[42] and has been shown to work well in comparative immunomicroscopic systems.[41] All chromogens and fluorescent-labeled compounds should be handled as potentially hazardous reagents.

Enzyme immunomicroscopic procedures

Several enzyme immunohistochemical procedures are available and these are diagrammatically summarized in *Figures 1–5.* Once the enzyme has been localized at the antigen site by any of these procedures, the techniques for development of the substrate chromogen reaction product are the same regardless of the immunohistochemical technique chosen.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.



Figs. 1–4. Diagrams of four different immunoperoxidase methods to detect IgG in epimembranous immune complexes deposited in membranous glomerulonephritis. **Fig. 1**, direct technique; **Fig. 2**, indirect technique; **Fig. 3**, unlabeled peroxidase-antiperoxidase (PAP) technique; and **Fig. 4**, protein A modification of PAP technique.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

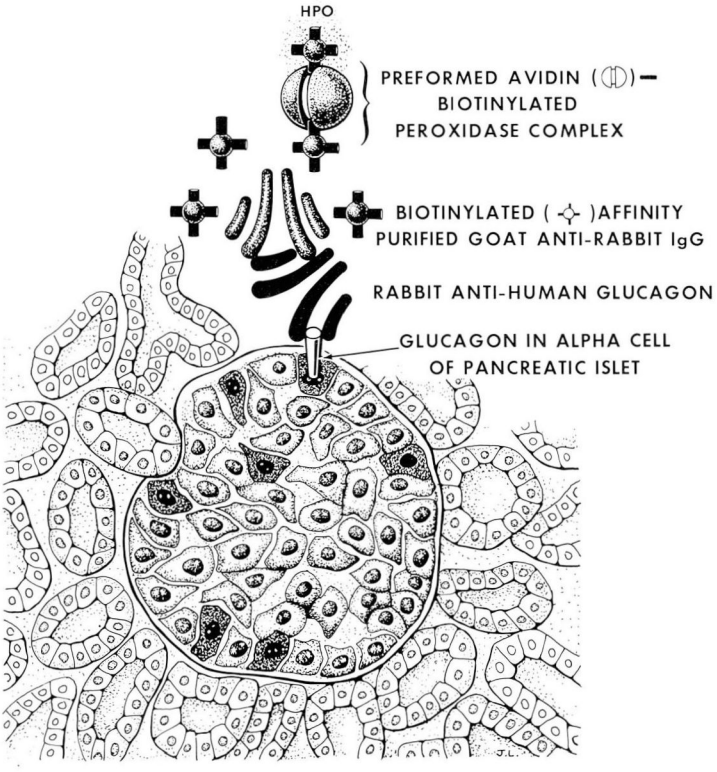


Fig. 5. Diagram of biotin-avidin "ABC" technique to detect glucagon within pancreatic alpha islet cells. Biotinylated affinity purified goat anti-rabbit IgG secondary antibody links the rabbit antiglucagon primary antibody to a preformed complex of avidin and biotinylated horseradish peroxidase.

Enzyme-labeled antibody methods

Direct method. The direct technique is the simplest immunomicroscopic procedure *(Fig. 1)*. The reagent consists of a specific antibody conjugated with enzyme. This enzyme-antibody conjugate is overlaid directly on the hydrated tissue section. Duration of incubation varies with the individual immunomicroscopic system. After washing of excess reagent from the tissue surface with an isotonic buffer system, the enzyme-substrate color reaction product is developed with one of the chromogens currently available.

The direct procedure that uses cryostat frozen sections is currently the technique of choice for studying renal tissue. Sensitivity of this procedure, although not as high as that for peroxidase-anti-peroxidase (PAP) procedure, is adequate for most clinical tissue studies. The direct technique also works well for detection of intracellular and surface membrane-associated immunoglobulins in lymphoproliferative disorders.[13]

Indirect method. The indirect immunoperoxidase (IMP) procedure does not differ from its immunofluorescent (IF) counterpart with respect to the basic technique *(Fig. 2)*. The primary unconjugated antibody binds specifically to its antigen in the tissue, and after washing off excess primary antibody from the surface of the tissue, the peroxidase-labeled secondary antibody is applied. Subsequently, the enzyme color reaction product is developed. Since the secondary antibody is labeled

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

with the enzyme, a color reaction product identifies the antigen focus in the tissue.

Unlabeled antibody methods

Triple antibody bridge method. For this method, primary antibody and antiperoxidase antibody are raised in the same animal, e.g., rabbit. A bridge antibody, e.g., anti-rabbit IgG, is applied in sequence after the primary antibody and before the addition of the antiperoxidase to the tissue surface. This secondary antibody "bridges" the primary and secondary antibody by virtue of its specificity for the immunoglobulin class in the primary and tertiary reagents. Finally, peroxidase is applied to the tissue section and the reaction product developed. Use of this particular reagent has been virtually eliminated by availability of the sensitive PAP unlabeled technique, which uses a preformed soluble PAP complex.

Unlabeled PAP method. The unlabeled PAP procedure is illustrated in *Figure 3.* This particular method differs from the triple antibody method only in that the tertiary reagent consists of a soluble complex of peroxidase and antiperoxidase. Excellent commercial sources of PAP are available. This particular procedure is generally more sensitive than the other available methods. However, the sensitivity of the labeled (indirect) antibody technique with the use of affinity-purified antibodies is about equal to that of the PAP method.[44] In some systems, the sensitivity of the PAP procedure approaches that of radioimmunoassay with useful working dilutions of the primary antibody approaching 1:100,000.[45] Rabbit PAP systems employ in sequence primary rabbit antibody against the tissue antigen in question, a bridge antibody consisting of goat or swine anti-rabbit IgG, and the soluble rabbit PAP complex. Similarly, the goat PAP system consists of a goat primary antibody, a bridge antibody, e.g., rabbit anti-goat IgG, and a soluble goat PAP complex.

Protein A modification of PAP method. Protein A from *Staphylococcus aureus* (SPA) has been shown to bind the Fc portion of IgG molecules of several species.[46] This particular reagent can be used as a conjugate with peroxidase as a "labeled secondary antibody" as a consequence of its Fc IgG binding.[47] Also, SPA can be substituted for bridge antibodies, e.g., goat anti-rabbit IgG or rabbit anti-goat IgG in the unlabeled PAP procedure *(Fig. 4).*[47–49] However, there are differences in relative avidity of SPA for the PAP complexes of different animal species; for example, rabbit and guinea pig PAP bind more completely than goat or rat PAP.[47–49]

Biotin/avidin lectin method

Recent evidence suggests that biotin/avidin enzyme immunohistochemistry compares favorably with established IMP techniques *(Fig. 5).*[50] The recently developed ABC lectin immunohistochemical system has been found to be 8 to 40 times more sensitive than the unlabeled PAP method, yields immunostained sections having negligible or no background staining, and is cost effective (about 5% of cost of average PAP procedure).[51] The ABC system uses in sequence unconjugated primary antibody, biotinylated affinity purified secondary antibody, and a preformed complex of avidin and biotinylated horseradish peroxidase as the tertiary reagent. The extraordinary sensitivity and specificity of this method are due to at least three factors: (1) avidin has high binding affinity for biotin; (2) the avidin-biotin binding reaction is essentially irreversible; and (3) unlike the second antibody of a PAP system (which must be present in excess since one of its two

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

potential antibody binding sites must be available to bind the PAP complex), biotinylated secondary bridge antibodies can be used in low concentrations since the biotin is already linked to the antibody.

Each of these methods has certain advantages and disadvantages. The indirect labeled and unlabeled antibody techniques, while offering increased sensitivity, require multiple procedural steps and involve additional reagents. Also, in some immunohistochemistry systems, increased sensitivity may be gained at the expense of specificity.

Background staining: the problem of endogenous peroxidases and pseudoperoxidases.

Both IF and IMP procedures are associated with certain predictable artifacts. Autofluorescence of certain materials in tissue must be recognized and interpreted for individual sections. Pseudoperoxidases such as hemoglobin and naturally occurring endogenous peroxidases in human tissue are similarly a source of misleading background staining in enzyme-labeled preparations. One of two approaches can be used to circumvent this problem. First, controls consisting of tissue not exposed to the specific primary antibody, but allowed to incubate with the substrate chromogen solution, will allow visualization of these endogenous peroxidases, and comparison can be made with specific immunostained sections. However, a more acceptable alternative is to destroy or consume the endogenous peroxidase either by preincubation of tissue sections with methanol H_2O_2 or by trypsin, protease, or pronase digestion.[9,10,52–56] Proteolytic enzyme pretreatment appears to enhance antigenicity by a mechanism that is not well established.

Nonspecific binding of antisera

Although methanolic H_2O_2 or enzyme pretreatment destroys or consumes endogenous peroxidases, the nonspecific absorption of heterologous serum to the tissue occasionally yields a problem in background staining. These problems can be eliminated by a combination of prolonged incubation with high dilutions of the primary antibody ($\geq$1:1000) and preincubation of rehydrated sections in nonimmune serum.[13]

Relative merits of immunomicroscopic methods

The disadvantages and advantages of fluorochromatic and enzyme-labeled techniques have been the subject of extensive debate. Since many of the initial IMP studies were done on paraffin-embedded tissues, for some time IF was thought to be a procedure most suited to frozen section material and IMP for fixed paraffin-embedded material. It is now known that either IF or IMP techniques are readily applied to fixed or frozen tissue sections. The initial lack of correlation observed between IF and IMP in studying lymphoproliferative disorders in frozen section material now appears to be a consequence of the methodology employed or antibody concentrations.[11,12,24] Also, the earlier problems encountered in differentiating granularity and linearity in renal biopsy specimens[57,58] were not observed in an evaluation of large numbers of kidney biopsies studied by comparative IF, IMP, and electron microscopy.[59–61]

Certain advantages are accorded the IMP procedure as compared with IF. IF preparations fade with repeated examination and storage, but immunohistochemical preparations yielding stable color reaction products do not fade.[13]

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

Also, it is not usually possible to visualize well simultaneously the immunostained antigen and adjacent tissue morphology in IF preparations. Conversely, IMP preparations are readily adaptable to a variety of counterstains enabling the observer to (1) more precisely locate the tissue antigen, and (2) evaluate such additional parameters as inflammatory response to the antigen. An IF microscope is necessary for examination of the IF preparations, and photographic documentation is necessary.

Previously, valid disadvantages were also accorded IMP procedures. These included the potential carcinogenic nature of the chromogens used with IMP, and the lack of reliable commercial reagents. Both of these objections are no longer valid, since diaminobenzidine (3,3′, 4,4′-Tetraaminodiphenylether · 4 HCl or 4,4′-oxybis-o-phenylenediamine) may not be a carcinogen[31] and at least one chromogen, HYR, is now available, which has no currently identified health hazard and yields excellent results. Several manufacturers currently distribute antibody-enzyme conjugates of excellent quality. Objections relating to the more complex nature of IMP procedures are no longer valid, since the direct technique using enzyme conjugates can be used for most studies that employ immunohistochemistry, i.e., renal diseases and lymphoproliferative disorders. The additional time required to develop the substrate chromogen reaction product is no longer than the additional time required for photography and cataloging of photographic slides for IF.

It is ideal to have the capability of doing both IF and IMP procedures. This allows the pathologist versatility in the selection of the appropriate procedure.

> It seems that the resistance to change from immunofluorescence to immunoperoxidase techniques for the routine examination of renal biopsy material cannot be explained in scientific terms but depends largely on emotional ties to a system which has been established for a considerable number of years.[60]

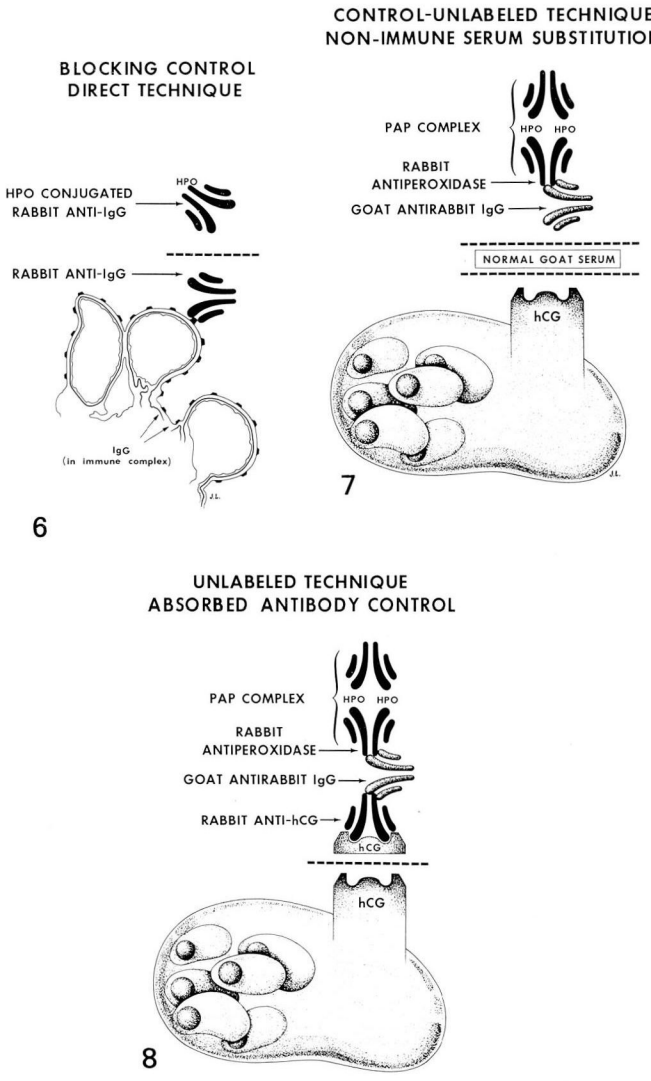
Quality assurance

Quality control of both IF and IMP reagents is an essential and often neglected part of fluorescent and enzyme immunohistochemistry. Commercially available antibody should not be assumed to be monospecific or of adequate immunoreactivity. The specificity and sensitivity of each reagent purchased should be evaluated when received. Each laboratory should have a protocol for evaluating all new antibodies entering the laboratory. The antisera should be dated when received, and evaluated by Ochterlony immunodiffusion, immunoelectrophoresis, competitive binding radioimmunoassay or immunohistochemistry with the use of preabsorption and postabsorption with antigen control tissues that have been well characterized with respect to the appropriate antigen. Many commercial antisera have package inserts that attest to the reliability of the reagents. However, there may be significant interlot variation and the reagent immunoreactivity may be altered by environmental factors during shipping. Individual techniques should be performed regularly to assure continued competence by technical personnel and adequate performance of reagents. A detailed record should be kept of all quality assurance tests and documentation of corrective actions taken.

Even when excellent standardization and characterization of antibody have been completed, rigorous in-run controls are necessary for valid interpreta-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

tion of results. Several types of controls that may be used are summarized in *Figures 6–8*. The simplest control is omission of the primary antibody and substitution of nonimmune serum such as normal goat serum or normal swine serum for the primary antibody. This technique serves as a control for recog-



Figs. 6–8. Diagrams of three types of controls for immunohistochemistry. **Fig. 6**. Preincubation of tissue section with unconjugated antibody followed by addition of labeled antibody does not result in staining, since tissue antigen binding sites are occupied by the first reagent. **Fig. 7**. Substitution of nonimmune normal heterologous serum for the primary antibody is applicable to any immunohistochemical procedure, detects nonspecific binding of secondary or tertiary reagents, and profiles endogenous peroxidase staining. **Fig. 8**. In vitro preabsorption of primary antibody with antigen in question, followed by addition of filtered or centrifuged antiserum-antigen mixture to the section, is also applicable to any immunohistochemical procedure and is the best available negative control. This type of control detects lack of primary antibody specificity, as well as nonspecific binding of secondary or tertiary reagents and endogenous peroxidase activity.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

nition of binding of secondary and tertiary antibodies to the tissue and for endogenous peroxidase in indirect and PAP methods, but cannot adequately assess monospecificity of the primary antibody. The direct IMP procedure can be controlled by preincubation with unlabeled antibody, preferably from the same antisera lots from which the conjugate was prepared. This type of control effectively blocks the labeled conjugate from reaching the antigen and although satisfactory for the direct procedure, cannot be applied to indirect or unlabeled modifications of the technique. The most reliable control for all IMP methods is an absorbed antibody control, in which the primary antibody is preincubated with exogenous antigen, thus binding all the available antibody-reacting sites. When the supernatant from the centrifuged mixture of bound antibody-antigen is applied to the tissue section, antibody is not available for the reaction and immunostaining does not occur.

Preabsorption may be necessary to remove nonspecific reactants or reactions with related antigens in the tissue. Not uncommonly, secondary antibodies, e.g., goat anti-rabbit IgG, or rabbit anti-goat IgG, will cross react with human immunoglobulins. In such instances, absorption with purified human gamma globulin followed by centrifugation of the antibody is necessary to assure specificity of the secondary antibody. For every procedure done on a day-to-day basis and for each tissue analyzed, an in-run control should be included for adequate verification of positive or negative results. Use of affinity purified antibodies may help insure specificity.

Finally, when a new commercial antibody is purchased or antibody is made available from other sources, checkerboard titrations with varying combinations of antibody dilutions can be used on control tissue sections to evaluate the optimal dilutions of each reagent.

Diagnostic applications

Immunohistochemical procedures have contributed greatly to the understanding of normal physiology and functional organization of many animal and human biologic systems. This paper will be restricted to reviewing the clinical diagnostic applications of IMP methods *(Figs. 9–16)*.

Renal immunohistochemistry

The value of immunomicroscopy in delineating deposition of immunoglobulin and complement components or autoantibody in glomeruli of patients with various autoimmune diseases is well established. Once a tool of academic interest, immunomicroscopy is now an essential diagnostic method that must be applied to every renal biopsy specimen. Patterns of IF have been shown to be highly reproducible and predictive histopathologically.[62]

Comparative studies of IMP and IF of glomerulonephritis were initially favorable.[8] Subsequently, studies of kidney biopsy specimens with the use of the direct technique and enzyme conjugates were associated with unacceptable background staining, and in some cases a distinction between linear and granular color reaction product was difficult.[57,58] Both IF and direct IMP procedures were compared to the unlabeled IMP technique, and the specificity and sensitivity of the unlabeled PAP procedure were demonstrated to be comparable to those of IF.[58] However, the length of the procedure and the expense of additional reagents make use of the PAP procedure for frozen renal tissue a poor choice.

In the past few years, improved tech-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

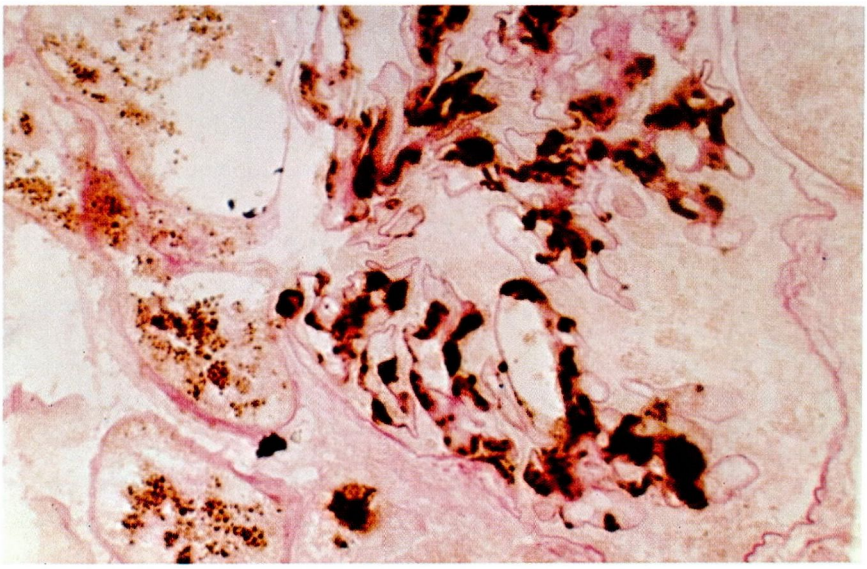


Fig. 9. Photomicrograph, IgA nephropathy, demonstrating confluent granular deposits of IgA in mesangial areas, (IMPAS × 160).

niques of enzyme conjugation have resulted in better commercial antibodies. Excellent reagents can be obtained from several commercial sources for identification of immunoglobulins and complement components. Three recent large series have described IMP results that compare favorably with IF performed on the same cases.[59–61] Immunostained frozen sections can be counterstained with periodic acid Schiff to locate more precisely sites of antigen deposition *(Fig. 9)*.[59]

Paraffin-embedded renal tissue, whether fixed in formalin or precipitative fixatives under the best of conditions, may not be adequate for demonstration of extracellular immune complexes or autoantibodies with standard immunohistochemistry. Since trypsin and pronase have been shown to enhance detection of tissue antigens, recent attempts have been made to use enzyme pretreatment for IF or IMP detection of extracellular immune complexes in paraffin-embedded tissue.[63–65] Similar results can be obtained with IMP with the use of enzyme-digested sections.[9,65,66] MacIver et al[9] have suggested that inconsistent detection of complement in previous studies may be a function of over trypsinization of tissue sections. These investigators demonstrated clear separation of granular and linear staining patterns, precise localization of deposits within glomeruli using posttrypsinization IMP, and a concordance rate with IF of 81%.[9] Optimum conditions for detection of complement were shown to be 0.05% trypsin for 40 minutes, a concentration also sufficient to detect immunoglobulin heavy chains and C1q.

For the present time, fresh tissue should still be used as the tissue of choice for renal immunomicroscopy. With the availability of excellent commercial antibodies and well-established techniques, the direct IMP procedure is probably the technique of choice. It is

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

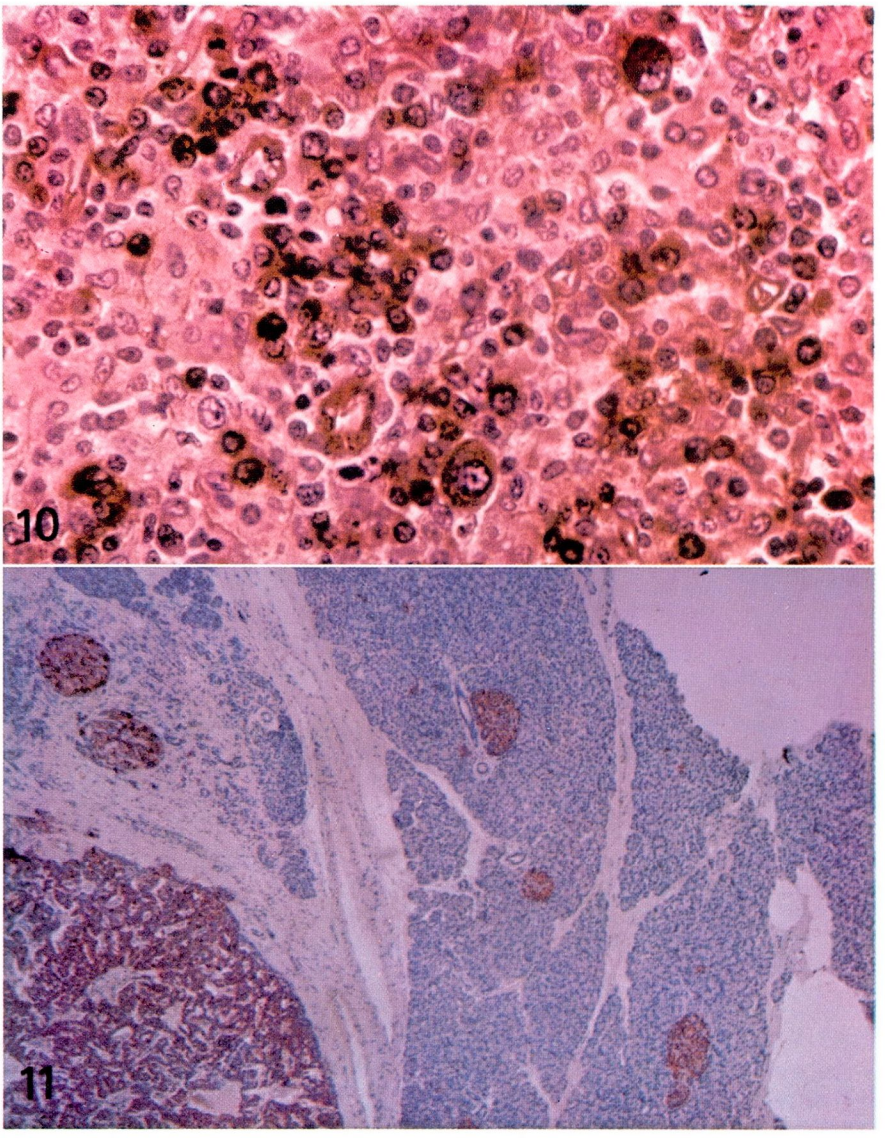


Fig. 10. Photomicrograph, B-cell immunoblastic sarcoma arising in plasmacytoid lymphocytic lymphoma, lymph node. The neoplastic cells (brown-black cytoplasm) are immunostained for kappa light chains. A serial section was negative for lambda light chains. Direct immunoperoxidase technique, counterstained with hematoxylin and eosin, (× 400).

Fig. 11. Photomicrograph, beta cell pancreatic apudoma. The neoplasm and adjacent normal islets contain immunoreactive insulin. Unlabeled PAP technique was used employing aminoethylcarbozole as the chromogen, (× 64).

not clear at this time whether trypsinized deparaffinized paraffin-embedded tissue will be acceptable as an immunomicroscopic preparation for most forms of glomerular disease, since in the hands of some investigators, enzyme pretreatment yields variable tissue digestion and inconsistent immuno-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

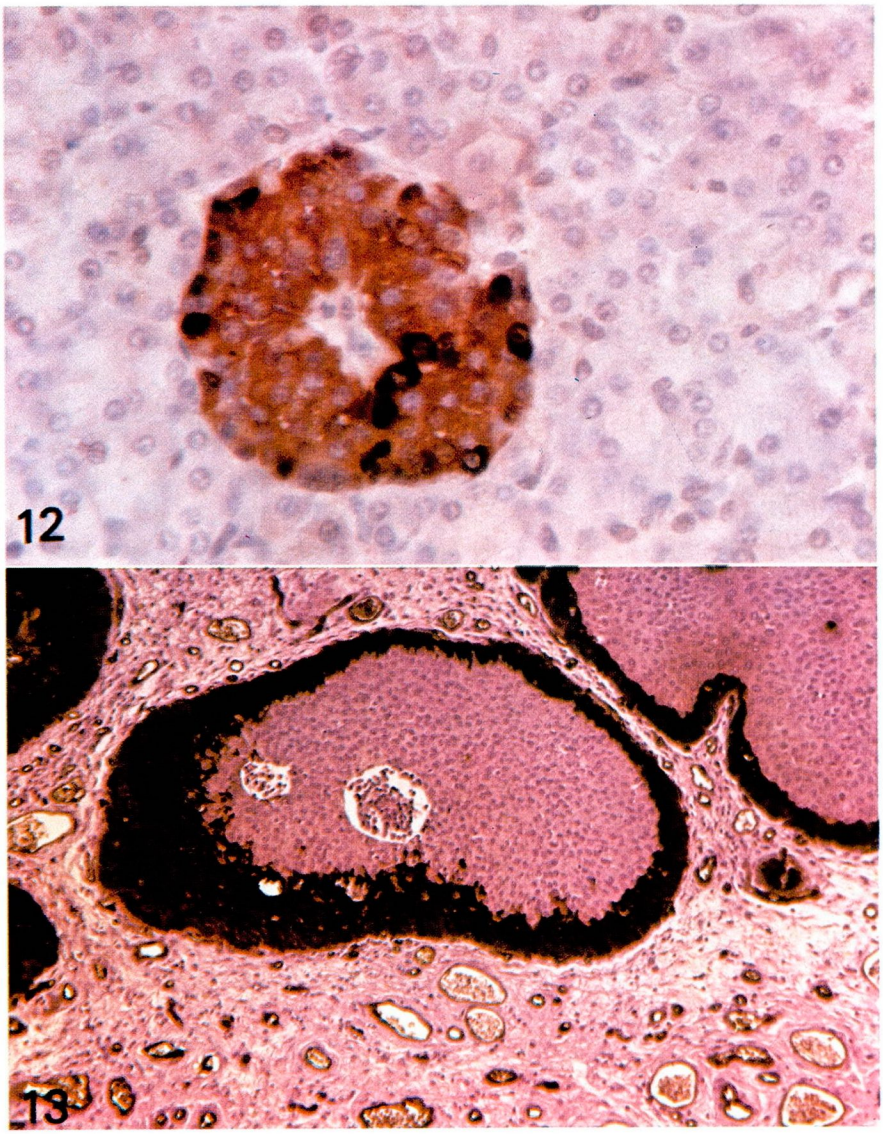


Fig. 12. Photomicrograph, double label study using the unlabeled PAP technique demonstrating glucacon (black) and insulin (red) within the same pancreatic islet, (× 160).

Fig. 13. Photomicrograph, bladder biopsy. Superficial transitional cell carcinoma at right extending over the residual nonneoplastic urothelium does not express blood group A antigen. Endothelial cells, erythrocytes, nonneoplastic urothelium express blood group A antigen (brown-black). Indirect immunoperoxidase technique, counterstained with hematoxylin and eosin, (× 160).

staining patterns due to poorly understood mechanisms that may include formation of antibody or protein moities of different antigenicity.[44]

Lymphoproliferative diseases

Immunohistochemistry has contributed greatly to our understanding of the

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

organization of the immune system and to architectural and functional alterations in its various components in a variety of disease states. The technology of immunohistochemistry has evolved parallel with increased knowledge about lymphoid neoplasia and has contributed significantly to the understanding of the nature of proliferating cells of malignant lymphoma. Immunohistochemistry of malignant lymphomas will eventually play a role similar to that of immunomicroscopy in evaluating renal disease.

Paradoxically, these techniques have contributed both to understanding and confusion regarding this group of entities. Thus, while documenting the presence of monoclonal cytoplasmic immunoglobulins in many non-Hodgkin's lymphomas,[14,67–72] these techniques as applied to paraffin-embedded tissues have yielded polyclonal staining of B-cell lymphomas in some reports.[14,68] Such observations are not in agreement with the clonal premise upon which most cancer immunology is based.[23,73–75]

Studies emphasizing the immunologic basis for classification of lympho-

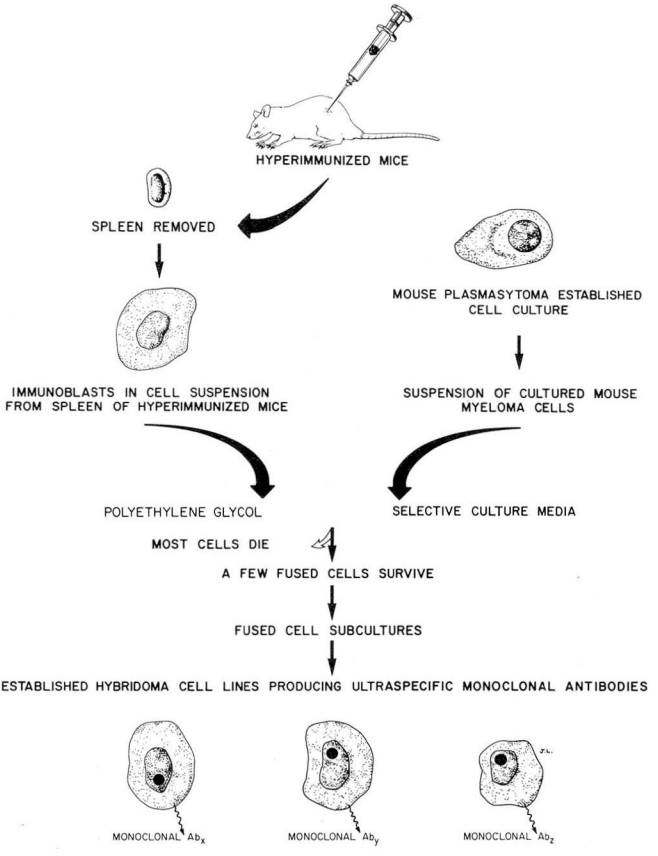


Fig. 14. Diagram of procedure used to obtain monoclonal hybridoma antibodies. Immunoblasts from hyperimmunized mice are fused with cultured mouse plasmacytoma cells in the presence of polyethylene glycol. Although most of the cells die, a few cells survive which contain the genetic content of both the stimulated immunoblasts and mouse myeloma cells. The fused cells are subcultured and cloned, reinjected into mouse peritoneal cavity, and ultraspecific monoclonal antibody harvested as ascitic fluid.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

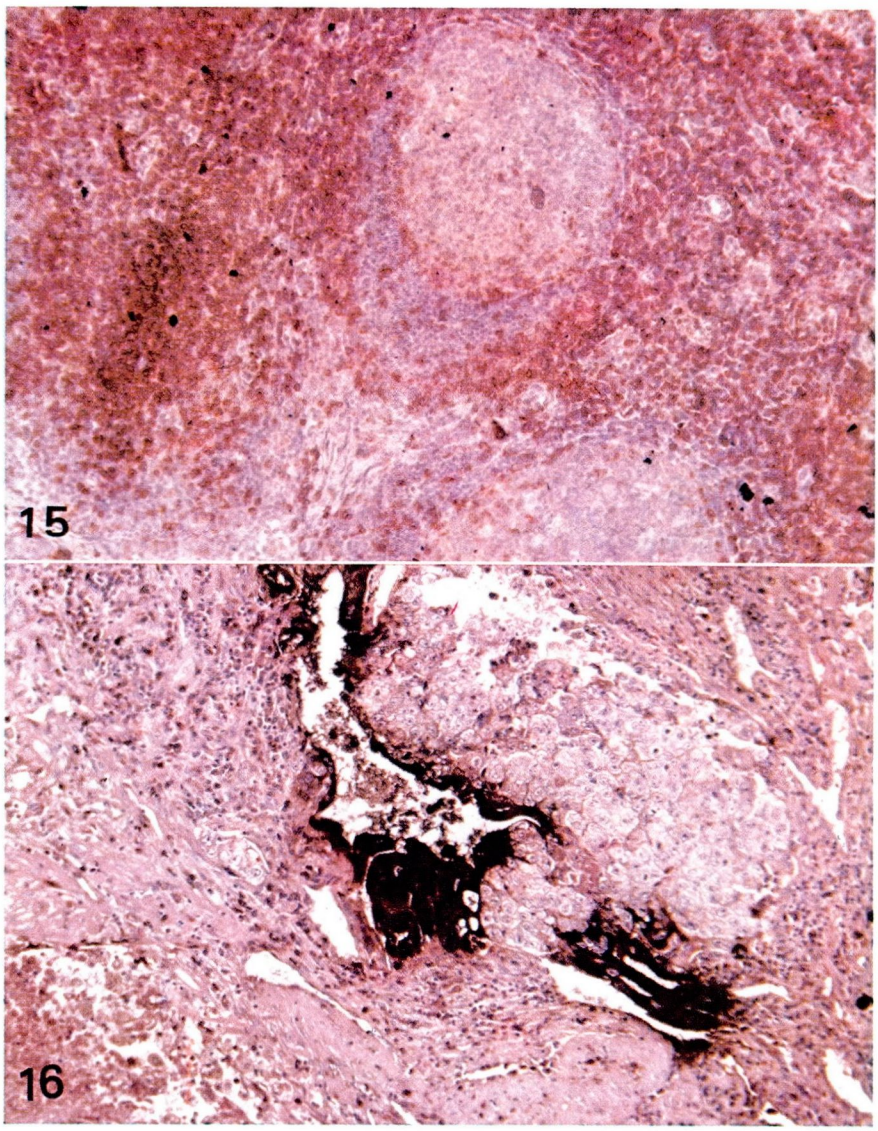


Fig. 15. Photomicrograph, reactive lymphoid hyperplasia, lingual tonsil. Red staining interfollicular T helper/inducer lymphocytes are identified using aminoethylcarbazole as the chromogen. Biotin-avidin "ABC" technique using mouse monoclonal hybridoma primary antibody specific for inducer/helper T lymphocytes, with methylene blue counterstain, (× 64).

Fig. 16. Photomicrograph, malignant mixed germinal neoplasm of testis. Neoplastic syncytia trophoblasts are immunostained for chorionic gonadotropin. Indirect immunoperoxidase technique using mouse monoclonal hybridoma primary antibodies specific for beta subunit of human chorionic gonadotropin, peroxidase conjugated affinity purifed goat anti-mouse IgG, and hematoxylin and eosin counterstained, (× 160).

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

mas attempt to draw parallels between the components of the immune system and the morphologic diversity of non-Hodgkin's lymphomas. Of the several different classifications available for subtyping of non-Hodgkin's lymphoma, only the Lukes-Collins classification,[69] which is currently available is directly dependent upon the identification of T- and B-cell marker expression by the neoplasm. However, immunologic data can be added to the Rappaport morphologic classification.[70]

Initial functional characterization of lymphomas was done principally by cell suspension (CS) studies.[75] With the use of CS techniques, classification as to T, B, or non-T/non-B origin can be effected in a relatively large number of cases.[73,74] In recent years, information regarding the reliability of CS studies has accumulated. In a number of B-cell lymphomas marking monoclonal with cryostat frozen section immunohistochemistry, polyclonality in CS has been observed.[76–78] This apparent discrepancy may be due to several factors, most likely selective loss of tumor cells or sampling error resulting in contamination of the suspension with nonneoplastic lymphocytes, particularly in nodular lymphomas in which a significant percentage of the lymphoid parenchyma may be spared by the neoplasm.

When the sensitive PAP immunohistochemical technique developed by Sternberger was initially applied to lymphoproliferative disorders, the use of immunologic markers to characterize non-Hodgkin's lymphomas was viewed as an academic curiosity rather than a clinically useful tool by many pathologists. Recent evidence suggests that when non-Hodgkin's lymphomas are approached from the standpoint of Lukes-Collins classification and interpreted in conjunction with surface marker analysis, data of distinct prognostic significance are obtained for subsets of lymphomas.[79] However, immunohistochemical study of paraffin tissue from patients with multiple myeloma has shown phenotypic expression of both kappa and lambda light chains in cells from patients with well-characterized circulating monoclonal immunoglobulins.[68] Thus, the initial enthusiasm for immunohistochemistry was tempered by these apparent anomalous staining patterns that violated basic concepts regarding monoclonality of B-cell neoplasia.[80]

A similar evolution of understanding of immunohistochemistry as applied to non-Hodgkin's lymphomas has been observed.[81–104] The spurious immunostaining patterns observed in a significant number of cases evaluated by paraffin-embedded immunohistochemistry raised serious questions about the validity of results obtained in this manner.[11,14,105] Initially, it was suggested that such results were a consequence of diclonal immunoglobulin production by the non-Hodgkin's lymphoma,[14] an explanation that is not in concurrence with the overwhelming body of evidence for monoclonality in human B-cell lymphomas.[23,106–108]

When results of frozen section and paraffin-embedded immunohistochemistry are compared, it becomes clear that the negative or spurious immunostaining patterns associated with paraffin-embedded tissue are probably a consequence of processing.[11,12,24,25] Currently, cryostat frozen section immunohistochemistry (CFSIH) provides the most sensitive and specific procedure for detection of monoclonal cell populations, since most non-Hodgkins lymphomas demonstrate monoclonal immunostaining with CFSIH.[11,15,23,25] However paraffin-embedded techniques provide su-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

perior morphology in the minority of cases that mark in monoclonal fashion *(Fig. 10)*. Optimally, both frozen and paraffin-embedded tissues should be evaluated for each case. CFSIH is also helpful in delineating physiologic domains of lymphoid subpopulations, defining the nature of nonneoplastic lymphoid infiltrates, and in detecting malignant cellular populations in histologically reactive lymph nodes.[109-111]

Immunoglobulin negative non-Hodgkin's lymphomas consist of unusual lymphomas of T-lymphocytic and dedifferentiated B-lymphocytic origin, true null lymphomas of non-T non-B cytogenesis, and neoplastic proliferations of true tissue macrophages. More precise characterization of these unusual lymphoma subtypes can be accomplished by identifying immunohistochemistry profiles of lymphocyte differentiation antigens and elaborated substances *(Table 2)*. The growing availability of hybridoma monoclonal antibodies specific for lymphocyte subpopulations is increasing the accuracy with which determinations of cytogenesis are made.[112] Differentiated B-lymphocytic lymphomas are characterized by monoclonal surface membrane-associated immunoglobulin (CS or CFSIH), cytoplasmic immunoglobulins as detected by immunohistochemistry procedures on paraffin-embedded tissues in some cases, Ia and Ia-like antigens, and J piece expression.[11,113-118] Recognition of T-cell lymphomas has been previously based upon cytochemical expression of acid alpha-naphthyl acetate esterase or acid phosphatase activity in neoplastic cells.[119] However, monoclonal hybridoma antibodies monospecific for subsets of T lymphocytes are now available and are helpful in the recognition of these unusual lymphomas.[120-126] With the use of monoclonal antibodies and CFSIH, immunohistochemical phenotypes can be identified for most large cell lymphomas.[127-129]

Recognition of neoplastic proliferations of true tissue macrophages is perhaps the most difficult diagnostic challenge at present. Much of the current problem is a consequence of various morphologic criteria used and the arbitrary distinctions that have been established to distinguish between malignant histiocytosis and histiocytic lymphoma of true tissue macrophage origin. True tissue macrophages with maturation will express alpha-naphthyl acetate esterase activity in diffuse pattern, and are associated with the expression of muramidase and alpha-1-antitrypsin, Ia antigen, and polyclonal cytoplasmic immunoglobulins.[130-137] However, in poorly differentiated variants of malignant histiocytosis, the neoplastic cells may not express these proteins.[134,135] The most definitive evidence for tissue macrophage origin will be the identification of antigens peculiar to monocyte/macrophage differentiation on neoplastic cells with the use of monoclonal antibodies.

Terminal transferase, an enzyme present in lymphoblasts but absent in myeloblasts, may be helpful in subtyping the acute leukemas.[138,139] Both biochemical and IF techniques are currently used to identify the enzyme. Reliable immunohistochemistry methods for in situ demonstration of terminal transferase in tissue have not been developed to our knowledge. Results of initial studies of a cross-reacting antibody that preferentially immunostains myeloblasts in tissue suggest that this marker may also be helpful in subtyping leukemias.[140]

Since the monoclonal nature of B-cell non-Hodgkin's lymphomas has been well established, use of objective methods has been advocated to distinguish

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

Table 2. Immunohistology of lymphoproliferative disorders

	Ig	JP	Ia	OKT_3 Pan	OKT_8 Sup	OKT_4 Ind
Reactive hyperplasia						
Follicles						
Light zone	PC	+	+	OC	–	OC
Dark zone	PC	+	+	–	–	–
Sinus	PC, E	–	+	–	–	–
Paracortex	OC, DC	–	OC	+	+	+
Non-Hodgkin's lymphomas						
B cell lymphomas	MC	+	V	–	–	–
With Fc receptors	E	+	V	–	–	–
T cell lymphomas	–	–	V	+	+*	+*
With Fc receptors	E	–	V	+	+*	+*
Dedifferentiated B cell						
Lymphomas	–	–	+	–	–	–
With Fc receptors	E	–	+	–	–	–
Hodgkin's disease						
Plasma cells	PC	+	–	–	–	–
Reactive tissue macrophages	PC	–	+	–	–	–
Neoplastic cells	PC	–	NWE	NWE	NWE	NWE

Abbreviations: Ig = surface and/or cytoplasmic immunoglobulins (best assessed with cryostat frozen section immunohistochemistry). JP = J piece, OKI = monoclonal antibody specific for Ia antigen, OKT3. Pan = monoclonal antibody specific for all peripheral blood T lymphocytes, OKT8. Sup = monoclonal antibody specific for suppressor/cytotoxic T lymphocytes, OKT4. IND = monoclonal antibody specific for inducer/helper T lymphocytes, PC = polyclonal (both κ and λ light chains present), + = present, – = absent, OC = occasional cells, DC = dendritic cells, NWE = not well established, V = variable, MC = monoclonal (only one light chain present, either κ or λ), Fc = receptors for Fc portion of immunoglobulin), E = surface-associated immunoglobulin which can be eluted with acidic buffer.

* T cell malignant lymphoproliferations react with either T Supp. or T inducer/helper monoclonal antibody, depending on differentiation of the neoplasm.

reactive lymphoid hyperplasias from non-Hodgkin's lymphomas by characterizing surface immunoglobulin phenotypes.[141–144] Since CS may yield spurious results in non-Hodgkin's lymphomas, possibly due to contamination with nonneoplastic populations or selective loss of tumor cells, CSFIH should be used to help determine the biologic potential of the lymphoproliferative disorders.[141–144]

The origin of Reed-Sternberg cells in Hodgkin's disease has been the subject of extensive debate. For years the preferential involvement of the lymph node sinus and interfollicular zone and defects in cellular immunity were interpreted as evidence for T-cell or tissue macrophage origin. Immunohistochemistry studies by Taylor[14] and others[145–148] showed that Reed-Sternberg cells contain polyclonal IgG. These observations are consistent with a tissue macrophage origin, the cytoplasmic immunoglobulin probably representing engulfed exogenous polyclonal immunoglobulin. Tissue culture cell lines derived from Hodgkin's disease display cytochemical and immunologic features of macrophages, and when transplanted into experimental animals produce tumors with morphology resembling Hodgkin's disease.[149] Furthermore, CS from tissues involved by Hodgkin's disease contain Reed-Sternberg cells that have polyclonal cytoplasmic IgG, and

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

actively bind and internalize labeled exogenous immunoglobulins.[150] Although a tissue macrophage origin of Hodgkin's disease appears likely, definitive evidence could be obtained with immunohistochemistry staining with the use of lymphocyte-monocyte differentiation monoclonal antibodies. To our knowledge, such a study has not been done.

Endocrine systems

IMP procedures have contributed greatly to our understanding of the C-cell neoplasms and preneoplastic state of the thyroid gland. Wolfe et al[151] have delineated the distribution of C cells in the normal gland and in the thyroid gland of patients at risk for hereditary medullary carcinoma. When evaluated in this fashion, it has been shown that the middle and upper portions of the lateral thyroid lobes show marked increases and clustering of calcitonin-containing cells in this disease. The immunohistochemical demonstration of thyroglobulin within well-differentiated tumors of the thyroid gland of both papillary and follicular types has been shown to be helpful in confirmation of thyroidal origin, since tumors of nonthyroidal histogenesis examined did not show thyroglobulin synthesis.[152,153]

Immunohistochemistry studies have been helpful in evaluation of both normal and neoplastic pituitary tissue. It has been shown that adenomas of the adenohypophysis, although they may be tinctorially homogeneous, are immunohistochemically heterogeneous.[154] Immunohistochemistry studies are helpful in delineating the presence of neoplastic cells containing the hormone circulating in the patient. Thus, the documentation of prolactin in resection tissue from the anterior pituitary of a patient with hyperprolactinemia as assessed by radioimmunoassay is a helpful confirmatory study.[155,156] Study of the pituitary tissue of acromegalic patients has documented the presence of growth hormone within the neoplastic tissue, an observation corroborating radioimmunoassay results.[157] However, Fukaya et al[157] have also demonstrated occasional immunoreactive cells positive for prolactin and luteinizing hormone in an adenoma removed from a patient with acromegaly, raising questions about the significance of identifying other hormones within a particular tumor. Such immunoreactive cells may represent residual normal pituitary tissue. Conversely, these observations may suggest that pituitary adenomas are polyclonal neoplastic proliferations with secretion of one hormone dominating the clinical presentation. Recent cases of pituitary adenomas with ultrastructural and immunohistochemical evidence for heterogeneous cell populations have been described that were eosinophilic in tinctatorial differentiation but heterogeneous in their elaboration of growth hormone and prolactin.[158,159] It has been suggested that at least some of these described cases may involve technical problems and may not actually represent a stem-cell neoplasm.[160] Immunohistochemistry may prove to be especially helpful in the confirmation of hormonal homogeneity in small specimens of tissue removed as microadenomas from patients with Cushing's syndrome. Currently, this confirmation is based upon standard histochemical and clinical correlations.[161] Study of pituitary adenomas occurring in association with MEN I syndrome has confirmed that most of the adenomas are of either prolactin or growth hormone type.[162,163] In the rate occurrence of pituitary carcinoma metastatic to extracranial sites, immunohistochemistry techniques may offer confirmatory evidence for pituitary

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

origin of the neoplasm.[164] Of greater interest is the immunoreactivity demonstrable in chromophobe adenomas of the adenohypophysis. These tumors are usually characterized by multiple hormonally positive cells for most of the hormones endogenous to the anterior pituitary.[165] Immunohistochemistry techniques may also prove helpful in the characterization of "hypoplasias" or preadenomatous states.[166] Other applications of immunohistochemistry in the study of pituitary disease include the delineation of decreased hormonal synthesis or storage in gonadotrophs in patients with hemochromatosis.[167] Confirmation of elaboration of adenohypophyseal hormones by ovarian teratomas, oat cell carcinomas, and other extrapituitary tumors may also be done with the use of immunohistochemistry techniques.[168–171]

The use of immunohistochemistry in evaluation of the endocrine pancreas has yielded some interesting results *(Figs. 11, 12)*. In experimental animals, the distribution and relative frequency of different immunoreactive cell populations have been documented with IMP and IF.[172] Human pancreatic endocrine tumors are characterized by distinct ultrastructural features that allow subclassification of pancreatic apudomas.[173] Immunohistochemistry techniques have been used to identify the distribution of insulin-positive cells in hyperinsulinemic hypoglycemia of infancy,[174] to document the presence of hormones such as glandular kallikrein and cholecystokinin-pancreozymin within islet-cell populations[175,176] and to profile immunohistochemically the hormonal content of islet-cell tumors[177] *(Fig. 11)*.

Kurman et al[178–180] have used immunohistochemistry to localize elaborated steroid molecules in tumors of the ovary and the testis. With these techniques, both testosterone and estradiol were identified in Sertoli-Leydig cells and in primitive spindled cells in these tumors.[169–172] Estradiol was localized in granulosa cells and in luteinized theca cells, and nonluteinized stromal cells were negative for steroids.[178–181] Immunohistochemistry techniques may thus prove helpful in the subclassification of ovarian tumors based upon the predominant hormone elaborated, and also in the confirmation of gonadal stromal origin when such neoplasms occur in an extragonadal location.[180,181]

When carcinoid tumors are evaluated with immunohistochemistry techniques, positive immunoreactivity for multiple hormones is usually identified.[182] However, somatostatin immunoactivity ordinarily predominates and is usually associated with immunostaining for other hormones such as gastrin or calcitonin.[182] Most of the tumors studied in this fashion have been clinically silent with respect to hormonal elaboration.

The emergence of immunohistochemistry techniques specific for prostatic acid phosphatase have proved to be very helpful in the confirmation of prostatic origin of secondary metastasis.[183–187] Nadji et al[188] have also recently shown that an antibody can be raised against specific tumor antigens of prostatic carcinoma rather than the elaborated acid phosphatase product. The antibody thus derived is specific only for carcinoma of prostate histogenesis.

Oncodevelopmental antigens

Oncodevelopmental antigens are a group of substances that are produced early in fetal life, but which disappear with fetal maturation. These substances may reappear in the bloodstream in association with a malignant neoplasm apparently through derepression of the

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

genes responsible for production of these markers. It has been shown that the sensitive PAP technique is satisfactory to demonstrate the presence of human chorionic gonadotropin (HCG) in placental syncytial trophoblast and neoplastic trophoblastic elements.[189–191] Radioimmunoassay of beta subunit HCG is helpful in monitoring patients with gestational trophoblastic and gonadal germinal neoplasia.[192–196] Ideally, RIA baseline follow-up measurements are used in conjunction with immunohistochemical study of initially resected tumor tissue. This approach permits a precise morphologic and immunohistochemistry characterization of the tumor for definitive subtyping, and suggests which serum markers will be most useful for therapeutic monitoring.[197–200] The amount of choriocarcinomatous differentiation can be best assessed with immunohistochemistry for HCG and the degree of endodermal sinus and embryonal differentiation best assessed with α-fetoprotein immunohistochemistry. These techniques may also be helpful in confirming germinal nature of neoplasms occurring in an extragonadal location such as the intracranial vault.[201,202] However, these markers are by no means specific for germinal neoplasia since they may occur in a variety of nongonadal neoplasm.[203–211] Although perhaps of limited usefulness in confirming a germinal origin for a particular neoplasm, monitoring of markers may provide an indication of therapeutic success.[212]

Similar results have been observed for carcinoembryonic antigen (CEA). The immunohistochemistry method works well for the detection of CEA in tissue.[213] However, the elaboration of this oncodevelopmental antigen by a variety of neoplasms makes diagnostic usefulness limited. CEA expression has been suggested as a useful diagnostic tool in the assessment of lung tumors, since mesotheliomas have been shown with immunofluorescence not to express CEA, whereas bronchogenic carcinomas are associated with CEA elaboration.[214] Peeripheral serologic measurements of CEA may be useful in monitoring response to therapy in patients with breast, stomach, and colorectal cancer.[215–221] A distinction between benign colonic mucosa and dysplastic or frankly carcinomatous changes within the bowel mucosal tissue of patients with ulcerative colitis is theoretically possible with CEA immunostaining, but staining patterns are inconsistent.[222,223] It has also been shown that IMP techniques are able to identify certain CEA-positive cervical carcinomas before ovarian CEA concentration is elevated.[224] Van Nagell et al[225] have shown the usefulness of immunohistochemistry identification of CEA expression by ovarian cystadenocarcinomas and follow-up serologic measurements to assess therapeutic success. The presence of CEA seems to correlate best with mucinous rather than serous differentiation of ovarian neoplasms.[226–228]

An antibody reacting with gp52, a 52,000-dalton glycoprotein of the mouse mammary tumor virus has been shown to immunostain selectively breast carcinoma cells, not reacting with the normal breast tissue or other malignancies.[229] Autoantibodies having specificity for the same virus have been shown to occur in sera of breast cancer patients.[230]

Blood group antigens

The expression of ABO blood group antigens by different human tissues has been recognized for many years. Normal urothelium, for example, expresses ABO antigen in agreement with the phenotype of the patient's red blood cells. In

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

most urothelial neoplasia, the capacity to express the ABO blood group antigen is lost as the neoplasm becomes more aggressive.[231-234] In some superficial transitional cell carcinomas of the bladder, the natural history of the disease is not characterized by aggressive biologic potential, and thus it would be useful to have a means to identify this particular group of patients who require less aggressive therapeutic measures.[232] With the mixed red cell agglutination test, as assessed by passive red blood cell immunoadherence, it is possible to identify neoplasms that retain the ability to express blood group antigens on the tumor cells. The expressin of ABO antigen on neoplastic urothelium seems to indicate a less aggressive biologic potential, i.e., the tumor does not become invasive.[231-234] Passive red cell immunoadherence has also been used to identify secretion of fetal blood group antigens on polyps of the distal colon, and the loss of ABO isoantigen expression in histologically benign lesions and in mammary carcinoma.[235,236] ABO antigens in tissue can also be detected by indirect immunohistochemistry *(Fig. 13)*.

Infectious agents

Immunohistochemistry techniques are readily adaptable to the detection of hepatitis-B surface antigen in fixed and frozen tissue.[237-241] These techniques can be used to confirm the presence of surface and core hepatitis-B antigen in hepatic cirrhoses or hepatocellular carcinomas, although the relationship between the presence of the antigen and the pathogenesis of the disease remains problematic.[241-244] Recently, it has been shown that hepatitis surface antigen has an affinity for free and bound horseradish peroxidase, an observation that mandates strict use of controls in the procedure.[245] With IF, immunohistochemistry techniques have been used to identify the causative agent of non-A, non-B hepatitis in tissue that may also prove useful in IMP studies.[246]

Antisera specific for certain parasites, polyoma viruses, herpes virus, and varicella/zoster virus have been effective in the retrospective identification of infectious agents in tissue with the use of IF or IMP procedures.[247-253]

Other antigens

Other antigens detected in tissue with immunohistochemistry include the distribution of laminin,[254] ligandin,[255] and Factor VIII coagulation factor.[256] Gliofibrillary acidic protein can be used to characterize glial cell populations, and other intracranial mass lesions.[257-260] However, an inverse relationship between the degree of anaplasia and the intensity of immunostaining with anti-GFA antibody has been observed; thus, in poorly differentiated neoplasms, the technique may have limited usefulness.[258] Neoplastic Paneth cells have been identified with lysozyme antisera in an unusual variant of gastric carcinoma,[261] the presence of actin has been documented in meyloepithelial cells in the breast and other tissues of smooth muscle cell origin,[262,263] fetal red cells identified in placental intervillous thrombi,[264] myoglobin in normal and neoplastic human skeletal muscle,[265] keratin in a variety of normal human tissues,[266,267] and basement membrane antigen has been demonstrated in Wilm's tumor.[268]

Immunohistochemistry techniques have also been shown to be useful in serologic studies to detect antinuclear factor in serum,[269] cell surface antigens in CS,[270] thymus leukemia antigen expression on lymphoid cells,[271] and antilymphocyte antibodies.[272]

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

The future of enzyme immunohistochemistry

The future of diagnostic and investigational immunohistochemistry will be influenced by several things. The role of this specialized technique in the diagnostic surgical pathology laboratory will increase substantially as the techniques become more widely accepted and as more systems for application are developed. However, as the techniques become more widely used, the necessity for good controls and standardization of reagents and techniques become even more important.[45,273]

The federal government will undoubtedly play some role in the practical daily use of immunohistochemistry in clinical laboratories. Classification of benzidine as a carcinogen and subsequent strict regulations governing its use have had considerable bearing on the use of this reagent in cytochemistry. As outlined earlier in this paper, alternative chromogens are available. However, many chromogens currently in use in the United States, other than benzidine and fluorescent compounds have not been fully investigated with regard to their neoplastogenic potential. A framework for decision-making by the federal government regarding potential human carcinogens has been recently outlined.[274]

The immunohistochemistry detection of specific tumor antigens associated with one particular type of tumor offers great promise. Such antigens have been identified in association with ovarian carcinomas, melanoma, breast as evaluated by an antigen cross-reacting with mouse mammary tumor-related antigens, human cervical squamous cell carcinoma, and mesothelioma.[275-279]

The role of external photoscanning with radiolabeled antibody tracers will continue to grow. Immunohistochemistry may be helpful in the initial immunologic characterization of such tumors before noninvasive scanning procedures.[280,281]

Prolactin receptors have been profiled with the use of IMP methodology,[282,283] and the potential exists to develop peroxidase-labeled histochemical procedures for the detection of a variety of receptors including estrogen, progesterone, and testosterone.

Perhaps the most exciting development in immunohistochemistry relates to hybridoma technology. Since Köhler and Milstein's[284,285] reports of the successful fusion of specific antibody producing cells in culture with tumor cell lines, the applications of this particular biotechnology have increased greatly.[286] Hybridomas are produced by the fusion of a myeloma tumor cell line maintained in tissue culture with cell lines derived from splenic immunoblasts removed from hyperimmunized animals *(Fig. 14)*. Selective tissue cultures yield cloned hybridoma cell lines that elaborate monoclonal ultraspecific antibody. As long as the tissue cell lines can be maintained in culture, production of a standardized antibody is guaranteed. The fused cells may be inserted into mouse peritoneum where functioning hybridoma plasmacytomas grow, and ascitic fluid containing the monoclonal antibody can subsequently be harvested. Monclonal antibodies have been made with specificity for lymphocyte differentiation antigens *(Fig. 15)*, tumor specific antigens, α-fetoprotein, CEA, beta subunit of human chorionic gonadotropin *(Fig. 16)*, and many other substances,[276-279] and have contributed greatly to understanding of lymphocyte maturation and immunopathology of neoplasia.[287-305]

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

Summary

Immunohistochemical procedures have contributed greatly to our understanding of disease processes and have become a necessary tool in the evaluation of many disease states. Initial detection systems utilized IF markers. Enzyme immunohistochemical techniques developed during the past decade have circumvented many of the problems inherent in IF procedures.

This paper outlines the technical aspects and clinical diagnostic applications of enzyme-labeled immunohistochemistry. The availability of monoclonal antibodies and the adaptation of these reagents to immunohistochemistry systems will contribute greatly to further understanding of disease processes and will have continued utility in clinical diagnosis.

References

1. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. Proc Soc Exp Biol Med 1941; **47:** 200–2.
2. Coons AH, Kaplan MH. Localization of antigens in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. J Exp Med 1950; **91:** 1–30.
3. Nakane PK, Pierce GB, Jr. Enzyme-labeled antibodies: preparation and application for the localization of antigens. J Histochem Cytochem 1966; **14:** 929–31.
4. Sternberger LA. Immunocytochemistry. 2nd ed. New York: John Wiley & Sons, 1978.
5. Wisdom GB. Enzyme immunoassay. Clin Chem 1976; **22:** 1243–55.
6. Suffin SC, Muck KB, Young JC, Lewin K, Porter DD. Improvement of the glucose oxidase immunoenzyme technic. Use of a tetrazolium whose formazan is stable without heavy metal chelation. Am J Clin Pathol 1979; **71:** 492–6.
7. Boorsma DM, Streefkerk JG. Periodate or glutaraldehyde for preparing peroxidase conjugates? J Immunol Methods 1979; **30:** 245–55.
8. Murphy WM, Deodhar SD, Cawley LP. Use of horseradish peroxidase in identification of serum antibodies and immune complexes. Clin Chem 1973; **19:** 1370–3.
9. MacIver AG, Giddings J, Mepham BL. Demonstration of extracellular immunoproteins in formalin-fixed renal biopsy specimens. Kidney Int 1979; **16:** 632–6.
10. Huang SN, Minassian H, More JD. Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. Lab Invest 1976; **35:** 383–90.
11. Warnke R, Pederson M, Williams C, Levy R. A study of lymphoproliferative diseases comparing immunofluorescence with immunohistochemistry Am J Clin Pathol 1978; **70:** 867–75.
12. Warnke R. Alteration of immunoglobulin-bearing lymphoma cells by fixation. J Histochem Cytochem 1979; **27:** 1195–6.
13. Taylor CR. Immunoperoxidase techniques. Practical and theoretical aspects. Arch Pathol Lab Med 1978; **102:** 113–21.
14. Taylor CR. An immunohistological study of follicular lymphoma, reticulum cell sarcoma, and Hodgkin's disease. Eur J Cancer 1976; **12:** 61–75.
15. Tubbs RR, Sheibani K, Weiss RA, Sebek BA. Frozen section immunohistochemistry of malignant lymphomas using the direct immunoperoxidase procedure (abstract). Lab Invest 1980; **42:** 156.
16. Burns J, Hambridge M, Taylor CR. Intracellular immunoglobulins: A comparative study on three standard tissue processing methods using horseradish peroxidase and fluorochrome conjugates. J Clin Pathol 1974; **27:** 548–57.
17. Arnold W, Kalden JR, VonMayersback H. Influence of different histologic preparation methods on preservation of tissue antigens in the immunofluorescent antibody technique. Ann NY Acad Sci 1975; **254:** 27–34.
18. Miller HRP. Fixation and tissue preservation for antibody studies: A review. Histochem J 1972; **4:** 305–20.
19. Feltkamp VTM. Preparation of tissues and cells for immunohistochemical processing. Ann NY Acad Sci 1975; **254:** 21–6.
20. Davenport WD, Ball CR. Observation on the results of specific histochemical techniques and empirical staining methods on several tissue/organ types using a variety of fixing fluids. Histopathology 1979; **3:** 321–7.
21. Taylor CR. Immunohistological observations upon the development of reticulum cell

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

sarcoma in the mouse. J Pathol 1976; **118:** 201–19.

22. Elias JM, Chandor S, Miller F. Fixative effects on tissue antigenicity. Abstract presented at the spring meeting of The Histochemical Society, New Orleans, June 1980.
23. Levy R, Warnke R, Dorfman RF, Haimovich J. The monoclonality of human B-cell lymphomas. J Exp Med 1977; **145:** 1014–28.
24. Tubbs RR, Sheibani K, Sebek BA. Immunohistochemistry versus immunofluorescence for non-Hodgkin's lymphoma. Am J Clin Pathol 1980; **73:** 144–5.
25. Tubbs RR, Sheibani K, Weiss BA, Sebek BA. Immunohistochemistry of fresh frozen lymphoid tissue using the direct immunoperoxidase technique. Am J Clin Pathol 1981; **75:** 172–4.
26. Culling CF, Reid PE, and Sinnott NM. The effect of various fixatives and trypsin digestion upon the staining of routine paraffin-embedded sections by the peroxidase-anti-peroxidase and immunofluorescent technique. J Histochem 1980; **3:** 10–9.
27. Straus W. Factors affecting the cytochemical reaction of peroxidase with benzidine and stability of the blue reaction product. J Histochem Cytochem 1964; **12:** 462–9.
28. Mesulam MM. The blue reaction product in horseradish peroxidase neurohistochemistry: Incubation parameters and visibility. J Histochem Cytochem 1976; **24:** 1273–80.
29. Mesulam MM, Rosene DL. Differential sensitivity between blue and brown reaction products for HRP neurohistochemistry. Neuroscience Letters 1977; **5:** 7–14.
30. Occupational Safety and Health Administration Standards. Part 1910-1010. Title 29. Code of Federal Regulations, Federal Register, 1979: 3825–45.
31. Griswold DP, Casey AE, Weisburger EK, Weisburger JH. The carcinogenicity of multiple intragastric doses of aromatic heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res 1968; **28:** 924–33.
32. Mesulam MM, Rosene DL. Sensitivity in horseradish peroxidase neurohistochemistry: A comparative and quantitative study of nine methods. J Histochem Cytochem 1979; **27:** 763–6.
33. Lechago J, Sun NJ, Weinstein WM. Immunoperoxidase—Immunofluorescence combination for the simultaneous detection of two different antigens in the same tissue section (abstract). Lab Invest 1979; **40:** 268.
34. Sternberger LA, Joseph SA. The unlabeled antibody method. Contrasting color staining of paired pituitary hormones without antibody removal. J Histochem Cytochem 1979; **27:** 1424–9.
35. Joseph SA, Sternberger LA. The unlabeled antibody method. Contrasting color staining of β-lipoprotein and ACTH-associated hypothalamic peptides without antibody removal. J Histochem Cytochem 1979; **27:** 1430–7.
36. Lewin KJ, Suffin SC, Porter DD, Muck KB, Young JC. The glucose oxidase immunoenzyme technique (abstract). Lab Invest 1979; **40:** 269.
37. Banks PM. Diagnostic applications of an immunoperoxidase method in hematopathology. J Histochem Cytochem 1979; **27:** 1192–4.
38. Tubbs RR, Velasco M, Benjamin SP. Immunocytochemical identification of human chorionic gonadotropin. Arch Pathol Lab Med 1979; **103:** 534–6.
39. National Cancer Institute Carcinogenesis Technical report series. Bioassay of 3-amino-9-ethylcarbazole hydrochloride for possible carcinogenicity. 1978; No. **93:** 1–180.
40. Hanker JS, Yates PE, Metz CB, Rustioni A. A new specific sensitive and non-carcinogenic reagent for the demonstration of horseradish peroxidase. Histochem J 1977; **9:** 789–92.
41. Tubbs RR, Sheibani K. Chromogens for immunohistochemistry (letter). J Histochem Cytochem 1981; **29:** 684.
42. Reiner A, Gamin P. On noncarcinogenic chromogens for horseradish peroxidase histochemistry. J Histochem Cytochem 1980; **28:** 187–8.
43. Sonlag JM. Carcinogenicity of substituted benzenediamines (phenylenediamines) in rats and mice. JNCI 1981; **66:** 591–602.
44. Heyderman E. Immunoperoxidase technique in histopathology—applications, methods, and controls. J Clin Pathol 1979; **32:** 971–8.
45. Petrusz P, DiMeo P, Ordronneau P, Weaver C, Keefer DA. Improved immunoglobulin-enzyme bridge method for light microscopic demonstration of hormone-containing cells of the rat adenohypophysis. Histochemistry 1975; **46:** 9–26.
46. Vronvall G, Frommel D. Definition of staphylococcal protein A reactivity for human immunoglobulin G fragments. Immunochemistry 1970; **7:** 124.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

47. Dubois-Dalq M, McFarland H, McFarlin D. Protein-A peroxidase: A valuable tool for the localization of antigens. J Histochem Cytochem 1977; **25:** 1201–06.
48. Celio MR, Lutz H, Binz H, Fey H. Protein A in immunoperoxidase techniques. J Histochem Cytochem 1979; **27:** 691–3.
49. Notani GW, Parsons JA, Erlandsen SL. Versatility of staphylococcus aureus protein A in immunocytochemistry. Use in unlabeled antibody enzyme system and fluorescent methods. J Histochem 1974; **27:** 1438–44.
50. Guesdon JL, Ternynck T, and Avrameas S. The use of avidin-biotin interaction in immunoenzymatic techniques. J Histochem Cytochem 1979; **27:** 1131–9.
51. Hsu SM, Raine L, Fanger H. A comparative study of the PAP method and avidin-biotin-complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981; **75:** 734–8.
52. Fink B, Loepfe E, Wyler R. Demonstration of viral antigen in cryostat sections by a new immunoperoxidase procedure eliminating endogenous peroxidase activity. J Histochem Cytochem 1979; **27:** 1299–1301.
53. Straus W. Inhibition of peroxidase by methanol and by methanol-nitroferricyanide for use in immunoperoxidase procedures. J Histochem Cytochem 1971; **19:** 682–8.
54. Weir EE, Pretlow TG, Pitts A, Williams EE. Destruction of endogenous peroxidase in order to locate cellular antigens by peroxidase labeled antibodies (letter). J Histochem Cytochem 1974; **22:** 51–4.
55. Afroudakis AP, Liew CT, Peters RL. An *immunoperoxidase technic* for the demonstration of the hepatitis B surface antigen in human livers. Am J Clin Pathol 1976; **65:** 533–9.
56. Denk H, Syre G, Weirich E. Immunomorphologic methods in routine pathology. Application of immunofluorescence and the unlabeled antibody-enzyme (peroxidase-anti-peroxidase) technique to formalin fixed paraffin embeded kidney biopsies. Beitr Pathol 1977; **160:** 187–94.
57. Davey FR, Busch GJ. Immunohistochemistry of glomerulonephritis using horseradish peroxidase and fluorescein-labeled antibody: A comparison of two technics. Am J Clin Pathol 1970; **53:** 531–6.
58. Elias JM, Miller F. A comparison of the unlabeled enzyme method with immunofluorescence for the evaluation of human immunologic renal disease. Am J Clin Pathol 1975; **64:** 464–71.
59. Tubbs RR, Gephardt G, Valenzuela R, Deodhar SD. An approach to immunomicroscopy of renal disease with immunoperoxidase and periodic-acid-Schiff counterstain (*IMPAS stain*). Am J Clin Pathol 1980; **73:** 240–4.
60. Turner DR, Wilson DM, Lake A, Heaton JM, Leibowitz S, Cameron JS. An evaluation of the immunoperoxidase technique in renal biopsy diagnosis. Clin Nephrol 1979; **11:** 13–7.
61. Sheibani K, Tubbs RR, Gephardt GN, McMahon JT, Valenzuela R. Comparison of alternative chromogens for renal immunohistochemistry. Human Pathol 1981; **12:** 349–54.
62. Valenzuela R, Deodhar S. Atlas on interpretation of immunomicroscopic pattern in renal and skin diseases. Am Soc Clin Pathol. In press.
63. Qualman SJ, Keren DF. Immunofluorescence of deparaffinized trypsin-treated renal tissues. Preservation of antigens as an adjunct to diagnosis of disease. Lab Invest 1979; **41:** 483–9.
64. Choi YJ, Reiner L. Immunofluorescence of renal lesions in paraffin-embedded and fresh-frozen sections. Am J Clin Pathol 1980; **73:** 116–9.
65. Curran RC, Gregory J. The unmasking of antigens in paraffin sections of tissue by trypsin. Experientia 1977; **33:** 1400–1.
66. Turner DR, Wilson D, Cameron JS. Peroxidase-labelled IgG and complement in plastic embedded human renal tissue, in First International Symposium on Immunoenzymatic Techniques, *INSERM* Symposium No. 2 Feldmann G, Druet P, Bignon J, Avrameas S. eds., Amsterdam, North-Holland Publishing Company, 1976: 105–8.
67. Taylor CR, Russell R, Chandor S. An immunohistologic study of multiple myeloma and related conditions using an immunoperoxidase method. Am J Clin Pathol 1978; **70:** 612–22.
68. Taylor CR, Burns J. The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase labelled antibody. J Clin Pathol 1974; **27:** 14–20.
69. Lukes RJ. The immunologic approach to the pathology of malignant lymphomas. Am J Clin Pathol 1979; **72:** 657–69.
70. Mann RB, Jaffe ES, Berard CW. Malignant lymphomas—A conceptual understanding

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

of morphologic diversity. Am J Pathol 1979; **94:** 105–92.
71. Taylor CR. The nature of Reed-Sternberg cells and other malignant reticulum cells. Lancet 1974; **2:** 802–7.
72. Pinkus GS, Said J. Specific identification of intracellular immunoglobulin in paraffin sections of multiple myeloma and macroglobulinemia using an immunoperoxidase technique. Am J Pathol 1977; **87:** 47–58.
73. Aisenberg AC, Wilkes BM, Long JC, Harris NL. Cell surface phenotype in lymphoproliferative disease. Am J Med 1980; **68:** 206–13.
74. Pinkus GS, Said JW. Characterization of non-Hodgkin's lymphomas using multiple cell markers. Immunologic, morphologic and cytochemical studies of 72 cases. Am J Pathol 1978; **94:** 349–80.
75. Green I, Jaffe ES, Shevach EM, Edelson RL, Frank MM, Berard CW. Determination of origin of malignant reticular cells by the use of surface membrane markers: The Reticuloendothelial system—IAP monograph, No. 16, Rebuck JW and Berard CW, eds. Baltimore: Williams and Wilkins, 1974.
76. Gajl-Peczalski KJ, Kersey JH, Bloomfield C, Frizzera G. The value of combined CS and tissue frozen section studies in surface marker evaluation of non-Hodgkin's malignant lymphomas (abstract). Lab Invest 1979; **40:** 254.
77. Harris NL, Poppema S. Detection of surface immunoglobulin in malignant lymphomas with the immunoperoxidase technique (abstract). Lab Invest 1981; **44:** 27A.
78. Tubbs RR, Sheibani K, Weiss R, Sebek BA, Deodhar SD. Tissue immunomicroscopic evaluation of monoclonality of B cell lymphomas. Comparison with cell suspension studies. Am J Clin Pathol. In Press.
79. Bloomfield CD, Gajl-Peczalska KJ, Frizzera G, Kersey JH, Goldman AI. Clinical utility of lymphocyte surface markers combined with the Lukes-Collins histologic classification in adult lymphoma. N Engl J Med 1979; **301:** 512–18.
80. Mukai K, Rosai J. Application of Immunoperoxidase Techniques in Surgical Pathology. Progr Surg Pathol Vol. I, Fenoglio CM, Wolff M, eds. New York: Masson Publishing, 1980.
81. Papadimitriou CS, Muller-Hermelik U., Lennert K. Histologic and immunohistochemical findings in the differential diagnosis of chronic lymphocytic leukemia of B-cell type and lymphoplasmacytic/lymphoplasmacytoid lymphoma. Virchows Arch (Pathol Anat) 1979; **384:** 149–58.
82. Johansen P, Jensen MK. Enzymecytochemistry and immunohistochemistry in monoclonal gammopathy and reactive plasmacytosis. Acta Path Microbiol Scand 1980; **88:** 377–82.
83. Taylor CR. Immunohistological approach to tumor diagnosis. Oncology 1978; **35:** 189–97.
84. Mori N, Masafumi ABE, Kojima M. Study of malignant lymphomas from the aspect of immunoglobulin production. Acta Pathol Jpn 1979; **29:** 705–22.
85. Halliday D, Davey FR, Marucci AA. Detection of intracellular immunoglobulin in nodular lymphomas. Am J Clin Pathol 1978; **69:** 587–93.
86. Morgan TW, Banks PM. Large cell neoplasia: An evaluation of criteria for the distinction of lymphoid from epithelial malignancies (abstract). Lab Invest 1979; **40:** 273.
87. Li CY, Harrison EG. Histochemical and immunohistochemical study of diffuse large-cell lymphomas. Am J Clin Pathol 1978; **70:** 721–32.
88. Sun NC, Fishkin BG, Nies KM, Glassy EF, Carpentier C. Lymphoplasmacytic myeloma. An immunological, immunohistochemical and electron microscopic study. Cancer 1979; **43:** 2268–78.
89. Scott FE, Dupont PA, Webb JW. Plasmacytoma of the stomach. Diagnosis with the aid of the immunoperoxidase technique. Cancer 1978; **41:** 675–81.
90. Mancilla R, Davis GL. Nonsecretory multiple myeloma. Immunohistologic and ultrastructural observations on two patients. Am J Med 1977; **63:** 1015–22.
91. Rambaud JC, Modigliani R, Nguyen Phuoc BK, et al. Non-secretory alpha-chain disease in intestinal lymphoma (letter). N Engl J Med 1980; **303:** 53.
92. Levitt LJ, Dawson DM, Rosenthal DS, Moloney WC. CNS involvement in the non-Hodgkin's lymphomas. Cancer 1980; **45:** 545–52.
93. Clausen PP, Jacobson M, Johansen P, Thommesen N. Immunohistochemical demonstration of intracellular immunoglobulin in formalin fixed, paraffin embedded sections, as staining method in diagnostic work. Acta Pathol Microbiol Scand (C), 1979; **87:** 308–12.
94. Woda BA, Knowles DM. Nodular lymphocytic lymphoma eventuating into diffuse histiocytic lymphoma. Immunoperoxidase detection of monoclonality. Cancer 1979; **43:** 303–7.
95. Vernon S, Voet RL, Naeim F, Waisman J.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

Nodular lymphoma with intracellular immunoglobulin. Cancer 1979; **44:** 1273–9.
96. Haghighi P, Tabei Z, Kharazmi A, Gerami S, Abadi P, Haghsenass M. Immunoperoxidase study in α-chain disease. Arch Pathol Lab Med 1978; **102:** 555–7.
97. Knowles DM, Shevchuk M. Pleomorphic reticulum cell sarcoma, monoclonal gammopathy and amyloidosis. An immunoperoxidase study. Cancer 1978; **41:** 1883–9.
98. Al-Saleem T, Al-Qadiry W, Issa F, King J. The immunoselection technic in laboratory diagnosis of alpha heavy-chain disease (letter). Am J Clin Pathol 1979; **72:** 132–3.
99. Morris JA, Bird CC. Ultrastructural and immunohistological study of immunoblastic sarcoma developing in child with immunoblastic lymphadenopathy. Cancer 1979; **44:** 171–82.
100. Isaacson P. Middle east lymphoma and alpha-chain disease. An immunohistochemical study. Am J Surg Pathol 1979; **13:** 431–41.
101. Isaacson P, Wright DH, Judd MA, Jones DB, Payne SV. The nature of the immunoglobulin-containing cells in malignant lymphoma: An immunoperoxidase study. J Histochem Cytochem 1980; **28:** 761–70.
102. Pangalis GA, Nathwani BN, Rappaport H. Detection of cytoplasmic immunoglobulin in well-differentiated lymphoproliferative diseases by the immunoperoxidase method. Cancer 1980; **45:** 1334–9.
103. van den Tweel JG, Taylor CR, Parker JW, Lukes RJ. Immunoglobulin inclusions in non-Hodgkin's lymphomas. Am J Clin Pathol 1978; **69:** 306–13.
104. Taylor CR. Immunohistologic studies of lymphomas past, present and future. J Histochem Cytochem 1980; **28:** 777–87.
105. Callihan TR, Braylan RC, Farnham R, Jaffe ES, Soban EJ, Berard CW. Correlation between immunohistochemistry and cell surface markers in diffuse large cell (histiocytic) lymphomas (abstract). Lab Invest 1979; **40:** 244.
106. Nisonoff A, Hopper JE, Spring SB. The antibody molecule. New York: Academic Press, Inc., 1975: 542.
107. Warnke R, Levy R. Immunopathology of follicular lymphomas. A model of β-lymphocyte homing. N Engl J Med 1978; **298:** 481–6.
108. Warnke R, Levy R, Pederson M, Dorfman RF. Tissue section immunofluorescence in the investigation of lymphoproliferative diseases (abstract). Lab Invest 1979; **40:** 291.
109. Stein H, Bonk A, Tolksdorf G, Lennert K, Rodt H, Gerdes J. Immunohistologic analysis of the organization of normal lymphoid tissue and non-Hodgkin's lymphomas. J Histochem Cytochem 1980; **28:** 746–60.
110. Tubbs RR, Sheibani K, Weiss RA, Lee V, Sebek BA, Valenzuela R. Immunohistochemistry of Warthin's tumor. Am J Clin Pathol 1980; **74:** 795–7.
111. Palutke M, Schnitzer B, Mirchandani I, Tabaczka P, So K, Garrillo G. Monoclonal lymphoid populations in lymph nodes with reactive hyperplasia (abstract). Lab Invest 1981; **44:** 60A.
112. Cooper MD. Immunologic analysis of lymphoid tumors. N Engl J Med 1980; **302:** 964–5.
113. Knowles DM, Halper JP. Ia antigen expression by human malignant lymphomas: Correlation with conventional lymphoid markers (abstract). Lab Invest 1980; **42:** 129.
114. Billing R, Rafizadeh B, Drew I, Hartman G, Gale R, Terasaki P. Human β-lymphocyte antigens expressed by lymphocytic and myelocytic leukemia cells. J Exp Med 1976; **144:** 167–78.
115. Yamanaka N, Ishii Y, Koshiba H, Mikuni C, Konno M, Kikuchi K. A study of surface markers in acute lymphocytic leukemia by using anti-T and anti-B lymphocyte sera. Cancer 1978; **42:** 2641–7.
116. Halper JP, Knowles DM, YiWang C. Ia antigen expression by human malignant lymphomas: Correlation with conventional lymphoid markers. Blood 1980; **55:** 373–82.
117. Isaacson P. Immunochemical demonstration of J Chain: A marker of B-cell malignancy. J Clin Pathol 1979; **32:** 802–7.
118. Mestecky J, Preud'homme JL, Crago SS, Mihaesco E, Prchal JT, Okos AJ. Presence of J chain in human lymphoid cells. Clin Exper Immunol 1980; **39:** 371–85.
119. Collins RD, Waldron JA, Glick AD. Results of multiparameter studies of T-cell lymphoid neoplasms. Am J Clin Pathol 1979; **72:** 699–707.
120. Warnke R, Levy R. Detection of T and B cell antigens with hybridoma monoclonal antibodies: A biotin-avidin-horseradish peroxidase method. J Histochem Cytochem 1980; **28:** 771–6.
121. Reinherz EL, Moretta L, Roper M, et al. Human T-Lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies: A comparison. J Exp Med 1980; **151:** 969–74.
122. Reinherz EL, Kung PC, Goldstein G, Schlossman SF. A monoclonal antibody with

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

selective reactivity with functionally mature human thymocytes and all peripheral human T cells. J Immunol 1979; **123:** 1312–7.

123. Kung PC, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T-cell surface antigens. Science 1979; **206:** 347–9.
124. Reinherz EL, Kung PC, Goldstein G, Schlossman SF. A monoclonal antibody reactive with the human cytotoxic/suppressor T-cell subset previously defined by a heteroantiserum termed TH_2. J Immunol 1980; **124:** 1301–7.
125. Reinherz EL, Kung PC, Goldstein G, Schlossman SF. Separation of functional subsets of human T-cells by a monoclonal antibody. Proc Natl Acad Sci USA 1979; **76:** 40612–5.
126. Royston I, Majda JA, Baird S, Meserve B, Griffiths J. Monoclonal antibody for human T-lymphocytes: Identification of normal and malignant T-cells. Blood 1979; **54**(Suppl I): 106a.
127. Warnke R, Miller R, Grogan T, Pederson M, Dilley J, Levy R. Immunologic phenotypes in 30 patients with diffuse large cell lymphoma. N Engl J Med 1980; **303:** 293–300.
128. Aisenberg AC, Wilkes BM. Unusual human lymphoma pheotype defined by monoclonal antibody. J Exp Med 1980; **152:** 1126–31.
129. Tubbs RR, Weiss RA, Savage RA, Sebek BA, Weick JK. Determination of immunologic phenotypes of large cell lymphoma using monoclonal antibodies (abstract). Lab Invest 1981; **44:** 69A.
130. Tubbs RR, Sheibani K, Sebek BA, Savage RA. Malignant histiocytosis. Ultrastructural and immunocytochemical characterization. Arch Pathol Lab Med 1980; **104:** 26–9.
131. Isaacson P, Wright DH. Intestinal lymphoma associated with malabsorption. Lancet 1978; **1:** 67–70.
132. Buchner SA, Rufli T. Malign histiozytose mit hautmanifestationen. Enzymzytochemische und immunzytologische. 1980; Nr. II, 14. Marz, 373–7.
133. Tubbs RR, Sheibani K, Savage RA, Sebek BA. Muramidase an immunohistochemical marker of malignant histiocytosis (letter). Human Pathol 1979; **10:** 483.
134. Meister P, Nathrath W. Immunohistochemical markers of histiocytic tumors. Human Pathol 1980; **11:** 300–1.
135. Mendelsohn G, Eggleston JC, Mann RB. Relationship of lysozyme (muramidase) to histiocytic differentiation in malignant histiocytosis. An immunohistochemical study. Cancer 1980; **45:** 273–9.
136. Meister P, Huhn D, Nathroth W. Malignant histiocytosis. Immunohistochemical characterization on paraffin embedded tissue. Virchows Arch (Pathol Anat) 1980; **385:** 233–46.
137. Ree HJ, Song JY, Leone LA, Crowley JP, Fanger H. Occurrence and patterns of muramidase containing cells in Hodgkin's disease, non-Hodgkin's lymphomas and reactive hyperplasia. Human Pathol 1981; **12:** 49–59.
138. Strass SA, Schumacher HR, Keneklis TP, Bollum FJ. Terminal deoxynucleotidyl transferase immunofluorescence of bone marrow smears. Am J Clin Pathol 1979; **72:** 898–903.
139. Janossy G, Hoffbrand AV, Greaves MF, et al. Terminal transferase enzyme assay and immunological membrane markers in the diagnosis of leukemia: A multiparameter analysis of 300 cases. Br J Haematol 1980; **44:** 221–34.
140. Pattengale PK, Taylor CR, Engvall E, Ruoslahti E. Direct tissue visualization of normal cross-reacting antigen in neoplastic granulocytes. Am J Clin Pathol 1980; **73:** 351–5.
141. Knowles DM, Jakobiec F. Orbital lymphoid neoplasms: A clinicopathologic study of 60 patients (abstract). Lab Invest 1980; **42:** 129.
142. Brubaker DB, Whiteside TL. Differentiation between benign and malignant human lymph nodes by means of immunologic markers. Cancer 1979; **43:** 1165–76.
143. Astarita RW, Minckler D, Taylor CR, et al. Orbital and adnexal lymphomas. Am J Clin Pathol 1980; **73:** 615–21.
144. Tubbs RR, Sheibani K, Weiss R, Sebek BA. Reactive lymphoid hyperplasia versus non-Hodgkin's lymphoma: Role of immunohistochemistry (abstract). Am J Clin Pathol 1980; **74:** 501.
145. Poppema S, Elema JD, Halie MR. The significance of intracytoplasmic proteins in Reed-Sternberg cells. Cancer 1978; **42:** 1793–1803.
146. Garvin AJ, Spicer SS, Parmley RT, Munster AM. Immunohistochemical demonstration of IgG in Reed-Sternberg and other cells in Hodgkin's disease. J Exp Med 1974; **139:** 1077–83.
147. Anagnostou D, Parker JW, Taylor CR, et al. Lacunar cells of nodular sclerosing Hodgkin's disease. An ultrastructural and immu-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

nohistologic study. Cancer 1977; **39:** 1032–43.
148. Parmley RT, Spicer SS, Morgan SK, Grush OC. Hodgkin's disease and myelomonocytic leukemia. An ultrastructural and immunocytochemical study. Cancer 1976; **38:** 1188–98.
149. Kaplan HS, Gartner S. Sternberg-Reed giant cells of Hodgkin's disease: Cultivation in vitro, heterotransplantation and characterization as neoplastic macrophages. Int J Cancer 1977; **19:** 511–25.
150. Kadin ME, Stites DP, Levy P, Warnke R. Exogenous immunoglobulin and macrophage origin of Reed-Sternberg cells in Hodgkin's disease. N Engl J Med 1978; **299:** 1208–14.
151. Wolfe HJ, Melvin KE, Cervi-Skinner S. C cell hyperplasia preceding medullary thyroid carcinoma. N Engl J Med 1973; **289:** 437–41.
152. Burt A, Goudie RB. Diagnosis of primary thyroid carcinoma by immunohistological demonstration of thyroglobulin. Histopathology 1979; **3:** 279–86.
153. Bocker W, Dralle H, Husselmann H, Bay V, Brassow M. Immunohistochemical analysis of thyroglobulin synthesis in thyroid carcinomas. Virchows Arch (Pathol Anat) 1980; **385:** 187–200.
154. Halmi NS, Duello T. Acidophilic pituitary tumors. A reappraisal with differential staining and immunocytochemical techniques. Arch Pathol Lab Med 1976; **100:** 346–51.
155. Veldhuis JD, Green JE, Kovacs E, Worgul TJ, Murray FT, Hammond JM. Prolactin-secreting pituitary adenomas. Association with multiple endocrine neoplasia, Type I. Am J Med 1979; **67:** 830–6.
156. Carter JN, Tyson JE, Tolis G, VanVliet S, Faiman C, Friesen HG. Prolactin-secreting tumors and hypogonadism in 22 men. N Engl J Med 1978; **299:** 847–52.
157. Fukaya T, Kageyama N, Kuwayama A, et al. Morphofunctional study of pituitary adenomas with acromegaly by immunoperoxidase technique and electron microscopy. Cancer 1980; **45:** 1598–1603.
158. Horvath E, Kovacs K, Singer W, et al. Acidophil stem cell adenoma of the human pituitary (abstract). Lab Invest 1980; **42:** 122.
159. Duello T, Halmi N. Acidophil stem cell adenomas of the pituitary. Arch Pathol Lab Med 1978; **102:** 439.
160. Horvath E, Kovacs K, Singer W, Ezrin C, Kerenyi NA. Acidophil stem cell adenoma. Arch Pathol Lab Med 1977; **101:** 594–9.
161. Tyrrell JB, Brooks RM, Fitzgerald PA, Cofoid PB, Forsham PH. Cushing's disease. Selective trans-sphenoidal resection of pituitary microadenomas. N Engl J Med 1978; **298:** 753–8.
162. Levine JH, Sagel J, Rosebrock G, et al. Prolactin-secreting adenoma as part of the multiple endocrine neoplasia-Type I. (Men-I) syndrome. Cancer 1979; **43:** 2492–6.
163. Horvath E, Kovacs K. Histologic, immunocytologic and fine structural findings in pituitary adenomas associated with the multiple endocrine neoplasia syndrome (MENS) (abstract). Lab Invest 1979; **40:** 261.
164. Mukai K, Wolseth DG, Bonner RA, Oppenheimer JH. Pituitary carcinoma with liver metastasis: An immunohistochemical study (abstract). Lab Invest 1980; **42:** 138.
165. Hassoun J, Charpin C, Jaquet P, et al. Analogies immunocytocehmi ques des adenomas hypophysaires de la maladie de cushing et des adenomes "non fonctionnels" (adenomas chromophobes) de l'hypophyse. Annals d'endocrinologie (Paris), 1979; **40:** 559–60.
166. Landot AM. Pituitary adenomas. Clinicomorphologic correlations. J Histochem Cytochem 1979; **27:** 1395–7.
167. Bergeron C, Kovacs K. Pituitary siderosis. A histologic, immunocytologic and ultrastructural study. Am J Pathol 1978; **93:** 295–310.
168. McKeel DW, Askin FB. Ectopic hypophyseal hormonal cells in benign cystic teratoma of the ovary. Arch Pathol Lab Med 1978; **102:** 122–8.
169. Skrabanek P, Powell D. Unifying concept of non-pituitary ACTH-secreting tumors. Evidence of common origin of neural-crest tumors, carcinoids, and oat-cell carcinomas. Cancer 1978; **42:** 1263–9.
170. Spark RF, Connolly PB, Gluckin DS, White BA, Sacks B, Landsberg L. ACTH secretion from a functioning pheochromocytoma. N Engl J Med 1979; **301:** 416–8.
171. Cohle SD, Tschen JA, Smith FE, et al. ACTH-secreting carcinoma of the breast. Cancer 1979; **43:** 2370–6.
172. Falkmer S. Immunocytochemical studies of the evolution of islet hormones. J Histochem Cytochem 1979; **27:** 1281–2.
173. Larsson LI. Endocrine pancreatic tumors. Human Pathol 1978; 9:401–16.
174. Jaffe R, Hashida Y, Yunis EJ. Pancreatic pathology in hyperinsulinemic hypoglycemia of infancy. Lab Invest 1980; **42:** 356–66.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

175. Grube D, Maier V, Raptis S, Schlegel W. Immunoreactivity of the endocrine pancreas. Evidence for the presence of cholecystokinin-pancreozymin within the A-cell. Histochemistry 1978; **56:** 13–35.
176. Ole-MoiYoi O, Pinkus GS, Spragg J, Austen KF. Identification of human glandular kallikrein in the beta cell of the pancreas. N Engl J Med 1979; **300:** 1289–94.
177. Nieuwenhuijzen Kruseman AC, Knijnenburg G, Erutel de la Riviere G, Bosman FT. Morphology and immunohistochemically-defined endocrine function of pancreatic islet cell tumors. Histopathology 1978; **2:** 389–99.
178. Kurman RJ, Andrade D, Goebelsmann U, Taylor CR. An immunohistological study of steroid localization in Sertoli-Leydig tumors of the ovary and testis. Cancer 1978; **42:** 1772–83.
179. Kurman RJ, Goebelsmann U, Taylor CR. Steroid localization in granulosa theca tumors of the ovary. Cancer 1979; **43:** 2377–84.
180. Taylor CR, Kurman RJ, Warner NE. The potential value of immunohistologic techniques in the classification of ovarian and testicular tumors. Human Pathol 1978; **9:** 417–27.
181. Maurer R, Taylor CR, Schmucki O, Hedinger CE. Extratesticular gonodal stromal tumor in the pelvis. A case report with immunoperoxidase findings. Cancer 1980; **45:** 985–90.
182. Dayal Y, O'Brian DS, Wolfe HJ, Reichlin S. Carcinoid tumors: A comparison of their immunocytochemical hormonal profile with morphologic and histochemical characteristics (abstract). Lab Invest 1980; **42:** 111.
183. Leav I, Savage P, Rule A, De Lellis RA, Merk FB. Immunohistochemical detection of human prostatic acid phosphotase (abstract). Lab Invest 1979; **40:** 267.
184. Jobsis Ac, DeVries GP, Anholt RR, Sanders GT. Demonstration of the prostatic origin of metastases. An immunohistochemical method for formalin-fixed embedded tissue. Cancer 1978; **41:** 1788–93.
185. Nadji M, Tabei SZ, Castro A, Chu TM, Morales AR. Prostatic origin of tumors. An immunohistochemical study. Am J Clin Pathol 1980; **73:** 735–9.
186. Skinner MS, Seckinger D. Evaluation of beta-subunit chorionic gonadotropin as an aid in diagnosis of trophoblastic disease. Ann Clin Lab Sci 1979; **9:** 347–52.
187. Manley PN, Mahan DE, Bruce AW, Kipkie GF, Franchi L. Postradiotherapy biopsy of prostatic adenocarcinoma: A light and immunohistochemical study (abstract). Lab Invest 1980; **42:** 132.
188. Nadji M, Tabei SZ, Castro A, Ming Chu T, Wang MC, Morales AR. Prostatic specific antigen. An immunohistologic marker for prostatic neoplasms. Cancer. In Press.
189. Yorde DE, Hussa RO, Garancis JC, Pattillo RA. Immunocytochemical localization of human choriogonadotropin in human malignant trophoblast. Model for human choriogonadotropin secretion. Lab Invest 1979; **40:** 391–8.
190. Nieumenhuijzen-Kruseman AC, VanLent M, Blom AH, Lauw GP. Choriocarcinoma in mother and child, identified by immunoenzyme histochemistry. Am J Clin Pathol 1977; **67:** 679–83.
191. Sheibani K, Tubbs RR, Velasco ME, Benjamin S. Immunocytochemical identification of human chronic gonadotropin (hCG) (abstract). Lab Invest 1979; **40:** 284A.
192. Von Eyben FE. Biochemical markers in advanced testicular tumors. Serum lactate dehydrogenase, urinary chorionic gonadotropin and total urinary estrogens. Cancer 1978; **41:** 648–52.
193. Stepanas AV, Samaan NA, Schultz PN, Holoye PY. Endocrine studies in testicular tumor patients with and without gynecomastia. A report of 45 cases. Cancer 1978; **41:** 369–76.
194. Schultz H, Sell A, Norgaard-Pedersen, Arends J. Serum alpha-feto-protein and human chorionic gonadotropin as markers for the effect of postoperative radiation therapy and/or chemotherapy in testicular cancer. Cancer 1978; **42:** 2182–6.
195. Javadpour N, McIntire KR, Waldmann TA. Human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) in sera and tumor cells of patients with testicular seminoma. A prospective study. Cancer 1978; **42:** 2768–72.
196. Javadpour N. Serum and cellular biologic tumor markers in patients with urologic cancer. Human Pathol 1979; **10:** 557–68.
197. Javadpour N. Significance of elevated serum alpohafetoprotein (AFP) in seminoma. Cancer 1980; **45:** 2166–8.
198. Kurman RJ, Scardino PT, McIntire KR, Waldmann TA, Javadpour N. Cellular localization of alpha-fetoprotein and human chorionic gonadotropin in germ cell tumors of the testis using an indirect immunoperox-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

idase technique. A new approach to classification utilizing tumor markers. Cancer 1977; **40:** 2136–51.
199. Javadpour N, McIntire KR, Waldmann TA, Bergman SM. The role of alpha-fetoprotein and human chorionic gonadotropin in seminoma. J Urol 1978; **120:** 687–90.
200. Talerman A, Haije WG, Baggerman L. Serum alphafetoprotein (AFP) in diagnosis and management of endodermal sinus (yolk sac) tumor and mixed germ cell tumor of the ovary. Cancer 1978; **41:** 272–8.
201. Stachura I, Mendelow H. Endodermal sinus tumor originating in the region of the pineal gland. Ultrastructural and immunohistochemical study. Cancer 1980; **45:** 2131–7.
202. Norgaard-Pedersen B, Lindholm J, Albrechtsen R, Arends J, Diemer NH, Riishedo J. Alpha-fetoprotein and human chorionic gonadotropin in a patient with a primary intracranial germ cell tumor. Cancer 1978; **41:** 2315–20.
203. Borkowski A, Muquardt C. Human chorionic gonadotropin in the plasma of normal, nonpregnant subjects. N Engl J Med 1979; **301:** 298–302.
204. Gerber MA, Thung SN, Euzenir S. Emergence of heterogeneous cell populations in hepatocellular carcinoma (abstract). Lab Invest 1979; **40:** 256.
205. Odell WD, Wolfsen AR. Hormones from tumors: Are they ubiquitous? Am J Med 1980; **68:** 317–8.
206. Franchimont P, Zangerle PF. Present and future clinical relevance of tumour markers. Eur J Cancer 1977; **13:** 637–46.
207. Rutanen EM, Seppala M. The HCG-beta subunit radioimmunoassay in nontrophoblastic gynecologic tumors. Cancer 1978; **41:** 692–6.
208. Hattori M, Fukase M, Yoshimi H, Matsukura S, Imura H. Ectopic production of human chorionic gonadotropin in malignant tumors. Cancer 1978; **42:** 2328–33.
209. Bellet D, Arrang JM, Contesso G, Caillaud JM, Bohuon C. Localization of the β-subunit of human chorionic gonadotrophin on various tumors. Eur J Cancer 1980; **16:** 433–9.
210. Bender RA, Weintraub BD, Rosen SW. Prospective evaluation of two tumor-associated proteins in pancreatic adenocarcinoma. Cancer 1979; **43:** 591–5.
211. Hattori M, Imura H, Matsukuba, S, et al. Multiple-hormone producing lung carcinoma. Cancer 1979; **43:** 2429–37.
212. Woo KB, Waalkes TP, Ahmann DL, Tormey DC, Gehrke CW, Oliverio VT. A quantitative approach to determining disease response during therapy using multiple biologic markers. Application to carcinoma of the breast. Cancer 1978; **41:** 1685–1703.
213. Primus FJ, Wang RH, Sharkey RM, Goldenberg DM. Detection of carcinoembryonic antigen in tissue sections by immunoperoxidase. J Immunol Methods 1975; **8:** 267–76.
214. Wang NS, Huang SN, Gold P. Absence of carcinoembryonic antigen-like material in mesothelioma. An immunohistochemical differentiation from other lung cancers. Cancer 1979; **44:** 937–43.
215. Falkson HC, Van der Watt JJ, Portugal MA, Falkson G. Carcinoembryonic antigen in patients with breast cancer. An adjunctive tool to monitor response and therapy. Cancer 1978; **42:** 1308–13.
216. Wahren B, Lidbrink E, Wallgren A, Eneroth P, Jajicek J. Carcinoembryonic antigen and other tumor markers in tissue and serum or plasma of patients with primary mammary carcinoma. Cancer 1978; **42:** 1870–8.
217. Ellis DJ, Speirs C, Kingston RD, Brookes VS, Leonard J, Dykes PW. Carcinoembryonic antigen levels in advanced gastric carcinoma. Cancer 1978; **42:** 623–5.
218. Ejeckan GC, Huang SN, McCaughey WT, Gold P. Immunohistopathological study on carcinoembryonic antigen (CEA)-like material and immunoglobulin A in gastric malignancies. Cancer 1979; **44:** 1606–14.
219. Evans JT. Carcinoembryonic antigen in prognosis of colorectal cancer (letter). N Engl J Med 1978; **299:** 1369.
220. Al-Sarraf M, Baker L, Talley RW, Kithier K, Vaitkevicius VK. The value of serial carcinoembryonic antigen (CEA) in predicting response rate and survival of patients with gastrointestinal cancer treated with chemotherapy. A Southwest Oncology group study. Cancer 1979; **44:** 1222–5.
221. Jubert AV, Talbott TM, Maycroft TM. Characteristics of adenocarcinomas of the colorectum with low levels of preoperative plasma carcinoembryonic antigen (CEA). Cancer 1978; **42:** 635–9.
222. Isaacson P. Tissue demonstration of carcinoembryonic antigen (CEA) in ulcerative colitis. Gut 1976; **17:** 561–7.
223. O'Brien MJ, Burke B, Zamcheck M, Gottlieb LS. An assessment of diagnostic value of immunocytochemical demonstration of CEA in benign and malignant colonic mucosa (abstract). Lab Invest 1979; **40:** 276.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

224. Rutanen EM, Lindgren J, Sipponen P, Stenman UH, Saksela E, Seppala M. Carcinoembryonic antigen in malignant and nonmalignant gynecologic tumors. Cancer 1978; **42:** 581–90.
225. VanNagell JR, Donaldson ES, Gay EC, Sharkey RM, Rayburn P, Goldenberg DM. Carcinoembryonic antigen in ovarian epithelial cystadenocarcinomas. The prognostic value of tumor and serial plasma determinations. Cancer 1978; **41:** 2335–40.
226. Horne CH, Towler CM, Milne GD. Detection of pregnancy specific β_1-glycoprotein in formalin-fixed tissues. J Clin Pathol 1977; **30:** 19–23.
227. Marchand A, Fenoglio CM, Pascal R, Richart RM, Bennett S. Carcinoembryonic antigen in human ovarian neoplasms. Cancer Res 1975; **35:** 3807–10.
228. Heald J, Buckley CH, and Fox H. An immunohistochemical study of the distribution of carcinoembryonic antigen in epithelial tumours of the ovary. J Clin Pathol 1979; **32:** 918–26.
229. Spiegelman S, Keydar I, Mesa-Tejada R, et al. Possible diagnostic implications of a mammary tumor virus related protein in human breast cancer. Cancer 1980; **46:** 879–92.
230. Witkin SS, Sarkar NH, Kinne DW, Good RA, Day NK. Antibodies reactive with the mouse mammary tumor virus in sera of breast cancer patients. Int J Cancer 1980; **25:** 721–5.
231. Johnson JD, Lamm DL. Prediction of bladder tumor invasion with the mixed cell agglutination test. J Urol 1980; **123:** 25–8.
232. Cummings KB. Carcinoma of the bladder: Predictors. Cancer 1980; **45:** 1849–55.
233. Limas C, Lange P, Fraley EE, Vessella RL. A, B, H antigens in transitional cell tumors of the urinary bladder. Correlation with the clinical course. Cancer 1979; **44:** 2099–107.
234. Weinstein RS, Alroy J, Farrow GM, Miller AW, Davidsohn I. Blood group isoantigen deletion in carcinoma in situ of the urinary bladder. Cancer 1979; **43:** 661–8.
235. Cooper HS, Cox J, Patchefsky AS. Immunohistologic study of blood group substances in polyps of the distal colon. Expression of a fetal antigen. Am J Clin Pathol 1980; **73:** 345–50.
236. Strauchen JA, Bergman SM, Hanson TA. Expression of A and B tissue isoantigens in benign and malignant lesions of the breast. Cancer 1980; **45:** 2149–55.
237. Theodoropoulos G, Nakopoulou L, Repanti M, Papcharalmpous N, Melissinos K. Detection of hepatitis B surface antigen in fixed tissues of patients with cirrhosis and hepatoma. Virchows Arch (Pathol Anat) 1979; **382:** 293–300.
238. Burns J. Immunoperoxidase localisation of hepatitis B antigen (HB) in formalin-paraffin processed liver tissue. Histochemistry 1975; **44:** 133–5.
239. Wu PC, Lam KC. Cytoplasmic hepatitis B surface antigen and the ground glass appearance in hepatocellular carcinoma. Am J Clin Pathol 1979; **71:** 229–34.
240. Sumithran E. Methods for detection of hepatitis B surface antigen in paraffin sections of liver: A guideline for their use. J Clin Pathol 1976; **30:** 460–3.
241. Tapp E, Jones M. HbsAg and HBcAg in the livers of asymptomatic hepatitis B antigen carriers. J Clin Pathol 1977; **30:** 671–7.
242. Krugman S, Overby LR, Mushahwar IK, Ling CM, Frosner GG, Deinhardt F. Viral hepatitis, Type B; studies on natural history and prevention re-examination. N Engl J Med 1979; **300:** 101–6.
243. Norkrans G, Frosner G, Iwarson S. Determination of HBeAg by radioimmunoassay prognostic implication in hepatitis B. Scand J Gastroenterol 1979; **14:** 289–93.
244. Trichopoulos D. Hepatitis B virus and hepatocellular carcinoma (letter). Lancet 1979; **1:** 1192.
245. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol 1980; **73:** 626–32.
246. Kabiri M, Tabor E, Gerety RJ. Antigen-antibody system associated with non-A, non-B hepatitis detected by indirect immunofluorescence. Lancet 1979; **2:** 221–4.
247. Willaert E, Stevens AR, Healy GR. Retrospective identification of *Acanthamoeba culbertsoni* in a case of amoebic meningoencephalitis. J Clin Pathol 1978; **31:** 717–20.
248. Culbertson CG. Soil ameba infection; specific indirect immunoenzymatic (peroxidase) staining of formalin-fixed paraffin sections. Am J Clin Pathol 1975; **63:** 475–82.
249. Capron A, Dugimont JC, Fruit J, Bout D. Application of immunoenzyme methods in diagnosis of human parasitic diseases. Ann NY Acad Sci 1975; **254:** 331.
250. Benjamin DR, Ray GC. Use of immuno-

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

peroxidase on brain tissue for the rapid diagnosis of herpes encephalitis. Am J Clin Pathol 1975; **64:** 472–6.

251. Hansen BL, Hansen GN, Vestergaard BF. Immunoelectron microscopic localization of herpes simplex virus antigens in infected cells using the unlabeled antibody-enzyme method. J Histochem Cytochem 1979; **11:** 1455–61.
252. Gerber MA, Shah KV, Thung SN, Zu Rhein G. Immunohistochemical demonstration of common antigen of polyomaviruses in routine histologic tissue sections of animals and man. Am J Clin Pathol 1980; **73:** 794–7.
253. Drew WL, Mintz L. Rapid diagnosis of varicella-zoster virus infection by direct immunofluorescence. Am J Clin Pathol 1980; **73:** 699–701.
254. Foidart JM, Bere EW, Yaar M, et al. Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab Invest 1980; **42:** 336–42.
255. Campbell JA, Kirsch RE. Immunohistological localization of ligandin in human tissues. Cancer 1980; **45:** 503–10.
256. Nadji M, Gonzalez MS, Castro A, Morales AR. Factor VIII-related antigen: An endothelial cell marker (abstract). Lab Invest 1980; **42:** 139.
257. Duchesne PY, Gheuens J, Brotchi J, Gerebtzoff. Normal and reactive astrocytes: A comparative study by immunohistochemistry and by a classical histological technique. Cell Mol Biol 1979; **24:** 237–9.
258. Velasco ME, Dahl D, Roessmann U, Gambetti P. Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms. Cancer 1980; **45:** 484–94.
259. Bender BL, Yunis EJ. Ultrastructural histochemical and immunohistochemical studies of the central nervous system lesions of tuberous sclerosis (abstract). Lab Invest. 1980; **42:** 169.
260. Jurco S, Harvey DG. The nature of the stromal cell in hemangioblastomas: Immunohistochemical studies (abstract). Lab Invest 1980; **42:** 127.
261. Heitz PV, Wegman W. Identification of neoplastic paneth cells in an adenocarcinoma of stomach using lysozyme as a marker and electron microscopy. Virchows Arch (Pathol Anat) 1980; **386:** 107–16.
262. Bussolati G, Alfani V, Weber K, Osborn M. Immunocytochemical detection of actin in fixed and embedded tissue: Its potential use in routine pathology. J Histochem Cytochem 1980; **28:** 169–73.
263. Bussolati G. Actin-rich (myoepithelial) cells in lobular carcinoma in situ of the breast. Virchows Arch (Cell Path) 1980; **32:** 165–76.
264. Kaplan CG, Blanc WA, Elias JM. Identification of fetal red cells in placental thrombi by peroxidase-antiperoxidase (PAP) immunoperoxidase (abstract). Lab Invest. 1980; **42:** 173.
265. Mukai K, Rosai J, Halloway BE. Localization of myoglobin in normal and neoplastic human skeletal muscle cells using an immunoperoxidase method. Am J Surg Pathol. 1979; **3:** 373–6.
266. Schlegel R, Banks-Schlegel S, Pinkus GS. Immunohistochemical localization of keratin in normal human tissues. Lab Invest 1980; **42:** 91–6.
267. Battifora H, Sun TTB, Rahu R, Rao S. The use of antikeratin antiserum in tumor diagnosis (abstract). Lab Invest 1980; **42:** 100.
268. Franklin W, Ringus J. Basement membrane antigen in Wilms' tumor (abstract). Lab Invest. 1980; **42:** 118.
269. Dorling J, Johnson GD, Webb JA, Smith ME. Use of peroxidase-conjugated antiglobulin as an alternative to immunofluorescence for the detection of antinuclear factor in serum. J Clin Pathol 1971; **24:** 501–5.
270. Bross KJ, Pangalis GA, Staatz CG, Blume KG. Demonstration of cell surface antigens and their antibodies by the peroxidase-antiperoxidase method. Transplantation 1978; **25:** 331–4.
271. Jeng MW, Finegold MJ, Basch RS, Lamm ME. Demonstration of thymusleukemia (TL) antigens on mitochondria of lymphoid cells by immunoelectron microscopy. Lab Invest 1978; **38:** 41–4.
272. MacPherson BR, Kottmeyer ME. Detection of anti-lymphocyte antibodies using the immunoperoxidase antiglobulin technic. Am J Clin Pathol 1977; **68:** 347–50.
273. De Lellis RA, Sternberger LA, Mann RB, Banks PM, Nakane PK. Immunoperoxidase technics in diagnostic pathology. Am J Clin Pathol 1979; **71:** 483–8.
274. Calkins DR, Dixon RL, Gerber CR, Zarin D, Ommen GS. Identification, characterization, and control of potential human carcinogens: A framework for Federal decision-making. JNCI 1980; **64:** 169–76.
275. Burton RM, McGrew TL, Barrows GH, et al. Occurrence of a thermostable antigen of ovarian carcinoma in normal tissues and

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

secretions. Cancer 1979; **43:** 2385–91.
276. Stuhlmiller GM, Boylston JA, Seigler HF, Fetter BF. Immunodiagnosis of melanoma using chimpanzee antihuman melanoma antiserum. Am J Clin Pathol 1977; **67:** 573–9.
277. Branwood AM, Mesa-Tejada R, Keydar I, et al. Clinical-pathologic correlation in patients with breast carcinoma expressing immunohistochemically detectable mouse mammary tumor virus-related antigens (abstract). Lab Invest. 1979; **40:** 242.
278. Kato H, Miyaughi F, Morioka H, Fujino T, Torigoe T. Tumor antigen of human cervical squamous cell carcinoma. Correlation of circulating levels with disease progress. Cancer 1979; **43:** 585–90.
279. Singh G, Whiteside TL, Dekker A. Immunodiagnosis of mesothelioma. Use of antimesothelial cell serum in an indirect immunofluorescence assay. Cancer 1979; **43:** 2288–96.
280. Goldenberg DM, Deland F, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N Engl J Med 1978; **298:** 1384–8.
281. Mach JP, Carrell S, Forni M, Ritschard J, Donath A, Alberto P. Tumor localization for radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. A critical evaluation. N Engl J Med 1980; **303:** 5–10.
282. Partrige RK, Hahnel R. Prolactin receptors in human breast carcinoma. Cancer 1979; **43:** 643–6.
283. Witorsch RJ. The application of immunoperoxidase methodology for the visualization of prolactin binding sites in human prostate tissue. Human Pathol 1979; **10:** 521–32.
284. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; **256:** 495–7.
285. Kohler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur J Immunol 1976; **6:** 511–9.
286. Wade N. Hybridomas: A potent new biotechnology. Science 1980; **208:** 692–3.
287. Tsung YK, Milunsky A, Alpert E. Secretion by a hybridoma of antibodies against human α-Fetoprotein (letter). N Engl J Med 1980; **302:** 180.
288. Accolla RS, Carrel S, Mach JP. Monoclonal antibodies specific for carcinoembryonic antigen and produced by two hybrid cell lines. Proc Natl Acad Sci 1980; **77:** 563–6.
289. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci 1980; **77:** 1588–92.
290. Thompson JJ, Herlyn M, Stephewski Z, Koprowski H, Elder DE, Clark WH. First use of monoclonal antibodies to detect melanoma-related antigens in tissue sections (abstract). Lab Invest 1981; **44:** 66A.
291. Reinherz EL, Weiner HL, Hauser SL, Cohen JA, Distaso JA, and Schlossman St. Loss of suppressor T cells in active multiple sclerosis—analysis with monoclonal antibodies. N Engl J Med 1980; **303:** 125–9.
292. Hoffman RA, Kung PC, Hansen WP, Goldstein G. Simple and rapid measurement of human T Lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA 1980; **77:** 4914–7.
293. Terhorst C, Van Agthoven A, Reinherz E, Schlossman S. Biochemical analysis of human T lymphocyte differentiation antigens T4 and T5. Science 1980; **209:** 520–1.
294. Reinherz EL, Kung PC, Goldstein G, Schlossman SF. Further characterization of the human inducer T cell subset defined by monoclonal antibody. J Immunol 1979; **123:** 2894–6.
295. Reinherz EL, Kung PC, Breard JM, Goldstein G, Schlossman SF. T cell requirements for generation of helper factor(s) in Man: Analysis of the subsets involved. J Immunol 1980; **124:** 1883–7.
296. Reinherz EL, Moretta L, Roper M, Breard JM, Mingari MC, Cooper MD, Schlossman SF. Human T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. J Exp Med 1980; **151:** 969–74.
297. Reinherz EL, Morimoto C, Penta AC, Schlossman SF. Regulation of B cell immunoglobulin secretion by functional subsets of T lymphocytes in man. Eur J Immunol 1980; **10:** 570–2.
298. Van Wauwe JP, DeMey JR, Goossens JG. OKT3: A monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. J Immunol 1980; **124:** 2708–13.
299. Reinherz EL, Kung PC, Goldstein G, Schlossman ST. A monoclonal antibody with selective reactivity with functionally mature human thymocytes and all peripheral human T cells. J Immunol 1979; **123:** 1312–7.
300. Breard J, Reinherz EL, Kung PC, Goldstein G, Schlossman SF. A monoclonal antibody

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.

reactive with human peripheral blood monocytes. J Immunol 1980; **124:** 1943–8.

301. Kung PC, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. Science 1979; **206:** 347–9.
302. Reinherz EL, O'Brien C, Rosenthal P, Schlossman SF. The cellular basis for viral-induced immunodeficiency: Analysis of monoclonal antibodies. J Immunol 1980; **125:** 1269–74.
303. Reinherz EL, Kung PC, Pesando JM, Ritz J, Goldstein G, Schlossman SF. Ia determinants on human T cell subsets defined by monoclonal antibody. J Exp Med 1979; **150:** 1472–82.
304. Morimoto C, Reinherz EL, Abe T, Homma M, Schlossman SF. Characteristics of anti-T cell antibodies in systemic lupus erythematosus: Evidence for selective reactivity with normal suppressor cells defined by monoclonal antibodies. Clin Immunol Immunopathol 1980; **16:** 474–84.
305. Reinherz EL, Schlossman SF. Regulation of the immune response-inducer and suppressor T-lymphocyte subsets in human beings. N Engl J Med 1980; **303:** 370–3.

Downloaded from www.ccjm.org on April 28, 2024. For personal use only. All other uses require permission.