DAVID J. DABBS

Diagnostic Immunohistochemistry

THERANOSTIC AND GENOMIC APPLICATIONS

Third Edition

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This book is dedicated to the patients we serve
and to my colleagues in pathology and oncology,
especially those who inspire me in very special ways.

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FOREWORD

Many are the "special" techniques that pathologists have used over the years to confirm, complement, and refine the information obtained with their "old faithful" armamentarium; that is, formalin fixation, paraffin embedding, and hematoxylin-eosin staining. These special techniques have come and gone, their usual life cycle beginning with an initial period of unrestrained enthusiasm, turning to a phase of disappointment, and finally leading to a more sober and realistic assessment. Many of these methods have left a permanent mark on the practice of the profession, even if often this was not as deep or wide-ranging as initially touted. These techniques include special stains, tissue culture, electron microscopy, immunohistochemistry, and molecular biology methods. Much was expected of the first three, and infinitely more is anticipated of the last, but it is fair to say that as of today no special technique has influenced the way that pathology is practiced as profoundly as immunohistochemistry, or has come even close to it. I don't think it would be an exaggeration to speak of a revolution, particularly in the field of tumor pathology. Those of us whose working experience antedated diagnostic immunohistochemistry certainly feel that way. The newer generations of pathologists who order so glibly an HMB-45 or a CD31 stain to identify melanocytes and endothelial cells, respectively, have very little feeling for the efforts made to achieve those identifications in the past. The virtues of the technique are so apparent and numerous as to make it as close to ideal as any biologic method carried out in human tissue obtained under routine (which usually means under less than ideal) conditions can be. To wit: It is compatible with standard fixation and embedding procedures; it can be performed retrospectively in material that has been archived for years; it is remarkably sensitive and specific; it can be applied to virtually any immunogenic molecule; and it can be evaluated against the morphologic backgrounds with which pathologists have long been familiar.

As with many other breakthroughs in medicine, immunohistochemistry started with a brilliant yet disarmingly simple idea: to have antibodies bind the specific antigens being sought and to make those antibodies visible by hooking to them a fluorescent compound. All subsequent modifications, such as the use of nonfluorescent chromogens, the amplification of the reaction, and the unmasking of antigens, merely represented technical improvements, although certainly not ones to be minimized. It is because of these technical advances that the procedure spread beyond the confines of the research laboratories and is now applied so pervasively in pathology laboratories throughout the world. Alas, it has its drawbacks. Antigens once believed to be specific for a given cell type were later found to be expressed by other tissues; cross-reactions may occur between unrelated antigens; nonspecific absorption of the antibody may supervene; entrapped non-neoplastic cells reacting for a particular marker may be misinterpreted as part of the tumor; and—most treacherously—antigen may diffuse out of a normal cell and find its way inside an adjacent tumor cell. Any of these pitfalls may lead to a misinterpretation of the reaction and a misdiagnosis. Ironically, this may lead to a final mistaken diagnosis after an initially correct interpretation of the hematoxylin-stained slides. A good protection against this danger is a thorough knowledge of these pitfalls and how to avoid them. An even more important safeguard is a solid background in basic anatomic pathology that will allow the observer to question the validity of any unexpected immunohistochemical result, whether positive or negative. There is nothing more dangerous (or expensive) than a neophyte in pathology making diagnoses on the basis of immunohistochemical profiles in disregard of the cytoarchitectural features of the lesions. Alas, this is true of any other special technique applied for diagnostic purposes to human tissue, molecular biology being the latest and most blatant example. However, when applied selectively and judiciously, immunohistochemistry is a notably powerful tool, in addition to being refreshingly cost effective. As a matter of fact, pathologists can no longer afford to do without it, one of the reasons being that failure to make a diagnosis because of the omission of a key immunohistochemical reaction may be regarded as grounds for a malpractice action.

Any listing of the virtues of immunohistochemistry would be incomplete if it did not include the visual pleasure derived from the examination of this material. I am only half kidding when making this remark. There is undoubtedly an aesthetic component to the practice of histology, as masters of the technique such as Pio del Rio Hortega and Pierre Masson liked to point out. It is sad that these superb artists of morphology left the scene without having had the opportunity to marvel at the beauty of a well-done immunohistochemical preparation. As their more fortunate heirs, let us enjoy this excellent book, edited by one of the foremost experts in the application of the immunohistochemical technique and written by a superb group of contributors—a book that summarizes in a lucid and thorough fashion the current knowledge in the field, in terms of both the technical aspects and the practical applications.

The first edition of this book, published in 2002, rapidly became one of the standard works in the field. The second edition featured a more standardized format,

a wider coverage of organ systems, and an extensive update of markers. It incorporated a large number of useful tables listing the various antibody groups, an algorithmic approach to differential diagnosis, and key diagnostic points for all the major subjects. Special attention was paid to the detailed description of the so-called predictive-type markers (such as HER2/neu in breast carcinoma and CD117 in GIST), which are playing an increasingly important role in the evaluation of tumors by the pathologist.

In this third edition, a new chapter has been added that describes, in a simplified and condensed fashion, the rationale, technology, and applications of molecular anatomic pathology techniques to aid the surgical pathologist in acquiring a basic understanding of these molecular tests.

A new, very timely chapter on immunocytology has been included by Dr. Chivukula, which discusses proper cytologic technique for fixation and processing specimens obtained for hormone receptors and HER2/neu testing.

Overall, each organ-based chapter addresses the state-of-the-art body of knowledge and is summarized in bulleted format for ease of understanding.

There are several new completely rewritten chapters with new authors, all of them experts in their respective fields, including N. Volkan Adsay, Jonathan Epstein, Alyssa M. Krasinskas, Alvin W. Martin, George Netto, and Yuri E. Nikiforov. The latest recommendations for proper fixation and processing of hormone receptor testing are authoritatively discussed by Dr. Clive R. Taylor.

The title of this new third edition has been changed to *Diagnostic Immunohistochemistry: Theranostic and Genomic Applications* to emphasize the fact that immunohistochemistry is no longer used solely for diagnosis. Rather, the growing body of knowledge of cancer genomics, transcriptomics, and the new therapeutic armamentarium of biologics forces pathologists to be cognizant of the emerging field of therapeutic and genomic applications of immunohistochemistry. Accordingly, each chapter of the book includes a synoptic coverage of theranostic and genomic applications. As a result, each organ-based chapter provides detailed information on how gene-based disease can be diagnosed through the microscope with immunohistochemistry. In a similar vein, the presence or absence of markers predictive of the beneficial effects of targeted therapies is determined, launching the age of theranostic immunohistochemistry.

Last but not least, each chapter provides a bridge to new molecular anatomic pathology menus for pathologists, in order to empower them with additional diagnostic modalities whenever immunohistochemistry falls short.

In summary, the authors have again brilliantly succeeded in producing an authoritative, comprehensive, and updated book that pathologists will find next to indispensable as a theoretical backbone for the various methods discussed and of invaluable assistance in their daily work.

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PREFACE

The title of this third edition of *Diagnostic Immunohistochemistry* has been lengthened to include the terms "Theranostic and Genomic Applications." Fundamentally, the continuing challenge of this book is to assemble the vast body of knowledge of immunohistochemistry into a work that has meaning for the diagnostic surgical pathologist. The discipline of immunohistochemistry for the surgical pathologist has been evolving rapidly since the first edition of this book, and it can further be broken down into subsets of theranostic and genomic applications. The diagnostic aspect of immunohistochemistry in surgical pathology is straightforward. Pathologists use this tool to assign lineage to neoplasms that include carcinomas, melanomas, lymphomas, sarcomas, and germ cell tumors. The term "theranostics" is used to describe the proposed process of diagnostic therapy for individual patients—to test them for possible reactions to a new medication and/or to tailor a treatment for them based on a test result. Theranostics is a rapidly emerging field in oncology, and pathologists need to be prepared to serve oncologic patients with a vast and emerging array of individualized patient therapies. The prototype for understanding the concept of theranostics is hormone receptor testing for breast cancer and HER2/neu analysis. These were among the first and most widely known immunohistochemical tests with theranostic applications. With the proper application of these immunohistochemical tests, individualized therapy in the form of selective estrogen receptor modulation therapy for the patient with an estrogen-receptor positive breast carcinoma can be designed. Trastuzumab is administered for the patient with a HER2-positive breast carcinoma.

In addition, the genomic application of immunohistochemistry (i.e., genomic immunohistochemistry) is a tool for the surgical pathologist to facilitate recognition of specific genomic aberrations in the patients' tissues by identifying (or not identifying) the presence or absence of specific proteins or immunohistochemical profiles that directly imply, or connote, a specific genomic abnormality, aberration, or gene signature. A prototype for genomic application could be immunohistochemical testing for microsatellite instability in colorectal carcinomas, where the surgical pathologist applies antibodies to detect proteins for MLH1, MSH2, MSH6, or PMS2. The presence or absence of this protein is in essence a genetic test, a direct genomic application for immunohistochemistry. A genetic signature application might include the identification of basal-like breast carcinoma, in which the signature profile typically is a high-grade ER, PR and HER2 negative, CK5 positive, CK14 positive, CK17 positive, variably EGFR positive tumor. Furthermore, immunohistochemical surrogate markers for gene expression profiles for breast carcinomas can further identify the gene expression profile subsets of carcinomas as luminal A, luminal B, and HER2 categories.

It becomes clear that immunohistochemistry is a powerful tool with overlapping features among diagnostic, theranostic, and genomic applications. Theranostic applications may also be genomic, and genomic immunohistochemistry may also be theranostic. These categories admittedly are artificial and simplistic but give the surgical pathologist and the student of surgical pathology a conceptual framework for recognition of the enormous power of the immunohistochemical test.

Molecular testing in surgical pathology has many important diagnostic, theranostic, and genomic applications as well, but it is the immunohistochemistry platform that lays the groundwork for our understanding of what is normal and what is diseased in tissue by virtue of the direct visualization of molecular morphology.

In this edition, most chapters have been completely revised, and there are several new authors. There is a new chapter on molecular anatomic pathology, with new authorships in non-Hodgkin lymphoma; immunohistology of the gastrointestinal tract; immunohistology of the pancreas, bile ducts, gallbladder, and liver; and immunohistology of the genitourinary system. An additional new chapter on immunocytology is patterned after the chapter that appeared in the first edition.

Each chapter format may include subsections that discuss relevant theranostic and genomic applications of immunohistochemistry. These are included to highlight to the pathologist that these important applications go beyond traditional diagnostic immunohistochemistry for individual organ systems.

Immunohistochemistry has undergone a tremendous change, with new stresses and demands throughout the last decade. A critical factor affecting the surgical pathologist/immunohistochemist is the proper standardization of procedures in the laboratory to assure the highest quality immunohistology for diagnostic, theranostic, and genomic applications. Recent recommendations by the CAP-ASCO and additional new recommendations for hormone receptor testing have highlighted the importance of proper standardization of procedures and internal and external quality assurance programs.

Once again, the challenge of putting this work together has been to assure that the base of knowledge in each chapter is relevant and robust long after the ink has dried. The contributions of expert authors in each discipline are unique to this work. The continuing goal of this book is to provide a reference for pathologists who practice contemporary surgical pathology and cytopathology.

With few exceptions, each chapter is designed to be a stand-alone work. Inherent in this design is a body of information that is reproduced and redundant throughout each chapter. Each chapter is comprehensive in a diagnostic sense, which should limit the need to do extensive cross-checking to other chapters. Each section is punctuated by key diagnostic points that summarize the section and that serve as a rapid summary reference for the most important points in that section.

The positive feedback on this work continues to grow exponentially. I welcome personally any feedback regarding this book, no matter how small, even to point out typographical errors or informational errors. Please contact me at ddabbs@upmc.edu or dabbsihc@gmail.com.

My special thanks go to the dedicated investigators and pathologists across the globe who have given me feedback on this work.

DAVID J. DABBS, MD

HOW TO USE THIS BOOK

The first chapter of this book details the techniques and development of immunohistochemistry. This includes critically important updates of standardization as applied to theranostic testing, especially hormone receptor testing. The new second chapter on diagnostic molecular anatomic pathology is to be used as a reference guide to understanding molecular anatomic tests mentioned throughout this textbook. Molecular anatomic pathology has grown exponentially over the past decade, and the discipline supplies critically important testing that supplements diagnostic, theranostic, or genomic applications beyond immunohistochemistry. Each chapter, where relevant, will have subsections titled "Beyond Immunohistochemistry: Anatomic Molecular Diagnostic Applications."

The third chapter, "Immunohistology of Infectious Diseases," has been completely restructured. The remaining chapters continue as an organ system approach to diagnostic, theranostic, and genomic applications of immunohistochemistry. Each chapter has a liberal number of "immunohistograms" depicting immunostaining patterns of tumors, along with numerous tables, well structured for easy reference. Diagnostic algorithms are used where relevant. Many areas of the text are punctuated by summary "Key Diagnostic Points." Diagnostic pitfalls are also cited where particularly relevant.

To maintain constant terminology throughout the chapters, the following abbreviations in the text and tables are used unless otherwise specified:

+, the result is almost always strong, diffusely positive;
S, sometimes positive;
R, rarely positive, and if so, rare cells are positive;
N or a (-), negative result.

These are exciting times indeed for the discipline of immunohistochemistry as well as for the rapidly evolving molecular tests that will significantly affect patients' lives. This work should be viewed as a focal point, a punctuation mark in the continuous quality improvement of the knowledge base for immunohistochemistry for surgical pathologists.

DAVID J. DABBS, MD

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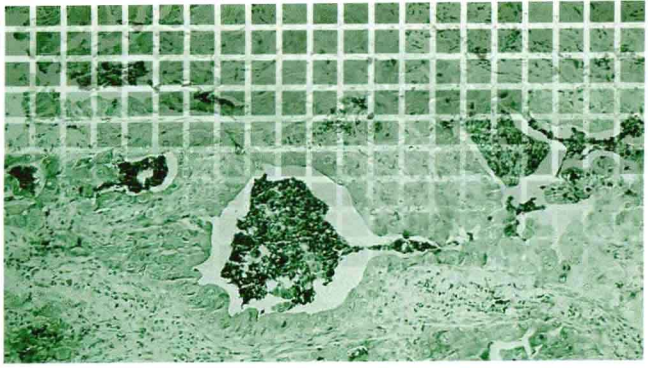

1

Techniques of Immunohistochemistry: Principles, Pitfalls, and Standardization

Clive R. Taylor • Shan-Rong Shi • Nancy J. Barr

INTRODUCTION

Immunohistochemistry (IHC), or immunocytochemistry, is a method for localizing specific antigens in tissues or cells based on antigen-antibody recognition; it seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light microscopic level. IHC has a long history, extending more than half a century from 1940, when Coons developed an immunofluorescence technique to detect corresponding antigens in frozen tissue sections.[1] However, only since the early 1990s has the method found general application in surgical pathology.[2-5] A series of technical developments led eventually to the wide range of IHC applications in use today. The enzymatic label (horseradish peroxidase), developed by Avrameas[6] and by Nakane and colleagues,[7] allowed visualization of the labeled antibody by light microscopy in the presence of a suitable colorogenic substrate system. In Oxford, Taylor and Burns developed the first successful demonstration of antigens in routinely processed formalin-fixed paraffin-embedded (FFPE) tissues.[5] A critical issue in the early development of immunoperoxidase techniques was related to the need to achieve greater sensitivity. Greater sensitivity would facilitate staining of FFPE tissues—from a simple one-step direct conjugate method to multiple-step detection techniques such as the peroxidase antiperoxidase (PAP), avidin-biotin conjugate (ABC), and biotin-streptavidin (B-SA) methods—and would eventually lead to amplification methods (such as tyramide) and highly sensitive "polymer-based" labeling systems.[4,8-20] We will describe these methods in detail later in this chapter.

As the IHC method has evolved, its use in diagnostic pathology has expanded such that the use of one or more IHC "stains" is routine in surgical pathology, especially with respect to tumor diagnosis and classification. Furthermore, IHC has been adapted to the identification and demonstration of both prognostic and predictive markers, with corresponding requirements for semi-quantitative reporting of results. The widespread use of IHC and the demands for comparison of qualitative and semi-quantitative findings among an increasing number of laboratories have resulted in a growing focus on method reproducibility and have led to a new emphasis upon standardization. This standardization serves as an underlying theme of this chapter, and we will discuss it in detail in the Quality Control and Standardization section.

The development of the hybridoma technique[21] facilitated the development of IHC and the manufacture of abundant, highly specific monoclonal antibodies, many of which found early application in staining of tissues. Initial success in cryostat sections was eventually extended to routinely processed paraffin, celloidin, or other plastic-embedded tissue sections. Only when the IHC technique became applicable to routine FFPE tissue sections did it usher in the "brown revolution."[22] The critical significance of rendering the IHC technique suitable for routine paraffin sections was illustrated in 1974 by Taylor and

Burns, who showed that it was possible to demonstrate at least some antigens in routinely processed tissue.[5] These initial studies led to serious attempts by pathologists to improve the ability to perform IHC staining on FFPE sections.[5,23-28] Although great effort has been expended in the search for alternative fixatives (formalin substitutes) to preserve antigenicity without compromising preservation of morphologic features, no ideal fixatives have been found to date. Larsson states, "An ideal immunocytochemical fixative applicable to all antigens may never be found."[29] In addition, preservation of morphologic features is not comparable with formalin fixation, causing problems in interpretation and diagnosis.

Enzyme digestion was introduced by Huang as a pretreatment to IHC staining to "unmask" some antigens that had been altered by formalin fixation.[30] However, the enzyme digestion method, while widely applied, did not improve IHC staining of many antigens, a subject well reviewed by Leong and colleagues.[31] Another drawback of enzyme digestion was that it proved difficult to control the optimal "digestion" conditions for individual tissue sections when stained with different antibodies. These difficulties in standardization provided a powerful incentive for the development of a new technique, with the requirements that it should be more powerful, more widely applicable, and easier to use than enzyme digestion. In addition, it should enhance immunohistochemical staining of routine FFPE tissue sections in a reproducible and reliable manner. The antigen-retrieval (AR) technique, based on a series of biochemical studies by Fraenkel-Conrat and coworkers,[32-34] was developed by Shi and associates in 1991.[35-40] In contrast to enzyme digestion, the AR technique is a simple method that involves heating routinely processed paraffin sections at high temperature (e.g., in a microwave oven) before IHC staining procedures. An alternative method that does not use heating was developed for celloidin-embedded tissues.[36-38] The intensity of IHC staining was increased dramatically after AR pretreatment,[39-43] as evidenced by more than 100 articles published subsequently. Various modifications of the AR technique have been described; the majority of these use different buffer solutions as the AR solution in place of metal salt solutions, which may have a potentially toxic effect.[39,40,43-54] Worldwide application of AR-IHC in pathology has validated the feasibility of AR-IHC and expanded its use in molecular morphology, while raising some basic questions and practical issues that are subject to ongoing evolution of the method.[2,3,33,40,55-61]

It is the authors' view that there is no immediate prospect of replacing formalin in "routine" surgical pathology. Even if there was agreement as to a superior fixative, the logistics of converting all laboratories nationwide, yet alone worldwide, are formidable. Formalin is thus what we have to work with for the foreseeable future. For this reason, in this chapter we will focus on IHC as applied to archival FFPE tissue sections for diagnostic pathologic study. In addition to basic principles and practical technical issues, the limitations and pitfalls of IHC are discussed, with the intention of providing "food for thought" in the further development of IHC, particularly with respect to standardization and ultimately quantitative IHC applications.

BASIC PRINCIPLES OF IMMUNOHISTOCHEMISTRY

Surgical pathologists have long recognized their fallibility, although they have not always publicized it.[2,3,26] They have, however, sought more certain means of validating morphologic judgments. A variety of "special stains" were developed to facilitate cell recognition and diagnosis; most of these early stains were based on chemical reactions of cell and tissue components in frozen sections (histochemistry). In certain circumstances, these histochemical stains proved to be of critical value in specific cell identification. More often, they served merely to highlight or emphasize cellular or histologic features that supported a particular interpretation without providing truly specific confirmation. When the new field of immunohistochemistry was created by combining immunology with histochemistry, a wide variety of truly specific special stains were generated. This subject, which has been discussed in thousands of papers, will be discussed in this book.

The aims of IHC are akin to those of histochemistry. Indeed, IHC builds on the foundations of histochemistry; it does not replace histochemistry but rather serves as a valuable adjunct that greatly extends the variety of tissue components that can be demonstrated specifically within tissue sections or other cell preparations. As emphasized by pioneers in this field of functional morphology, "the object of all staining is to recognize microchemically the existence and distribution of substances which we have been made aware of macrochemically."[62] The basic critical principle of IHC, as with any other special staining method, is a sharp visual localization of target components in the cell and tissue, based on a satisfactory signal-to-noise ratio. Amplifying the signal while reducing non-specific background staining (noise) has been a major strategy to achieve a satisfactory result that is useful in daily practice.

After more than two decades, advances in IHC have provided a feasible approach to performing immunostaining on routinely processed tissues, such that this method is now "routine" for the performance of IHC "special stains" in surgical pathology laboratories using FFPE tissues (see Appendix 1A). However, demands for improved reproducibility and for quantification have led to a growing recognition that IHC has the potential to be more than just a special stain. If properly controlled in all aspects of its performance, IHC can provide a tissue-based immunoassay with the reproducibility and quantitative characteristics of an ELISA (enzyme-linked immunosorbent assay) test, which not only detects the presence of an "analyte" (protein or antigen) but also provides an accurate and reliable measure of its relative or real amount (see Quality Control and Standardization section).

ANTIBODIES AS SPECIFIC STAINING REAGENTS

An antibody is a molecule that has the property of combining specifically with a second molecule, termed the *antigen*. Further, the production of antibody by an

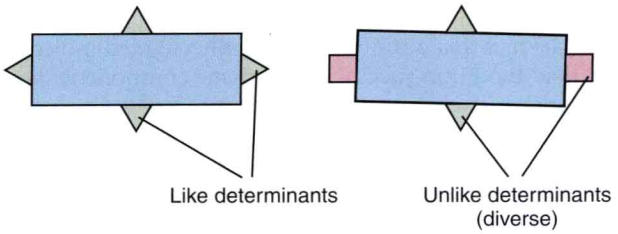

FIGURE 1.1 Antigens and antigenic determinants. An antigenic molecule may be considered to consist of an immunologically "inert" carrier component and one or more antigenic determinants of like type *(left)* or diverse types *(right)*. *From Taylor CR, Cote RJ, eds. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist. 3rd ed. Philadelphia: Elsevier; 2005:6.*

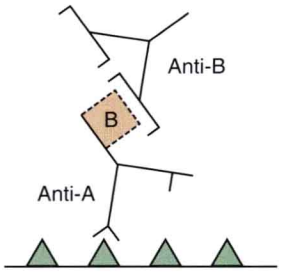

FIGURE 1.2 Antibodies as antigens. Anti-A antibody binds specifically to antigen A in the tissue section. Antigen B (B) is depicted as a second antigenic determinant that is part of the anti-A molecule; anti-B antibody, made in a second species, will bind to this determinant. Thus anti-B (the so-called secondary antibody) can be used to locate the site of binding of anti-A (the primary antibody) in a tissue section. *From Taylor CR, Cote RJ, eds. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist. 3rd ed. Philadelphia: Elsevier; 2005:9.*

animal is induced specifically by the presence of antigen; this forms part of the basic immune response. Antigen-antibody recognition is based on the three-dimensional structure of protein (or other antigen), which may be compromised by formalin-induced modification of protein conformation ("masking") and restored in part by AR. We will discuss this process later in this chapter.

Antibodies are immunoglobulin molecules consisting of two basic units: a pair of light chains (either a kappa or a lambda pair) and a pair of heavy chains (gamma, alpha, mu, delta, or epsilon). An antigen is any molecule that is sufficiently complex that it maintains a relatively rigid three-dimensional profile and is foreign to the animal into which it is introduced. Good antigens are proteins and carbohydrates that are sufficiently complex to possess a unique three-dimensional "charge-shape" profile. In fact, such molecules may bear more than one unique three-dimensional structure capable of inducing antibody formation (Fig. 1.1). Each of these individual sites on a molecule may be termed an *antigenic determinant* (or *epitope*), being the exact site on the molecule with which the antibody combines. For a protein, the term *epitope* corresponds to a cluster of amino acid residues that binds specifically to the paratope of an antibody.[63] Although it is part of the protein, an epitope cannot be recognized independently of its paratope partner.[63] Antigenic determinants (epitopes) may be classified as continuous and discontinuous. The former are composed of a continuum of residues in a polypeptide chain, whereas the latter consist of residues from different parts of a polypeptide chain, brought together by the folding of the protein conformation.[64] This is an interesting issue that may reflect the variable influence of formalin fixation on antigenicity, and variations in the effectiveness of AR.

Antibody molecules are proteins; thus any rigid part of an antibody molecule may itself serve as the antigenic determinant to induce an antibody. IHC techniques exploit the fact that immunoglobulin molecules can serve both as antibodies (binding specifically to tissue antigens) and as antigens (providing antigenic determinants to which secondary antibodies may be attached) (Fig. 1.2).

Evaluation of an antibody for use in IHC is based on two main points: the sensitivity and the specificity of the antibody-antigen reaction for IHC. The development of the hybridoma technique[21] provided an almost limitless source of highly specific antibodies. Monoclonal antibodies do not guarantee antigen specificity; however, since different antigens may share similar or cross-reactive epitopes, the "practical" specificity reflected by IHC is excellent for most monoclonal antibodies. In contrast, a "polyclonal antibody" is an antiserum that contains many different molecular species of antibody having varying specificities against the different antigens (or antigenic determinants) used to immunize the animal. It is important to remember that polyclonal antibodies may also include varying amounts of antibodies to a whole range of antigens (including bacteria and viruses) that the immunized animal encountered before its use as a source of antibody. As a result, polyclonal antibodies often give more non-specific background staining in slides than encountered using monoclonal antibodies. By the same token, however, the presence of a mixture of different antibodies may on occasion confer an advantage to the use of polyclonal antibodies in the staining of certain "hard-to-detect" antigens in fixed tissues. For these reasons, the use of highly purified antigen preparations to produce high-affinity conventional polyclonal antibodies (antisera), which are then subjected to multiple absorption procedures to maximize specificity, is of value for certain applications. However, note that immunodiffusion assays used by manufacturers in the assessment of such antisera specificity may fail to detect "trace" antibody specificities; this becomes apparent only when the antiserum is applied to tissue sections containing many different antigens.

Comparison of sensitivity and specificity between polyclonal and monoclonal antibodies indicates that polyclonal antibody may be more sensitive but less specific than monoclonal antibody. The reason may be that polyclonal antibody (actually a composite of many antibodies) may recognize several different binding sites (epitopes) on a single protein (antigen), whereas a monoclonal antibody recognizes only a single type of epitope. Sophisticated amplification techniques, coupled with the use of the AR technique, have reduced the practical importance of this distinction. Although the specificity of monoclonal antibody is, as noted, not absolute because of cross-reactivity with non-target molecules,[65] most commercially available monoclonal antibodies are