CURRENT Surgical Pathology

WARHOL

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Current Surgical Pathology

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To Tamara and Larisa

Preface

This text is intended to serve as both an introduction and a manual of current usage for the newer technologies that have been introduced into diagnostic surgical pathology. Its proposed audience is the practicing pathologist in a general or community hospital and the resident in training. Experience indicates that these individuals are intrigued by the newer methodologies but are uncertain as to the specific impact on one's practice. Immunoperoxidase techniques, in particular, have produced a small revolution in diagnostic pathology. Like most revolutions, however, this one has produced confusion and skepticism. The myriad diagnostic reagents, the cryptic terminology, and the conflicting reports in the literature have contributed to this cynicism.

The general pathologist faces certain common problems that modern technology is capable of solving, provided that one has an understanding of the techniques and their limitations. As indicated by the chapter titles, a frequent problem in surgical pathology is the correct classification of tumors. The primary purpose of this text is to allow the pathologist to develop a diagnostic scheme, or algorithm, that will solve the problems relevant to his or her practice.

Although there are individual texts that discuss electron microscopy, immunocytochemistry, and flow cytometry, there is no text that compares the relative merits of these techniques. Both the relative usefulness and complementary aspects of these different techniques have not been adequately addressed. The first part of the text is designed to provide a working introduction to the techniques of immunocytochemistry, electron microscopy, flow cytometry, and recombinant DNA technology as applied to diagnostic pathology. Rather than overburden the reader with technical details, this section is designed to explain the basic principles of the techniques and to emphasize both their merits and their disadvantages. The reader should then develop an understanding of why these techniques are potentially useful to the pathologist's practice.

The second section emphasizes the problem-oriented approach to surgical pa-

thology. Each of the individual chapters examines a common diagnostic dilemma. The relative merits and limitations of the techniques are discussed in relation to the specific problems. Questions such as "When is electron microscopy preferable to immunoperoxidase?" and "What immunoperoxidase reagents should one stock?" are addressed in this section. An example of a discussion would be why electron microscopy is superior to immunoperoxidase for the diagnosis of mesotheliomas.

In keeping with the working manual format of the text, some of the information will be contained in charts and tables. This allows for ready reference. Particularly for immunoperoxidase, as superior reagents are made available, they can be substituted for reagents on the chart. I hope that this will prevent obsolescence.

MICHAEL J. WARHOL, M.D.

Acknowledgments

The ideas and concepts in this text are the result of my association with many gifted and dedicated pathologists. In particular, I would like to acknowledge Dr. Joseph Mackie Corson, Chief of Surgical Pathology at the Brigham and Women's Hospital, and Professor of Pathology at Harvard Medical School. Dr. Corson was my first teacher in surgical pathology. The prospective problem-solving approach I advocate in the text was first proposed to me by Dr. Corson, and inspired by the way the Surgical Pathology Service at the Brigham is run. Dr. Corson always emphasizes that the pathologist is a physician whose primary responsibility is to the patient. Therefore, it is important to handle the specimen in whatever way will yield the most useful information for patient care.

The other person who has had a profound influence on my thinking is Dr. Geraldine S. Pinkus, Chief of Hematopathology at the Brigham and Women's Hospital, and Associate Professor of Pathology at Harvard Medical School. Dr. Pinkus introduced me to immunoperoxidase, and taught me what I know about that subject. Dr. Pinkus brought rigorous scientific discipline to surgical pathology, and has been a force in its transition from an art to a science. To both of these individuals I am forever grateful.

Contents

Chapter 1

Introduction

The emergence of a biotechnology with practical applicability to diagnostic pathology has created turmoil among surgical pathologists. A schism has developed between traditionalists and those who actively embrace the newer diagnostic methods. The controversy has been intensified by the rapid growth in the use of immunoperoxidase techniques. Many remain skeptical about the efficacy of this methodology. Others do not know how immunoperoxidase relates to other, more established diagnostic methods such as electron microscopy and the even newer techniques such as flow cytometry. These conflicts are unfortunate, because both traditional and newer methods are useful when they are applied to the appropriate problem. Use of the new technology has reinforced the fundamental, but often neglected, principle of surgical pathology, that of prospective thinking and planning. One should approach the surgical specimen as a problem to be solved and, preferably, should define that problem before, not after, processing the specimen. It is then possible to select whatever methodology is most appropriate. In almost every situation, one of the methodologies chosen will be routine histologic examination with the preparation of a hematoxylin and eosin (H&E) slide. For routine cases, this is usually all that is necessary. For more complicated cases, the H&E slide is preliminary. The surgical pa-

thologist must then develop a diagnostic algorithm useful to his or her own individual practice. It is hoped that this text will serve as an aid in developing such an algorithm.

It is unfortunate that many pathologists approach pathology retrospectively. They regard the microscopic slide as the definitive diagnostic tool from which all pertinent information can be extracted. Although the analysis of histologic sections is both enjoyable and intellectually challenging, the accuracy of this method, even in the hands of the most skilled observer, is unacceptable in the current era of modern therapy. One should remember that the euphemism "good eye" is often the refuge of the intellectually destitute.

Advances in oncology have had a great impact on surgical pathology. The development of radiation therapy and medical oncology in particular have placed a premium on accurate diagnoses. The realization that different types of tumors respond differently to different kinds of therapy has made accurate tumor classification essential. When surgery was the only available method of therapy, tumor taxonomy was largely a pedantic exercise. If the surgery was not curative, it was of little consolation to the patient to die correctly classified. Effective alternatives to surgery have enhanced the importance of surgical pathologists but have also increased their burden of responsibility. One can no longer find safe refuge with such diagnoses as "reticulum cell sarcoma" and "undifferentiated malignant tumor." Although surgical pathologists may lament the passing of this more innocent, halcyon era, they can derive consolation from knowing that they are providing a greater service to the patient. A correct initial diagnosis ensures that the patient will receive the correct therapeutic regimen.

The availability of newer diagnostic methods requires that the pathologist exercise his or her authority as a physician and acquire the relevant clinical information about the patient. Classic observations including the duration of symptoms, the clinical distribution of disease, and radiographic findings provide the framework for the prospective approach we are advocating. It is important that the pathologist be aware of the list of possibilities in the clinical differential diagnosis. If the final diagnosis is outside this differential list, the pathologist must be prepared to explain the discrepancy. These traditional methods are still essential but not completely adequate.

The prospective approach begins before, not after, surgery. A simple perusal of the next day's surgical schedule should alert the pathologist to many diagnostic problems. One's suspicions can then be confirmed with a conversation with the clinician, a review of the patient's record, or a consultation with the radiologist. Avoiding the

pitfall of simply dumping every specimen into formalin prevents the irretrievable loss of valuable pieces of information. Sometimes simply culturing the specimen will provide all the necessary information.

The purpose of this text is to help the pathologist develop an approach relevant to his or her practice. The creation of a diagnostic algorithm will permit the exploitation of the available technology. Implicit in the development of this diagnostic algorithm is the integration of the various biotechnologies pertinent to diagnostic pathology. The pathologist should understand how the technologies complement one another, and which technique is preferable. Undoubtedly, some of the reagents discussed in this text will have become obsolete by the time of publication. The specific reagents are less important than the general technique and the logic behind the diagnostic approaches, and the logic that developed the diagnostic approach. The clinical problems discussed will not disappear, and it is hoped that this text will retain its vitality and continue to remain relevant to the practice of current, enlightened surgical pathology.

Chapter 2

Immunocytochemistry

INTRODUCTION

Immunocytochemistry, particularly the immunoperoxidase technique, has had a revolutionary impact on surgical pathology. Histologic stains were initially a by-product of the chemical dyes that were developed in central Europe at the end of the nineteenth and the beginning of the twentieth centuries. The ability of acidic and basic dyes to form ionic bonds with their counterparts was found to be just as applicable for tissues as textiles. These observations lead to the development of stains with greater specificity for certain tissue constituents. Chemical stains have been used for many years and are particularly useful in defining the extracellular matrix. Conventional chemical stains, however, have a limited specificity. They are generally not very helpful in distinguishing different cell types.

In contrast with chemical stains, antibodies are highly specific reagents. Although the exquisite specificity of antibodies had been defined by Landsteiner many years ago, a technology needed to be developed before these unique proteins could be used in clinical medicine. What was lacking was a detection system sufficiently sensitive to detect minute quantities of material. The effective use of antibodies in the clinical laboratory began with the radioimmunoassay technique of Berson and Yalow. This technique utilized the high

energy generated by the radioactive decay to produce a sensitive detection system. Although radioactive compounds can be used in histologic material and their presence detected by exposure of a photographic emulsion, this technique is too complicated and time-consuming to be used routinely.

Just as light emission methods and enzyme methodologies have replaced radioimmunoassay in the chemistry laboratory, these detection systems found their way into histology in the form of immunofluorescence and immunoperoxidase. The introduction of these methodologies has had a profound impact on diagnostic pathology. These techniques conceptually altered the way the pathologist approaches a diagnostic problem. The interpretation of a hematoxylin and eosin (H&E) slide has a large subjective component, influenced tremendously by the pathologist's experience. Immunocytochemistry adds objectivity to this process. Either an antigen is present or it is not. The interpretation of immunocytochemical staining is not quite that straightforward. Assessment of positivity still remains somewhat subjective. Nevertheless, immunocytochemistry has produced a quantum improvement in diagnostic accuracy.

IMMUNOFLUORESCENCE

The immunofluorescence technique of Coons was the first method that permitted the use of antibodies to detect antigens on tissue samples. Antibody is tagged with a fluorescent marker, which generates light against a dark background. The capacity of the eye to distinguish light from dark provides the necessary sensitivity. Immunofluorescence methodology is illustrated in Figure 2–1. There are two types of immunofluorescence techniques: the direct method, in which the fluorescent probe is coupled directly to the antibody, and the indirect method, in which the fluorescent probe is coupled to a secondary antibody, reactive against the primary antibody. Although the direct method would appear to offer the advantages of a simple, one-step technique, it requires numerous labile and expensive reagents. The indirect method allows the standardization of one or possibly two detection reagent systems that are capable of reacting against a variety of primary antisera. In such a system, the secondary antibody that is tagged with the fluorescent probe is a species-specific antibody. This antibody should react with any primary antibody raised in the same species. If one is using only primary antisera raised in rabbits, one needs only one type of fluorescently tagged antirabbit secondary antiserum.

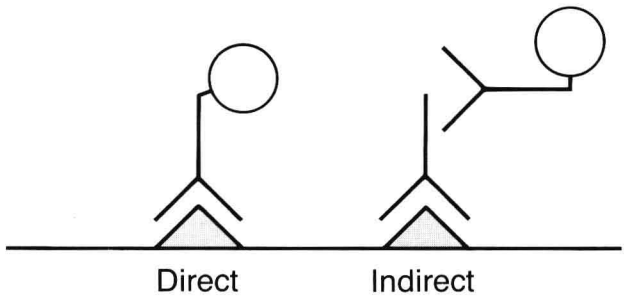

Figure 2–1. A comparison between direct and indirect immunofluorescence techniques. The direct technique binds the fluorescein molecule directly to the antibody probe and would seem to have the advantage of being a simple, one-step technique. However, reagent preparation is costly and complicated. In contrast, the indirect immunofluorescence technique utilizes a secondary antibody that is directed against a species epitope of the primary antibody. Therefore, the primary antibody also serves as an antigen in this system. If one has primary antibodies that all are raised in the same species, i.e., the rabbit, this system allows for a simpler catalog of reagents, since only one type of secondary antibody is required. This method also has potentially greater sensitivity, as it is possible that one molecule of the primary antibody can bind more than one secondary antibody.

Despite certain advantages of sensitivity, immunofluorescence techniques have not gained widespread popularity in diagnostic pathology. Currently, they are used primarily for the evaluation of renal biopsies and the detection of certain infectious agents. Immunofluorescence techniques have intrinsic limitations that render them unattractive for surgical pathology. The lability of the fluorescent probe prevents having a permanent slide record of the immune reaction. Although this limitation can theoretically be overcome by repeating the reaction on the slide, this is cumbersome and adds another level of complexity to the technique. The major limitation is the lack of detail inherent with a darkfield technique. Although it is easy to recognize a positive reaction, it is difficult to localize the specificity of the reaction. This is a particular problem during the evaluation of tumors. Most tumors are a heterogeneous mixture of tumor cells and residual normal tissue. One must be able to identify individual cells in order to diagnose tumors. Only then can one determine whether a specific antigen is present or absent.

A myth concerning immunofluorescence is that it can be used only with frozen tissue. The requirement for frozen tissue is a function of the antigen being probed, rather than the detection system

being used. If the antigen is not denatured by chemical fixation, the immunofluorescence procedure works perfectly well on fixed tissue.

IMMUNOPEROXIDASE TECHNIQUES

The method that has had by far the greatest impact on diagnostic pathology is the immunoperoxidase method of Sternberger. This method is illustrated in Figure 2–2. The sensitivity of this method results from the several multiplication steps present. The primary antibody (Ab) itself serves as an antigen that can then bind more than one "secondary" antibody or "bridge" antibodies. The horseradish peroxidase–antiperoxidase complex also serves as an antigen that is bound by the bridge antibody. Therefore, the antibody reactions themselves produce a multiplication effect. The most significant multiplication is produced by the peroxidase enzyme. This enzyme can theoretically reduce an infinite number of substrate molecules, producing a visually apparent product. The popularity that this technique enjoys is because it closely resembles conventionally fixed and stained tissue. This similarity permits direct comparisons. The ambiguity of darkfield immunofluorescence is eliminated, and the specific cells in question can be positively identified. The dramatic impact that immunocytochemistry can make on diagnostic pathology is illustrated by the case shown in Figure 2–3. For many years, thymomas

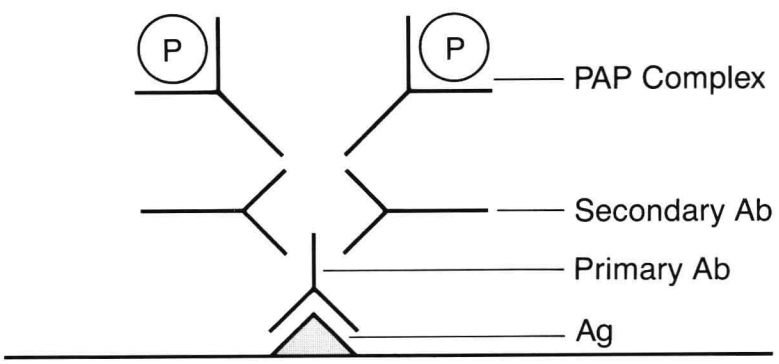

Figure 2–2. The immunoperoxidase technique of Sternberger. This method has multiple steps, all of which offer potential multiplication. The primary antibody (Ab) can bind more than one secondary antibody. These secondary antibodies form a "bridge" between the primary antibody and the peroxidase-antiperoxidase (PAP) complex. The final multiplication results from the enzymatic activity of the peroxidase enzyme. This can reduce many molecules of substrate, creating a visible precipitate. These multiplication steps result in great sensitivity.