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Immunohistochemistry: Forging the links between immunology and pathology

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Abstract

The technique of immunohistochemical staining allows the visualization of epitopes in situ in histological tissue sections. A series of innovations in the methods and reagents and the introduction of mechanization have enhanced the ease and technical reliability of this technique resulting in widespread application in veterinary diagnostics and research. This brief overview will highlight some of the applications for immunohistochemical staining with an emphasis on the use of the technique in diagnostic veterinary medicine, particularly for the detection of infectious disease agents.

Keywords: Immunohistochemistry; Diagnostics; Skin disease; Infectious agents; Tumors

1. Introduction

Immunohistochemical (IHC) staining is a laboratory technique that utilizes specific antibodies for visualization of the quantity, tissue distribution and cellular location of immunogenic epitopes in histological tissue sections. The concept was introduced as early as the 1940s when fluorescein dye (visible under ultraviolet light) was tagged to antibodies directed against pneumococci for identification of this organism with specific anti-serum (Coons et al., 1941). This method, often abbreviated IFA for ''immunofluorescence assay'' has been widely used for the detection of specific pathogens, viral as well as bacterial and protozoal in ''fresh''/unfixed tissues in both human and veterinary medicine.

Since its inception, the ease, accessibility and reliability of IHC staining has increased enormously due to a series of advances in methodology. The introduction of ''enzyme-'', rather than fluorescein-labeled antibodies enabling IHC staining to be viewed with the light microscope arguably had the greatest impact (Nakane and Pierce, 1967). This innovation removed IHC techniques from the exclusive purview of specialized laboratories with fluorescence microscopes to the realm of a much wider group of scientists

Abbreviations: IHC, immunohistochemistry; BVDV, bovine viral diarrhea virus; IFA, immunofluorescence assay; FeLV, feline leukemia virus; ABC, avidin–biotin complex; PMWS, porcine postweaning mortality and wasting syndrome; PCVII, porcine circovirus II; CWD, chronic wasting disease

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and histopathologists. The discovery of antigen retrieval methods (Huang et al., 1976) and highly amplified detection methods (Hsu et al., 1981) were instrumental in allowing IHC methods to be used in conjunction with tissues subjected to tissue fixatives such as formalin and thus increased the applicability for routine diagnostics. The final development that has advanced routine use of IHC methods was the innovation of robotic staining systems. These machines have removed the tedium and increased the reliability of IHC methods. Together these advances in technique have resulted in the use of IHC in many research and diagnostics laboratories as integral parts of the routine technique repertoire. This brief review will touch on some of the many applications for IHC in veterinary immunology and pathology with examples primarily of work from the Diagnostic Immunology Laboratory of Prairie Diagnostic Services at the Western College of Veterinary Medicine at the University of Saskatchewan, Saskatoon, Canada (Haines and Clark, 1991; Haines and Chelack, 1991).

2. Application of immunohistochemistry in diagnostic pathology

Many of the first reports of applications for IHC staining were of detection of immunoglobulin in tissue sections from patients with suspect immune-mediated skin disease, and as technical improvements developed these were often first reported for this application (Burnham et al., 1963; Michel et al., 1973). In particular, the use of antigen retrieval methods and the amplified detection methods that enabled IHC to be used in formalin-fixed sections has been instrumental in improving the utility and reliability of the test for diagnosing these patients (Berti et al., 1983; Haines et al., 1987). This improved reliability is attributable to the use of serial sections from a single biopsy for determination of the histological appearance of the lesions and for demonstration of the presence and pattern of immunoglobulin deposition. The ability to make this correlation assists in confirming the pathogenesis of these autoantibody-induced disease processes and increases the confidence of the clinician and histologist in the diagnosis. As veterinarians gain more experience with responses to various therapies among the plethora of immune-mediated diseases, the ability to accurately distinguish disorders such as bullous pemphigoid, systemic lupus eythematosus and the various pemphigus disorders will be of increasing importance. More recently, as the antigenic targets for the immune-mediated skin diseases have become better understood, IHC methods have been used to demonstrate changes in the patterns of these molecules in lesional tissues. The best example is alteration in the pattern of desmosglein 1 expression in patients with pemphigus foliaceous; this antigen is the target for antibody-mediated destruction in a significant number of both human and veterinary patients. Antibody binding to this antigen (part of the desmosome involved in cell to cell adhesion), prompts the phenomena of antibody-induced patching, capping with subsequent shedding and/or internalization. Demonstration of these alterations in distribution of desmosglein can be used to study pathogenesis of these lesions as well as to assist in confirmation of the diagnosis (Carlotti et al., 1993; Steeves et al., 2002).

While the demonstration of autoantibody deposition in immune-mediated skin disease is the historical focus for IHC diagnostics, infectious disease diagnosis is currently the most widely used application of the technique in veterinary diagnostic medicine. The classical technique, using fluorescein labeled antibody is still the basis for specific pathogen identification in fresh/frozen tissues in most virology and bacteriology laboratories. Through the use of antigen retrieval methods and amplified detection systems such as the avidin–biotin complex (ABC) immuno-peroxidase methods, the applicability of IHC to formalin fixed tissues has increased the convenience and the spectrum of applications for identifying specific pathogens in tissues. There are specific antibodies available either commercially (Linscott, 2002) or from researchers or government agencies to most of the known pathogens of importance in veterinary medicine. The reagents for production and amplification of the visible signal (i.e. the secondary antibodies directed to immunoglobulin and avidin–biotin complex reagents) are widely available in ‘‘kit’’ format and of excellent quality.

IHC staining has been proven to be rapid and reliable to readily detect a wide array of agents, bacterial, viral and protozoal, including those that are nonviable or inherently difficult to isolate or culture.