The Detection of Bovine Respiratory Syncytial Virus in Formalin Fixed Bovine Lung with Commercially Available Monoclonal Antibodies and Avidin Biotin Complex Immunohistochemistry

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ABSTRACT

Eight commercially available monoclonal antibodies directed against respiratory syncytial virus antigens were tested for ability to detect bovine respiratory syncytial virus (BRSV) antigen in formalin fixed, paraffin embedded bovine lung using avidin-biotin complex immunohistochemical staining. Monoclonal antibodies from clone 18B2 purchased from Biosoft, Paris, France and those from clone 8G12 purchased from the Department of Veterinary Sciences, University of Nebraska, Lincoln, Nebraska stained BRSV antigen in infected bovine lung with acceptable background staining of uninfected tissues. This method offers advantages over other techniques for BRSV diagnosis in that fresh tissue is not required and all reagents may be purchased commercially.

RÉSUMÉ

Cette expérience portait sur huit anticorps monoclonaux contre les angigènes du virus respiratoire syncytial bovin; elle visait à déterminer s'ils pouvaient détecter ce virus dans du tissu pulmonaire bovin formolé et enrobé dans de la paraffine, à l'aide de la coloration immunohistochimique avec le complexe avidine-biotine. Des anticorps monoclonaux, issus du clone 18B2 et achetés chez Biosoft, Paris, France, ainsi que du cline 8G12 et achetés du département des sciences vétérinaires de l'université du Nebraska, Lincoln, Nebraska, colorèrent l'antigène précité dans le tissue pulmonaire infecté et firent ressortir, de façon acceptable, le tissu sain. Cette méthode semble par conséquent plus avantageuse que d'autres, pour la recherche du virus respiratoire syncytial bovin. En effet, elle ne nécessite pas l'utilisation de tissu frais et tous les raéctifs sont disponibles sur le marché.

Bovine respiratory syncytial virus (BRSV) is a pathogen of economic importance to the livestock industry (1). The diagnosis of this disease is usually based upon clinical signs, gross lesions, histological findings of syncytial cell bronchiolitis and alveolitis, and immunohistochemical demonstration of viral antigen in sections of affected tissues (1-3).

Immunohistochemical detection of microorganisms in tissue sections relies upon the availability of an antiserum which specifically binds to antigens expressed by the organism. Usually these antisera are sera from experimental animals immunized with the target microorganism. Increasingly however, hybridoma-derived monoclonal antibodies are replacing these polyclonal reagents in immunohistochemical tests. Monoclonal antibodies offer several advantages over polyclonal reagents, the most pertinent of which may be the capability for standardization of immunohistochemical test methods. If polyclonal antibodies are used the method will vary among different laboratories and within laboratories over time as antisera from different individual animals are depleted. Monoclonal antibodies of identical specificity are available in unlimited quantities providing an opportunity for standardization of immunohistochemical methods both within and between laboratories.

An avidin biotin complex (ABC) immunoperoxidase technique for the detection of BRSV antigen in formalin fixed tissue sections has been reported using polyclonal rabbit antisera (4,5). The present report describes evaluation of eight commerically available monoclonal antibodies directed against human or bovine RSV for the detection of BRSV in formalin fixed tissues with the ABC immunoperoxidase method.

The tissues examined included formalin-fixed, paraffin-embedded bovine lung from an animal with naturally occurring acute BRSV infection. The diagnosis was based upon clinical signs, histological evidence, and BRSV antigen demonstration by immunofluorescent staining of cryostat sections using a polyclonal rabbit antiserum. Eight commercially available monoclonal antibodies (Table I) were substituted for the polyclonal rabbit antisera to BRSV in the ABC immunoperoxidase stain (5). Each antibody preparation was applied to the tissue sections in

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TABLE I. Evaluation of Commercial Monoclonal Antibodies to Respiratory Syncytial Virus for Detection of Bovine Respiratory Syncytial Virus in Formalin Fixed Tissue Sections of Bovine Lung

Antibody Clone (dilution)	Specific Staining	Background Staining
18B2 (1/100)	++++	—
850 (1/100)	+++	++
858 (1/100)	+++	++
?*	—	—
Imagen	—	—
02-610-95	—	+
7C2 (1/100)	+++	+++
8G12 (1/200)	++++	+

Antibody sources: 18B2: Biosoft, Paris, France; 850 and 858: Chemicon Int. Inc., El Segundo, California; ?* manufacturer refused information about the clone: Whittaker Bioproducts, Walkersville, Maryland; Imagen: Boots-Celltech, Slough, Berkshire, United Kingdom; 02-610-95: York Biological Int., Stony Brook, New York; 7C2: Institut Armand-Frappier, Laval, Quebec; 8G12: Dr G. Anderson, University of Nebraska, Lincoln, Nebraska

serial twofold dilutions ranging from undiluted to 1/200. Binding of the monoclonal antibodies to the tissue sections was detected with 1/200 dilution of biotin labelled horse anti-murine IgG (Vector Laboratories Inc., Burlingame, California) followed by avidin-biotin-peroxidase complex solution (Vector Laboratories Inc., Burlingame California) then peroxidase substrate (3,3′-diaminobenzadine, Electron Microscopic Supplies, Fort Washington, Pennsylvania). Controls included sections in which there was omission of the RSV monoclonal antibody from the staining sequence or substitution of an irrelevant monoclonal antibody of the same isotype. In addition, specificity was demonstrated by applying the staining sequence to formalin-fixed bovine lung tissue sections from a normal animal and from an animal experimentally infected with bovine herpesvirus type 1 (BHV-1). The intensity of the staining was arbitrarily assessed on a scale of 0-4+. The stains were graded independently and blindly by two observers (DMH and BJC).

Dark, chocolate brown specific staining of some cells was present in formalin-fixed, paraffin-embedded tissues infected with BRSV and immunostained with monoclonal antibodies 18B2, 850 and 858, 7C2, and 8G12. Other RSV monoclonal antibody preparations failed to show specific staining (Table I). Specific staining was present as fine granular cytoplasmic staining of individual cells with accentuation of the cell surface and was especially prominent in cells forming syncytia within bronchioles and/or alveoli (Fig. 1). Nonspecific background staining was apparent as diffuse brown staining of all cells including connective tissues in BRSV infected tissues, in normal bovine lung and in BHV-1 infected tissues. The background staining varied from indiscernible with monoclonal antibodies 18B2 to that which almost completely obscured specific staining with antibodies of the 7C2 clone (Table I). There was no specific staining when the monoclonal antiserum to RSV was omitted from the staining sequence or when an irrelevant monoclonal antibody was substituted for the RSV antibody. There was no specific staining of normal bovine lung tissue sections or of sections from an animal infected with BHV-1 with most monoclonal antibodies, however, antibodies of the 8Gl2 clone stained large intracytoplasmic inclusions in mucus glands and within epithelial cells of associated large airways. This staining was sporadically observed in bronchial epithelium in both BRSV infected and uninfected tissues. These cells do not contain BRSV upon EM examination (D. Bryson, personal communication) and the staining likely reflects detection of a cross-reacting epitope on an unrelated molecule.

Seven commercial companies (6) market antibodies from the 18B2 clone developed in France by Pothier (7) which detects a nucleocapsid protein of human respiratory syncytial virus (HRSV). The 18B2 antibody was found in the present study to be highly effective in detection of the viral antigen of BRSV in formalin fixed and paraffin embedded tissues without background staining of other tissues. Two other monoclonal antibodies directed against HRSV, designated 850 and 858, were also effective in the detection of BRSV but the background staining of uninfected tissues was considerably greater than with the 18B2 preparation and might interfere with accurate interpretation. Three other monoclonal antibodies against HRSV were ineffective in the demonstration of BRSV. There are two equally likely explanations for the failure of these reagents to detect BRSV in formalin fixed tissues. Formalin fixation may have altered

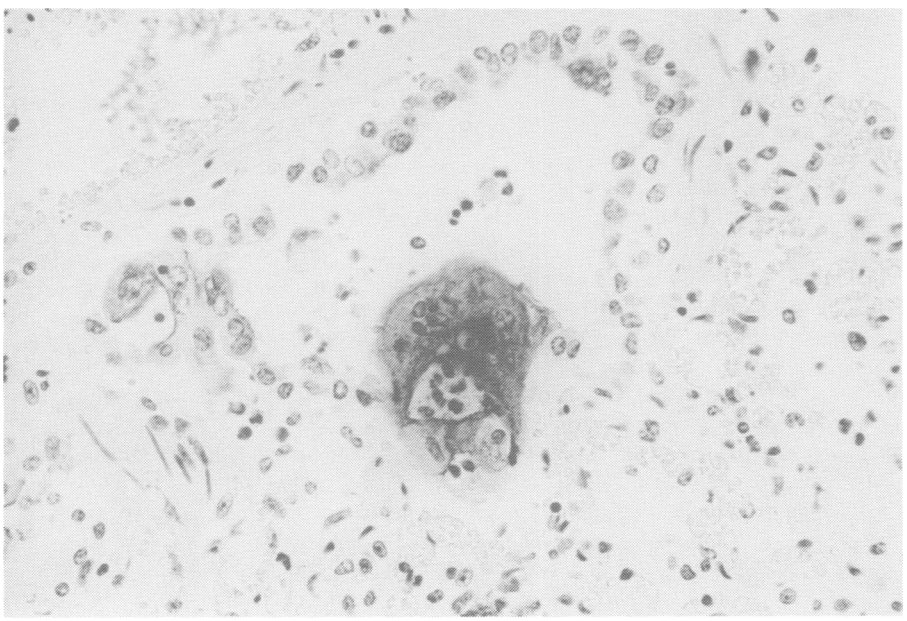

Fig. 1. Detection of bovine respiratory syncytial virus in formalin fixed and paraffin embedded bovine lung tissue section with monoclonal antibody cone 8G12 and an ABC immunoperoxidase method. The positive staining is dark brown, the section is counterstained with hematoxylin.

the antigenic sites in the tissue sections such that they were no longer recognized by the monoclonal antibody. An additional explanation for failure of antibodies to HRSV to detect BRSV may be differences in epitopes expressed on HRSV and BRSV. Other studies have shown that some but not all monoclonal antibodies cross-react to detect antigens common to both viruses (8). Two antibodies raised to BRSV were tested, these antibodies were effective in the detection of BRSV in this study, however both resulted in higher background staining than did the 18B2 antibody. The background staining of the 8G12 clone was considered acceptable while that of 7C2 interfered with interpretation of the specific staining. The sporadic staining of mucus glands and associated bronchial epithelial cells with antibody 8G12 is disconcerting; however it is distinct from and should not be confused with that of the syncytial cells which are characteristic of BRSV infection.

The ABC immunoperoxidase method described by Bryson et al (5) enables detection of BRSV antigens in routinely fixed sections. The present study has shown that commercially available monoclonal antibodies to RSV can be used in place of polyclonal antibodies to detect BRSV in formalin fixed tissues. The availability of these antibodies enables standardization of this technique in other laboratories.

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