Immunofluorescence Staining of Adenovirus in Fixed Tissues Pretreated with Trypsin

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We describe an immunofluorescence staining procedure for adenovirus in Formalin-fixed, paraffin-embedded tissue sections pretreated with a 0.25% trypsin solution. In trypsinized tissue sections stained with fluorescein-labeled antibody to adenovirus, viral antigen was brightly fluorescent and easily detected each time specimens were processed, and the nonspecific background fluorescence was always minimal. Viral antigen was nonfluorescent or only weakly fluorescent in tissue sections not pretreated with trypsin. Because pathology laboratories usually receive Formalin-fixed tissues for examination, this rapid and practical procedure can be routinely used to extend the diagnostic capability of conventional histopathology.

The detection of virus or viral antigen in biopsy and autopsy specimens by immunofluorescence (IF) staining has usually been hindered because of the loss of antigenic reactivity after fixation. Recently, however, Huang et al. (3) enhanced IF staining of hepatitis B antigen in Formalin-fixed, paraffin-embedded tissues by first digesting the tissue sections with a weak trypsin solution. Johnson et al. (4) then adapted the trypsin digestion method for the IF identification of rabies virus in fixed, paraffin-embedded brain sections. In this report we describe the application of a similar trypsin digestion technique to the IF staining of adenovirus in Formalin-fixed tissues submitted to our laboratory for confirmation of adenoviral infection.

Formalin-fixed, paraffin-embedded tissues selected for IF studies were from the lung of a human with adenoviral pneumonia (8) and from the pancreas of three rhesus monkeys with adenoviral pancreatitis (1, 5). All tissues had been taken at autopsy and were examined retrospectively. The lung specimen had been fixed in 10% neutral, buffered Formalin for 48 h and then embedded in paraffin for 1 year before IF examination. The three specimens of pancreatic tissues had been embedded in paraffin for 9, 5, and 2 years, respectively. Those embedded for 9 years had remained in Formalin for 4 months, but the other two specimens had been fixed in Formalin for only 72 h before embedding. In each case, light and electron microscopic examination of the lung or pancreas revealed adenovirus infection (6, 7, 9), and adenovirus had been isolated in two instances. The pathology of adenovirus infection (5, 8) and the procedures for viral isolation and characterization (2, 5) have been reported in detail, so they will not be discussed here.

Tissues were sectioned at 4 to 6 μm as for routine histological staining, picked up on alcohol-cleaned glass microslides coated with Histostik solution (Accurate Chemical and Scientific Corp., Westbury, N.Y.), deparaffinized by two 2-min passages through xylene baths, rehydrated in graded alcohols (100 to 80%), and rinsed in phosphate-buffered saline free of Ca2+ and Mg2+. To enhance IF staining of adenovirus, tissue sections on microslides were then gently immersed in a solution of 0.25% trypsin in Hanks balanced salt solution free of Ca2+ and Mg2+ (GIBCO Laboratories, Grand Island, N.Y.) for 1 h at room temperature. Before immersion, the trypsin solution was removed from the refrigerator at 4°C, slowly brought to room temperature in a 60°C water bath, and adjusted to a pH of 7.6 with a 7% solution of sodium bicarbonate. After trypsin digestion, the sections were gently rinsed in phosphate-buffered saline free of Ca2+ and Mg2+ for 5 min. Fluorescein isothiocyanate-conjugated goat antiserum (group specific for adenovirus; lot no. 3-8556; MA Bioproducts, Walkersville, Md.) that had been diluted 1:40 was then applied to each tissue section with a small pipette, and the sections were kept in a moist chamber at room temperature for 30 min. Sections were then washed by two 5-min immersions in phosphate-buffered saline baths, cover slips were applied, and the sections were examined with a Leitz Orthoplan Ploem binocular microscope illuminated by a 100-W halogen lamp and equipped...
Section of lung tissue from a patient with adenoviral pneumonia. Brightly fluorescing nuclear and cytoplasmic adenoviral antigen is seen as light areas in (A) alveolar and (B) bronchiolar lining cells. ×600.

with an H2 filter block and a K480 filter. Positive substrate controls consisted of HEp-2 cells infected with adenovirus. Formalin-fixed, paraffin-embedded sections of histologically normal human lung and rhesus monkey pancreas served as negative controls. No indirect IF staining procedures were done, and tissues fixed in other solutions were not examined.

In tissue sections infected with adenovirus and treated with trypsin before IF staining, adenovirus antigen was strongly fluorescent each time slides were processed. Large amounts of green, brightly fluorescent antigen were seen in the nucleus and sometimes the cytoplasm of alveolar and bronchiolar epithelial cells of human lung (Fig. 1) and of acinar and ductal epithelial cells of rhesus monkey pancreas (Fig. 2). Many of the epithelial cells contained a solid, homogeneous mass of fluorescing material which evidently corresponded to the "smudge" cells created by nucleocytoplasmic fusion as seen by light microscopy (5, 8, 9). Other adenovirus-infected cells contained small, intensely fluorescent fragments that were predominantly intranuclear. Nonspecific fluorescence was always minimal, and background tissues appeared faint yellowish-brown. Control slides of adenovirus-infected HEp-2 cells were uniformly and strongly positive, whereas sections of histologically normal human lung and rhesus monkey pancreas showed little or no fluorescence and appeared yellowish-brown.

IF identification of adenovirus antigen in Formalin-fixed tissues can be routinely used in the laboratory to extend the diagnostic capability of conventional histopathology. The IF technique is particularly helpful when intranuclear inclusions in histological sections are suspected of being adenovirus but cannot be differentiated morphologically from inclusions formed during other viral infections, such as those due to cytomegalovirus. Ultrastructural studies are also helpful, but they take more time, are less specific, and are very restrictive for sampling as compared with IF techniques. Rapid identification of adenovirus by IF in routine histological sections has other advantages. Tedious, time-consuming, and costly culture procedures are eliminated, and the hazards of handling infectious materials are reduced because the virus is
inactivated by Formalin. Further studies on the rapid IF identification of other viral pathogens in Formalin-fixed tissues is certainly warranted.

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LITERATURE CITED

FIG. 2. Fluorescing adenoviral antigen in pancreatic acinar cells (arrows) from a rhesus monkey with necrotizing pancreatitis. The pancreas section was treated with trypsin solution and stained with fluorescein-labeled antibody to adenovirus. ×600.