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Short communication

Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies

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Abstract

Feline infectious peritonitis (FIP) virus antigen was demonstrated after methanol, ethanol or formalin fixation in paraffin-embedded tissues by means of monoclonal and polyclonal antibodies. The monoclonal antibody was induced by immunization with transmissible gastroenteritis virus. Polyclonal antibodies were obtained by purification on protein A-Sepharose of ascites fluid from a cat with FIP. Almost all cats diagnosed as suffering from FIP by postmortem and histological examination exhibited FIP virus (FIPV) antigen in macrophages in granulomas whereas FIPV antigen was only once demonstrable in another location.

Keywords: Cat disease; Feline infectious peritonitis (FIP); Feline coronavirus; Monoclonal antibodies

1. Abbreviations

FIP, feline infectious peritonitis; FIPV, feline infectious peritonitis virus; TGEV, transmissible gastroenteritis virus.

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2. Introduction

A definite diagnosis of feline infectious peritonitis (FIP) usually requires a post-mortem examination (Grahn, 1991). In this context, the application of enzyme-based immunohistological techniques allows the simultaneous evaluation of histopathological changes and in situ detection of virus, which greatly aids in diagnosis.

The purpose of this study was to evaluate immunoperoxidase techniques for in situ identification of FIP virus (FIPV) in paraffin-embedded tissue specimens obtained from cats with clinical and/or histopathological changes indicative of FIP. Ascites fluid from a cat with effusive FIP and a monoclonal antibody against porcine coronavirus (transmissible gastroenteritis virus, TGEV) were used as primary antiserum/antibody.

3. Materials and methods

3.1. Animals

One hundred and eight cats sent to the Department of Pathology, School of Veterinary Medicine, University of Giessen, Germany, and to the National Veterinary Institute, Oslo, Norway, for postmortem examination were investigated histologically for FIP-specific granulomas when gross pathology was indicative of FIP. These cats were also subjected to immunohistological examination for FIPV antigen in the focal lesions present as well as in various organs and tissues.

3.2. Antibodies

3.2.1. Polyclonal antibodies

The coronavirus antibody titre in ascites fluid of cats that had died of the effusive form of FIP was determined by an indirect immunofluorescence assay according to the method of Osterhaus et al. (1977). Antibodies were purified from filtered ascites fluid with an FIP titer of > 1:1600 by affinity chromatography on a protein A-Sepharose Cl-4B column (Pharmacia, Uppsala, Sweden) according to the method of Ey et al. (1978). Purified antibodies were coupled with horseradish peroxidase (purity grade 1, salt-free; Boehringer Mannheim, Mannheim, Germany) as described by Nakane and Kawaoi (1974).

3.2.2. Monoclonal antibodies

For the production of monoclonal antibodies BALB/c mice were immunized with the TGEV capsid protein p56 highly purified by preparative polyacrylamide gel electrophoresis as described elsewhere (Lutz et al., 1981). Hybridomas were selected by enzyme-linked immunosorbent assay using TGEV purified from cell cultures by ultracentrifugation over sucrose gradients. Antibody positive hybridomas were recloned three times and their specificity was tested by Western blotting under conditions described elsewhere (Lutz et al., 1988). Five hybridoma cultures that produced antibodies recog-
nizing p56 of TGEV and of FIPV (Strain UCD 2; kindly provided by N.C. Pedersen) were used for ascites induction in balb/C mice. The animals were inspected daily, also over weekends, and were euthanized when they began showing signs of discomfort. Ascites fluid was collected, filtered and stored at −70°C until use. One of the antibodies (63 I G9C4) was used in the present study.

3.4. Histology and immunohistology

Specimens were fixed in 10% phosphate buffered (pH 7.2) formalin, absolute methanol or absolute ethanol. Embedding in paraffin and staining with haematoxylin and eosin followed routine procedures for diagnostic histopathology. Different approaches were used to demonstrate FIPV antigen immunohistologically in tissues. Regardless of the method used, endogenous peroxidase was inhibited by incubation of the dewaxed sections in methanol containing 3% hydrogen peroxide for 30 min. The rehydrated sections were preincubated with undiluted swine serum for 10 min followed without washing steps by the first incubation with the specific antisera and antibody, respectively. All the antibody incubation steps were performed for 30 min at room temperature, followed each by three wash steps with Tris-buffered saline (TBS; 0.05 M Tris-HCl, pH 7.6, with 0.8% NaCl). The antibodies were diluted in 20% swine serum diluted in TBS. Visualization of bound peroxidase activity was demonstrated using diaminobenzidine according to Graham and Karnovsky (1966).

3.5. Direct immunoperoxidase method

For this method, antibodies purified from the ascites fluid of a cat that had died of effusive FIP (see above) were labelled with peroxidase and diluted 1:200 for use in the immunohistological incubation. Control group tissues were provided from tissues of cats without FIP alterations, including cats with peritonitis from other causes. Control serum was provided by the use of antibodies prepared as above from the ascites of a FIP-negative cat that had also tested negative for FIP antibodies.

3.6. Indirect immunoperoxidase method

The monoclonal antibody 63I was diluted 1:400 for the first incubation step, followed by peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako, Copenhagen, Denmark) diluted 1:100. Controls were as described above except that for the second type of controls an irrelevant murine IgG₂ monoclonal antibody directed against rat pyruvate kinase isoenzyme type L (Domingo et al., 1992) was used as primary antibody.

3.7. Avidin–biotin–peroxidase complex method

The avidin–biotin–peroxidase method was performed as described previously (Evensen, 1993) using the monoclonal antibody described above. Briefly, deparaffinized sections of formalin-fixed organ samples were pretreated with trypsin and the sections
were overlaid with phosphate buffered saline with bovine serum albumin (5 mg ml\(^{-1}\)) to block unspecific binding sites. The monoclonal antibody was diluted 1:10,000 (formalin-fixed specimens) or 1:20,000 (ethanol-fixed specimens), and incubated for 16–18 h at room temperature. Controls were performed as described above.

4. Results

A specific positive reaction was observed only in FIP-specific granulomas (Fig. 1), either in macrophages or in necrotic areas. In a single cat positive monocytes could be seen intravascularly in blood vessels of the choroid plexus of the brain. The reaction was granular in appearance and varied from being very intense and occurring in most macrophages of a given granuloma, to being so inconspicuous that it was seen only in a few cells and was detectable only when using the high-power objective (40 × ). The reaction pattern did not differ between the monoclonal antibody 63I and the polyclonal feline ascites antibodies. A positive reaction was obtained in 100 of the 102 cats with FIP; 98 cats were positive with the monoclonal antibody 63I as well as with the polyclonal feline ascites antibodies, while one cat each was positive only with one of the two types of antibodies used in this study. None of the cats without FIP investigated in this study (two with lymphosarcoma, two with adenocarcinoma metastasizing in the peritoneal cavity, one with hepatocellular adenoma and interstitial nephritis, one with necrotizing pancreatitis) exhibited any positive reaction. Positive staining of formalin-fixed specimens was dependent on use of buffered formalin and pretreatment with trypsin. Demasking of the epitope identified by the monoclonal antibody was best achieved after 90 min of trypsinization.

We could not demonstrate coronavirus antigen in epithelial cells of the intestinal tract in cats that had died of FIP. In one cat the intestinal epithelium was coronavirus
antigen-negative in an area where immunohistologically positive FIP granulomas in the intestinal wall had ulcerated into the lumen of the intestine, and where shedding of FIPV in the direct vicinity of epithelial cells would have been expected.

The nictitating membranes of 25 cats that had died of FIP and that were immunohistologically positive in granulomas at other locations were examined for FIP antigen. Twenty-two were completely negative. Two cats exhibited positive macrophages in tiny granulomas in the mesenchyma but the epithelial cells were negative. One cat showed a positive reaction in epithelial cells which was also present, however, in the control incubations and thus was not regarded as FIP-specific.

5. Discussion

FIPV antigen is demonstrable using antibodies purified from ascites fluid of a cat that had died of effusive FIP, as well as with monoclonal antibodies. Each type was able to visualize FIPV antigen in 97% of the histopathologically diagnosed FIP cases.

With one exception FIPV antigen could be seen only in FIP-specific granulomas. This result contradicts statements in the paper of Walter et al. (1989), whose results were also questioned by the same group itself in a later publication (Wichmann-Schauer, 1990).

FIPV antigen could not be demonstrated in epithelial cells of the nictitating membrane. Only in 8% of the cats which died of FIP could antigen be seen in infiltrating macrophages in the mesenchyma of the third eyelid. The differences to the results obtained by Höök (1989, 1991) may be due to the different antibodies and methods used.

Coronavirus antigen was never demonstrated in enterocytes in cats which died of FIP even when shedding of FIPV from ulcerated granulomas present in the intestinal wall in direct vicinity of the epithelial cells was to be expected. These facts do not support the hypothesis that the common pathogenesis of spontaneous FIP cases is a mutation of FIPV from feline enteric coronavirus (Evermann, 1991; Addie and Jarrett, 1992).

Immunohistological demonstration of FIPV antigen is not necessary for routine diagnosis of FIP at postmortem investigation, since histopathology yields results of similar reliability. Such an investigation may be helpful, however, in cases having infiltrates in tissues that are not clearly FIP-specific in histopathology. The rare cases with histopathological alterations typical for FIP which are immunohistologically negative for FIPV antigen remain challenging. They may be explained by methodological problems concerning the amount of virus present in the granulomas. In addition to technical difficulties, these cases could also be explained by coronaviruses immunologically unrelated to FIPV or TGEV, such as a coronavirus similar to the immunologically distant bovine coronavirus group.

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