Abstract

A female, 10-month old, mixed breed cat showed clinical signs of severe dehydration, purulent nasal discharge, dyspnea, severe crepitating lung sounds and no response to antibiotic treatment. Upon necropsy, histopathology revealed severe, diffuse, fibrinopurulent pleuropneumonia and numerous, homogeneous, amphophilic, intranuclear inclusion bodies in the pulmonary alveolar macrophages, bronchial and alveolar epithelium and bronchial glandular epitheliums. Immunohistochemical studies using 3 different monoclonal, anti-Feline Herpesvirus type 1 antibodies, revealed immunoreactivity of viral antigen in the lung that was associated with the histopathological changes. Monoclonal, anti-glycoprotein B, antibody showed the strongest immunoreactivity.

Keywords: cat, lung, Feline Herpesvirus type 1, immunohistochemistry
Introduction

Feline viral rhinotracheitis (FVR) was first reported in 1958. The virus has been classified in the subfamily Alphaherpesvirinae as a feline herpesvirus type 1 (FHV-1) (Maeda et al., 1995). The virus was considered to be the major cause of upper, respiratory tract, disease in cats. Common clinical signs included sudden the onset of conjunctivitis, rhinitis, serous to mucopurulent oculo-nasal discharges, coughing and sneezing. In natural cases, bronchial pneumonia, keratitis, corneal ulceration and panophthalmitis, ulcerative glossitis and abortion have been described (Carlson and Scott, 1978). In young cats, FHV-1 associated mortality can exceed 60% (Burgesser et al., 1999).

The conventional method for the diagnosis of herpesvirus infections is by detecting its cytopathic effects in tissue cultures. Herpes viral glycoproteins are very important in viral infections. These molecules play an important role in the attachment of the virion to the host cell, syncytial formation, cell-to-cell spread and the determination of the subtype specificity of the virus (Maes et al., 1984). Recently polymerase chain reaction assays, for active and latent infection, have been developed (Stiles et al., 1997; Burgesser et al., 1999; Suchy et al., 2000).

FHV-1 viral glycoproteins have been shown to be responsible for protective immunity after vaccination and are a critical part of the defense mechanism of the host against infection (Maes et al., 1984). The properties and function of FHV-1 glycoproteins have been described, including glycoprotein B(gB) and glycoprotein C(gC) (Maeda et al., 1995).
et al., 1998). FHV-1 gB induces high titers of virus-neutralizing antibodies in mice and shares common antigenic epitopes with *Herpes simplex* virus-1 gB and canine herpes virus gB (Maeda et al., 1998).

The purpose of this study was to demonstrate some uncommon lesions of FHV-1 infection in lungs by histopathological criteria and to compare the efficacy of 3 different monoclonal antibodies against FHV-1, using immunohistochemical techniques.

**Materials and methods**

Case history: A female, 10-month old, mixed breed cat with clinical signs of severe dehydration, purulent nasal discharge, dyspnea, severe lung crepitation sounds and no response to ampicillin treatment, was submitted to the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. A necropsy was performed and formalin-fixed samples of lung, liver, spleen, kidney and brain were sliced into 0.5 cm. thick serial sections, embedded in paraffin wax and submitted for routine histopathologically processing. The blocks were sectioned 5-6 µm. thick and stained with Hematoxylin and Eosin (H&E).

**Immunohistochemistry**

Immunohistochemical identification of feline rhinotracheitis viral antigen using the peroxidase-anti-peroxidase (PAP) method was performed on formalin-fixed, paraffin embedded tissues, using silane-coated slides. Tissue sections were deparaffinized and hydrated by immersion in xylene, graded alcohols and distilled water. Endogenous peroxidase activity was blocked by incubating sections for 30 min in 2% hydrogen peroxide solution. Sections were incubated with skim milk, for blocking non specific reaction, for 20 min. Sections were incubated with 3 different monoclonal anti-Feline herpesvirus I glycoprotein antibodies (courtesy by Dr. K. Tsuchiya), clones 22 F-4 (glycoprotein B; gB), clones 35F-4 (glycoprotein C; gC) and clones WIGH strain (dilution 1: 1000) in PBS at 37°C for 1 hr. After another rinse in PBS, sections were consecutively incubated with commercial, peroxidase, conjugated, antibody polymer (Nichirei, Japan), stained slides in 3,3’-diaminobenzidine tetrahydrochloride solution (DAB) and counterstained with Mayer’s hematoxylin. Slide sections were dehydrated through immersion in ethyl alcohol and xylene and mounted with permount. The slides were observed under a light microscope.

**Result**

**Pathological findings**

At necropsy, a copious purulent discharge was observed in the nostril. The state of the carcass was fresh and markedly dehydrated. Fibrinopurulent exudate was found in trachea. All lobes of the lungs showed severe, diffuse, fibrinopurulent, pleuropneumonia. Fatty change was found in the liver and 3 *Toxocara cati* were found in the intestine together with a catarrhal enteritis.

Histopathologically, lungs showed a severe, fibrinopurulent, broncho-pleuropneumonia
**Figure 1**  severe suppurative pneumonia with accumulation of fibrin, pus cells, pulmonary alveolar macrophages and syncytial cells (lung, H&E, bar = 25 µm.)

**Figure 2**  Homogeneous amphophilic intranuclear inclusion body in syncytial alveolar macrophages (arrow head, H&E, bar = 50 µm.)

**Figure 3**  FHV-1 antigen positive in bronchial glandular epitheliums (arrow heads, IHC, anti-gB antibody, bar = 25 µm.)

**Figure 4**  FHV-1 antigen positive in both nuclei and cytoplasms of intact and degenerated pulmonary alveolar macrophages (arrow heads, IHC, anti-gB antibody, bar = 50 µm.)
characterized by fibrin, tissues debris, polymorphonuclear cells, pulmonary alveolar macrophages (PAM) and some syncytial cells (Figure 1). A number of homogeneous, amphophilic, intranuclear, inclusion bodies were found in PAM, bronchial and alveolar epithelial cells, and bronchial glandular epithelial cells (Figure 2). The liver showed evidence of neutrophils and mononuclear cells, infiltrated in portal triads and sinusoids. Centrilobular fatty degeneration and congestion were also found. The intestine was infiltrated with chronic inflammatory mononuclear cells and hyperplasia of the circular muscle layer was noted. No evidence of viral inclusion bodies could be detected in the liver, kidney or spleen.

**Immunohistochemistry:** Immuno-positive antigens were detected in both intranuclear and intracytoplasmic areas of infected cells. There were all kinds of antibodies, especially in the PAM (Figure 3.), bronchial and alveolar epithelium and the bronchial glandular epithelium (Figure 4.). Some endothelial cells in the lungs were also positive. The characteristic patterns of positive immunoreactivity for each of the antibodies were different. Anti-gB monoclonal antibody gave the best result for detection and was easily observed. Anti-gC monoclonal antibody and anti-WIGH strain antibody gave the weakly positive results. There was no positive immunostaining for FHV-1 detected in samples of the liver, spleen, kidney and brain.

**Discussion**

Based on the histopathological studies, common lesions of FHV-1 infections were focused in the upper respiratory tract, including ulcerative glossitis, with a Cowdry-type A intranuclear inclusion body (Sheilds and Gaskin, 1977). This unusual appearance of the FHV-1 lesion in the lungs revealed some characteristics of a herpesvirus infection, especially the homogeneous, eosinophilic, intranuclear, inclusion bodies in the bronchial and alveolar epithelial cells. Such progressive lesions of FHV-1 might be assisted severe immunosuppressive effects of other diseases, such as feline immunodeficiency virus, feline leukemiavirus, feline calicivirus, bacterial infections or chlamydial infections (Nakamura et al., 1999). As can be seen in other herpesvirus infections, FHV-1 can be detected in epithelial cells and in cells of mesenchymal origin, such as endothelial cells and macrophages indicating its cellular polytropisms (Suchy et al., 2000).

The immunohistochemical methods used in this study were rather simple, no special treatment for retrieving antigen was used, such as enzyme digestion or microwave treatment. Monoclonal anti-glycoprotein antibody gave the best results when compared with the whole virion of WIGH strain monoclonal antibody. The study using antibody against glycoproteins has been very useful for the detection of herpes viral antigen. Numerous FHV-1 glycoproteins were incorporated into membranes of infected cells and the virion envelope. These glycoproteins have been characterized and are typical for related herpesvirus
Studies of *Herpes simplex* virus type-1 glycoproteins has shown them to play an important role in membrane attachment, complement binding penetration of the virion into cells by cell, to cell spread, induction of virus-neutralizing antibodies and cell-mediated immunity. Glycoprotein gene clusters appears to be a feature throughout the *γ*-*Herpesvirinae* subfamily (Maes et al., 1984; Spatz et al., 1994).

Glycoprotein B monoclonal antibody showed a better positive reaction than glycoprotein C monoclonal antibody under the same condition. FHV-1 gB is one of the most conservative proteins among three herpesvirus subfamilies, such as α-, β- and γ-*Herpesvirinae*. Purified gB induces high titers of virus neutralizing antibodies in mice, it appears also to be one of the most important candidates for a subunit vaccine against FHV-1 infection (Maeda et al., 1998).

Acknowledgement

We would like to thank Dr. Tsuchiya Katoro, Nippon Institute of Biological Science, Japan for kindly providing FHV-1 antibodies.

References


