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Alveolar macrophages are the main target cells in feline calicivirus-associated pneumonia

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ABSTRACT

Feline calicivirus (FCV) is a pathogen of felids and one of the most common causative agents of feline upper respiratory disease (URD). Reports of natural FCV pneumonia in the course of respiratory tract infections are sparse. Therefore, knowledge on the pathogenesis of FCV-induced lung lesions comes only from experimental studies. The aim of the present study was to assess the type and extent of pulmonary involvement in natural respiratory FCV infections of domestic cats and to identify the viral target cells in the lung. For this purpose, histology, immunohistochemistry and RNA-in situ hybridisation for FCV and relevant cell markers were performed on diagnostic post-mortem specimens collected after fatal URD, virulent systemic FCV or other conditions. All groups of cats exhibited similar acute pathological changes, dominated by multifocal desquamation of activated alveolar macrophages (AM) and occasional type II pneumocytes with fibrin exudation, consistent with diffuse alveolar damage (DAD). In fatal cases, this was generally seen without evidence of epithelial regeneration. In cats without clinical respiratory signs, type II pneumocyte hyperplasia was present alongside the other changes, consistent with the post-damage proliferative phase of DAD. FCV infected and replicated in AM and, to a lesser extent, type II pneumocytes. This study shows that lung involvement is an infrequent but important feature of FCV-induced URD. AM are the main viral target cell and pulmonary replication site, and their infection is associated with desquamation and activation, as well as death via apoptosis.

Introduction

Feline calicivirus (FCV) is one of the most common causative infectious agents of feline upper respiratory disease (URD; Bannasch and Foley, 2005; Di Martino et al., 2007; Gaskell et al., 2012). URD is widespread and common in cats and presents with a variety of clinical signs, including conjunctivitis, rhinitis, oral ulcers and, occasionally, pneumonia (Hurley and Sykes, 2003; Radford et al., 2007, 2009; Pesavento et al., 2008; Gaskell et al., 2012). The mortality rate is usually low, but occasionally kittens develop fatal pneumonia (Love and Baker, 1972; Turnquist and Ostlund, 1997).

FCV is a member of the Caliciviridae, which possess a non-enveloped, positive-sense, single-stranded RNA genome of approximately 7.5 kb (Clarke and Lambden, 1997). The virus exhibits high genetic variability and there are a variety of natural strains (Radford et al., 2003) with variable cell tropism, pathogenesis and virulence, resulting in different clinical manifestations, such as self limiting URD, lameness due to acute synovitis (Dawson et al., 1994) and systemic disease caused by highly virulent strains, so-called virulent systemic (VS)-FCV (Hurley and Sykes, 2003; Radford et al., 2007; Pesavento et al., 2008). The genetic diversity of FCV is a consequence of changes in the hypervariable C and E regions that encode part of the capsid proteins involved in the generation of neutralising antibodies (Radford et al., 1999; Geissler et al., 2002). Animals develop a protective immune response against FCV (Kahn et al., 1975; Kahn and Hoover, 1976; Scott, 1977); however, continual genomic changes in the virus can lead to reinfections with different strains or closely related variants of the same strain, despite previous infection episodes (Johnson, 1992; Radford et al., 2003; Coyne et al., 2006b, 2007). Due to the genetic instability of FCV, effective disease prophylaxis has remained challenging (Radford et al., 1997) and, after years of vaccination programmes, infection is still widespread, leading to the search for new heterologous vaccines that can protect against more than one strain (Poulet et al., 2005; Radford et al., 2006).

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Moreover, in 1998, new highly virulent VS-FCV strains emerged. The first VS-FCV cases were observed in California (Pedersen et al., 2000), but further outbreaks have subsequently been reported from the USA (Schorr-Evans et al., 2003; Hurley et al., 2004; Pesavento et al., 2004) and, more recently, Europe (Coyne et al., 2006a; Schulz et al., 2011; Battilani et al., 2013; Velasco et al., 2013). The most frequent clinical signs are cutaneous oedema (mainly on the head and limbs), ulceration of the skin and mucosa, predominantly affecting the oral cavity, nares, pinnae and footpads, and clinical signs due to multi-organ necrosis, most commonly affecting the liver, but occasionally also the spleen, pancreas or lungs (Pedersen et al., 2000; Hurley and Sykes, 2003; Coyne et al., 2006a; Radford et al., 2007, 2009; Pesavento et al., 2008).

Reports of natural FCV pneumonia are sparse and our understanding of the pathogenesis of FCV-induced lung lesions is from experimental studies only. These used high doses (up to 2 x 10^4 median tissue culture infective dose) of virus propagated in tissue culture in an aerosol for the intranasal infection of kittens and young cats, which often resulted in pulmonary involvement (Holzinger and Kahn, 1970; Kahn and Gillespie, 1971; Hoover and Kahn, 1973, 1975; Love, 1975; Langloss et al., 1978a). The development and type of lung lesions was generally similar; after an initial phase of pneumocyte injury with exudation and neutrophil infiltration, proliferation of type II pneumocytes and desquamation of alveolar macrophages (AM) into the alveolar lumen was observed. Virus was detected by immunofluorescence in pneumocytes and AM (Holzinger and Kahn, 1970; Kahn and Gillespie, 1971). Some authors suggested a correlation between the type of virus inoculum and the clinical signs (Love, 1975; Ormerod et al., 1979), and it has since been accepted that the experimental studies overemphasise the relevance of FCV-associated pneumonia, since natural infection mainly occurs via the oronasal route (Radford et al., 2007; Gaskell et al., 2012). However, other authors have suggested that severe pneumonia might not be rare in naturally infected cats (Pesavento et al., 2008).

Definitive reports of pneumonia as a complication of severe, naturally acquired FCV-associated respiratory disease are very rare (Love and Baker, 1972; Turnquist and Ostlund, 1997). Furthermore, attempts to demonstrate the virus in the lungs to assess whether FCV or bacteria, such as *Bordetella bronchiseptica*, were the relevant pulmonary pathogens, have not been made (Turnquist and Ostlund, 1997). This study, which was initiated after acute pneumonia and FCV infection had been diagnosed post-mortem in a number of cats with URD, aimed to assess the type and extent of pulmonary involvement in natural respiratory FCV infections of domestic cats, and to identify the viral target cells in the lung. For this purpose, histopathology, immunohistochemistry (IH) and RNA-in situ hybridisation were employed on post-mortem specimens from cats with fatal URD, VS-FCV or other conditions.

Materials and methods

Animals and tissues

The study was performed on cats from Germany, the UK, Finland, Italy and Spain that had undergone full diagnostic post-mortem examinations. Tissue specimens had been collected for histological examination and, in some cases, for virological and bacteriological examinations (Tables 1–4). Four groups were included. Group 1 comprised eight cats with clinical histories of URD and pneumonia. FCV was isolated from the lungs by virus culture, and involvement of FCV in the pneumonia was confirmed by IH. Group 2 comprised five cats with URD and pneumonia. Virus culture was not performed, but FCV involvement was confirmed by IH. Group 3 comprised two cats without URD. These had been euthanased because of feline parvovirus infection, but exhibited histopathological findings consistent with FCV pneumonia, confirmed by FCV IH. Group 4 comprised four cats with VS-FCV; pulmonary specimens from these cats were examined by IH for the presence and distribution of FCV. Most of the cats in group 4 have since been reported as confirmed cases of VS-FCV in the UK (Coyne et al., 2006a), Italy (Battilani et al., 2013) and Spain (Velasco et al., 2013).

All lung tissue specimens were fixed in 10% non-buffered formalin for 24–72 h, followed by trimming and routine paraffin wax embedding. Specimens of tongue, larynx or nose were also examined when they showed gross lesions associated with URD. Other tissues/organs (spleen, liver, kidney, intestine, brain) were processed for histological examination, to identify or exclude any concurrent disease.

Table 1
Relevant gross and histological findings for group 1: Cats with upper respiratory disease (URD) and pneumonia, involvement of feline calicivirus (FCV) confirmed by isolation of FCV from the lungs and immunohistochemistry (IH) for FCV antigen.

Case	Breed	Age	Sex	Lung histopathology	Demonstration of FCV
1.1	ESH	11 years	F	Multifocal mild to moderate fibrin exudation and low numbers of AM/type II pneumocytes [a] in AL	IH: Scattered cells in AW (type II pneumocytes) and in AL (AM)
1.2	DSH	8 weeks	NK	Multifocal moderate to marked fibrin exudation and moderate numbers of often apoptotic [b] AM/type II pneumocytes with a few neutrophils in AL; bronchiolar lumen with similar content; moderate hyperaemia and perivascular oedema	IH: Moderate numbers of cells in AL, occasional cells in AW ISH (S, AS): Moderate number of cells in AL (IH, FHV: Negative)
1.3	DSH	12 weeks	F	Multifocal marked fibrin exudation and abundant AM/ type II pneumocytes in AL; bronchiolar lumen with similar content; diffuse fibrin exudation on pleura	IH: Low numbers of cells in AL ISH (S, AS): Low number of cells in AL
1.4	DSH	8 weeks	F	Multifocal moderate to marked fibrin exudation and abundant, occasionally apoptotic AM/type II pneumocytes in AL; moderate numbers of extravasated erythrocytes and erythrophagocytosis in AL; bronchioles with similar content and with focal loss and degeneration of epithelial cells and presence of macrophages	IH: Low to moderate numbers of cells in AL, some macrophages/degenerate epithelial cells in bronchiolar wall. ISH (S, AS): Low number of cells in AL
1.5	ESH	6 years	MN	Multifocally, low to moderate numbers of AM/type II pneumocytes in AL; marked alveolar oedema and occasional extravasated erythrocytes in AL	IH: Low numbers of cells in AL (IH, FHV: Positive, tongue; negative, lung)
1.6	MC	1 week	M	Multifocal mild to moderate alveolar fibrin exudation and desquamation of abundant, often apoptotic AM and type II pneumocytes; bronchioles with similar content	IH: Moderate numbers of desquamated cells in alveoli ISH (S, AS): Scattered cells in alveolar lumen
1.7	MC	12 weeks	F	Multifocal moderate fibrin exudation and abundant, often apoptotic AM and type II pneumocytes in AL, in focal area with extensive fibrin exudation and necrosis, and neutrophil infiltration	IH: Moderate numbers of cells in AL; cell free viral antigen in area with necrosis (IH, FHV: Negative)
1.8	DSH	4 weeks	M	Multifocal extensive fibrin exudation and abundant, often apoptotic AM/type II pneumocytes in AL, mild erythrocyte extravasation in AL.	IH: Scattered desquamated cells in AL

ESH, European shorthair; DSH, domestic shorthair; BSH, British shorthair; MC, Maine Coon; F, female; M, male; N, neutered; NK, not known; AL, alveolar lumina; AM, alveolar macrophages; AS, anti-sense probe; AW, alveolar wall; FHV, feline herpesvirus type 1; ISH, RNA-in situ hybridisation; S, sense probe.

[a] AM/type II pneumocytes, numerous CD18 positive AM and scattered individual SP-C positive type II pneumocytes.

[b] Apoptosis/apoptotic: confirmed by IH (cleaved caspase-3 positive).