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Feline leucocyte antigen class II polymorphism and susceptibility to feline infectious peritonitis

Diane D. Addie[a]*, Lorna J. Kennedy[c], Ruth Ryvar[b], K. Willoughby[d], R.M. Gaskell[b], W.E.R. Ollier[c], Pablo Nart[a], Alan D. Radford[b]

[a] *Institute of Comparative Medicine, Companion Animal Diagnostics, Bearsden Road, Bearsden, Glasgow, Scotland G61 1QH, UK*
[b] *Mammalian Immunogenetics Research Group, Faculty of Veterinary Science, University of Liverpool, Crown Street, Liverpool L69 7ZJ, UK*
[c] *Centre for Integrated Genomic Medical Research, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK*
[d] *Department of Veterinary Pathology, University of Edinburgh, Easter Bush, Edinburgh, UK*

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Summary There are four outcomes to feline coronavirus (FCoV) infection: the development of feline infectious peritonitis (FIP, which is immune-mediated), subclinical infection, development of healthy lifelong carriers and a small minority of cats who resist infection (Addie and Jarrett, Veterinary Record 148 (2001) 649). Examination of the FCoV genome has shown that the same strain of virus can produce different clinical manifestations, suggesting that host genetic factors may also play a role in the outcome of infection. FIP is most prevalent amongst pedigree cats, although how much of this is due to them living in large groups (leading to higher virus challenge and stress which predisposes to FIP) and how much is due to genetic susceptibility is not known. If host genetics could be shown to play a role in disease, it may allow the detection of cats with a susceptibility to FIP and the development of increased population resistance through selective breeding. The feline leucocyte antigen (FLA) complex contains many genes that are central to the control of the immune response. In this preliminary study, we used clonal sequence analysis or reference strand conformational analysis (RSCA) to analyse the class II FLA–DRB of 25 cats for which the outcome of FCoV exposure was known. Individual cats were shown to have between two and six FLA–DRB alleles. There was no statistically significant association between the number of alleles and the outcome of FCoV infection. No particular allele appeared to be associated with either the development of FIP, resistance to FCoV, or the carrier status. However, the analysis was complicated by apparent breed variation in FLA–DRB and the small number of individuals in this study.

Introduction

Feline coronavirus (FCoV) is a ubiquitous infection of cats which leads to persistent carrier status in 13% of infections and to the development of the fatal disease, feline infectious peritonitis (FIP), in less than 10%. The majority of cats infected with FCoV successfully eliminate the virus, but are susceptible to re-infection by the same or another strain (Addie et al., 2003). Since cats which have different outcomes are often infected with one

* Corresponding author: Tel.: +44-141-330-5786; fax: +44-141-330-5748
E-mail address: D.D.Addie@vet.gla.ac.uk (D.D. Addie).

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strain of FCoV, it is likely that genomic variation between cats also contributes to varied clinical outcome. There is some evidence that susceptibility to development of FIP in the cat has a somatic basis (Foley and Pedersen, 1996) and the apparent susceptibility of cheetahs to FIP has been ascribed to that species having gone through a genetic bottleneck in the past (O'Brien et al., 1987).

There is some evidence that cats developing FIP undergo a shift from Th1 to Th2 (Lutz, personal communication), and it is therefore possible that variation in the class II feline leucocyte antigen (FLA)-DRB could account for differences in response to FCoV infection. The FLA complex contains many genes that are central to the control of the immune response. As such, they are intimately involved in an individual's response to antigenic challenge following infection or vaccination. Many of the genes of the FLA are extremely polymorphic leading to individual variation in clinical phenotype. To date, studies on FLA polymorphism are limited and largely restricted to the class II DRB locus.

The number of FLA–DRB loci present in the cat is not yet clear: four genes have been identified by sequence analysis of the class II FLA region, of which one appeared to be a pseudogene (O'Brien et al., 2002). However, the number of genes may vary between individuals. A total of 25 DRB alleles have been characterised so far in cats although it is likely that others will be identified (LJ Kennedy, personal communication).

In this preliminary study, we have attempted to identify correlations between FLA–DRB genotype and FCoV status in individual cats.

Materials and methods

Thirty-three pet cats from the UK were studied including representatives of eight breeds. Seventeen were part of a larger cohort of cats involved in a FCoV excretion survey (Addie and Jarrett, 2001; Addie et al., 2003) and 20 were also included as part of study of FLA–DRB polymorphism (Kennedy et al., 2002). Samples from cats with FIP were naturally occurring cases submitted to University of Glasgow for diagnosis. One FIP case was a clinical case of one of the authors (DDA) at the University of Glasgow Veterinary School and his sibling was also tested. Eight cats were from a household with FIP, but whose FCoV status was not yet known. They are included because they supply data for another breed (Korat). Exon 2 of the FLA–DRB was amplified by PCR and polymorphisms detected either by clonal sequence analysis (Kennedy et al., 2002) or by reference strand mediated conformational analysis (RSCA; Arguello et al., 1998).

FCoV definitions are as follows:

FIP—the cat had histopathologically confirmed and/or clinically diagnosed FIP. For the latter, the cat had to fulfil the following criteria: be seropositive for FCoV antibodies by immunofluorescent antibody test; be hyperglobulinaemic; have an albumin:globulin ratio less than 0.7 and α1-acid glycoprotein level over 1500 μg/ml (Duthie et al., 1997); have an effusion with total protein over 35 g/l and albumin:globulin ratio <0.7 and white blood cell count less than 2×10^9/l comprising mainly neutrophils and macrophages; be lymphopenic (Paltrinieri et al., 2001); have a haematocrit less than 30% which is non-regenerative. Eight cats with FIP were included in this study.

FCoV carrier—a cat which has shed FCoV continuously for 9 months or over (Addie and Jarrett, 2001). Four carrier cats were included, one developed FIP.

Transient infection—a cat which has been exposed to FCoV, has seroconverted and shed virus, subsequently ceasing to shed FCoV and becoming seronegative (Addie and Jarrett, 2001). These cats may then be re-infected by the same or another strain of FCoV (Addie et al., 2003). Ten transiently infected cats were included.

Resistant—cats that have been exposed to FCoV infection but do not shed virus and do not develop high immunofluorescent antibody titres (although antibodies to the FCoV nucleocapsid protein can be detected by Western blotting). Three FCoV resistant cats were included in this study. These cats have been followed for over 7 years, where despite continual exposure to FCoV, they remain uninfected (Addie and Jarrett, 2001).

Results

Nineteen distinct alleles were identified amongst the 33 cats. Table 1 shows the distribution of these alleles amongst 25 cats with the four FCoV phenotypes described in this study. Considerable variation in the alleles was present in cats from different breeds (data not presented) which, together with the small numbers, made any more detailed analysis difficult. However, there appeared to be no clear association between FLA–DRB genotype and FCoV phenotype.

The number of alleles in individual cats ranged from two to six. Whilst there was a greater number

Table 1 FLA–DRB alleles found in cats of differing FCoV fates

FLA–DRB allele	Cats with FIP (*n*=8 cats)	Cats carriers (*n*=4)	Cats transiently infected (*n*=10)	Cats resistant (*n*=3)
0103	1			
0104	1		1	
0107	5	3	8	2
0201	2		2	
0203	2	1		1
0301	2	1	1	
0304	1		2	1
0403	4		2	1
0501	1	3	8	1
0504	4	1	2	
0511	?1			
051201	1			
040103		1		
rr3	2			1
rr7	1		1	
rr9	2	1		1
n05		2	6	1
n06	1			
n09	1			
Totals	32	13	33	9
Average No alleles per cat	4	3.25	3.3	3[a]

[a]However, one resistant cat had another allele that was not identified.

Table 2 Number of FLA–DRB alleles found in different cat breeds

Breed	Number of individuals	Number of alleles per individual (average)
Burmese	11	2–3 (2.8)
Korats	8	2–6 (4.4)
Domestic shorthaired	5	2–5 (4.2)
Maine Coon	3	3–5 (4.3)
Siamese	2	3–4 (3.5)
Balinese	2	4–5 (4.5)
Persian	1	5
British Silver Spotted Tabby	1	4

of alleles in the cats that developed FIP compared to the other groups (Table 1) this was not statistically significantly different (chi-squared, P=0.8) and may have reflected differences in the proportion of different breeds present in each group. Indeed, Burmese cats appeared to contain fewer alleles than other represented breeds (Table 2) and were over-represented in the transiently infected and carrier groups.

Discussion

The mechanisms by which FIP develop and by which most cats successfully eliminate FCoV infection are poorly understood. In human medicine, viral load and outcome of infection have been correlated with human leukocyte antigen (HLA) class II alleles: HLA-DRB1 homozygosity is associated with high load of human immunodeficiency virus (Zijenah et al., 2002); hepatitis C viral load is associated with HLA class II haplotypes (Fanning et al., 2001) and the HLA-DRB1*1301 allele was present in 46% of children with protracted hepatitis A infection (Fainboim et al., 2001). Our preliminary investigation has failed to link any particular FLA–DRB alleles with development of FIP, carrier status, transient infection or resistance. However, the numbers of cats included in this study was small and breed variations in the numbers and distribution of alleles further complicated the analysis.

In this study, we showed that the Burmese breed, which has been suggested to be particularly susceptible to FIP, appears to have fewer DRB alleles compared to other breeds. However, the two households from which the Burmese cats in this study were obtained did interbreed and as a result, could not be considered independent. It is therefore possible that our observations may be biased rather than being truly representative of the whole breed. In future studies, we aim to determine the extent of variation of FLA–DRB allele frequency and distribution between different cat breeds by targeted collection of unrelated representatives of individual breeds.

Overall, it appeared that cats who developed FIP had on average four alleles, while survivors had up to 3.3. However, this difference was not statistically significant. The low average number of alleles in survivors was likely to be in part due to the over representation of Burmese cats in this group. Clearly, more work needs to be carried out to establish whether there is a real difference in the number of alleles between the different FCoV groups.

The mechanism by which cats eliminate FCoV infection is unknown, neither is it known how FCoV manages to persistently infect some cats for life. In this study we have developed and applied methods for characterising FLA–DRB polymorphism in domestic cats. This is the first attempt to correlate disease outcome with the genotype of the host in the cat. RSCA in particular offers the possibility of high throughput gene typing in the cat. However, it is apparent from this and other studies that in order to identify associations with disease outcome, it will be necessary to pick breed-related controls. Recent work suggests that a class I response is protective for FIP (Kiss et al., 2004). In future studies, we aim to develop RSCA protocols for other FLA loci including FLA class I (Smith and Hoffman, 2001).

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