

Clearance of infection in cats naturally infected with feline coronaviruses is associated with an anti-S glycoprotein antibody response

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We have investigated by Western blotting the antibody responses against the three major structural proteins in cats naturally infected with feline coronaviruses that cleared virus infection (group I), established chronic asymptomatic infection (group II) or were sick (group III). The cats of group I developed an anti-S glycoprotein response that was, relative to the anti-M glycoprotein response, at least 30-fold higher than that of chronically infected cats from groups II and III. These results suggest that the anti-S glycoprotein response against antigenic domains revealed by Western blot is associated with clearance of the virus after natural infection, and is not a risk factor for the establishment of a chronic infection.

Feline infectious peritonitis (FIP) is a fatal immune-mediated coronavirus infection of cats which is caused by some strains of feline coronaviruses (FCoVs). FCoV can be divided into two serotypes (I and II) according to neutralization assays, epitope specificity of the spike glycoprotein S, and proximity to canine coronavirus (Fiscus & Teramoto, 1987; Hohdatsu *et al.*, 1991*b*; Motokawa *et al.*, 1995; Herrewegh *et al.*, 1995*b*; Motokawa *et al.*, 1996). Vaccination by the parenteral route with live vaccines has been unsuccessful. In fact, in most cases, vaccinated cats developed a faster and more severe disease after challenge than control cats (reviewed by Olsen, 1993). This 'facilitation' was linked to the antibody response against the S glycoprotein of the virus, as demonstrated with recombinant vaccines expressing this glycoprotein, but not the M glycoprotein or N protein (Vennema *et al.*, 1990, 1991; Gonin *et al.*, 1995). It is believed that this antibody-dependent enhancement of infection is due to the facilitation of macrophage infection by virus-antibody complexes through internalization after interaction with the Fc receptor (Hohdatsu

et al., 1991*a*). Nevertheless, this facilitation of infection has only been described in experimental conditions of challenge. Epidemiological data suggest that, in field conditions, seropositive cats develop a protective immunity against natural infection rather than an increased sensitivity (Addie *et al.*, 1995).

Preliminary observations showed that naturally seropositive cats, following isolation to avoid successive reinfections, follow two types of behaviour. Some of them maintain an antibody response for several months, suggesting a chronic infection. In others, a decrease in antibody titres is observed until a negative response is recorded, which may be a sign of elimination of the virus (V. Gonon, unpublished data). To analyse if such differences between virus-host interactions could be associated with immune recognition targeted to specific virus proteins, we planned to investigate the antibody response against the major structural proteins of the virus.

To this aim, 13 catteries from different areas of France that comprised 150 seropositive cats, with previous (eight catteries) or no (five catteries) recorded cases of FIP, were included in the protocol. All the cats were isolated from each other by their owners at the beginning of the protocol, so as to minimize superinfection events. The antibody responses were regularly screened (every 2 months) for 1 to 2 years by an immunofluorescence assay. Because, in certain cases, the owners did not maintain strict conditions of isolation, we discarded some samples and conserved only those of 90 strictly isolated cats, which were processed for further analysis. At the end of the observation period, we ranked the cats into two groups (I and II), depending on their antibody titres. Group I included the 42 cats that became antibody-negative. RT-PCR identification of virus sequences as described in Herrewegh *et al.* (1995*a*) was conducted using samples of blood and faeces from each cat at the end of the observation period, and gave negative results (data not shown). Group II included the 48 cats that did not show any evidence of decrease in antibody titres. RT-PCR analysis was conducted on samples from 36 cats at the end of the observation period and gave positive results in all cases. A sample of serum corresponding to the isolation date, or close to it, was used for Western blot analysis. The cats were ranked

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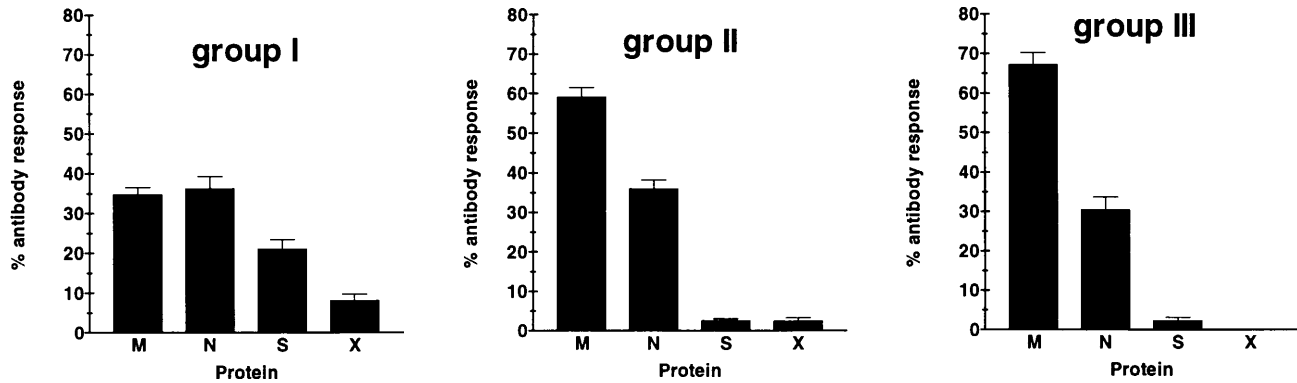


Fig. 1. Specific antibody response of cats. Reactivity of serum antibodies from cats of group I ($n = 42$), II ($n = 48$) and III ($n = 43$) against structural proteins of 79-1146 type II FCoV was tested by Western blot. The mean ratio of the intensity of each protein spot to the sum of the intensity of all the spots is depicted (\pm SEM, $P = 0.05$).

in groups I and II independently of previous records of clinically expressed FIP. To these two groups, we added a third group (III) of 43 naturally sick cats that were diagnosed as having the humid form FIP on the basis of symptoms, age and necropsy (when available). In this group, we tested by Western blot the sera sent by the practitioner for routine analysis.

Sera of cats from these three groups were analysed by Western blot as follows. The 79-1146 type II FCoV virus was amplified in Fcwf cells. The supernatant was clarified by low-speed centrifugation, then concentrated by ultracentrifugation. Virus proteins were resolved by SDS-PAGE in a 7.5% acrylamide gel, then transferred to a nitrocellulose membrane (Optitran BA-S83, Schleicher & Schuell). Sera diluted to 1 in 7 were incubated for 4 h at room temperature. Bound antibodies were revealed through the phosphatase alkaline reaction using alkaline-phosphatase conjugated anti-cat (H+L) antibodies (Jackson ImmunoResearch). The spot intensity was evaluated with NIH Image software after membrane scanning. A control positive serum, from a naturally infected cat, which gave clearly defined spots for each structural virus protein was used for the calibration of each membrane (definition of the basal line and location of each protein). (Glyco)proteins S, M and N were identified on the basis of their relative molecular mass (about 200, 25 and 50 kDa, respectively). Some sera also recognized a protein with a relative molecular mass of 60–69 kDa, possibly of cellular origin (labelled protein X). The absolute intensities of anti-S, -M and -N glycoprotein responses that reflected the absolute level of antibodies against the virus varied from cat to cat independently of their group of origin. So, the results were expressed as the ratio of the intensity of each virus protein spot to the sum of the intensity of all the spots.

Fig. 1 shows the results obtained for the cats of groups I, II and III. For the X protein, which was not recognized by all sera, the depicted value is the average between the responders and the non-responders. In all groups, the anti-N response corresponded to about 30% of the total antibody response, and could not be used to define a typology of cats. In contrast,

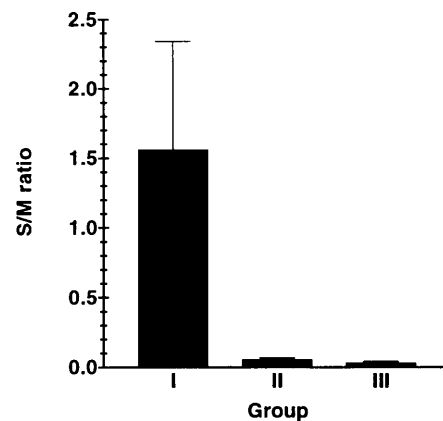


Fig. 2. For each cat, the ratio of the intensity of the anti-S to anti-M response was calculated. Means \pm SEM ($P = 0.05$) of each group are depicted.

antibody responses against M and S permitted us to define two categories, as summarized in Fig. 2, by the S/M ratio. The first category, characterized by a high S/M ratio (mean 1.6 ± 0.8 , $P = 0.05$) includes the cats from group I, which cleared virus infection. The second category, defined by a very low S/M ratio, includes the cats from groups II (mean 0.05 ± 0.01 , $P = 0.05$) and III (mean 0.03 ± 0.01 , $P = 0.05$), which established a chronic FCoV infection that eventually led to FIP disease. No obvious difference could be found between cats from groups II and III.

We have analysed the antibody responses of cats naturally infected with FCoV. We demonstrated here that cats that cleared a natural FCoV infection developed an antibody response against one or several domain(s) of the S glycoprotein, relative to the M glycoprotein, that was on average 30 times higher than that of cats either chronically infected or sick. Because our study was designed to investigate antibody responses following natural infection, we could not control all the variables that differed between groups. In particular, infecting strains and their virulence, doses of infection,

variation between cat breeds or lineages could not be matched between groups. Another limitation was that each serum was tested at a unique dilution which was chosen after preliminary calibration. Because it is likely that the intensity of the spots was not proportional to antibody concentration in all ranges of concentrations, this could under or overestimate the value of the S/M ratio. Nevertheless, a clear difference was seen between antibody responses from groups I and II/III. Because cats that cleared FCoV infection (group I) came from catteries that either experienced FIP cases or did not record any case of FIP, it is unlikely that the difference between groups I and II/III is linked solely to the virulence of the FCoV strain. We postulate that this difference reflects a more complex interaction between the virus and the host. This interaction could be different, for a given strain, from cat to cat for several reasons, including the genetic background of the host and the virus infecting dose (which might also explain the observed differences regarding the natural or experimental conditions of challenges).

At this time, it is not clear whether the anti-S antibody response is a mechanism or only a marker of virus clearance. To address this question, we plan to identify the domains of the S glycoprotein preferentially recognized by the antibodies from cats of group I and to test them in vaccination experiments. In fact, as this antibody response was identified by Western blot in denaturing conditions, it is likely that the antigenicity of corresponding domain(s) is not highly dependent upon the glycoprotein conformation. The antibody response against conformational epitopes was not investigated in this study.

The results presented here show that, in natural conditions, the anti-S response does not seem to be a risk factor in the development of FIP disease, which should challenge the widely accepted idea of the detrimental effect of the S glycoprotein in FIP recombinant vaccines.

References

- Addie, D. D., Toth, S., Murray, G. D. & Jarrett, O. (1995).** Risk of feline infectious peritonitis in cats naturally infected with feline coronavirus. *American Journal of Veterinary Research* **56**, 429–434.
- Fiscus, S. A. & Teramoto, Y. A. (1987).** Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. *Journal of Virology* **61**, 2607–2613.
- Gonin, P., Oualikene, W., Fournier, A., Soulier, M., Audonnet, J. C., Riviere, M. & Eloit, M. (1995).** Evaluation of a replication defective adenovirus expressing the feline infectious peritonitis membrane protein as a vaccine in cats. *Vaccine Research* **4**, 217–227.
- Herrewegh, A. A., De Groot, R., Cepica, A., Egberink, H. F., Horzinek, M. C. & Rottier, P. J. (1995 a).** Detection of feline coronavirus RNA in faeces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. *Journal of Clinical Microbiology* **33**, 684–689.
- Herrewegh, A. A., Vennema, H., Horzinek, M. C., Rottier, P. J. & De Groot, R. (1995 b).** The molecular genetics of feline coronaviruses: comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virology* **212**, 622–631.
- Hohdatsu, T., Nakamura, M., Ishizuka, Y., Yamada, H. & Koyama, H. (1991 a).** A study on the mechanism of antibody-dependent enhancement of feline infectious peritonitis virus infection in feline macrophages by monoclonal antibodies. *Archives of Virology* **120**, 207–217.
- Hohdatsu, T., Okada, S. & Koyama, H. (1991 b).** Characterization of monoclonal antibodies against feline infectious peritonitis virus type II and antigenic relationship between feline, porcine, and canine coronaviruses. *Archives of Virology* **117**, 85–95.
- Motokawa, K., Hohdatsu, T., Aizawa, C., Koyama, H. & Hashimoto, H. (1995).** Molecular cloning and sequence determination of the peplomer protein gene of feline infectious peritonitis virus type I. *Archives of Virology* **140**, 469–480.
- Motokawa, K., Hohdatsu, T., Hashimoto, H. & Koyama, H. (1996).** Comparison of the amino acid sequence and phylogenetic analysis of the peplomer, integral membrane and nucleocapsid proteins of feline, canine and porcine coronaviruses. *Microbiology and Immunology* **40**, 425–433.
- Olsen, C. W. (1993).** A review of feline infectious peritonitis virus: molecular biology, immunopathogenesis, clinical aspects, and vaccination. *Veterinary Microbiology* **36**, 1–37.
- Vennema, H., De Groot, R., Harbour, D. A., Dalderup, M., Gruffydd-Jones, T., Horzinek, M. C. & Spaan, W. J. (1990).** Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. *Journal of Virology* **64**, 1407–1409.
- Vennema, H., De Groot, R., Harbour, D. A., Horzinek, M. C. & Spaan, W. J. (1991).** Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. *Virology* **181**, 327–335.

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