Studies on Antibodies Against Feline Leukaemia Virus (FeLV) in Cat Sera and Rabbit Anti-FeLV Sera: Cross Reaction and Differences

(Accepted 2 May 1979)

SUMMARY

The indirect immunoferritin technique (IFT) that enables us to distinguish clearly whether an antibody reacts with a virus particle or only with the cell membrane, was used to study 25 cat sera and one rabbit anti-feline leukaemia virus (FeLV) serum using FL-74 cells as target. (1) All sera contained antibodies against FeLV even though 11 of the cats were viraemic at the same time; (2) from the effect of glutaraldehyde fixation of the FL-74 cells on the reaction with cat sera and the results of blocking experiments, it could be concluded that cat sera and rabbit anti-FeLV sera react partly with different antigenic specificities of FeLV, partly with the same antigens; and (3) the indirect membrane immunofluorescence test using FL-74 cells as target is not a good test to detect the presence of antibodies against feline oncornavirus-associated cell membrane antigen (FOCMA) because FL-74 cells produce a large quantity of FeLV and the fluorescence measured could be from antibodies against FeLV.

Tumours induced by feline sarcoma virus (FeSV) in cats can regress spontaneously (Snyder et al. 1970). A correlation between the presence of humoral antibodies to feline oncornavirus-associated cell membrane antigens (FOCMA) and resistance to the development of progressive malignant tumours following challenge with FeSV has been described (Essex et al. 1971a). The levels of anti-FOCMA detected in sera of virus-exposed cats have been shown to be predictive of tumour occurrence and growth. High anti-FOCMA titres appear to be protective against feline leukaemia virus (FeLV) infection and tumour development (Jarrett et al. 1974, 1975) or to be protective against tumour development only, without preventing viraemia (Olsen et al. 1976). High levels of anti-FOCMA were also found in sera of normal cats from high-leukaemia cluster households (Cotter et al. 1974; Essex et al. 1975; Charman et al. 1976).

To detect the presence of FOCMA antibody the indirect membrane immunofluorescence (IMI) test is commonly used with FL-74 cells as the target (Essex et al. 1971b). These cells produce a large quantity of FeLV. With the IMI test no distinction can be made between a positive reaction with the cell membrane only and/or reaction with the FeLV particles. However, the antibody response to FOCMA was found to be independent of the antibody response to the major envelope and core proteins of FeLV, gp70 and p30 (Stephenson et al. 1977). Exhaustive in vitro absorption of highly immune anti-FOCMA non-viraemic serum with gp70 and p30 also failed to remove FOCMA antibody activity (Stephenson et al. 1977). A non-producer mink cell line transformed by the Gardner–Arnstein strain of FeSV expresses high levels of FOCMA but not of the major virus structural proteins (Sliski et al. 1977). All these results suggest that FOCMA is distinct and separate from the FeLV structural proteins.

The present study was undertaken to analyse further the nature of FOCMA using the indirect immunoferritin technique (IFT) that enables us to distinguish clearly whether an antibody reacts with a virus particle or only with the cell membrane.
An established cell line of lymphoblastoid cells FL-74 was used. This cell line was kindly given by Dr O. Jarrett, Glasgow, and was originally established by Theilen et al. (1969) from a FeLV-induced lymphosarcoma. In addition, a feline kidney cell line (CRFK-F2; Crandell et al. 1973) and lymphocytes from a cat negative for FeLV antigens and anti-FOCMA in the IMI test, were used.

The source of sera used was as follows: (a) from 15 cats previously found positive in the IMI test using FL-74 cells as target cells, five were positive for FeLV antigens on peripheral blood smears examined by the indirect immunofluorescence antibody test (Hardy et al. 1973) without counterstaining. (b) From 10 cats vaccinated with FL-74 cells using a procedure previously described (Jarrett et al. 1975), six were positive for FeLV antigens on peripheral blood smears before vaccination. (c) A rabbit antiserum was produced against the Rickard strain of FeLV, as previously described for antiserum against mouse mammary tumour virus (Daams, 1970). This antiserum was absorbed with lyophilized foetal calf serum (150 mg/ml) and with lyophilized spleen and liver tissue (100 mg/ml each) from a cat negative for FeLV-antigen. It was also absorbed in vivo in a 6-month-old cat negative for FeLV-antigen.

Ferritin-labelled goat antisera to rabbit IgG and to cat IgG (Miles Laboratories Ltd, Slough, England; Microbiological Associates Inc., Bethesda, Md., U.S.A.) were prepared as previously described (Calafat et al. 1974) and used at a dilution of 1:20 and 1:10 respectively in Hanks' solution.

FL-74 cells \((2 \times 10^6)\) suspended in 0.1 ml Hanks' solution were incubated and agitated periodically for 90 min at room temperature or 37 °C in 0.1 ml specific antiserum at a concentration of 1:5 for rabbit anti-FeLV, absorbed in vitro and in vivo and a concentration varying from undiluted to 1:5 for the cat sera. The cells were washed twice with Hanks' solution and then incubated and periodically shaken for 75 to 90 min at room temperature or 37 °C with 0.2 ml of either ferritin-labelled goat antiserum to rabbit IgG or ferritin-labelled goat antiserum to cat IgG. After being washed three times the pellets were fixed in 3.5% glutaraldehyde in 0.1 M-cacodylate buffer, pH 7.2, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead hydroxide. The preparations were examined with a Philips electron microscope 300 or 301.

Two kinds of controls were used: (1) normal rabbit serum and normal cat serum, negative for FOCMA antibodies, instead of rabbit anti-FeLV serum and cat anti-FOCMA sera, respectively; and (2) CRFK-F2 cells or lymphocytes from a normal cat instead of FL-74 cells.

The 25 cat sera examined showed labelling on the virus envelope and some areas of the FL-74 cell membranes (Fig. 1a) but to a different extent for the various sera. However, a good correlation was found between the intensity of the tagging of the virus and the cell. No difference was observed between the sera from cats with and without FeLV antigens in the peripheral blood smears. The same results were obtained with rabbit anti-FeLV serum i.e. labelling of the virus envelope and FL-74 cell membrane (Fig. 1b).

Neither labelling of the FeLV particles nor the cell surface was observed in the control experiments: (1) FL-74 cells incubated with normal rabbit serum or normal cat serum, followed by incubation with ferritin-labelled goat anti-rabbit IgG or ferritin-labelled goat anti-cat IgG respectively (Fig. 1c); (2) CRFK-F2 cells and lymphocytes from a normal cat incubated with rabbit anti-FeLV or serum from a vaccinated cat followed by incubation with their corresponding conjugate.

In a preliminary study we observed in one cat serum a remarkable influence of glutaraldehyde fixation of the cells on the intensity of the reaction. We have extended this observation with 11 more cat sera from the different groups described above. When
Fig. 1. FL-74 cells (a) incubated with serum from a vaccinated cat and goat anti-cat IgG; (b) incubated with rabbit anti-FeLV serum and goat anti-rabbit IgG. In both cases C-types particles (arrows) and areas of the cell surface are labelled; (c) incubated with normal rabbit serum and goat anti-rabbit IgG. There is no labelling on the virus envelope and cell surface. (d) FL-74 cells fixed with glutaraldehyde incubated with serum from a vaccinated cat and goat anti-cat IgG. There is a considerable decrease of labelling on cell surface and virus envelope (C) compared with unfixed cells from (a).
Table 1. Summary of the results of the blocking experiment between cat sera and rabbit anti-FeLV sera on FL-74 cells

<table>
<thead>
<tr>
<th>First serum</th>
<th>Second serum</th>
<th>Ferritin conjugate</th>
<th>Labelling*</th>
<th>Cell surface</th>
<th>Virus surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>Rabbit anti-FeLV</td>
<td>Goat anti-rabbit IgG</td>
<td>++ + +</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>Cat</td>
<td>Rabbit anti-FeLV</td>
<td>Goat anti-rabbit IgG</td>
<td>-- --</td>
<td>-- --</td>
<td>-- --</td>
</tr>
<tr>
<td>Rabbit anti-FeLV</td>
<td>Cat</td>
<td>Goat anti-cat IgG</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Rabbit anti-FeLV</td>
<td>Cat</td>
<td>Goat anti-cat IgG</td>
<td>-- --</td>
<td>-- --</td>
<td>-- --</td>
</tr>
</tbody>
</table>

* Results expressed for labelling of cell surface: ++++, 1/3 to 1/2 of cell surface labelled mainly in a large patch; +, groups of ferritin covering less than 1/4 of cell surface. Labelling of virus envelope: ++++, labelled all round, > 50 grains of ferritin; +, partially labelled, about 20 grains of ferritin; --, not labelled.

FL-74 cells were fixed with 1% glutaraldehyde for 5 min before incubation with these cat sera (Fig. 1d) a considerable decrease of label on the cell membrane and the virus envelope was observed as compared with the reaction on unfixed cells (Fig. 1a). However, after fixation of the cells with glutaraldehyde before incubation with rabbit anti-FeLV serum, the intensity of the labelling was the same as with unfixed cells. In this case the distribution of the labelling was different due to the mobility of the antigens on the surface of the unfixed cells.

These results suggested that some antibodies, or the majority of the antibodies, of the cat sera and the antibodies of the rabbit anti-FeLV serum react with different antigenic specificities. To confirm these observations two kinds of blocking experiments were performed (Table 1): (a) FL-74 cells were first incubated with serum from two vaccinated cats and three non-vaccinated cats respectively (titre in IMI test between 64 and 512; one of these sera was from a FeLV positive cat), followed by incubation with rabbit anti-FeLV serum (titre in IMI test 160) and ferritin-labelled goat anti-rabbit IgG serum. The labelling on the cell membrane and the virus envelope was comparable with the labelling of these cells after incubation with anti-FeLV serum only, followed by ferritin-labelled goat anti-rabbit IgG. This means that the reaction with cat sera does not inhibit the further reaction with rabbit anti-FeLV serum. The controls, FL-74 cells incubated with these cat sera followed by ferritin-labelled goat anti-rabbit IgG, were negative; (b) FL-74 cells were first incubated with rabbit anti-FeLV, followed by incubation with serum from a vaccinated cat (titre in IMI test 256) and then ferritin-labelled goat anti-cat IgG. In different experiments, the concentration of rabbit anti-FeLV serum used was, respectively, two and four times higher than that of the cat serum, to ensure that we used enough anti-FeLV to react with all FeLV antigens present. The labelling on the cell membrane and the virus envelope at both concentrations of anti-FeLV was the same and was clearly lower than the labelling of these cells after incubation with only the cat serum followed by ferritin-labelled goat anti-cat IgG. This means that the reaction with rabbit anti-FeLV serum inhibited to a considerable extent the further reaction with the cat serum. The control, FL-74 cells incubated with rabbit anti-FeLV serum followed by ferritin-labelled goat anti-cat IgG was negative.

The 25 cat sera examined with IFT, using FL-74 cells as target, gave labelling on the virus envelope and some areas of the cell membrane. All these sera were also found to be positive in the IMI test using the same cells as target. The IFT and the IMI test are based on the same principle: incubation of living cells with a specific antiserum. The second antiserum is conjugated in the first test with ferritin and in the second with fluorescein. The IMI test using FL-74 cells as target was first developed by Essex et al. (1971b) and is commonly used.
to determine serum antibodies against FOCMA. The basis of the idea is that the observed fluorescence is localized exclusively on the cell membrane. However, the results of the IFT test show clearly that the ferritin labelling is not only on the cell membrane, but also on the virus envelope. This indicates, with the sera studied here, that the fluorescence in the IMI test is caused by binding of antiserum on the cell membrane and on the virus envelope. In these sera, antibodies were present against FeLV and perhaps also against FOCMA. It would be better to use non-producer cells as target cells in the IMI test, e.g. the mink foetal lung cell line transformed by FeSV (Sliski et al. 1977).

The effect of glutaraldehyde fixation of the cells and the blocking experiments show that some antibodies of the cat sera and some of the rabbit anti-FeLV serum react with different FeLV antigenic specificities. It is known that glutaraldehyde forms intra- and intermolecular cross-linkages with proteins and may thus change their tertiary structure with a concomitant loss of antigenicity (Hopwood, 1972). After glutaraldehyde fixation of FL-74 cells almost all antigens reacting with the cat sera have lost their antigenicity. However, no change in the amount of antigens reacting with rabbit anti-FeLV could be seen by the IFT. On the other hand, the results of the blocking experiments showed that cat sera and rabbit anti-FeLV serum cross-react with some antigens because the reaction of FL-74 cells with cat serum is partially inhibited by previous incubation with rabbit anti-FeLV serum. Rabbit anti-FeLV serum probably reacts with more antigens than the cat sera, since these could not inhibit or decrease the reaction of FL-74 cells with rabbit anti-FeLV serum whereas the reverse was possible.

In conclusion these experiments show: (1) that the 25 cat sera examined with IFT contain antibodies against FeLV even though 11 of these cats were viraemic at the same time. This is in accordance with the results of Russell & Jarrett (1978) who found in some sera of viraemic cats, neutralizing antibodies against FeLV (especially against FeLV of subgroup C). They postulated a relationship between the occurrence of antibodies to FOCMA and FeLV-C-neutralizing antibodies. (2) Cat sera and rabbit anti-FeLV sera react partly with different antigenic specificities of FeLV, partly with the same antigens. (3) The IMI test using FL-74 cells as target is no good for detecting the presence in a serum of antibodies against FOCMA because FL-74 cells produce large quantities of FeLV and the fluorescence measured could be from antibodies against FeLV.

We thank Dr A. Hekman for the ferritin-labelled antisera, and Mr H. Janssen, Mr N. Ong, Mr A. Riethorst and Mrs P. Spoorenberg for excellent technical assistance.

**Division of Virology**  
The Netherlands Cancer Institute  
Plesmanlaan 121, 1066 CX Amsterdam  
The Netherlands

---

**REFERENCES**


Short communications


(Received 25 January 1979)