FELINFO No. 2

Feline leukaemia prophylaxis

Our second FELINFO is dedicated to feline leukaemia which—to follow a modern terminology—should rather be named FAIDS (feline acquired immuno-deficiency syndsrome). Similar to AIDS in humans, feline leucosis is also caused by a retrovirus (FeLV) which has a devastating effect on the immune system; as a consequence normally innocuous and ubiquitous germs are no longer kept in check.

  Professor Dr V. Moennig's article concerning prophylactic aspects of feline leukaemia aims at a very hot topic. The Norden Laboratories in Lincoln, Nebraska (U.S.A.) have recently released a FeLV vaccine for the American market and small animal practitioners are issuing circulars to their clients explaining the possibilities of vaccination. From SmithKline Germany I understood that the vaccine will be available in the Federal Republic from January 1986 onwards. A second, parallel development takes place in Sweden and The Netherlands. This vaccine should contain so-called ISCOMS (immune stimulating complexes), which consist of subunits of feline leukaemia virus. The vaccine is still in an experimental stage, but preliminary results are promising. FELINFO will keep you informed about developments in this area.

  Starting with the present issue, a questions-and-answers column will be run; Dr Hans Lutz, Zürich, Switzerland, who was the author of the first FELINFO article on FIP, will be responsible for it. He is an expert in laboratory diagnostic methods (with special respect to immunological techniques) and has done experimental work on FeLV in Dr Thielen's laboratory in Davis, California.

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Feline leukaemia prophylaxis

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INTRODUCTION

Feline leukaemia is a widespread and serious infectious disease of the cat. The aetiological agent (feline leukaemia virus—FeLV) belongs to the large family of retroviruses which contains amongst others the viruses causing bovine leucosis and avian leucosis, of equine infectious anaemia and of the acquired immuno-deficiency syndrome (AIDS) of man.

PATHOGENESIS

In 1964 feline leukaemia was recognized as an infectious disease by Jarret *et al.* (1). The virus occurs in different subgroups, of which A and B are encountered in most of the clinical cases, whereas viruses of subgroup C have apparently arisen by recombination of viruses of the first two subgroups with the so-called endogenous, apathogenic retroviruses of felines. FeLV causes a wide spectrum of diseases, including proliferative conditions such as leukaemia and lymphosarcoma as well as degenerative syndromes such as anaemias and immunosuppression. In their consequences these are very similar to AIDS in humans. For this multitude of syndromes the expression 'feline leukaemia complex' has become popular.

Infection in the cat population is still widespread irrespective of the fact that different serological procedures for the demonstration of viraemic animals have been developed and applied since 1974 (2, 3). A constant source for new infections are viraemic, clinically healthy cats. These animals can excrete large virus quantities with their saliva or urine for years. Especially when carrier animals are kept together with other cats, the efficiency of virus transmission is very high since it occurs almost exclusively by direct contact. The portal of virus entry into the body is the oral or nasal cavity from where the agent is spread via the blood stream, resulting in infection of the bone marrow. From there viraemia and virus excretion proceeds. Young animals (less than six weeks of age) and old cats are at an especially high risk, particularly when they have frequent contacts with virus carriers. The course and result of an FeLV infection may be,

(1) transient viraemia resulting in a solid humoral immunity,
(2) high antibody titres without preceding viraemia,
(3) persistent viraemia without demonstrable antibody,
(4) persistent viraemia with concomitant high antibody levels.

In most cases the infection is followed by the development of a solid immunity. Studies in some populations have shown that up to 90 per cent of the cats may have antibodies against the virus.

IMMUNITY FOLLOWING NATURAL INFECTION

In order to better understand the humoral immune response directed against FeLV some explanations of the virion structure are necessary (Fig. 1). The genetic information of the virus—a single stranded RNA—as well as the viral enzyme 'reverse transcriptase' are enclosed in a protein capsid which consists of the protein p27 (mol.wt. 27,000) only. This internal viron structure, also called 'core', is surrounded by a lipid envelope. The envelope carries knob-like projections consisting of the glycoprotein gp70 (mol.wt. 70,000). Anchorage of the gp70 in the
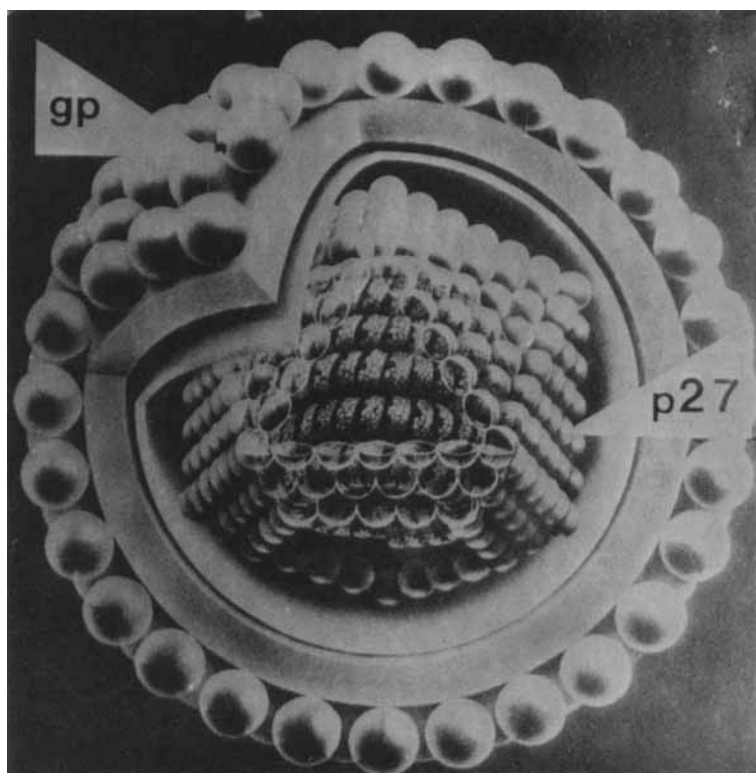

FIG. 1. Model of feline leukaemia virus. Arrows indicate the position of the viral glycoprotein complexes (gp) and the core protein (p27).

membrane occurs by disulphide bonds with the intramembrane p15E (mol.wt. 15,000). The intact gp70/p15E complex is referred to as gp85. The humoral immune response against FeLV is directed mainly against the viral surface structure gp70/85 and against the so-called 'feline oncornavirus-associated membrane antigen' (FOCMA). Antibodies against the first structures are virus neutralizing which means that they protect the animal from viraemia and its consequences. This immunity, however, can collapse when immune animals are constantly in contact with carrier animals which excrete large quantities of virus. Effective formation of antibodies against the virus is compromised by the structural and serological relationship of FeLV with the endogenous, apathogenic retroviruses of the cat mentioned above (4). Only the structures of FeLV which are different from those of the endogenous viruses are recognized as foreign by the cat's immune system and induce a response.

FOCMA is an FeLV-induced cell membrane antigen which is similar to the envelope glycoprotein of viruses belonging to subgroup C in its biochemical and serological properties. Antibodies directed against FOCMA protect the animal from FeLV-induced tumours. However, they do not protect against another infection with FeLV, against viraemia or the degenerative diseases of the feline leukaemia complex.

EXPERIMENTAL VACCINES

Since solid immunity follows infection under natural conditions, the perspective of active immunization against FeLV was not far-fetched. An effective vaccine should produce a sufficient and long lasting titre of neutralizing antibodies; in addition, antibodies directed against FOCMA and p15E would provide protection against tumours and immunosuppression. The numerous experiments to develop an efficient vaccine against FeLV infection have recently shown their first success: in the U.S.A. a preparation is presently being marketed for use by the small animal practitioner. Before discussing this product I should like to explain some principal experimental approaches in vaccine development which have been used or are presently being tested. A vaccine should contain viral gp70 and FOCMA as immunogenic substances. The membrane protein p15E should account for only a small fraction of the antigenic mass, since it has an immunosuppressive activity at higher concentrations (5). On the basis of these considerations the following immunogenic materials may be considered as vaccine preparations:
  (1) cells (virus-producing, live or inactivated)
  (2) virus (live or inactivated)
  (3) split products (viral or cellular surface structures; gp70/gp85, FOCMA)
  (4) synthetic antigens
  (5) products obtained by genetic engineering (antigens from pro- or eukaryotic cells after cloning of the respective viral genes)
Each of the above-mentioned strategies has been followed with varying success,

and the hope for the future is directed towards development of synthetic and genetically engineered vaccines.

CELLS

Cells of the lymphoblastoid line FL74 produce FeLV of all three subgroups and express FOCMA on their surface. This makes the line suitable for vaccination trials using intact live cells. Jarret *et al.* have performed pertinent experiments and were able to prove protection (6). All vaccinated animals withstood a challenge infection with high doses of virulent FeLV. Nevertheless, vaccines of this type are not practical. From the technical point of view it is impossible to produce and ship live viable cells on an industrial scale. In addition, principal considerations of safety plead against the FL74 cell being used as a vaccine. As mentioned before the virus belongs to the Retroviridae family. Members of this family are notorious for their ability to integrate their genetic information into chromosomal DNA of the host. Doing this the viruses may interact with host cellular genes and escape attack of the immune system. Vaccination with live virus-producing tumour cells would therefore come down to an uncontrollable genetic experiment which must be dismissed for ethical reasons.

In view of these safety considerations the FL74 cell was used in further experiments either inactivated or after lysis (6–8). Unfortunately, the cells had lost their immunogenic potential after the respective procedures of inactivation had been employed. In not a single case has a convincing protection been achieved.

VIRUSES

In parallel to their experiments with a cell vaccine, Jarrett *et al.* have tested live purified virus from FL74 cells (6); however, the immune response of the animals
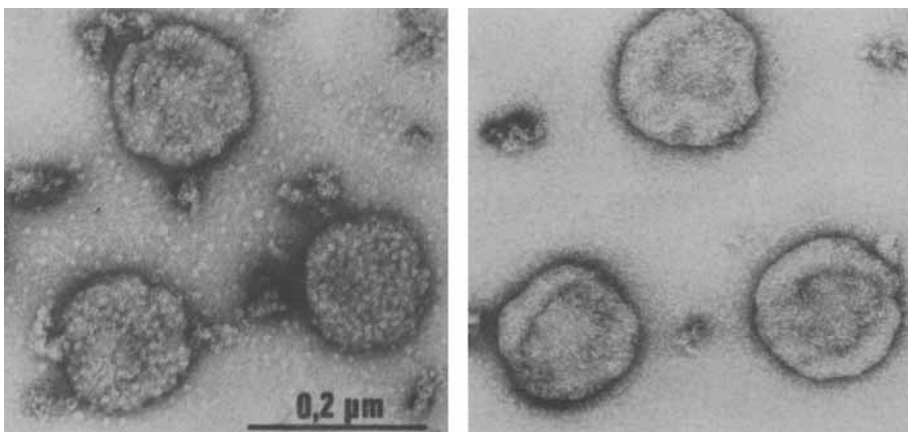

FIG. 2. Electron microscopical appearance of a retrovirus. In the left picture the viral surface is studded with the glycoprotein whereas the particle on the right has lost its glycoprotein and most of its immunogenicity.

was insufficient for effective protection. Although Pedersen *et al.* seems to have obtained better results using live virus, the same limitations as mentioned for cell vaccines apply (8).

Some retroviruses—amongst them the FeLV particle—have a labile surface substructure. The viral surface glycoprotein readily detaches from the virion, and the virus consequently loses its immunogenicity (Fig. 2). This disadvantage plays a decisive role in the production of inactivated virus preparations for vaccine purposes (9). Only when the surface glycoproteins of newly formed virus particles are anchored to the membrane, e.g. by formaldehyde fixation, inactivated viruses can be used for vaccination with a certain degree of success (8). A commercial vaccine based on this methodology has so far not reached the market.
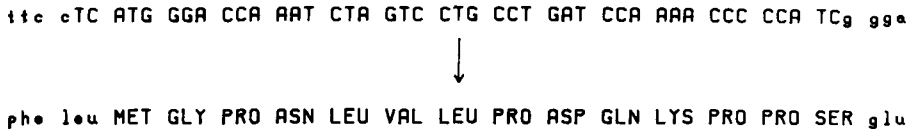
SPLIT VACCINES

When considering conventional vaccines, viral split products must be considered safe and effective. By removal of the viral nucleic acid from the preparation and limitation to the relevant proteins innocuity of the vaccine is assured. The first experiments for the development of a split vaccine against feline leukaemia were done some 10 years ago. Using the model of mouse leukaemia it had been shown previously that isolated viral glycoproteins are suitable for active immunization. Since isolated glycoproteins possess only low immunogenicity for animals of the homologous species (due to the existence of structurally similar endogenous retroviruses) high concentrations of antigenic mass had to be used for obtaining satisfactory protection (10, 11). These model experiments had no consequences for feline leukaemia since it would have been very expensive to produce the necessary quantities of gp70 of FeLV; when lower antigenic doses were applied—similar to the situation in the mouse—the experiments failed (12). Hunsmann *et al.* succeeded in improving the methodology by covalently binding gp70 with the p15E, resulting in the gp85. The linkage of the hydrophilic gp70 with the hydrophobic p15E lead to the formation of complex, rosette-like structures which showed strong immunogenicity—far stronger than monomeric gp70 (13). The results obtained with vaccines of this kind, however, are not uniform. Pedersen's group was unable to confirm the results from Hunsmann's laboratory (14). A possible explanation again is the use of too low doses of antigen. Also the immunosuppressive action of p15E may play a role.

Lewis *et al.* have chosen a different approach (15). They took advantage of the fact that under defined conditions FL74 cells secrete large quantities of gp70/85 and FOCMA into the culture medium. The antigens isolated from the cell supernatant are suitable for use as a vaccine. In experimental animals antibodies have been demonstrated against viral gp70, p15E and FOCMA; about 80 per cent of the vaccinees resisted a challenge infection. It should be noted that in contrast to earlier experiments also younger animals (older than 4 weeks of age) have been successfully vaccinated. A vaccine of this type has been marketed in the U.S.A. in this year and is currently being licenced for European countries.

SYNTHETIC ANTIGENS

Future vaccines against feline leukaemia will probably consist of a synthetic oligopeptide which represents an immunogenic fragment of the surface glyco-protein gp70. An experiment aiming in this direction has recently been described by Nunberg *et al.* (16). Using neutralizing monoclonal antibodies the authors have identified the relevant antigenic determinant on the gp70 of FeLV and elucidated its nucleotide and amino acid sequence (Fig. 3). A synthetic oligopeptide identical in sequence with the analysed peptide was subsequently tested in the animal with respect to its immunogenic and protective capacities. Experiments in other systems have shown that the immunogenicity of synthetic oligopeptides is often worse than that of the natural antigens. The choice of a suitable coupling reagent (a carrier, to which the small peptide is attached) and of an adjuvant is decisive for the success of vaccines of this kind. It remains to be shown whether they will play a role in the prophylaxis of feline leukaemia in the future.

ttc cTC ATG GGA CCA AAT CTA GTC CTG CCT GAT CCA AAA CCC CCA TCg gga

$$\downarrow$$

phe leu MET GLY PRO ASN LEU VAL LEU PRO ASP GLN LYS PRO PRO SER glu

J.H. NUNBERG et al.
PROC. NAT. ACAD. SCI. USA 81, 3675, (1984)

FIG. 3. The upper row of letters shows part of the nucleotide sequence from the envelope gene of FeLV (only the upper case letters—the lower case letters represent adjacent nucleotides from the vector). Each group of three nucleotides codes for one amino acid (lower row). The amino acid chain determined by this technique was synthesized; it was shown to bind neutralizing monoclonal antibodies.

GENETICALLY-ENGINEERED VACCINES

Parts of the genetic information of FeLV have been cloned in bacteria and yeast cells by different groups. Presently, major efforts are put into expression of the genes coding for gp70. These expression products are subsequently tested for their immunogenicity and the protection of cats. As mentioned for the synthetic peptides, the choice of the right adjuvant will play an important role.

CONCLUSIONS

Since keeping cats in a household has become more and more popular during recent years, feline leukaemia has developed into an uncontrollable risk due to its protracted course, nature and latent spread. First progress in controlling the disease

has been achieved some 10 years ago by the introduction of laboratory diagnostic tests. Using these, spread of the virus in the cat population could be limited to a certain extent.

Immunoprophylaxis by vaccination seemed to be impossible for many years. Promising developments have been reported only recently thanks to the progress in biotechnological methods and the experiences gained from other retroviral systems. In the U.S.A. a vaccine has been introduced and will be field tested by the veterinary practitioner. Industrial production of this vaccine is expensive and it remains to be shown how successful its application will be; also, new developments are expected in the near future which concern the protective capacity and the lowering of the production costs of this product.

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FELINFO Questions and Answers

As announced before, FELINFO should like to offer you an additional service. In this column we intend to help the veterinary practitioner by answering his questions which arise from his daily routine work as well as from incidental observations. We should like to start out with questions pertaining to infectious diseases, but also other problems will be dealt with. The questions-and-answers column is intended for all colleagues interested in feline disease, that is not only for the specialized feline practitioner but also the vet with a mixed practice.

We are prepared to answer urgent questions by phone, if necessary after consulting specialists in Europe and the United States; questions of a more general interest will be published in this column.

Please address your questions to 'FELINFO Questions and Answers', Dr Hans Lutz, Veterinärmedizinische Klinik, Universität Zürich, Winterthurer Str. 260, CH 8057 Zürich, Switzerland; for consultation by phone call on Mondays, Wednesdays and Fridays between 8.30 and 9.30 a.m., phone Switzerland 01 3651295.

Following our publication of the first FELINFO article on FIP 'the present state of knowledge' by H. Lutz, B. Hauser and M. C. Horzinek we have obtained the following reactions.

Question: In a household with seven adult pedigree cats and some kittens, three of the adult animals have anti-FIP titres of 1,600. The other animals have titres of 400 (1), 100 (2) and 25 (1), respectively. The kittens have not been tested. Are the animals with a titre of 1,600 at a higher risk to contract FIP than those with the lower titres?

Answer: This question cannot be answered with certainty. There are no reasons whatsoever to perform FIP titre determinations in the healthy animals. At the present time determination of FIP titres is of diagnostic importance only when the cat is suspected of developing FIP.

Question: In a boarding cattery with some 35 animals two FIP cases have been observed within 4 weeks. The cattery owners are afraid of an endemic during which most animals may succumb to FIP. Which measures are to be recommended?

Answer: We have no possibility at present to identify animals which excrete the virus. It must be emphasized that determination of titres cannot be used for this purpose. The owners should be told, however, that on a yearly basis only about 5 per cent of a cat population will contract FIP. Even under the most adverse conditions in large catteries the disease incidence rarely exceeds 10 per cent. The most sensible measure is improvement of the conditions such as avoidance of stress by allowing enough space, cleanliness, and varied and palatable diet.

Question: An owner has lost his animal as a consequence of a car accident. After one month he purchases a pedigree kitten 17 weeks of age. The owner is afraid of losing this cat, too, and he consequently does not allow it to roam outdoors. Four weeks after having brought this animal into his household the diagnosis of the wet form of FIP is made by the veterinarian. How is it possible that this kitten has been infected? Is it conceivable that the previous cat excreted the virus and that the home was still contaminated?

Answer: It appears very unlikely that FIP virus should have survived 4 weeks in the owner's home. It must be assumed that the kitten has been latently infected with the virus in the cattery where it came from. The disease is thought to be the consequence of the stress situation arising from changing the owner and the territory. There is very little a cat breeder can do to prevent such a situation; it must be assumed that there is hardly any breeding cattery in Europe where feline coronaviruses (the feline enteric and the feline infectious peritonitis viruses) do not occur.