STUDIES ON LATENT FELINE LEUKAEMIA VIRUS INFECTIONS

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by

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DECLARATION

The work recorded in this thesis was carried out in the department of Veterinary Pathology at the University of Glasgow between October 1983 and September 1986. All of the results were obtained by the author unless otherwise stated in the text.

I also certify that no part of this thesis has been submitted previously in any form to any university, but has been published in part in the following scientific papers.

Pacitti, A.M. and Jarrett, O. Duration of the latent state in feline leukaemia virus infections. The Veterinary Record 117, 472-474 (1985).

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LIST OF ABBREVIATIONS

AFC10	:	Alpha Minimal Essential Medium supplemented with 10% FCS, 2 mM L-glutamine, 400 units/ml penicillin-streptomycin and 10^{-6}M hydrocortisone succinate.
AFC20	:	Alpha Minimal Essential Medium supplemented with 20% FCS, 2mM L-glutamine, 400 units/ml penicillin-streptomycin and 10^{-6}M hydrocortisone succinate.
ATL	:	adult T-cell leukaemia.
BLV	:	bovine leukaemia virus.
BMSN	:	bone marrow supernatant fluid.
CDV	:	canine distemper virus.
c81	:	Clone 81 cells.
DFC10	:	DMEM supplemented with 10% FCS, 2mM L-glutamine and 400 units/ml penicillin-streptomycin.
DFC10-AL	:	DFC10 supplemented with 1% amphotericin B and 1% lincomycin hydrochloride.
DFC20-AL	:	DMEM supplemented with 20% FCS, 2mM L-glutamine, 400 units/ml pencillin-streptomycin, 1% amphotericin B and 1% lincomycin hydrochloride.
DMEM	:	Dulbecco's Modified Eagle's Minimal Essential Medium.
EDTA	:	ethylenediaminotetra-acetate.
EIA	:	equine infectious anaemia.

ELISA	:	enzyme-linked immunosorbant assay.
FCS	:	foetal calf serum.
FEA	:	feline embryo cells of the FEA line.
FEA/FeLV-A	:	FEA cells infected with FeLV-A.
FEA/FeLV-B	:	FEA cells infected with FeLV-B.
FeLV	:	feline leukaemia virus.
FeLV-A	:	feline leukaemia virus of subgroup A.
FeLV-A/Glasgow-1	:	FeLV-A of the Glasgow-1 strain.
FeLV-B	:	feline leukaemia virus of subgroup B.
FeLV-B/Sarma	:	FeLV-B of the Sarma strain.
fiu	:	focus inducing units.
FOCMA	:	feline oncornavirus-associated cell membrane antigen.
F-MuLV	:	murine leukaemia virus of the Friend strain.
G-MuLV	:	murine leukaemia virus of the Gross strain.
HIV	:	human immunodeficiency virus.
HSV	:	herpes simplex virus.
HTLV I	:	human T-lymphotropic virus type I.
IF	:	immunofluorescence.
ISCOM	:	immunostimulating complex.

LM medium	:	equal volumes of Leibovitz L-15 medium and McCoy's 5a medium supplemented with 10% FCS, 2mM L-glutamine, and 400 units/ml penicillin-streptomycin.
MCAs	:	monoclonal antibodies.
MuLV	:	murine leukaemia virus.
MV	:	maedi-visna.
PBS	:	phosphate buffered saline.
SPF	:	specific pathogen free.
SSPE	:	subacute sclerosing panencephalitis.
SV5	:	simian virus 5.
VNAs	:	virus neutralising antibodies.

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SUMMARY

The aims of this study were to identify latently infected cats; to determine the prevalence of latent infections in FeLV-positive multicat households and the duration of such infections; to investigate the clinical and epidemiological significance of these infections, in a closed multicat household; and to investigate the mechanisms involved in the control of latency.

Chapter 1 is a General Introduction which outlines the general biology and epidemiology of FeLV and describes in detail the previous work which led to the present study.

Chapter 2 describes the General Materials and Methods employed during the course of the study. Specialised methods are detailed at the beginning of each subsequent Chapter.

The next chapter, Chapter 3, describes the techniques developed to detect latent FeLV infection in cats. Latent virus was found in the bone marrow of cats which had virus neutralising antibodies (VNAs) and were therefore believed to have recovered from infection. Those methods which have been used in previous studies to detect these infections are then described, followed by an account of the methods used here. The latter involve the culture of aspirated bone marrow cells followed by the detection of reactivated virus by either virus isolation from cell culture fluids or by immunofluorescence (IF) techniques carried out on the cultured cells. IF was the more efficient method as it could detect viral antigen within one week of culture. These methods could not detect any virus or viral antigen prior to the culture of the bone marrow cells. Finally in this Chapter an attempt was made to quantitate the proportion of latently infected cells by the use of infectious centre assays. It was found that the proportion of these cells was low and ranged from 3 to 400 per million.

The prevalence and duration of latent infections in a closed, FeLV-exposed multicat household are discussed in Chapter 4. Nineteen SPF cats were housed together with 4 cats excreting FeLV. It was found that the proportion of ostensibly recovered cats (i.e. cats which had VNAs and were not viraemic) with latent infections was initially high, being approximately 50% 36 weeks after initial exposure to the viraemic cats. This proportion gradually decreased with time, the greatest decline occurring between 36 and 64 weeks after exposure. After 3 years only one of these cats still had a latent infection. Most latent infections are therefore short-lived with approximately 10% being long-term. Those cats which appeared to eliminate the latent virus were then believed to have truly recovered from infection. Possible reasons for the termination of latent infection are discussed.

Chapter 5 investigates the clinical and epidemiological significance of latency. Twenty-six latently infected cats were studied for periods of up to 4 years, and none developed any of the common FeLV-associated diseases. Latently infected cats therefore appeared to be no more at risk of developing these diseases than unexposed cats. The second part of this Chapter considers the possibility of horizontal or congenital transmission of infection from latently infected cats. After one year of exposure to 8 such cats, 8 susceptible FeLV-niaive cats showed no evidence of having been infected with the virus. Similarly the kittens of 7 of 8 latently infected queens had no evidence of congenital or post-natal infection from their mothers. However, the kittens of 4 consecutive litters of the eighth queen became persistently viraemic at 6-8 weeks of age and the source of the virus was found to be her milk. This queen herself subsequently became viraemic. The epidemiological importance of such cats for the control and eradication of FeLV from multicat households is discussed.

Attempts to determine the mechanisms which control latent infections are detailed in Chapter 6. It was believed that VNAs might be important since all latently infected cats possess these

antibodies and the culture of their bone marrow in the absence of VNAs resulted in virus reactivation. By contrast, it was found that when latently infected bone marrow cells were cultured in the continuous presence of cat sera containing VNA, virus reactivation was inhibited. Further it was found by the use of mouse monoclonal antibodies to FeLV proteins that the epitope involved in reactivation inhibition (RI) was that on the gp70 which is involved in neutralisation. The VNA and RI titres of the inhibiting antibodies were closely correlated ($r = 0.89$). Attempts were then made to elucidate the means by which neutralising antibody maintained latent infections. It was clear that the antibody was neither cytolytic nor cytotoxic since inhibition was reversible upon the removal of antibody, but it appeared from those experiments involving the mixing of bone marrow cells from a viraemic and an uninfected cat that the antibody prevented the spread of virus from the cells of the viraemic cat to those of the uninfected cat. Other possibilities of the antibody preventing the transcription of viral antigen or preventing the assembly or budding of virus at the cell membrane are discussed.

The General Discussion (Chapter 7) assesses the extent to which the aims of this study were achieved. Particular attention is paid to the mechanisms of control of other persistent viral infections including the paramyxoviruses, herpesviruses and other retroviruses. Methods by which further investigation into the controlling mechanisms of latent FeLV infection might be carried out are suggested.

CHAPTER 1

GENERAL INTRODUCTION

1. Feline leukaemia virus

a) Discovery of the virus

Feline leukaemia was first reported to be an infectious disease by Jarrett *et al.* (1964a) who inoculated a homogenate of the tumour from a field case of thymic lymphosarcoma into 4 newborn kittens by subcutaneous injection and found that they all developed lymphosarcoma. Electron microscopy carried out on the tissues of one of these kittens demonstrated the presence of virus-like particles in intracellular vescicles (Jarrett *et al.*, 1964b) which resembled the viruses known to cause leukaemia in mice and chickens.

Rickard *et al.* (1967) obtained a similar result and later reported that a virus-induced lymphocytic leukaemia was serially transmissible and 56 of 68 kittens which were inoculated with cell-free extracts developed lymphosarcoma (Rickard *et al.*, 1969). Kawakami *et al.* (1967) demonstrated virus particles in the plasma of cats with leukaemia. These authors also showed that the disease was transmissible when kittens were inoculated intraperitoneally with material from a leukaemic cat.

The virus demonstrated in these experiments, feline leukaemia virus (FeLV), was recognised as belonging to the family of viruses now known as retroviruses (Fenner, 1975-76).

b) Structure of FeLV

FeLV is a typical retrovirus. The RNA genome consists of 3 genes, *gag*, *pol* and *env*, arranged from the 5' to 3' end, which code for the structural proteins and the enzyme, reverse transcriptase (RT). The *gag* gene (group-specific antigen gene) codes for the polyprotein precursors of the internal core proteins with molecular weights of 27,000 (p27), 15,000 (p15), 12,000 (p12) and 10,000 (p10) (Graves and Velicer, 1974). These are similar in all FeLV isolates. The *pol* (polymerase) gene codes for the RT and the *env*

(envelope) gene codes for the polyprotein precursor of the envelope proteins. This precursor is cleaved to give a glycoprotein of 70,000 daltons (gp70) and an unglycosylated protein of 15,000 daltons (p15(E)). Variations in the env gene sequences give subgroup specificity to each isolate (see Section 1d).

Flanking the gag, pol and env genes at the 5' and 3' ends of the genome are regions containing sequences which regulate and enhance viral transcription.

In the virion the viral RNA is surrounded by a protective protein core consisting of p27, p15 and p10. These proteins are hexagonally arranged and also surround the RT (Scolnik et al., 1970). Outside this core is an inner protein coat consisting of p12. The outermost structure is the viral envelope which is a phospholipid membrane into which is inserted the gp70 and p15(E). The gp70 forms spikes on the exterior of the virus particle and is anchored into the membrane by the hydrophobic p15(E) (Bolognesi et al., 1978).

c) Replication of FeLV

After FeLV has adsorbed to and penetrated its host cell, the virus loses its envelope in order to release the RNA. A single stranded DNA copy of the RNA is then made using the viral RT. This single stranded DNA acts as a template for a double stranded DNA provirus. The provirus circularises and integrates into the host cell chromosome. Thus, the provirus becomes part of the cellular genome. This process can only occur in cells which are undergoing DNA synthesis (Temin, 1971). It should be noted that several copies of endogenous FeLV-like sequences occur in the cellular DNA of the domestic cat (Sarma et al., 1974; Niman et al., 1977; Okabe et al., 1976; Koshy et al., 1980; Soe et al., 1983).

Two species of mRNA are transcribed from proviral DNA: firstly, the whole genome (gag-pol-env) from which the gag and pol proteins are translated; and secondly, env which is transcribed

independently. A precursor, Pr65 gag, is made from which the gag proteins are produced. The env precursor Pr80 env is processed to gp70 and p15(E) (Neil and Onions, 1985).

The components of the virus envelope migrate to the cell surface and are inserted into the plasma membrane (Bolognesi et al., 1978) to form an evagination called the viral bud (Schäfer and Bolognesi, 1977; Bolognesi et al., 1978). The gag polyprotein precursor is then transported to this site. One end of this molecule is linked to the viral envelope and the other to the viral RNA (Bolognesi et al., 1978). This precursor is then cleaved to form the individual core proteins (p15, p12, p27 and p10) which align to form the internal structures of the virus. The env precursor is glycosylated and cleaved to produce gp70 and p15(E) (Bolognesi et al., 1978). The p15(E) is then further processed to give p12(E) (Neil et al., 1980a). When maturation is complete infectious virus particles are budded off without damaging the host cell.

d) Subgroups of FeLV

FeLV occurs in 3 distinct subgroups which are defined by interference; A (FeLV-A), B (FeLV-B) and C (FeLV-C) (Sarma and Log, 1971; 1973). The occurrence of the different subgroups in the field has been described in detail by Jarrett (1980). It is believed that the structure of the envelope glycoprotein, gp70, determines subgroup specificity.

The subgroup of any particular strain of FeLV is important in determining its host range in vitro. As described in Section 1e, FeLV-A is only capable of infecting feline cells, i.e. is ecotropic, whereas FeLV-B and FeLV-C also infect human cells, i.e. are xenotropic (Jarrett et al., 1973c).

Jarrett (1980) found that all strains of FeLV were antigenically closely related and shared group specific antigens.

Although Sarma and Log (1973) reported the occurrence of distinct subgroups by virus neutralisation, Russell and Jarrett (1978a) found that by using this technique there was extensive cross-reactivity between subgroups, since antibody which neutralised FeLV-A often neutralised FeLV-C, but to a lesser degree. Russell and Jarrett (1978a) also found that FeLV-A isolates were monotypic but that there was antigenic variation amongst FeLV-B and FeLV-C isolates.

In the field FeLV-A is by far the most common subgroup and is present in all isolates. FeLV-B and FeLV-C occur, but invariably FeLV-A is also present (Sarma and Log, 1971; 1973; Jarrett et al., 1978a). It appears that FeLV-B and FeLV-C viruses are recombinants between FeLV-A and cellular FeLV-related DNA sequences (Russell and Jarrett, 1978a; Stewart et al., 1986). Jarrett et al. (1978a) found that in healthy FeLV-positive cats, 65% had FeLV-A, 33% had FeLV-AB, and FeLV-C was only isolated from diseased cats.

The efficacy of replication of FeLV in the cat is related both to subgroup of the virus and age of the cat. For example, experimentally, FeLV-A will infect 100% of cats younger than 16 weeks of age, whereas FeLV-B will infect only 20% of newborn kittens and will not infect older kittens (Jarrett, 1980). Jarrett et al. (1978b) and Jarrett and Russell (1978) reported that FeLV-A and FeLV-B behaved differently in terms of transmission between cats and growth in the cat. They found that FeLV-A was readily transmitted between cats, but that FeLV-B could only be transmitted as a phenotypic mixture with subgroup A, i.e. FeLV-AB. These authors also found that FeLV-B had restricted growth in cats and that FeLV-A enhanced the growth of FeLV-B in vivo. FeLV-C appears to be intermediate in its behaviour in that it will infect 100% of newborn kittens (Onions et al., 1982), 50% of 2 week old kittens but will not infect 8 week old kittens (Jarrett, 1980). Jarrett et al. (1984) found that simultaneous infection with FeLV-A enhanced the growth of FeLV-C in the cat and overcame the age related resistance to FeLV-C.

There is some evidence that FeLVs of different subgroups are involved in specific types of disease as discussed in Section 2.

e) Growth of FeLV in cell culture

FeLV was first reported to grow in culture by Jarrett et al. (1968) who infected two cell lines with cell-free homogenates from field cases of lymphosarcoma. One of these lines was derived from combined spleen, thymus, liver, kidney and heart of newborn kittens (NK cells) and the other was established from the lungs of feline embryos (FL cells). Both of these cell lines consisted predominantly of fibroblasts and grew as cell monolayers. It was found that FeLV was capable of replicating continuously in homologous cell cultures without causing a cytopathic effect.

Theilen et al. (1969) succeeded in maintaining suspension cell cultures which continuously produced virus. These cells (FL74) were transformed lymphocytes derived from a renal lymphosarcoma.

Jarrett et al. (1973c) reported that although all FeLV isolates, irrespective of subgroup, grew in feline embryo (FE) cells, the subgroup of each isolate was important in determining its ability to grow in the cells of other species. They found that FeLV-B and FeLV-C grew in human embryonic lung (HEL) cells whereas FeLV-A did not (Jarrett et al., 1969). However isolates which contained both subgroups A and B in a phenotypic mixture were capable of infecting these cells (Jarrett et al., 1973c). FeLV-A, therefore, has a restricted host range.

f) Detection of FeLV in cell culture

The presence of FeLV in cell culture can be demonstrated in a number of ways including electron microscopy (Jarrett et al., 1968), interference (Fischinger and O'Connor, 1969), immunofluorescence (Hardy et al., 1973b), complement fixation (Sarma et al., 1971), radioactive labelling of particles having characteristic bouyant density (Jarrett et al., 1971) and virus isolation from culture fluids (Fischinger et al., 1974). The most common techniques currently in use are virus isolation and immunofluorescence.

2. Diseases associated with FeLV infection

FeLV is widespread in cat populations and excluding trauma is probably the most common single cause of death in adult cats (Jarrett, 1984; 1985a). A large number of diseases are associated with the virus which are listed in Table 1.1 and have been described in detail (Hardy, 1980a; Jarrett, 1984).

The diseases can be divided into two categories. Firstly, there are proliferative or malignant diseases. These include lymphosarcomas (alimentary, multicentric and thymic) and leukaemias (lymphatic, myeloid and erythroid). Of these, lymphosarcomas are much more commonly diagnosed. However FeLV is not isolated from all cats with lymphosarcoma (Laird et al., 1968; Hardy et al., 1980). For example, while 90% of cats with thymic lymphosarcoma and 60% of cats with multicentric lymphosarcoma are viraemic only 30% of cats with alimentary lymphosarcoma are viraemic. There is some evidence that cats with lymphosarcoma which are not viraemic at the time of diagnosis have had previous contact with FeLV (Russell and Jarrett, 1978b; Hardy et al., 1980). Cats with thymic lymphosarcoma are generally much younger than cats with other kinds of lymphosarcoma, with an average age of 2.5 years (Hardy, 1980a).

Secondly, there are degenerative diseases which include anaemias such as erythroid hypoplasia and haemolytic anaemia, bone marrow aplasia, immunosuppression and reproductive failure such as early foetal death or abortion.

Immunosuppression can occur in both kittens and adult cats. Kittens born to viraemic queens may develop thymic atrophy with T-lymphocyte depletion leading to a defective cell mediated immune response (Anderson et al., 1971; Hardy, 1980a). In adult cats immunosuppression is the most common cause of death associated with FeLV. Such cats are susceptible to a number of intercurrent diseases such as feline infectious peritonitis, feline infectious anaemia, chronic upper respiratory infections, enteric infections and septicaemia.

TABLE 1.1

Diseases associated with FeLV infection

Malignant haemopoietic diseases.

Lymphoid tumours	:	thymic lymphosarcoma
		multicentric lymphosarcoma
		alimentary lymphosarcoma
Leukaemias	:	myeloid leukaemia
		erythroid leukaemia
		lymphatic leukaemia

Non-malignant haemopoietic diseases.

Anaemia	:	haemolytic anaemia
		erythroid hypoplasia
Immunosuppression	:	thymic atrophy
Marrow aplasia		
Myelofibrosis and osteosclerosis		

Non-haemopoietic diseases.

Reproductive failure	:	foetal resorption
		abortion

FeLV-associated anaemia can occur as a primary result of virus infection, as in erythroid hypoplasia, or secondary to other primary effects of the virus, e.g. lymphosarcoma (Mackey et al., 1975; Hardy, 1980a). Other conditions which often occur secondary to erythroid hypoplasia are myelofibrosis and osteosclerosis (Hoover and Kociba, 1974; Onions et al., 1982).

There is some evidence that certain diseases are attributable to particular subgroups of the virus. In cats with lymphosarcoma, Jarrett et al. (1978a) found that 42% had FeLV-A and 58% had FeLV-AB. FeLV-C, however, is often associated with erythroid hypoplasia (Jarrett, 1980; Onions et al., 1982; Jarrett et al., 1984).

The pathogenesis of FeLV infection will be described in Section 5.

3. Source of FeLV

Cats which are persistently viraemic represent the major source of infection for susceptible cats. Hardy et al. (1973a) postulated that the saliva, blood and urine of such cats were possible sources of infectious virus and that saliva was the most likely of these. Francis et al. (1977) confirmed that virus was present in bodily secretions and that it was excreted continuously from the mouth of viraemic cats. Saliva containing virus is therefore the most important source of infection. As described in Chapter 5, FeLV can be transmitted in milk. The possibility of blood-sucking parasites such as fleas acting as vectors of the virus was considered by Hardy et al. (1973a) but this has never been proven to be important.

4. Transmission of FeLV

Transmission of the infection occurs in 2 main ways. Congenital transmission occurs when virus crosses the placenta to infect developing embryos. As mentioned in Section 2, reproductive

failure may follow congenital transmission. Any kittens born to a persistently viraemic queen, however, are themselves persistently viraemic (Jarrett and Russell, 1978) and generally succumb to one of the FeLV-associated diseases within 2 years.

The other major mode of transmission is by horizontal transfer of virus-containing saliva from persistently viraemic cats to susceptible cats (Hardy et al., 1973a). Jarrett et al (1973b) found that horizontal transmission of infection occurred within one month of mixing viraemic cats with susceptible cats. Although aerosolisation of oronasal secretions may transmit virus, it is likely that direct intimate contact between cats such as licking is necessary in order to transfer sufficiently high doses of virus to result in infection (Hoover et al., 1977a).

Indirect transmission, such as spread of virus by contaminated feed bowls or attendants' hands, is thought to be unimportant.

5. In vivo spread of FeLV

Oronasal administration of FeLV has been used experimentally to simulate natural infection (Hoover et al., 1972). Rojko et al. (1979a) used this method of infection to investigate the sites of initial virus replication and the spread of virus within the cat. Virus was detected in tissues by immunofluorescence.

Infection begins when virus enters and grows in the cells of the oropharynx and upper respiratory tract. Within 3 days virus can be detected in reticular cells and germinal centres of local lymph nodes and a small proportion of circulating lymphocytes and monocytes also contain virus. Dissemination of virus-infected lymphocytes then occurs and by 7-14 days these can be found in the bone marrow, in blood and lymphatic vessels in lymph nodes, in the thymus and in Peyer's patches. Viral antigen is concentrated in actively dividing cells in these tissues.

The virus then spreads to non-lymphoid haemopoietic tissues so that by 14-21 days viral antigen is detected in an increasing proportion of bone marrow cells, particularly myelomonocytic cells. The amount of antigen present in granulocytes increases as the cells mature. Circulating neutrophils and platelets by this time contain considerable quantities of viral antigen. The release of free virus particles into the blood coincides with the appearance of antigen-containing neutrophils and platelets and the growth of virus in the marrow (Hoover et al., 1977b).

Finally, from 28 to 56 days after infection the virus is spread to non-haemopoietic tissues throughout the body, particularly the epithelial cells of the oropharynx, salivary glands, bladder, intestine and pancreas. Infectious virus is then released from these organs.

6. Outcomes of infection with FeLV

Although the cycle of infection with FeLV as described by Rojko et al. (1979a) can take up to 56 days to reach completion, the outcome of the infection is often determined earlier. Russell and Jarrett (1978b) and Rojko et al. (1979a) found that when cats were experimentally inoculated with virus the outcome of the infection was determined within 28 days. However when cats which were susceptible to the virus were mixed with cats which were excreting virus and thus developed an infection, it could be as long as 10 weeks after mixing before the outcome was resolved (Russell and Jarrett, 1978b). The latter situation would mimic the conditions in the field where the interval between mixing and the development of sufficient socialising in order to transmit virus may be prolonged.

There are three possible outcomes of infection: productive persistent infection, recovery or latency. Productive persistence and recovery will be discussed in this Section and latency will be discussed in Section 10.

a) Productive persistent infection

This type of infection is characterised by viraemia when free infectious virus is present in the blood. Approximately half of the cats which were experimentally inoculated with virus when 16 weeks old developed persistent viraemia while the other half had a transient viraemia (Jarrett et al., 1982).

In persistently viraemic cats the virus has completed all of the stages of pathogenesis and is excreted in the saliva, urine and faeces. Such cats fail to mount an effective anti-viral immune response and are susceptible to the diseases described above. The longterm prognosis for persistently viraemic cats is very poor. Francis and Essex (1980) found that the average time from first diagnosis of viraemia until death was 22.6 months and McClelland et al. (1980) found in their extensive study that 83% of persistently viraemic pet cats were dead within 3.5 years of exposure compared to only 17% of uninfected cats and 16% of exposed, non-viraemic cats.

The duration of a transient viraemia can range from a few days to several weeks (Jarrett et al., 1982). Rojko et al. (1979a) suggested that the infection in these cats is suppressed before the virus begins to replicate in the marrow. However, these results are contrary to those of Jarrett et al. (1982) who found that even if a cat was only viraemic for a few days, infectious virus was isolated from its saliva, suggesting that the virus had become disseminated to the tissues before becoming suppressed.

Transiently viraemic cats suppress their infection and appear to recover by mounting an immune response as described below.

i) Detection of viraemia

Viraemia can be detected in several ways including immunofluorescence on blood smears to detect group specific (p27) antigen in the cytoplasm of platelets and neutrophils (Hardy et

al., 1973b); detection of p27 in blood by enzyme-linked immunosorbant assay (ELISA) (Lutz et al., 1983), and virus isolation from blood (de Noronha et al., 1977; Jarrett et al., 1982) and saliva (Francis et al., 1977; Jarrett et al., 1982). Jarrett et al. (1982) reported an unusual cat which had detectable virus in the saliva but not in the blood. This may have been due to a sampling error since virus had been isolated from its blood as well as from saliva on previous samplings and was again isolated subsequently. It is possible, however, that virus is occasionally present in saliva but not in blood since a similar case has recently been observed (H. Lutz and O. Jarrett; personal communication).

The methods used in this study to detect viraemia were ELISA (Leukassay F; C-Vet) and virus isolation from plasma. It should be noted that approximately 10% of cats which are positive on ELISA for the presence of p27 antigen in blood (antigenaemic) are not viraemic as determined by virus isolation (Jarrett, 1985b). The results obtained by ELISA were therefore always confirmed by virus isolation in this study.

b) Recovery

Cats which recover from infection with FeLV may or may not develop a transient antigenaemia and/or viraemia. Recovery is associated with the development of an immune response to FeLV.

Two types of antibodies are found in recovered cats. Firstly, virus neutralising antibodies (VNAs) may be present which inactivate virus in vitro and, by fixing complement, kill cells which are expressing virus (Grant et al., 1983). These antibodies are presumed to have a similar effect in vivo and are often detected in cats which have eliminated a transient viraemia (Jarrett et al., 1973b; 1977; Pedersen et al., 1977). Even small amounts of VNAs protect kittens from viraemia when they are challenged with large doses of virus (Jarrett et al., 1977).

The second antibody which is produced is against a cell membrane antigen, feline oncornavirus-associated cell membrane

antigen (FOCMA). FOCMA was originally defined as an antigen on the surface of the FL74 line of feline lymphosarcoma cells (Theilen et al., 1969) which reacted with sera from cats recovered from feline sarcoma virus infection (Essex et al., 1971b) or exposure to FeLV (Riggs, 1971). This antigen was originally believed by some to be a virus-coded transformation-specific antigen (Hardy et al., 1977) although now there is evidence that there may be a relationship between FOCMA and an envelope antigen of FeLV-C (Vedbrat et al., 1983). Anti-FOCMA antibodies are very common in cat sera (Jarrett et al., 1973a; Rogerson et al., 1975). They do not protect the cat against persistent infection since persistently viraemic cats often have low levels of these antibodies. However, Essex et al. (1971a) suggested that anti-FOCMA antibodies protect cats against progressive tumour development.

Anti-FOCMA antibodies are very common amongst free-range urban cats. Rogerson et al. (1975) found that 40% of urban cats had these antibodies compared to 6% of rural cats. This was confirmed by Russell and Jarrett (1978b) who found that 50% of urban cats had anti-FOCMA antibodies in their sera whereas only 4% had VNAs. Whatever the specificity and function of anti-FOCMA antibodies it is generally regarded that their presence in the serum of a cat is a sensitive indicator of exposure to the virus.

There is also some evidence for a cellular immune response being involved in recovery from FeLV infection (Rojko et al., 1982). These authors found that peripheral blood mononuclear leucocytes from latently infected cats were cytotoxic for autochthonous bone marrow cells which had been cultured and had reactivated infectious virus, but were not cytotoxic for freshly obtained marrow cells which did not express virus (see Section 10). There is no further information available on cellular immunity to FeLV infections although it is considered likely that it is important in eliminating FeLV-infected cells.

7. Factors which influence the outcome of FeLV infection

There are a number of factors which influence the outcome of infection with FeLV. Probably the most important is the age at which the cat is first exposed to the virus. Hoover et al. (1976) found that the susceptibility of cats to the infection decreased with age. Upon experimental inoculation of FeLV the percentage of cats which developed persistent viraemia was 100% of newborn kittens, 85% of kittens between 2 weeks and 2 months of age and only 15% of cats aged between 4 months and 1 year. The remaining cats became immune. Similar results were obtained by Rojko et al (1979a) who suggested that young cats may be more at risk to infection because they have a larger population of potential target cells in their bone marrow than adults.

The proportion of cats which becomes viraemic following FeLV infection may be a function of the dose of virus and the frequency of exposure to the virus (see Section 8 below). Large doses and frequent exposure, especially for young kittens, increase the probability of persistent viraemia (Hoover et al., 1976).

A defective immune system can also play a role in determining the outcome of infection. Cats which have intercurrent disease and may therefore be immunosuppressed, or cats which are iatrogenically immunosuppressed by prolonged or high doses of corticosteroids or other immunosuppressive drugs, have an increased risk of becoming persistently viraemic. Indeed, Rojko et al. (1979b) found that the natural age resistance to FeLV infection could be overcome by treating cats with adrenal corticosteroids and Schaller et al. (1978) found that the carcinogen, methylnitrosourea, could also overcome this age resistance.

8. Epidemiological situations of FeLV infections

As has been discussed previously, the incidence of infection with FeLV may be high within cat populations, with the virus being either horizontally transmitted in the saliva of persistently viraemic cats, or congenitally transmitted from mother to foetus.

There are two reasonably distinct epidemiological patterns of FeLV infection. The first involves closed multicat households such as breeding colonies. When a viraemic cat is introduced to these establishments almost all of the in-contact cats eventually become infected as indicated by the presence of anti-FOCMA antibodies in the sera of 90% of these cats (Hardy et al., 1973a). Kittens which are born in these households are exposed to the infection at a very early age and therefore run a high risk of developing viraemia. This outcome is also favoured by exposure of these kittens to frequent and high doses of the virus. The prevalence of persistent viraemia in these households is therefore very high. Jarrett et al. (1978a) reported that 40% of these cats were persistently viraemic and Hardy (1980b) found that 28% of cats in a household with FeLV were viraemic compared to 0% in households which had not been exposed to FeLV. The incidence of FeLV-related diseases is therefore also high but the majority of the cats which are not viraemic produce VNAs and are believed to be resistant to re-infection (Hardy et al., 1976a; Russell and Jarrett, 1978b). Russell and Jarrett (1978b) found that 42% of cats in these households had VNAs compared to only 4% of free range cats (see below).

In summary, in closed multicat households in which FeLV is enzootic, cats are exposed to frequent high doses of virus from a very early age. There is a high incidence of viraemia with associated disease and the prevalence of VNAs and anti-FOCMA antibodies is also high.

The second epidemiological situation involves free range cats in urban and suburban areas. Between 40% and 50% of these cats have anti-FOCMA antibodies (Rogerson et al., 1975; Russell and Jarrett, 1978b) indicating that exposure to the infection is frequent. However, the dose of virus transmitted is probably low compared to the situation described above. Urban cats are generally older than cats in multicat households when they are first exposed to the virus and therefore have developed an age-related resistance to the infection. Only 1% of urban cats are persistently viraemic (Hardy et al., 1976a) and only 4% have

VNAs in their serum (Russell and Jarrett, 1978b). The incidence of FeLV-related diseases in urban cats is therefore relatively low. It is possible that transiently viraemic cats which excrete virus for a short time are important in maintaining the cycle of infection in this community as well as the small number of persistently viraemic cats (Jarrett et al., 1982).

9. Control of FeLV infection

a) Test-and-removal programmes

Because of the rapid spread of FeLV through cats in multicat households and the incidence of persistent viraemia and disease which results, there is often a demand to adopt a means of control. An effective control programme which is in common use was first described by Hardy et al. (1976b) and involves a test and removal procedure.

In a single-cat household control is relatively easy. Once a cat has been identified as being viraemic it should be isolated from other cats in the area and retested after an interval of 3 months. Three months should be long enough to allow most transient infections to become suppressed. However, if the cat is again viraemic on this second test it should be kept in isolation from other cats or even destroyed since its life expectancy is very short (McClelland et al., 1980). The household should then be cleaned with normal household detergents in order to inactivate any virus present, the litter tray and feeding bowls should be either disinfected or discarded, and an interval of 30 days should elapse before another cat is introduced.

A similar procedure is adopted in multicat households. Viraemic cats are identified and isolated and the house should be cleaned as above. Both the viraemic and the non-viraemic cats should be retested after 3 months and any new cases of viraemia should also be isolated. Cats which are negative on the second test but were positive on the first test remain in isolation until

they are negative on two consecutive occasions. Cats which are negative on two consecutive tests can then be considered to be free from infection and can be bought or sold as desired. Cats which are positive on the two tets, however, should be eliminated from the household. All new cats which enter the house should be isolated and tested for FeLV and it would be advisable for owners of breeding stock to obtain a certificate stating that the cat is free from viraemia before going to stud.

Hardy et al. (1976b) thus successfully controlled the infection in many multicat households. After removal of viraemic cats, only 3 out of 657 uninfected cats became viraemic (0.46%) compared to 55 out of 284 (19.3%) which became viraemic when the viraemic cats were not removed. Similar results were obtained by Weijer and Daams (1978) using this method in the Netherlands.

b) Vaccination

It was thought for the following reasons that vaccination might be a possible method of controlling FeLV infections. Firstly, the virus is transmissible by contact (Hardy et al., 1973a; Jarrett et al., 1973b) and therefore the cycle of infection might be broken. Secondly, recovered cats develop neutralising antibodies (Jarrett et al., 1973b; 1977; Pedersen et al., 1977) and are resistant to reinfection with the virus. Thirdly, passively acquired antibody will protect cats from infection: maternally derived antibody protected kittens from challenge (Jarrett et al., 1977).

Experimental vaccines have been produced in several laboratories. Four types of vaccine have been described.

i) Vaccines derived from cells

The first attempts at vaccine production were by Jarrett et al. (1974) who described two cellularly derived vaccines. These consisted of either live or paraformaldehyde-inactivated lympho-

blastoid cells of the FL74 line (Theilen et al., 1969). It was found that both vaccines induced anti-FOCMA antibodies and that cats vaccinated with the live cells were protected against challenge. Cats vaccinated with the inactivated cells were not challenged and therefore it is not known whether or not this vaccine was protective.

ii) Vaccines derived from virus

Both live and inactivated virus produced by FL74 cells have been used as vaccines (Pedersen et al., 1979; 1986). Although a detectable antibody response was not found in vaccinated cats, 80% were found to be protected from challenge.

A similar type of vaccine was produced using the virus from tumour cells of the F422 line (Rickard et al., 1969). This vaccine consisted of paraformaldehyde-inactivated virus, and like the FL74 vaccine described above, rendered 80% of the cats resistant to challenge in the absence of a detectable antibody response (Mahmoud, 1984).

iii) Subunit vaccines

The first subunit vaccine was produced by Salerno et al. (1978) using the gp70 from purified virus. Again, there was no antibody response in these cats. Unfortunately the results of these experiments are inconclusive since the cats used in the study were too old to be effectively challenged.

Pedersen et al. (1986) produced a similar gp70 subunit vaccine which was found to be ineffective because rather than protecting cats it resulted in an enhancement of the challenge.

A second type of subunit vaccine consisting of the gp70/85 of virus produced by either FL74 or F422 cells has been developed (Osterhaus et al., 1985). This vaccine was prepared by combining gp70/85 from disrupted purified FeLV with an immunostimulating

complex (ISCOM) derived from saponin. This vaccine resulted in 100% protection from challenge and is as yet the only vaccine which has induced a detectable neutralising antibody response in the vaccinates. It is likely that the success of this vaccine is due to the efficient way in which the ISCOM presents the gp70 to the immune system.

iv) Soluble tumour cell vaccine

This type of vaccine was produced by culturing FL74 cells in serum-free medium which resulted in the cell surface components being shed into the culture fluid (Olsen et al., 1976). These components were then inactivated, concentrated and used as a vaccine. Again, there was no detectable neutralising antibody response produced in these cats. These inactivated FL74 cell surface components are the basis of a commercial vaccine which is currently available in the United States and some European countries (Leukocell; Norden) and is claimed to give between 70 and 80% protection against challenge.

10. FeLV latency

Until recently it was thought that there were only two outcomes of infection with FeLV; persistent viraemia or recovery. However, Post and Warren (1980) discovered that a third possible outcome was latency. These authors treated 4 non-viraemic cats with corticosteroids daily for 21 days in an attempt to reactivate a latent infection. One of these cats had been viraemic initially but had eliminated its viraemia after passive immunotherapy, and another 2 of the cats had been exposed to viraemic cats and were presumed to have recovered from infection. The fourth cat was not known to have been exposed to FeLV.

After treatment infectious virus was isolated from the blood of 2 of these cats, although 3 were positive for antigen by ELISA and all 4 were positive for antigen in neutrophils by immuno-fluorescence.

This work showed that cats which appear to have recovered from infection may harbour a latent infection which in some cases can be reactivated by corticosteroid therapy.

These results were confirmed by Rojko et al. (1982) when viraemia was produced in all of 5 latently infected cats by the use of corticosteroid therapy twice weekly for 4 weeks. The viraemia was persistent in only 2 of these cats. These authors also found that virus could be reactivated and isolated from cultured bone marrow of 8 out of 10 exposed immune cats compared to none of 4 unexposed cats. However virus could not be isolated from freshly obtained uncultured marrow.

By carrying out immunofluorescence on cultured marrow Rojko et al. (1982) found that viral antigen was present in differentiating myelomonocytic precursor cells but not in mature macrophages or granulocytes. The site of the latent virus was therefore identified as being in the bone marrow, probably in myelomonocytic precursor cells.

The possible involvement of latent FeLV in lymphosarcomas in non-viraemic cats has been studied (Rojko et al., 1982; Madewell and Jarrett, 1983). The results from the 2 groups of workers differ. Rojko et al. (1982) isolated virus which had been reactivated from cultured marrow from both of 2 cats whereas Madewell and Jarrett (1983) failed to isolate virus from 2 similar cases. The possible role of latent FeLV in these tumours is therefore unresolved.

Madewell and Jarrett (1983) reported that FeLV could be reactivated from the cultured bone marrow cells of 9 of 16 ostensibly recovered cats 36 weeks after exposure to viraemic cats. All 9 of these cats had VNAs and anti-FOCMA antibodies. At any one time therefore a considerable proportion of the cats in a FeLV-positive multicat household may harbour latent infections.

11. Aims of the present study

Because of the potentially large numbers of latent infections in multicat households it was of importance to investigate the significance of these infections and this is the subject of this thesis. The major aims of the study were:

a) to develop a reliable method to identify latently infected cats,

b) to determine the prevalence and duration of the latent state in multicat households following exposure of cats to natural FeLV infection,

c) to determine the clinical and epidemiological significance of latency, particularly in multicat households, and

d) to investigate the factors which maintain FeLV in the latent state.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Cells

Monolayer cell cultures used in this study were feline embryo fibroblast cells of the FEA strain (Jarrett et al., 1973c) and FEA cells chronically infected with FeLV of subgroup A (FEA/FeLV-A) or subgroup B (FEA/FeLV-B) (Jarrett et al., 1972) which were continuously expressing infectious virus. The FEA/FeLV-A cells were infected with the Glasgow-1 strain (FeLV-A/Glasgow-1) (Jarrett et al., 1972) and the FEA/FeLV-B cells were infected with the Sarma strain (FeLV-B/Sarma). Clone 81 (c81) cells were feline cells derived from the CCC line which carry the genome of a murine sarcoma virus (Fischinger et al., 1974).

The feline tumour cell line, FL74, which was derived from a renal lymphosarcoma (Theilen et al., 1969) was used. This culture is composed of transformed T-lymphocytes which express FeLV of subgroups A, B and C and grow in stationary suspension.

Cell culture

Cells were routinely grown in 25 cm^2, 80 cm^2 or 175 cm^2 plastic flasks (Nunc) in an atmosphere of 5% CO_2 in air and were incubated at 37°C. Cells grown in plates were cultured in a LEEC GA2 incubator with a fully humidified atmosphere of 5% CO_2 in air at 37°C.

The foetal calf serum (FCS) (Gibco) used for culturing cells was heated to 56°C for 30 minutes in order to remove complement.

a) Monolayer cells

FEA, FEA/FeLV-A, FEA/FeLV-B and c81 cells were grown in Dulbecco's modified Eagle's Minimal Essential Medium (DMEM) (Gibco) supplemented with 10% FCS, 2mM L-glutamine and 400 units/ml of penicillin-streptomycin (DFC10).

Three times per week the culture fluid was removed and the adherent cell monolayers were dispersed by rinsing three times in a solution of 0.01% trypsin in 0.02% EDTA. The cells were resuspended in DFC10 and subcultured at a ratio of 1:4.

b) Suspension cells

FL74 cells were grown at a concentration of 8 x 10^5/ml in a medium consisting of equal volumes of Leibovitz L-15 medium (Gibco) and McCoys 5a medium (Gibco) supplemented with 10% FCS, 2mM L-glutamine and 400 units/ml of penicillin-streptomycin (LM medium). They were subcultured and resuspended at this concentration twice weekly.

Experimental cats

Cats which were free from infection with FeLV were bred in a closed colony and were housed in huts, either in groups or individually. Up to 8 cats were housed in a hut with a floor area of approximately $3m^2$, and between 9 and 20 cats were housed in a hut with a floor area of approximately $7.5m^2$. Fig. 2.1 shows examples.

Infection of cats

The virus used to infect cats was FeLV-A/Glasgow-1. The dose of virus and the route of administration for each cat used in this study are summarised in Table 2.1 and described in detail in each Chapter.

Production of FeLV-A/Glasgow-1 for infection of cats

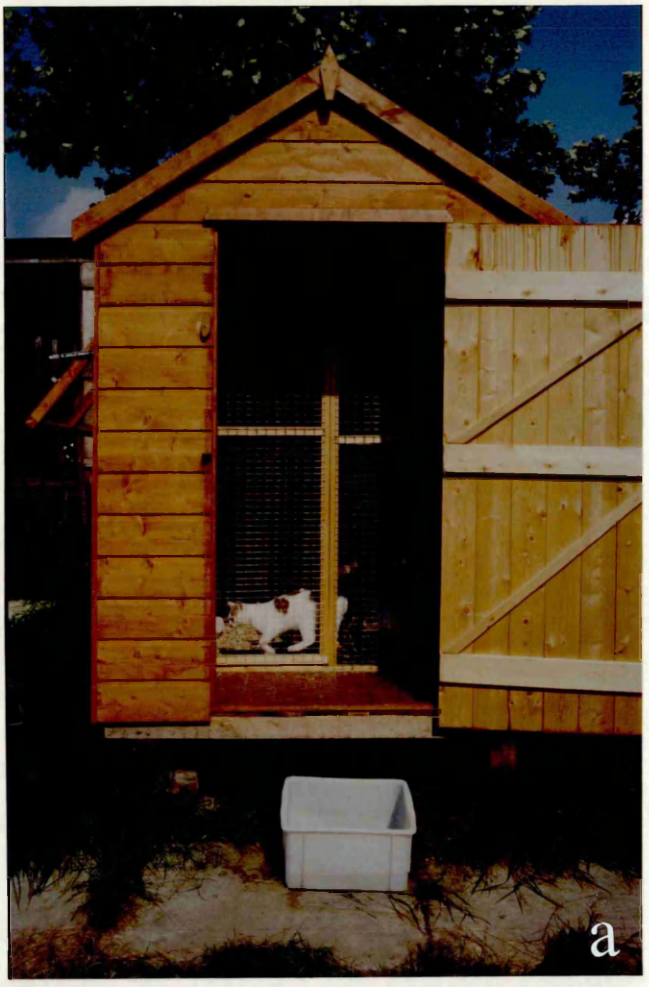
The virus was produced from FEA/FeLV-A cells which were chronically infected with FeLV-A/Glasgow-1.

One million FEA/FeLV-A cells were plated in 9 cm sterile plastic petri dishes (Nunc) in 10 ml of DFC10 supplemented with 1%

FIG. 2.1 Examples of cat huts.

a. To house up to 8 cats.

b. To house between 9 and 20 cats.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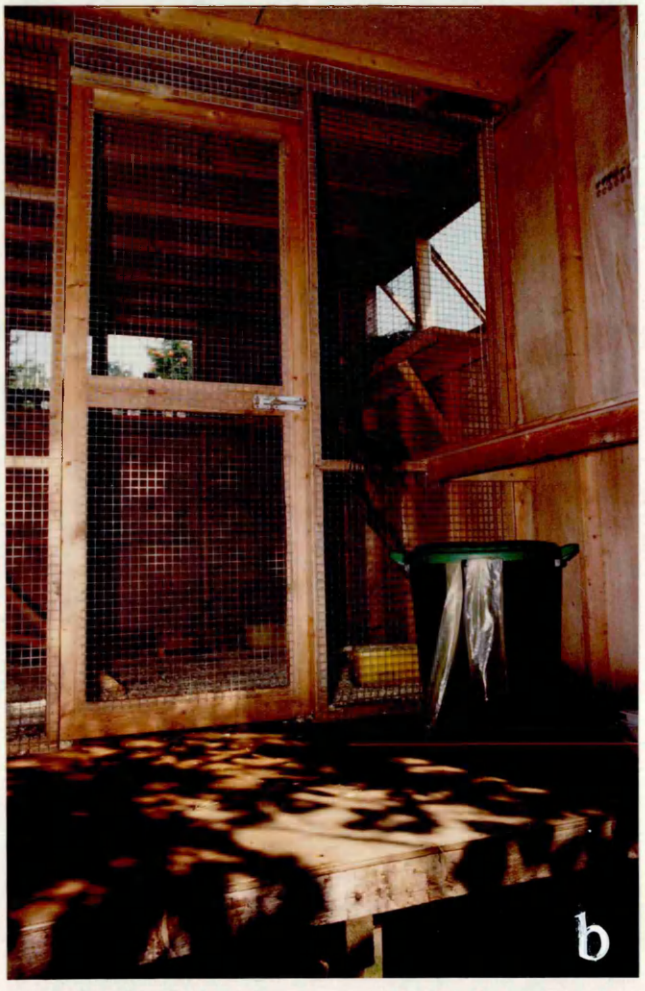

b

TABLE 2.1 Cats used in this study

Cat No.	Virus status[1]	Method of infection
1	V	Natural exposure FeLV-A/Glasgow-1
2	V	" "
3	V	" "
4	L	" "
5	L	" "
6	L	" "
7	L	" "
8	L	" "
9	L	" "
10	L	" "
11	L	" "
12	L	" "
13	R	" "
14	R	" "
15	R	" "
16	R	" "
17	R	" "
18	R	" "
19	R	" "
20	L → R	Intraperitoneal FeLV-A/Glasgow-1 8×10^4 fiu
21	L	Oronasal FeLV-A/Glasgow-1 1×10^6 fiu
22	L	" "
23	L	" "
24	L	" "
25	V	" "
26	V	" "
27	V	Vaccinated → oronasal FeLV-A/Glasgow-1 1×10^6 fiu
28	R	" "
29	L	" "

TABLE 2.1 (Continued)

Cat No.	Virus status[1]	Method of infection
30	L	Vaccinated → oronasal FeLV-A/Glasgow-1 1×10^6 fiu
31	L	" " "
32	L	Natural exposure FeLV-A/Glasgow-1
33	T	Natural exposure to cats with latent
34	T	FeLV-A/Glasgow-1
35	T	" " "
36	T	" " "
37	T	" " "
38	T	" " "
39	T	" " "
40	T	" " "
41	L	Oronasal FeLV-A/Glasgow-1 1×10^5 fiu
42	L	" " " "
43	L	Vaccinated → oronasal FeLV-A/Glasgow-1 1×10^6 fiu
44	L	" " "
45	L	" " "
46	L	Oronasal FeLV-A/Glasgow-1 1×10^6 fiu
47	L	" " "
48	L	" " "
49	U	
50	U	
51	U	
52	V	Oronasal FeLV-A/Glasgow-1 1×10^6 fiu
53	V	" " "
54	R	Oronasal FeLV-A/Glasgow-1 1×10^5 fiu
55	R	" " "
56	R (field)	Natural exposure FeLV
57	R (field)	"
58	R (field)	"

TABLE 2.1 (Continued)

Cat No.	Virus status[1]	Method of infection
59	R (field)	Natural exposure FeLV
60	U	
61	U	
62	V	Oronasal FeLV-A/Glasgow-1 1×10^6 fiu
63	V	Subcutaneous FeLV-A/Glasgow-1 1.3×10^6 fiu
64	U	

Key to Table 2.1

1. Virus status: L = latently infected; V = viraemic;
R = recovered; U = uninfected;
T = tracer

amphotericin B and 1% lincomycin hydrochloride (DFC10-AL). These plates were incubated at 37°C.

After 48 hours the culture fluid was harvested and spun at 2000 rpm for 10 minutes. The supernatant fluid was then passed through a 0.45 µm sterile filter (Nalgene). The virus was concentrated in an Amicon hollow fibre concentrator (CH4) using an H1 MP01 cartridge with a nominal molecular weight cut-off of 100,000.

The concentrated virus was then distributed into vials and stored at -70°C prior to use.

Virus infectivity assay

To determine the infectivity of each batch of virus a focus assay was used.

A mixture of 2 x 10^5 FEA cells and 2 x 10^4 c81 cells was plated in 5 cm sterile plastic petri dishes (Nunc) in 2 ml of DFC10-AL. These cultures were incubated overnight at 37°C.

The virus was recovered from -70°C and fivefold dilutions were made in DFC10-AL supplemented with 4 µg/ml of polybrene in order to aid virus adsorption. The medium was removed from the plates and an inoculum of 1.0 ml of virus dilution was added. Each dilution was tested in duplicate. The virus was adsorbed for 90 minutes at 37°C after which the medium was replaced with 4 ml of DFC10-AL.

The plates were then incubated for a further 3-4 days when the medium was replaced with 4 ml of fresh DFC10-AL. After 7-10 days, the foci in plates which contained approximately 20 foci were counted. From these counts the titre of virus was obtained and expressed as focus inducing units (fiu) per ml.

Blood samples

Blood samples were obtained from experimental cats by jugular venepuncture. Samples were taken into heparin coated tubes for detection of viral antigen and infectious virus, and into plain tubes for serological tests. Plasma and serum were separated by centrifugation at 2000 rpm for 10 minutes. Plasma was stored at -70°C and serum at -20°C.

Detection of viral antigen

FeLV p27 antigen was detected in the plasma of cats using an enzyme-linked immunosorbant assay (ELISA) kit (Leukassay-F; C-Vet). The test was carried out according to the methods of Jarrett et al. (1982). A volume of 50 µl of plasma was tested.

Detection of infectious virus

Infectious FeLV in the plasma of cats and in cell culture fluids was detected by a modification of the virus isolation test described by Jarrett et al. (1982). This test resembles the focus assay described above. A mixture of 2 x 10^5 FEA cells and 2 x 10^4 c81 cells was plated in 5 cm petri dishes in 2 ml DFC10-AL with 4 µg/ml of polybrene which were incubated overnight at 37°C. A volume of between 0.5 ml and 1 ml of sample was added and incubated at 37°C for 90 minutes to allow adsorption of virus. The medium was then replaced with 4 ml of DFC10-AL and incubated for 3 days. After this time, the medium was again replaced with a fresh 4 ml of DFC10-AL and the plates were incubated for a further 3-4 days. The presence of infectious virus was observed as foci of transformed cells on a background of a confluent FEA/c81 cell monolayer. Fig. 2.2 shows photographs of uninfected FEA/c81 cell mixture (a), and a FEA/c81 cell mixture transformed by FeLV (b).

Detection of virus neutralising antibodies

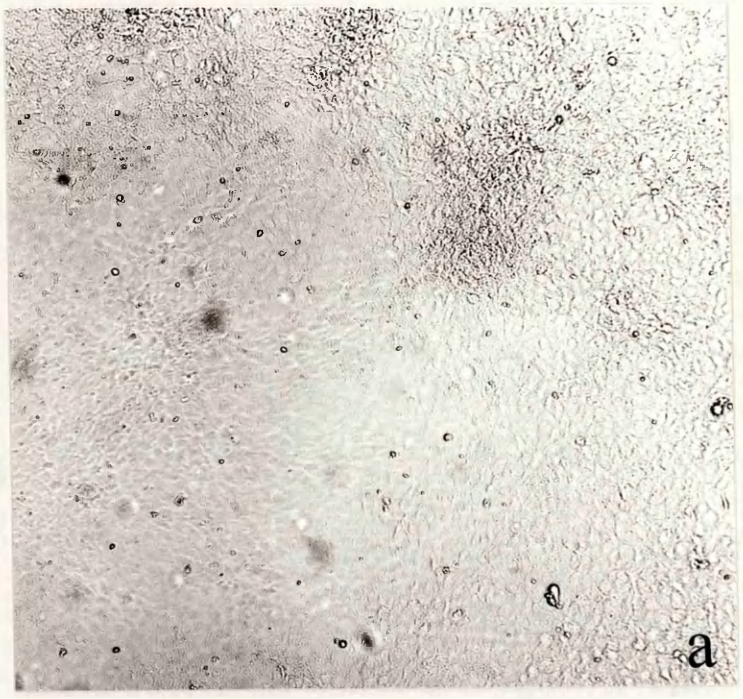
Virus neutralising antibodies (VNAs) to FeLV-A in the serum of

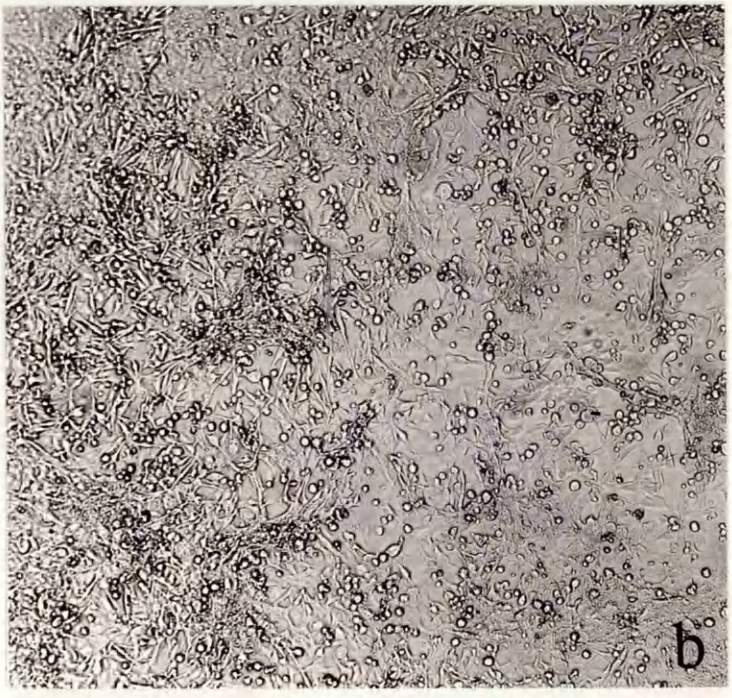
FIG. 2.2 Virus isolation.

a. An uninfected FEA/c81 cell monolayer.

b. A FEA/c81 cell monolayer transformed with FeLV.

Magnification x 120


a


b

cats and in mouse monoclonal antibodies (MCAs) to FeLV proteins were detected by a modification of the methods described by Russell and Jarrett (1978a).

A volume of 0.5 ml of medium containing 1 x 10^4 FEA/FeLV-B cells was plated into each well of 24-well sterile plastic multiwell trays (Nunc). The medium was DMEM supplemented with 20% FCS, 2mM L-glutamine, 400 units/ml of penicillin-streptomycin, 1% amphotericin B and 1% lincomycin hydrochloride (DFC20-AL). The cells were incubated for 2 hours at 37°C. Twelve twofold serum dilutions were made in Leibovitz L-15 medium supplemented with 5% FCS, 2 mM L-glutamine, 400 units/ml of penicillin-streptomycin, 1% amphotericin B and 1% linocmycin hydrochloride (dilution medium). These dilutions started at a concentration of 1:2 and 50 µl volumes were made of each in sterile plastic round-bottomed 96-well microtitre plates (Nunc). A volume of 50 µl of a FeLV-A/Glasgow-1 pseudotype of Moloney murine sarcoma virus (Russell and Jarrett, 1976) was added to each well. Twelve twofold dilutions of the virus itself were included as a control. The plates were then incubated at 37°C for 2 hours to allow virus neutralisation to take place.

After the incubation period, a volume of 25 µl of the reaction mixture was added to the wells containing FEA/FeLV-B cells. The cells were then incubated as before. After 24 hours the medium was removed and 8 x 10^4 FEA cells in 1 ml of DFC20-AL were added to each well. The plates were then incubated for a further 3-4 days. The presence of virus was identified as foci of transformation on the cell sheet. The VNA titre was taken as the highest dilution of serum which caused a 75% reduction in the number of foci compared to the virus control.

Detection of anti-FOCMA antibodies

FOCMA is an antigen demonstrable on the plasma membrane of FeLV-infected leukaemic cells (Essex et al., 1971b). The presence

of antibodies directed against this antigen in cat serum is believed to be a sensitive indicator of exposure to FeLV. Anti-FOCMA antibodies were detected by a live cell immunofluorescence (IF) test using the FL74 cell line. The method described here is a modification of that described by Essex et al. (1971b).

FL74 cells were washed twice in serum-free Leibovitz L-15 medium by centrifugation at 1000 rpm for 5 minutes and were suspended in fresh medium at a concentration of 2 x 10^6/ml. A volume of 25 µl of this suspension was placed in each 7 mm well of a 96-well teflon-coated glass slide (Henley-Essex). The slides were incubated at 37°C in a moisture chamber for 30 minutes to allow the cells to adhere to the glass.

Eight doubling dilutions in 25 µl volumes of the sera to be tested were made in serum-free Leibovitz L-15 medium in 96-well U-bottomed plastic microtitre plates. When the FL74 cells had adhered to the glass 25 µl volumes of each serum dilution were added and the slides were incubated as above for 1 hour.

After this time the slides were gently washed twice in phosphate buffered saline (PBS) at room temperature. The slides were then flooded (approximately 30 µl/well) with goat-anti-cat IgG conjugated with fluorescein isothiocyanate (Miles) at a dilution of 1:30 in serum-free Leibovitz L-15 medium. After a further incubation of 1 hour the slides were washed as above and kept moist in PBS. They were examined for membrane fluorescence under a Leitz Orthoplan microscope with an ultraviolet light source using a x 25 water immersion objective. The antibody titre was taken as the highest dilution of serum in which more than approximately 25% of the cells showed membrane fluorescence (Fig. 2.3).

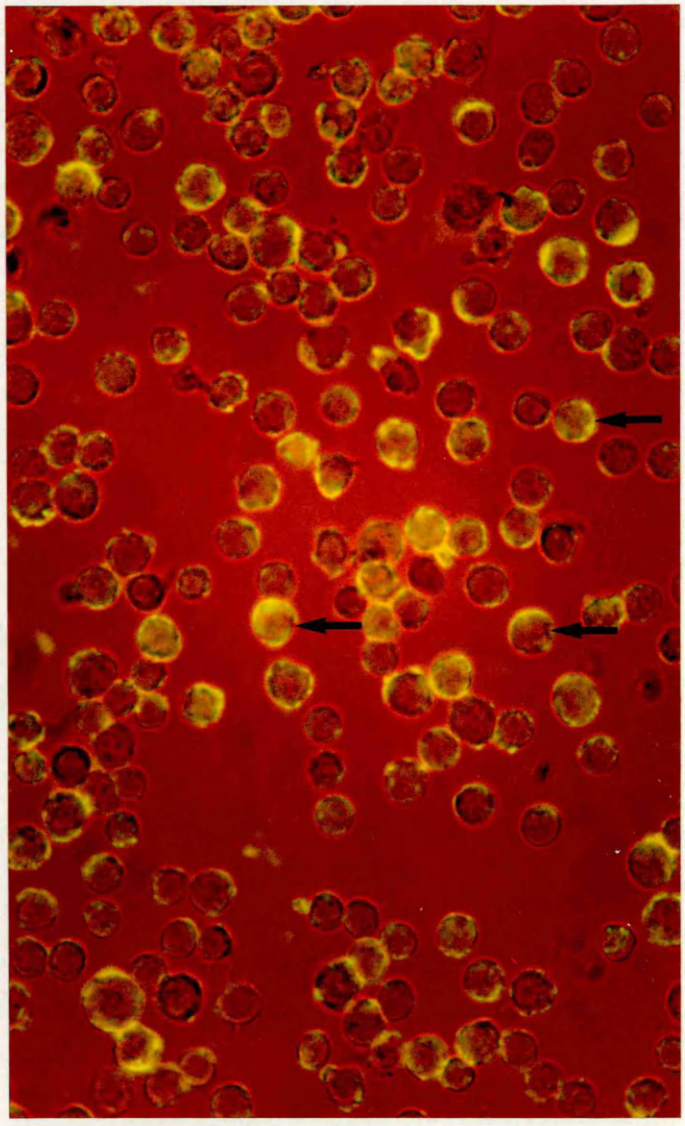
Oropharyngeal swabs

Saliva was obtained for detection of infectious virus in the mouth of cats. The tonsillar area was carefully swabbed with a sterile wooden cotton-tipped swab (Exogen). The tip was broken off

FIG. 2.3 Demonstration of anti-FOCMA antibodies in a cat serum by the presence of membrane IF on FL74 cells. The photographs were taken using both visible and ultraviolet light sources.

The arrows point to cells showing membrane fluorescence.

Magnification x 250



into a sterile plastic bottle (Sterilin) containing 3 ml Leibovitz L-15 medium supplemented with 5% FCS, 2 mM L-glutamine, 400 units/ml of penicillin-streptomycin, 1% amphotericin B and 1% lincomycin hydrochloride (transport medium). Gross debris was removed by centrifugation of the fluid at 2000 rpm for 10 minutes and the supernatant was stored at -70°C prior to testing for infectious virus.

CHAPTER 3

DETECTION OF LATENCY

Latent FeLV infections may be detected by treating latently infected cats with high doses of corticosteroids. Virus becomes reactivated in a proportion of the cats which develop a persistent viraemia. It was in this way that Post and Warren (1980) discovered the latent state. In their study 4 apparently recovered cats were treated with 1 mg of dexamethasone and 5 mg of prednisolone daily for 21 days, except on days 10, 11 and 12 when no drugs were given. Two of these cats became viraemic as determined by virus isolation from plasma.

Rojko et al. (1979b) suggested that the establishment of regressive (recovery) versus progressive (viraemia) infection in vivo was sensitive to corticosteroid therapy and that cats which were thus treated had an increased risk of developing viraemia. The effect of corticosteroids on latent FeLV infections was tested both in vivo and in vitro (Rojko et al., 1982). Five latently infected cats were treated with 10 mg/kg of methylprednisolone twice weekly for 4 weeks and 2 of them became persistently viraemic. Pedersen et al. (1984) produced persistent viraemia in only 5 out of 49 cats with known latent infections when they were treated with 10 mg/kg of methyprednisolone once weekly for 4 weeks. These authors also suggested that the ease of reactivation was dependent on the strain of virus with which the cats were infected. Cats which were infected with the Rickard strain became viraemic more frequently than cats infected with the Snyder-Theilen strain.

It is clear that high doses of corticosteroids can reactivate latent infections in some cases to produce viraemia although the mechanism of reactivation is unclear. Two of the undesirable effects of long-term corticosteroid therapy are the occurrence of degenerative changes in the bone marrow and immunosuppression. Therefore the fact that latent FeLV can be reactivated from marrow following such therapy may be due to these side effects or may be

due to a primary effect on virus expression. The two- or threefold increase in virus production reported by Rojko et al. (1982) when latently infected cells were treated with corticosteroids in vitro, was thought to be due to a direct effect on viral transcription.

The most reliable and commonly used method to detect latent FeLV infections is the culture of aspirated bone marrow cells which leads to reactivation of the latent virus (Rojko et al., 1982; Madewell and Jarrett, 1983; Pedersen et al., 1984). Virus cannot be detected in freshly aspirated marrow. Upon culture of the marrow, reactivated virus has been detected in three ways: firstly, by isolation of infectious FeLV which is released into the culture fluids (Rojko et al., 1982; Madewell and Jarrett, 1983; Pedersen et al., 1984); secondly, by detection of FeLV p27 antigen in the culture fluids by ELISA (Pedersen et al., 1984) and thirdly, by detection of p27 in the cytoplasm of infected cells by fixed cell IF (Rojko et al., 1982).

In this study latently infected animals were detected by the culture of aspirated bone marrow. Corticosteroid therapy was not used due to the low proportion of latently infected cats which reactivate virus (Pedersen et al., 1984). It would appear that the chances of achieving reactivation are much less than the chances of causing other secondary infections resulting from the immunosuppression produced by prolonged and high doses of corticosteroids (N.C. Pedersen, personal communication).

In the present study freshly aspirated latently infected marrow was tested for the presence of FeLV by virus isolation in a similar way to that described by Madewell and Jarrett (1983). In addition two other methods were used: live cell IF which detects FeLV gp70 antigen on the cell surface and fixed cell IF which detects p27 in the cytoplasm. After culture reactivated virus was detected in the culture fluids by virus isolation and by detection of p27 antigen in the cytoplasm of cells by fixed cell IF. Also, to obtain a quantitative assessment, the proportion of latently infected cells in the marrow was determined by an infectious centre assay.

Collection of bone marrow cells

Cats were heavily sedated prior to bone marrow biopsy by an intramuscular inoculation of 25 mg/kg ketamine hydrochloride (Vetalar: Parke Davis). The fur covering the gluteal region was closely clipped and the skin was disinfected with 0.5% Hibitane (ICI) in 70% methanol. Femoral shaft bone marrow biopsies were obtained via the trochanteric fossa (Fig. 3.1) by aspiration through sterile 18 gauge disposable Illinois pattern bone marrow biopsy needles (Kormed) (Fig. 3.2) using 10 ml syringes. The marrow was collected into 3 ml of Alpha Minimal Essential Medium (Gibco) supplemented with 10% FCS, 2 mM L-glutamine, 400 units/ml penicillin-streptomycin, 10^{-6}M hydrocortisone succinate (AFC10) and 1% preservative-free heparin (Pularin) (collection medium).

Culture of bone marrow cells for detection of latent FeLV

Single cell suspensions of bone marrow cells were prepared in 10 ml of collection medium by passing the cells through a 21 gauge needle and then through a 23 gauge needle. The suspension was centrifuged for 10 minutes at 2000 rpm. The bone marrow supernatant (BMSN) was decanted and stored at -70°C for future virus isolation. The cell pellet was resuspended in 10 ml of 0.83% ammonium chloride and incubated on ice for 5 minutes in order to lyse the red blood cells. Following a second centrifugation at 2000 rpm for 10 minutes the supernatant was discarded and the cell pellet was resuspended in 10 ml Alpha Minimal Essential Medium supplemented with 20% FCS, 2mM L-glutamine, 400 units/ml penicillin -streptomycin and 10^{-6}M hydrocortisone succinate (AFC20). The cells were then counted and were cultured in 10 ml AFC20 at a concentration of 2 x 10^6/ml in 25 cm^2 plastic flasks. Duplicate cultures were prepared for each biopsy and were incubated at 37°C.

FIG. 3.1 Femoral shaft bone marrow biopsy. Sedation was achieved using ketamine hydrochloride. The biopsy needle gains entry to the femoral shaft via the trochanteric fossa. Bone marrow is aspirated using a 10 ml syringe.

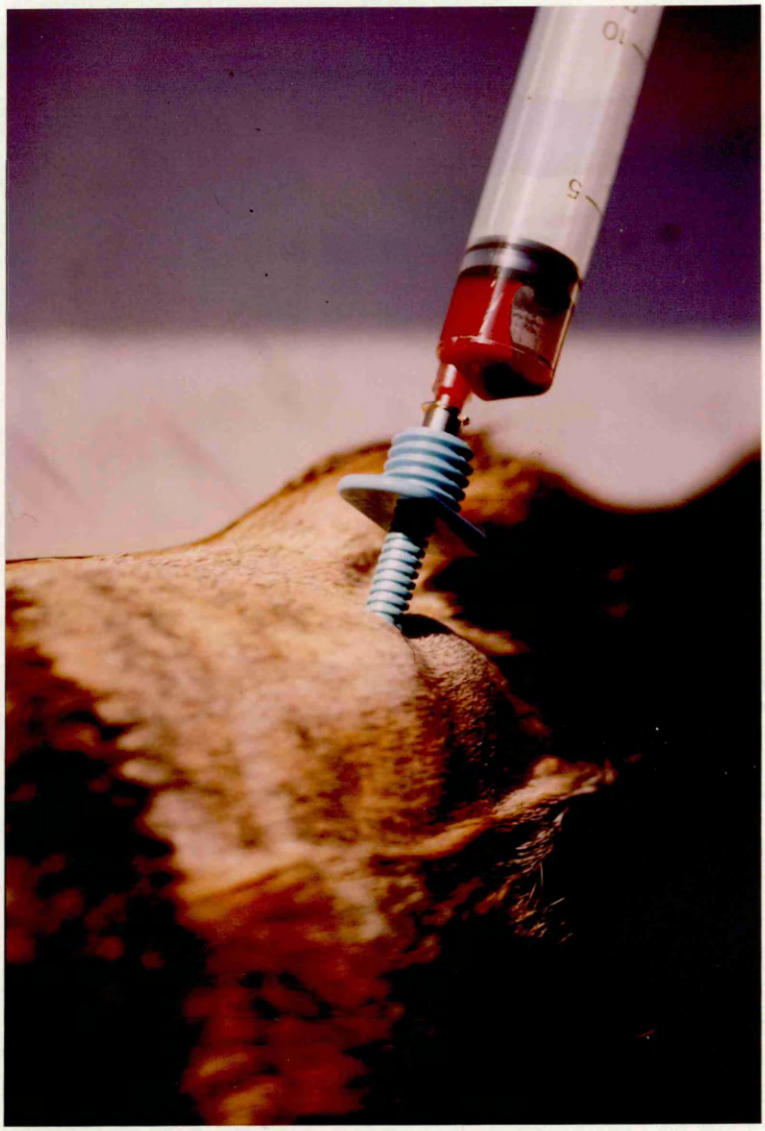
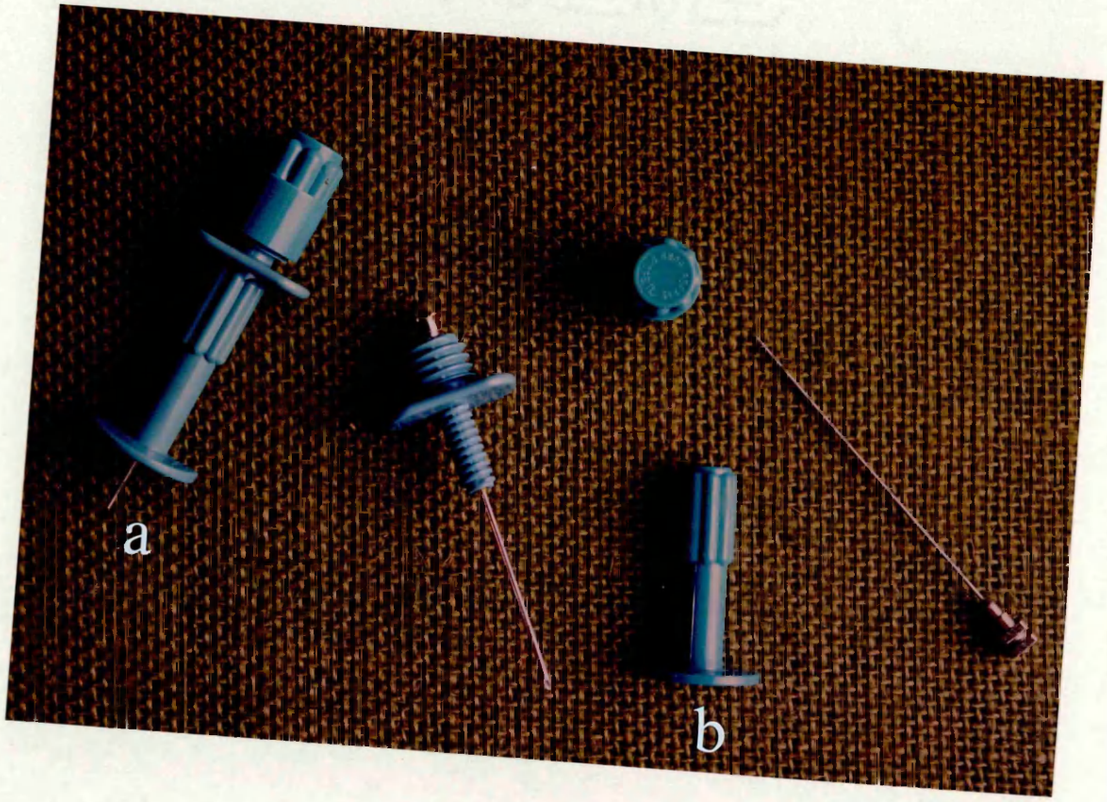


FIG. 3.2 Bone marrow biopsy needle.

a. Entire needle.

b. Fragmented needle showing individual components.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The cultures were fed each week by replacing 5 ml of medium with fresh AFC20. The medium which was removed was stored at -70°C prior to testing for infectious virus.

IF on bone marrow prior to culture

a) Live cell IF

Single cell bone marrow suspensions were washed twice in serum-free Leibovitz L-15 medium and were resuspended in this medium at a concentration of 2 x 10^6/ml. A volume of 25 µl of this suspension was placed on each 7 mm well of an 8-well teflon coated glass slide (Flow Laboratories). The slides were incubated at 37°C in a moisture chamber for 30 minutes to allow the cells to adhere to the glass. A mouse MCA which neutralises FeLV-A (46II E2; Vedbrat et al., 1983) was used at a dilution of 1:100 in Leibovitz L-15 medium and a volume of 25 µl was then added to each well. The slides were incubated as above for 1 hour and were then gently washed twice in PBS for 2 minutes. A volume of 25 µl of sheep anti-mouse Ig linked to fluorescein isothiocyanate (Amersham International) at a dilution of 1:100 in Leibovitz L-15 medium was added to each well and the slides were incubated for 1 hour at 37°C. The slides were then washed as above and the cells were examined for the presence of membrane IF as described for the anti-FOCMA antibody test. The test described above detects the envelope glycoprotein (gp70) on the surface of cells which are expressing FeLV.

b) Fixed cell IF

Bone marrow cell suspensions were prepared as above and resuspended in serum-free Leibovitz L-15 medium at a concentration of 2 x 10^6/ml. Three drops of this suspension were spun onto glass slides at 1500 rpm for 5 minutes in a Cytospin II cytocentrifuge (Shandon). The cells were then dried, fixed in absolute methanol at room temperature for 5 minutes, dipped in water and dried.

A volume of 25 µl of rabbit anti-FeLV serum (see below) which was diluted 1:40 in PBS was added to each cytospin preparation. The slides were then incubated at 37°C in a moisture chamber for 2 hours. Following this incubation the slides were washed twice in PBS at room temperature for 2 minutes, dipped in water and dried.

A volume of 25 µl of donkey anti-rabbit IgG conjugated to fluo -rescein isothiocyanate (Amersham International) diluted 1:40 in PBS was added to each slide which was then incubated as above for 1 hour. The slides were again washed in PBS, dipped in water and dried. The cells were examined for the presence of cytoplasmic FeLV antigen under a microscope with an ultraviolet light source as above. This test detects antigen in the cytoplasm of cells which are actively infected with FeLV.

Production of rabbit anti-FeLV antibody

Antibody to FeLV gag proteins was obtained from O. Jarrett. Antisera were raised in 4 rabbits immunised with purified FeLV-A/F422 (Rickard et al., 1969) disrupted with triton X-100. Antibodies were purified from pooled sera by affinity chromatography using a column of Sepharose 4B linked to FeLV-A/F422 disrupted by NP40.

Culture of bone marrow cells for IF

Single cell suspensions of marrow were prepared as described above in AFC20 at a concentration of 2×10^6/ml. A volume of 0.3 ml of this suspension was plated into each well of 8-well Flaskettes (Lab-Tek) (Fig. 3.3) and incubated at 37°C. At 3-day intervals half of the medium was removed and replaced with fresh AFC20. Bone marrow was grown in this manner for 10 days after which time the presence of viral antigen could be detected in the cytoplasm of latently infected cells by fixed cell IF.

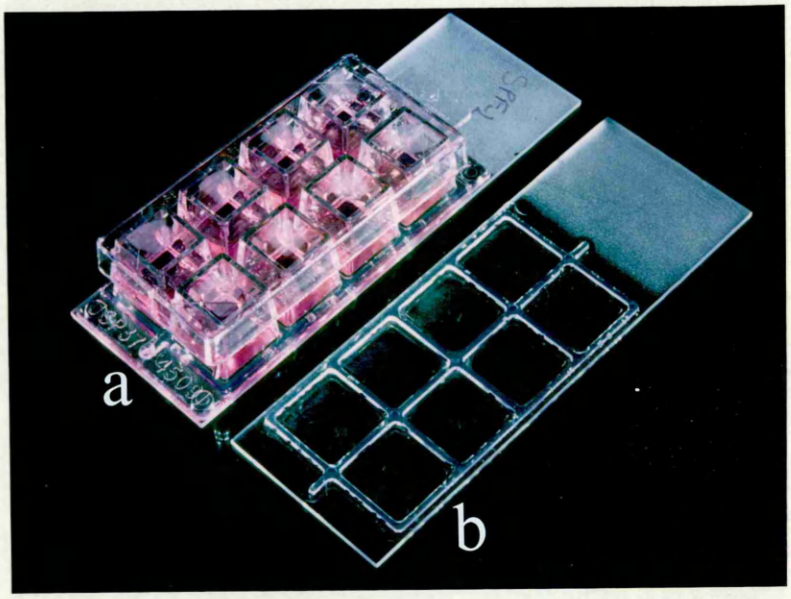
Preparation of Flaskette cultures for IF

The culture fluid was carefully pipetted from the chambers of

FIG. 3.3 Photograph of an 8-well Flaskette.

a. Flaskette in use for cell culture showing individual chambers.

b. Flaskette slide prepared for IF after removal of the chamber walls.


a
b

the Flaskette. The plastic walls of the chambers were removed leaving the bone marrow cells adherent to the glass slide. The slides were gently washed twice in PBS at room temperature for 2 minutes and then fixed in absolute methanol at room temperature. They were then dipped in water and air dried. Fixed cell IF was carried out on the Flaskette slides using 50 µl volumes of reagents per well.

Proportion of latently infected cells in the bone marrow

a) Experimental cats

Bone marrow was taken from 8 latently infected cats in order to assess the proportion of cells in the marrow which was harbouring latent virus. Four of these cats (cats 21-24) had received an oronasal dose of 1 x 10^6 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium at 16-20 weeks of age. Three cats (cats 29-31) had received an experimental FeLV vaccine which was given by intramuscular injection at 8 and 11 weeks of age. Briefly, this vaccine was made from FeLV-A/F422 inactivated with 0.02% paraformaldehyde and adjuvanted with aluminium hydroxide and incomplete Freund's adjuvant (Mahmoud, 1984). The cats were then challenged oronasally with 1 x 10^6 fiu of FeLV-A/Glasgow-1 at 14 weeks of age. These cats did not become viraemic but were latently infected. The eighth cat, cat 32, was born in the multicat household detailed in Chapter 4 and after exposure to the viraemic donor cats developed a latent infection.

One recovered cat (cat 28) and 3 viraemic cats (cats 25-27) were included as controls. Cats 27 and 28 had received the experimental FeLV vaccine and had been challenged as described above. Cat 27 developed a persistent viraemia, whereas cat 28 resisted challenge, had no evidence of latent infection and was therefore said to be "recovered". Cats 25 and 26 had been given 1 x 10^6 fiu of FeLV-A/Glasgow-1 oronasally in 1 ml Leibovitz L-15 medium at 16-20 weeks of age. The sources of infection of these animals are summarised in Table 2.1.

b) Infectious centre assay

A mixture of 4 x 10^5 FEA cells and 4 x 10^4 c81 cells were co-cultivated in 5 cm petri dishes in a volume of 3 ml DFC10-AL and incubated for 4 hours at 37°C. Single cell suspensions of bone marrow were prepared as described above but were finally suspended at a concentration of 4 x 10^6/ml in DFC10-AL. Five tenfold dilutions were made and 1 ml of each dilution was added to the FEA/c81 assay plates. Duplicate plates were used for each cell concentration.

The plates were incubated for 3 days after which time the medium was removed and replaced with a fresh 4 ml DFC10-AL. After a further incubation for 3 days the plates were examined for the presence of foci of transformed cells, which were counted.

The reproducibility of each assay was assessed by plating FEA/FeLV-A cells onto the FEA/c81 mixture. The FEA/FeLV-A cells were known to be continuously producing virus. Five twofold dilutions were made with a highest concentration of 50 cells/ml. The assay proceeded as above.

Culture of bone marrow cells

Bone marrow cells grew as an adherent cell monolayer. After one week in culture small colonies of fibroblasts developed which gradually coalesced, so that by 2 weeks in culture the fibroblasts were confluent. Also by this time large granulated cells were present and areas of more mature myeloid cells could be identified (Fig. 3.4). The presence of mature myeloid cells indicated that myeloid stem cells in the marrow were capable of differentiation in vitro. Cultures were maintained for 4 weeks.

Evidence for lack of FeLV expression in freshly aspirated bone marrow from latently infected cats

Detection of FeLV expression in freshly aspirated bone marrow cells from latently infected cats was attempted in 3 ways: by virus isolation from the BMSN; by live cell IF to detect gp70 on the surface of cells, and by fixed cell IF to detect p27 in the cytoplasm of cells. A comparison of the results obtained from carrying out these tests on marrow from latently infected or viraemic cats is given in Table 3.1.

Infectious virus was not isolated from the BMSN of any of 26 latently infected cats used in this study but was isolated from all of 8 viraemic cats used. In 12 of these cats live cell IF failed to detect gp70 on the surface of latently infected cells and fixed cell IF failed to detect p27 in the cytoplasm. By contrast, between 1 and 10% of cells from viraemic cats were expressing gp70 (O. Jarrett; personal communication) and almost all cells contained p27 (Fig. 3.5).

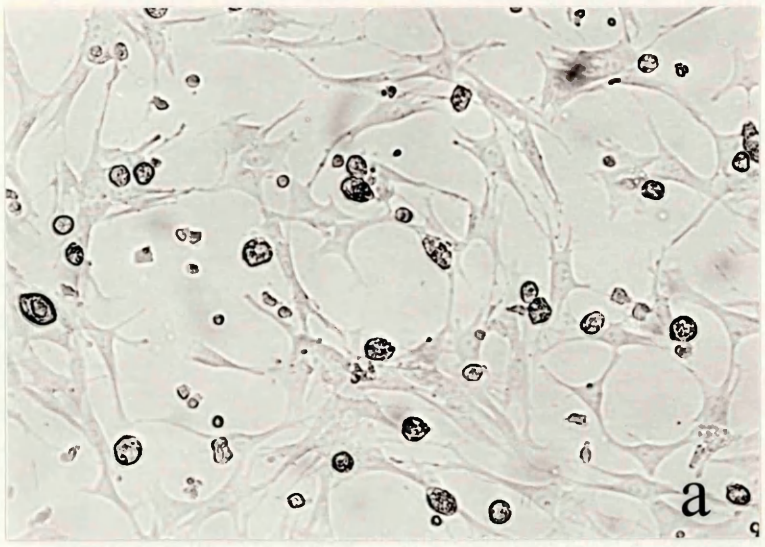
FIG. 3.4 Bone marrow culture.

a. Sparse colony of fibroblasts visible after one week in culture.

b. Confluent monolayer of fibroblasts after 2 weeks in culture. The photograph also demonstrates the presence of large granulated cells (arrowed).

c. Colony of differentiated myeloid cells (arrowed) seen after 2 weeks in culture.

Magnification x 100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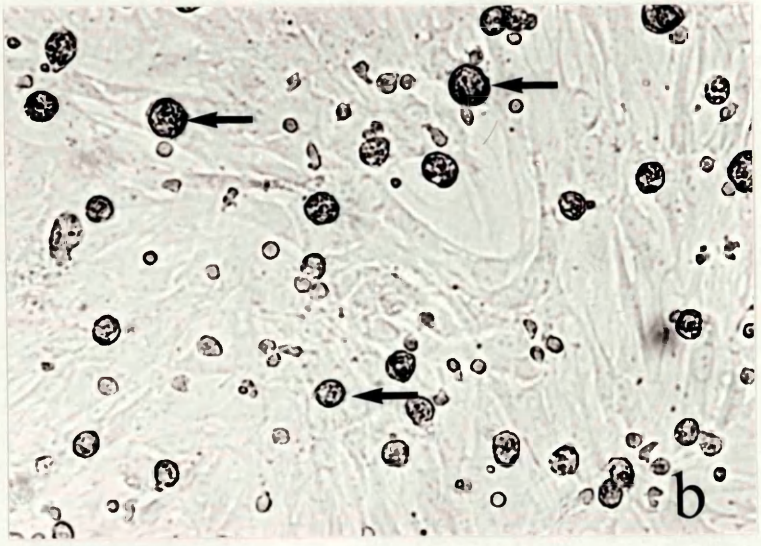
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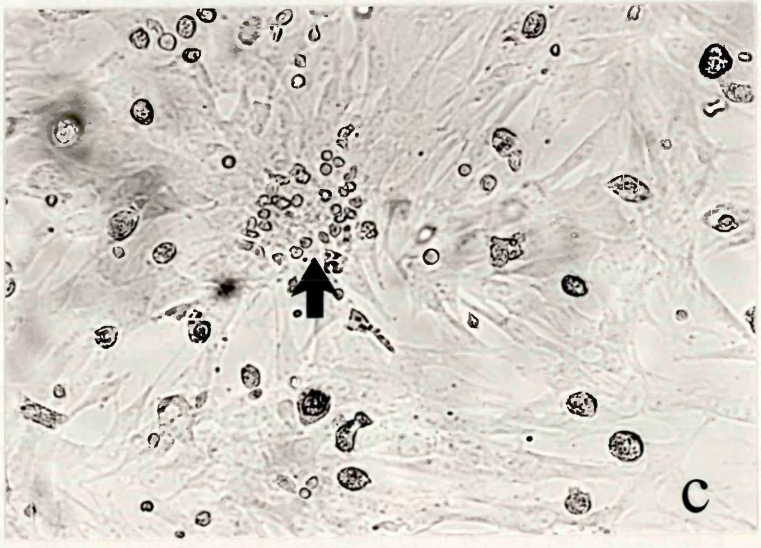

c

TABLE 3.1

Detection of FeLV expression in freshly aspirated bone marrow from latently infected versus viraemic cats

Test used	Virus status: Latently infected	Viraemic
Virus isolation[1] from BMSN	-	+
Live cell IF[2] gp70 detection	0	1 - 10%
Fixed cell IF[2] p27 detection	0	≒ 100%

[1] + virus isolated
- virus not isolated

[2] Figures given are percentage of total cells showing IF

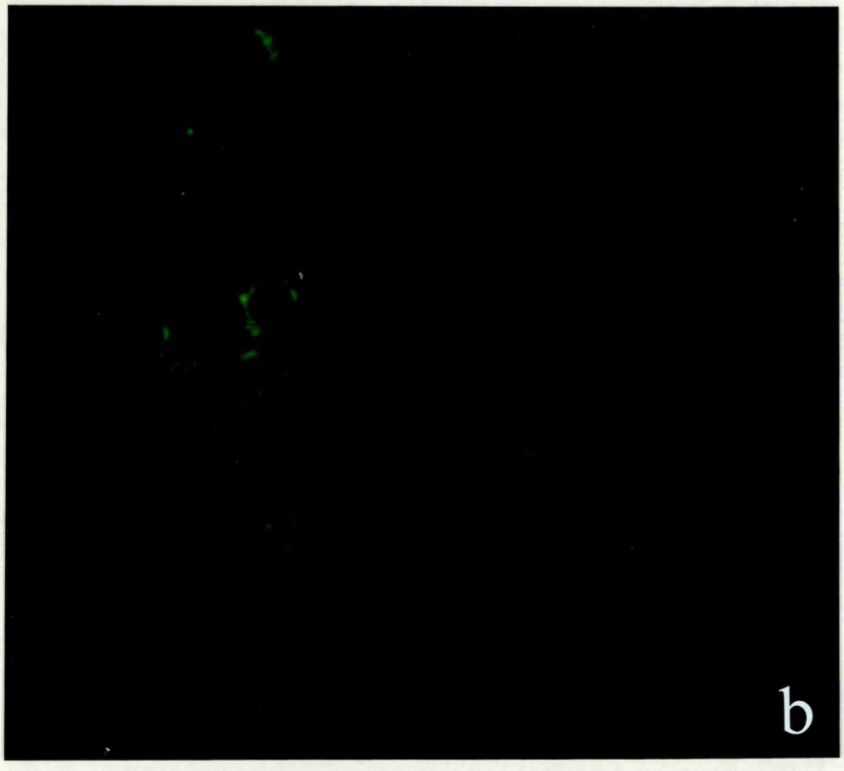
FIG. 3.5 IF on freshly aspirated bone marrow from a viraemic cat.

a. Live cell IF demonstrating the presence of gp70 on the cell surface. The arrow indicates a positive cell.

b. Fixed cell IF demonstrating the presence of p27 in the cytoplasm of cells. Nearly all of the cells are positive.

Magnification x 500


a


b

Evidence for reactivation of FeLV from bone marrow cells from latently infected cats following culture

a) Virus isolation from culture fluids

Samples of cell culture fluid were tested at weekly intervals for the presence of infectious virus. In almost all cases it was found that latent virus was reactivated and could be isolated from the culture fluid within 2 weeks of establishment of the culture. It was extremely unlikely that the bone marrow harboured a latent infection if reactivation had not occurred after 4 weeks in culture. Table 3.2 shows the results of a typical experiment in which bone marrow from the cats in the multicat household described in Chapter 4 were cultured and tested at weekly intervals for the presence of infectious virus. These results were obtained from biopsies taken 88 weeks after the cats were naturally exposed to FeLV (see Chapter 4). Cats 2 and 3 were persistently viraemic since infectious virus could be isolated from their plasma and BMSN, and 9 cats (cats 5, 6, 7, 11, 12, 15, 17, 18 and 19) were believed to have recovered from exposure since infectious virus could not be detected in plasma, BMSN or in cell culture fluids after culture for 4 weeks.

The remaining 6 cats (cats 4, 8, 9, 10, 14 and 16) were believed to be latently infected since infectious virus could not be detected in plasma or BMSN but could be detected after culture of bone marrow. Three of these latter 6 bone marrow cultures contained reactivated virus within 1 week of culture, 2 within 2 weeks, and the sixth within 3 weeks. Both the latently infected and recovered cats had VNAs to FeLV-A in their sera.

b) Fixed cell IF

After a latently infected marrow had been in culture for 6 days group specific antigen could be detected in individual cells by IF. The proportion of antigen-containing cells gradually increased so that by 8 days small foci of infection could be seen

TABLE 3.2

Detection of latently infected bone marrow

			Virus isolation from bone marrow culture[1]								
			Week 1		Week 2		Week 3		Week 4		
Cat No.	V	BMSN	A	B	A	B	A	B	A	B	VNA
2	+	+	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+	+	+	-
4	-	-	+	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-	-	+
6	-	-	-	-	-	-	-	-	-	-	+
7	-	-	-	-	-	-	-	-	-	-	+
8	-	-	+	+	+	+	+	+	+	+	+
9	-	-	+	+	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-	+			+
11	-	-	-	-	-	-	-	-	-	-	+
12	-	-	-	-	-	-	-	-	-	-	+
14	-	-	-	-	+	+	+	+	+	+	+
15	-	-	-		-		-		-		+
16	-	-	-	-	+	+	+	+	+	+	+
17	-	-	-	-	-	-	-	-	-	-	+
18	-	-	-	-	-	-	-	-	-	-	+
19	-	-	-	-	-	-	-	-	-	-	+

[1] A and B are duplicate cultures made from one biopsy.

V = virus isolation from blood

BMSN = virus isolation from bone marrow collecting medium

\+ = virus isolated

\- = virus not isolated.

VNA = presence of virus neutralising antibodies to FeLV-A.

Where no result is given the test was not done.

and by 10 days most of the cells contained antigen. Fig. 3.6 shows IF carried out on a latently infected bone marrow after being cultured for 8 and 10 days.

Proportion of latently infected cells in the bone marrow

Each infectious centre was interpreted as representing one infected cell. In every assay essentially all of the FEA/FeLV-A cells plated registered as infectious centres indicating that the assay was an effective method of detecting virus-producing cells.

The number of infectious centres produced per 1 x 10^6 bone marrow cells plated in the assay are shown in Table 3.3. The proportion of infectious centres produced by latently infected marrow was found to be very low and variable between cats, ranging from 3 to 400 per 1 x 10^6 cells plated (0.0003 to 0.04%). The bone marrows from viraemic cats produced many infectious centres, ranging from 6 x 10^3 to 1.2 x 10^5 per 1 x 10^6 cells plated (0.6 to 12%). Infectious centres were not found in the marrow from the recovered cat. The foci produced by the latently infected bone marrow were the same size as those produced by the marrow of the viraemic cat and by FEA/FeLV-A cells indicating that under these conditions reactivation of latent virus had occurred within a short time.

Fig. 3.7 shows the foci produced in an infectious centre assay by 3 tenfold dilutions of a latently infected bone marrow.

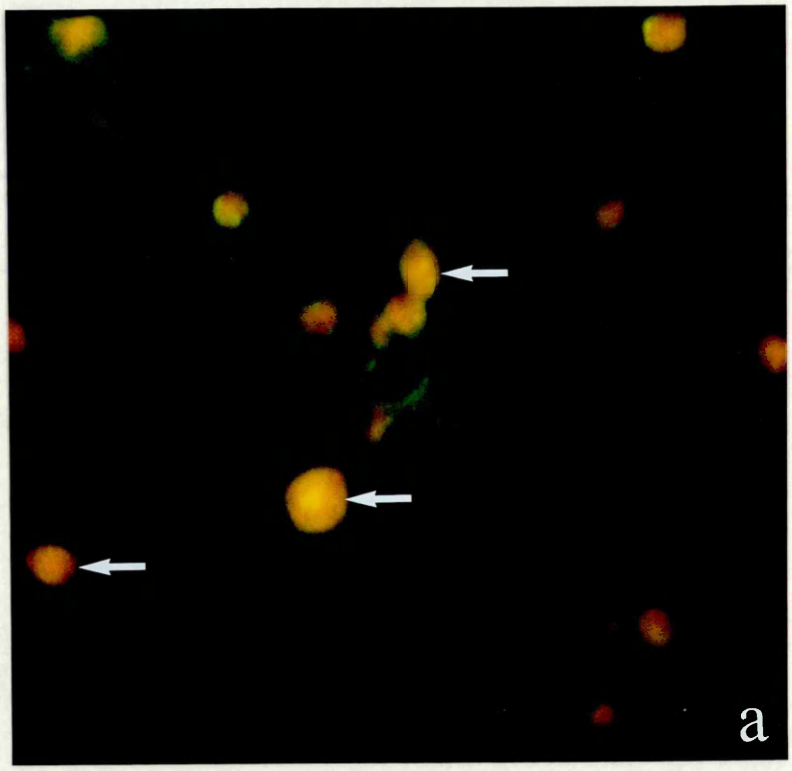
When the infectious centre assay was being carried out the FeLV status of each cat involved was determined by virus isolation from plasma, BMSN and bone marrow culture fluids. Neither the latently infected or recovered cats had virus in plasma or BMSN. Virus was isolated from marrow culture fluids of latently infected cats but not from recovered cats. Plasma, BMSN and marrow culture fluids were all positive for the presence of virus in the viraemic cats.

FIG. 3.6 Fixed cell IF on cultured bone marrow from a latently infected cat.

a. Demonstration of a focal area of FeLV p27 antigen after 8 days in culture. This preparation was counterstained with 0.2% carbol chromotrope in water in order to remove non-specific fluorescent granules from myeloid cells which are arrowed.

b. After 10 days in culture virtually all of the cells contain p27 antigen.

Magnification x 250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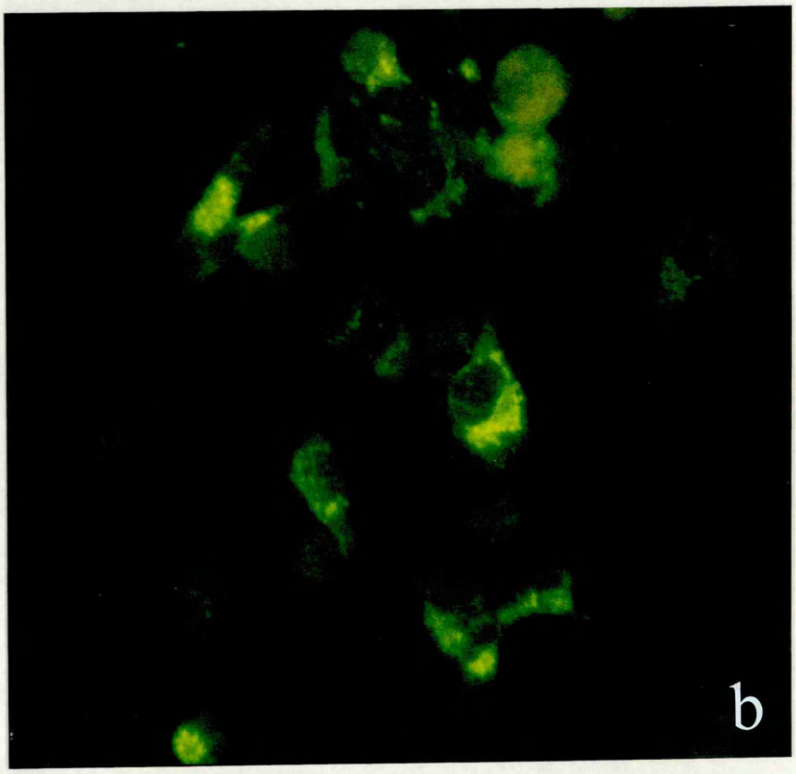

b

TABLE 3.3

Proportion of infected cells in the bone marrow

Cat No.	Status[1]	No. of infectious centres per 10^6 cells
21	L	36
22	L	3
23	L	60
24	L	8
25	V	1.2×10^5
26	V	6×10^3
27	V	1×10^5
28	R	≤ 1
29	L	8
30	L	200
31	L	90
32	L	400

[1] L = latently infected
V = viraemic
R = recovered

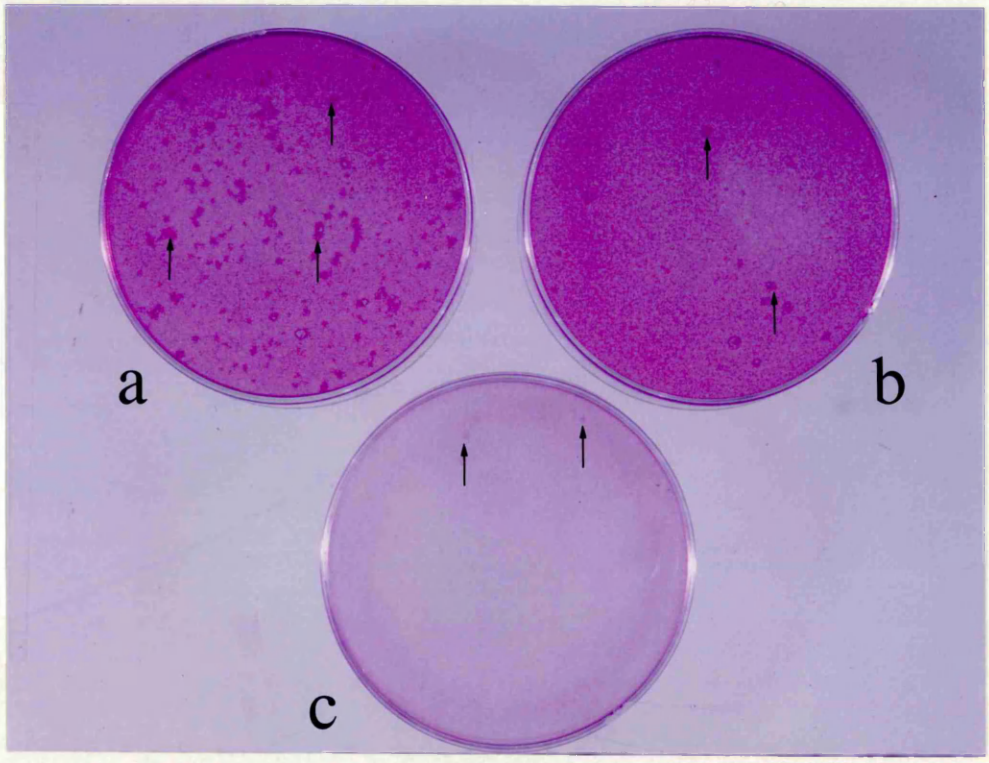
FIG. 3.7 Foci produced in an infectious centre assay by 3 tenfold dilutions of a latently infected bone marrow. Foci are arrowed.

a. 4×10^6 cells plated.

b. 4×10^5 cells plated.

c. 4×10^4 cells plated.

The plates were stained with 1% crystal violet in 10% formalin and 5% methanol.


a
b
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DISCUSSION

In this study the culture of aspirated bone marrow was found to be an efficient and reliable method of identifying latently infected cats. Previously Rojko et al. (1982) and Madewell and Jarrett (1983) had found that infectious FeLV could not be isolated from the BMSN of freshly aspirated marrow, but could be isolated from the culture fluid after cultivation of the marrow. In the present study bone marrow from a total of 26 latently infected cats were thus tested and in none of these was virus isolated from the BMSN. In contrast, virus was isolated from BMSN of all of 8 viraemic cats. Upon culture of the latently infected marrow, virus was reactivated and isolated from the culture fluids of all 26 cats tested. If virus reactivation had not occurred within 4 weeks of culture the cat was assumed to have recovered from infection. In previous studies bone marrow cultures were maintained for up to 12 weeks to find the minimum incubation period required for detecting reactivated virus. No culture was found to subsequently release virus if it were negative 3 weeks after initiation (O. Jarrett, personal communication). Pedersen et al. (1984) found that virus was reactivated within 6 to 87 days of culture. However reactivation occurred after 87 days in only one case and in the majority of cases this occurred within 6 to 21 days (N.C. Pedersen, personal communication). It is possible that the appearance of virus after 87 days was as a result of contamination from other cultures.

The absence of p27 in the cytoplasm or gp70 on the surface of uncultured latently infected cells, as determined by fixed or live cell IF respectively, confirmed that an active FeLV infection was not present. In contrast, virtually all of the nucleated cells in the marrow from viraemic cats contained p27, and between 1 and 10% of these cells were expressing gp70 on their surface.

Fixed cell IF proved to be a rapid method of detecting latency since after only 8 days in culture small foci of cells containing p27 were seen. These cells appeared to be fibroblasts and by 10

days after the onset of culture, when a confluent monolayer had developed, most of the cells contained p27. However, as will be discussed in Chapter 6, IF is a relatively insensitive assay compared to the infectious centre assay described here.

Rojko et al. (1982) also used fixed cell IF to detect p27 in latently infected bone marrow cells after culture. These authors carried out the test on cells which had been resuspended and cytocentrifuged rather than in situ and it is unclear how long the cells had been in culture before examination. However, the antigen was found in 20% of the cells which were identified as proliferating myelomonocytic precursor cells. Antigen was not present in differentiated macrophages or mature granulocytes.

Attempts have been made to reactivate latent virus from sites other than bone marrow (Rojko et al., 1982). Virus could not be reactivated from peripheral blood mononuclear leucocytes or from peritoneal macrophages. The only evidence to suggest that latent FeLV exists in extramedullary sites is that low titres of virus were isolated from lymph node T-cells following stimulation with the T-cell mitogen, staphylococcal Protein A.

An infectious centre assay was used in the present study in an attempt to determine the proportion of cells in the marrow which are latently infected. Each assay was proven to be an effective method of detecting infected cells since almost all of the chronically infected FEA/FeLV-A cells which were known to continuously express virus registered as infectious centres. The results obtained from plating bone marrow cells in these assays indicated that the proportion of cells in the marrow which harbour latent FeLV is very low, ranging from 0.0003% to 0.04%. These figures however may be underestimates since it is possible that not every infected cell will register as an infectious centre. The presence of latent FeLV in marrow from each of these cats was confirmed by virus isolation from marrow culture fluids. The fact that there are so few latently infected cells in the marrow may lead to difficulties in detection, particularly if the cat is

beginning to recover by eliminating its latent infection (see Chapter 4 Discussion).

Infectious centres were not found in the bone marrow from the recovered cat but it is possible that the number of latently infected cells was below the lower limit of detection. This is unlikely, however, since virus was not isolated from bone marrow culture fluids from this cat during 4 weeks of incubation.

In contrast, between 0.6% and 12% of cells in bone marrow from viraemic cats registered as infectious centres. These figures compare well with those found by detection of gp70 on the surface of these cells prior to culture where between 1 and 10% of cells were found to be expressing antigen, and presumably virus (O. Jarrett; personal communication).

Although the infectious centre assay is of limited usefulness unless a relatively high proportion of cells carry a latent infection, it generally correlated well with the other methods used in this study to detect latency and had the great advantage of providing a degree of quantitation of this state.

CHAPTER 4

PREVALENCE AND DURATION OF LATENCY IN A CLOSED MULTICAT HOUSEHOLD

INTRODUCTION

When a cat which is excreting FeLV is introduced into a closed household of susceptible cats the virus rapidly spreads to the other cats. The incidence of persistent viraemia in such households is very high. Jarrett et al. (1978a) found that 40% of these cats had persistent viraemia and this was confirmed in a later study when approximately 30% of the cats in these households were found to be viraemic compared to less than 1% of free range cats (Hardy, 1980b). The proportion of cats in these households which are immune, i.e. have VNAs, is also much greater than in free range cats. In a study carried out by Russell and Jarrett (1978b) 42% of the cats in FeLV-infected multicat households had neutralising antibodies compared to only 4% of free range cats.

There are several reasons for the high incidence of infection in these households: cats are generally exposed to the virus at a much younger age than free range cats; they are exposed to frequent and high doses of the virus, and exposure can be prolonged.

After the discovery of latency (Post and Warren, 1980), it was of interest to investigate the prevalence of this state amongst the ostensibly recovered immune cats in these FeLV-infected multicat households. Such an experimental household was initiated when 4 persistently viraemic cats were housed with 19 16-week-old uninfected kittens (Madewell and Jarrett, 1983). The initial results of this experiment have been published (Madewell and Jarrett, 1983) but will be summarised here, and the events which took place subsequent to this publication will be described more fully.

It is known that recovered cats which have VNAs are resistant to re-infection and that viraemia is life-long, therefore it was of interest to discover the duration of the latent state and if latently infected cats which also have VNAs were resistant to reinfection.

In this chapter the prevalence of latency in the multicat household initiated by Madewell and Jarrett (1983), and the duration of these latent infections is described.

Experimental cats

The cats used in this experiment were derived from a multicat household which had been established previously (Madewell and Jarrett, 1983). Nineteen 16-week-old uninfected kittens (cats 1-19) were housed with 4 6-month-old cats which were excreting FeLV. These latter 4 donor cats became persistently viraemic 3 weeks after receiving an intraperitoneal inoculation of 1.8×10^5 fiu of FeLV-A/Glasgow-1 at 8 weeks of age.

At intervals of 4 months after the onset of continuous exposure to the donor cats blood and bone marrow samples were obtained from cats 1-19 to test for evidence of FeLV infection. Monitoring continued over a period of 3 years. The present author was responsible for this experiment from 64 weeks after exposure to the donor cats. By this time the donor cats had been removed from the house.

RESULTS

Prevalence of latency

Blood and bone marrow samples were taken from the cats at the times indicated in Table 4.1. Plasma samples were tested for the presence of FeLV antigen by ELISA and infectious virus by virus isolation. Serum was tested for VNAs and anti-FOCMA antibody. Bone marrow cultures in duplicate were maintained for 4 weeks and tested for the presence of reactivated virus at weekly intervals as described in Chapter 3. The results are given in Table 4.1.

Thirty-six weeks after initial exposure to the 4 donor cats, the 19 cats could be divided into 3 distinct groups.

The cats in the first group (cats 1-3) were persistently viraemic. FeLV antigen was demonstrated in their plasma by ELISA and infectious virus was isolated. Infectious virus was also detected in the bone marrow collecting medium prior to culture, and in the medium after culture. These cats did not develop VNAs although cat 2 had low levels of anti-FOCMA antibodies.

In the second group (cats 4-12) infectious virus was not isolated from plasma although cat 4 had detectable antigen. Bone marrow collecting medium from these cats did not contain infectious virus, but virus was detected in the medium after the marrow had been in culture. These cats were said to be latently infected. All had VNAs and anti-FOCMA antibodies.

The third group consisted of cats 13-19. Neither viral antigen nor infectious virus was demonstrated in their plasma. Infectious virus was not detected in bone marrow collecting medium, or in medium after the marrow was cultured. Like the second group of cats, these cats all had VNAs and anti-FOCMA antibodies and were therefore believed to have recovered from infection.

TABLE 4.1

Latency in cats following contact exposure to FeLV

Cat	Weeks after exposure																													Cat
	36*					64					88					110					138					160					
No.	E	V	B	VNA	F	E	V	B	VNA	F	E	V	B	VNA	F	E	V	B	VNA	F	E	V	B	VNA	F	E	V	B	VNA	F	No.
1	+	+	+	0	0	+	+	+	0	0	Dead																			1
2	+	+	+	0	16	+	+	+	0	2	+	+	+	0	0	+	+	+	0	2						+	+	+	0		2
3	+	+	+	0	0	+	+	+	0	2	+	+	+	0	0	+	+	+	0	2						+	+	+	0		3
4	+	−	+	8	64	−	−	+	8	8	−	−	+	2	4	−	−	+	8	8	+	−	+	4	16	+	+	+	0		4
5	−	−	+	32	256	−	−	−	32	32	−	−	−	32	64	−	−	+	64	64	−	−	+	16	128	−	−	−	32	16	5
6	−	−	+	64	32	−	−	−	16	32	−	−	−	64	64	−	−	−	64	64	−	−	−	8	32	−	−	−	16	32	6
7	−	−	+	128	64	−	−	−	128	32	−	−	−	64	16	−	−	−	64	128	−	−	−	16	64	−	−	−	0	128	7
8	−	−	+	64	128	−	−	+	16	32	−	−	+	64	16	−	−	+	64	32	−		−	16	256	−	−	+	64	64	8
9	−	−	+	8	32	−	−	+	32	64	−	−	+	64	64	−	−	+	64	64	Dead										9
10	−	−	+	32	8	−	−	−	16	16	−	−	+	64	8	−	−	−	32	32	−	−	−	8	64	−	−	−	32	32	10
11	−	−	+	64	16	−	−	−	32	64	−	−	−	64	128	−	−	−	64	128	−	−	−	16	256	−	−	−	64	32	11
12	−	−	+	128	128	−	−	+	32	128	−	−	−	64	64	−	−	−	64	128	−	−	−	8	64	−	−	−	64	32	12
13	−	−	−	64	16	−	−	−	32	16	Dead																			13
14	−	−		8	16	−	−	−	8	8	−	−	+	16	4	−	−	−	16	64	−		−	8	16	−	−	−	16	8	14
15	−	−	−	8	8	−	−	−	4	4	−	−	−	8	8	−	−	−	2	16	−	−	−	4	16	−	−	−	2	8	15
16	−	−	−	64	4	−	−	−	64	32	−	−	+	64	8	−	−		64	64	−		−	8	256			−	64	64	16
17	−	−	−	32	16	−	−	−	64	8	−	−	−	64	16	−	−	−	64	32	−	−	−	16	32	−	−	−	16	64	17
18	−	−	−	16	32	−	−	−	16	32	−	−	−	32	32	−	−	−	64	32	−	−	−	16	64			−	32	16	18
19	−	−	−	2	2	−	−	−	8	8	−	−	−	2	2	−	−	−	4	32	−	−	−	2	4	−	−	−	4	2	19

E = ELISA antigen in blood.
V = Virus isolation from blood
B = Virus isolation from bone marrow culture
\+ = Virus isolated
− = Virus not isolated
VNA = Virus neutralising antibody titre
F = Anti-FOCMA antibody titre
Where no result is given the test was not done.
* Results from Madewell and Jarrett (1983).

The blood samples taken at 64, 88 and 110 weeks after initial exposure showed that the VNA and anti-FOCMA antibody titres for each cat remained very stable. At around 110 weeks several of the latently infected and recovered cats were rehoused for the purposes of breeding in order to determine whether or not any transmission of FeLV occurred between latently infected cats and their kittens. Details of this experiment are given in Chapter 5. The viraemic cats (cats 2 and 3) were also removed from the multicat household at this time and rehoused together. During the interval between rehousing and the next sampling at 138 weeks there was a fourfold decrease in the cats' VNA titres. Serum samples taken at 160 weeks after exposure showed a slight increase in VNA titres.

Duration of latency

The proportion of cats which were not viraemic, i.e. were ostensibly recovered, but which had latent infections in their marrow was initially high (56% at 36 weeks after infection). It was found, however, that with time this proportion decreased with the exception of the 88 week sampling when there was a transient increase in the proportion of cats with latent infections. Those cats which no longer had detectable virus in their marrow had recovered from infection. After 160 weeks only 1 of the non-viraemic cats (cat 8) had a latent infection (8%). This cat was still latently infected after 210 weeks. The figures are shown in Table 4.2 and are given as the percentage of the non-viraemic cats still alive at each sampling which had latent infections at the times indicated.

These results show that the latent state is relatively short-lived with few infections lasting longer than 2.5 years.

Cat 4, which was one of the latently infected queens used in the breeding experiments mentioned above, was intermittently positive by ELISA for the presence of FeLV antigen. At 160 weeks she reactivated her latent infection and became persistently viraemic. At this time her VNA levels, which had always been low, had dropped to 0. This cat will be discussed in detail in Chapter 5.

TABLE 4.2

Proportion of cats with latent infections at intervals following exposure

Weeks after exposure	Cats with latent infection	
	Proportion[1]	Percent
0	0/19	0
36	9/16	56
64	4/16	25
88	6/15	40
110	4/15	27
138	2/14	14
160	1/13	8
210	1/13	8

[1] The proportion of non-viraemic cats still alive at each sampling which had latent infections.

Three of the cats died during the course of the experiment. Cat 1 was persistently viraemic and developed ulcerative colitis and died between 64 and 88 weeks after exposure. Cat 9 was latently infected and died between 110 and 138 weeks after exposure as a result of a perforated pyometra. The third cat (cat 13) was recovered and died as a result of a blocked urethra between 64 and 88 weeks after exposure.

DISCUSSION

The cats in the multicat household described here were infected by the horizontal transmission of virus as occurs in the field.

Thirty-six weeks after exposure to the donor cats 3 out of the 19 cats were persistently viraemic. This represents a very low proportion, only 15%, compared to that observed in other multicat households in the field which have been studied where the proportion was 30-40% (Jarrett et al., 1978a; Hardy, 1980b). A probable reason for the low incidence of viraemia in the present study is that the cats were 16 weeks old when first exposed to the virus and had therefore developed an age resistance to the infection (Hoover et al., 1976), compared to the situation in the field where kittens are born into these households and exposed to the virus at a very early age.

The 3 viraemic cats did not make VNAs therefore did not have a protective immune response. However, anti-FOCMA antibodies which are believed by some authors to protect against tumour development (Essex et al., 1971a) were detected in two of these cats. The 16 remaining cats in the house had all been infected with the virus by 36 weeks from initial exposure since they all had anti-FOCMA antibodies. These cats were also immune as determined by the presence of VNAs in their sera.

At this time virus could be reactivated from the bone marrow of 9 of the 16 cats (56%). These 9 cats were therefore assumed to have latent infections. The remaining 7 cats were believed to have recovered since virus could not be isolated from their cultured marrow. As time went on, the proportion of latently infected cats decreased, with the greatest decrease occurring between 36 and 64 weeks after exposure. Pedersen et al. (1984) also found that the proportion of cats with latent infections decreased with time but in this latter study the greatest decrease occurred after 28

weeks. In the present study after 160 weeks only 8% of the immune cats still had latent infections.

Those cats which no longer had reactivatable virus in their marrow were thought to have recovered. The fact that there was an increase in the proportion of latent infections at 88 weeks after exposure may be indicative of a sampling error on the previous occasion because, as discussed in Chapter 3, the use of the infectious centre assay showed that very few cells in the marrow contain latent virus. It may therefore be possible to obtain an aspirate which does not contain any infected cells.

The factors responsible for eliminating a latent infection resulting in recovery are as yet unknown. Rojko et al. (1982) found that latent virus was present in immature but not differentiated myeloid cells. It is possible, therefore, that latency ends when infected precursor cells have differentiated to maturity. The more primitive the infected cells are and the greater their numbers, the longer the latent infection might last. If this were the case, then one possibility is that in those cats which have a long-term latent infection the virus is present either in myeloid stem cells or in longer-lived cells such as lymphocytes.

It is also possible that an immune mechanism is responsible for eliminating latent infections. For instance, neutralising antibody, which is present in all latently infected cats, may kill any virus which is expressed or may kill those cells which express it. Similarly, cytotoxic T-cells may have a role. Latency may therefore end when all of these cells have been killed. The possible factors which control latency are discussed in Chapter 6.

In the present study all of the latently infected and recovered cats were resistant to reinfection from the viraemic cats. Their antibody levels remained stable throughout the experiment with the exception of a fourfold decrease in their VNA titres observed between 110 and 138 weeks. This may be due to the removal of the viraemic cats and therefore the removal of the

challenge at this time. The VNA titres tended to increase again at 160 weeks and so the variation observed could merely indicate individual assay variation.

The only exception was cat 4 which spontaneously reactivated her latent infection to become persistently viraemic. This cat had been intermittently antigenaemic, had had very low levels of VNAs and may always have been on the verge of viraemia. She became viraemic after being exposed to several litters of viraemic kittens and she will be discussed in more detail in Chapter 5.

In summary, the prevalence of latent FeLV infections in multicat households can be very high following the introduction of viraemic cats. In view of these results it is likely that a large proportion of the cats in the extensive study carried out by McClelland et al. (1980) had latent infections. However, latency is usually short-lived with approximately 10% of the infections lasting more than 3 years, and the cats are generally immune and resistant to reinfection upon continuing exposure to the virus.

CHAPTER 5

CLINICAL AND EPIDEMIOLOGICAL SIGNIFICANCE OF LATENCY

INTRODUCTION

Cats which are persistently viraemic with FeLV are at risk of developing a number of diseases associated with the virus (see Chapter 1, Section 2). In a prospective study of cats exposed to FeLV McClelland et al. (1980) found that 83% of the viraemic cats in multicat households were dead within 3.5 years compared to only 17% of cats in uninfected households. Cats which had been exposed to the virus but which had developed a protective antibody response, and were therefore immune, were no more at risk of developing FeLV-associated disease than unexposed cats.

As discussed in the previous Chapter, a considerable proportion of the non-viraemic cats in a FeLV-infected multicat household may harbour latent infections. In this Chapter the results of experiments to assess the clinical and epidemiological significance of latency are described. Initially the following four questions were posed. First, are latently infected cats susceptible to any of the FeLV-associated diseases? Secondly, can these cats spontaneously reactivate virus to become viraemic? Thirdly, is there horizontal transmission of virus from latently infected to susceptible cats? Fourthly, do latently infected queens transmit infection to their kittens, either before or after birth?

Horizontal transmission

Eight 4-month-old uninfected kittens (cats 33-40) were used as tracer cats and were housed together with 8 latently infected cats (cats 41-48) to determine whether or not FeLV was horizontally transmitted from latently infected cats.

a) Source of latently infected cats

The latently infected cats developed their infections in a variety of ways as summarised in Table 2.1. Cats 41 and 42 became infected after receiving an oronasal dose of 1×10^5 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium at 16 weeks of age. Cats 43, 44 and 45 were inoculated with an experimental FeLV vaccine and challenged as described in Chapter 3. These cats resisted viraemia but became latently infected. Cats 46, 47 and 48 were unvaccinated control cats which had been challenged oronasally with 1×10^6 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium at 14 weeks of age.

Congenital transmission

a) Source of cats

The latently infected and recovered cats in the multicat household described in Chapter 4 were studied for the possible transmission of infection to their kittens either before or after birth. In addition, a tom cat (cat 20) which became latently infected after receiving an intraperitoneal dose of 8×10^4 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium at 9 weeks of age, was used.

b) Collection of bone marrow from newborn kittens

Newborn kittens were anaesthetised by enclosing them in a small perspex box containing a pad of cotton wool which had been

soaked in halothane (Fluothane, ICI). The fur was clipped and the gluteal area was disinfected as described in Chapter 3. Femoral bone marrow aspirates were taken using a 21 gauge needle and a 5 ml syringe. The marrow was taken into collection medium and cultured as described in Chapter 3.

c) Collection of milk

Mild sedation of the cat was achieved by giving 10 mg/kg ketamine hydrochloride by intramuscular injection. A volume of 0.3 ml of oxytocin-S (Intervet) was also given intramuscularly to aid the collection of milk. The fur around the mammary glands was closely clipped and the skin was disinfected with 0.5% Hibitane and allowed to dry. Milk was then manually expressed and approximately 1 ml was collected into a sterile 10 ml plastic centrifuge tube.

d) Preparation of milk for virus isolation

The milk was diluted to 3 ml in Leibovitz L-15 medium and was centrifuged at 2000 rpm for 10 minutes to pellet the cells. The supernatant was then collected and passed through a 0.45 μm filter and was stored at -70°C in aliquots of 1 ml prior to being tested for the presence of FeLV.

Clinical significance of latency

The incidence of ill-health in cats which had had latent FeLV infections for up to 4 years was very low. Table 5.1 lists all of the latently infected cats which were studied, giving the duration of study and medical history. Only one cat in the multicat household, cat 9 became ill. She developed a pyometra and subsequently died.

Epidemiological significance of latency

a) Horizontal transmission

Blood and bone marrow samples were taken from the tracer cats before the start of the experiment. The cats were all free from antigenaemia, viraemia and latent infection and none of those which were tested had VNAs. Serum samples taken from the latently infected cats prior to the start of the experiment were positive for the presence of VNAs.

At intervals of 14 to 16 weeks blood and bone marrow samples were taken from all 16 cats. Plasma was tested for the presence of FeLV antigen and infectious virus, and bone marrow was cultured in order to detect latent infections. Serum was tested for the presence of VNAs. The results are given in Table 5.2.

None of the tracer cats developed latent or active infection with FeLV and none developed neutralising antibodies to the virus.

Cats 41-48 had detectable VNAs throughout the experiment. However at the 54 week sampling 2 of these cats (cats 44 and 48) had eliminated their latent infections.

TABLE 5.1

Medical history of latently infected cats

Cat No.	Duration of study (months)	Medical history
4	36	Healthy
5	36	"
6	36	"
7	36	"
8	48	"
9	24	Pyometra: died
10	36	Healthy
11	36	"
12	36	"
20	36	"
21	9	"
22	9	"
23	9	"
24	9	"
29	24	"
30	24	"
31	24	"
32	24	"
41	18	"
42	18	"
43	18	"
44	18	"
45	18	"
46	18	"
47	18	"
48	18	"

TABLE 5.2

Attempts to demonstrate horizontal transmission of FeLV from cats with latent infections

Cat	Pre-contact				Weeks after exposure: 14				30				43				54			
No.	E	V	B	VNA	E	V	B	VNA	E	V	B	VNA	E	V	B	VNA	E	V	B	VNA
Tracer cats																				
33	–	–	–		–	–	–	0	–	–	–	0	–		–	0	–	–	–	0
34	–	–	–	0	–	–	–	0	–		–	0	–	–	–	0	–	–	–	0
35	–	–	–		–	–	–	0	–	–	–	0	–	–	–	0	–	–	–	0
36	–	–	–	0	–	–	–	0	–	–	–	0	–	–	–	0	–	–	–	0
37	–	–	–		–	–	–	0	–		–	0	–	–	–	0	–	–	–	0
38	–	–	–		–	–	–	0	–		–	0	–	–	–	0	–	–	–	0
39	–	–	–	0	–	–	–	0	–		–	0	–	–	–	0	–	–	–	0
40	–	–	–		–	–	–	0	–		–	0	–	–	–	0	–	–	–	0
Latently infected cats																				
41	–	–	+	64	–	–	+	64	–	–	+	32	–		+	512	–	–	+	32
42	–	–	+	128	–	–	+	64	–		+	64	–	–	+	128	–	–	+	32
43	–	–	+		–	–	+	64	tr	–	+	32	tr	–	+	512	tr	–	+	32
44	–	–	+	2048	–	–	+	1024	–	–	+	64	–	–	+	2048	–	–	–	512
45	–	–	+	2048	–	–	+	1024	–		+	128	–	–	+	128	–	–	+	512
46	–	–	+	4	–	–	+	32	–		+	64	–	–	+	512	–	–	+	1024
47	–	–	+	256	–	–	+	8	–	–	+	4	–		+	16	–	–	+	8
48	–	–	+	128	–	–	+	32	–	–		8	–	–	+	2	–	–	–	8

E = ELISA antigen in blood.
V = Virus isolation from blood
B = Virus isolation from bone marrow culture
\+ = Virus isolated
– = Virus not isolated
VNA = Virus neutralising antibody titre
tr = trace of colour in ELISA
Where no result is given the test was not done

b) Congenital transmission

i) First litters

Eight queens in the multicat household detailed in Chapter 4 gave birth to their first litters between 64 and 88 weeks after initial exposure to FeLV. Of these, 5 had latent infections at that time (cats 4, 8, 10, 14 and 16) and 3 had recovered from infection (cats 12, 18 and 19). At parturition blood samples were taken from the mothers to test for the presence of viral antigen and infectious virus, and two kittens from each litter were sacrificed. Blood samples from these kittens were also tested for the presence of viral antigen and infectious virus and their bone marrow was cultured to determine whether or not congenital latent infections were present. None of the mothers or kittens tested had antigen or virus in their plasma. The kittens were therefore believed to be free from congenital active infection.

The bone marrow cultures from these kittens were grown for at least 4 weeks, with the exception of those from the kittens of cat 4 which became contaminated with bacteria at an early stage. All of the cultures were tested at weekly intervals for the presence of reactivated virus and were found to be negative.

Blood samples which were taken from the remaining kittens of each litter at 12 weeks of age showed that one of the kittens of cat 4 became viraemic.

ii) Second litters

In the light of these results cat 4 and another latently infected queen (cat 8) which was chosen at random from the other queens, were removed from the multicat household and rehoused together with 3 tom cats. Two of these toms, cats 5 and 6, were from the multicat household and had recovered from infection and the third, cat 20, was latently infected.

Four kittens were born to each of the queens and they were reared together in the presence of the toms. The first blood and bone marrow samples were not taken until the kittens of cat 4 (4B1 - 4B4) were 15 weeks of age and those of cat 8 (8B1 - 8B4) were 18 weeks of age. It is therefore not known if congenital active or latent infections were present in these kittens.

By the time of first sampling 4 kittens (4B1, 4B2, 4B3 and 8B1) were viraemic and one (8B2) was antigenaemic. Two of the kittens (4B4 and 8B2) had virus in their bone marrow but not in their blood. The results are shown in Table 5.3. Blood samples were taken from the kittens over the following 5-6 weeks. There was little change in their virus status. Kittens 4B4 and 8B2 remained free from viraemia with 8B2 eliminating its antigenaemia; kitten 8B4 remained completely free from antigenaemia and viraemia. At 22 weeks of age kitten 8B3 who had been free from infection until that time became antigenaemic.

Serum samples which were taken at each bleeding were tested for the presence of VNAs and anti-FOCMA antibodies. The results are shown in Table 5.3. The only kittens to develop VNAs were 4B4 and 8B2, and all eventually developed anti-FOCMA antibodies.

Blood samples were taken at regular intervals from the adults and at no time was viraemia detected in any of them. Cat 4, however, was intermittently antigenaemic. At the time of last bleeding oropharyngeal swabs were taken. None of the adults had infectious virus in the saliva. VNA and anti-FOCMA antibody titres remained constant over the course of the experiment. The results are given in Table 5.3.

iii) Third litters

Approximately one week prior to kittening cats 4 and 8 were removed from the tom cats and rehoused in individual huts so that their litters could be reared in isolation from each other. Within the first seven days of life blood and bone marrow samples were

TABLE 5.3 Transmission of FeLV to kittens of queens with latent infections

	Age of kittens in weeks																				
Cat	15					18					20				22						
No.	E	V	B	VNA	F	E	V	B	VNA	F	E	V	VNA	F	E	V	B	VNA	F	M	
4B1	+	+	+	0	32	+	+		0	8	+	+	0	8							
4B2	+	+	+	0	32	+	+		0	16	+	+	0	128							
4B3	+	+	+	0	16	+	+		0	0	+	+	0	4							
4B4	-	-	+	32	32	-	-		8	16	-	-	16	128							
8B1						+	+	+		16	+	+	0	256	+	+		0	128		
8B2						+	-	+		64	-	-	16	256	-	-		8	128		
8B3						-	-	-		0	-	-	0	0	+	-		0	64		
8B4						-	-	-		0	-	-	0	0	-	-		0	16		
4						+	-		4	4	tr	-	4	2	-	-	+	4	16	-	
8						-	-		>64	16	-	-	32	32	-	-	+	32	32	-	
5						-	-		>64	32	-	-	32	64	-	-	-	16	16	-	
6						-	-		>64	16	-	-	32	32	-	-	-	32	32	-	
20						-	-		64	16	-	-	32	32	-	-	+	16	32	-	

E = ELISA antigen in blood.
V = Virus isolation from blood
B = Virus isolation from bone marrow culture
\+ = Virus isolated
\- = Virus not isolated
VNA = Virus neutralising antibody titre
F = Anti-FOCMA antibody titre
M = Virus isolated from mouth
tr = trace of colour on ELISA
Where no result is given the test was not done

taken from the kittens. None of the kittens had antigenaemia, viraemia or latent infection at that time but VNAs and anti-FOCMA antibodies were detected in their blood. Blood samples and oropharyngeal swabs were taken from the kittens twice weekly and from the queens once weekly. Second bone marrow biopsies were taken when the kittens were 7 weeks old. The results are shown in Table 5.4.

By 8 weeks of age 2 of 3 of the kittens of cat 4 (4C2 and 4C3) had developed antigenaemia and viraemia and the third (4C1) had detectable virus in its bone marrow. At this time VNAs could no longer be detected in the serum of these kittens although they still had anti-FOCMA antibodies. Two weeks later kitten 4C1 became viraemic. Infectious virus could not, however, be isolated from the saliva of these kittens. Although cat 4 was again intermittently antigenaemic she was never viraemic and virus could not be isolated from her saliva.

The kittens of cat 8 remained completely free from antigenaemia, viraemia, and latent infection. VNAs could be detected in their sera until they were 8 weeks old and anti-FOCMA antibodies were detected throughout the experiment.

iv) Fourth litter

An uninfected tom cat (cat 49) was housed with cat 4 and sired her fourth litter. He remained in the house during her subsequent pregnancy and lactation. Blood samples were obtained from the kittens (4D1 - 4D3) prior to suckling and they were all found to be negative for viral antigen and infectious virus. A 12-day-old kitten (4D4) from an uninfected queen (cat 60) was fostered on cat 4 when this litter was born. Blood samples were taken weekly and oropharyngeal swabs were taken on alternate days from mother and kittens. Bone marrow biopsies were obtained when kittens 4D1, 4D2 and 4D3 were 7 days and 45 days old.

TABLE 5.4

Transmission of FeLV to isolated litters of queens with latent infections

Age of kittens (weeks)

Cat	Pre-kittening						0			4					8						12				
No.	E	V	B	VNA	F	M	E	V	B	E	V	VNA	F	M	E	V	B	VNA	F	M	E	V	VNA	F	M
4	-	-	+	4	16	-				+	-	8	32	-	+	-		4	32	-					
4C1								-	-	-	-	4	8	-	-	-	+	0	8	-	+	+	0	256	-
4C2								-	-	-	-	8	8	-	+	+	+	0	32	-	+	+	0	4	-
4C3								-	-	-	-	8	8	-	+	+	+	0	4	-	+	+	0	32	-
8	-	-	+	64	32	-	-	-		-	-	16	64	-	-	-		32	64	-	-	-	32	64	-
8C1							-	-	-	-	-	16	8	-	-	-	-	4	8	-	-	-	0	4	-
8C2							-	-	-	-	-	2	4	-	-	-	-	0	4	-	-	-	0	8	-
8C3								-	-	-	-	4	8	-	-	-	-	2	8	-	-	-	0	32	-
8C4							-	-	-	-	-	4	16	-	-	-	-	8	8	-	-	-	0	4	-

E = ELISA antigen in blood.
V = Virus isolation from blood
B = Virus isolation from bone marrow culture
\+ = Virus isolated
\- = Virus not isolated
VNA = Virus neutralising antibody titre
F = Anti-FOCMA antibody titre
M = Virus isolated from mouth
Where no result is given the test was not done.

The results from sampling this litter are summarised in Table 5.5. There was no evidence of congenital active or latent infection in any of the kittens. At one week of age the kittens of cat 4 had detectable VNAs and anti-FOCMA antibodies whereas her foster kitten had not. This latter kitten developed viraemia within 3 weeks of fostering, but kittens 4D1 - 4D3 did not become viraemic until 6 weeks of age. By this time kittens, 4D1, 4D2 and 4D3 had no detectable VNAs, and kitten 4D4 had developed anti-FOCMA antibodies. FeLV was isolated from the saliva of the viraemic kittens.

Infectious virus was not detected in the blood or saliva of cat 4 at any time. Two milk samples were taken from her when her kittens were 42 and 52 days old. Infectious virus was isolated from both of these.

After the kittens were removed from the house, blood samples and oropharyngeal swabs were taken regularly from cat 4. It was found that cat 4 spontaneously reactivated her latent infection to become persistently viraemic 11 weeks after kittening.

TABLE 5.5

Transmission of FeLV from latently infected queen to a fostered kitten

Cat	Age of kittens (weeks)																								
	0		1						3					6							8				
No.	E	V	E	V	B	VNA	F	M	E	V	VNA	F	M	E	V	B	VNA	F	M	E	V	VNA	F	M
4	–	–						–					–	+	–		2	16	–	+	–	2	32	–
4D1	–	–	–	–	–	4	8	–	–		2	0	–	+	+	+	0	0	+	+	+	0	64	+
4D2	–	–	–	–	–	4	16	–	–		0	4	–	+	+	+	0	4	–	+	+	0	128	
4D3	–	–	–	–	–	2	16	–	–			2	–	+	+	+	0	4	+	+	+	0	128	+
4D4			–	–	–	0	0	–	+	+	0	0	+	+	+	+	0	8	+	+	+	0	128	+

E = ELISA antigen in blood.
V = Virus isolation from blood
B = Virus isolation from bone marrow culture
\+ = Virus isolated
– = Virus not isolated
VNA = Virus neutralising antibody titre
F = Anti-FOCMA antibody titre
M = Virus isolated from mouth
Where no result is given the test was not done.

DISCUSSION

In this study 26 latently infected cats were studied for periods ranging from 9 months to 4 years. None developed any of the diseases commonly associated with FeLV, although one, cat 9, died of an unrelated cause. This figure represents only 4% and is in sharp contrast to the situation with persistently viraemic cats, where 83% were dead within 3.5 years of natural exposure to infectious FeLV (McClelland et al., 1980). From the present results it is clear that a significant proportion of the cats in that study which were believed to have "recovered" from infection with FeLV must have had latent infections, but only 50 out of 321 (16%) died during the 3.5 year period. Similarly, Pedersen et al. (1984) found that only 12% of FeLV "recovered" cats developed disease over periods ranging from 6 months to 6 years. Again, a proportion of these cats presumably had latent infections. Cats with latent FeLV infections therefore do not appear to be at risk of developing FeLV-associated diseases.

Since there is epidemiological evidence that some of the non-viraemic cats with lymphosarcoma have had previous exposure to FeLV (Hardy et al., 1980), the possibility of latent FeLV infection being involved in such cases has been considered. Rojko et al. (1982) found that latent virus was reactivated from cultured bone marrow cells, but not from blood or tumour cells, from 2 FeLV-negative lymphosarcoma cases. This is in contrast to the results of Madewell and Jarrett (1983) who were unable to demonstrate latent FeLV in bone marrow cells of 2 FeLV-negative cats with lymphosarcoma or myeloid leukaemia. The possible role of latent FeLV in virus-negative lymphosarcomas is therefore unresolved.

Cats which are persistently viraemic with FeLV transmit the infection horizontally to susceptible cats through virus-containing saliva (Hardy et al., 1973a). The possibility of horizontal transmission of FeLV from latently infected cats was studied in an

experiment in which susceptible tracer cats were housed together with cats known to have latent infections.

During one year of exposure there was no evidence of horizontal transmission of virus from the latently infected to the tracer cats. None of the tracer cats developed active or latent infections or VNAs. All of the latently infected cats maintained VNA titres and none spontaneously reactivated its infection to become viraemic. Indeed, by the end of the experiment only 6 of these cats were still latently infected, the other 2 (cats 44 and 48) having eliminated their infections. This is in contrast to the situation with viraemic cats where there is evidence of horizontal transmission to susceptible cats within one month of exposure (Jarrett et al., 1973b).

From the results of this study it appears that, unlike persistently viraemic cats, latently infected cats do not horizontally transmit FeLV infection. This conclusion agrees with the preliminary findings of Madewell and Jarrett (1983) that there was no transmission of infection from one latently infected cat (cat 20 in the present study) to 6 susceptible kittens during 8 months of observation.

Although the tracer cats used in this experiment were not as susceptible as kittens under 8 weeks of age to developing persistent viraemia, they would, however, be susceptible to infection and would therefore be expected to seroconvert and become immune if transmission of infection had occurred from the latently infected cats. In order to find whether these cats would develop VNAs when challenged with the virus, they have now been housed with three persistently viraemic cats. This experiment is in progress and will not be included in this thesis.

The other major mode of transmission of FeLV is by the infection in utero of foetuses of persistently viraemic queens. All kittens born to such queens are themselves persistently viraemic (Jarrett and Russell, 1978). Eight latently infected

queens were therefore studied for the possible transmission of infection to their kittens either before or after birth.

From the results of the study of the first litters it was clear that none of the kittens which were sacrificed at birth had acquired a congenital viraemia. Although it is impossible to determine whether or not the kittens of cat 4 were harbouring congenital latent infections, it is known by the absence of reactivated virus in the bone marrow cultures from the sacrificed kittens of other litters that they were all free from congenital latent infection. By 12 weeks of age one of the remaining kittens of cat 4 was persistently viraemic although the source of its infection was not established.

The second litters from cats 4 and 8 confirmed the transmission of virus to the kittens of both litters since by the time of first sampling 4 of the 8 kittens were viraemic and another 2 were latently infected. The source of the infection was still unidentified and it is unknown whether or not congenital infections had occurred.

As expected, none of the viraemic kittens had VNAs while the 2 latently infected kittens had antibodies. It is likely that these antibodies were actively acquired after exposure to the virus since passively acquired colostral antibody which has a half-life of 9.5 to 15 days (Scott et al., 1970; Jarrett et al., 1977) would have disappeared by that time. Interestingly, an antibody response was not detected in kittens 8B3 or 8B4 until 23 weeks of age when they were found to have anti-FOCMA antibodies indicating exposure to FeLV. It is possible that the comparatively late exposure of these kittens to the virus was due to a lack of socialising since intimate contact between cats is necessary for the transmission of infection (Hoover et al., 1977a).

Although infectious virus was not detected in the blood or saliva of any of the adults, cat 4 was found to be intermittently antigenaemic. Therefore it seemed likely that this cat was the source of the infection.

The results of studying the third litters of cats 4 and 8, which were reared in isolation from each other, showed that congenital infection was not present in any of the kittens and confirmed the belief that cat 4 was the source of infection since her kittens became viraemic between 7 and 8 weeks after birth. By this time colostral antibodies could not be detected. The fact that virus was not isolated from the saliva of these kittens possibly represents a sampling error. The kittens of cat 8, however, remained free from active or latent infection even after their colostral VNA levels had dropped to undetectable levels. This result suggested that in the previous litter, these kittens had been infected by horizontal spread of virus from the kittens of cat 4.

Again, cat 4 had not been viraemic but had been antigenaemic and it appeared that she infected her kittens post-natally although it was still unknown how the infection occurred.

It was believed that passively acquired colostral VNAs protected the kittens of cat 4 from viraemia at an earlier age. Therefore when her next litter, which was sired by an uninfected tom cat (cat 49), was born, a 12-day-old normal kitten (4D4) was fostered on her. Since this kitten had no passively acquired FeLV antibodies from its own mother, and by 12 days of age would no longer be capable of absorbing antibody from the colostrum of cat 4 (Brambell, 1970), it would be fully susceptible to FeLV. In fact this kitten became viraemic within 3 weeks of fostering while cat 4's own kittens (4D1-4D3) did not become viraemic until 6 weeks of age when again the occurrence of viraemia coincided with the disappearance of passively acquired VNAs from the sera of the kittens. Thus the protective effect of these antibodies in the first few weeks of life was confirmed.

It may be significant that cat 4 differed from the other latently infected cats in the study in two ways: firstly she was intermittently antigenaemic, and secondly, she had low levels of VNAs. These may be predisposing factors for the transmission of infection from latently infected cats.

The source of the infection was found to be the milk of cat 4 since infectious virus was isolated from 2 samples taken at 42 and 52 days post-partum. Although FeLV has previously been demonstrated in the milk of viraemic cats (Hardy et al., 1976a) this is the first demonstration of virus in the milk of a latently infected cat. It is possible therefore that cats which are antigenaemic and not detectably viraemic but which have latent infections actually produce infectious virus at extramedullary sites such as the mammary gland. Approximately 10% of cats which are antigenaemic are not viraemic (Jarrett et al., 1982) and it is possible that some of these have latent infections. Cats like these could complicate the control and eradication of FeLV from multicat households and could explain the reappearance of viraemic cats which has been reported in such households after long periods without overt infection (Rojko et al., 1982; O. Jarrett, personal communication).

In what may be a similar situation Lutz et al. (1980) isolated FeLV in cultures of the foetal cat cell line (fcwf-4) inoculated with the ultracentrifuged pellet of urine from an antigenaemic, but non-viraemic cat. However virus was not isolated from cultures of fcwf-4 cells which had been exposed to bone marrow from this cat although it is not recorded for how long the bone marrow was cultured. This cat therefore may or may not have had a latent bone marrow infection.

It is clear from the results of the present study that congenital transmission of infection from latently infected cats did not occur since none of the kittens had active or latent infections at birth. By contrast, Rojko et al. (1982) found evidence of congenital latent infections in kittens born to 2 latently infected queens. However that study is flawed since the queens had been re-exposed to FeLV by receiving an intraperitoneal inoculation of virus during the first week of gestation. It is therefore impossible to know if the congenital infection in the kittens was a result of true transmission from their mothers, or was a consequence of the virus given to the mothers during pregnancy.

Pedersen et al. (1984) have also studied the possible transmission of infection from latently infected queens to their kittens. Twenty-nine of 30 kittens had no evidence of congenital infection. The remaining kitten, which was one of a litter of 3, was claimed to be viraemic at birth, although the first blood samples were not obtained until the kittens were 2 weeks of age. The litter-mates of this kitten then became infected at 10-13 weeks of age by what was believed to be the horizontal transmission of virus from the viraemic kitten. It is possible that these kittens like those of cat 4, were protected from viraemia at an earlier age by maternally derived antibody, although this is not known since antibody titres were not investigated. The mother of these kittens (cat P) was never antigenaemic, and presumably not viraemic, during pregnancy of lactation. Interpretation of these results poses problems because if like cat 4, cat P was producing infectious virus at an extramedullary site such as the uterus it is difficult to explain why infection was transmitted to only one of 3 foetuses. If only small amounts of virus were being produced in the uterus which were undetectable in the blood of the mother the probability of infection may have been such that only one foetus in the litter became infected. The fact that each foetus in the cat has a separate blood supply and that these blood supplies are not interconnected may then explain why the infection did not spread from foetus to foetus.

Alternatively the situation which occurred with this queen could be similar to that in avian leukosis virus infections where only a proportion of the embryos in the eggs of hens which are viraemic but which also have antibodies become infected (Spencer et al., 1977; Payne et al., 1982). Those embryos which do not become infected are believed to be protected by maternal antibody in the yolk. If cat P produced very small amounts of virus locally in the uterus or placenta it is possible that 2 of the foetuses were protected by antibody.

Pedersen et al. (1984) suggested that the foetus of cat P may have been infected by the transfer of latently infected maternal

leucocytes across the placenta. This may be possible since Gruffydd-Jones (1982) demonstrated that congenital infection occurred after implantation and not in the ovum. Against this interpretation however is the fact that Rojko et al. (1982) failed to reactivate virus in circulating leucocytes in latently infected cats.

Since the first blood samples were not taken from the kittens of cat P until 2 weeks of age, it is possible that infection occurred post-natally and not congenitally as claimed. The source of infection may have been the milk, although if this were the case one might expect all 3 kittens to become viraemic within a few days of each other when maternally derived antibody had waned, as found in the present study. Although transmission of infection did occur from cat P to her kittens, it is unclear if infection was congenital or post-natal and the source of infection remains unresolved.

As discussed in Chapter 4, all latently infected cats have VNAs and most are thus resistant to reinfection with FeLV. However one cat in the present study, cat 4, spontaneously reactivated her latent infection to become persistently viraemic 11 weeks after her fourth litter was born. Reactivation of latency in vivo has previously only been reported when cats were treated with high and prolonged doses of corticosteroids (Post and Warren, 1980; Rojko et al., 1982; Pedersen et al., 1984). Cat 4 received no such therapy. She had been intermittently antigenaemic for approximately 2.5 years, had always had low levels of VNAs and was producing infectious virus in her mammary glands. She may therefore have always been on the verge of becoming viraemic, and she eventually did so only after being exposed to 4 of her own litters of viraemic kittens and after being subjected to several stressful manipulations during her final pregnancy and lactation. It is possible, of course, that she always had a very low-grade viraemia which could not be detected by the methods of virus isolation used in this study. When viraemia became established her VNAs dropped below the levels of detection.

Cats which thus spontaneously reactivate latent infections to become viraemic are a potential source of infection for susceptible cats.

Pedersen et al. (1984) placed 400 cats which had "recovered" from experimental FeLV infection in homes throughout the United States. Two of these cats became persistently viraemic within 6 months of housing. One of the cats was exposed to a FeLV-positive companion but the other was not known to have been re-exposed to the virus. There is no conclusive evidence that these cats had really "recovered" before housing since VNA titres were not reported. However, if these cats did have VNAs it is possible that titres were low and that like cat 4 they reactivated latent FeLV infections upon challenge with viraemic cats, although this is impossible to determine with certainty.

In summary, most latent FeLV infections appear to be clinically and epidemiologically insignificant. However, a minority of cats like cat 4 which are antigenaemic, have low levels of VNAs and are latently infected, may be producing infectious virus at extramedullary sites such as the mammary glands. These cats are therefore a potential source of infection not only for their own kittens but for other susceptible cats in the household. Some of these cats may also spontaneously reactivate their latent infection to become viraemic. Such cats would complicate the control of FeLV in closed multicat households such as breeding colonies, and could be reponsible for the reappearance of viraemic cats in these households months or even years after the virus was believed to have been eradicated (Rojko et al., 1982; O. Jarrett, personal communication).

CHAPTER 6

MECHANISMS OF THE CONTROL OF LATENCY

INTRODUCTION

In the previous three Chapters the duration and significance of latent FeLV infections were investigated. The present Chapter describes experiments which were carried out in cell culture in an attempt to understand how latent infections are established and maintained in cats.

Exposure of cats to FeLV may result in a persistent infection or in apparent recovery. When cats are experimentally inoculated with the virus the outcome is determined within 28 days of infection (Russell and Jarrett, 1978b; Rojko et al., 1979a) and within about 70 days when naturally exposed to viraemic cats excreting the virus (Russell and Jarrett, 1978b). One of the factors which is related to the outcome of infection is whether or not the animal develops a neutralising antibody response. Cats which "recover" from infection produce VNAs and are resistant to reinfection, whereas cats which are persistently viraemic do not have serum VNAs.

However, as was found in the present study many of these "recovered" cats which have VNAs actually have a latent infection in their bone marrow. Although most latent infections last for a short period, as described in Chapter 4 approximately 10% of these infections are longterm.

It was believed that VNAs might have a role in the control and maintenance of latency for the following reasons. First, some latent infections can be reactivated in vivo by treating cats with prolonged and high doses of corticocosteroids (Post and Warren, 1980; Rojko et al., 1982; Pedersen et al., 1984) although it is not known if reactivation is due to immunosuppression or to a direct effect of the corticosteroids on viral transcription (Rojko et al., 1982) or effects on the bone marrow cells themselves.

Secondly, as found in the present study and by Madewell and Jarrett (1983) all latently infected cats have VNAs. Also, it is known from the present study that spontaneous reactivation of the latent infection in cat 4, which resulted in persistent viraemia, coincided with the disappearance of VNAs from her serum.

Thirdly, as found in the present study and by others (Rojko et al., 1982; Madewell and Jarrett, 1983; Pedersen et al., 1984) reactivation of virus occurs upon culture of latently infected marrow in the absence of VNAs.

The possible role of VNAs in the control and maintenance of latent FeLV infections was investigated in 3 ways. First, experiments were carried out to simulate in culture the conditions which might occur in the cat. Bone marrow cells from latently infected cats were cultured in the presence of cat sera from cats with latent infections, cats which had recovered from FeLV exposure, viraemic cats or unexposed cats. Reactivation of virus was found to be inhibited by sera containing VNAs. Secondly, the epitope which was involved in inhibition of viral reactivation was defined in similar experiments using mouse MCAs to FeLV proteins. Thirdly, attempts were made to understand the mechanisms by which antibody appeared to control reactivation.

Reactivation inhibition (RI)

Inhibition of the reactivation of latent virus was attempted by culturing bone marrow cells from 4 latently infected cats in Flaskettes in the presence of various cat sera and mouse MCAs to FeLV proteins. The cats used were cats 8, 30, 31 and 32 (see Table 2.1).

All cat sera and MCAs were heated to 56°C for 30 minutes before use to inactivate complement.

a) Cat sera

Bone marrow cells, prepared as in Chapter 3, were suspended at a concentration of 3.3 x 10^6/ml in AFC10. A volume of 0.2 ml of this suspension was added to each well of the Flaskette. An equal volume of cat serum which had already been diluted 1:2 in AFC10 was added to make a final concentration of 1:4 of cat serum in culture. The cells were incubated and were fed at 3-day intervals by replacing 0.2 ml of the culture fluid with a fresh 0.2 ml volume of the appropriate cat serum. The cells were grown in the continuous presence of the antibody for 10 days after which time IF was carried out to test for the presence of viral antigen. Bone marrow cells which were grown in the absence of antibody were included as a control.

The cat sera used were from cats 8, 30, 31, 32, 41, 42, 43, 44, 45 and 47 which were latently infected and cats 13, 14, 15, 16, 17 and 18 which had recovered from infection (see Table 2.1). Control sera were from 2 uninfected cats (cats 50 and 51) and 2 viraemic cats (cats 52 and 53). Cats 52 and 53 were unvaccinated control cats in the experimental FeLV vaccine experiment detailed in Chapter 3. These cats were challenged oronasally at 14 weeks of age with 1 x 10^6 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium.

b) Mouse MCAs

Bone marrow was grown as above but in the presence of mouse MCAs to FeLV proteins. These included 4 MCAs which neutralised FeLV-A (46II E2, I9 B4, 3-17, 6-15) (Vedbrat et al., 1983; Weijer et al., 1986), 6 which neutralised FeLV-BC (A415, C5E5, C8B11, C9E10, C13B5, C16C2) (Grant et al., 1983), 6 non-neutralising MCAs to FeLV-A which were directed against gp70 (C1G10, C10B7, G13D8, G14F5, 1 III B1, 1 III C6) (Grant et al., 1983) and 2 MCAs to p27 (24IA3, 24IB3) (Lutz et al., 1983). Monoclonal antibodies A415, C5E5, C8B11, C9E10, C13B5, C16C2, C1G10, C10B7, G13D8, G14F5, 1 III B1 and 1 III C6 had been purified on protein A-sepharose columns by Dr. C.K. Grant and reconstituted in PBS at a concentration of 1 mg/ml. The remaining MCAs were unpurified ascitic fluid with an unknown concentration of antibody.

Those MCAs which neutralised FeLV-A were diluted 1:50 in AFC10 to make a final concentration of 1:100 in culture, and those MCAs which did not neutralise FeLV-A were used at a dilution of 1:10 in AFC10 to make a final concentration of 1:20 in culture. Cultures were maintained as above and IF was again carried out after 10 days. Bone marrow cells which were grown in the absence of antibody were included as a control.

Titration of antibodies against latently infected bone marrow

Eight doubling dilutions of 12 cat sera and 4 MCAs which were effective in RI were made in AFC10. The dilutions chosen for each antibody spanned its VNA titre to FeLV-A. The 12 cat sera included 4 from latently infected experimental cats (cats 41, 42, 43 and 45), 4 from cats which had recovered from experimental infection (cats 16, 20, 54 and 55) and 4 from cats which had recovered from natural exposure in the field (cats 56-59).

The experimental cats had been infected in a variety of ways as shown in Table 2.1. Cat 20 had eliminated its latent infection at the time of the present experiment. Cats 54 and 55 recovered

from infection after receiving an oronasal dose of 1 x 10^5 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium at 16 weeks of age. Serum from an uninfected cat (cat 51) was included as a control.

The MCAs used were 46II E2, 3-17A, 3-17B and I9 B4. Antibodies 3-17A and 3-17B were different batches of the same MCA.

Bone marrow cells from 2 latently infected cats (cats 41 and 42) were grown as above in the presence of 0.2 ml of the antibody dilutions. These cats had both developed latent infections following an oronasal dose of 1 x 10^5 fiu of FeLV-A/Glasgow-1 in 1 ml Leibovitz L-15 medium. Again, cultures were maintained as before, with IF being carried out after 10 days. The reciprocal of the highest dilution at which an antibody was still effective in completely inhibiting reactivation was called the RI titre.

Role of antibody in RI

It was important to determine whether or not those antibodies which were effective in RI exerted their effect by preventing the production of virus, by killing cells which began to produce virus or by preventing the spread of reactivated virus throughout the culture so that it could not be detected by IF.

Three experiments were carried out in an attempt to distinguish between these alternatives. In the first, a volume of 0.2 ml of bone marrow cells from a latently infected cat (cat 45) at a concentration of 3.3 x 10^6/ml in AFC20 was grown in the presence of neutralising antibody as described above. The antibody used was serum from either a recovered cat (cat 12) or cat 45 itself, both of which had VNA titres of 128. The cultures were maintained as before with volumes of 0.2 ml of the antibodies being added for the first time to the bone marrow at day 0, 3 or 6 after initiation of the culture. The antibodies were used at a dilution of 1:15 to make a final concentration of 1:30 in culture. After antibody had been added to the cells, they were then grown in the continuous presence of this antibody for the remainder of the

experiment. Where no antibody was used the bone marrow cells were grown in a volume of 0.4 ml of AFC20. Fixed cell IF was carried out after 10 days.

The second experiment was similar to the first, involving the same bone marrow and sera. In this experiment, however, cultures were established in the presence of antibodies which were then removed 0, 3 or 6 days after the initiation of the culture. When antibody was removed after 3 or 6 days all of the original culture fluid was replaced with a volume of 0.4 ml of AFC20 in order to eliminate antibody. The cells were then grown in the absence of antibody for the remainder of the experiment and again IF was carried out on day 10.

In both of these experiments bone marrow cells from a viraemic cat (cat 62) and an uninfected cat (cat 61) were treated in a similar manner to the cells of cat 45 and were included as controls. Cat 62 became infected after receiving an oronasal dose of 1×10^6 fiu of FeLV-A/Glasgow-1 in 1 ml of Leibovitz L-15 medium at 14 weeks of age.

In the third experiment bone marrow from a viraemic cat (cat 63) which became infected after receiving a subcutaneous dose of 1.3×10^6 fiu of FeLV-A/Glasgow-1 in 1 ml of Leibovitz L-15 medium at 8 weeks of age and an uninfected cat (cat 64) were co-cultivated for 10 days in varying proportions in the continuous presence of serum from either cat 12 or cat 45 as described above. The ratio of bone marrow cells of cat 63 to those of cat 64 were 1:10; 1:50; 1:100; 1:500; 1:1000 and 1:5000. Wells containing only cells of cat 63 or 64 were also included. The cultures were maintained as before with IF being carried out after 10 days. Similar cultures grown in the absence of antibody were included as controls. Each of these three experiments was done in duplicate.

Reactivation inhibition

a) Cat sera

As shown in Table 6.1, all of the cat sera which contained VNAs to FeLV-A, i.e. the sera from latently infected or recovered cats, were effective in inhibiting reactivation of latent virus as determined by the absence of viral antigen upon IF of the bone marrow cultures. The sera from the uninfected and viraemic cats which contained no VNAs did not inhibit virus reactivation. The results were independent of the source of the bone marrow cells. Fig. 6.1 shows IF carried out on bone marrow which had been cultured for 10 days in the presence of cat sera which either did not contain (Fig. 6.1a) or did contain (Fig. 6.1b) VNAs to FeLV-A.

b) Mouse MCA

Those MCAs which neutralised FeLV-A were effective in inhibiting reactivation of latent virus, whereas those which neutralised FeLV-BC were not. Likewise, the non-neutralising MCAs directed against FeLV-A gp70 or those against the core protein, p27, were unable to inhibit virus reactivation, even when used at high concentrations. A list of the MCAs used and their efficacy in RI is given in Table 6.2.

Titration of antibodies against latently infected bone marrow

The RI titres of the cat sera and MCAs which were used in a series of dilutions on the bone marrow cells of 2 latently infected cats are listed in Table 6.3. Also shown are the titres of these antibodies as determined by virus neutralisation.

TABLE 6.1

Reactivation inhibition of FeLV latency with cat sera

Cat serum	Cat numbers	VNA	RI
Autologous latent	8, 30, 31 or 32	+	+
Heterologous latent	41, 42, 43, 44, 45, 47	+	+
Heterologous recovered	13, 14, 15, 16, 17, 18	+	+
Viraemic	52, 53	-	-
Uninfected	50, 51	-	-

VNA = virus neutralising antibodies to FeLV-A

RI = reactivation inhibition activity

\+ = present

\- = absent

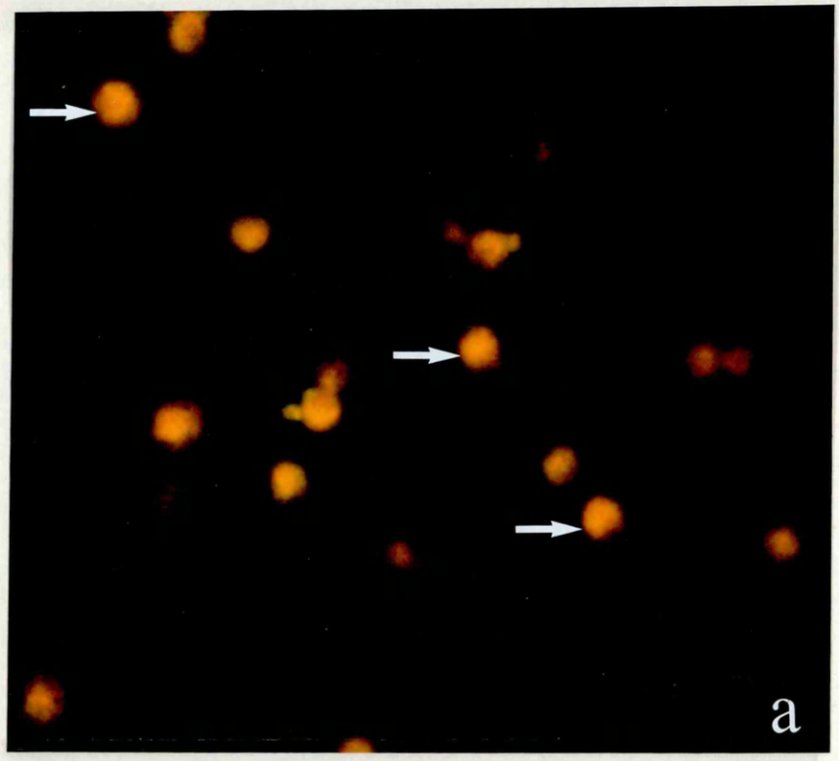
FIG. 6.1 Fixed cell IF carried out on latently infected bone marrow after being cultured for 10 days in the continuous presence of cat serum.

a. The cat serum used contained VNAs to FeLV-A. No viral p27 antigen can be detected.

b. The cat serum used did not contain VNAs to FeLV-A. Virtually all of the cells contain p27 antigen.

The preparations were counterstained with 0.2% carbol chromotrope in water in order to remove non-specific fluorescent granules in myeloid cells which are arrowed.

Magnification x 250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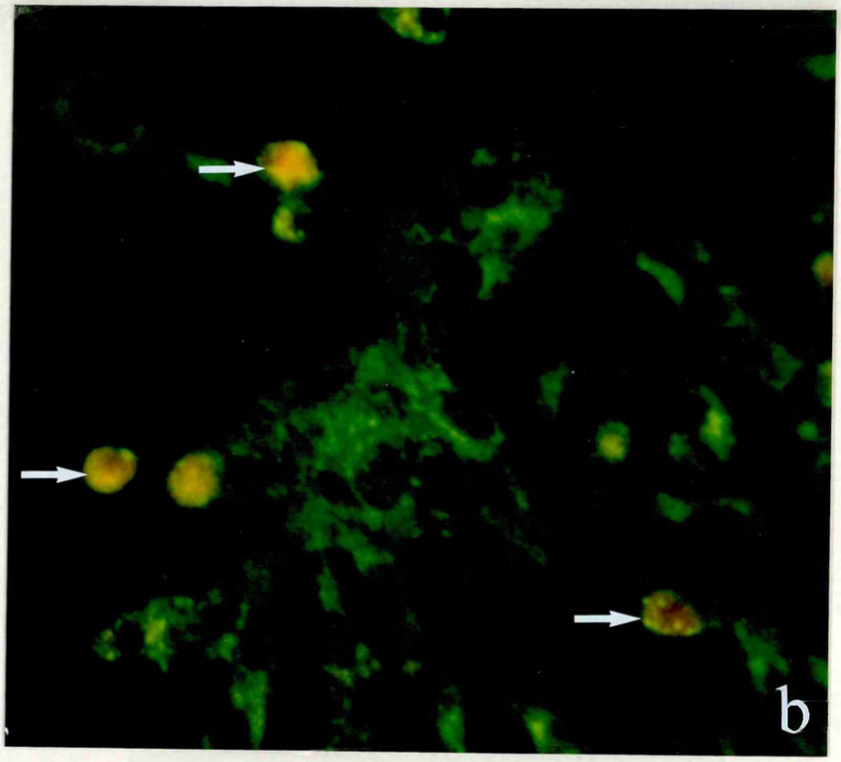

b

TABLE 6.2

Reactivation inhibition of FeLV latency with MCAs

MCA	Specificity	VNA	RI
46II E2	gp70 FeLV-A neutralising	+	+
I9 B4	"	+	+
3-17	"	+	+
6-15	"	+	+
A4 I5	gp70 FeLV-A non-neutralising	-	-
C5 E5	"	-	-
C8 B11	"	-	-
C9 E10	"	-	-
C13 B5	"	-	-
C16 C2	"	-	-
C1 G10	gp70 FeLV-BC neutralising	-	-
C10 B7	"	-	-
G13 D8	"	-	-
G14 F5	"	-	-
1III B1	"	-	-
1III C6	"	-	-
24I A2	p27 non-neutralising	-	-
24I B3	"	-	-
None		-	-

VNA = virus neutralising antibodies to FeLV-A

RI = reactivation inhibition activity

\+ = present

\- = absent

TABLE 6.3

Virus neutralising and reactivation inhibition titres of antibodies

Antibody	VNA titre	RI titre Cat 41	RI titre Cat 42
Monoclonal			
46II E2	12800	8000	16000
3-17A	1024	2048	
3-17B	2048		2048
19 B4	512	4096	
Latent cat			
41	128	64	256
42	128	256	512
43	64	64	512
45	512	512	128
Recovered experimental cat			
16	1024	2048	2048
20	256	512	256
54	128	512	32
55	128	64	64
Recovered field cat			
56	128	128	64
57	512	256	4096
58	32	128	
59	64	512	2048
Uninfected cat			
51	0	0	0

VNA = virus neutralising antibody to FeLV-A

RI = reactivation inhibition

Where no result is given the test was not done.

Correlation of RI and VNA titres

The RI and VNA titres of the antibodies used were plotted as shown in Fig. 6.2. They were found to have a correlation coefficient of 0.89.

Role of antibody in RI

In the first 2 experiments identical results were obtained when using the serum of either cat 12 or cat 45.

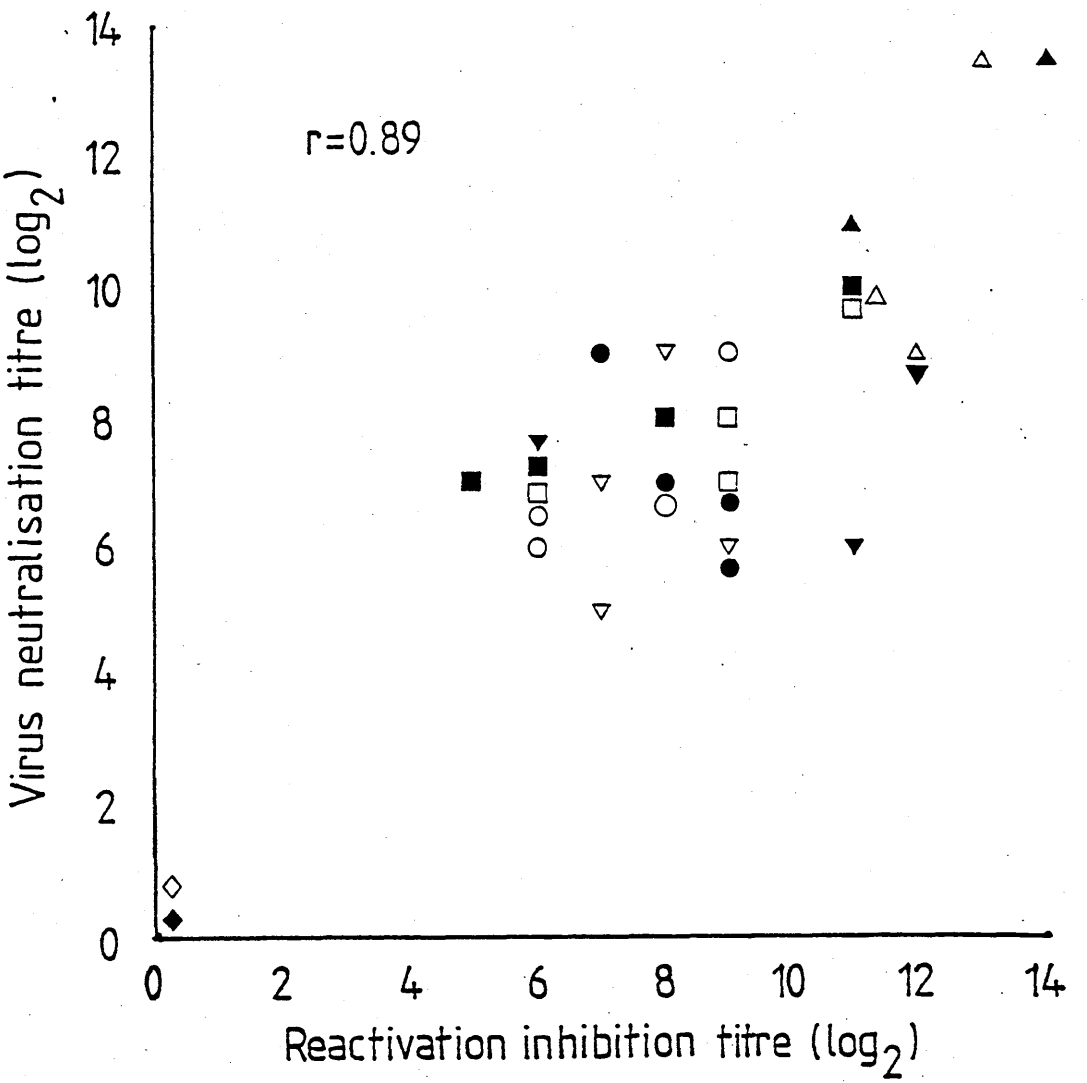
When antibody was added to the latently infected marrow on day 0 or day 3, antigen could not be detected when IF was carried out on day 10. However, when addition of antibody was delayed until day 6, small foci of cells containing antigen could be detected on day 10.

When the latently infected marrow was grown in the absence of antibody for 10 days, as expected virus reactivation occurred and antigen could be detected in almost all of the cells. When the cells were grown in the presence of antibody for 3 days followed by its absence for 7 days small foci of infected cells were observed. However, when the cells were grown in the presence of antibody for 6 days followed by its absence for only 4 days, antigen was not detected.

Similar experiments carried out on bone marrow cells from the uninfected cat failed to detect antigen. When bone marrow from the viraemic cat was used virtually all of the cells in every instance contained antigen, even when antibody was continuously present for 10 days.

The presence of antibody did not appear to have a non-specific cytotoxic effect since there was no difference in the appearance of the cells which had been grown in its continuous presence or absence for 10 days.

FIG. 6.2 Correlation between titres of antibody in virus neutralisation and reactivation inhibition.



	Cat 41	Cat 42
Neutralising MCA to FeLV-A	△	▲
Recovered experimental cat serum	□	■
Latent experimental cat serum	○	●
Recovered field cat serum	▽	▼
Unexposed cat serum	◇	◆

The third experiment was designed to determine whether or not serum from latently infected cats containing VNAs could prevent the spread of FeLV through bone marrow cultures. This experiment, involving the co-cultivation of bone marrow cells from a viraemic and an uninfected cat, proved difficult to quantitate when IF was carried out after 10 days in culture, but unequivocal qualitative results were obtained which are summarised in Table 6.4.

In the control cultures which were grown in the absence of antibody, those wells which contained only cells from the viraemic cat contained large quantities of free antigen and antigen could be demonstrated in the cytoplasm of virtually every cell. "Free" antigen was seen as light punctate fluorescence over the glass surface of the culture well and was presumed to represent the p27 which is shed from FeLV infected cells in large quantities, probably in the form of a glycosylated gp40 precursor (Neil et al., 1980b). Similar results were obtained when the ratio of cells from the viraemic cat to those from the uninfected cat was decreased until this ratio was 1:500. At this ratio fewer antigen-containing cells were detected and no free antigen was evident. At ratios of 1:1000 and 1:5000, or where all of the cells were from the uninfected cat, no cells with antigen could be detected.

When the cells were co-cultivated in the presence of antibody for 10 days the same results were obtained when using the serum of either cat 12 or cat 45. Where the culture contained only cells from the viraemic cat essentially all contained antigen and free antigen was present in the culture. However, where the ratio of cells from the viraemic to the uninfected cat was 1:10 only focal areas of cells containing antigen were seen. It was possible to detect individual cells containing antigen adjacent to cells which did not contain antigen. Where the ratio was 1:50 very few cells contained antigen, and only occasional individual antigen-containing cells were detected at 1:100. Where there were fewer than 1:100 cells from the viraemic cat, no antigen was detected.

TABLE 6.4

Co-cultivation of bone marrow cells from a viraemic and an uninfected cat in the presence or absence of neutralising antibody

Ratio of cells viraemic:uninfected	No antibody	Neutralising antibody from cat 12 or cat 45
All viraemic	Antigen in all cells Free antigen	Antigen in all cells Free antigen
1 : 10	"	Antigen in foci of cells No free antigen
1 : 50	"	Very few cells with antigen
1 : 100	"	Occasional cells with antigen
1 : 500	Fewer cells with antigen No free antigen	No antigen
1 : 1000	No antigen	"
1 : 5000	"	"
All uninfected	"	"

Although it was not feasible to count the antigen-containing cells it was clear that as the number of cells from the viraemic cat which was originally cultured decreased, then the number of cells which contained antigen after 10 days in culture in the continuous presence of antibody also decreased.

DISCUSSION

Initial experiments described in this Chapter showed that the reactivation of latent FeLV from cultured bone marrow could be inhibited by culturing the bone marrow cells in the continuous presence of sera from latently infected or recovered cats, and that sera from persistently viraemic or uninfected cats were not effective. The fact that sera from latently infected and recovered cats contain VNAs and those from viraemic and uninfected cats do not strengthen the belief that these antibodies may have a role in the maintenance of latent infections.

In order to identify the determinant with which these antibodies reacted, mouse MCAs to FeLV proteins were used in the same manner as the cat sera. Those MCAs which reacted with a non-neutralising epitope of FeLV-A gp70 or the neutralising epitope of FeLV-BC gp70 or the core protein, p27, were not effective in RI. It is significant that the cats used in this study were infected with FeLV-A since those MCAs which reacted with the neutralising epitope of FeLV-A gp70 inhibited the reactivation of virus from cultured latently infected bone marrow cells. This would suggest that the epitope involved in RI is the same as that which is involved in virus neutralisation and that VNAs may be at least in partial control of latency in vivo. The RI and VNA titres of the antibodies which were effective in RI were correlated and gave a correlation co-efficient of 0.89. This result suggested that the two assays measured the same activity and reinforced the belief that VNAs played a role in the control of latency.

Attempts were then made to determine the role of neutralising antibody in RI. The possibilities considered were that the antibody prevented viral expression in latently infected cells, killed cells which began to produce virus, and/or prevented the spread of reactivated virus throughout the culture by virus neutralisation.

In order to study the possibility that antibody killed cells from which virus had been reactivated a set of experiments was initiated where antibody was either added or removed from latently infected bone marrow cells at various times after the onset of culture. IF was carried out on day 10 in order to detect reactivated virus in the form of cytoplasmic antigen. When latently infected bone marrow cells were cultured in the absence of antibody for 3 days followed by its presence for 7 days there was no detectable antigen in the cells. This finding could be interpreted in a number of ways. Either reactivation did not occur within 3 days or if it did, then the presence of antibody for a further 7 days caused a reversal of this phenomenon. A third possibility is that reactivation did occur, but that the antibody then prevented spread of virus throughout the culture, and therefore prevented its detection by IF, because as shown by infectious centre assays in Chapter 3, the proportion of cells with latent infections is very small and individual antigen-containing cells may not be detected by IF.

When latently infected bone marrow was grown for 6 days in the absence of antibody and then in its presence for 4 days foci of cells containing antigen could be detected. Therefore it was obvious that reactivation of virus had occurred within 6 days and was not reversed by the addition of antibody for the subsequent 4 days. It is possible that reversion might have occurred if antibody had been present for longer than 4 days. However, from the results obtained with the bone marrow cells from the viraemic cat it would appear that once an infection had become established and cells were producing virus it could not be suppressed with the concentrations of antibody used here, since even after the continuous presence of antibody for 10 days, antigen was still detected in these virus-producing cells. Although quantitation of antigen production was not attempted the intensity of fluorescence obtained appeared similar when bone marrow cells from the viraemic cat were grown in the continuous presence or absence of antibody for 10 days. Therefore, the detection of foci of infected bone marrow cells from the latently infected cat after being cultured for 6 days without antibody followed by 4 days with antibody, may

be a reflection of the fact that this antibody could not suppress viral expression in cells which had an established infection. Alternatively it could merely indicate that the spread of virus throughout the culture was prevented by the neutralisation of reactivated virus.

Other experiments showed that when latently infected bone marrow cells were grown in the presence of antibody for 3 days and then in its absence for 7 days, foci of infected cells could be seen indicating that within 3 days the antibody had not killed the latently infected cells and that they were still capable of producing virus when the antibody was removed. However, the antibody did have an effect on viral production within these 3 days because only small foci of infected cells were present in contrast to the situation in which cells were grown in the absence of antibody for 10 days when virtually all of the cells contained antigen.

When bone marrow cells were grown in the presence of antibody for 6 days and then in its absence for 4 days no infected cells were detected. This could be because these 4 days were not long enough to allow viral reactivation after 6 days of inhibition, or it may be due to antibody-mediated killing of cells which began to reactivate virus. However, this latter possibility appears to be unlikely since the presence of antibody in bone marrow cell cultures from the viraemic cat which were producing virus had no apparent cytolytic effect. It is possible, therefore, that reactivation may have occurred from the latently infected bone marrow cells if they had been grown in the absence of antibody for longer than 4 days since, as described above, antigen could not be detected when these cells were grown in the absence of antibody for 3 days followed by its presence for 7 days, but could be detected when they were grown in the absence of antibody for 6 days followed by its presence for 4 days. These results would suggest that the crucial time for reactivation of latent virus under these conditions is between 3 and 6 days after the onset of culture.

From the results obtained with bone marrow cells from the uninfected cat it appeared that the antibody did not have a non-specific cytotoxic effect since cells grown in either the continuous presence or absence of antibody for 10 days appeared equally healthy. The lack of infected cells in these cultures proved that the fluorescence obtained in the cultures from the latently infected and viraemic cats was specific.

It would appear therefore that neither a cytolytic nor a cytotoxic effect was involved in RI and that once an infection became established, as in cells from the viraemic cat, the antibody had no inhibitory effect on viral expression. However, it was still unclear if this antibody prevented the spread of virus or had a direct effect on viral production. In an attempt to answer this question an experiment was carried out involving the co-cultivation of varying proportions of bone marrow cells from a viraemic and an uninfected cat in the continuous presence of antibody for 10 days. From previous results it was known that the antibody was not non-specifically cytotoxic, that it was not cytolytic for virus-producing cells, and that it did not appear to suppress viral production in cells with established infection. Therefore, by mixing virus-producing with non-producing cells it was hoped to determine whether or not the antibody prevented the spread of virus throughout the culture. If this were the case, then where the ratio of producing cells to non-producing cells was 1 to 10 then it would be expected that 10% of the cells would contain antigen after 10 days in culture, whereas if the antibody were not having this effect it would be expected that the infection would have spread to the non-producing cells, thus resulting in more than 10% of the cells containing antigen.

However, in practice the experiment proved difficult to quantitate. From the results obtained when the cell mixtures were grown in the absence of antibody it was evident that IF was not as sensitive an assay for detecting infected cells as was the infectious centre assay described in Chapter 3. In the experiments described here it is clear that the limit of detection by IF of

cells which contain antigen lies between 1:500 and 1:1000 whereas the infectious centre assay detected as few as 3 infected cells per million.

Nevertheless when the mixtures of bone marrow cells from the viraemic and the uninfected cats were grown in the presence of antibody it appeared that this antibody inhibited the spread of infection from the cells of the viraemic to those of the uninfected cat since as the number of the former which were originally cultured decreased, then so did the number of cells which contained antigen after 10 days in culture.

By contrast, when the cells were grown in the absence of antibody the infection spread to the cells from the uninfected cat, and it was not until the proportion of cells from the viraemic to those from the uninfected cat was 1:500, that it was obvious that the infection had not spread throughout the culture within 10 days.

The fact that individual cells containing antigen could be seen adjacent to cells which did not when grown in the presence of antibody also indicated that the antibody prevented the spread of infection.

These results may suggest therefore that a "latent" FeLV infection in vivo might not be a true latent infection but might represent a low-grade active infection present in a small proportion of bone marrow cells, with VNAs preventing virus from spreading to other cells in the bone marrow and from entering the blood. This may explain why some cats, like cat 4 described previously, appear to be "latently" infected but have viral antigen in their blood and are producing infectious virus at extramedullary sites, and may confirm the suggestion that these cats are on the verge of becoming viraemic, as was proven in the case of cat 4.

It is probable that other immumnological mechanisms as well as VNAs are also involved in the control of latency. Rojko et al. (1982) found that cytotoxic T-cells reacted with autologous

latently infected bone marrow cells after virus had been reactivated upon their culture, but not on freshly aspirated bone marrow cells which were not expressing FeLV antigens. Therefore a cell mediated immune response may also participate in the control of latency by removing cells which express virus.

Another possibility is that an intact complement system may be important in the maintenance of latent infections. Kraut et al. (1985) treated 5 latently infected cats with cobra venom factor which causes a depletion of complement. Before treatment none of the cats was positive for antigen in circulating blood leucocytes or in freshly aspirated marrow. However one week after treatment, 3 of these cats became positive for antigen in circulating leucocytes and in fresh bone marrow cells, thus indicating an increase in viral antigen production. At this time these cats had decreased levels of complement.

From these results it is probable that the control of latent FeLV infections in vivo is complex, involving a combination of neutralising antibody production, cell mediated immunity and complement.

Although the results obtained in the present study show that neutralising antibody prevents the spread of virus from latently infected cells in vitro it is still unknown whether or not it has a direct effect on individual cells by inhibiting the release of virus. Ways in which these alternatives might be distinguished and the possible significance of the results of the present study for other persistent viral infections are discussed in Chapter 7.

CHAPTER 7

GENERAL DISCUSSION

The aims of the studies described here were to determine the prevalence and duration of latent infection in cats exposed to FeLV, to assess the clinical and epidemiological consequences of latency and to define the mechanisms by which the latent state is maintained. In this Chapter the extent to which these aims were achieved is discussed and where appropriate further studies to investigate questions raised in the present work are suggested. Particular attention is paid to the mechanisms by which latency may be controlled in vivo.

Cats with latent FeLV infections were identified by the culture of freshly aspirated bone marrow cells which resulted in reactivation of the latent virus. Infectious virus could then be detected in cell culture fluids by virus isolation, usually within 2 weeks of the initiation of the culture. An immunofluorescence technique was developed which could detect viral antigen in the cytoplasm of bone marrow cells after only 1 week of culture. Thus, culturing of bone marrow cells proved to be a rapid and effective method of identification of latently infected cats.

Another method of identifying such cats involves treating them with corticosteroids resulting in virus reactivation, and viraemia (Post and Warren, 1980; Rojko et al., 1982; Pedersen et al., 1984). However, this method is relatively insensitive since only a proportion of the cats develops viraemia, and only after prolonged administration of high doses of the drugs. Corticosteroid therapy is also an inhumane method of detecting latency because the resulting viraemia is obviously undesirable since, if persistent, would predispose to the FeLV-related diseases detailed in Chapter 1. It is for this reason, however, that it would be important to determine the effects on latent infections of the routine use of corticosteroids in veterinary practice in the therapy of inflammatory conditions or autoimmune disease.

It would also be of interest to investigate the effect of megestrol acetate (Ovarid; Glaxovet) which is widely used not only as a contraceptive but also in the therapy of diseases such as flea-bite hypersensitivity and eosinophilic granulomata. The

mechanism by which this drug affects the latter conditions is not known, but it is believed to have a corticosteroid-like activity since it will produce adrenocortical suppression and atrophy in normal cats (Chastain et al., 1981). The possible influence of therapeutic doses of both corticosteroids and megestrol acetate on latent infections should be the subject of a future study.

From the studies of the experimental multicat household detailed in Chapter 4 it was found that in such FeLV-infected households the proportion of the non-viraemic cats which harbour latent infections may be high at any one time. It was also shown by the use of infectious centre assays that the proportion of latently infected cells in the marrow is variable between cats, but low. A remaining question is which cells in the marrow contain the latent virus. Rojko et al. (1982) suggested that latent virus was present in immature but not in differentiated myeloid cells although this has not been demonstrated unequivocally. The fact that there are so few cells which harbour the latent infection makes their identification difficult. However, it may be possible to enrich for the latently infected cells by fractionating the bone marrow cells on a cell sorter (FACS II; Becton Dickinson). The individual fractions could then be cultured and the presence of reactivated virus detected as previously described for whole bone marrow. Identification of the cells which produce virus would then depend on morphology by histological staining or on colony assay (Onions et al., 1982) since there are as yet no markers for the different types of feline myeloid cells. From the results of the infectious centre assays carried out in the present study it appears that virus reactivation occurs quickly because the size of the transformed foci produced by bone marrow cells from latently infected cats was similar to those produced by marrow cells from viraemic cats or by chronically infected fibroblasts (FEA/FeLV-A) both of which continuously produce virus. Therefore, it would not be unreasonable to attempt identification of latently infected cells on morphological grounds since they are unlikely to alter in the time necessary for viral reactivation to occur.

Alternatively, cells in which virus is reactivated could be sorted by IF. The bone marrow could be cultured in order to allow virus reactivation to occur. The cells could then be resuspended and the surface of virus-expressing cells labelled with a mouse MCA to FeLV gp70. Labelled cells could then be separated on a cell sorter. Identification of the cells would again be by morphology or colony assay.

What is clear from the present study is that the majority of latent infections are short-lived, being eliminated within 2.5 years of exposure to the virus. What is unclear is the mechanism by which latency is terminated. From the findings of Rojko et al. (1982) it is possible that latent virus is present in myeloid precursor cells and that latent infection ends when these cells have differentiated, have completed their natural life span and have been removed from circulation. The duration of a latent infection would then be dependent on the type and the number of cells which were infected initially. It might be expected that latency would last longer if a large number of immature cells were infected than if infection involved only a few cells which were reaching the final stages of maturation. It is possible that the small proportion of longer-lived latent infections (like cat 8) is a reflection of the virus being present in very primitive stem cells which are not eliminated, or even in lymphocytes which are themselves long-lived.

An alternative explanation is that latency ends as a result of an immunological response. Neutralising antibody which is present in all latently infected cats may kill any virus which is produced and/or kill any cell which begins to express virus. On the other hand cell mediated immunity may be responsible for killing virus-producing cells. If either of these events does occur then it is likely that latency ends when all of the infected cells have been killed.

However, the possibility that latency does not end must not be forgotten. The methods of virus isolation from bone marrow culture fluids, fixed cell IF on cultured bone marrow cells and infectious centre assays used in this study may not be sufficiently sensitive to detect infected cells. This problem will be discussed in more detail later.

The next objective of this study was to determine the mechanisms which were responsible for initiating and controlling latent infections. For the reasons discussed in Chapter 6 it was thought that VNAs probably had a role in the maintenance of latency in vivo.

From the experiments involving the culture of latently infected bone marrow cells in the continuous presence of cat serum it was found that those sera which contained VNAs from either latently infected or recovered cats inhibited the reactivation of latent virus. By the use of mouse MCAs to FeLV proteins it was determined that the epitope with which this antibody reacted was that on the gp70 which is also involved in virus neutralisation. It was therefore concluded that, at least in vitro, antibody which neutralised the infecting virus had a role in the maintenance of latency. This was further confirmed when it was found that the virus neutralising and reactivation inhibition titres of these antibodies were closely correlated suggesting that the two assays were measuring the same activity and that VNAs were important in the control of latent infections in vivo.

When neutralising antibody was either added to or removed from latently infected bone marrow cells at varying times after the onset of culture, it was found that the inhibitory effect of the antibody on virus reactivation was reversible but that once reactivation had occurred and the cells were expressing p27, the addition of antibody had no such effect. Similarly, antibody could not stop virus production from bone marrow cells from viraemic cats or from chronically infected FEA/FeLV-A cells.

In summary, neutralsing antibody inhibited reactivation of FeLV from latently infected bone marrow cells in vitro. This effect was reversible, but if virus were allowed to reactivate prior to antibody addition, then inhibition did not occur.

The mechanism by which neutralising antibody inhibits virus reactivation is not yet known. It is possible that the antibody has an effect on individual latently infected cells by binding to gp70 expressed on the cell surface and inhibiting virus production. The action of the antibody binding to this antigen may initiate a transmembrane signal which prevents further expression of viral proteins. The operation of this mechanism would obviously require the continuous transcription and translation of the env gene in order that gp70 would be expressed on the cell surface. However, it is not known if such transcription occurs. It would be necessary to use the technique of in situ hybridisation which detects viral RNA in individual cells to determine whether or not transcription of any of the FeLV proteins occurred (Harper et al., 1986).

From the results of the present study neither p27 nor gp70 were detected by fixed or live cell IF in uncultured freshly aspirated bone marrow cells from latently infected cats. However, as described in Chapter 6 IF proved not to be a very sensitive method of detecting antigen-producing cells, being capable of detecting only between 1:500 and 1:1000 cells which produced p27. Approximately 1 to 10% of these cells produce gp70 (O. Jarrett, personal communication). The results of infectious centre assays discussed in Chapter 3 showed that the numbers of latently infected cells ranged from 3 to 400 per million cells. Therefore, even if viral antigen production did occur, it would not be possible to detect it by the methods of IF described in this study. The detection of gp70 by IF may be further compromised by the presence of bound autologous antibody.

The method of in situ hybridisation has proven to be a much more sensitive method of detecting transcribed RNA in uncultured

cells. For example, Harper et al. (1986) showed that this technique detected as few as 1 in 100,000 lymphocytes in peripheral blood or lymph nodes which contained the RNA of human immunodeficiency virus (HIV). This technique is currently being developed in order to detect transcription of FeLV genes in bone marrow cells from latently infected cats. It is possible that differential transcription of these genes occurs since env can be transcribed independently of gag and pol (Neil and Onions, 1985). It would therefore be possible for gp70 to be expressed in the absence of any of the gag proteins. Thus, gp70 may be expressed on the cell surface, and the action of neutralising antibody binding to it may then initiate a signal which prevents further transcription of viral genes.

Alternatively, the entire FeLV genome may be transcribed and the binding of antibody to the gp70 may inhibit assembly of the viral proteins and final maturation and budding of the virus, thus preventing the production of infectious virus particles.

It is known that other viruses can persist in a non-productive state and the mechanisms involved in the persistence of some of these have been studied. Probably the most fully investigated are the paramyxoviruses and in particular measles virus.

Following an acute infection, measles virus can persist in brain tissue of individuals in the presence of a fully competent immune system and in later life can cause subacute sclerosing panencephalitis (SSPE) (Joseph and Oldstone, 1975). It was suggested that the virus persisted in SSPE patients because neutralsing antibody bound to viral antigens expressed on the cell surface caused a rearrangement of these antigens (Joseph and Oldstone, 1975; Lampert et al., 1975; Oldstone, 1977). This phenomenon is known as "antigenic modulation" and was first described by Boyse et al. (1963).

In vitro studies showed that cells which were chronically infected with measles virus could be lysed in the presence of

optimal levels of anti-viral IgG and complement and that it was the alternative rather than the classical complement pathway which was initiated (Joseph et al., 1975). However, when these cells were grown in the presence of antibody with moderately decreased levels of complement, they were not lysed (Joseph et al., 1975; Gorman and Lachmann, 1982). Instead, antigenic modulation occurred as demonstrated by the stripping of viral antigen from the cell surface. Therefore, if complement levels were depleted the cells escaped the immune system. Similar findings have also been reported by Fujinami and Oldstone (1985).

The suggestion of Joseph and Oldstone (1975) and Oldstone (1977) that antigenic modulation occurred in vivo in SSPE patients is plausible because high levels of antibody but not complement were present in the cerebrospinal fluid of these patients. This was later confirmed by Gorman et al. (1980) who found that patients with SSPE had greatly enhanced levels of antiviral antibody in the cerebrospinal fluid although this was noted to be part of a general systemic hyperimmune response to the virus. Infected cells thus escape from immune destruction and the virus persists. The ways in which measles virus persists and escapes immunosurveillance have been reviewed by Oldstone and Fujinami (1982).

The modulation which occurred in vitro peaked at 24 to 34 hours after the addition of antibody. There was no re-expression of viral antigen when antibody was present and once modulation had occurred the addition of complement or cytotoxic T-cells had no effect (Gorman and Lachmann, 1982). However, this phenomenon was reversible, as demonstrated by the reappearance of viral antigen upon removal of the antibody (Fujinami and Oldstone, 1980; Gorman and Lachmann, 1982) although the rate at which this happened depended on how long the antibody had been present (Joseph and Oldstone, 1975).

The molecular mechanisms involved in the antigenic modulation of measles virus have been investigated. Fujinami and Oldstone (1980) found that there was a decrease in the expression of viral

polypeptides on the surface of acutely infected cells upon the addition of antibody. These authors suggested that alterations in the polypeptides may lead to abberations in the alignment of viral proteins and synthesis of virus at the membrane (Fujinami and Oldstone, 1979; 1980).

Joseph and Oldstone (1975) reported that in acute measles infections cells are killed due to the expression of a viral protein, the fusion protein, on the cell surface which fuses infected cells which are subsequently lysed. In persistent measles infection, where antigenic modulation occurs, there is a decrease in the expression of the fusion protein, therefore the infected cells do not fuse and are not killed. Thus the infection persists (Fujinami and Oldstone, 1980).

Interestingly the binding of antibody to viral antigens on the cell surface not only resulted in antigenic modulation but also interfered with the expression of viral antigens inside the cell. There was a decrease in the production of viral haemolysin and phosphoprotein upon the addition of antibody (Fujinami and Oldstone, 1979). It is not known if these proteins are even transcribed under these conditions. Such a mechanism would require a transmembrane signal but what this might be is also unknown.

In summary, measles virus persists in its host in the presence of high titres of neutralising antibody which binds to and causes modulation of viral antigens expressed on the cell surface. The binding of antibody also alters the expression of viral antigens inside the cell although it is not known how this is initiated. These processes are reversible upon the removal of antibody.

Another paramyxovirus, canine distemper virus (CDV) which is very closely related to measles virus can also persist in its host. Like measles virus, in acute infections the infected cells are lysed by the activation of the alternative complement pathway by the binding of antiviral antibody to viral antigens expressed on the cell surface (Gorman, 1983). As observed in persistent measles

virus infections, in persistent CDV infections there are decreased levels of complement and the binding of antibody to cell surface viral antigen causes antigenic modulation (Gorman and Lachmann, 1982). The infected cells thus escape the host's immune system and the virus persists.

Other paramyxoviruses have been found to persist in human bone marrow cells. These include simian virus 5 (SV5) and human parainfluenza viruses types 1 and 3 (Goswami et al., 1984). Prior to culture SV5 antigen could be demonstrated in individual bone marrow cells by IF, but upon culture for 5-7 days the antigen spread to neighbouring cells to produce foci of infection. However, the infection did not become generalised and degeneration of the cells occurred as the antigen spread. The parainfluenza viruses did not spread. The presence of neutralising antibody to SV5 in the sera of patients correlated well with the detection of antigen in the bone marrow cells. Therefore it has been suggested that antigenic modulation may occur to prevent spread of virus in vivo and allow the virus to persist (Russell and Goswami, 1984). However, the prevention of the spread of antigen through cultured bone marrow cells by the addition of neutralising antibody has not been attempted.

Thus, paramyxovirus can persist in the host and it is thought that the mechanism of persistence involves the binding of neutralising antibody to cell surface viral antigens resulting in antigenic modulation.

The persistence of herpes simplex virus (HSV) has also been studied in detail. HSV may form a latent infection which in man can be reactivated by stimuli such as pyrexia, skin trauma and ultraviolet light (Wildy et al., 1982). It has long been thought that the latent virus resides in neurones and this was confirmed by McLennan and Darby (1980). Latent HSV can be detected by co-cultivating cells from infected ganglia with susceptible cells (Stevens and Cook, 1971; Openshaw et al., 1979) leading to reactivation of the virus which can then be detected by the presence of a characteristic cytopathic effect. Detection of

infected cells has been attempted in a number of other ways. Immunofluorescence carried out on fresh ganglia of 50 latently infected mice failed to detect viral antigen (McLennan, 1981). Therefore if there is expression of antigen it is below the levels of detection by this method. However, the use of infectious centre assays on disaggregated ganglia showed that only 0.1% of cells in the ganglia were infected (Walz et al., 1976). Therefore, even if antigen were expressed it would be unlikely to be detected in so few cells by IF. McDougall and Galloway (1978) detected viral mRNA by in situ hybridisation in many neurones although it is believed that these cells may have been undergoing virus reactivation at that time.

Stevens and Cook (1974) suggested that neutralising antibody controlled latent HSV infections. However, this was not confirmed since when mice were infected with virus and were given rabbit hyper-immune anti-herpesvirus serum between 3 and 144 hours after infection they developed latent infections within 1 to 2 days which remained in over 90% of the mice even after the antibody had disappeared, approximately 2 months after administration (Sekizawa et al., 1980). Latent virus could be reactivated by trauma to the inoculation site and neutralising antibodies reappeared in the sera of the mice at this time. Since this antibody was not detected while the virus was latent Sekizawa et al. (1980) concluded that expression of viral antigen either did not occur during latency or it occurred at levels too low to initiate a detectable neutralising antibody response. Neutralising antibody therefore appeared to be unimportant in the control of latent HSV infections. However, it cannot be ruled out that non-neutralising antibody plays a role in the maintenance of these infections (Nash, 1981). It has also been suggested that cell mediated immunity may play a part here since this is important for the protection of the host against acute infection (Lodmell et al., 1973; Oakes, 1975) and patients undergoing recrudescence had decreased cell mediated immune responses (Wilton et al., 1972; Shillitoe et al., 1977).

On the other hand HSV latency may occur because the virus is present in non-permissive cells (Sekizawa et al., 1980) or it may

be that it is maintained by a low level of virus replication or intermittent spontaneous reactivation (Scriba, 1975; Hill and Blyth, 1976).

The molecular biology of HSV latency has recently been reviewed by Cantin et al. (1985). It has been found that almost the entire genome is present in the brain tissue of latently infected people (Fraser et al., 1981) and mice (Rock and Fraser, 1983) although it is not known in what form the genome is present. It may exist as a free molecule of linear DNA (Kastoukoff et al., 1981) or as a circular molecule of DNA (Rock and Fraser, 1983). Alternatively, it may be molecularly rearranged or integrated into the cellular DNA (Puga et al., 1984). However, Fenner et al. (1974) postulated that integration was unnecessary for latency to occur since neurones do not divide. Puga et al. (1978) suggested that latency occurred due to a block in transcription of the genome and it was later confirmed that limited transcription occurred (Galloway et al., 1982).

In summary, HSV can persist in the neurones of its host. There appears to be no expression of viral antigens during latency although a variety of stimuli can cause reactivation of the virus. Neutralising antibody is not necessary for the maintenance of latency although non-neutralising antibody and cell mediated immunity may be important. The entire viral genome is present in the host cell although its form is unknown, and only limited transcription occurs.

The other major group of viruses known to persist in a silent non-productive form are the retroviruses. There are 3 subgroups of these viruses: the lentiviruses or slow viruses which include maedi-visna (MV) virus of sheep and equine infectious anaemia (EIA) virus; the oncoviruses which include FeLV, bovine leukaemia virus (BLV) and human T-lymphotropic virus type I (HTLV-I); and the spumaviruses, like feline syncytium-forming virus. The murine leukaemia viruses (MuLV) and avian leukosis virus also belong to the oncovirus subgroup of retroviruses and are more closely related

to FeLV than the others. It is unknown if the murine and avian viruses persist in their hosts in a latent form as does FeLV. However, some work has been carried out in vitro on cell lines infected with Gross MuLV (G-MuLV) and Friend MuLV (F-MuLV) and is discussed below.

The pathogenesis of lentiviruses has recently been reviewed (Haase, 1986) but relatively little is known about the mechanisms by which these viruses persist in vivo. In culture, MV virus undergoes rapid replication and spreads throughout the culture resulting in cytolysis (Thormar, 1963). However, in vivo, the infection was non-lytic and the virus persisted in the lungs, central nervous system and haemopoietic system of the host (Brahic et al., 1981). The host develops neutralising antibodies which presumably prevent the dissemination of the virus through the body (Petursson et al., 1976). Haase et al. (1977) reported that the virus persisted in cells in a latent form and it was suggested that the production of infectious virus was blocked at the level of transcription (Brahic et al., 1981).

A major characteristic of MV virus is that it undergoes antigenic drift to produce variant viruses (Narayan et al., 1977a). The variant viruses arise due to point mutations in the env gene (Scott et al., 1979; Clements et al., 1980) but do not replace the original strain. Instead, it was found that accumulation of variant viruses occurred and that the host eventually made neutralising antibodies to each one (Clements and Narayan, 1985). Since the original strain of virus remained in its host, it was clear that antigenic drift was not essential for virus persistence (Petursson et al., 1985). Indeed, Thormar et al. (1983) found that in longterm infections in vivo the appearance of antigenic variants was a rare event, although this did occur.

It was suggested that infected cells escaped immunosurveillance due to insufficient antigen production (Haase et al., 1977). Neither humoral nor cellular immunity appeared to play a significant role in the maintenance of latency because immuno-

suppression in the host did not lead to recrudescence of infection (Nathanson et al., 1976; Narayan et al., 1977b).

Antigenic drift also occurs with EIA virus (Kono et al., 1973) and like MV virus the variants are due to point mutations on the env gene (Montelaro et al., 1984; Salinovich et al., 1986). However, EIA virus differs from MV virus as it undergoes cycles of replication and each new variant is refractory to the antibody which neutralised previous variants (Kono et al., 1973; Salinovich et al., 1986). Another characteristic of EIA virus is that non-neutralising antibody binds to the virus forming immune complexes which remain infectious. The virus is thus protected from neutralising antibody and therefore persists in the form of complexes (Mohanty and Dutta, 1981).

The molecular mechanisms by which the lentiviruses, in particular, MV virus, persist remain largely unknown and the role of antibody in their persistence is unclear.

The second subgroup of retroviruses, the oncoviruses, are perhaps a little more understood. As mentioned previously, it is not known if a latent state exists in animals exposed to the avian and murine viruses but in vitro studies on the maintenance of MuLV infections have been carried out.

Cells which were chronially infected with G-MuLV were killed in vitro by the presence of neutralising antibody plus complement. However, in the presence of antibody but in the absence of complement lysis did not occur, but antigenic modulation resulted (Ioachim and Sabbath, 1979) rather like the situation with measles virus. There was redistribution of both viral particles inside the cell as well as antigen expressed on the cell membrane. It has been reported that this antigenic modulation affected the degree of malignancy of infected cells (Ioachim et al., 1974; Ioachim, 1976) and such cells would escape the host's immune reponse. Antibody-induced antigenic modulation in vitro was reversible (Ioachim and Sabbath, 1979). The mechanisms by which antibody

prevents the production of infectious virus is unknown. The binding of antibody to viral antigen expressed on the cell membrane may act as a signal to stop further antigen synthesis; it may decrease the rate at which antigen is produced or it may result in selective deletion of specific antigens.

The growth of F-MuLV-induced erythroleukaemia cells in vitro could be stopped by the addition of goat antiserum to the envelope glycoprotein, gp71 (Genovesi and Collins, 1983). As with G-MuLV it is believed that antigenic modulation occurs (Genovesi et al., 1977; Genovesi and Collins, 1983). This effect was found to be independent of complement and was demonstrated to be due to the presence of virus-specific IgG. Initially, specific modulation of gp71 occurred, followed by non-specific modulation of other viral antigens. These effects were reversible depending on the duration and concentration of antibody in the cultures (Genovesi and Collins, 1983). For example, antigen re-expression occurred rapidly if antibody had been present for up to 48 hours, whereas the presence of antibody for 72 hours or longer resulted in a lag phase prior to the re-expression of viral antigens.

The mechanism by which this antibody has its effect is believed to be due to its prevention of cells from entering the G_2/M phase of the cell cycle. Antibody to the group or type specific determinants on the gp71 were capable of causing cytostasis and the action of antibody binding to antigen was the critical factor (Genovesi and Collins, 1983).

Anti-gp71 antibody was used in vivo to protect mice from infection with F-MuLV. The antibody induced active humoral and cell mediated immune responses (Collins et al., 1979; Genovesi et al., 1983) and it was suggested that such passive immunotherapy decreased the titre of infectious virus below the level necessary to induce immunosuppression and thus allowed the animal to mount an active immune response (Genovesi et al., 1982).

Antibody to the major envelope glycoprotein of MuLV therefore appears to have an inhibitory effect on viral expression in vitro and would appear to maintain the virus in a non-productive state. It would be of great interest to learn if such a silent non-productive state occurred in vivo, not only of MuLV, but also if avian leukosis virus.

Both HTLV-I and BLV which are closely related, cause latent infections in their hosts. HTLV-I is the aetiological agent of adult T-cell leukaemia (ATL). The viral genome is integrated in the DNA of the leukaemic T-cells of these patients (Yoshida et al., 1984) although there is no expression of viral antigen in vivo (Miyoshi et al., 1982). However, when peripheral blood leukocytes from patients with ATL and from healthy carriers who have anti-HTLV-I antibodies were cultured there was spontaneous reactivation of virus and antigen expression occurred (Tochikura et al., 1985). After 3 days in culture approximately 2% of the cells contained antigen but virus gradually spread to 20% by 7 days and by 50 days all of the cells expressed antigen.

When these cells were cultured with anti-HTLV-I antibody from patients with ATL, healthy carriers or HTLV-I-positive monkeys, the reactivation of virus was inhibited. This effect was not permanent and was not due to cytotoxicity because re-expression of virus occurred even after the continuous presence of antibody for up to 45-60 days. It was shown that it was antibody which exerted this inhibitory effect since pretreatment of the anti-HTLV-I serum with protein-A abolished the effect (Tochikura et al., 1985). However, MCAs to viral antigens were not used, so that it is not known with which epitope this human antibody reacted, although it is believed to be the viral envelope glycoprotein. The antibody was less effective in inhibiting reactivation if a proportion of the cells already contained antigen and it had no effect on cell lines which were continuously expressing virus. Tochikura et al. (1985) suggested that antigen expression both in vitro and in vivo was regulated by antibody and that the binding of antibody to genome-positive cells initiated a signal which inhibited expression.

BLV causes enzootic bovine leukosis and persistent lymphocytosis. Like HTLV-I but unlike FeLV and MuLV, BLV infects only lymphocytes, and as in HTLV-I viral antigens are not expressed in vivo. However, complete infectious virus was detected as early as 3 hours after initiation of cultures of infected lymphocytes (Baliga and Ferrer, 1977; Kettmann et al., 1980). Gupta and Ferrer (1982) showed that the expression of the major core protein, p25, could be inhibited by a non-immunoglobulin plasma protein in short-term cultures of infected lymphocytes. This protein was present in the plasma of a majority of BLV-infected cattle but not in uninfected cattle or cattle infected with other viruses and it was capable of preventing p25 expression even if cells already contained this antigen. When the protein was removed from the cultures, however, re-expression occurred (Gupta and Ferrer, 1982).

The protein also blocked the expression of p25 in BLV-infected fibroblast cell lines of both bovine and non-bovine origin but had no effect on cell lines infected with either FeLV or Rauscher-MuLV. It was therefore demonstrated that the protein was BLV-specific (Gupta et al., 1984). It was found that the protein was in plasma of infected cattle but not in serum, it was not interferon and had a molecular weight of approximately 150,000. Using cloned BLV DNA in dot blot hybridisation Gupta et al. (1984) claimed that the expression of the genome was blocked at the level of transcription. These authors concluded that the inhibition of p25 expression was not caused by antibody. Attempts to inhibit expression of this antigen in cultured lymphocytes from BLV-infected cattle by mouse MCAs to a variety of BLV proteins, including the envelope glycoprotein, failed (A. Pacitti, O. Jarrett and A. Burny, unpublished results).

Interestingly, Tsukiyama et al. (a; submitted for publication) discovered a platelet derived factor in the plasma of both BLV-infected and uninfected cattle which inhibited the effect of the protein described above, i.e. it reversed the inhibition of p25 expression in cultured lymphocytes induced by the plasma protein. This effect was irreversible. This platelet derived factor

increased p25 expression and was shown to be a mitogen to bovine lymphocytes and to both bovine and murine fibroblasts (Tsukiyama et al. b; submitted for publication). It appeared to have similar properties to platelet derived growth factor. It is unknown if the factor exerted its effect by stimulating the cell cycle or by stimulating viral transcription.

The mechanisms involved in the maintenance of latent HTLV-I and BLV infections therefore remain controversial. The two viruses are very closely related and it is difficult to explain why antibody should play a role in the maintenance of latent HTLV-I infection, at least in vitro, but not with BLV.

Latent FeLV infetions appear to be maintained by mechanisms similar to those which are involved in the persistence of the paramyxoviruses and HTLV-I since anti-viral antibody inhibits the reactivation of virus in vitro. It has been shown by the use of MCAs to FeLV proteins that antibody to the neutralising epitope on the gp70 is necessary for this effect although the molecular mechanisms involved remain to be established. It is possible that antigenic modulation occurs as in measles virus infection but if this were to occur expression of gp70 would be necessary. Attempts so far to demonstrate gp70 on latently infected cells have failed but the use of a more sensitive technique such as in situ hybridisation will perhaps clarify this matter. Other immunological effector mechanisms may be involved such as complement dependence or cell mediated immunity as has been suggested for the control of latent HSV infection.

On the other hand, truly latent FeLV infections may not exist. Instead, what is regarded as latency may be a limited productive infection with release of amounts of virus in the blood below the levels of detection of the methods used in this study. Any virus released may be neutralised by antibody so that the production of infectious virus is limited to areas such as the bone marrow and epithelial cells. From the experiments described in Chapter 6 it was found that neutralising antibody appeared to

prevent the spread of virus in vitro from bone marrow cells from a viraemic cat to those of an uninfected cat.

This phenomenon of limited viral production may be possible in vivo since, as described by Rojko et al. (1979a), virus enters the mouth and grows in the cells of the oropharynx and upper respiratory tract. Virus then infects circulating lymphocytes which are disseminated to the bone marrow and lymphatic tissues throughout the body. Subsequent spread to non-lymphoid tissues occurs and virus can be found in myelomonocytic cells in the marrow, in circulating neutrophils and platelets, and finally in epithelial cells in a variety of tissues. The host may simultaneously develop an immune response which limits the infection to the bone marrow and the epithelia.

Indeed, cats with such infections have been reported. In the present study cat 4 was "latently" infected but produced infectious virus in her mammary glands, presumably in epithelial cells. Non-viraemic cats which have infectious virus in their saliva have been described (Jarrett et al., 1982; H. Lutz and O. Jarrett, personal communication) and Lutz et al. (1980) isolated virus from the urine of a non-viraemic cat, although it is unknown if any of these cats had latent infections.

It is therefore not unreasonable to suspect that "latently infected" cats actually produce infectious virus at extramedullary sites which would not be detected by the routine diagnostic techniques used in the field.

Such "latently infected" cats would complicate the control of the spread of FeLV and its eradication from multicat households by test-and-removal programmes and may be of particular importance in closed households such as breeding colonies. Cats like cat 4 described in the present study, who produced virus in her milk and thus infected her kittens, may explain the reports of the reappearance of FeLV in households which were believed to have been freed of the virus. For these reasons it would be of interest to

examine those cats which remained in these households for the presence of latent infections after this method of eradication had been used.

The continuation of the study of the control of latent FeLV infections will lead to a more complete understanding of other "latent" retroviral infections, including those which affect man such as HTLV-I. In addition, elucidation of the molecular mechanisms by which anti-gp70 antibody influences reactivation of latent FeLV infections should help us to understand how other persistent viral infections are maintained.

REFERENCES

Anderson, L.J., Jarrett, W.F.H., Jarrett, O. and Laird, H.M. Feline leukemia virus infection of kittens: mortality associated with atrophy of the thymus and lymphoid depletion. Journal of the National Cancer Institute 47, 807-817 (1971).

Baliga, V. and Ferrer, J.F. Expression of bovine leukemia virus and its internal antigen in blood lymphocytes. Proceedings of the Society of Experimental Biology and Medicine 156, 388-391 (1977).

Bolognesi, D.P., Motelaro, R.C., Frank, H. and Schäfer, W. Assembly of type C oncornaviruses: A model. Science 199, 183-186 (1978).

Boyse, E.A., Old, L.J. and Leull, S. Antigenic properties of experimental leukemias. II. Immunological studies in vivo with C57BL/6 radiation-induced leukemias. Journal of the National Cancer Institute 31, 987-995 (1963).

Brahic, M., Stowring, L., Ventura, P. and Haase, A.T. Gene expression in visna virus infection of sheep. Nature 292, 240-242 (1981).

Brambell, F.W.R. Transmission of passive immunity from mother to young. In: Frontiers of Biology. Eds: A. Neuberger and E.L. Tatum. pp.154-159 Elsevier/North Holland (1970).

Cantin, E.M., Puga, A. and Notkins, A.L. Molecular biology of herpes simplex virus latency. In: Concepts in Viral Pathogenesis. Eds: A.L. Notkins and M.B.A. Oldstone. pp.172-177 Springer-Verlag (1985).

Chastain, C.B., Graham, C.L. and Nichols, C.E. Adrenocortical suppression in cats given megestrol acetate. American Journal of Veterinary Research 42, 2029-2035 (1981).

Clements, J.E. and Narayan, O. Immune selection of virus variants. In: Concepts in Viral Pathogenesis. Eds: A.L. Notkins and M.B.A. Olstone. pp.152-157 Springer-Verlag (1985).

Clements, J.E., Pedersen, F.S., Narayan, O. and Haseltine, W.A. Genomic changes associated with antigenic variation of visna virus during persistent infection. Proceedings of the National Academy of Sciences. (USA) 77, 4454-4458 (1980).

Collins, J.J., Snyderman, R., Genovesi, E.V. and Sanfilippo, F. Viral antigens as targets for leukemia immunotherapy. In: Virus-Lymphocyte Interactions: Implications for Disease. Ed: M. Proffitt. pp.91-105 Elsevier/North Holland (1979).

Essex, M., Klein, G., Snyder, S.P. and Harrold, J.B. Feline sarcoma virus induced tumours: correlation between humoral antibody and tumour regression. Nature 233, 195-196 (1971a).

Essex, M., Klein, G., Snyder, S.P. and Harrold, J.B. Antibody to feline oncornavirus associated cell membrane antigen in neonatal cats. International Journal of Cancer 8, 384-390 (1971b).

Fenner, F. The classification and nomenclature of viruses. Summary of results of meetings of the International Committee on Taxonomy of Viruses in Madrid. Intervirology 6, (i) 1-12 (1975-76).

Fenner, F., McAuslan, B.R., Mims, C.A., Sambrook, J. and White, D.O. In: Biology of Animal Viruses. pp.452-478 Academic Press (1974).

Fischinger, P.J., Blevins, C.S. and Namura, S. Simple quantitative assay for both xenotropic murine leukaemia and ecotropic feline leukaemia viruses. Journal of General Virology 14, 177-179 (1974).

Fischinger, P.J. and O'Connor, T.E. Viral infection across species barriers: Reversible alteration of murine virus for growth in cat cells. Science 165, 714-716 (1969).

Francis, D.P. and Essex, M. Epidemiology of feline leukemia. In: Feline Leukemia Virus. Eds: W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp.127-131 Elsevier/North Holland (1980).

Francis, D.P., Essex, M. and Hardy, W.D., Jr. Excretion of feline leukaemia virus by naturally infected pet cats. Nature 269, 252-254 (1977).

Fraser, N.W., Lawrence, W.C., Wroblewska, Z., Gilden, D.H. and Koprowski, H. Herpes simplex type 1 DNA in human brain tissue. Proceedings of the National Academy of Sciences (USA) 78, 6461-6465 (1981).

Fujinami, R.S. and Oldstone, M.B.A. Anti-viral antibody reacting on the plasma membrane alters measles virus expression inside the cell. Nature 279, 529-530 (1979).

Fujinami, R.S. and Oldstone, M.B.A. Alterations in expression of measles virus polypeptides by antibody: Molecular events in antibody-induced antigen modulation. Journal of Immunology 125, 78-85 (1980).

Fujinami, R.S. and Oldstone, M.B.A. Antibody initiates virus persistence: Immune modulation and measles virus infection. In: Concepts in Viral Pathogenesis. Eds: A.L. Notkins and M.B.A. Oldstone. pp.187-193 Springer-Verlag (1985).

Galloway, D.A., Fenoglio, C. and McDougall, J.K. Limited transcription of the HSV genome when latent in human sensory ganglia. Journal of Virology 41, 686-691 (1982).

Genovesi, E.V. and Collins, J.J. In vitro growth inhibition of murine leukemia cells by antibody specific for the major envelope glycoprotein (gp71) of Friend leukemia virus. Journal of Cellular Physiology 117, 215-229 (1983).

Genovesi, E.V., Linvat, D. and Collins, J.J. Immunotherapy of murine leukemia. VII. Prevention of Friend leukemia virus-induced immunosuppression by passive serum therapy. Intrnational Journal of Cancer 30, 609-624 (1982).

Genovesi, E.V., Linvat, D. and Collins, J.J. Immunotherapy of murine leukemia. VIII. Efficacy of passive serum therapy of Friend leukemia virus-induced disease in immunocompromised mice. Journal of the National Cancer Institute 70, 311-322 (1983).

Genovesi, E.V., Marx, P.A. and Wheelock, E.F. Antigenic modulation of Friend virus erythroleukemic cells in vitro by serum from mice with dormant erythroleukemia. Journal of Experimental Medicine 146, 520-534 (1977).

Gorman, N.T. The interaction of cells persistently infected with canine distemper virus with antiviral antibody and complement. Cellular Immunology 77, 242-248 (1983).

Gorman, N.T., Habicht, J. and Lachmann, P.J. Intracerebral synthesis of antibodies to measles and distemper virus in patients with SSPE and multiple sclerosis. Clinical and Experimental Immunology 39, 44-52 (1980).

Gorman, N.T. and Lachmann, P.J. (1982). In vitro modulation of viral cell surface glycoproteins by antiviral antibody in the presence of complement. Clinical and Experimental Immunology 50, 507-514 (1982).

Goswami, K.K.A., Cameron, K.R., Russell, W.C., Lange, L.S. and Mitchell, D.N. Evidence for the persistence of paramyxoviruses in human bone marrows. Journal of General Virology 65, 1881-1888 (1984).

Grant, C.K., Ernisse, B.J., Jarrett, O. and Jones, F.R. Feline leukemia virus envelope gp70 of subgroups B and C defined by monoclonal antibodies with cytotoxic and neutralising functions. Journal of Immunology 131, 3042-3048 (1983).

Graves, D.C. and Velicer, L.F. Properties of feline leukemia virus. I. Chromatographic separation and analysis of the polypeptides. Journal of Virology 14, 349-365 (1974).

Gruffydd-Jones, T. Some aspects of reproductive physiology and pathology in cats. Ph.D. Thesis. University of Bristol (1982).

Gupta, P. and Ferrer, J.F. Expression of bovine leukemia virus genome is blocked by a non-immunoglobulin protein in plasma from infected cattle. Science 215, 405-407 (1982).

Gupta, P., Kashmiri, S.V.S. and Ferrer, J.F. Transcriptional control of the bovine leukemia virus genome: role and characterization of a non-immunoglobulin plasma protein from bovine leukemia virus-infected cattle. Journal of Virology 50, 267-270 (1984).

Haase, A.T. Pathogenesis of lentivirus infections. Nature 322, 130-136 (1986).

Haase, A.T., Stowring, L., Narayan, O., Griffin, D. and Price, D. Slow persistent infection caused by visna virus: role of host resistance. Science 195, 175-177 (1977).

Hardy, W.D., Jr. Feline leukemia virus diseases. In: Feline Leukemia Virus. Eds: W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp.3-31. Elsevier/North Holland (1980a).

Hardy, W.D., Jr. The virology, immunology and epidemiology of the feline leukemia virus. In: Feline Leukemia Virus. Eds: W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp.33-78. Elsevier/North Holland (1980b).

Hardy, W.D., Jr., Hess, P.W., MacEwen, E.G., McClelland, A.J., Zuckerman, E.E., Essex, M., Cotter, S.M. and Jarrett O. Biology of feline leukemia virus in the natural environment. Cancer Research 36, 582-588 (1976a).

Hardy, W.D., Jr., Hirshaut, Y. and Hess, P. Detection of feline leukemia virus and other mammalian oncornaviruses by immunofluorescence. In: Unifying Concepts in Leukemia. Eds: R.M. Dutcher and L. Chieco-Bianchi. pp.778-799 Karger (1973b).

Hardy, W.D., Jr., McClelland, A.J. and Zuckerman, E.E. Development of virus non-producer lymphosarcoma in pet cats exposed to FeLV. Nature 288, 90-92 (1980).

Hardy, W.D., Jr., McClelland, A.J., Zuckerman, E.E., Hess, P.W., Essex, M., Cotter, S.M., MacEwen, E.E. and Hayes, A. Prevention of contagious spread of FeLV and the development of leukaemia in pet cats. Nature 263, 326-328 (1976b).

Hardy, W.D., Jr., Old, L.J., Hess, P.W., Essex, M. and Cotter, S.M. Horizontal transmission of feline leukaemia virus. Nature 244, 266-269 (1973a).

Hardy, W.D., Jr., Zuckerman, E.E., MacEwen, E.G., Hayes, A.A. and Essex M. A feline leukaemia virus and sarcoma virus induced tumour-specific antigen. Nature 270: 240-251 (1977).

Harper, M.E., Marselle, L.M., Gallo, R.C. and Wong-Staal, F. Detection of HTLV-III-infected lymphocytes in lymph nodes and peripheral blood from AIDS patients by in situ hybridization. Proceedings of the National Academy of Sciences (USA) 83, 772-776 (1986).

Hill, T.J. and Blyth, W.A. An alternative theory of herpes-simplex recurrence and a possible role for prostaglandins. Lancet 1976 1, 397-398 (1976).

Hoover, E.A. and Kociba, G.J. Bone lesions in cats with anemia induced by feline leukemia virus. Journal of the National Cancer Institute 53, 1277-1284 (1974).

Hoover, E.A., McCullough, C.B. and Griesmer, R.A. Intranasal transmission of feline leukemia. Journal of the National Cancer Institute 48, 973-983 (1972).

Hoover, E.A., Olsen, R.G., Hardy, W.D., Jr. and Schaller, J.P. Horizontal transmission of feline leukemia virus under experimental conditions. Journal of the National Cancer Institute 58, 433-434 (1977a).

Hoover, E.A., Olsen, R.G., Hardy, W.D., Jr., Schaller, J.P. and Mathes, L.E. Feline leukemia virus infection: age-related variation in response of cats to experimental infection. Journal of the National Cancer Institute 57, 365-369 (1976).

Hoover, E.A., Olsen, R.G., Mathes, L.E. and Schaller, J.P. Relationship between feline leukemia virus antigen expression in blood and bone marrow and viral infectivity in blood, bone marrow, and saliva of cats. Cancer Research 37, 3707-3710 (1977b).

Iaochim, H.L. The stromal reaction of tumors: An expression of immune surveillance. Journal of the National Cancer Institute 57, 465-475 (1976).

Ioachim, H.L. and Sabbath, M. Redistribution and modulation of Gross murine leukemia virus antigens induced by specific antibodies. Journal of the National Cancer Institute 62, 169-180 (1979).

Ioachim, H.L., Keller, S., Sabbath, M. and Dorsett, B. Antigenic expression as a determining factor of tumor growth in Gross virus lymphoma. Progress in Experimental Tumor Research 19, 284-296 (1974).

Jarrett, O. Natural occurrence of subgroups of feline leukemia virus. In: Viruses in Naturally Occurring Cancers. Eds: M. Essex, G. Todaro and H. zur Hausen. pp.603-611 Cold Spring Harbor Laboratory (1980).

Jarrett, O. Pathogenesis of feline leukaemia virus related diseases. In: Mechanisms of Viral Leukaemogenesis. Eds: J.M. Goldman and O. Jarrett. pp.135-154 Churchill-Livingstone (1984).

Jarrett, O. Feline leukaemia virus. In: Feline Medicine and Therapeutics. Eds: E.A. Chandler, C.J. Gaskell, A.D.R. Hilbery. pp.271-283 Blackwell Scientific (1985a).

Jarrett, O. Feline leukaemia virus. In Practice (Supplement to The Veterinary Record) 7, 125-126 (1985b).

Jarrett, O., Golder, M.C. and Stewart, M.F. Detection of transient and persistent feline leukaemia virus infections. The Veterinary Record 110, 225-228 (1982).

Jarrett, O., Golder, M.C., Toth, S., Onions, D.E. and Stewart, M.F. Interaction between feline leukaemia virus subgroups in the pathogenesis of erythroid hypoplasia. International Journal of Cancer 34, 283-288 (1984)

Jarrett, O., Hardy, W.D., Jr., Golder, M.C. and Hay, D. The frequency of occurrence of feline leukaemia virus subgroups in cats. International Journal of Cancer 21, 334-337 (1978a).

Jarrett, O., Laird, H.M. and Hay, D. Growth of feline leukaemia virus in human cells. Nature, 224, 1028-1029 (1969).

Jarrett, O., Laird, H.M. and Hay, D. Restricted host range of a feline leukaemia virus. Nature 238, 220-221 (1972).

Jarrett, O., Laird, H.M. and Hay, D. Determinants of the host range of feline leukaemia viruses. Journal of General Virology 20, 169-175 (1973c).

Jarrett, O., Laird, H.M., Hay, D. and Crighton, G.W. Replication of cat leukaemia virus in cell cultures. Nature 219, 521-522 (1968).

Jarrett, O., Pitts, J.D., Whalley, J.M., Clason, A.E. and Hay, J. Isolation of the nucleic acid of feline leukemia virus. Virology 42, 317-320 (1971).

Jarrett, O. and Russell, P.H. Differential growth and transmission in cats of feline leukaemia viruses of subgroups A and B. International Journal of Cancer 21, 466-472 (1978).

Jarrett, O., Russell, P.H. and Hardy, W.D., Jr. The influence of virus subgroup on the epidemiology of feline leukaemia virus. In: Advances in Leukemia Research 1977. Eds. P. Bentvelzen, J. Hilgersand and D.S. Yohn. pp.25-28 Karger (1978b).

Jarrett, O., Russell, P.H. and Stewart, M.F. Protection of kittens from feline leukaemia virus infection by maternally derived antibody. Veterinary Record 101, 304-305 (1977).

Jarrett, W.F.H., Crawford, E.M., Martin, W.B. and Davie, F. Leukaemia in the cat. A virus-like particle associated with leukaemia (lymphosarcoma). Nature 202, 567-568 (1964b).

Jarrett, W.F.H., Essex, M., Mackey, L., Jarrett, O. and Laird, H. Antibodies in normal and leukemic cats to feline oncornavirus-associated cell membrane antigens. Journal of the National Cancer Institute 51, 261-263 (1973a).

Jarrett, W., Jarrett, O., Mackey, L., Laird, H., Hardy, W., Jr. and Essex, M. Horizontal transmission of leukemia virus and leukemia in the cat. Journal of the National Cancer Institute 51, 833-841 (1973b).

Jarrett, W.F.H., Mackey, L.J., Jarrett, O., Laird, H.M. and Hood, C. Antibody response and virus survival in cats vaccinated against feline leukaemia. Nature, 248, 230-232 (1974).

Jarrett, W.F.H., Martin, W.B., Crighton, G.W., Dalton, R.G. and Stewart, M.F. Leukaemia in the cat. Transmission experiments with leukaemia (lymphosarcoma). Nature 202, 566-567 (1964a).

Joseph, B.S., Cooper, W.L. and Oldstone, M.B.A. Immunologic injury of cultured cells infected with measles virus. I. Role of IgG antibody and the alternative complement pathway. Journal of Experimental Medicine 141, 761-774 (1975).

Joseph, B.S. and Oldstone, M.B.A. Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. Journal of Experimental Medicine 142, 864-876 (1975).

Kastoukoff, L., Long, C., Doherty, P.C., Wroblewska, Z. and Koprowski, H. Isolation of virus from brain after immune suppression of mice with latent herpes simplex. Nature 291, 432-433 (1981).

Kawakami, T.G., Theilen, G.H., Dungworth, D.L., Munn, R.J. and Beall, S.G. "C"-Type viral particles in plasma of cats with feline leukemia. Science 158, 1049-1050 (1967).

Kettmann, R., Marbain, G., Cleuter, Y., Portetelle, D., Mammerickx, M. and Burny, A. Genomic integration of bovine leukemia provirus and lack of viral RNA expression in the target cells of cattle with different response to BLV infection. Leukemia Research 4, 509-519 (1980).

Kono, Y., Kobayashi, K. and Fukunaga, K. Antigenic drift in equine infectious anemia virus in chronically infected horses. Archives für Virusforschung 41, 1-10 (1973).

Koshy, R., Gallo, R.C. and Wong-Staal, F. Characterization of the endogenous feline leukemia virus-related DNA sequences in tissues of virus negative leukemic animals. Virology 103, 434-445 (1980).

Kraut, E.H., Rojko, J.L., Olsen, R.G. and Tuomari, D.L. Effects of cobra venom factor treatment on latent feline leukemia virus infection. Journal of Virology 54, 873-875 (1985).

Laird, H.M., Jarrett, O., Crighton, G.W. and Jarrett, W.F.H. An electron microscopic study of virus particles in spontaneous leukemia in the cat. Journal of the National Cancer Institute 41, 867-878 (1968).

Lampert, P.W., Joseph, B.S. and Oldstone, M.B. Antibody-induced capping of measles virus antigens on plasma membrane studied by electron microscopy. Journal of Virology 15, 1248-1255 (1975).

Lodmell, D.L., Niwa, A., Hayashi, K. and Notkins, A.L. Prevention of cell-to-cell spread of herpes simplex virus by leukocytes. Journal of Experimental Medicine 137, 706-720 (1973).

Lutz, H., Jarrett, O. and Suter, P.F. A one-step ELISA for the rapid detection of feline leukaemia virus infection using monoclonal antibodies; further investigations. In: Immunoenzymatic Techniques. Ed: S. Avrameas. pp.363-368 Elsevier (1983).

Lutz, H., Pedersen, N.C., Higgins, J., Harris, C.W. and Theilen, G.H. Quantitation of p27 in the serum of cats during natural infection with FeLV. In: Feline Leukaemia Virus. Eds: W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp.497-505. Elsevier/ North Holland (1980).

McClelland, A.J., Hardy, W.D., Jr. and Zuckerman, E.E. Prognosis of healthy FeLV infected cats. In: Feline Leukaemia Virus. Eds: W.D. Hardy, Jr., M. Essex, and A.J. McClelland. pp.121-126 Elsevier/North Holland (1980).

McDougall, J.K. and Galloway, D.A. Detection of viral nucleic acid sequences using in situ hybridization. In: Persistent Viruses. ICN/UCLA Symposium on Molecular and Cellular Biology, XI. Eds: J.G. Stevens, G.J. Todaro and C.F. Fox. pp.181-188 Academic Press (1978).

Mackey, L., Jarrett, W., Jarrett, O. and Laird, H.M. Anemia associated with feline leukemia virus infection in cats. Journal of the National Cancer Institute 54, 209-217 (1975).

McLennan, J.L. Studies on herpes simplex virus latency. Ph.D. Thesis. University of Cambridge (1981).

McLennan, J.L. and Darby, G. Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. Journal of General Virology 51, 233-243 (1980).

Madewell, B.R. and Jarrett, O. Recovery of feline leukaemia virus from non-viraemic cats. The Veterinary Record 112, 339-342 (1983).

Mahmoud, J.S. Studies on immunity to feline leukaemia virus. Ph.D. Thesis. University of Glasgow (1984).

Miyoshi, I., Taguchi, H., Fujishita, M., Niiya, K., Kitagawa, T., Ohtsuki, Y. and Akagi, T. Asymptomatic type C virus carriers in the family of an adult T-cell leukemia patient. Japanese Journal of Cancer Research (GANN) 73, 332-333 (1982).

Mohanty, S.B. and Dutta, S.K. Equine Viruses. In: Veterinary Virology. Eds: S.B. Mohanty and S.K. Dutta. pp. 156-184 Lea and Fabiger (1981).

Montelaro, R.C., Parekh, B., Orrego, A. and Issel, C.J. Antigenic variation during persistent infection by equine infectious anemia virus, a retrovirus. Journal of Biological Chemistry 259, 10539-10544 (1984).

Narayan, O., Griffin, D.E. and Chase, J. Antigenic shift of visna virus in persistently infected sheep. Science 197, 376-378 (1977a).

Narayan, O., Griffin, D.E. and Silverstein, A.M. Slow virus infection: replication and mechanisms of persistence of visna virus in sheep. Journal of Infectious Diseases 135, 800-806 (1977b).

Nash, A.A. Antibody and latent herpes simplex infections. Immunology Today 2, 19-20 (1981).

Nathanson, N., Panitch, H., Palsson, P.A., Petursson, G. and Georgsson, G. Pathogenesis of visna. II. Effect of immunosuppression upon early central nervous system lesions. Laboratory Investigation 35, 444-451 (1976).

Neil, J.C. and Onions, D.E. Feline leukaemia viruses: molecular biology and pathogensis. Anticancer Research 5, 49-64 (1985).

Neil, J.C., Onions, D.E. and Jarrett, O. Polypeptides of feline leukaemia virus: identification of p15E and p12E. Journal of General Virology 50, 455-460 (1980a).

Neil, J.C., Smart, J.E., Hayman, M.J. and Jarrett, O. Polypeptides of feline leukemia virus: a glycosylated gag-related protein is released into culture fluids. Virology 105, 250-253 (1980b).

Niman, H.L., Gardner, M.B., Stephenson, J.R. and Roy-Burman, P. Endogenous RD-114 virus genome expression in malignant tissues of domestic cats. Journal of Virology 23, 578-586 (1977).

de Noronha, F., Poco, A., Post, J.E. and Rickard, C.G. Virus isolation test for feline leukemia virus. Journal of the National Cancer Institute 58, 129-131 (1977).

Oakes, J.E. Role for cell mediated immunity in the resistance of mice to subcutaneous herpes simplex infection. Infection and Immunity 12, 166-172 (1975).

Okabe, H., Twiddy, E., Gilden, R.V., Hatanaka, M., Hoover, E.A. and Olsen, R.G. FeLV-related sequences in DNA from a FeLV-free cat colony. Virology 69, 798-801 (1976).

Oldstone, M.B.A. Role of antibody in regulating virus persistence: modulation of viral antigens expressed on the cell's plasma membrane and analysis of cell lysis. In: Development of Host Defences. Eds: M.D. Cooper and D.H. Dayton. pp.223-235 Raven Press (1977).

Oldstone, M.B.A. and Fujinami, R.S. Virus persistence and avoidance of immune surveillance: how measles viruses can be induced to persist in cells, escape immune assault and injure tissues. In: Virus Persistence. 33rd Symposium of the Society for General Microbiology. Eds: B.W.J. Mahy, A.C. Minson and G.K. Darby. pp.185-202 Cambridge University Press (1982).

Olsen, R.G., Hoover, E.A., Mathes, L.E., Heding, L. and Schaller, J.P. Immunization against feline oncornavirus disease using a kitted tumor cell vaccine. Cancer Research 36, 3642-3646 (1976).

Onions, D.E., Jarrett, O., Testa, N., Frassoni, F. and Toth, S. Selective effect of feline leukaemia virus on early erythroid precursors. Nature 296, 156-158 (1982).

Openshaw, H., Puga, A. and Notkins, A.L. Latency and reactivation of herpes simplex virus in sensory ganglia of mice. Developments in Immunology 7, 301-306 (1979).

Osterhaus, A., Weijer, K., UytdeHaag, F., Jarrett, O., Sundquist, B. and Morein, B. Induction of protective immune response in cats by vaccination with feline leukemia virus iscom. Journal of Immunology 135, 591-596 (1985).

Payne, L.N., Holmes, A.E., Howes, K., Pattison, M., Pollock, D.L. and Waters, D.E. Further studies on the eradication and epizootiology of lymphoid leukosis virus infection in a commercial strain of chickens. Avian Pathology 11, 145-162 (1982).

Pedersen, N.C., Johnson, L., Birch, D. and Theilen, G.H. Possible immunoenhancement of persistent viremia by feline leukemia virus envelope glycoprotein vaccines in challenge-exposure situations where whole inactivated virus vaccines were protective. Veterinary Immunology and Immunopathology 11, 123-148 (1986).

Pedersen, N.C., Meric, S.M., Ho, E., Johnson, L., Plucker, S. and Theilen, G.H. The clinical significance of latent feline leukemia virus infection in cats. Feline Practice 14, 32-48 (1984).

Pedersen, N.C., Theilen, G., Keane, M.A., Fairbanks, L., Mason, T., Orser, B., Chen, C. and Allison, C. Studies of naturally transmitted feline leukemia virus infection. American Journal of Veterinary Research 38, 1523-1531 (1977).

Pedersen, N.C., Theilen, G.H. and Werner, L.L. Safety and efficacy studies of living and killed feline leukemia virus vaccines. American Journal of Veterinary Research 40, 1120-1126 (1979).

Petursson, G., Lutley, R., Palsson, P.A., Goergsson, G. and Nathanson, N. Antigenic variation of visna virus in relation to virus persistence and disease progression. In: Agriculture: Slow viruses in sheep, goats and cattle. Eds: J.M. Sharp and R. Hoff-Jorgensen. pp.21-25 Commission of the European Communities (1985).

Petursson, G., Nathanson, N., Georgsson, G., Panitch, H. and Palsso, P.A. Pathogenesis of visna I. Sequential virologic, serologic and pathologic studies. Laboratory Investigation 35, 402-412 (1976).

Post, J.E. and Warren, L. Reativation of latent feline leukemia virus. In: Feline Leukemia Virus. Eds: W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp.151-155 Elsevier/North Holland (1980).

Puga, A., Cantin, E.M., Wohlenberg, C., Openshaw, H. and Notkins, A.L. Different sizes of restriction endonuclease fragments from the terminal repetitions of the herpes simplex type 1 genome latent in trigeminal ganglia of mice. Journal of General Virology 65, 437-444 (1984).

Puga, A., Rosenthal, J.D., Openshaw, H. and Notkins, A.L. Herpes simplex DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. Virology 89, 102-111 (1978).

Rickard, C.G., Barr, L.M., de Noronha, F., Dougherty, E., III and Post, J.E. C-type virus particles in spontneous lymphocytic leukemia in a cat. Cornell Veterinarian 57, 302-307 (1967).

Rickard, C.G., Post, J.E., de Noronha, F. and Barr, L.M. A transmissible virus-induced lymphocytic leukemia of the cat. Journal of the National Cancer Institute 42, 987-1014 (1969).

Riggs, J.L. An immunofluorescent test for feline leukemia and sarcoma virus antigens and antibodies.Journal of the American Veterinary Medical Association 158, 1085-1087 (1971).

Rock, D.L. and Fraser, N.W. Detection of HSV-1 genome in central nervous system of latently infected mice. Nature 302, 523-525 (1983).

Rogerson, P., Jarrett, W. and Mackey, L. Epidemiological studies on feline leukaemia virus infection. I. A serological survey of urban cats. Internatinal Journal of Cancer 15, 781-785 (1975).

Rojko, J.L., Hoover, E.A., Mathes, L.E., Krakowa, S. and Olsen, R.G. Influence of adrenal corticosteroids on the susceptibility of cats to feline leukemia virus infection. Cancer Research 39, 3789-3791 (1979b).

Rojko, J.L., Hoover, E.A., Mathes, L.E., Olsen, R.G. and Schaller, J.P. Pathogenesis of experimental feline leukemia virus infection. Journal of the National Cancer Institute 63, 759-765 (1979a).

Rojko, J.L., Hoover, E.A., Quackenbush, S.L. and Olsen, R.G. Reactivation of latent feline leukaemia virus infection. Nature 298, 385-388 (1982).

Russell, P.H. and Jarrett, O. An improved assay for feline leukaemia virus pseudotypes of murine sarcoma virus. Journal of General Virology 31, 139-144 (1976).

Russell, P.H. and Jarrett, O. The specificity of neutralising antibodies to feline leukaemia viruses. International Journal of Cancer 21, 768-778 (1978a).

Russell, P.H. and Jarrett, O. The occurrence of feline leukaemia virus neutralising antibodies in cats. International Journal of Cancer 22, 351-357 (1978b).

Russell, W.C. and Goswami, K.K.A. Antigenic relationships in the paramyxoviridae - implications for persistent infections in the central nervous system. In: Viruses and Demyelinating Diseases. Eds: C.A. Mims, M.L. Cuzner and R.E. Kelly. pp.89-99 Academic Press (1984).

Salerno, R.A., Lechman, G.D., Larson, V.M. and Hilleman, M.R. Feline leukemia virus envelope glycoprotein vaccine: preparation and evaluation of immunizing potency in guinea-pigs and cats. Journal of the National Cancer Institute 61, 1487-1493 (1978).

Salinovich, O., Payne, S.L., Montelaro, R.C., Hussain, K.A., Issel, C.J. and Schnorr, K.L. Rapid emergence of novel antigenic and genetic variants of equine infectious anemia virus during persistent infection. Journal of Virology 57, 71-80 (1986).

Sarma, P.S., Gilden, R.V. and Huebner, R.J. Complement-fixation test for feline leukemia and sarcoma viruses (COCAL test). Virology 44, 137-145 (1971).

Sarma, P.S. and Log, T. Viral interference in feline leukemia-sarcoma complex. Virology 44, 352-358 (1971).

Sarma, P.S. and Log, T. Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. Virology 54, 160-170 (1973).

Sarma, P.S., Sharar, A., Tseng, J., Price, P.J. and Gardner, M. Studies on the prevalence of endogenous type C virus RD114 in cats. Proceedings of the Society for Experimental Biology and Medicine 145, 757-762 (1974).

Schäfer, W. and Bolognesi, D.P. Mammalian C-type oncornaviruses: Relationships between viral structure and cell surface antigens and their possible significance in immunological defense mechanisms. Contemporary Topics in Immunobiology 6, 127-167 (1977).

Schaller, J.P., Olsen, R.G. and Mathes, L.E. Increased susceptibility to feline leukemia virus in cats exposed to methylnitrosourea. Cancer Research 38, 996-998 (1978).

Scolnik, E., Rands, E., Aaronson, S.A. and Todaro, G. RNA-dependent DNA polymerase activity in five RNA viruses: Divalent cation requirements. Proceedings of the National Academy of Sciences (USA) 67, 1789-1796 (1970).

Scott, F.W., Csiza, C.K. and Gillespie, J.H. Maternally derived immunity to feline panleukopenia. Journal of the American Veterinary Medical Association 156, 439-453 (1970).

Scott, J.V., Stowring, L., Haase, A.T., Narayan, O. and Vigne, R. Antigenic variation in visna virus. Cell 18, 321-327 (1979).

Scriba, M. Herpes simplex virus infection in guinea pigs: an animal model for studying latent and recurrent herpes simplex virus infection. Infection and Immunity 12, 162-165 (1975).

Sekizawa, T., Openshaw, H., Wohlenberg, C. and Notkins, A.L. Latency of HSV in absence of neutralising antibody: Model for reactivation. Science 210, 1026-1028 (1980).

Shillitoe, E.J., Wilton, J.M.A. and Lehner, T. Sequential changes in cell-mediated immune responses to herpes simplex virus after recurrent herpetic infection in humans. Infection and Immunity 18, 130-137 (1977).

Soe, L.H., Devi, B.G., Mullins, J.I. and Roy-Burman, P. Molecular cloning and characterization of endogenous feline leukemia virus sequences from a cat genome library. Journal of Virology 46, 829-840 (1983).

Spencer, J.L., Crittenden, L.B., Burmester, B.R., Okazaki, W. and Witter, R.L. Lymphoid Leukosis: Interrelations among virus infections in hens, eggs, embryos and chicks. Avian Diseases 21, 331-345 (1977).

Stevens, J.G. and Cook, M.L. Latent herpes simplex virus in spinal ganglia of mice. Science 173, 843-845 (1971).

Stevens, J.G. and Cook, M.L. Maintenance of herpetic latent infection. An apparent role for anti-viral IgG. Journal of Immunology 113, 1685-1693 (1974).

Stewart, M.A., Warnock, M., Wheeler, A., Wilkie, N., Mullins, J.I., Onions, D.E. and Neil, J.C. Nucleotide sequences of a feline leukemia virus subgroup A envelope gene and long terminal repeat and evidence for the recombinational origin of subgroup B viruses. Journal of Virology 58, 825-834 (1986).

Temin, H.M. Mechanism of cell transformation by RNA tumor viruses. Annual Reviews of Microbiology 8, 639-648 (1971).

Theilen, G.H., Kawakami, T.G., Rush, J.D. and Munn, R.J. Replication of cat leukaemia virus in cell suspension cultures. Nature 222, 589-590 (1969).

Thormar, H. The growth of visna virus in monolayer cultures of sheep cells. Virology 19, 273-278 (1963).

Thormar, H., Barshatzky, M.R., Arnesen, K. and Kozlowski, P.B. The emergence of antigenic variants is a rare event in long-term visna virus infection in vivo. Journal of General Virology 64, 1427-1432 (1983).

Tochikura, T., Iwahashi, M., Matsumoto, T., Koyangi, Y., Hinuma, Y. and Yamamoto, N. Effect of human serum anti-HTLV antibodies on viral antigen induction in in vitro cultured peripheral lymphocytes from adult T-cell leukemia patients and healthy virus carriers. International Journal of Cancer 36, 1-7 (1985).

Tsukiyama, K., Onuma, M. and Izawa, H. Effect of platelet-derived factor on expression of bovine leukemia virus genome. Submitted for publication a).

Tsukiyama, K., Onuma, M., Odawara, T., Mikami, T., Kawakami, Y. and Izawa, H. Mitogenic activity of bovine platelet-derived factor on bovine leukemia virus-infected lymphocytes. Submitted for publication b).

Vedbrat, S.S., Rasheed, S., Lutz, H., Gonda, M.A., Ruscetti, S., Gardner, M.B. and Prensky, W. Feline oncornavirus-associated cell membrane antigen: a viral and not a cellularly coded transformation-specific antigen of cat lymphomas. Virology 124, 445-461 (1983).

Walz, M.A., Yamamoto, H. and Notkins, A.L. Immunological response restricts number of cells in sensory ganglia infected with herpes simplex virus. Nature 264, 554-556 (1976).

Weijer, K. and Daams, H. The control of lymphosarcoma/leukaemia and feline leukaemia virus. Journal of Small Animal Practice 19, 631-637 (1978).

Weijer, K., UytdeHaag, F.G.C.M., Jarrett, O., Lutz, H. and Osterhaus, A.D.M.E. Post-exposure treatment with monoclonal antibodies in a retrovirus sytem: failure to protect cats against feline leukemia virus infection with virus neutralizing monoclonal antibodies. International Journal of Cancer 38, 81-87 (1986).

Wildy, P., Field, H.J. and Nash, A.A. Classical herpes latency revisited. In: Virus Persistence. 33rd Symposium of the Society for General Microbiology. Eds: B.W.J. Mahy, A.C. Minson and G.K. Darby. pp.133-167 Cambridge University Press (1982).

Wilton, J.M.A., Ivanyi, L. and Lehner, T. Cell-mediated immunity in Herpesvirus hominis infections. British Medical Journal 1, 723-726 (1972).

Yoshida, M., Seiki, M., Yamaguchi, K. and Takatsuki, K. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proceedings of the National Academy of Sciences. (USA) 81, 2534-2537 (1984).