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Clinical and pathological findings in feline immunodeficiency virus experimental infection

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ABSTRACT

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A study is described of the clinical and pathological findings in 20 specific pathogen free cats infected when 1 year old with feline immunodeficiency virus and monitored over 12 months. Cats were divided into two groups (A and B). The clinical and clinicopathological features were studied in Group A. In Group B, at 1, 2, 4, 9 and 12 months post infection two cats were necropsied. Clinically all cats developed generalised lymphadenopathy, six cats were neutropenic and five cats lymphopenic. Three cats became febrile with conjunctivitis and anterior uveitis and one of these cats ultimately developed jaundice. Postmortem examinations confirmed a generalised lymphadenopathy involving peripheral and visceral lymph nodes with concurrent stimulation of splenic white matter and mucosal lymphoid tissue of the digestive tract and conjunctiva. Within the lymph nodes there was a reactive follicular hyperplasia accompanied by a paracortical hyperplasia with an increased paracortical vascularity. Unusual features were the presence of lymphoid follicles in the bone marrow, thymus and parathyroid tissue. In addition, aggregates of lymphoid cells were found within salivary glands, kidneys, sclera and choroid of the eye. One cat developed a lymphosarcoma affecting the liver and kidneys at 36 weeks post infection. The cat with jaundice had a cholangitis with marked biliary epithelial hyperplasia.

ABBREVIATIONS

FIV, feline immunodeficiency virus; SPF, specific pathogen free.

INTRODUCTION

Feline immunodeficiency virus (FIV) infection is common in cat populations throughout the world (Hosie et al., 1989; Ishida et al., 1989; Shelton et al., 1989) and is a significant cause of disease (Hosie et al., 1989). The virus

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was first isolated in 1987 from cats with an immunodeficiency syndrome (Pedersen et al., 1987). In the field, infection is associated with various non-specific chronic diseases (Hopper et al., 1989; Hosie et al., 1989), cytopenias (Shelton et al., 1990a) or tumours (Shelton et al., 1990b).

Current clinical, immunological and pathological studies of experimental infection are related to findings within the first 18 months (Yamamoto et al., 1988; Ackley et al., 1990; Dow et al., 1990) and little is yet known of the development of long-term clinical and pathological effects. In these studies of the primary phase of FIV infection kittens 7–14 weeks of age have been used and this may not be representative of the situation in the field where infection in adults is more likely. The present study presents the clinical and pathological findings over a 12 month period in 20 young adult cats experimentally infected with the Glasgow-8 strain of FIV (FIV/GLA-8).

MATERIALS AND METHODS

Experimental animals

Two groups of 15 11–14-month-old cats were used. In Group A, the clinical and clinicopathological features were studied; in Group B the necropsy findings were analysed. Both groups comprised 10 infected cats (A1–A10, B1–B10) and five uninfected control cats (A11–A15, B11–B15). The cats were specific pathogen free (SPF) and maintained for the experiment in isolated infected or control groups. They were negative for feline leukemia virus (Petchek FeLV antigen detection kit; IDEXX Laboratories, Slough, UK) and antibodies to feline coronavirus.

Experimental design

Cats were infected intraperitoneally with 2000 cell culture infectious doses of FIV-GLA-8. At Week 8 after infection cats were confirmed FIV antibody positive by enzyme-linked immunosorbent assay (FIV antibody detection kit; IDEXX) and virus was isolated by methods previously described (Hosie and Jarrett, 1990).

In Group A, all cats were clinically examined initially twice weekly for 1 month then weekly for 4 months and finally monthly for 7 months. Rectal temperatures were noted on each occasion and an estimate of the sizes of the popliteal and submandibular lymph nodes was recorded. Cats that were noted to be abnormal (febrile or dull) were examined daily, and ampicillin (Ampifen; Mycofarm, Cambridge, UK) was administered to cats with temperatures greater than 40°C. Blood samples were obtained to determine haematological and blood biochemical profiles initially biweekly to 4 months and then monthly for 8 months. Blood was obtained by jugular venepuncture into

potassium EDTA and lithium heparin. Total circulating leucocyte values were determined using a Roche ABX automatic haematology analyser (Roche, Welwyn Garden City, UK) Coulter counter and the differential count for each sample was calculated from a standard 200 cell count performed on a smear stained by the May–Grunwald–Giemsa method. The absolute numbers of neutrophils and lymphocytes were calculated from the total leucocyte counts and differential counts. Total circulating leucocyte values, neutrophil and lymphocyte counts from control animals at each sampling were used as normal ranges. Using a dry chemistry analyser (Vetest 8008) values were obtained for urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, albumin, globulin, cholesterol and triglycerides. Direct bilirubin levels were determined by Jendrassilk/Grof reaction (Bilirubin test, Roche, Welwyn Garden City, UK). Cats were weighed at various intervals.

In Group B, cats were examined clinically on a routine basis and in detail before infection, 2 weeks prior to postmortem and on the day of postmortem. Full haematological and blood biochemical profiles were determined at these times. Two infected and one control cat were killed at 1, 2, 4, 9, and 12 months post infection and received a detailed postmortem examination. Sections of submandibular, retropharyngeal, mediastinal, mesenteric, paraortic and carcase lymph nodes, tonsil, spleen, thymus, mouth epithelium, salivary gland, oesophagus, stomach, small intestine, large intestine, pancreas, liver, heart, trachea, lungs, bone marrow, kidney, bladder, adrenal, thyroid, eye, skin, brain and spinal cord were examined. Tissues were fixed in 10% neutral buffered formalin and post-fixed in mercuric chloride–formalin. Tissue blocks were embedded in paraffin wax; sections were cut at 6 μm and stained with Meyer's haematoxylin and eosin. Selected sections were also stained with Gordon Sweet's reticulin stain and Masson's trichrome stain.

RESULTS

Clinical findings

All ten infected cats in Group A developed generalised lymph node enlargement commencing 3–6 weeks after infection. The nodes were enlarged for approximately 35 weeks but in seven cats the popliteal lymph nodes remained enlarged up to 12 months, the termination of the experiment.

In Group A, three of the cats (3A, 4A, 6A) developed pyrexia (39.4°C, 39.8°C, 40.2°C) 5–6 weeks after infection which lasted for 1–7 days and in one case recurred 1 month later. In Group B, two of the cats (5B, 6B) became febrile 5 and 9 weeks after infection and one remained febrile until postmortem at 6 weeks after infection. Fever was accompanied by dullness, conjunctivitis and anterior uveitis with photophobia. The cat in Group B (5B) with a non-resolving pyrexia developed profound anorexia, weight loss, anterior abdominal pain and jaundice. It was destroyed on humane grounds and nec-

ropsied. With the exception of this case there was no significant weight loss recorded in this group of cats.

Haematology

In Group A, six of the cats developed a neutropenia (less than 1.8×10^9 l^{-1}) identified at only one sampling in four of the cats but present for up to 18 weeks in two of the cats. In four of the cats neutropenia occurred at 5 weeks post infection and it occurred regularly in association with a leucopenia. Also in Group A, five of the cats developed lymphopenia (less than $2.9 \times 10^9 l^{-1}$) identified again in one sampling or lasting for periods of 8 weeks. In four of the cats lymphopenia commenced 2–5 weeks after infection and reoccurred once to six times during a 12 month period.

In Group B, at postmortem 50% of cases had a neutropenia (4B, 5B, 6B, 7B, 9B), 20% (5B, 7B) had a leucopenia and 10% had a lymphopenia (5B). In addition, lymphoblastic cells were present in one case (7B) which had a hepatorenal lymphosarcoma (Callanan et al., 1992).

In both groups, the periods of pyrexia and dullness were accompanied by leucopenia with neutropenia and a return to normal health was associated with a return to normal haematological parameters.

Biochemistry

The blood biochemical results were in general unremarkable but some individual cats developed hyperproteinaemia with hyperglobulinaemia. Case 5B had hyperbilirubinaemia primarily the result of raised direct bilirubin blood concentrations.

Pathology

At postmortem examination of the uninfected control cats (11B–15B) one cat (11B) had enlarged mesenteric and colonic lymph nodes. No other abnormalities were detected in the control cats.

At postmortem examination all of the infected cats had a generalised lymphadenopathy involving visceral as well as carcase lymph nodes. The nodes were uniformly enlarged (two to three times normal) and there was distinct cortical and medullary differentiation. Splenic white pulp expansion was evident (Fig. 1) and the gut associated lymphoid tissue was prominent. Case 5B was dehydrated and jaundiced. Case 7B had well-defined pale tan masses in the renal cortices of both kidneys and a solitary mass in the liver.

Microscopic examination of the lymphoid tissues confirmed that the generalised lymphadenopathy was a result of expansion of both the B- and T-cell regions of the cortex of the lymph node. There was prominent and irregular follicular expansion with attenuation of the mantle zone (Fig. 2) and zoning

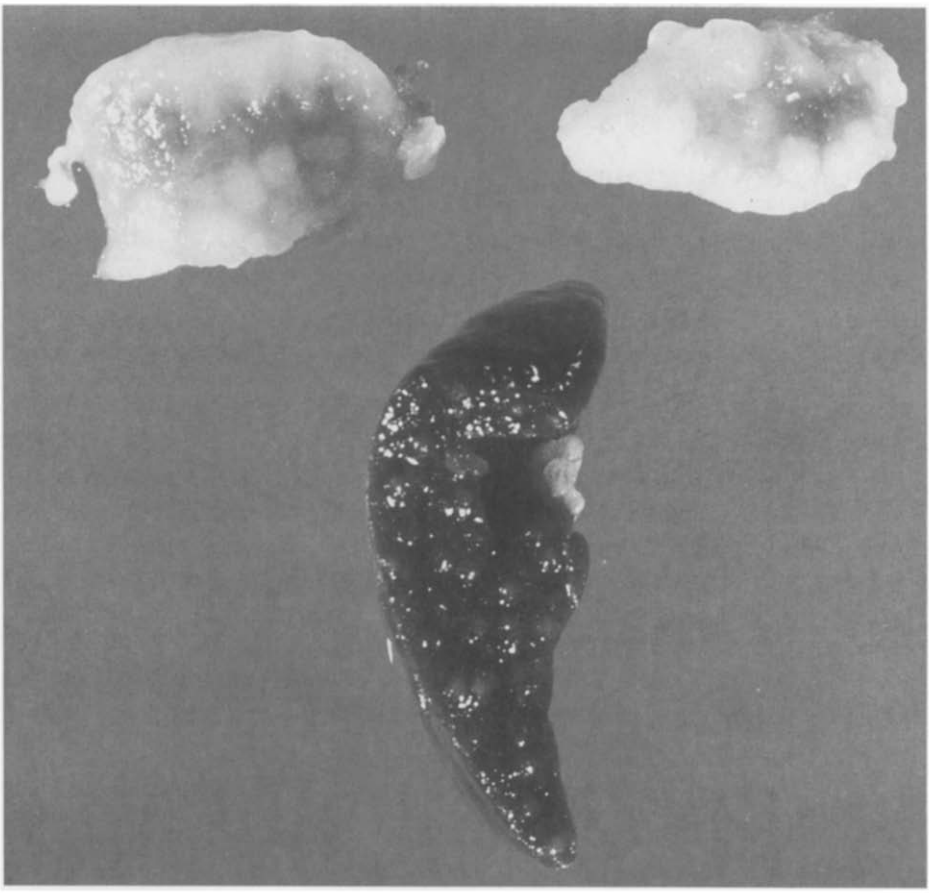

Fig. 1. Case 2B: cross-sections of submandibular lymph node and spleen showing irregular cortical expansion in the node and prominent white pulp in the spleen.

of the germinal centres. Occasionally, germinal centres contained aggregates of small lymphocytes. The parafollicular regions were also expanded and in the early stages of infection were composed predominantly of mature lymphocytes. However, in later stages the population appeared less dense and larger immature cells were more prominent. In addition the parafollicular vasculature (high endothelial blood vessels) became prominent.

Expansion of B- and T-cell areas within the lymph nodes was accompanied by similar expansions in the spleen and mucosal associated lymphoid tissues. The lymphoid tissues of the conjunctiva, stomach, small intestines, large intestines, lung and bladder were prominent. The thymus, although undergoing normal physiological atrophy, developed numerous follicles similar to the germinal centres of the B-cell areas at its corticomedullary junction. Histological examination of the bone marrow core samples revealed that haemo-

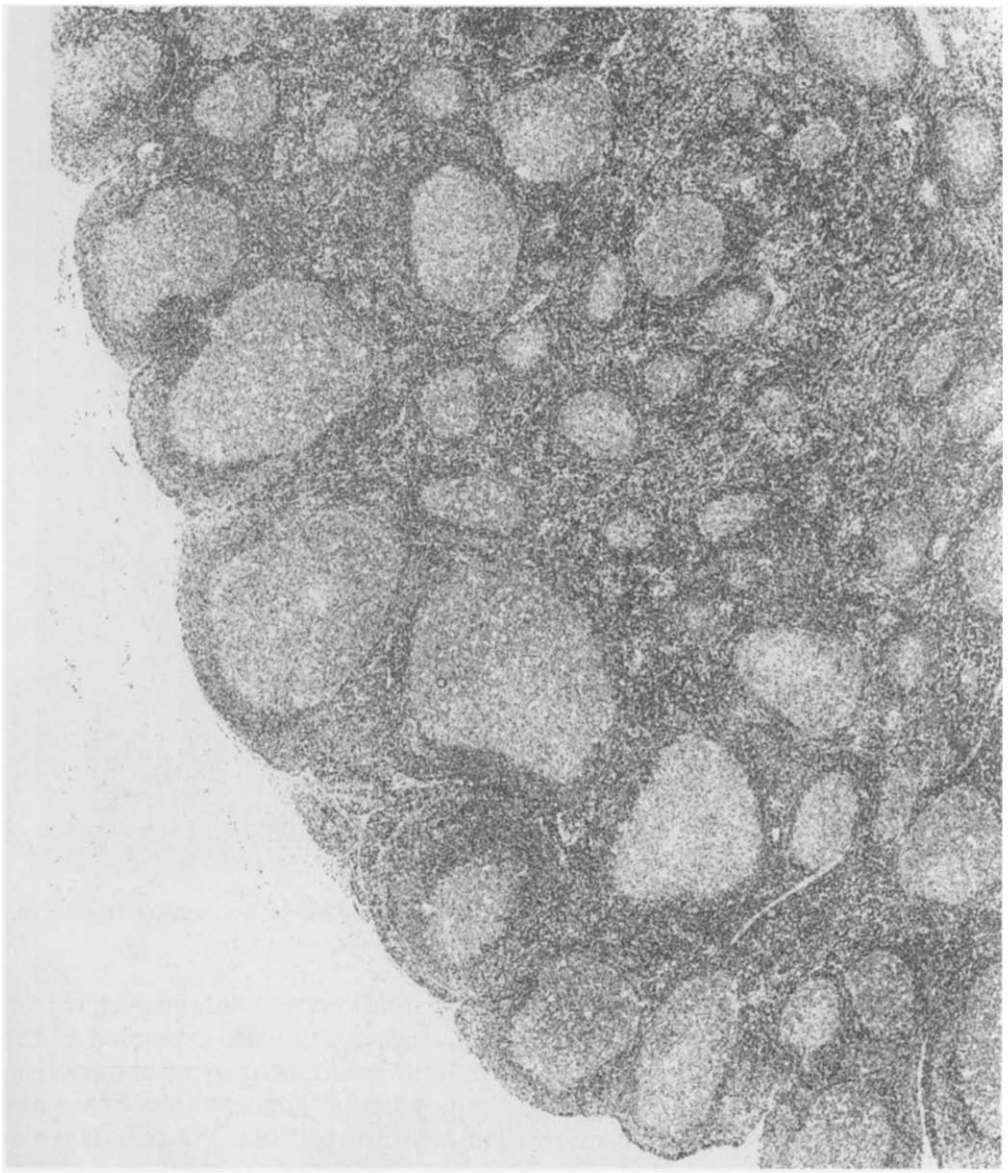

Fig. 2. Case 2B: prominent and irregular follicular expansion in the cortex of submandibular lymph node. (H&E×100).

poiesis was normal with all cell lines represented at all stages of differentiation. The myeloid/erythroid ratios were within normal range and varied from 1.2:1 to 2.4:1. In the cases where neutropenia had been recorded just prior

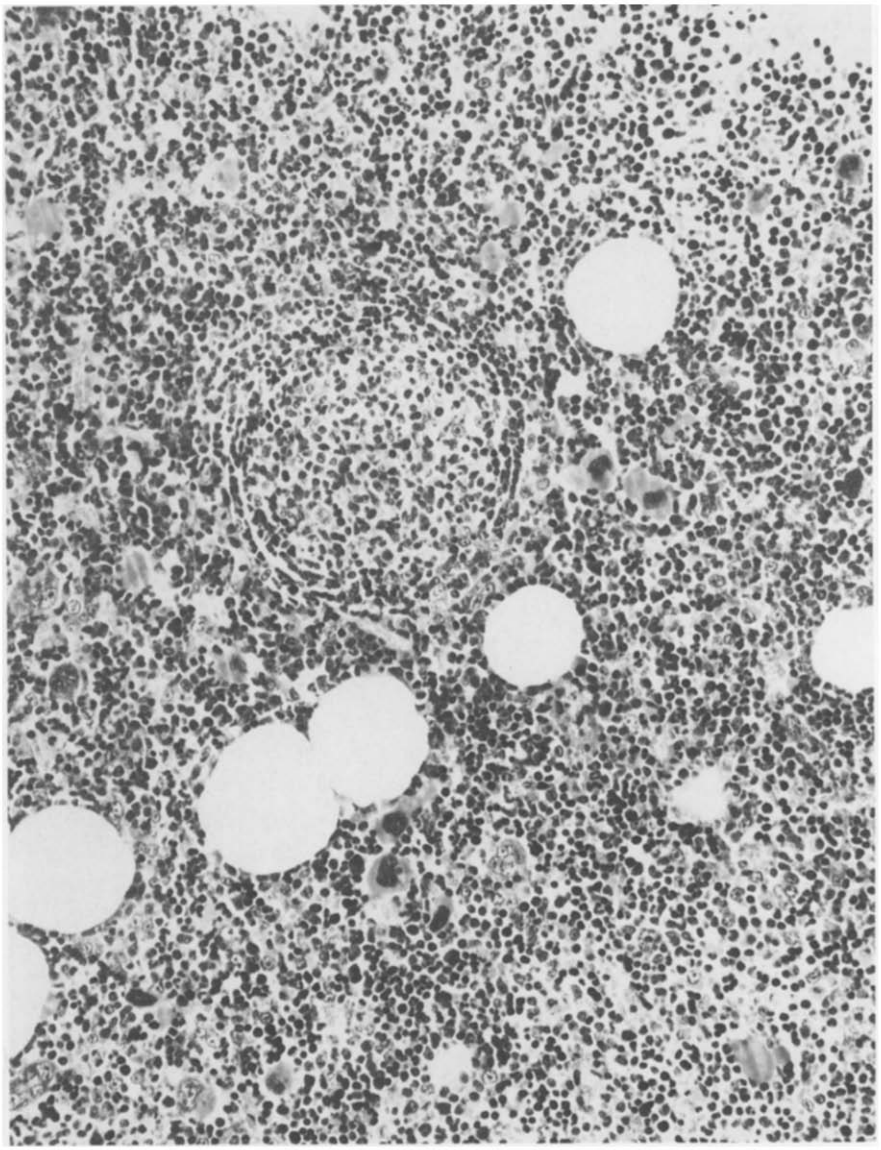

Fig. 3. Case 6B: section of bone marrow with a lymphoid follicle. (H&E×660).

to death, the bone marrow reserve pool of mature neutrophils was partially or totally depleted and the remaining granulocyte precursors were predominantly immature, i.e. they showed a 'left shift'. In one cat (5B) with leucopenia and neutropenia granulopoiesis was abnormal with a maturation arrest

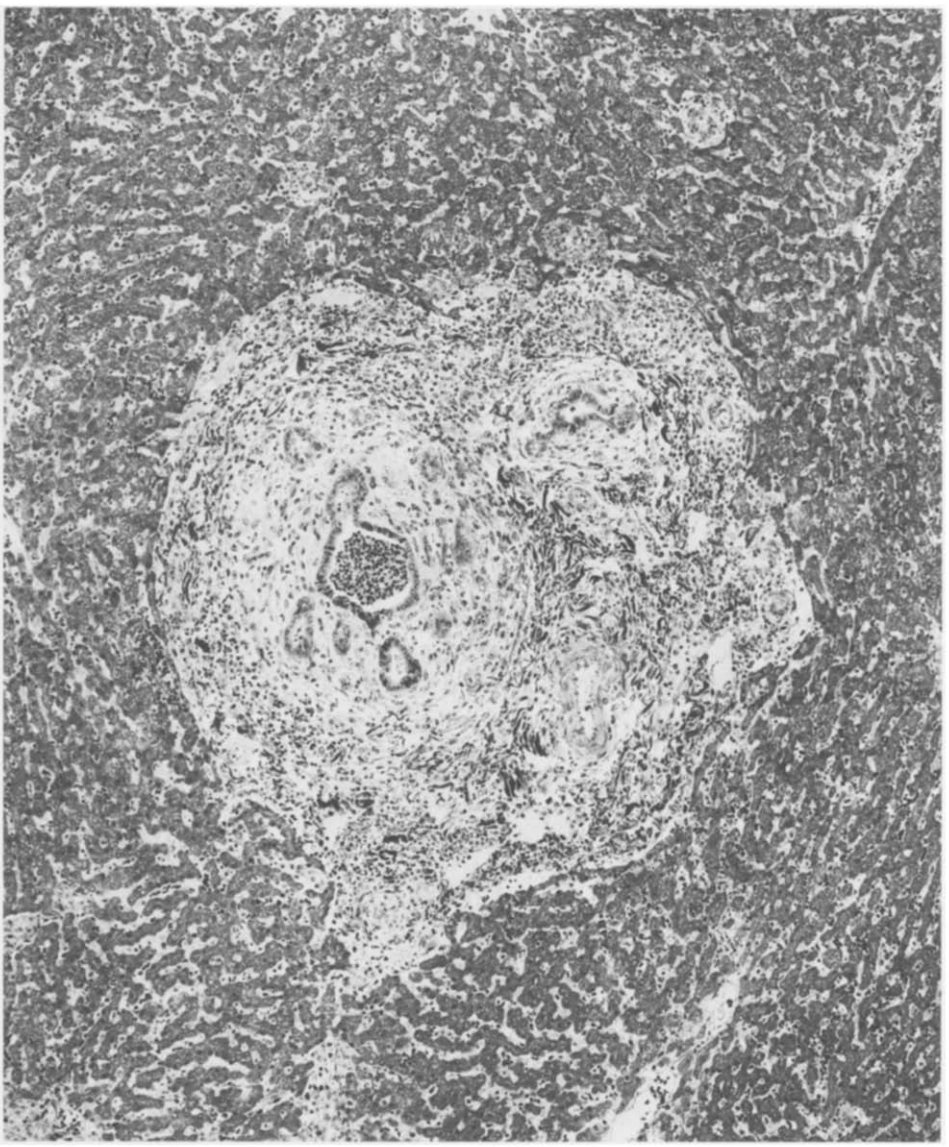

Fig. 4. Case 5B: portal triad with peribiliary fibrosis, irregular bile duct proliferation and plugging of bile ducts by polymorphonuclear leucocytes. (H&E×250).

and vacuolation of precursor cells. Three cats (6B, 7B, 9B) in this study had lymphoid follicles present in their bone marrow (Fig. 3). These follicles were well organised with normal cell morphology. Lymphoid follicles were also

found in the calyx of the kidneys and in one cat (4B) in the parathyroid gland. Aggregates of lymphoid cells were also noted in the perivascular tissue of the choroid and sclera of the eye and around interlobular ducts of the salivary gland.

Case 5B had a dramatic cholangitis with biliary duct hyperplasia and peribiliary fibrosis and microabscessation (Fig. 4). In case 7B, the lesions in the kidney and liver were confirmed to be lymphosarcoma (Callanan et al., 1992).

DISCUSSION

Infection of young adult SPF cats with FIV-GLA-8 causes a syndrome characterised by generalised lymphadenia which commenced 3–6 weeks after infection and could last for greater than 12 months. Coexisting with this generalised lymphadenopathy were episodes of neutropenia, lymphopenia, pyrexia, dullness and anorexia. In addition, one cat became jaundiced with a severe cholangitis and a second cat developed a lymphosarcoma. Yamamoto et al. (1988) have also described a similar primary phase following infection in young kittens characterised by fever, neutropenia and generalised lymphadenopathy. In both experiments mortalities were recorded but with such diverse pathological findings that a common link to FIV was not clear. Yamamoto et al. (1988) described one kitten with a necrotising and pyogranulomatous vasculitis centred around the caecum and mesenteric lymph nodes and a second cat which died from a myeloproliferative disorder.

As observed in previous studies (Yamamoto et al., 1988) the fever and depression in our experimental cats was responsive to antibiotic therapy except for one case (5B) which developed jaundice and cholangitis. It is of interest to note that in our unrelated but similar experimental FIV infections, two cats presented with similar clinical signs of unresponsive fever, neutropenia and leucopenia and ultimately were killed. These two cases were also hyperbilirubinaemic prior to death but unlike 5B they had a generalised hepatic degeneration (unpublished data).

Although the above clinical signs relate to experimental infection there appears to be no direct relationship between the dose of virus administered and the severity of clinical signs. It has been shown previously that a kitten infected by its mother developed these clinical signs (Callanan et al., 1991a) and it is therefore probable that cats naturally infected with FIV must go through similar periods of lymphadenopathy with a proportion of these cats developing pyrexia and dullness. The viral aetiology of such cases could easily be discounted by most veterinarians because of the rapid response to conventional antibiotic therapy.

Haematological abnormalities and myelodysplasia are known to occur in human AIDS patients (O'Hara, 1989). Many studies have been performed to determine the effect of HIV infection on the bone marrow cells but the

pathogenic mechanisms remain unclear (Folks, 1991). The direct or indirect role of FIV infection in the development of feline haematological disorders is not yet known. In this experiment, neutropenia appeared to be consumptive. In Case 5B, the profound neutropenia and lymphopenia was the result of toxic bone marrow suppression and stress. A direct effect of FIV infection on granulopoiesis could not however be ruled out.

To date, limited information is available on the histopathological changes in experimental FIV infections. Yamamoto et al. (1988) observed that lymphadenopathy during the initial stages of FIV infection was a result of lymphoid hyperplasia and follicular dysplasia. Dow et al. (1990) reported on the histopathology of the brain in natural and experimental infected cats and showed that FIV is neurotropic and causes lesions consisting predominantly of perivascular mononuclear cell infiltrates. In this study, lymphadenopathy was also the result of generalised B- and T-cell stimulation. In addition, there was involvement of the non-lymphoid organs with the presence of perivascular aggregates of lymphoid tissue which in many cases became fully formed mature lymphoid follicles.

FIV is a T-lymphotropic virus and studies have centred around the alteration of T-cell subsets, notably the T-helper cells (CD4) (Ackley et al., 1990). There is, however, a major activation of B-cells, and histopathological changes in the primary phase of infection are predominantly B-cell associated. The mechanisms of B-cell stimulation and their antigen and epitope specificity to FIV, as well as the ultimate fate of B-cell aggregates in non-lymphoid organs, have yet to be determined.

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