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Cytokine production by cats infected with feline immunodeficiency virus: a longitudinal study

C. E. LAWRENCE, J. J. CALLANAN, B. J. WILLETT & O. JARRETT *Department of Veterinary Pathology, University of Glasgow, Bearsden, Glasgow*

SUMMARY

The immune responsiveness of cats naturally or experimentally infected with feline immunodeficiency virus (FIV) was studied. Peripheral blood mononuclear cells (PBMC) from naturally infected, symptomatic animals displayed depressed proliferation and interleukin-2 (IL-2) production in response to mitogens, which was accompanied by a significant increase in IL-1, IL-6 and tumour necrosis factor (TNF) production. Longitudinal studies were performed over a period of 4 years in experimentally infected animals. The responses of cells from these cats to concanavalin A (Con A) were consistently less than those from uninfected cats throughout the period but, owing to variation between cats, were significantly lower on only a few occasions. By contrast, the responses of cells to pokeweed mitogen (PWM) were severely affected and declined progressively throughout the 4-year period. In general, responses to Con A but not PWM could be restored by the addition of exogenous IL-2. The decline in immune responsiveness was concurrent with a decline in feline (f)$CD4^+$ cells and an inversion in the CD4 : CD8 ratio. Peak production of IL-1, IL-6 and TNF coincided with periods of depressed immune responses. Additionally, immunodeficient responses and elevated levels of proinflammatory cytokines were concurrent with the presence of clinical signs. We conclude that, like human immunodeficiency virus (HIV), FIV infection results in significant perturbation of the immune response. Responses to PWM appear to correlate with disease progression which suggests that the CD3 pathway is affected in the earlier stages of the disease and that additional activation pathways such as CD2 may not be affected until the animal enters the acquired immune deficient syndrome (AIDS) stage of the disease.

INTRODUCTION

The role of cytokines in human immunodeficiency virus (HIV) infection, the causative agent of acquired immune deficient syndrome (AIDS),[1] has been extensively studied. HIV infection induces major changes in cytokine production which may be crucial for the pathogenesis of the disease.[2] In addition, the cytokines induced also appear to enhance viral replication[3] and the range of cytokines produced may influence the outcome of infection.[4]

Feline immunodeficiency virus (FIV) is a lentivirus of cats resembling HIV in morphology, protein structure, tropism for T lymphocytes and associated immune dysfunction, including decreased responses to mitogens, decreased interleukin-2 (IL-2) production and a decline in numbers of $CD4^+$ T cells.[5–8] The disease progression in cats infected with FIV is similar to that in people with HIV infection. Thus, there is an acute phase of infection characterized by neutropenia, pyrexia, dullness and generalized lymphadenopathy. After this period, cats enter an asymptomatic phase lasting several years which may ultimately lead to clinical immunodeficiency resembling the AIDS-related complex (ARC) and AIDS stages of HIV infection.[9–12] Many of these clinical signs are compatible with the effects of cytokines such as IL-1, IL-6, and tumour necrosis factor (TNF) and determination of the role cytokines play in the immunopathology of experimentally induced FIV infection may be useful in elucidating the mechanisms resulting in the immunological defects of lentiviral infections.

In order to characterize the possible role of cytokines in the development of the immune dysfunction accompanying FIV infection, we examined the immune responses of naturally infected animals in the AIDS-like stage of the disease by assessing the response of their peripheral blood mononuclear cells (PBMC) to mitogens and measuring production of the cytokines IL-1, IL-2, IL-6 and TNF. The PBMC displayed severely reduced proliferation and IL-2 production in response to mitogens, which were accompanied by elevated levels of IL-1, IL-6 and TNF.

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Abbreviations: AIDS, acquired immune deficient syndrome; Con A, concanavalin A; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus; IL, interleukin; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; rhIL-2, recombinant human IL-2; SN, supernatant; SPF, specific pathogen-free; TNF, tumour necrosis factor.

Correspondence: Dr C. E. Lawrence, Department of Veterinary Pathology, University of Glasgow, Bearsden, Glasgow G61 1QH, UK.

To investigate the longitudinal immune responsiveness and the role of cytokines in the development of immunodeficiency, we also examined a group of experimentally infected cats over a period of 4 years.

MATERIALS AND METHODS

Animals

Ten 11–14-week-old specific pathogen-free (SPF) cats were inoculated intraperitoneally (i.p.) with 2×10^3 cat infectious doses of the Glasgow-8 strain of FIV (FIV/GL-8).[13] Five age-matched cats were kept as uninfected controls. Blood samples were collected into heparinized tubes every 2 months. Five symptomatic naturally infected animals, two symptomatic experimentally infected animals and two naturally infected asymptomatic animals killed in our veterinary hospital were also examined. Controls were represented by five healthy SPF animals and three field case animals.

Sample preparation

PBMC were isolated from preservative-free heparinized blood by density gradient centrifugation on Lymphoprep (Nycomed, Birmingham, UK). The cells were washed twice with phosphate-buffered saline (PBS) and once with RPMI-1640 (Gibco, Paisley, UK) supplemented with 10% heat-inactivated fetal calf serum (FCS; Tissue Culture Services, Buckingham, UK) penicillin (100 IU/ml), streptomycin (100 μg/ml; Gibco), L-glutamine (2 mM; Gibco) and 2-mercaptoethanol (2×10^{-5} M; Sigma, Poole, UK), designated as complete medium (CM).

Mitogen-induced proliferation

Cells were cultured in triplicate at 1×10^5 cells/well in a final volume of 200 μl in 96-well flat-bottomed microtitre plates (Costar, High Wycombe, UK), with concanavalin A (Con A; 7·5 μg/ml; Sigma) or pokeweed mitogen (PWM; 1 μg/ml; Sigma) in the presence or absence of rhIL-2 (100 IU/ml). Cells were incubated for 96 hr at 37° in a 5% CO_2 humidified atmosphere and proliferation was measured by [^{3}H]thymidine incorporation (0·5 μCi/well; Amersham Int., Amersham, UK), during the final 16 hr of culture.

Supernatant preparation

1×10^7 cells were incubated in a final volume of 2 ml in 24-well tissue culture plates (Costar) and various exogenous stimuli were added. Con A was used at 7·5 μg/ml or lipopolysaccharide (LPS) at 10 μg/ml (Sigma). Following incubation for 24 hr at 37° in a 5% CO_2 humidified atmosphere, the culture fluids were harvested, cell debris removed by centrifuging for 5 min at 13 000 r.p.m. on an MSE benchtop microfuge, and the supernatants stored at −70° until required.

Assays for feline cytokines

Since assays were not available for feline cytokines, we adapted bioassays originally developed for the measurement of murine IL-1, IL-2, IL-6 and TNF.

Assay for feline IL-1

The murine T-cell subline D10(N4)M[14] was used to detect feline IL-1. Cell growth was maintained in CM with the addition of 3 μg/ml Con A, 40 IU/ml rhIL-2 and 400 pg/ml recombinant murine (rm) IL-1β, and the cells were subcultured 1 : 10 every 3–4 days.

In the assay, serial threefold dilutions of the samples in triplicate were made in flat-bottomed microtitre plates (Costar), using CM to give a final volume of 100 μl. Similar dilutions of the standard (including negative control) were also made in which the initial concentration of rmIL-1β was 270 pg/ml. D10 cells were used on their fourth day of culture, washed three times in CM and resuspended at a concentration of 5×10^4 cells/ml in CM containing 6 μg/ml Con A and 60 U/ml rhIL-2. One hundred microlitres were added to each well of the microtitre plate. The plates were then incubated for 72 hr at 37° in a 5% CO_2 humidified atmosphere. Cell growth was determined by [^{3}H]thymidine incorporation (0·5 μCi/well) in the final 6 hr of incubation. The units of IL-1 were determined by probit analysis at 50% of the rmIL-1β standard.

Assay for feline IL-2

Cells of the murine CTLL line[15] were cultured in CM; growth was maintained by the addition of 100 U/ml rhIL-2, and they were subcultured 1 : 10 every 2–3 days. Assays were carried out as described previously.[16]

Assay for feline IL-6

The IL-6-dependent murine hybridoma line cell line B9 was used.[17] The cells were cultured in CM, growth was maintained by the addition of 1 U/ml rhIL-6, and they were subcultured 1 : 10 every 2–3 days.

To assay IL-6, threefold serial dilutions of sample and standard (initial concentration 9 U/ml), including a negative control, were made in a flat-bottomed microtitre plate to give a final volume of 100 μl in CM. Two-day-old cells were washed twice in medium, resuspended at a concentration of 5×10^4 cells/ml in CM and added in a volume of 100 μl to each well of the microtitre plate. After 3 days culture at 37° in a humidified CO_2 incubator, cell growth was determined by [^{3}H]thymidine incorporation (0·5 μCi/well) in the final 6 hr of incubation. The units of IL-6 were determined by probit analysis at 50% of the rhIL-6 standard.

Assay for feline TNF

The assay for TNF utilized the cytotoxic action of the cytokine on the murine fibroblast cell line, L929.[18] Cells were maintained in CM. Cells were subcultured every 4–5 days using 0·01% trypsin to disperse the culture, and were diluted 1 : 5. Assays were carried out as described previously.[16]

Lymphocyte subset analysis

Feline $CD4^+$ and $CD8^+$ lymphocyte subsets were quantified as described previously.[19]

Statistical analysis

For analysis of the data from the experimentally infected cats, a two-sample Student's *t*-test was used to identify statistical differences between control and infected animals by comparing the average of each control and each infected animal over all the time points examined. Data are presented as responses relative to control values. Differences between control, asymptomatic and symptomatic field cases were compared using the Kruskal–Wallis analysis of variance.

RESULTS

We examined the proliferative responses and cytokine production by PBMC from healthy and symptomatic cats that had been naturally or experimentally infected with FIV.

Sick cats

Nine FIV-infected cats were examined. Seven were naturally infected of which five were seriously ill and two were clinically well (asymptomatic). Three uninfected healthy pet cats served as controls. The other two infected cats were SPF cats that had been inoculated with a vaccine which induced accelerated and enhanced signs of disease 9 weeks following experimental inoculation of FIV.[20]

The clinical findings of the symptomatic animals were weight loss, anorexia, jaundice, pyrexia, gingivitis, dullness, behavioural problems, stomatitis, dermatitis, anaemia, leucopenia, hyperglobulinaemia, lymphadenopathy, tapeworms, roundworms and flea infestation. Although the cats in the asymptomatic phase were generally healthy, one was anaemic with hyperglobulinaemia and lymphadenopathy and the other had chronic gingivitis, aniscoria, hyperglobulinaemia, lymphadenopathy, tapeworms and roundworms. The control animals comprised three uninfected healthy field case animals and five age-matched SPF control animals, which were killed at the end of an experimental vaccine trial.

Responses of PBMC from terminally ill FIV-infected cats to mitogens. The responses of PBMC from terminally ill cats to Con A and PWM were assessed (Table 1). PBMC from both naturally and experimentally infected symptomatic animals showed significantly depressed responses to Con A in the presence or absence of IL-2. Cells from the asymptomatic animals only had depressed responses in the absence of rhIL-2.

Table 1. Proliferative responses of PBMC from uninfected and FIV-infected cats to the mitogens Con A and PWM in the presence and absence of 100 U/ml rhIL-2

	Uninfected cats		FIV-infected cats		
Sample	Field case (n = 3)	SPF (n = 5)	Asymptomatic field case (n = 2)	Symptomatic experimental (n = 2)	Symptomatic natural (n = 5)
Responses to mitogens					
Con A (7·5 μg/ml) (c.p.m.)	39 340 ± 875	32 873 ± 2775	10 191 ± 6817*	62 ± 1*†	143 ± 77*†
Con A + IL-2 (7·5 μg/ml; 100 U/ml) (c.p.m.)	52 225 ± 3447	41 132 ± 2813	22 482 ± 12 943	183 ± 115*†	1678 ± 984*†
PWM (1 μg/ml) (c.p.m.)	9072 ± 3621	14 217 ± 1536	7616 ± 2602	46 ± 5*†	89 ± 27*†
PWM + IL-2 (1 μg/ml; 100 U/ml) (c.p.m.)	17 914 ± 7473	21 488 ± 2106	15 085 ± 8942	77 ± 19*†	551 ± 380*†
Cytokine production					
IL-2 (Con A SN) (IU/ml)	46·1 ± 9·7	41·05 ± 8·4	37·5 ± 14·5	6·9 ± 1·4*†	2·92 ± 1·8*†
IL-1 (LPS SN) (pg/ml)	376 ± 136	677 ± 135	2148 ± 656*	7433 ± 992*†	5999 ± 1622*†
IL-6 (LPS SN) (IU/ml)	8·3 ± 4	2·36 ± 0·45	32·3 ± 7·5*	102 ± 8*†	113 ± 56*†
TNF (plasma) (IU/ml)	1·5 ± 0·4	1·76 ± 0·74	4·3 ± 0·8*	47·8 ± 5·3*†	52·9 ± 14·1*†

Cytokine production by Con A- or LPS-stimulated PBMC from FIV-infected and uninfected cats. Results are presented as means ± 1 SE. Statistical comparison was performed using Student's t-test.

* Significantly different from both uninfected field case cats and SPF cats ($P < 0·05$).

† Significantly different from asymptomatic field case cats ($P < 0·05$). There was no significant difference between the two groups of uninfected cats or between the two groups of symptomatically infected cats.

A similar pattern was found in response to PWM but asymptomatic animals also had depressed proliferation, even in the presence of exogenous rhIL-2. There was no significant difference between the responses of healthy field or experimental uninfected cats to either Con A or PWM in the presence or absence of IL-2.

Cytokine production by PBMC from terminally ill FIV-infected cats. Production of IL-2 by PBMC was reduced in both experimentally and naturally infected cats, but the levels in asymptomatic cases were not as profoundly depressed as those of the symptomatic cases. Production of IL-1, IL-6 and TNF was substantially elevated in all of these animals, with even higher levels in both symptomatic cases than in asymptomatic cases (Table 1). There were no significant differences in cytokine production between the healthy field case animals and the SPF animals.

Experimental infection

As our studies of naturally infected cats had indicated defects in both proliferation and cytokine production in response to mitogens, we assessed the onset and duration of immunodeficiency in FIV-infected cats. Ten 11–14-week-old kittens were infected with FIV and their responses to mitogens and cytokine production were monitored at intervals over a period of 4 years.

Responses of PBMC of experimentally FIV-infected cats to mitogens. The responses of PBMC from experimentally infected cats to Con A in the presence of exogenous IL-2 (Fig. 1a) were significantly depressed compared to the uninfected controls between 12 and 24 weeks post-infection, and again at 152 weeks post-infection. Responses to Con A

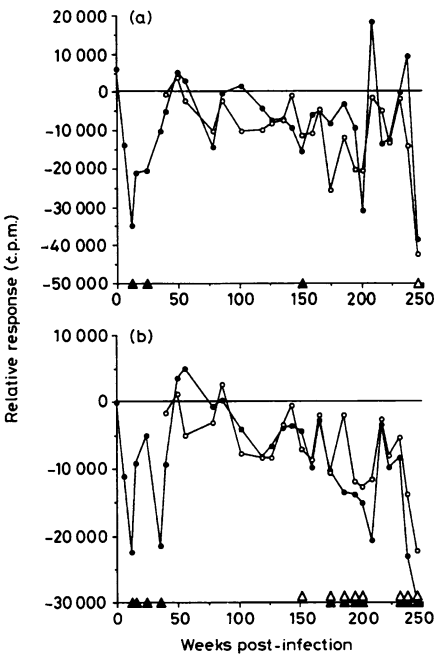

Figure 1. Proliferative responses of feline PBMC to the mitogens Con A (a) and PWM (b) in the presence (●) and absence (○) of 100 U/ml rhIL-2. Results are presented as responses of infected cats relative to control cats. Statistical comparison of uninfected versus FIV-infected cats was performed using Student's *t*-test. (▲) Significantly different in the presence of IL-2, $P < 0{\cdot}05$; (△) significantly different in the absence of IL-2, $P < 0{\cdot}05$.

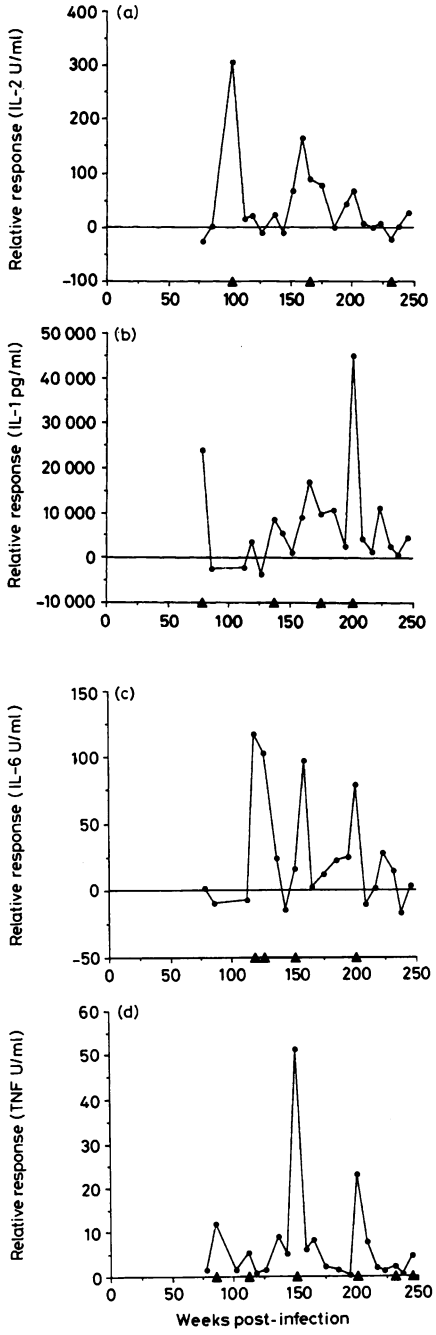

Figure 2. Cytokine production by stimulated feline PBMC. (a) IL-2 was assayed in the supernatants of Con A-stimulated PBMC using CTLL target cells. (b) IL-1 was assayed in the supernatants of LPS-stimulated PBMC using D10(N4)M cells. (c) IL-6 was assayed in the supernatants of LPS-stimulated PBMC using B9 target cells. (d) TNF was measured in plasma using L929 target cells. Results are presented as responses of infected cats relative to control cats. Statistical comparison of uninfected versus FIV-infected cats was performed using Student's *t*-test. (▲) Significant $P < 0{\cdot}05$.

Table 2. T-lymphocyte subsets in FIV-infected and uninfected SPF cats at various time points after infection

Weeks post-infection	Uninfected cats			FIV-infected cats		
	Feline $CD4^+$ (cells/ml)	Feline $CD8^+$ (cells/ml)	CD4:CD8	Feline $CD4^+$ (cells/ml)	Feline $CD8^+$ (cells/ml)	CD4:CD8
67	1450 ± 470	1000 ± 280	1·32 ± 0·27	1110 ± 190	1510 ± 220	0·74 ± 0·05*
119	1450 ± 457	1405 ± 531	1·081 ± 0·26	692 ± 129*	1118 ± 206	0·598 ± 0·07*
157	1304 ± 281	995 ± 149	1·435 ± 0·51	583 ± 178*	757 ± 182	0·754 ± 0·024*
243	1367 ± 497	1330 ± 417	1·039 ± 0·183	435 ± 50*	658 ± 110*	0·764 ± 0·141*

Peripheral blood T lymphocytes were quantified by flow cytometry and absolute cell numbers calculated from the lymphocyte count; ratios were calculated by dividing the absolute CD4 number by that for CD8. Cell numbers are expressed as cells/mm^3 ± 1 SE. Statistical comparison of uninfected versus infected was performed using Student's *t*-test.

* Significant $P < 0·05$.

were depressed at 246 weeks post-infection in the absence of exogenous IL-2. From 40 weeks post-infection the addition of exogenous IL-2 appeared to increase the responses of infected animals to levels approaching control animals, inasmuch as there were no significant differences between the responses of cells from infected or control animals.

Depressed responses of PBMC from infected cats to PWM in the presence of exogenous IL-2 (Fig. 1b) were apparent 12–36 weeks, 102 and 166 weeks, 175–201 weeks and again 232–246 weeks post-infection. In this case the addition of exogenous IL-2 did not significantly elevate the responses of control animals. Responses to PWM by the cells of infected animals in the absence of IL-2 were significantly depressed compared to control animals at 152, 175–201 and 232–246 weeks post-infection. Over the whole of the 4-year period there was a progressive decline in the response to PWM.

No data are available for the responses of cells to mitogens in the absence of IL-2 prior to 40 weeks post-infection, as insufficient cells could be obtained from the young cats for the assay. Data on cytokine responses are also absent as the assays were developed during the study.

Cytokine production by experimentally FIV-infected cats. IL-2 production by PBMC following Con A stimulation was reduced in infected animals at 78 and 232 weeks post-infection, but was significantly elevated 102 and 166 weeks post-infection (Fig. 2a). Further analysis revealed that cells from the animals producing elevated levels of IL-2 were also secreting substantial quantities in the absence of Con A (data not shown).

IL-1 production (Fig. 2b) by LPS-stimulated PBMC from infected animals was significantly elevated in comparison with controls at 78, 137, 175 and 201 weeks after infection. The peak production of IL-1 correlated with the depressed responses to Con A and PWM.

IL-6 production by LPS-stimulated PBMC from infected cats was significantly elevated at 119, 127, 152 and 201 weeks after infection (Fig. 2c). Again, these events coincided with depressed responses to mitogens and elevated IL-1 levels.

Levels of plasma TNF (Fig. 2d) were significantly elevated 86, 119, 152, 201, 232 and 246 weeks post-infection, at a time when levels of IL-1 and IL-6 production were at their greatest and when responses to both Con A and PWM were depressed.

Lymphocyte subset analysis. Feline $CD4^+$ and $CD8^+$ lymphocyte subsets were quantified in the peripheral blood of uninfected and infected cats at 67, 119, 157 and 243 weeks post-infection (Table 2). Infected cats had significantly reduced $CD4^+$ cell numbers compared to the controls at 119, 157 and 243 weeks post-infection, while $CD8^+$ cell numbers were only depressed at the last time point. The CD4:CD8 ratio was significantly depressed at each time point examined.

Clinical signs of cats experimentally infected with FIV. All of the infected animals developed generalized lymph node enlargement between 3 and 6 weeks post-infection, which lasted for up to 1 year. All cats had periods of fever and dullness, sometimes accompanied by periods of gingivitis and conjunctivitis. Five cats developed pyrexia and fever, which was accompanied by dullness, conjunctivitis and gingivitis between 5 and 24 weeks post-infection. All cats had periods of leukopenia consisting of neutropenia and lymphopenia. All were hyperglobulinaemic and some also had hyperpróteinaemia and hyperbilirubinaemia.

DISCUSSION

The results from this study support the hypothesis that cytokines contribute to the immunopathogenesis of lentiviral infections. PBMC from clinically ill infected animals had significantly depressed IL-2 production and proliferative responses to both PWM and Con A that coincided with significantly enhanced production of IL-1, IL-6 and TNF. Experimental infection of cats with FIV resulted in periods of increased production of IL-1 and IL-6 produced by stimulated PBMC, and of elevated plasma TNF which coincided with periods of immunodepression, as assessed by depressed IL-2 production and proliferative responses by PBMC in response to both Con A and PWM. Feline $CD4^+$ and $CD8^+$ numbers also declined, resulting in an inversion in the CD4:CD8 ratio. Clinical signs including gingivitis, conjunctivitis, lymphadenopathy and fever accompanied periods of immunodeficiency in both asymptomatic and symptomatic infected cats.

These results extend the findings of our previous study on the early stage of infection, in which we demonstrated defective immune responses and elevated plasma TNF in cats as early as 20 days following experimental infection with FIV.[16] Like other studies which have demonstrated depressed immune responses in both symptomatic and asymptomatic infected animals,[6,7,21,22] we show that during the asymptomatic stage of

the infection the response to Con A appears to fluctuate, while that to PWM appears to decline progressively. By continuing to study our experimentally infected cats we expect in the future to determine the pattern of responses accompanying the progression of the disease from an asymptomatic state to a symptomatic state.

Elucidation of the alteration in cytokine production following FIV infection is a crucial step in determining the immunopathological mechanisms involved in lentiviral disease progression. Acute, primary HIV-1 infection is characterized by low IL-2 production and increased secretion of IL-1β and IL-6. This is accompanied by $CD4^+$ lymphocytopenia and elevated numbers of $CD8^+$ and natural killer (NK) cells.[23] Increased levels of IL-1, IL-6 and TNF during infection with HIV may be responsible for some of the clinical signs of the disease such as hypergammaglobulinaemia, B-cell malignancies, influenza-like symptoms, fever and wasting.[24–26] Furthermore, these molecules are also important in the control of viral replication: TNF increases viral replication by activating NF-kB, a transcription factor, while both IL-1 and IL-6 appear to synergize with TNF to increase viral replication further.[3]

Like patients infected with HIV, cats infected with FIV display hypergammaglobulinaemia,[27] polyclonal B-cell activation, autoantibody production[28] and an increased incidence of lymphosarcoma,[29] all of which could be attributed to increased IL-6 production. Elevated levels of IL-1, IL-6 and TNF in conjunction with depressed levels of IL-2 production are also associated with fever, wasting, and influenza-like symptoms, many of which were present in infected animals and are characteriztic signs of FIV infection.

Unlike HIV, in which IL-2 production becomes depressed, IL-2 levels in FIV-infected cats were elevated in asymptomatic, experimental animals and a number of animals was also found to be producing IL-2 constitutively despite depressed numbers of $CD4^+$ lymphocytes. The reasons for this are unclear. Despite increased IL-2 production, and relatively preserved responses to Con A, it was clear that PWM-induced proliferation was significantly depressed. Indeed, there appeared to be a progressive decline in the responsiveness to PWM over the time period examined.

During infection with HIV it has been proposed that immune dysfunction is a result of a defect in the CD3 activation pathway in the asymptomatic phase, and that during the AIDS stage the CD2 activation pathway is additionally affected. Binding of a ligand to CD3 induces cells from the resting G_0 phase to the activated G_1 phase. However, a second signal delivered by binding to CD2, or the addition of exogenous IL-2, is required for the induction of cellular proliferation.[30] A similar perturbation of the immune response may be occurring during FIV infection, as depressed responses to PWM accompanying the asymptomatic stage is characteriztic of a defect in the CD3 activation pathway. Decreased responses to both Con A and PWM and depressed IL-2 production suggest that an additional activation pathway, such as CD2, was also affected during the symptomatic stages.

The factors responsible for initiating a switch from asymptomatic to symptomatic disease expression in lentiviral infections have yet to be resolved. Recent evidence has suggested that a change in the tropism of the virus may be important.[31,32] Alternatively, it has been proposed that changes in T-helper type-1 (Th1) and Th2 T-helper cell responses may mediate disease progression during HIV infection.[33] Patients with Th1-type responses, characterized by secretion of IL-2 and interferon-γ (IFN-γ) remain asymptomatic whereas those that switch from Th1 to Th2, as indicated by a fall in IL-2 production and rise in IL-4 production, go on to develop AIDS.[2] It has also been demonstrated that responses to HIV antigen by the cells of patients in the symptomatic stages of the disease, which could not be restored by the addition of exogenous IL-2 *in vitro*, were restored by the addition of exogenous IL-12.[34] It was suggested that this cytokine was acting to push the response from Th2 (symptomatic) back to Th1 (asymptomatic), as it promotes Th1 cell expansion by suppressing macrophage IL-10 production.[35]

Similar mechanisms of immune dysfunction, involving a switch from Th1 to Th2 helper T-cell responses, may be occurring during FIV infection. We have shown that cats in the terminal stages of infection produce low levels of IL-2 (a Th1 cytokine) and high levels of IL-6 (a Th2 cytokine), whereas those with high levels of IL-2 and low levels of IL-6 remain asymptomatic. However, further studies monitoring changes in levels of IL-4, IFN-γ and antibody isotype during infection with FIV are required before a definite role for T-helper subsets in the immunopathology of FIV infection can be determined.

In conclusion, we suggest that, like HIV, FIV produces a significant perturbation of cytokine production that may be responsible for the immune dysfunction and immunologically mediated pathology associated with this disease. Additionally, this study shows that FIV is an excellent model for HIV infection as the role of cytokines in lentiviral infection can be studied on a longitudinal basis in the absence of opportunistic infections. These findings may also be of clinical use. Firstly, monitoring of cytokine levels in cats infected with FIV may be useful in predicting the onset of the clinical signs of the disease, so that decisions may be made about the management of the cat. Secondly, the FIV system may be useful for developing techniques for immunotherapeutic intervention. For example, antibodies and/or antagonists to cytokines may be useful in decreasing viral replication and subsequent infection of cells and also in preventing the clinical effects of elevated cytokine levels such as fever, wasting and generation of autoantibodies. Finally, these results should be taken into consideration when designing potential vaccines where it may be important to produce vaccines that preferentially activate a protective Th1 response, as stimulation of macrophages and/or Th2 cells may accelerate or enhance disease upon challenge, as has already been demonstrated with certain FIV vaccines.[20]

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