

Feline Immunodeficiency Virus (FIV) Infection in the Cat as a Model for HIV Infection in Man: FIV-Induced Impairment of Immune Function

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ABSTRACT

To assess the value of feline immunodeficiency virus (FIV) infection as a model for human immunodeficiency virus (HIV) infection in man, we studied the impairment of certain immunological functions following natural or experimental FIV infection. Proliferative responses of peripheral blood mononuclear cells (PBMC) from symptomatic and asymptomatic cats after naturally or experimentally acquired FIV infection, induced by activation with the mitogens concanavalin A, pokeweed mitogen, or lipopolysaccharide or by stimulation with human interleukin-2 (IL-2), were significantly lower than the proliferative responses found with PBMC from noninfected control cats. Also IL-2 production levels of mitogen-activated PBMC from naturally infected symptomatic cats were significantly reduced. These data confirm that the pathogenesis of FIV infection in the cat, like HIV infection in man, is characterized by a serious malfunction of the immune system.

INTRODUCTION

SINCE THE DISCOVERY OF human immunodeficiency virus type 1 (HIV-1) as the cause of acquired immunodeficiency syndrome (AIDS) in 1983,^{1,2} it was realized that there is an urgent need for animal models to study the pathogenesis of HIV-1 infection and possibilities for interventional strategies. Apart from chimpanzees and gibbons, no animal species can be infected with HIV-1, and these apes do not develop clinical symptoms similar to AIDS upon experimental infection.^{3,4} However, it has been shown that certain monkey species can be infected with HIV-2 and with a number of simian immunodeficiency viruses (SIV), and that they may subsequently develop an AIDS-like syndrome (for review see Ref. 5). Recently, a lentivirus of the cat, feline immunodeficiency virus (FIV), has been identified in cats with an AIDS-like syndrome.⁶ Since cats are less expensive and easier to handle than monkeys, the FIV

infection model may be of special interest for testing the potential of antiviral drugs and vaccination strategies in relation to HIV infection in man.

The knowledge about the pathogenesis of FIV infections is limited at present (for review see Refs. 7,8). Although infection under natural circumstances seems to be associated with the development of chronic disease symptoms eventually leading to AIDS-like disease, it is difficult to reproduce the disease by experimental infection of cats.⁹ Therefore, we now have undertaken studies regarding the functioning of the immune system of cats following natural or experimental infection with FIV. The progression to AIDS in man following HIV-1 infection is characterized by the gradual impairment of *in vivo* and *in vitro* immune functions.¹⁰⁻¹² Specifically, loss of mitogen-induced lymphocyte proliferation is seen shortly after infection in asymptomatic individuals.^{13,14} In this report, we present studies on mitogen and interleukin-2- (IL-2) induced proliferative re-

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sponses and IL-2 production of peripheral blood mononuclear cells (PBMC) of cats after infection with FIV.

MATERIALS AND METHODS

FIV-infected and control animals

Four different groups of cats were used in these studies. Fifteen private household cats were identified as seropositive for FIV in an enzyme-linked immunosorbent assay (ELISA).¹⁵ Five of these cats (3–9 years old), designated Group S, showed clinical signs of chronic disease suggestive of feline AIDS.⁹ The other ten (2–6 years old), designated Group A, were healthy at the time of sampling. Twelve specific pathogen-free (SPF) cats (1.5–3 years old), designated Group E, had previously been experimentally infected subcutaneously with a Dutch field isolate of FIV ($n = 10$) or the Petaluma strain ($n = 2$),⁶ 12 to 30 months before sampling. These animals were negative for antibodies against a number of viruses which commonly infect cats, including feline leukemia virus (FeLV), feline panleukopenia virus (FPV), feline calici virus (FCaV), feline herpes virus-1 (FHV-1), feline syncytium-forming virus (FeSFV), and feline corona virus (FCV) as demonstrated in regular serological screening procedures. The last group consisting of 25 private household cats (1.5–10 years old) seronegative for FIV, was designated Group C and served as a noninfected control group. Comparison of the data obtained in Group C for proliferative responses and IL-2 production upon mitogen stimulation to data obtained previously in our lab for a group of SPF cats ($n = 4$) demonstrated no significant differences between these two groups (data not shown). Therefore, only non-SPF cats were used as controls in these assay systems.

Sampling

Blood samples were collected once from cats of Group S and Group A. From 12 experimentally infected cats (Group E) blood samples were collected monthly for determination of lymphocyte counts and once, 0.5 to 2 years after infection, to assess the mitogen and IL-2-induced proliferation and IL-2 production of PBMC. Peripheral blood mononuclear cells (PBMC) were isolated from preservative-free heparinized blood by density gradient centrifugation on Ficoll-Isopaque. The cells were washed three times with RPMI-1640 (Gibco, Grand Island, NY), supplemented with penicillin (100 IU/ml), streptomycin (100 μ g/ml), L-glutamine (2 mM), and β -mercaptoethanol ($2 \cdot 10^{-5}$ M), hereafter designated as culture medium (CM). The lymphocyte counts from the experimentally infected cats were determined monthly. The lymphocyte counts from the three other groups of cats were determined once.

Mitogen-induced proliferation

PBMC (10^5 cells/well) were cultured at 37°C, 5% CO₂, in a humid environment in CM, supplemented with 10% heat-inactivated calf bovine serum (FCS) with concanavalin A (ConA) (5 μ g/ml) (Flow Laboratories) or pokeweed mitogen (PWM) (1 μ g/ml) (Flow Laboratories) for 72 h or lipopolysaccharide (LPS) (50 μ g/ml) (*Escherichia coli* 0.27:B8, Difco

Laboratories) for 96 h in round-bottomed tissue culture plates using unstimulated cells as a control. The optimum concentration of mitogens and time of culture were first defined by dose-response curve. Proliferative responses were measured by [³H]-thymidine incorporation (1 μ Ci/culture). Results presented are the mean of triplicate wells. Differences were found significant for $p < 0.01$ as calculated according to the Student's *t*-test.

Proliferative response to exogenous IL-2

The response of PBMC to exogenous IL-2 was tested using a method similar to the one described by Goitsuka et al.¹⁶ PBMC (10^6 cells/ml) were incubated with ConA (2 μ g/ml) for 1 h at 37°C in CM, supplemented with 10% FCS in 12 \times 75 mm culture tubes (Falcon, catnr. 2085, Becton and Dickinson, E. Rutherford, NJ). The cells were washed three times with CM and cultured for 96 h with 100 IU recombinant human IL-2/ml (rIL-2) (Boehringer, Mannheim, Germany) in 96 wells flat-bottomed tissue culture plates. Sixteen hours before harvesting, the cells were pulsed with [³H]-thymidine to measure their rate of proliferation, using unstimulated cells as control. The stimulation index was calculated by dividing the cpm of the IL-2-stimulated culture by the cpm of the unstimulated culture. Differences were found significant for $p < 0.01$ as calculated according to Student's *t*-test.

IL-2 production

Production of IL-2 was measured using the method described by Goitsuka et al.¹⁶ PBMC (10^5 /well) were cultured with or without ConA (10 μ g/ml) in CM supplemented with 10% FCS in 96-well round-bottomed culture plates. After 24 h, the culture supernatants were tested for the presence of IL-2, using an IL-2-dependent cloned murine cytotoxic T-cell line (CTLL): CTLL cells ($5 \cdot 10^3$), in the logarithmic phase, were seeded into 96 wells of round-bottomed microtiter plates. Twofold serial dilutions of the culture supernatants were added. After three days the cells were pulsed with [³H]-thymidine and after 16 h harvested and counted in a scintillation counter (LKB). The units of IL-2 were determined by probit analysis at 50% of the rIL-2-standard (Boehringer). In preliminary experiments optimal conditions for cell concentration, dosage of mitogens, and culture time were determined. Differences were found significant for $p < 0.01$ as calculated according to Student's *t*-test.

RESULTS

Mitogen-induced proliferative responses of PBMC

To assess the effect of FIV infection on the immune system, we studied the proliferative responses to various mitogens of PBMC from symptomatic and asymptomatic cats infected with FIV. The data are presented in Figure 1. ConA-induced proliferative responses of PBMC from five naturally infected cats with clinical symptoms (Group S) were significantly lower than the responses of PBMC from 25 control cats (Group C). Also for 10 naturally and 12 experimentally infected FIV seropositive cats without apparent clinical symptoms (Groups A and E), significantly lower proliferative responses were found. The geometric

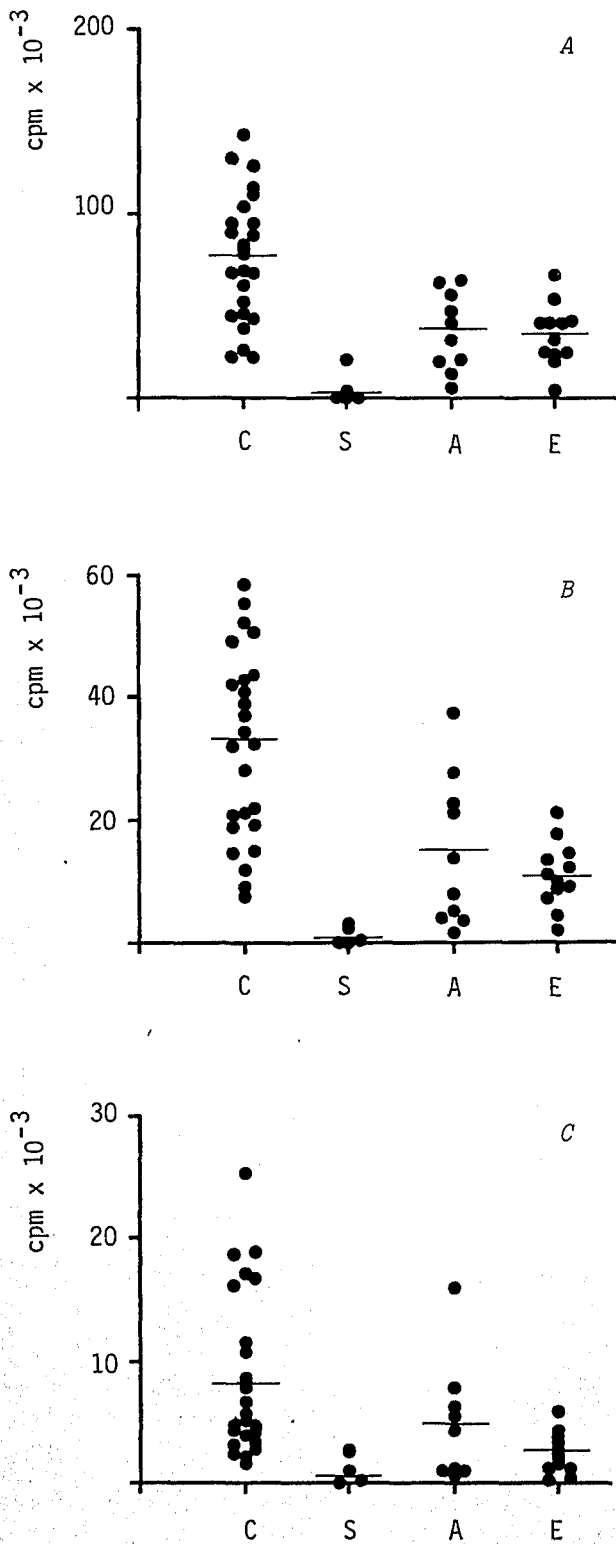


FIG. 1. Mitogen-induced proliferative responses of PBMC from the control (C), naturally infected symptomatic (S), and naturally infected asymptomatic (A) and experimentally infected asymptomatic (E) cats. Proliferative responses induced by ConA (A), PWM (B), and LPS (C) are expressed as counts per minute of tritiated thymidine incorporation.

means of the values found for the control group, the symptomatic, and the two asymptomatic seropositive groups were 75,769, 5,151, 35,834, and 33,908 cpm, respectively (Fig. 1A). Similar results were found for PWM- and LPS-induced proliferative responses: PBMC from the 5 naturally infected cats with clinical symptoms and from the 10 naturally and the 12 experimentally infected asymptomatic cats showed significantly lower PWM- and LPS-induced proliferative responses than the PBMC from the 25 control cats. The geometric means of the values found upon stimulation with PWM for the control group, the symptomatic, and the two asymptomatic seropositive groups were 31,918, 1,159, 14,348, and 10,829 cpm, respectively (Fig. 1B). Following stimulation with LPS the geometric means of the values found for the control group, the symptomatic, and the asymptomatic seropositive groups were 8,399, 1,286, 4,802, and 2,382 cpm, respectively (Fig. 1C).

IL-2 production and proliferative responses to exogenous IL-2

The impaired responses to mitogens in FIV-infected cats could be due either to reduced IL-2 production or to a reduced response to the IL-2 produced. Therefore, IL-2 production and proliferative responses to exogenous IL-2 by PBMC from cats were measured.

ConA-induced production of IL-2 by PBMC from the 5 naturally infected cats with clinical symptoms and from the 12 experimentally infected cats was significantly lower than the production of IL-2 by PBMC from the 25 control cats. No significant differences were found when the ConA-induced production of IL-2 by PBMC from the 10 naturally infected cats without apparent clinical symptoms were compared with the IL-2 production by PBMC from the 25 control cats. The geometric means of the IL-2 production found for the control group, the symptomatic seropositive group, and the two asymptomatic groups were 13, 1, 16, and 9 IU IL-2/ml, respectively (Fig. 2). No IL-2 production (< 0.2 U/ml) could be measured in the unstimulated cultures.

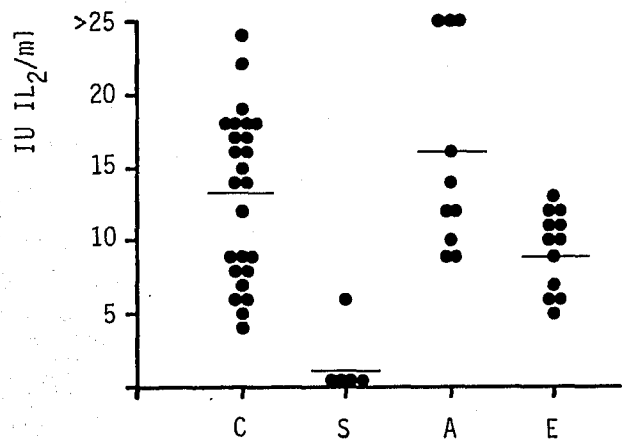


FIG. 2. IL-2 production, after stimulation with ConA, of PBMC from control (C), naturally infected symptomatic (S), naturally infected asymptomatic (A), and experimentally infected (E) cats. Results are expressed as international units (IU) of IL-2 per ml.

The proliferative responses of ConA-activated PBMC from the 5 naturally infected cats with clinical symptoms to a saturating amount of human recombinant IL-2 (100 IU IL-2/ml) were significantly lower than the responses of the PBMC from the 25 control cats. Also, for PBMC from the 10 naturally and the 12 experimentally infected asymptomatic cats, significantly lower proliferative responses to IL-2 were found. The geometric means of the control group increased from 5,917 cpm to 25,088 cpm upon cultivation in the presence of IL-2 (Fig. 3A). For the naturally and experimentally infected seropositive nonsymptomatic cats, these values increased from 1,153 to 12,958 cpm and from 3,466 to 18,522 cpm, respectively.

The geometric mean cpm of the PBMC from naturally infected symptomatic cats was 139 cpm and did not increase significantly when the cells were cultured in the presence of IL-2. The stimulation indices of these cultures were 4.2, 11.3,

5.4, and 1.4, respectively. Similar results were obtained when PBMC were not activated with ConA prior to the addition of IL-2, although the differences in proliferative responses found between the respective groups were less pronounced but the stimulation indices remained the same (Fig. 3B).

Development of lymphopenia

All 5 of the naturally infected cats with clinical symptoms (Group S), showed lymphopenia ($< 1.5 \times 10^{12}$ lymphocytes/L blood) at the time of sampling. No lymphopenia was found in the naturally FIV-infected cats without clinical symptoms (Group A). Of the experimentally infected cats, only the two animals that were infected with the Petaluma strain showed lymphopenia, developing within four months after infection,¹⁷ and which persisted throughout the two years of observation. Similar lymphopenia was never observed in the cats experimentally infected with the Dutch FIV strain during a followup period of 2 years (manuscript in preparation). All the mitogen- and IL-2-induced proliferative responses and the ConA-induced IL-2 production of the PBMC of these two cats were significantly lower than those of the nonlymphopenic animals in group E.

DISCUSSION

We have studied *in vitro* parameters of the feline immune system following natural or experimental infection with FIV. Proliferation responses of PBMC from symptomatic and asymptomatic cats after natural or experimental infection with FIV, induced by activation with mitogens or human IL-2, were significantly lower than those of noninfected cats. Similarly, IL-2 production levels of PBMC from symptomatic and experimentally infected asymptomatic cats were significantly lower, whereas the production of IL-2 by PBMC from asymptomatic naturally infected cats was unchanged. It may be concluded that the immune function of the symptomatic, and also of the asymptomatic FIV seropositive cats studied, was seriously impaired. All of these parameters are generally also reduced in HIV seropositive individuals soon after infection.¹⁸⁻²³ The data of our studies are in line with earlier findings, showing that alterations in the immune system in FIV seropositive cats parallel those in HIV seropositive individuals as exemplified by changes CD4/CD8 ratios^{24,25} and lymph node morphology (W. Jarrett, personal communication).

Since most of the FIV seropositive cats in these studies were privately owned spontaneously infected animals, in which the moment of FIV infection was not known, we could not conclude whether the differences in the responses found between individual animals were related to the duration of infection. The data obtained from the 12 experimentally infected cats that were still asymptomatic (followup time: two years) showed that loss of immune function occurs within, at most, 6 months after infection, before the onset of clinical symptoms. Apparently, an early sign of immunological dysfunction was a reduced responsiveness to IL-2, since no longitudinal data were available from these animals this could not further be substantiated. The fact that ConA-activated cells of infected cats show a reduced response to exogenous IL-2 while the stimulation indices of these cells for Groups C, A, and E stay the same suggests a

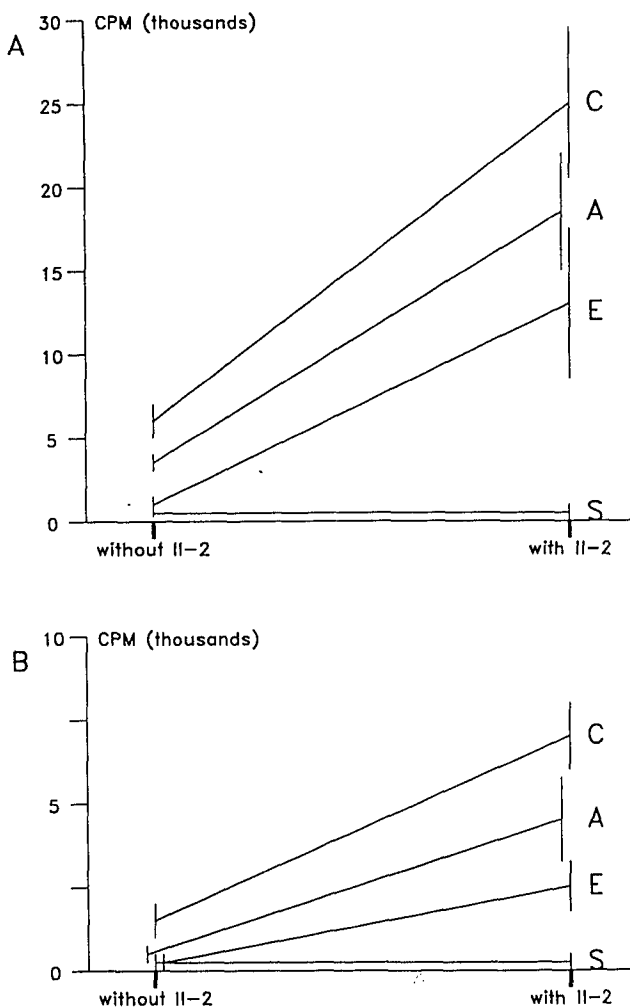


FIG. 3. Proliferative responsiveness of PBMC from the four groups of cats, in cultures with and without recombinant human IL-2 (100 IU/ml). Proliferative responses after stimulation with ConA (A) or without ConA (B) are expressed as counts per minute of tritiated thymidine incorporation. Control group (C), naturally infected symptomatic (S), and naturally infected asymptomatic (A) cats, experimentally infected cats (E).

reduction of the number of cells responding rather than a reduced response per cell. Further studies are needed to clarify this issue. The development of lymphopenia in two of the experimentally infected cats without apparent clinical symptoms, within four months after experimental infection plus the observation that the proliferative responses and the IL-2 production of the PBMC of these two cats was significantly lower than those of the other cats in this group, indicate that either there are individual differences in susceptibility for the development of malfunction of the immune system upon infection, or that strain differences of FIV play a role in the pathogenicity of the virus.

Since secondary infections play a major role in the pathogenesis of human AIDS, it was decided to study whether differences could be found between the respective groups, with regard to virus infections that are commonly found in cats. The experimentally infected cats (Group E) all remained free from these virus infections during the course of these experiments, as shown by serological screening. Serological studies in the symptomatic (Group S) and asymptomatic (Group A) cats, that had naturally acquired FIV infection, revealed no significant differences between these two groups: as expected, serum antibodies to FPV, FCaV, FHV, FeSFV, and FCV were present in the majority of the cats of both groups. None of the cats in this study tested positive for FeLV, an important cofactor in the development of feline AIDS following FIV infection.²⁴

Although more longitudinal studies are needed to define the factors that determine the clinical course of experimental FIV infection of the cat, it may be concluded on the basis of the data presented, the clinical manifestations, and immunological alterations found in symptomatic cats, that the course of FIV infection in the cat resembles that of HIV infection in man. This underlines the value of the FIV model for the development of intervention strategies for AIDS.

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