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Suppression of feline immunodeficiency virus replication *in vitro* by a soluble factor secreted by CD8$^+$ T lymphocytes

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SUMMARY

Mitogen-activated lymphoblasts isolated from the blood and lymph nodes, but not the spleen, of domestic cats acutely infected with the Petaluma or Glasgow$_8$ isolates of feline immunodeficiency virus (FIV), suppressed the replication of FIV in the MYA-1 T-cell line in a dose-dependent manner. This effect was not limited to the homologous isolate of FIV. The suppressor activity declined with progression to chronic infection, with lower levels of activity detectable only in the lymph nodes. Immunization of domestic cats with whole inactivated FIV vaccine elicited profound suppressor activity in both the blood and lymph nodes. The suppressor activity was associated with the CD8$^+$ T-cell subpopulation, the effect did not appear to be major histocompatibility complex-restricted, and was mediated by a soluble factor(s). This activity may be associated with the control of virus replication during both the asymptomatic stages of FIV infection, and in the protective immunity observed in cats immunized with whole inactivated virus vaccines.

INTRODUCTION

Infection with human immunodeficiency virus (HIV-1) is characterized by the rapid development of HIV-1-specific major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes (CTL), which recognize a variety of structural and non-structural viral antigens.[1,2] The association between high levels of virus-specific CTL detected prior to the development of virus-neutralizing antibodies and reduction in early viraemia,[3,4] coupled with the observation of a decline in CTL precursor frequency with progression to clinical disease,[5,6] has led to the conclusion that the virus-specific CTL response has a pivotal role to play in controlling infection. This belief is reinforced by the detection of virus-specific CTL in seronegative children born to HIV-infected mothers,[7–9] and in seronegative sex workers repeatedly exposed to the virus[10] and yet who remain uninfected.

CD8$^+$ T cells can also control HIV infection without lysing the infected cell.[11,12] This non-cytolytic cellular immune response involves suppression by CD8$^+$ T cells of HIV replication in CD4$^+$ T cells and macrophages.[12,13] Such activity has been demonstrated previously either by cocultivating CD8$^+$ T cells with an individual's own CD4$^+$ T cells, termed the 'endogenous assay', or by coculturing the patient's CD8$^+$ T cells with autologous or allogeneic CD4$^+$ T cells infected *in vitro* with a known isolate of HIV, termed the 'acute infection assay'.[13] The antiviral activity is not MHC-restricted and involves the release of soluble factors. A number of cytokines have been implicated including the β-chemokines RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory proteins -1α and -1β (MIP-1α; MIP-1β,[14] macrophage-derived chemokine (MDC)[15] and interleukin-16 (IL-16).[16] In addition, another soluble factor, which does not share identity with any known cytokine or chemokine, termed CD8$^+$ T-cell antiviral factor (CAF)[11,17] has also been described. Several studies have revealed the occurrence of a similar antiviral activity in non-human primate models of acquired immune deficiency syndrome (AIDS).[18–21]

Unlike simian immunodeficiency virus (SIV) or HIV-2 infection of non-human primates, infection of domestic cats with feline immunodeficiency virus (FIV) represents lentiviral infection in its natural host. This system therefore represents an ideal system in which to characterize the immune responses associated with the control of viral replication during the asymptomatic phase of infection in a natural host.[22] Furthermore, the feline model is unique in the ability to induce protective immunity in cats by immunization with whole inactivated virus (WIV) vaccines,[23–25] thus allowing the dissection of the vaccinal protective immune response. As in HIV infection, a virus-specific CTL response is observed following experimental infection with FIV prior to the onset of humoral immunity.[26,27] Vaccination also elicits both FIV Gag- and Env-specific CTL responses,[28] and recent studies suggest that Env-specific CTLs are important in the long-term maintenance of protection.[29,30] Therefore, as in HIV and SIV infections, cellular immunity has an important role to play in FIV immunity. However, the contribution played by non-cytolytic CD8$^+$ T cells is less well understood in FIV infection. CD8$^+$

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T-cell-mediated antiviral activity has been described in cats chronically infected with the $NCSU_1$ isolate of FIV,[31] suggesting that this activity may be involved in the maintenance of the asymptomatic state in these animals.

In the present study, we developed an acute infection assay to measure suppression of FIV replication *in vitro*. Is suppressor activity involved in the control of virus in infected cats? To address this question we determined the suppression observed in cats acutely and chronically infected with FIV. We examined the lymphoid distribution of this activity, and compared this with the situation in unexposed, non-infected cats. Does suppressor activity contribute to vaccinal protection? Suppressor activity was measured in the lymphoid tissues of cats following immunization with whole inactivated virus vaccines to determine its role in the protection observed. Finally, we demonstrate that this activity is mediated at least in part by a soluble factor(s) secreted by $CD8^+$ T cells which acts in a non-MHC-restricted manner to suppress FIV replication.

MATERIALS AND METHODS

Experimental animals

The 11 adult, outbred, specific pathogen-free domestic cats selected for this study were serologically negative for FIV. Two cats were vaccinated by subcutaneous (s.c.) inoculation on three occasions, 3 weeks apart with 250 μg of cell-free FIV inactivated by treatment with 0·5% paraformaldehyde. A combination of threonyl muramyl dipeptide (tMDP) and SAF-M was used as an adjuvant (kindly provided by Chiron Corporation, Emeryville, CA). This vaccine, prepared from the culture fluid of the FL4 feline lymphoblastoid cell line, which is persistently infected with FIV_{PET},[32] has been previously shown to protect cats from challenge with homologous virus.[23] These cats resisted homologous virus challenge and were examined 12 weeks later. Seven cats were experimentally infected with FIV by intraperitoneal (i.p.) inoculation of either 25 cat infective doses 50% (CID_{50}) of FIV_{PET}, 10 CID_{50} of FIV_{GL-8}, or 4000 CID_{50} of FIV_{GL-8}. Two adult specific pathogen-free cats which were not exposed to FIV antigens were included as normal controls.

Tissues were prepared from all animals 3–4 months following either vaccination or infection. This corresponded with the acute stage of infection. The two cats infected with 4000 CID_{50} FIV_{GL-8} were allowed to develop a chronic infection and were subsequently examined 22 months after challenge.

Preparation of lymphocyte effector cells

Mononuclear cells were prepared from the blood, spleen, and lymph nodes of all cats. At post-mortem examination, blood was collected into an equal volume of Alsevers' solution (Scottish Antibody Production Unit, Carluke, UK), and mononuclear cells were isolated by centrifugation over Ficoll-Paque (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Samples of spleen and lymph nodes were also collected, and a single-cell suspension of these tissues was prepared by gentle manual homogenization. Mononuclear cells were again isolated by centrifugation over Ficoll-Paque and were washed twice and stored in liquid nitrogen until use in the assays.

In vitro *infection of MYA-1 cells with FIV*

MYA-1 cells[33] were infected *in vitro* by incubating 5×10^5 cells with 1 ml cell-free supernatant from either FIV_{PET}- or FIV_{GL-8}-infected MYA-1 cells, corresponding to one tissue culture infective dose 50% ($TCID_{50}$) of either isolate of FIV, in a 2054 Falcon tube (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) for 1 hr at 37°. The cells were then washed twice and resuspended in 1 ml fresh RPMI-1640 medium (Gibco Biocult, Paisley, UK) containing 10% fetal bovine serum (Biological Industries Ltd, Cumbernauld, UK), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 IU of penicillin per ml, and 100 μg of streptomycin per ml (complete RPMI-1640 medium) supplemented with 5% culture supernatant from the Ltk^-IL-2.23 cell line producing human recombinant IL-2 (a kind gift from T. Miyazawa, University of Tokyo, and M. Hattori, University of Kyoto) for use in the assays.

Detection of antiviral activity in mononuclear cells

The capacity of mononuclear cells from the blood, spleen and lymph nodes of FIV-vaccinated or FIV-infected cats to suppress the replication of FIV *in vitro* was assessed. The cells were stimulated *in vitro* with 7·5 μg/ml concanavalin A (Con A; Sigma Chemical Co., Poole, UK) for 72 hr and thereafter maintained in complete RPMI-1640 medium supplemented with 5% culture supernatant from the Ltk^-IL-2.23 cell line. Assays were performed by adding the appropriate number of Con A-stimulated lymphoblasts to 5×10^5 MYA-1 cells previously infected with 1 $TCID_{50}$ of either FIV_{PET} or FIV_{GL-8}, in flat-bottomed 24-well tissue culture plates (Falcon, Becton Dickinson Labware) to give ratios of lymphoblasts to infected MYA-1 cells of 4:1, 2:1, and 1:1, in a final volume of 2 ml complete RPMI-1640 medium. The cell mixtures were then incubated in a humidified atmosphere containing 5% CO_2. At intervals of 3–4 days, 0·5 ml samples of culture supernatant fluids were collected and replaced with fresh medium. Samples were stored at −20° until assayed by enzyme-linked immunosorbent assay (ELISA) for the presence of FIV p24 antigen (PetChek, IDEXX Corp., Portland, ME). Cultures were maintained for up to 14 days.

Depletion of T-cell subsets from effector populations

To determine the phenotype of the cells responsible for the observed suppression of FIV replication by mononuclear cells derived from the lymph nodes, $CD4^+$ or $CD8^+$ T cells were depleted from the effector cell population by incubating lymph node cells with mouse monoclonal anti-fCD4 antibody (vpg 39) or mouse monoclonal anti-fCD8 antibody (vpg 9)[34] for 30 min at 4°. Labelled cells were then washed once, and the $CD4^+$ or $CD8^+$ T-cell fraction was removed by incubation with magnetic beads coated with sheep anti-mouse immunoglobulin G1 (IgG1) antibody (Dynabeads M-450, Dynal AS, Oslo, Norway).

Detection of soluble antiviral factors

To assess the involvement of soluble factors in the antiviral activity observed in the lymph nodes, Con A-stimulated lymphoblasts were cocultivated with FIV-infected MYA-1 cells in 24-well tissue culture plates in which the two cell populations were separated by a 0·4-μM high pore density polyethylene terephthalate track-etched membrane (Falcon, Becton

Dickinson Labware) in a total volume of 2 ml complete RPMI-1640 medium supplemented with 5% culture supernatant from the Ltk$^-$IL-2.23 cell line. The cultures were incubated at 37° in a humidified atmosphere containing 5% CO_2, and the culture supernatant fluids were collected and assayed by ELISA for the presence of FIV p24 antigen.

RESULTS

Lymphocytes from acutely infected cats suppress FIV replication *in vitro*

To assess the capacity of the mitogen-activated lymphoblasts obtained from different lymphoid organs during the acute stage of FIV infection to suppress the replication of FIV *in vitro*, mononuclear cells were prepared from the blood, spleen and lymph nodes of three cats, 12 weeks following experimental infection with 25 CID_{50} FIV_{PET}. The cells were stimulated *in vitro* for 72 hr with Con A and were then cocultured with FIV_{PET}- or $FIV_{GL\text{-}8}$-infected MYA-1 cells. Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA.

Viral replication was initially observed at day 7 in control cultures of MYA-1 cells infected *in vitro* with FIV_{PET} and cultured alone (Fig. 1a, c and e). Co-cultivation of FIV_{PET}-infected MYA-1 cells with mitogen-activated lymphoblasts from blood or lymph nodes of FIV_{PET}-infected cats suppressed FIV replication. The degree of suppression varied depending on the effector to target ratio (E:T) examined, with complete suppression at the highest E:T ratios (4:1 and 2:1; $P<0{\cdot}05$ on day 7 and 10 by two way *t*-test) and partial suppression at the lowest E:T ratio (1:1) as indicated by the detection of FIV p24 in the culture supernatants. By day 7 following *in vitro* infection, viral replication was observed at the lowest E:T ratio (1:1), although the optical density (OD) values measured were lower than those control cultures indicating that virus replication was suppressed by up to 46% at this E:T ratio. However, this effect was not observed when splenic lymphoblasts were co-cultivated with FIV_{PET}-infected cells. In these cultures, viral replication was observed at similar levels to that in control cultures. By day 10, higher levels of FIV p24 were detectable in cultures containing splenocytes suggesting that FIV replication was enhanced in the presence of splenic lymphoblasts.

To assess the specificity of the suppressor activity,

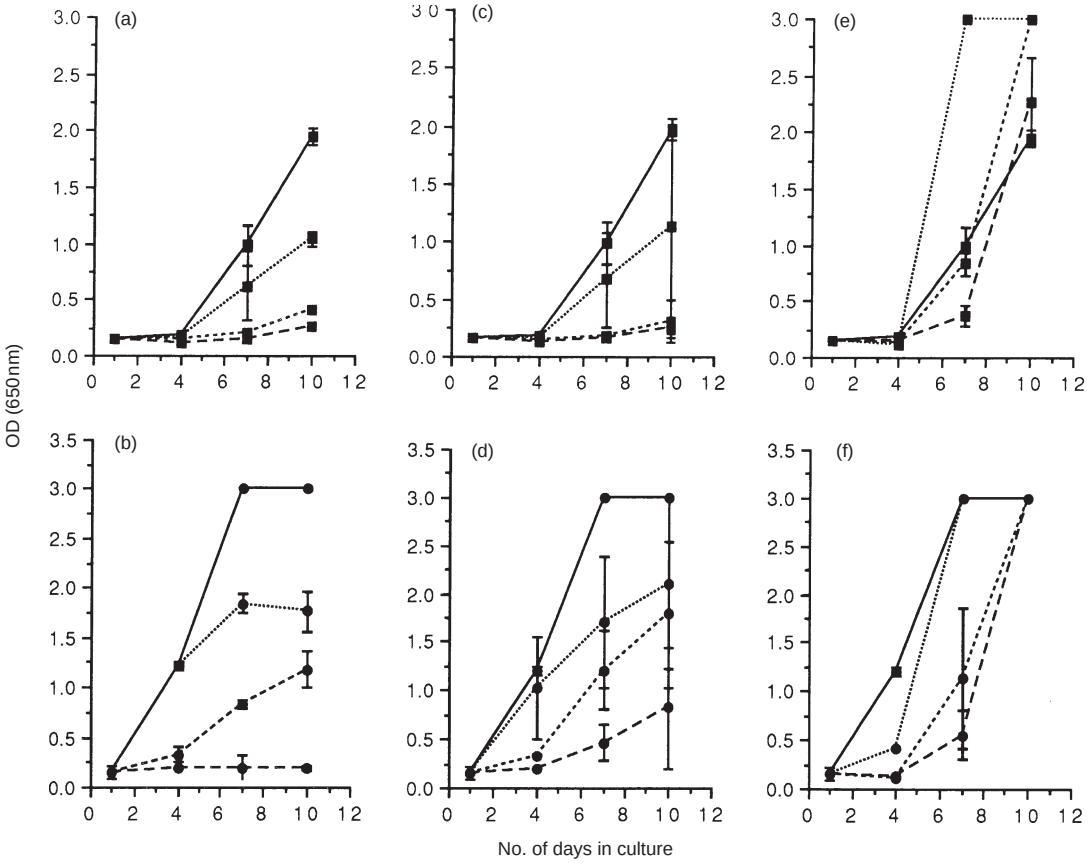

Figure 1. FIV-specific suppressor activity in acutely infected cats. Mitogen-activated lymphoblasts were prepared from the blood (PBMC; a and b), lymph nodes (LNC; c and d), and spleen (e and f) of three cats, 12 weeks postinfection with FIV_{PET}, and were cocultured with either FIV_{PET}-infected (■) or $FIV_{GL\text{-}8}$-infected (●) MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·). Control cultures contained FIV-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the OD values at 650 nm for the lymphoid tissues of each individual cat. Control cultures with FIV-infected MYA-1 cells were performed in duplicate, and are represented as the mean OD values at 650 nm for the three cats ± 2 SD, error bars are not discernible where the SD is small.

mitogen-activated lymphoblasts from the FIV_{PET}-infected cats were cocultivated with FIV_{GL-8}-infected MYA-1 cells. Replication of FIV_{GL-8} was detected earlier (day 4) in control cultures containing MYA-1 cells infected *in vitro* with FIV_{GL-8}, compared to similar control cultures containing FIV_{PET}-infected MYA-1 cells (day 7, see above), and higher levels of p24 were detected in the culture supernatant indicative of increased viral replication (Fig. 1b,d,andf). Despite the higher levels of viral replication observed in the FIV_{GL-8}-infected cultures, cocultivation with lymphoblasts from the blood or lymph nodes of FIV_{PET}-infected cats did significantly down-regulate FIV replication in a dose-dependent manner ($P<0{\cdot}05$ at all E:T ratios on day 7, and at highest E:T ratio on day 10 by two way *t*-test). This effect became less noticeable at the lowest E:T ratio, presumably reflecting the greater viral burdens in the cultures. In contrast to the experiments using FIV_{PET}-infected MYA-1 cells, splenic lymphoblasts were capable of suppressing FIV_{GL-8} replication at days 4 and 7 by 90% and 81%, respectively, at an E:T ratio of 4:1. This effect was lost by day 10 in culture.

Control experiments were designed to determine whether the observed suppression of FIV replication by blood and lymph node lymphoblasts was elicited by FIV_{PET} infection, or whether it represented an innate form of immunity. Thus, lymphoblasts were prepared from the blood and lymph nodes of two age-matched uninfected cats which had not been exposed to FIV antigens. Suppression was only observed in cultures containing blood lymphocytes at the highest E:T ratio of 4:1, and this was not statistically significant (Fig. 2a). In all other cultures, including those containing lymph node lymphoblasts, FIV replication was comparable to control cultures containing FIV_{PET}-infected MYA-1 cells alone, indicating that although suppressive activity was observed in lymphoblasts prepared from uninfected cats, the activity was lower than that observed in the acutely infected cats. These results suggest that there may be an up-regulation of the suppressive activity associated with prior exposure to FIV.

To determine whether the observed suppression of FIV replication was a feature of FIV_{PET} infection alone, experiments were performed in which blood and lymph node lymphoblasts from two FIV_{GL-8}-infected cats prepared at 16 weeks following experimental infection, were cocultivated with FIV_{GL-8}-or FIV_{PET}-infected MYA-1 cells (Fig. 3). In cultures of PBMC, suppression of FIV_{PET} replication was observed at the highest E:T ratios (4:1 and 2:1; $P<0{\cdot}05$ at E:T ratio of 4:1, but not significant at E:T ratio of 2:1 by two way *t*-test) at day 7 (87% suppression) and day 10 (61% suppression), and suppression of FIV_{GL-8} was observed at the highest E:T ratios on day 7 (81% suppression at 4:1). However, no suppression was observed in cultures of lymph node cells with either FIV_{PET} or FIV_{GL-8}-infected MYA-1 cells. This is in marked contrast to the results observed with lymph node cells from cats acutely infected with FIV_{PET}, where suppression of both FIV_{PET} and FIV_{GL-8} was observed (Fig. 1).

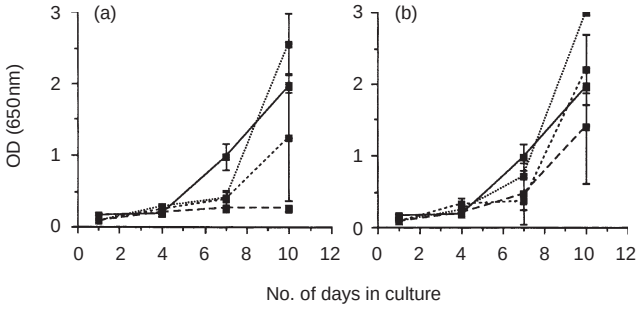

Figure 2. FIV-specific suppressor activity in normal cats. Mitogen-activated lymphoblasts were prepared from the blood (a) and lymph nodes (b) of two normal, specific pathogen-freecats which had never been exposed to either infectious FIV or FIV antigens, and were cocultured with FIV_{PET}-infected MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·). Control cultures contained FIV_{PET}-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm for the two cats ± 2 SD, error bars are not discernible where the SD is small.

Suppressor cell activity in chronically FIV-infected cats

The previous experiments clearly demonstrate that a population of cells capable of down-regulating FIV replication in MYA-1 T cells *in vitro* is present in the blood and lymph nodes of cats, and that this activity is increased during the acute stage of FIV infection. This effect is dose-dependent, is not MHC-restricted, and the activity is not limited to the homologous isolate of the virus. To determine whether this cell population persisted during the chronic, asymptomatic phase of infection, lymphoblasts were prepared from the blood, spleen and lymph nodes of two cats 22 months following experimental infection with FIV_{GL-8} and analysed for their FIV suppressor activity.

Lymph node lymphoblasts from chronically infected cats were capable of suppressing FIV_{PET} and FIV_{GL-8} replication at the highest E:T ratio on day 7 (80% suppression of FIV_{PET}, 92% suppression of FIV_{GL-8}) and day 10 (81% suppression of FIV_{PET}, 85% suppression of FIV_{GL-8}). In contrast, suppression of FIV replication was not observed in cultures of lymphoblasts derived from either the blood or spleen, using either FIV_{PET} or FIV_{GL-8}-infected MYA-1 cells as targets (Fig. 4). Interestingly, co-cultivation with splenic lymphoblasts did not induce the enhancement of FIV replication observed during acute FIV_{PET} infection (Fig. 1e,f).

WIV vaccination elicits FIV-specific suppressor cells

To determine whether WIV vaccination could elicit similar suppressor cell activity to that observed during acute FIV infection, the experiments were repeated with lymphoid tissues from 2 WIV vaccinated cats 15 weeks following the final immunization. Lymphoblasts from both the blood and the lymph nodes of the WIV-vaccinated cats suppressed the replication of either FIV_{PET} or FIV_{GL-8} *in vitro* (Fig. 5). Lymphoblasts derived from the blood completely suppressed FIV_{PET} replication, with no FIV p24 detected in the culture supernatants at any time during the study, at any E:T ratio, $P<0{\cdot}05$ at E:T=4:1 and 2:1 on day 10. Replication of FIV_{GL-8} was also suppressed, although low levels of FIV p24 could be detected in the lowest E:T ratio of 1:1, nevertheless this suppression was statistically significant ($P<0{\cdot}05$ by two-tailed *t*-test). However, FIV_{PET} replication was not down-regulated by splenic lymphoblasts (Fig. 5e), but partial suppression of FIV_{GL-8} was observed at the highest E:T ratios

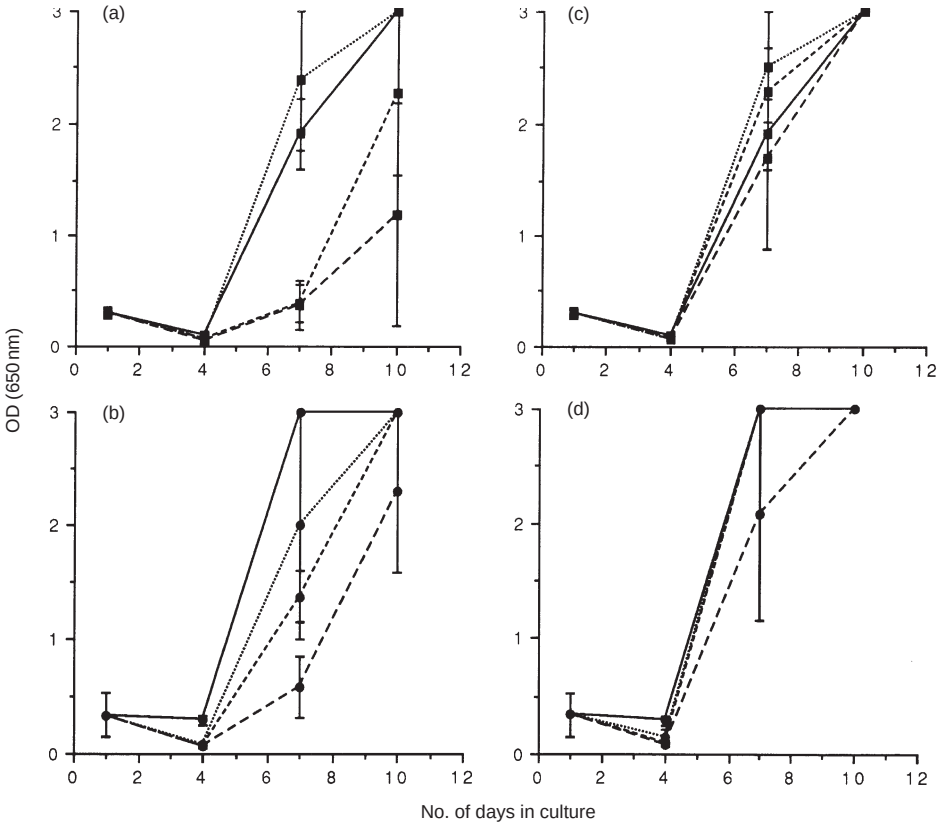

Figure 3. FIV-specific suppressor activity during acute infection with FIV_{GL-8}. Mitogen-activated lymphoblasts were prepared from the blood (a and b) and lymph nodes (c and d) of two cats, 16 weeks postinfection with FIV_{GL-8}, and were cocultured with either FIV_{PET}-infected (■) or FIV_{GL-8}-infected (●) MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·). Control cultures contained FIV-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm measured for the two cats ± 2 SD, error bars are not discernible where the SD is small.

on day 7 (65% suppression, E:T=4:1) and day 10 (47% suppression, E:T=4:1).

Suppression of FIV replication is associated with $CD8^+$ T cells

In the preceding experiments, inhibition of virus replication was consistently observed when lymph node cells from acutely infected cats and WIV vaccinates were cocultivated with MYA-1 T cells infected with either FIV_{GL-8} or FIV_{PET}. To determine the cell phenotype responsible for the suppressor activity, the experiments were repeated using lymphoblasts derived from cats either acutely infected with FIV_{PET}, or immunized with WIV vaccine from which either the $CD4^+$ or $CD8^+$ T-cell subpopulations had been depleted by immunomagnetic bead separation.

Co-cultivation of lymph node lymphoblasts with FIV-infected MYA-1 cells again suppressed the replication of FIV when compared to control cultures containing only FIV-infected MYA-1 cells. Depletion of $CD8^+$ T cells from the effector cells abrogated the suppressor effect (Fig. 6), whereas suppression was still observed in cultures depleted of $CD4^+$ T cells indicating that $CD8^+$ T cells were mediating the suppression. This effect was observed with both FIV_{PET} or FIV_{GL-8} infected target MYA-1 T cells.

Suppressor effect is mediated by a soluble factor

Our previous studies have shown that MHC class I-restricted FIV-specific CTL responses mediated by $CD8^+$ T cells are elicited in cats following either experimental infection or immunization with WIV vaccines.[28,29] Although the activity described in the present report also appears to be mediated by $CD8^+$ T cells, it does not appear to be MHC-restricted since the MYA-1 T cells are unlikely to be histocompatible with all of the cats examined in this study. To formally exclude the involvement of FIV-specific CTL in the suppression of FIV replication described in this report, experiments were performed in which the effector lymphoblasts and the FIV-infected MYA-1 T cells were separated by a 0·4-μm semi-permeable membrane allowing the free passage of soluble molecules, but preventing direct cell-to-cell contact required for cytolysis by CTL.

Suppression of FIV_{PET} and FIV_{GL-8} replication was observed with lymph nodes from cats acutely infected with FIV_{PET}. Suppression of FIV replication, in a dose-dependent manner, was still observed following separation of the effector and target cells by a semi-permeable membrane, implicating the involvement of a soluble factor in the observed down-regulation of virus replication (Fig. 7). The inhibition of

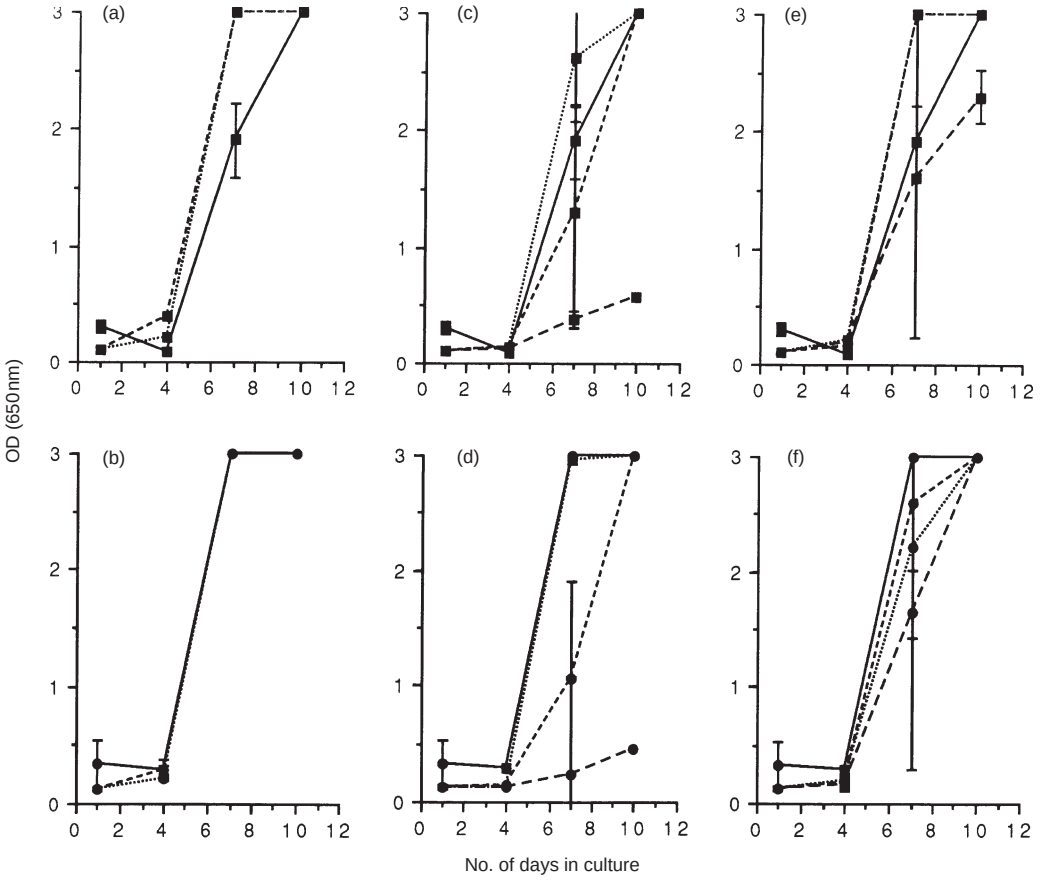

Figure 4. FIV-specific suppressor activity in chronically infected cats. Mitogen-activated lymphoblasts were prepared from the blood (a and b), lymph nodes (c and d), and spleen (e and f) of two cats, 22 months following infection with FIV_{GL-8}, and were cocultured with either FIV_{PET}-infected (■) or FIV_{GL-8}-infected (●) MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·). Control cultures contained FIV-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm for the two cats ± 2 SD, error bars are not discernible where the SD is small.

FIV_{GL-8} replication was lost at all E:T ratios examined by day 10 of culture, whereas replication of FIV_{PET} was still suppressed at the higher E:T ratios (4:1 and 2:1) at this time-point.

DISCUSSION

Animal models of human lentivirus infection have an important role to play in the determination of the immune correlates of vaccine-induced protection, and in the control of virus replication during the asymptomatic stage of infection. Studies of FIV in domestic cats have revealed that both humoral immune responses, such as virus neutralizing antibodies,[23] and cell-mediated immunity, such as virus-specific CTL,[28–30] represent important components of the host's protective immune response. Recently, another cell-mediated immune response, non-cytolytic antiviral $CD8^+$ T cells, has been implicated in the control of FIV replication during the asymptomatic phase of infection.[31] In the present study we developed an acute infection assay based in the *in vitro* infection of the feline T lymphoblastoid cell line, MYA-1, with equivalent amounts of either FIV_{PET} or FIV_{GL-8}. Using this assay we investigated the contribution of suppressor activity to the control of viral replication during the asymptomatic stage of infection by comparing the capacity of mitogen-stimulated $CD8^+$ T cells from cats acutely or chronically infected with FIV to suppress virus replication *in vitro*. These responses were compared with cats immunized with whole inactivated virus vaccines to determine the contribution of suppressor activity to vaccinal immunity.

Following acute experimental infection with FIV_{PET} and, to a lesser extent FIV_{GL-8}, a suppressor cell population was demonstrated in the blood and lymph nodes capable of inhibiting FIV replication *in vitro*. A similar phenomenon has been observed in asymptomatic HIV-infected individuals[12] with dose-dependent suppression of virus replication mediated by $CD8^+$ T cells. That activity was associated with a block in virus production rather than destruction of the virus-infected $CD4^+$ T lymphocytes and macrophages themselves. Furthermore, $CD8^+$ lymphocytes from SIV-infected, but not uninfected, rhesus macaques block SIV replication in peripheral blood lymphocytes.[21] The inhibition of FIV replication observed in the acutely infected cats was not limited to the homologous isolate of FIV, with suppression of both FIV_{PET} and FIV_{GL-8} by the lymphoblasts from FIV_{PET}-infected cats. When splenic lymphoblasts were examined they were unable

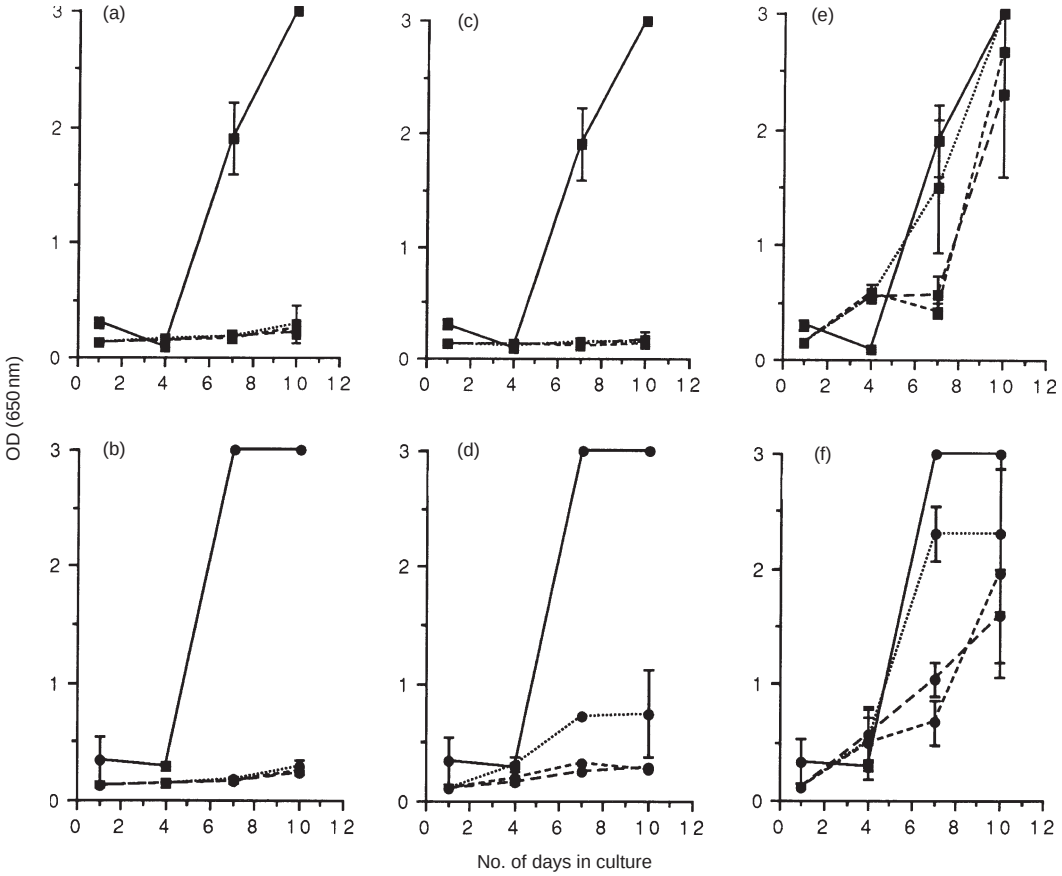

Figure 5. FIV suppressor cell activity in WIV-vaccinated, protected cats. Mitogen-activated lymphoblasts were prepared from the blood (a and b), lymph nodes (c and d), and spleen (e and f) of two cats immunized with whole inactivated virus vaccine on three occasions, 3 weeks apart, 15 weeks following the final inoculation, and were cocultured with either FIV_{PET}-infected (■) or FIV_{GL-8}-infected (●) MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·). Control cultures contained FIV-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm measured for the two cats ± 2 SD, error bars are not discernible where the SD is small.

to to suppress FIV replication, and enhancement was observed in some situations. At present, the reason for the observed enhancement of FIV replication with splenic lymphoblasts is unknown, although the spleens of domestic carnivores do contain high numbers of γδ T cells, with up to 33% of T cells in the canine spleen expressing the γδ phenotype (P. Moore, personal communication), and it is possible that these two observations may be linked.

In the present study, the suppressor activity measured during the acute stage of infection did not appear to persist into the more chronic stage of infection. Twenty-two months after infection with FIV_{GL-8}, suppressor activity was no longer observed in the blood, and only the highest E:T ratios of lymph node lymphoblasts demonstrated high levels (80–92%) of suppression. This observation may reflect the much higher viral loads achieved following infection with FIV_{GL-8} compared to FIV_{PET} (M.J. Hosie, personal communication), and also the more rapid replication rate of FIV_{GL-8} *in vitro* (Fig. 1). The decline in antiviral activity with disease progression following FIV_{GL-8} infection suggests that this activity may contribute to the control of viraemia during asymptomatic infection. This would agree with observations of HIV-infected individuals, where $CD8^+$ T-cell antiviral activity appears during the acute stage of disease, persists during the asymptomatic phase of disease, and then deteriorates with progression to clinical illness.[12,35,36] However, our results contrast with those of Jeng and co-workers who were unable to detect $CD8^+$ T-cell anti-FIV activity in the blood of cats acutely infected with the $NCSU_1$ isolate of FIV. Such activity only became detectable during the asymptomatic phase of infection at 22–44 weeks, and the activity persisted in a proportion of the long-term infected cats (95–105 weeks p.i.).[31] The differences between these two studies may be attributed to the different FIV isolates, which have different characteristics *in vitro* and *in vivo* (M.J. Hosie, personal communication), used to infect the cats.

It is of interest that we observed antiviral activity prior to infection or exposure to viral antigens (Fig. 2). Our data suggest that the $CD8^+$ T cell antiviral activity described may reflect an innate form of antiviral immunity which is up-regulated following exposure to viral antigens. However, since we have not characterized the factor(s) responsible for the observed down-regulation of FIV replication observed in this study, it is not clear whether the same mechanisms are

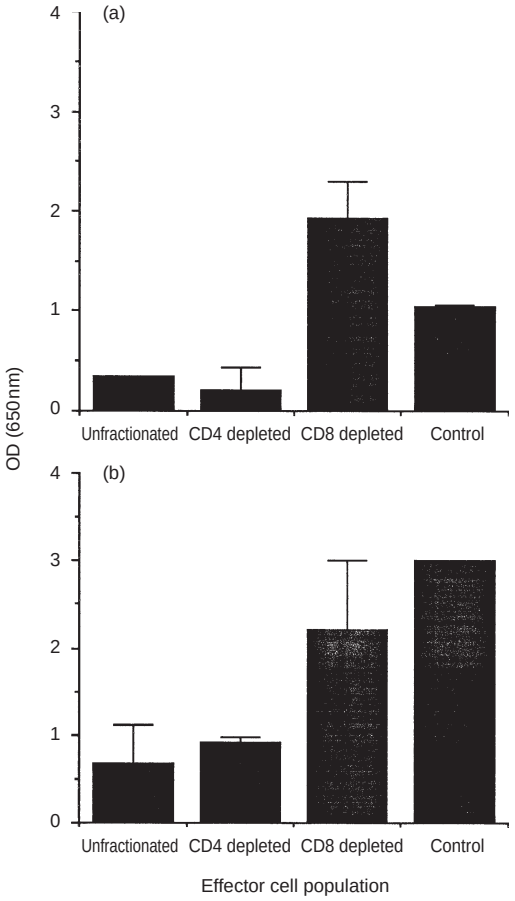

Figure 6. FIV-specific suppression is associated with $CD8^+$ T cells. Mitogen-activated lymphoblasts were prepared from the lymph nodes of three cats, 12 weeks postinfection with FIV_{PET} (a), and two WIV-vaccinated, protected cats (b). To determine the cell phenotype responsible for the virus-specific suppression, the $CD4^+$ or $CD8^+$ T-cell subpopulation was depleted by labelling with appropriate monoclonal antibodies and immuno-magnetic bead separation. Control cultures contained FIV-infected MYA-1 cells alone. Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm measured in individual samples for either the three infected cats or two vaccinated cats, respectively, $\pm$ 2 SD, error bars are not discernible where the SD is small.

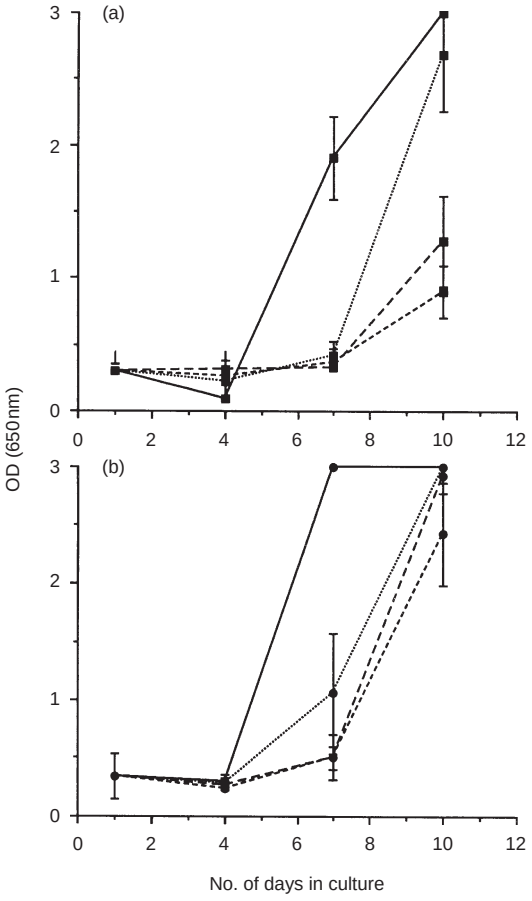

Figure 7. FIV-specific suppressor effect is mediated by a soluble factor. Mitogen-activated lymphoblasts were prepared from the lymph nodes of three cats, 12 weeks postinfection with FIV_{PET}, and were cocultured with either $FIV_{PET\text{-}infected}$ (a) or $FIV_{GL\text{-}8}$-infected (b) MYA-1 cells at ratios of 4:1 (---), 2:1 (– – –), and 1:1 (· · ·), separated by a 0·4-μm semi-permeable membrane. Control cultures contained FIV-infected MYA-1 cells alone (——). Replication of FIV was indicated by the detection of viral p24 in the culture supernatant by ELISA. The results shown represent the mean OD values at 650 nm measured for the three cats $\pm$ 2 SD, error bars are not discernible where the SD is small.

involved in uninfected, unexposed cats as in FIV-infected or vaccinated, protected cats. Certainly $CD8^+$ anti-HIV activity has been observed in uninfected, unexposed humans using both endogenous[13] and acute infection assays,[37] although higher ratios of $CD8^+$ cells to target cells are required *in vitro* to demonstrate this activity compared to $CD8^+$ cells from infected individuals. Likewise non-human primates, including baboons and chimpanzees, also possess a constitutive cell-mediated immune mechanism for the control of HIV replication.[14,18]

Previous studies on cell-mediated immunity to FIV have focussed on virus-specific cytotoxic $CD8^+$ T cells. From these studies we know that FIV-specific cytotoxicity is detected in the blood as early as 2 weeks following experimental infection[26,27] preceding seroconversion and the clearance of circulating virus. As infection progresses, CTL activity appears in the lymph nodes and declines in the peripheral blood, presumably reflecting the compartmentalization of the virus to these sites during infection.[29] Therefore, non-cytolytic and cytolytic immune responses may both be involved in the control of FIV replication, and both are mediated by $CD8^+$ T cells. It is possible, however, that the two activities may be associated with different subpopulations within the $CD8^+$ T-cell fraction. The rapid expansion of $CD8^{low}$ T cells has been documented following FIV infection.[34,38,39] This T-cell subpopulation shares some features with natural killer cells, and has recently been shown to express either $CD8\alpha\alpha$ homodimers or, or to have decreased expression of the $CD8\beta$ chain.[40] Our future studies will focus on the potential antiviral properties of these $CD8^{low}$ T cells.

To formally exclude the possibility that the suppression of FIV replication observed *in vitro* was not associated with either virus-specific lymphocytoxicity or a mixed lymphocyte reaction (MLR), both of which require intimate cell-to-cell contact, the $CD8^+$ effector T cells were separated from the

virus-infected target cells by a 0·4-μm permeable membrane. Separation of the cell populations failed to block the antiviral activity (Fig. 7) indicating the involvement of a soluble factor. Furthermore, the activity was not MHC-restricted, since all of the cats used in the study were outbred, and the MYA-1 cells did not share a common source with any of the experimental cats. In contrast, all of the $CD8^+$ CTL activity described to date appears to be MHC-restricted.[26–30]

No attempt was made to characterize the soluble factors associated with the antiviral activity described in this report. However, a number of molecules have been shown to be associated with this activity in HIV-infected individuals, including MIP-1α, MIP-1β and RANTES,[14] MDC,[15] IL-16,[16] and another unidentified soluble factor, CAF.[11] Indeed it has been shown that $CD4^+$ T cells from individuals that produce higher levels of the C-C chemokines MIP-1α, MIP-1β and RANTES are relatively more resistant to HIV-1 infection.[41]

One unique feature of the FIV model is the ability to induce virus-specific protection by immunization with WIV vaccines, thus allowing elucidation of the immune correlates of protection. Recent studies have revealed that WIV vaccination elicits FIV-specific CTL activity[28] in addition to virus-neutralizing antibodies.[23] FIV Env-specific CTLs appear also to be involved in the long-term protection induced by vaccination.[29,30] In this report we demonstrate that WIV vaccination also elicits potent antiviral suppressor cell activity in the blood and lymph nodes (Fig. 5), which may represent an invaluable component of the host's protective immunity, and indicate that WIV vaccination elicits a broad spectrum of virus-specific immune reactivity resulting in protection of which noncytolytic $CD8^+$ T cells represent a fraction.

In summary, experimental FIV infection of domestic cats is associated with the induction of $CD8^+$ T cells capable of suppressing viral replication *in vitro* in a non-MHC-restricted manner. The effect is not limited to the homologous viral isolate and is mediated, at least in part, by a soluble factor(s). Progression to later disease is accompanied by a decline in suppressor activity. Inoculation of cats with WIV vaccines elicits potent suppressor cell activity in the blood and lymph nodes, suggesting that this activity may represent an important component of the host's protective immunity. Future studies will focus on the kinetics of the suppressor activity following experimental infection and attempt to correlate this activity with the induction of virus-specific CTL responses, and viral loads *in vivo*, allowing rational decisions to be made on vaccine design based on a comprehensive understanding of the protective cellular immune mechanisms *in vivo*.

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