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Involvement of cytolytic and non-cytolytic T cells in the control of feline immunodeficiency virus infection

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Abstract

The appearance of non-cytolytic T cells that suppressed feline immunodeficiency virus (FIV) replication in vitro, and FIV-specific cytotoxic T cell (CTL) responses was compared in a group of seven, specific pathogen free (SPF) domestic cats following primary infection with the Glasgow$_8$ isolate of FIV (FIV$_{GL\text{-}8}$). FIV proviral burdens were quantified in the blood and lymphoid tissues by real-time PCR. Non-cytolytic T cell suppression of FIV replication was measured by co-cultivating lymphoblasts prepared from the cats at different time-points during infection with FIV-infected MYA-1 cells in vitro. Non-cytolytic suppressor activity was detected as early as 1 week after infection, and was evident in all the lymphoid tissues examined. Further, this activity was present in subpopulations of T cells in the blood with normal (CD8^{hi}) or reduced (CD8^{lo}) expression of the CD8 molecule, and temporal modulations in non-cytolytic suppressor activity were unrelated to the circulating CD8$^+$ T cell numbers. Virus-specific CTL responses, measured by ^{51}Cr release assays, were not detected until 4 weeks after infection, with the emergence of FIV-specific effector CTLs in the blood. Throughout infection the response was predominantly directed towards FIV Gag-expressing target cells, and by 47 weeks after infection CTL responses had become localised in the lymph nodes and spleen. The results suggest that both non-cytolytic T cell suppression of FIV replication and FIV-specific CTL responses are important cellular immune mechanisms in the control of FIV replication in infected asymptomatic cats.

Keywords: Feline immunodeficiency virus; CD8$^+$ T cells; Cytotoxic T cells

1. Introduction

Feline immunodeficiency virus (FIV) is a naturally occurring lentivirus of domestic cats with world-wide distribution. Primary infection of cats is associated with a protracted asymptomatic phase of several months or years that in some cats culminates in the development of immunosuppression or neoplasia. Understanding how the host controls virus replication and dissemination following lentiviral infection aids the rational design of safe and effective vaccines. Following infection with FIV the host mounts a vigorous virus-specific cytotoxic T cell (CTL) response which precedes the development of virus-specific humoral immunity (Song et al., 1992; Beatty et al., 1996). In addition, non-cytolytic CD8$^+$ T cells, which suppress virus replication in a non-MHC-restricted manner by the secretion of soluble factors, have been described during acute and asymptomatic stages of FIV infection (Jeng et al., 1996; Hohdatsu et al., 1998;

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Flynn et al., 1999; Choi et al., 2000). Studies aimed at elucidating the immune correlates of vaccinal immunity have revealed that virus neutralising antibodies, virus-specific CTLs, and $CD8^+$ T cells capable of suppressing FIV replication, are all involved, either acting alone or in concert (Flynn et al., 1995, 1996, 1999; Hosie and Flynn, 1996). Furthermore, we have recently shown that it is feasible to induce protective immunity in cats by inoculation with an FIV DNA vaccine based on replication-defective yet essentially full length proviral genome (Hosie et al., 1998; Flynn et al., 2000). Protection was observed in the absence of a virus-specific humoral response. However high levels of virus-specific CTLs were detected in the peripheral blood, implicating this immune mechanism in the observed protection. Human immunodeficiency virus (HIV)-1 infection is also characterised by the rapid development of virus-specific CTL and non-cytolytic $CD8^+$ T cell responses (Koup et al., 1994; Borrow et al., 1994; Mackewicz et al., 1994a,b; Levy et al., 1996). Epidemiological studies have highlighted the very significant role that strong and persistent cell-mediated immune responses have in the control of retroviral replication and in the maintenance of the symptom-free state (Koup et al., 1994; Borrow et al., 1994; Rinaldo et al., 1995; Ogg et al., 1998; Paxton et al., 1996). These observations have resulted in attempts to modify the outcome of infection in human patients by the adoptive transfer of $CD8^+$ T cells of predetermined viral antigen specificity (Koenig et al., 1995; Brodie et al., 1999). Such immunotherapeutic strategies may prove to be an invaluable adjunct to chemotherapeutic regimes which recent reports suggest are unlikely to completely eliminate reservoirs of virus within the host (Finzi et al., 1999).

Infection with certain isolates of FIV is associated with the appearance of a subset of $CD8^+$ T cells in the peripheral circulation characterised by diminished expression of surface CD8 (Willett et al., 1993). Recently these $CD8^{lo}$ T cells have been shown to express either CD8 α/α homodimers or relatively lower amounts of the β subunit of the CD8 heterodimer than conventional $CD8^+$ T cells (Shimojima et al., 1998). The function of cells of this phenotype has not been completely elucidated, although their appearance during the acute phase of infection may be associated with the development of non-cytolytic viral suppressor factors. $CD8^+$ T cells from HIV infected individuals release soluble factors which inhibit HIV replication in vitro in a non-MHC-restricted manner and loss of this activity appears to correlate with disease progression (Mackewicz et al., 1991). The nature of the factors involved has been controversial with claims for interleukin (IL)-16 (Baier et al., 1995), several chemokines (RANTES, MIP-1α, MIP-1β) (Cocchi et al., 1995), macrophage-derived chemokine (MDC) (Pal et al., 1997), or further uncharacterised factors (Levy et al., 1996) however there is clearly substantial evidence for the activity.

In the present study we compare the emergence, duration and lymphoid distribution of virus-specific CTL and non-cytolytic cell-mediated immune responses during infection with the $Glasgow_8$ isolate of FIV ($FIV_{GL\text{-}8}$). The virus-specific cellular immune responses were compared with the FIV proviral burden in lymphoid tissues, as assessed by real-time PCR, throughout infection. Both $CD8^{hi}$ and $CD8^{lo}$ T cells in the blood were capable of suppressing FIV replication in vitro. The results suggest that novel intervention strategies should aim to elicit both virus-specific CTL responses and non-cytolytic responses in vivo to afford complete protection from infection.

2. Materials and methods

2.1. Experimental animals

The seven 30 week old, outbred, specific pathogen free (SPF) domestic cats selected for this study were serologically negative for FIV. Cats were inoculated intraperitoneally (ip) with 25 cat infective doses 50% (CID_{50}) of a stock of $FIV_{GL\text{-}8}$ prepared in the feline IL-2 dependent lymphoblastoid cell line, Q201.

2.2. Preparation of mononuclear cells

Peripheral venous blood was collected at regular intervals from all animals during the study and at post-mortem examination of the infected cats at 1, 4, 8, 47 (2 animals) and 113 (2 animals) weeks post infection (pi). Blood was collected into an equal volume of Alsevers' solution and mononuclear cells (PBMC) were prepared by centrifugation over Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden). Additionally lymphocytes were prepared from a pool of peripheral

(submandibular, retropharyngeal, prescapular, and popliteal) lymph nodes, mesenteric lymph nodes, and spleen. Cells were cryopreserved until use in the assays.

2.3. Determination of FIV proviral load

FIV proviral load was determined by quantitative Taqman PCR using an ABI Prism 7700 Sequence Detection System (PE Biosystems, Warrington, Cheshire, UK) together with FIV *gag* specific primers and probe as described previously (Leutenegger et al., 1999; Pedersen et al., 2001). The sensitivity of this technique was determined to be 10 copies of provirus within 1 μg genomic DNA (Leutenegger et al., 1999). DNA was extracted from mononuclear cells derived from peripheral blood, lymph nodes and spleen using a QIAamp blood mini kit (Qiagen, Crawley, West Sussex, UK). The plasmid pBlunt-zero (Invitrogen, Groningen, The Netherlands) containing the FIV *gag* gene was used as a standard for the PCR and the copy number of this standard was calculated by OD_{260} estimation and confirmed by gel electrophoresis. A five-fold dilution series was made in PCR grade water with 30 μg/ml calf thymus DNA (Life Technologies, Inchinnan, UK) as a carrier. To allow accurate estimation of the proviral load per 10^6 mononuclear cells, a further Taqman PCR designed to measure total sample DNA was performed using primers and probe specific for the *rRNA* gene (Klein et al., 2000). Standards for this PCR were derived from MYA-1 (Miyazawa et al., 1989) genomic DNA. The DNA was quantified by measuring the absorbance at OD_{260} and a dilution series was prepared in PCR grade water as before. Yeast RNA (Roche, Lewes, E. Sussex, UK) replaced calf thymus DNA as a carrier.

2.4. Detection of FIV-specific CTL activity

CTL activity was determined directly in the peripheral blood, lymph nodes and spleen. Target cells were autologous or allogeneic skin fibroblasts derived from biopsy material collected prior to infection (Flynn et al., 1995). The target cells were labelled with ^{51}Cr and infected with recombinant vaccinia viruses expressing FIV Gag (Flynn et al., 1995) or Env (Stephens et al., 1992) or wild-type vaccinia virus as a control. Effector cells (>99% viable) were added at *E*:*T* ratios of 50:1, 25:1 and 12.5:1. Microcytotoxicity assays were then performed as described previously (Flynn et al., 1996).

2.5. In vitro infection of MYA-1 cells with FIV

MYA-1 cells were infected in vitro by incubating 5×10^5 cells with 1 ml cell-free supernatant from $FIV_{GL\text{-}8}$ 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 h at 37 °C. The cells were then washed twice and resuspended in 1 ml fresh RPMI 1640 medium (Gibco Biocult, Paisley, UK) containing 10% foetal bovine serum (Biological Industries, Cumbernauld, UK), 2 mM L-glutamine 5×10^{-5} M 2-mercaptoethanol, 100IU of penicillin/ml, and 100 μg of streptomycin/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.

2.6. Detection of anti-viral activity in mononuclear cells

The capacity of mononuclear cells from the blood, spleen, and lymph nodes of FIV-infected cats to suppress the replication of FIV in vitro was assessed. The cells were first stimulated in vitro with 7.5 μg/ml Con A (Sigma Chemical, Poole, UK) for 72 h. Assays were then 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 $FIV_{GL\text{-}8}$, 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, and 1:1, in a final volume of 2 ml complete RPMI 1640 medium supplemented with 5% culture supernatant from the Ltk^-IL-2.23 cell line. The cell mixtures were then incubated at 37 °C 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 °C until assayed by ELISA for the presence of FIV p24 antigen (PetChek, IDEXX, Portland, ME). Cultures were maintained for up to 14 days.

2.7. Cell staining for flow cytometry

Mononuclear cells prepared from the peripheral blood, lymph nodes and spleen and the proportion of $CD4^+$ and $CD8^+$ T cells determined. Briefly, 2×10^6 cells were incubated with 50 μl mouse monoclonal anti-CD4 Ab (vpg 39) or mouse monoclonal anti-CD8 Ab (vpg 9) (Willett et al., 1993) for 30 min at 4 °C. Labelled cells were then washed, and bound monoclonal Ab visualised with a 1:20 dilution of an anti-mouse Ig–FITC conjugate (DAKO, UK). After incubation for a further 30 min at 4 °C, the cells were washed again and analysed on an EPICS (Beckman-Coulter, Emeryville, CA).

3. Results

3.1. In vivo dissemination of FIV following challenge

The FIV proviral burden was determined in the blood, peripheral and mesenteric lymph nodes and spleen at 1, 4, 8, 47, and 113 weeks pi by quantitative Taqman PCR (Table 1). Using this highly sensitive technique, which is estimated to detect as few as 10 copies of provirus within 10 μg genomic DNA (Leutenegger et al., 1999), FIV proviral DNA was detected as early as 1 week following exposure to $FIV_{GL\text{-}8}$ in the peripheral and mesenteric lymph nodes, and in the spleen. By 4 weeks pi, four copies of FIV proviral DNA could also be detected per 10^5 mononuclear cells in the blood. Peak FIV proviral burdens were observed at 8 weeks following infection in all lymphoid tissues examined, and the highest loads were detected in the blood and peripheral lymph nodes (1155.5 and 1065.6 copies of proviral DNA per 10^6 mononuclear cells, respectively). Thereafter, proviral burdens declined in all tissues. This effect was most noticeable in the blood and peripheral lymph nodes, although noted in the other tissues.

3.2. FIV-specific CTL responses

To correlate different aspects of virus-specific cell-mediated immune function during acute and chronic experimental infection with FIV, virus-specific CTL responses and the non-cytolytic T cell suppression of FIV replication in vitro were measured in the lymphoid tissues of cats at 1, 4, 8, 47, and 113 week pi.

Virus-specific effector CTL responses were first detected at 4 weeks pi, with the emergence of FIV Gag-specific effector CTL responses in the peripheral blood (Fig. 1). This was followed at 8 weeks pi with the detection of low levels of Gag-specific lysis in the mesenteric lymph nodes (Fig. 1). At 47 weeks pi, representing the asymptomatic phase of infection, two animals were examined. CTL responses were detected in the peripheral lymph nodes and spleen of both cats, although the magnitude of the response varied markedly between the cats. CTLs were also detected in the peripheral blood at this time, although at very much lower levels of specific lysis. The response was skewed towards recognition of FIV Gag-expressing targets, and no CTL activity was detected in the mesenteric lymph nodes of either cat at 47 weeks pi.

Table 1
FIV proviral load in lymphoid tissues[a]

Tissue	Weeks pi						
	1	4	8	47	47	113	113
No. of copies of FIV proviral DNA per 10^6 mononuclear cells							
Peripheral blood	0.0	0.4	1155.5	185.8	nd[b]	181.3	171.9
Peripheral LN[c]	0.8	0.2	1065.6	62.6	58.2	37.7	34.2
Mesenteric LN[c]	0.7	1.0	97.3	65.6	24.3	18.8	14.6
Spleen	0.2	0.0	85.2	51.0	8.9	18.3	30.6

[a] FIV proviral load was determined by quantitative Taqman PCR. DNA was extracted from mononuclear cells derived from blood, peripheral lymph nodes, mesenteric lymph nodes and spleen at several time-points following infection. The sensitivity of this technique was determined to be 10 copies of provirus within 1 μg genomic DNA.

[b] Not done. Due to technical reasons only one sample of blood collected 47 weeks after infection was available for analysis.

[c] Lymph node.

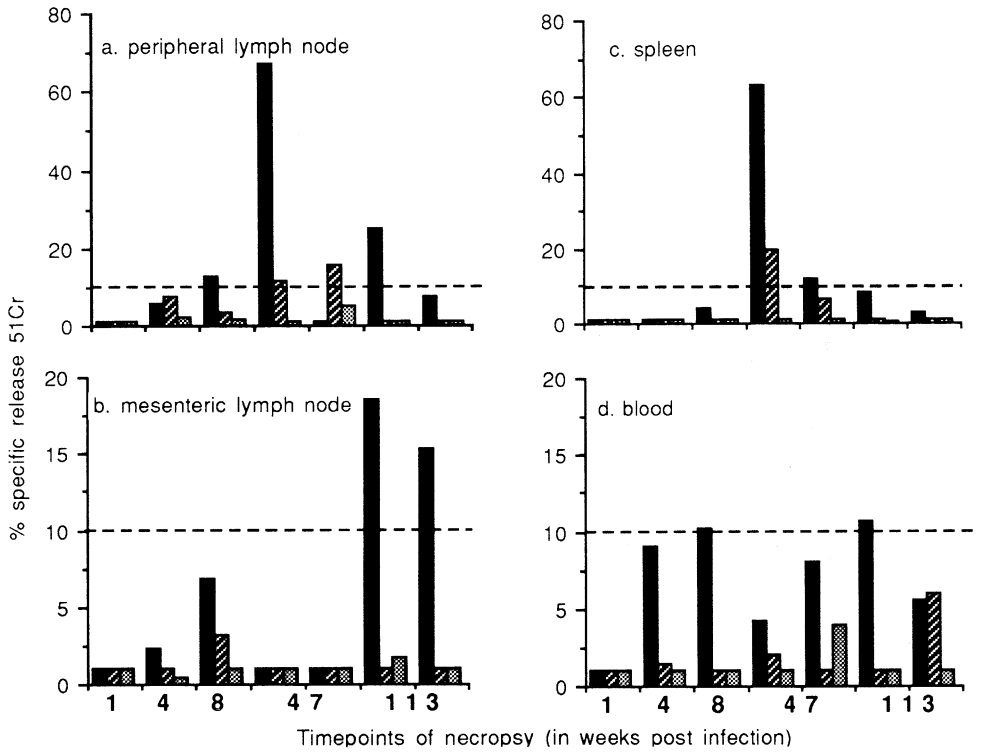

Fig. 1. FIV-specific CTL responses during FIV_{GL-8} infection. FIV-specific effector CTL responses were measured directly in mononuclear cells prepared from the peripheral lymph nodes (a), mesenteric lymph nodes (b), spleen (c) and blood (d) of a total of seven cats. Samples were collected from one cat at 1, 4 and 8 weeks pi, and from two cats at 47 and 113 weeks pi with FIV_{GL-8}. Autologous or allogeneic skin fibroblasts infected with either recombinant vaccinia viruses expressing FIV Gag (■), FIV Env (▨), or wild-type vaccinia virus (▩), and labelled with ^{51}Cr were used as targets in the assay. The results shown represent the mean release of ^{51}Cr into the culture supernatant from triplicate cultures at an *E*:*T* ratio of 25:1 after 4 h incubation at 37 °C. The values for the recognition of allogeneic targets (which were less than 10%) have been subtracted.

Tissues were next examined in two cats at 113 weeks pi, representing a more chronic phase of infection. At this time, CTL responses were detected in all of the lymphoid tissues examined in both cats. The activity detected was highest in the peripheral and mesenteric lymph nodes, with only very low levels of specific lysis observed in the blood and spleen. As observed at 47 weeks pi, the CTL activity in both animals was preferentially directed towards FIV Gag-expressing target cells.

3.3. Lymphoid distribution of FIV suppressor activity

Examination of the temporal co-evolution of CTL and non-cytolytic T cell suppression of FIV replication during FIV_{GL-8} infection, revealed a very significant (>80% compared to FIV-infected MYA-1 cells cultured alone) suppression of FIV replication by non-cytolytic T cells which was evident in all the lymphoid tissues examined as early as 1 week pi (Fig. 2). This effect was dependent on the number of mononuclear cells added to the cultures, as shown by the decreased activity observed at the lower ratios of mononuclear cells to FIV-infected MYA-1 cells. An increase in the level of suppression was evident at week 4 pi. However, by week 8 pi this activity had decreased in all tissues. This decrease in the suppression of FIV replication was most marked in the lymph nodes, with suppression undetectable in the peripheral LNC cultures (Fig. 2). By 47 weeks pi suppressor activity was again detected in all lymphoid tissues of the two cats at this time, although the levels of suppression observed

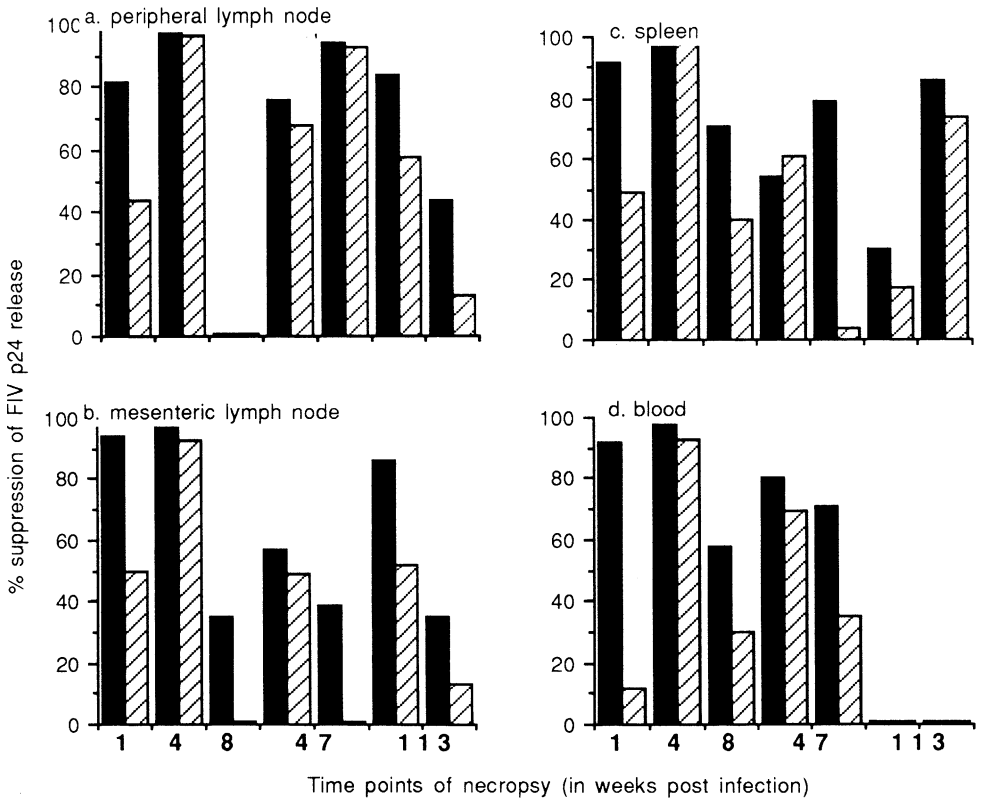

Fig. 2. Lymphoid distribution of FIV suppressor cell activity. Mitogen-activated lymphoblasts were prepared from the peripheral lymph nodes (a), mesenteric lymph nodes (b), spleen (c) and blood (d) of a total of seven cats at 1, 4, 8, 47 (2 cats) and 113 (2 cats) weeks pi with FIV_{GL-8} and were co-cultivated with FIV-infected MYA-1 cells at ratios of 4:1 (■) 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 at day 10 by ELISA. The results shown represent the % suppression of FIV replication for duplicate cultures calculated with reference to FIV-infected MYA-1 cells cultured alone.

were lower than those detected at week 4 pi. The decline in suppressor T cell activity was further noted in the lymphoid tissues from both cats examined at week 113 pi. At this time, suppressor T cell activity was undetectable in the blood.

3.4. Longitudinal analysis of FIV suppressor activity in blood

Due to the outbred nature of the cats used in this study, it is feasible that the modulations in the cell-mediated suppression of FIV replication observed in the lymphoid tissues throughout infection may merely represent individual variations in host immune response to the virus, rather than modulations occurring as a result of the prolonged interaction of the host immune system with the virus. To overcome this potential disadvantage, a longitudinal analysis of the suppressor activity in the blood was performed on the two cats which survived until the termination of this study at 113 weeks pi. The results are shown in Fig. 3. In both cats suppressor activity was detectable in the blood prior to challenge, with PBMC able to suppress the production of FIV p24 by 9 and 35%, respectively. Following infection, this activity appeared to increase, resulting in over 90% suppression of viral replication at 4 weeks pi. This activity then declined, and became undetectable at 24 weeks pi. This decline was also observed in all the other lymphoid tissues examined (see Fig. 2). A second peak

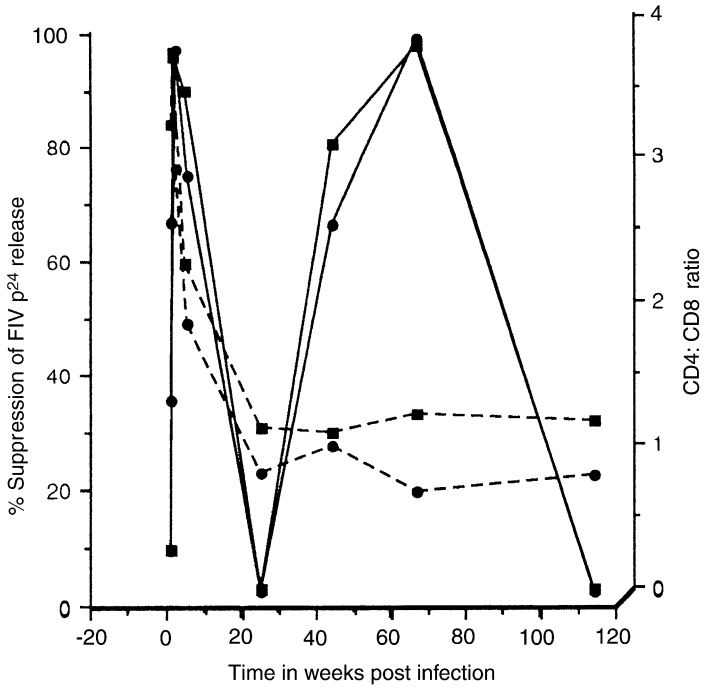

Fig. 3. Longitudinal analysis of FIV suppressor activity in the blood. Mitogen-activated lymphoblasts were prepared from the blood of two cats (■ and ●) at intervals prior to and following infection with FIV_{GL-8}, and were co-cultivated with FIV-infected MYA-1 cells at a ratio of 4:1. Replication of FIV was indicated by the detection of viral p24 in the culture supernatant at day 10 by ELISA. The results shown represent the % suppression of FIV replication for triplicate cultures calculated with reference to control cultures of FIV-infected MYA-1 cells alone. The ratio of CD4:CD8 T cells in the blood is shown (- - -).

in suppressor activity was observed in the blood between 40 and 80 weeks pi, before declining to baseline levels at the termination of the study. The modulations of suppressor activity observed in the blood correlated with neither the absolute numbers of $CD4^+$ or $CD8^+$ T cells, nor with the CD4:CD8 T cell ratio is shown in Fig. 3.

3.5. $CD8^{lo}$ T cells suppress FIV replication during FIV infection

The previous experiments clearly demonstrate that virus-specific cell-mediated immune responses are associated with the control of FIV replication during acute, asymptomatic and chronic stages of infection. Infection with FIV_{GL-8} is known to be associated with the appearance of $CD8^{lo}$ T cells in the blood (Willett et al., 1993). Do these $CD8^{lo}$ T cells contribute to the control of FIV replication observed in vitro? To address this issue, populations of either $CD8^{lo}$ or $CD8^{hi}$ T cells were prepared from the blood of two cats at week 66 pi, and the capacity of each to suppress FIV replication in vitro was assessed. The two cats with the highest levels of circulating $CD8^{lo}$ T cells (data not shown) were selected for this experiment to facilitate the preparation of maximal numbers of T cell subsets by flow cytometry.

As shown in Fig. 4, the $CD8^+$ T cell population in the blood was comprised of two subpopulations of varying fluorescence intensity. The two subpopulations were prepared by flow cytometry and the purity of the sorted cells confirmed. The $CD8^{lo}$ T cell preparations contained <1.1% $CD8^{hi}$ T cells, and the $CD8^{hi}$ T cell population contained <1.9% $CD8^{lo}$ cells. Unsorted $CD8^+$ T cells from the two FIV-infected cats were capable of suppressing FIV replication in vitro. Analysis of the sorted $CD8^{lo}$ or $CD8^{hi}$ T cells from the blood of both cats revealed that both subpopulations had the capacity to suppress FIV replication in vitro.

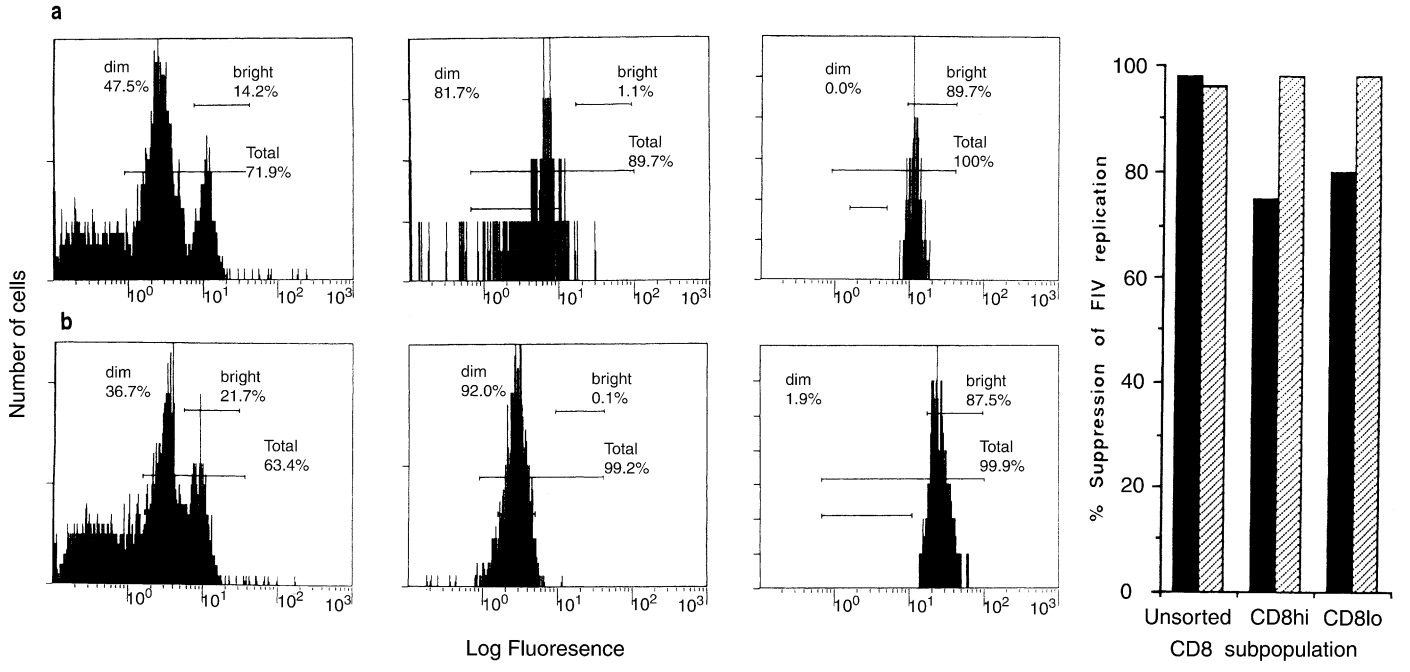

Fig. 4. $CD8^{hi}$ and $CD8^{lo}$ T cells suppress FIV replication during asymptomatic FIV infection. A gate was set around the lymphocyte fraction of PBMC on the basis of forward and 90° light scatter. Populations of $CD8^{hi}$ and $CD8^{lo}$ T cells were prepared by flow cytometry from the blood of 2 cats 66 weeks pi with $FIV_{GL\text{-}8}$ (a and b; ■ and ▨). Purified cells, and an aliquot of unsorted cells were stimulated with Con A and co-cultivated with FIV-infected MYA-1 cells at a ratio of 2:1. Control cultures contained FIV-infected MYA-1 cells alone. Replication of FIV was indicated by the detection of viral p24 in the culture supernatant at day 10 by ELISA. The results shown represent the % suppression of FIV replication for duplicate cultures calculated with reference to FIV-infected MYA-1 cells cultured alone.

4. Discussion

Elucidation of the primary virus-specific effector mechanisms associated with control of viral replication and dissemination following lentiviral infection are crucial to the rational design of novel, effective vaccines. FIV infection of domestic cats is a well recognised animal model for AIDS vaccine development (Willett et al., 1997). Previous studies have shown that virus-specific CTL responses are detectable before virus-specific humoral immunity (Song et al., 1992; Beatty et al., 1996; Flynn et al., 1996). However, little is known of other cellular immune mechanisms of viral control, and how they compare with FIV-specific cytotoxicity. In the present report we have compared the temporal co-evolution of non-cytolytic T cells, capable of suppressing FIV replication in vitro with FIV-specific CTLs following experimental infection of cats with the virulent, $Glasgow_8$ isolate of FIV ($FIV_{GL\text{-}8}$). The results demonstrate that T cell-mediated suppression of FIV replication precedes the elicitation of FIV-specific CTL responses.

Non-cytolytic T cells capable of suppressing the replication of FIV represented the first detectable antiviral immune response in cats following $FIV_{GL\text{-}8}$ infection. This activity was detectable in peripheral and mesenteric lymph nodes, spleen and blood as early as 1 week following infection with $FIV_{GL\text{-}8}$ with the capacity to suppress FIV replication in vitro by up to 96% compared to control cultures of FIV-infected MYA-1 cells. In contrast, virus-specific CTL responses, were not detected until the fourth week of infection, and at this time were detected only in the blood. It was not until later in infection that virus-specific CTL activity appeared lymph nodes and spleen. Lysis of target cells expressing FIV Gag or Env antigens was detected following infection, although there was preferential recognition of Gag-expressing targets throughout the period of investigation. The reason for this skewing of the immune response towards recognition of Gag, which is in agreement with previous studies remains unclear (Beatty et al., 1996; Flynn et al., 1996).

The cell-mediated suppression of FIV replication was observed to peak at week 4 pi, corresponding to the emergence of FIV-specific CTLs, followed by a decline at week 8 pi. A second peak was then observed at week 47 pi in both cats examined. The pattern of FIV suppressor activity varied in the two cats examined 113 weeks pi, however it was very clear that this activity was no longer present in the blood, which agrees with our previous findings following infection with $FIV_{GL\text{-}8}$ where it was not possible to detect suppression of FIV replication in the blood at 22 months pi (Flynn et al., 1999). Longitudinal analysis of the FIV suppressor activity in the blood of two cats also showed the apparent bi-phasic pattern of FIV suppression observed in the other lymphoid tissues. The modulations of suppressor activity observed in the blood correlated with neither the absolute numbers of $CD4^+$ or $CD8^+$ T cells, nor with the CD4:CD8 T cell ratio is shown in Fig. 3. None of the cats in this study displayed any clinical signs relating to infection with FIV. It has been reported in HIV-1-infected patients that the increased incidence of opportunistic infections is associated with defects in both the natural (Lopez et al., 1983, 1984) and adaptive (Rinaldo et al., 1995; Carmichael et al., 1993) components of the immune system. Clearly a longer study period would be required to determine whether the lack FIV suppressor activity in the blood of cats at 113 weeks pi would be associated with the onset of clinical disease.

Do non-cytolytic suppressor cells and FIV-specific CTLs contribute to the control of viral replication in vivo? To address this question, the distribution of FIV proviral DNA was quantified in the lymphoid tissues throughout the study and compared with the virus-specific cellular immune responses. By analogy with studies on virus kinetics following HIV-1 infection, the relatively high proviral burden maintained in the blood until the termination of the study was rather surprising, as it is recognised that HIV-1 proviral burdens are low in the blood of human patients during the asymptomatic phase of the disease (Ogg et al., 1998) with sequestration of HIV-1 to lymph nodes (Embretson et al., 1993; Pantaleo et al., 1993). Our findings may reflect the intraperitoneal route of challenge used in this study, coupled with the use of a highly pathogenic isolate of FIV. No temporal association was evident between proviral FIV load and the emerging virus-specific CTL activity in the blood. However, more proviral DNA copies were detected in blood than any other tissue examined which correlated inversely with the FIV-specific CTL activity detected in blood. Peak viral loads observed in the blood, and other lymphoid tissues at week 8 pi,

co-incided with a drop in FIV suppressor activity. Presently, it is not possible to deduce whether the decline in FIV suppressor activity caused viral burdens to escalate, or whether the high levels of viral replication merely overwhelmed the host immune response.

Recently, a number of studies have attempted to further elucidate the phenotype of the T cells responsible for mediating the non-cytolytic suppression of FIV replication in vitro. Following experimental infection of cats with the NCSU1 isolate of FIV, $CD8^+$, L-selectin$^-$ ($CD62L^-$) T cells which also have increased expression of CD44, CD49d, and CD18 (Bucci et al., 1998) have been shown to be associated with the non-cytolytic suppression of FIV replication. Infection with $FIV_{GL\text{-}8}$ is associated with the appearance of an unusual subset of $CD8^+$ T cells in the blood characterised by diminished expression of surface CD8. To determine the contribution of this cell type to suppression of FIV replication, purified populations of $CD8^{lo}$ or $CD8^{hi}$ were prepared by flow cytometry from the blood of cats during asymptomatic infection and analysed for their capacity to modulate the replication of FIV in vitro. The results revealed that both $CD8^{hi}$ and $CD8^{lo}$ T cell subpopulations prepared from FIV-infected cats had the capacity to suppress FIV replication in vitro. Due to the limited availability of these purified blood-derived cells, it was not possible to determine whether these cells also had FIV-specific CTL activity. The ability of this cell-mediated immune activity to up-regulate more rapidly than virus-specific humoral immune or CTL responses following exposure to viral antigens further suggests that this activity may represent an innate type of immunity.

No attempt has been made to identify the factor(s) involved in the control of virus replication observed in this study, although it is clear from previous studies that the activity is mediated by soluble factors. Studies on the Petaluma isolate of FIV (FIV_{PET}) have shown that the human β-chemokines RANTES, MIP-1α, MIP-1β, and MCP-1 could not prevent infection of CrFK cells or T lymphocytes, whilst human SDF-1α could inhibit FIV_{PET} infection of CrFK cells but not T cell lines, depending on the incubation conditions (Hosie et al., 1998). In contrast, studies on another American isolate of FIV (FIV_{PPR}), which has 91% homology with FIV_{PET} at the amino acid level, have revealed that human MIP-1α did suppress FIV replication in PBMC at a concentration of 500 ng/ml, whereas human SDF-1α used at the same concentration actually caused increased expression of FIV rather than suppression (Choi et al., 2000). Clarification on the role of feline β-chemokines in $CD8^+$ T cell-mediated suppression of FIV replication will be reliant upon the availability of these recombinant feline proteins in the future. However, such efforts to characterise the factors involved may be confounded by studies on HIV demonstrating that several soluble factors may act in concert to produce the suppressive effect (Cocchi et al., 1995).

In conclusion, exposure of the host immune system to FIV results in the up-regulation of non-cytolytic T cells capable of suppressing virus replication in vitro, this is followed by the appearance of FIV-specific effector CTLs in the blood and lymphoid tissues. Both of these cellular immune mechanisms contribute to the control of viral replication in vivo and studies on lentiviral vaccination should take account of these immune responses when assessing efficacy criteria.

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