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Temporal association of large granular lymphocytosis, neutropenia, proviral load, and FasL mRNA in cats with acute feline immunodeficiency virus infection

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Abstract

During acute feline immunodeficiency virus-CpG_{gamma} (FIV C-PG) infection, we observed that cats develop LGL lymphocytosis concurrent with a marked neutropenia that is temporally associated with the rise and fall of FIV-C-PG proviral loads. Large granular lymphocytes (LGLs), generally considered to be analogous to natural killer (NK) cells, can also be highly cytolytic CD8/CD57 T cells. Neutropenia has been reported during acute human immunodeficiency virus (HIV-1) infection, but there is a paucity of information describing the pathogenesis of this condition. During HIV-1 infection, LGLs have been shown to be both CD16⁺ NK cells and CD8⁺/CD57⁺ T cells, but have not been associated with neutropenia. However, neutropenia with concurrent LGL lymphocytosis has been demonstrated in both LGL leukemia and common variable immunodeficiency syndrome in people, and in both syndromes, an increase in soluble Fas ligand (FasL) has been associated with neutrophil apoptosis leading to neutropenia. Flow cytometric analysis demonstrated increases in CD56 and CD8 peripheral blood cell surface expression during acute FIV-C-PG infection. Expression of FasL mRNA was increased at the same time points as these peripheral hematologic abnormalities, and also decreased as FIV-C-PG proviral load reached set point. We describe an interesting temporal association between innate immune responses and viral load during acute FIV-C-PG infection, which has similarities to HIV-1 infection and other immune dyscrasias of people, and which may contribute to the neutropenia and LGL lymphocytosis during FIV-C-PG infection.

Keywords

FIV; Large Granular Lymphocytes; Neutropenia

1. Introduction

Transient severe neutropenia has been reported relatively commonly in cats (*Felis catus*), less commonly in humans (*Homo sapiens*), and very rarely in rhesus macaques (*Macaca mulatta*) during acute FIV, HIV-1 and simian immunodeficiency virus (SIVmac251) infections

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Conflict of Interest

The authors have no conflict of interest associated with this study

(Babadoko et al., 2008; Colson et al., 2005; Elbim et al., 2008; Levine et al., 2006; Linenberger et al., 1995), respectively, and is associated with more severe disease progression compared to non-neutropenic, lentivirus-infected animals or people. The mechanisms underlying neutropenia in these conditions is poorly understood and likely multifactorial.

Constitutive neutrophil apoptosis is complex and can involve a variety of intracellular and extracellular molecules, including FasL, reactive oxygen species (ROS), cysteine-aspartic acid proteases (caspases), and cytosolic calcium-dependent cysteine proteases (calpains) (Luo and Loison, 2008). Neutrophils purified from HIV-1-infected individuals were shown to have increased Fas and FasL expression, which when exposed to a cross-linking Fas antibody, underwent apoptosis that correlated with viral loads (Salmen et al., 2004). A spontaneous form of neutrophil apoptosis that did not correlate with viral load was also detected that was linked to oxidative stress during HIV-1 infection (Salmen et al., 2007). A calpain-dependent, caspase-independent neutropenia was recently reported during acute SIVmac251 infection in rhesus macaques, suggesting perhaps that caspase-mediated FasL mechanism of apoptosis is not involved in neutropenia induced by SIVmac251 infection (Elbim et al., 2008).

Neutropenia associated with increases in soluble FasL have been demonstrated in cases of LGL leukemia (Burks and Loughran, 2006; Liu et al., 2000) and common variable immunodeficiency syndrome (Holm et al., 2006), both of which have LGL lymphocytoses. LGLs in these diseases have been shown to express variable cell surface markers, including CD56, CD8/CD57, CD16 and are thought to have natural killer (NK) cell or cytotoxic T cell properties (Prochorec-Sobieszek et al., 2008; Pulik et al., 1997; Smith et al., 2000). During HIV-1 infection, LGLs have been shown to be both CD16⁺ NK cells and CD8⁺/CD57⁺ T cells (Ghali et al., 1990; Kronenberg et al., 2001; Smith et al., 2000), but associated neutropenia has not been reported.

Finally, bone marrow lentivirus infection with destruction or growth inhibition of myeloid precursor cells has been implicated as mechanism of neutropenia during FIV (Petaluma) and HIV-1 infections (Beebe et al., 1992; Fujino et al., 2008; Linenberger et al., 1995; Moses et al., 1996).

In this study, we describe a temporal association between innate immune responses (large granular lymphocyte counts, neutrophil counts and FasL expression) and viral loads during acute FIV-C-PG infection that has similarities with HIV-1 and other non-viral-associated immunological dyscrasias of people, and which may contribute to the neutropenia and granular lymphocytosis in some cats with FIV-C-PG. These findings may lead to a more in depth understanding of the role of innate immunity in modulating lentiviral disease.

2. Materials and Methods

2.1. Viral Stocks

FIV-C-PG was recovered from the retropharyngeal lymph node of a cat with a molecularly cloned FIV-C-PG by culture with MYA-1 cells. Reverse transcriptase (RT) activity of culture supernatants was monitored and virus was harvested by slow speed (200–500xg) centrifugation using a Beckman GPR centrifuge with a GH 37 rotor (Beckman Coulter, Fullerton, CA) to remove cell debris at peak RT activity. The sham inoculum (medium) was similarly prepared from culture supernatant of uninfected MYA-1 Cells (Terwee et al., 2008).

2.2. Animals

Five specific-pathogen-free (SPF) cats were obtained from a breeding colony at Colorado State University and were inoculated IV with 1 ml of a previously characterized FIV-C-PG stock that had been diluted 1:100 in 0.9% NaCl (Terwee et al., 2008). Animals were part of a bigger

study and had been randomized by gender and were housed in isolation rooms in an AAALAC-international accredited animal facility. All procedures were approved by the CSU Institutional Animal Care and Use Committee prior to initiation of studies.

2.3. Large granular lymphocyte, CD56 and CD8 α enumeration

Blood samples were collected in EDTA by venipuncture of the jugular or cephalic vein on the day of FIV-C-PG infection and every one to four weeks for 77 weeks. Total white and red blood cell counts were measured using a Coulter Z1 (Coulter, Miami, FL). Differential cell counts were performed manually and LGL counts (see section 3.1 and figure 1 for identification criteria) were recorded as a percentage of the nucleated cells after 100 nucleated cells were counted. Percentages of lymphocytes positive for CD8 α and CD56 were determined by flow cytometry using a monoclonal antibody to feline CD8 α (Southern Biotech, Birmingham, AL) and to feline CD56 antibody (gift of M Shimojima; (Shimojima et al., 2003)). Two $\times 10^5$ to 1×10^6 peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood over a ficoll gradient and then incubated for 20–60 minutes at room temperature with CD8 α antibody at 5 μ g/ml or CD56 antibody at a 1:2000 dilution in flow buffer (PBS containing 2% FBS and 0.2% sodium azide). Cells were then washed twice in flow buffer, resuspended in 100 μ l of FITC-labeled sheep anti-mouse IgG (Sigma, St. Louis, MO) at 10 μ g/mL in flow buffer and incubated for 20–60 minutes at room temperature in the dark. After incubation, cells were washed twice, and samples resuspended in 300 μ L of buffer with 0.01% propidium iodide added to exclude dead cells. Samples were then immediately analyzed on a Coulter EPICS XL MCL flow cytometer (Beckman Coulter, Miami, FL). The discriminator was set based on forward scatter to eliminate small particles and a total of 5000 cells in the lymphocyte gate were collected. To determine the percentage of cells stained with each antibody, gates were set based on the isotype controls (unconjugated mouse IgG1, Southern Biotec) diluted similarly to that of the antibody, followed by incubation with a secondary sheep anti-mouse IgG FITC such that 1% or fewer cells were positive.

List mode files were analyzed using FlowJo (Tree Star Inc., San Carlos, CA). Total lymphocyte counts were calculated by multiplying the total white blood cell count by the percentage of lymphocytes in the sample as determined by the differential count and then by the percentage of lymphocytes expressing CD8 α . Percent of CD56 $^+$ cells was reported as a percentage of the cells analyzed in the lymphocyte gate.

2.4. Viral Copy Number

Peripheral blood mononuclear cells (PBMCs) were purified from heparinized whole blood using a Histopaque (Sigma, St. Louis, MO) gradient according to the product insert. DNA was extracted from one million PBMCs using the Qiaamp blood mini DNA kit (Qiagen, Valencia, CA). DNA was eluted with 50–200 μ l of buffer. Primers and probe for FIV-C-PG gag (Pedersen, et al., 2001) were used to quantify FIV-C-PG in plasma. The sensitivity of detection is a minimum of 5 copies. Copy number was normalized to the cellular house-keeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), to calculate copy number per extracted cell.

2.5. Fas Ligand Expression

FasL expression was quantified by real time PCR using the method of Mizuno et al. (Mizuno et al., 2003). Five to ten million PBMC (purified as above) were dissolved in Trizol (Sigma, St. Louis, MO) at 10 million cells/ml. cDNA was reverse transcribed from RNA which had been purified by extracted phenol:chloroform extraction and ethanol precipitation. Cytokine expression for FasL was quantified relative to the gene GAPDH, using the formula $2^{-\Delta CT}$, where ΔCT represents the cycle when the cytokine threshold is reached minus the cycle when

the GAPDH threshold is reached. All samples collected on a given study day were prepared and tested together.

2.6. Statistical Analysis

Statistical analysis comparing FasL mRNA expression, LGL counts, CD56 expression, CD8 lymphocyte counts, neutrophil counts and proviral loads over time was measured by repeated-measures analysis of variance (ANOVA) after data was log transformed to establish a Gaussian distribution using Prism version 4.0b software for Macintosh, (GraphPad, Inc. San Diego, CA, 2004). The Student's T test was used for paired comparisons of the data (Microsoft Excel, Microsoft Corporation, Redmond, WA.). Statistical significance was considered to have occurred when a *P* value was < 0.05. Pearson product-moment correlations (*r*) between CD56 and CD8 α expression and LGL counts were performed using JMP7.0.1 software for Macintosh (SAS, Cary, NC).

3. Results

3.1. Identification of LGLs in peripheral blood of animals during acute FIV-C-PG infection that coincided with FIV-C-PG proviral loads

LGLs can be differentiated from normal lymphocytes in that they are larger, have eccentric nuclei, more abundant cytoplasm and fine intracytoplasmic azurophilic granules compared to normal lymphocytes (Fig. 1). Absolute LGLs in circulation began to increase 2 weeks post FIV-C-PG infection (not statistically significant) and returned to near normal values between days 49 and 77 post infection (PI), coinciding with a similar increase and decrease in FIV-C-PG proviral loads that was highly significant (Fig. 2, *p* < 0.0001). Statistically significant changes were appreciated on days 21 (*p* = 0.0335), 28 (*p* = 0.0214), 35 (*p* = 0.0105), 49 (*p* = 0.0030), and 77 (*p* = 0.0034), post FIV-C-PG infection when compared to LGL counts prior to infection (Day 0).

3.2. CD8 α^+ lymphocytes increase concurrently with LGL during acute FIV-C-PG infection, but remain increased as LGLs decrease

LGLs have been reported to be either CD8 $^+$ cytotoxic T cells or CD56 $^+$ NK cells (Prochorec-Sobieszek et al., 2008; Pulik et al., 1997; Smith et al., 2000). Leukocytes from whole blood were labeled with anti-CD8 α and anti-CD56. CD8 α^+ lymphocytes increased significantly with proviral loads (*p* < 0.0001). Compared to day 0, significant CD8 α^+ lymphocyte increases were not seen until 49 days PI (*p* = 0.005) and remained elevated at day 77 PI (Fig. 2A, *p* < 0.001). Although the concurrent increase of CD8 α^+ lymphocytes and LGLs was significant (*p* = .0005), linear correlation was only moderate between the two variables (Fig. 2B, *r* = 0.5033).

3.3 CD56 is expressed at low concentration on circulating PBMC and the percent of CD56 $^+$ lymphocytes increase concurrently and correlates linearly with the LGL lymphocytosis

CD56 cell surface expression was low on PBMC as demonstrated in Fig 3A. This finding correlates with dim CD56 expression on highly cytotoxic CD56 $^+$ NK cells in the peripheral blood of acutely infected HIV-1 individuals (Alter et al., 2007). The percent of CD56 $^+$ circulating lymphocytes increased concurrently with LGLs and the increase was considered highly significant (Fig. 3B, *p* = 0.0024). The percent of CD56 $^+$ cells/peripheral blood lymphocytes also showed higher correlation with LGL/ μ l of blood (Fig. 3C, *r* = 0.7033) than did the number of lymphocytes expressing CD8 α^+ / μ l of blood (Fig. 2B, *r* = 0.5033). The percent of CD56 $^+$ cells also increased significantly with increasing proviral loads during acute FIV-C-PG infection (data not shown, *p* < 0.0001), however, these cells were not significantly increased over baseline until day 49 (*p* = 0.0250).

3.4. A temporal relationship of neutropenia and increasing FasL mRNA expression on PBMC exists during the rise and fall in proviral loads during acute FIV-C-PG infection

Acute FIV-C-PG infection has been shown to be associated with a transient neutropenia that is more marked during infection with virulent strains (Pedersen et al., 2001). In this study, neutrophil counts began to decrease between 21 and 28 days post-FIV-C-PG infection and reached a nadir at 35 days PI, and was significantly associated with increasing proviral loads (Fig. 4A, $p < 0.0001$). Conversely, we observed a significant increase in LGLs by 21 days PI that continued to rise as the neutropenia worsened, but then declined as peripheral neutrophil counts began to rebound (Fig. 4B, $p < 0.0001$). FasL mRNA expression in PBMC also increased and decreased concurrently with the appearance of LGLs ($p = 0.0096$) and the development of the neutropenia ($p = 0.0002$) (Fig. 4B), suggesting a correlation between these parameters. The neutropenia reached statistical significance at 14 days ($p = 0.0429$), 49 days ($p = 0.0124$) and 77 days ($p = 0.040$) PI.

4. Discussion

Neutropenia occurs in up to 44% of HIV-1-infected individuals. During acute and chronic phases of HIV-1 infection, neutropenia has been considered a marker for severity of disease, rapidity of progression, and immunological deterioration (Babadoko et al., 2008; Colson et al., 2005; Elbim et al., 2008; Levine et al., 2006; Linenberger et al., 1995). Additionally, neutropenia has been documented as a consequence of the most severe forms of the FIV-C-PG disease (Pedersen et al., 2001). Despite the relationship between neutrophil loss and disease progression, the mechanisms underlying neutropenia in these conditions have not been determined.

Mechanisms of neutrophil apoptosis during lentiviral infections are likely multifactorial. Oxidative stress has been shown to be responsible for increased spontaneous neutrophil apoptosis in HIV-1-infected individuals in an intrinsic apoptotic manner that is caspase-3 dependent and caspase-8 independent (Salmen et al., 2007), however, recent work by Elbim et al., demonstrated that neutrophil apoptosis in rhesus macaques acutely infected with SIVmac251 was caspase-independent, suggesting that mechanisms other than an intrinsic pathway also contribute to neutrophil apoptosis and neutropenia during lentiviral infection (Elbim et al., 2008). Neutrophils showed an increased susceptibility to undergo apoptosis following the cross-linking of Fas with anti-Fas Ab in HIV-1 infected individuals, and this characteristic correlated with both viral load and increased neutrophil Fas/FasL expression (Salmen et al., 2004). In addition, intravenous anti-FasL given prior to and during the acute stages of SIVmac239 infection lead to attenuated disease, and although neutrophils were not monitored, this finding suggests that FasL plays an important role in establishment of disease severity during acute lentivirus infection (Salvato et al., 2007). In support of this mechanism for neutrophil apoptosis, increased FasL was found in the sera of HIV-1-infected individuals (Sabri et al., 2001).

LGLs are generally considered to be analogous to natural killer (NK) cells, however, they are also found to be highly cytolytic CD8/CD57 T cells (Chattopadhyay et al., 2009; Prochorec-Sobieszek et al., 2008; Pulik et al., 1997; Timonen et al., 1979). Neutropenia has previously been identified in humans as a consequence of LGL leukemia and common variable immunodeficiency disease where there is a polyclonal expansion of LGLs. In both syndromes increased soluble FasL has been demonstrated in sera (Burks and Loughran, 2006; Holm et al., 2006). During HIV-1 infection, LGLs were shown to be both CD16⁺ NK cells and CD8⁺/CD57⁺ T cells (Ghali et al., 1990; Kronenberg et al., 2001; Smith et al., 2000). Polyclonal expansions of CD8⁺/CD57⁺ LGLs have been described in HIV-1 patients that were shown to be cytolytic in response to infection (Chattopadhyay et al., 2009; Le Priol et al., 2006), however, Leu7⁺2⁺ cells (analogous to CD57⁺/CD8⁺ cells) were found to have depressed cytotoxic

responses in HIV-1-infected individuals (Gupta, 1986). CD8⁺/CD57⁺ cytotoxic cells were identified in peripheral circulation of cats with and without FIV-C-PG infection; however, morphology of these cells was not assessed (Smith et al., 2000; Zhao et al., 1995). In HIV-1 and SIVmac239 infections, CD16⁺ NK cells were shown to be the most cytolytic subset of NK cells (Kottlilil et al., 2007; Pereira et al., 2008), and have been shown to undergo accelerated deletion during disease progression. These observations indicate that LGLs and NK cells and/or CD8⁺ T cells, may play an important yet ill-defined role in lentiviral pathogenesis (Alter and Altfield, 2009; Levy, 2006).

Bone marrow suppression has been implicated as a cause of neutropenia in FIV-C-PG infection; Kubes et al demonstrated impaired neutrophil function in response to bacterial stimulants (Kubes et al., 2003) that could be restored with GM-CSF administration (Heit et al., 2006). GM-CSF and G-CSF have been shown to improve neutrophil counts in chronically infected HIV-1 positive individuals (Hewitt and Morse, 1992; Wong, 1999). FIV-C-PG has been shown to replicate to high titer in bone marrow (Troth et al., 2008). These findings suggest that lentiviral impairment of neutrophil progenitor cells may also contribute to neutropenia observed during early infection.

The appearance of LGLs that occurred concurrent with increased PBMC FasL mRNA expression correlates with reports of increased soluble FasL expression in the sera of patients with LGL leukemia and common variable immunodeficiencies that exhibit LGL lymphocytosis (Burks and Loughran, 2006; Holm et al., 2006; Liu et al., 2000). A recent report demonstrated differential expression of a CD16⁺ on CD56⁺ cytolytic NK cells between SIVmac239-resistant Sooty mangabeys (high expression) and SIVmac239-susceptible rhesus macaques (low expression) during acute SIV infection that did not correlate with viral load (Pereira et al., 2008). In addition, puma lentivirus (PLV-1695)-infected cats, that are PLV-resistant with high viral titers, do not have increases in LGLs during acute infection (personal observation). We have demonstrated a correlation between the expression of CD56 and CD8α⁺ on lymphocytes and the appearance of LGL in the peripheral blood. CD56 expression may more closely correlate with LGL expression than CD8α⁺ because fewer time points were analyzed. This observation will be expanded in future studies with CD56 and other reported markers for LGLs. Another interesting observation is that neutrophils appear to rebound while the proviral loads continue to increase. This might be explained by the findings that both CD8⁺/CD57⁺ T cells and CD4⁺ NK cells lose their cytotoxic capabilities during HIV-1 infection and that CD4⁺ NK cells were reported to be productively infected by HIV-1 (Bernstein et al., 2009; Gupta, 1986). It would be interesting to further investigate cell surface markers expressed on LGLs identified in this study to more completely determine the function and phenotype of these cells. Determination of FasL mRNA expression on LGLs and evaluation of serum FasL levels would allow validation of the mechanisms for neutropenia suggested by data presented in this study. FasL mRNA expression should also be evaluated in other cell subsets that have been shown to contain abundant intracellular FasL released in response to immune stimulation, such as monocytes (Kiener et al., 1997).

In this study, we demonstrate a temporal pattern of LGL lymphocytosis associated with severe neutropenia and increased PBMC FasL mRNA expression that is correlated with FIV-C-PG proviral loads. This association creates compelling circumstantial evidence for at least one mechanism of neutrophil apoptosis during acute FIV-C-PG infection and implies that cells of the innate immune system play a more important role in the pathogenesis of FIV-C-PG infection than has been previously described. Mechanisms underlying neutrophil apoptosis that result in severe and transient neutropenia during acute lentivirus infections require greater exploration given the close association noted here and by others between neutropenia and disease severity.

A previous study demonstrated that heat-inactivated sera from FIV-C-PG-infected cats inhibited growth of autologous myeloid progenitors, but did not inhibit growth of myeloid progenitors from uninfected cats. Since no inhibitory activity was found when the sera from acutely infected cats added to uninfected bone marrow cultures, the authors' presumed that the progenitors were more susceptible to growth inhibition, but did not conduct additional studies (Linenberger et al., 1995). Our data supports the hypothesis that increased Fas expression on neutrophils and/or their myeloid progenitors, and increased soluble Fas ligand in the peripheral blood during acute FIV-C-PG infection maybe responsible for neutrophil apoptosis leading to neutropenia. This proposed mechanism is analogous to Fas/FasL work of Salmen et al. (Salmen et al., 2004) and Sabri et al. (Sabri et al., 2001) described above.

This study identifies, for the first time, an LGL lymphocytosis during acute FIV-C-PG infection that is associated with both neutropenia and increased FasL mRNA in PBMC, and which presents a potentially important, yet unidentified, mechanism of neutropenia in cats with FIV-C-PG infection that may correlate with circumstantial evidence in other lentivirus infections.

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References

- Alter G, Altfeld M. NK cells in HIV-1 infection: evidence for their role in the control of HIV-1 infection. *J Intern Med* 2009;265:29–42. [PubMed: 19093958]
- Alter G, Teigen N, Ahern R, Streeck H, Meier A, Rosenberg ES, Altfeld M. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. *J Infect Dis* 2007;195:1452–1460. [PubMed: 17436225]
- Babadoko AA, Aminu SM, Suleiman AN. Neutropenia and human immunodeficiency virus-1 infection: analysis of 43 cases. *Niger J Med* 2008;17:57–60. [PubMed: 18390135]
- Beebe AM, Gluckstern TG, George J, Pedersen NC, Dandekar S. Detection of feline immunodeficiency virus infection in bone marrow of cats. *Vet Immunol Immunopathol* 1992;35:37–49. [PubMed: 1337401]
- Bernstein HB, Wang G, Plasterer MC, Zack JA, Ramasastry P, Mumenthaler SM, Kitchen CM. CD4+ NK cells can be productively infected with HIV, leading to downregulation of CD4 expression and changes in function. *Virology* 2009;387:59–66. [PubMed: 19251297]
- Burks EJ, Loughran TP Jr. Pathogenesis of neutropenia in large granular lymphocyte leukemia and Felty syndrome. *Blood Rev* 2006;20:245–266. [PubMed: 16530306]
- Chattopadhyay PK, Betts MR, Price DA, Gostick E, Horton H, Roederer M, De Rosa SC. The cytolytic enzymes granzyme A, granzyme B, and perforin: expression patterns, cell distribution, and their relationship to cell maturity and bright CD57 expression. *J Leukoc Biol* 2009;85:88–97. [PubMed: 18820174]
- Colson P, Foucault C, Mokhtari M, Tamalet C. Severe transient neutropenia associated with acute human immunodeficiency virus type 1 infection. *Eur J Intern Med* 2005;16:120–122. [PubMed: 15833679]
- Elbim C, Monceaux V, Mueller YM, Lewis MG, Francois S, Diop O, Akarid K, Hurtrel B, Gougerot-Pocidalo MA, Levy Y, Katsikis PD, Estaquier J. Early divergence in neutrophil apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates. *J Immunol* 2008;181:8613–8623. [PubMed: 19050281]
- Fujino Y, Horiuchi H, Mizukoshi F, Baba K, Goto-Koshino Y, Ohno K, Tsujimoto H. Prevalence of hematological abnormalities and detection of infected bone marrow cells in asymptomatic cats with feline immunodeficiency virus infection. *Vet Microbiol*. 2008
- Ghali V, Castella A, Louis-Charles A, Agranovsky E, Croxson ST. Expansion of large granular lymphocytes (natural killer cells) with limited antigen expression (CD2+, CD3–, CD4–, CD8–, CD16

- +, NKH-1-) in a human immunodeficiency virus-positive homosexual man. *Cancer* 1990;65:2243–2247. [PubMed: 2346908]
- Gupta S. Abnormality of Leu 2+7+ cells in acquired immune deficiency syndrome (AIDS), AIDS-related complex, and asymptomatic homosexuals. *J Clin Immunol* 1986;6:502–509. [PubMed: 3097061]
- Heit B, Jones G, Knight D, Antony JM, Gill MJ, Brown C, Power C, Kubes P. HIV and other lentiviral infections cause defects in neutrophil chemotaxis, recruitment, and cell structure: immunorestorative effects of granulocyte- macrophage colony-stimulating factor. *J Immunol* 2006;177:6405–6414. [PubMed: 17056572]
- Hewitt RG, Morse GD. Granulocyte-macrophage colony-stimulating factor and zidovudine in the treatment of neutropenia and human immunodeficiency virus infection. *Pharmacotherapy* 1992;12:455–461. [PubMed: 1492010]
- Holm AM, Tjonnfjord G, Yndestad A, Beiske K, Muller F, Aukrust P, Froland SS. Polyclonal expansion of large granular lymphocytes in common variable immunodeficiency - association with neutropenia. *Clin Exp Immunol* 2006;144:418–424. [PubMed: 16734610]
- Kiener PA, Davis PM, Rankin BM, Klebanoff SJ, Ledbetter JA, Starling GC, Liles WC. Human monocytic cells contain high levels of intracellular Fas ligand: rapid release following cellular activation. *J Immunol* 1997;159:1594–1598. [PubMed: 9257817]
- Kottilil S, Jackson JO, Reitano KN, O'Shea MA, Roby G, Lloyd M, Yang J, Hallahan CW, Rehm CA, Arthos J, Lempicki R, Fauci AS. Innate immunity in HIV infection: enhanced susceptibility to CD95-mediated natural killer cell death and turnover induced by HIV viremia. *J Acquir Immune Defic Syndr* 2007;46:151–159. [PubMed: 17558334]
- Kronenberg A, Seebach JD, Bossart W, Weber R. Polyclonal proliferation of large granular lymphocytes during cytomegalovirus primary infection in a human immunodeficiency virus-infected patient receiving antiretroviral therapy. *Clin Infect Dis* 2001;33:E34–E36. [PubMed: 11486299]
- Kubes P, Heit B, van Marle G, Johnston JB, Knight D, Khan A, Power C. In vivo impairment of neutrophil recruitment during lentivirus infection. *J Immunol* 2003;171:4801–4808. [PubMed: 14568958]
- Le Priol Y, Puthier D, Lecureuil C, Combadiere C, Debre P, Nguyen C, Combadiere B. High cytotoxic and specific migratory potencies of senescent CD8+ CD57+ cells in HIV-infected and uninfected individuals. *J Immunol* 2006;177:5145–5154. [PubMed: 17015699]
- Levine AM, Karim R, Mack W, Gravink DJ, Anastos K, Young M, Cohen M, Newman M, Augenbraun M, Gange S, Watts DH. Neutropenia in human immunodeficiency virus infection: data from the women's interagency HIV study. *Arch Intern Med* 2006;166:405–410. [PubMed: 16505259]
- Levy JA. HIV pathogenesis: knowledge gained after two decades of research. *Adv Dent Res* 2006;19:10–16. [PubMed: 16672543]
- Linenberger ML, Beebe AM, Pedersen NC, Abkowitz JL, Dandekar S. Marrow accessory cell infection and alterations in hematopoiesis accompany severe neutropenia during experimental acute infection with feline immunodeficiency virus. *Blood* 1995;85:941–951. [PubMed: 7849316]
- Liu JH, Wei S, Lamy T, Epling-Burnette PK, Starkebaum G, Djeu JY, Loughran TP. Chronic neutropenia mediated by fas ligand. *Blood* 2000;95:3219–3222. [PubMed: 10807792]
- Luo HR, Loison F. Constitutive neutrophil apoptosis: mechanisms and regulation. *Am J Hematol* 2008;83:288–295. [PubMed: 17924549]
- Mizuno T, Goto Y, Baba K, Momoi Y, Endo Y, Nishimura Y, Masuda K, Ohno K, Tsujimoto H. Quantitative analysis of Fas and Fas ligand mRNAs in a feline T-lymphoid cell line after infection with feline immunodeficiency virus and primary peripheral blood mononuclear cells obtained from cats infected with the virus. *Vet Immunol Immunopathol* 2003;93:117–123. [PubMed: 12814697]
- Moses AV, Williams S, Heneveld ML, Strussenberg J, Rarick M, Loveless M, Bagby G, Nelson JA. Human immunodeficiency virus infection of bone marrow endothelium reduces induction of stromal hematopoietic growth factors. *Blood* 1996;87:919–925. [PubMed: 8562963]
- Pedersen NC, Leutenegger CM, Woo J, Higgins J. Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats. *Vet Immunol Immunopathol* 2001;79:53–67. [PubMed: 11356250]
- Pereira LE, Johnson RP, Ansari AA. Sooty mangabeys and rhesus macaques exhibit significant divergent natural killer cell responses during both acute and chronic phases of SIV infection. *Cell Immunol* 2008;254:10–19. [PubMed: 18640666]

- Prochorec-Sobieszek M, Rymkiewicz G, Makuch-Lasica H, Majewski M, Michalak K, Rupinski R, Warzocha K, Maryniak R. Characteristics of T-cell large granular lymphocyte proliferations associated with neutropenia and inflammatory arthropathy. *Arthritis Res Ther* 2008;10:R55. [PubMed: 18474096]
- Pulik M, Lionnet F, Genet P, Petitdidier C, Jary L, Fourcade C. CD3+ CD8+ CD56- clonal large granular lymphocyte leukaemia and HIV infection. *Br J Haematol* 1997;98:444–445. [PubMed: 9266947]
- Sabri F, De Milito A, Pirskanen R, Elovaara I, Hagberg L, Cinque P, Price R, Chiodi F. Elevated levels of soluble Fas and Fas ligand in cerebrospinal fluid of patients with AIDS dementia complex. *J Neuroimmunol* 2001;114:197–206. [PubMed: 11240032]
- Salmen S, Montes H, Soyano A, Hernandez D, Berrueta L. Mechanisms of neutrophil death in human immunodeficiency virus-infected patients: role of reactive oxygen species, caspases and map kinase pathways. *Clin Exp Immunol* 2007;150:539–545. [PubMed: 17956581]
- Salmen S, Teran G, Borges L, Goncalves L, Albarran B, Urdaneta H, Montes H, Berrueta L. Increased Fas-mediated apoptosis in polymorphonuclear cells from HIV-infected patients. *Clin Exp Immunol* 2004;137:166–172. [PubMed: 15196258]
- Salvato MS, Yin CC, Yagita H, Maeda T, Okumura K, Tikhonov I, Pauza CD. Attenuated disease in SIV-infected macaques treated with a monoclonal antibody against FasL. *Clin Dev Immunol* 2007;93462. [PubMed: 18317535]
- Shimajima M, Nishimura Y, Miyazawa T, Kato K, Tohya Y, Akashi H. CD56 expression in feline lymphoid cells. *J Vet Med Sci* 2003;65:769–773. [PubMed: 12939502]
- Smith PR, Cavenagh JD, Milne T, Howe D, Wilkes SJ, Sinnott P, Forster GE, Helbert M. Benign monoclonal expansion of CD8+ lymphocytes in HIV infection. *J Clin Pathol* 2000;53:177–181. [PubMed: 10823134]
- Terwee JA, Carlson JK, Sprague WS, Sondgeroth KS, Shropshire SB, Troyer JL, VandeWoude S. Prevention of immunodeficiency virus induced CD4+ T-cell depletion by prior infection with a non-pathogenic virus. *Virology* 2008;377:63–70. [PubMed: 18499211]
- Timonen T, Saksela E, Ranki A, Hayry P. Fractionation, morphological and functional characterization of effector cells responsible for human natural killer activity against cell-line targets. *Cell Immunol* 1979;48:133–148. [PubMed: 509533]
- Troth SP, Dean AD, Hoover EA. In vivo CXCR4 expression, lymphoid cell phenotype, and feline immunodeficiency virus infection. *Vet Immunol Immunopathol* 2008;123:97–105. [PubMed: 18295345]
- Wong RJ. Treatment of HIV-related neutropenia. *Am J Health Syst Pharm* 1999;56:S17–S20. [PubMed: 10613382]
- Zhao Y, Gebhard D, English R, Sellon R, Tompkins M, Tompkins W. Enhanced expression of novel CD57+CD8+ LAK cells from cats infected with feline immunodeficiency virus. *J Leukoc Biol* 1995;58:423–431. [PubMed: 7561518]

Figure 1.

Figure 2.

Figure 3.

Figure 4.