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Large granular lymphocytes are universally increased in human, macaque, and feline lentiviral infection

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ABSTRACT

Large granular lymphocytes (LGLs) have only been anecdotally reported in HIV infection. We previously reported an LGL lymphocytosis in FIV-infected cats associated with a rise in FIV proviral loads and a marked neutropenia that persisted during chronic infection. Extensive immunophenotyping of peripheral blood mononuclear cells in cats chronically infected with FIV were identified LGLs as CD8lo$^+$FAS$^+$; this cell population expanded commensurate with viral load. CD8lo$^+$FAS$^+$ cells expressed similar levels of interferon-γ compared to CD8lo$^+$FAS$^+$ cells from FIV-naive control animals, yet CD3ε expression, which was increased on total CD8$^+$ T cells in FIV-infected cats, was decreased on CD8lo$^+$FAS$^+$ cells. Down-modulation of CD3 expression was reversed after culturing PBMC for 3 days in culture with ConA/IL-2. We identified CD8lo$^+$FAS$^+$ LGLs to be polyclonal T cells lacking CD56 expression. Blood smears from HIV-infected individuals and SIVmac239-infected rhesus macaques revealed increased LGLs compared to HIV/SIV negative counterparts. In humans, there was no correlation with viral load or treatment and in macaques the LGLs arose in acute SIV infection with increases in viremia. This is the first report describing and partially characterizing LGL lymphocytosis in association with lentiviral infections in three different species.

1. Introduction

The importance of CD8$^+$ T cells in control of lentiviral infection of humans, macaques and cats has been apparent for decades (Flynn et al., 1999; Jeng et al., 1996; Kannagi et al., 1988; Walker et al., 1986). Studies of different CD8$^+$ subset function in innate and adaptive immune responses has indicated that central memory CD8$^+$ T cells are the CD8$^+$ cell subset with the most *in vitro* viral suppressive activity (Buckheit et al., 2012; Lopez et al., 2011; Mendoza et al., 2012; Ndhlovu et al., 2012).

Historically, large granular lymphocytes (LGLs) have been considered either NK cells or CD3$^+$ cells that participate in antibody-dependent cytotoxicity (Chan et al., 1986). LGLs represent 10–15% of the peripheral blood mononuclear cell (PBMC) population in healthy individuals (Loughran, 1993). This low percentage of LGLs has made detailed analysis difficult and thus, most information about LGLs is derived from studies on patients with LGL leukemia (Alekshun and Sokol, 2007). LGLs have only been anecdotally reported during HIV infection, and have usually been associated with neoplasia (Boveri et al., 2009; Pulik et al., 1997). However, a study of HIV-infected patients reported that LGLs persisted between 6 and 30 months and had a consensus phenotype in PBMC of activated CD8$^+$ T cells expressing CD57. LGLs in these patients represented polyclonal T cells (Smith et al., 2000).

We have previously reported that FIV-infected cats had a LGL lymphocytosis that was temporally associated with neutropenia, increased PBMC-associated FasL mRNA and decreased in PBMC FIV proviral loads (Sprague et al., 2010). We report here that these LGLs correlated with cells that expressed low surface CD8 and FAS, that were polyclonal T cells and that expressed similar intracellular interferon-γ in FIV-infected animals compared to FIV-naive control animals. These cells also expressed decreased surface CD3epsilon

Abbreviations: LGLs, large granular lymphocytes; ConA, concanavalin A; IL-2, interleukin; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; EDTA, ethylene diamine tetra acetic acid; NK, natural killer; PBMC, peripheral blood mononuclear cell.

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(CD3ε) levels in FIV-infected animals compared to FIV-naive controls and this decreased expression was upregulated via cytokine rescue. Most interestingly, we found that LGLs arise during acute SIV infection in macaques and are detectable and elevated during HIV infection in humans, documenting the importance and presence of these cells during lentiviral infections in three different species.

2. Materials and methods

2.1. Animals

Blood of cats from two different studies were included in the overall study design. Two chronically infected cats were originally infected at 6 months of age with an IV inoculation of 1 ml of a previously characterized FIV-C-PG (Terwee et al., 2008). Blood from these cats was collected in EDTA by venipuncture and was used for the CD8lo$^+$FAS$^+$ cell phenotypic characterization; flow sorting studies and CD8lo$^+$FAS$^+$ cell PCR for TCR receptor and immunoglobulin rearrangement studies. Additionally, six cats were infected with an IV inoculation of 1 ml of FIV-C-PG. These cats were 6 months of age at time of infection and blood samples were collected in EDTA by venipuncture on the day of FIV-C-PG infection and during acute infection at 1, 2 and 4 weeks PI. The blood of these cats was used for the CD8lo$^+$FAS$^+$ cell and LGL correlation studies and for the cell culture studies to evaluate CD3ε up-regulation. In addition, blood from four age matched FIV-naïve cats were used for the flow cytometric studies of CD8lo$^+$FAS$^+$ cells. All cats were specific-pathogen-free (SPF) and the chronically infected cats were 3–4 years of age at the time of study. None of the cats were given any other vaccinations and all cats were maintained in an AAALAC International approved animal facility at Colorado State University (CSU). All procedures were approved by the CSU Institutional Animal Care and Use Committee prior to initiation. Eight macaques, maintained at the Tulane National Primate Research Center, were infected intravenously with SIVmac239 according to standard procedures as part of another study performed in 2007 (Stump, 2008). EDTA blood was collected by venipuncture every 10 days to 2 weeks for approximately 3 months and blood smears were made and stored for later examination of LGLs.

2.2. Human blood smears

Blood was collected by venipuncture from eight individuals with HIV infection to evaluate blood smears for the presence of LGLs. All individuals provided written consent prior to participating in this study, and all studies were approved by the Poudre Valley Health System Institutional Review Board. HIV status was determined by screening tests using an ADVIA Centaur HIV 1/O/2 Enhanced immunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY). Blood smears were examined for the presence of LGLs. Blood smears from nine HIV-negative controls were also evaluated. The pathologist (Sprague) was blinded to the infection status when reading the slides. White blood cell (WBC) counts, CD4 T cell counts, viral loads and treatment regimens were also provided.

2.3. Large granular lymphocyte enumeration

Absolute LGLs counts of all species were determined by multiplying the blood smear differential counts by total WBC counts. Total white blood cell counts were measured using a Coulter Z1 (Coulter, Miami, FL). Differential cell counts were performed manually and LGL counts were recorded as a percentage of the nucleated cells after 100 nucleated cells were counted.

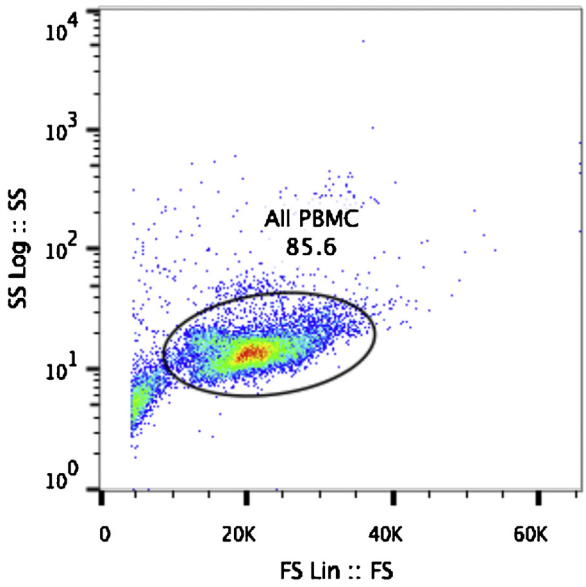

Fig. 1. Scatterplot of PBMC isolated with FIcoll-Hypaque showing gating strategy for detecting large granular lymphocytes. The plot is from 1 cat and is representative of 6 cats.

2.4. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated over a Histopaque 1.077 gradient (Sigma, St. Louis, MO) according to manufacturer's instructions. Percentages of cells positive for CD8lo$^+$FAS$^+$ and CD8$^+$CD57$^+$ expression were determined by flow cytometry using a directly conjugated monoclonal antibody (Ab) to feline CD8α (Southern Biotech, Birmingham, AL), a polyclonal Ab to feline FAS (R&D systems, Minneapolis, MN), and a cross-reactive directly conjugated monoclonal Ab to human CD57 (Beckman–Coulter, Brea, CA). CD3ε expression on CD8lo$^+$FAS$^+$ cells was examined using a FITC-conjugated monoclonal anti-feline CD3ε Ab recognizing the CD3 surface receptor (gift of T. Miyazawa; 1:100 dilution). Intracellular interferon-gamma (IFN-γ) expression was examined in CD8lo$^+$FAS$^+$ cells using a polyclonal anti-feline IFN-γ Ab (R&D systems). For multicolor flow cytometry using IFN-γ, surface Abs were incubated with cells followed by fixation and permeabilization and staining with IFN-γ according to manufacturer's instructions (BD Perm/Wash, BD Biosciences, San Jose, CA). For the FAS polyclonal antibody, labeling with fluorochrome prior to staining cells was accomplished using a Zenon antibody labeling kit (Life Technologies, Grand Island, NY). A directly conjugated anti-human CD56 (NCAM; Biolegend, San Diego, CA; 1:20 dilution) that has been shown to cross-react with feline CD56 Ab was used to determine if the CD8$^+$FAS$^+$ cells expressed this NK marker (Simoes et al., 2012). Two × 10^5 to 1 × 10^6 PBMC were used per reaction and cell Fc receptors were blocked using goat serum (MP Biomedicals, Solon, OH) at a 1:10 dilution and incubated for 30 min at 4 °C. After washing cells, cells were incubated for 30 min at room temperature in triplicate with the CD8α Ab at a 1:100 dilution, the Fas Ab at 1:200 dilution and the CD57 Ab at 1:5 dilution in flow buffer (PBS containing 5% FBS and 0.2% sodium azide). The CD56 Ab was added to the mixture of antibodies in some experiments. Cells were then washed three times in flow buffer and re-suspended in 200 μl of buffer with 1% paraformaldehyde for fixation. Samples were then analyzed on a DAKO Cyan ADP (Beckton–Dickinson, Brea, CA). Gates were set to eliminate small particles, neutrophils and eosinophils using forward and side scatter (Fig. 1). A total of between 5000 and 20,000 gated cells were analyzed, depending on the starting volume of cells. Isotype-matched mouse immunoglobulins (Igs) were used