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Veterinary Immunology and Immunopathology 122 (2008) 16–24
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Oxidative stress during acute FIV infection in cats

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Received 21 June 2007; received in revised form 9 August 2007; accepted 6 November 2007

Abstract

Oxidative stress is thought to contribute to the pathogenesis of HIV infection in humans. For example, CD4$^+$ T cells are particularly affected in HIV patients and oxidative stress may also contribute to impairment of neutrophil function in HIV/AIDS patients. Since cats infected with FIV develop many of the same immunological abnormalites as HIV-infected humans, we investigated effects of acute FIV infection on oxidative stress in cats. Cats were infected with a pathogenic strain of FIV and viral load, changes in neutrophil number, total blood glutathione, malondiadehye, antioxidant enzyme concentrations, and reduced glutathione (GSH) concentration in leukocytes were measured sequentially during the first 16 weeks of infection. We found that superoxide dismutase and glutathione peroxidase concentrations in whole blood increased significantly during acute FIV infection. In addition, neutrophil numbers increased significantly during this time period, though their intracellular GSH concentrations did not change. In contrast, the numbers of CD4$^+$ T cells decreased significantly and their intracellular GSH concentration increased significantly, while intracellular GSH concentrations were unchanged in CD8$^+$ T cells. However, by 16 weeks of infection, many of the abnormalities in oxidative balance had stabilized or returned to pre-inoculation values. These results suggest that acute infection with FIV causes oxidative stress in cats and that CD4$^+$ T cells appear to be preferentially affected. Further studies are required to determine whether early treatment with anti-oxidants may help ameliorate the decline in CD4$^+$ T cell number and function associated with acute FIV infection in cats.
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Keywords: Cat; Superoxide dismutase; Glutathione; FIV; Monochlorobimane

1. Introduction

Oxidative stress plays a significant role in the pathogenesis and progression of human immunodeficiency virus (HIV) infection. Reduced glutathione (GSH), the predominant endogenous antioxidant in mammalian cells, is significantly reduced in lymphocytes of patients with HIV. Decreased GSH levels are associated with enhanced HIV replication, an increase in T-cell apoptosis, increased DNA damage, and functional impairment of CD4$^+$ T cells. Low GSH concentrations are also correlated with decreased survival times in AIDS patients (Herzenberg et al., 1997; James, 1997; Walmsley et al., 1997; Losa and Graber, 2000). Antioxidants are being studied as potential adjunctive therapies in the treatment of HIV-infected individuals (Muller et al., 2000; Nakamura et al., 2002). For example, glutathione supplementation *in vitro* increases T cell proliferation and suppresses the release of tumor necrosis factor-alpha from peripheral blood mononuclear cells from HIV-infected patients receiving highly active retroviral therapy (Muller et al., 2000; Aukrust et al., 2003).

Glutathione homeostasis relies on the activity of a number of antioxidant enzymes, including superoxide

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0165-2427/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2007.11.004

dismutase, catalase, glutathione peroxidase, and glutathione reductase. Patients with HIV have been shown to have variable differences in erythrocyte glutathione peroxidase and superoxide dismutase (Look et al., 1997; Treitinger et al., 2000; Gil et al., 2003). These differences may represent effects of the stage or severity of infection, or simply be a reflection of different degrees of compensatory changes in antioxidant enzyme activity when patients are challenged by oxidative stress.

Though oxidative stress is known to occur in humans infected with HIV, the level of oxidative stress that develops in FIV-infected cats has not been previously investigated. Because of their unique metabolism, cats are especially prone to the development of oxidative stress. For example, we reported recently that intracellular GSH concentrations were significantly increased in neutrophils and decreased in $CD4^+$ and $CD8^+$ T cells from cats infected with a pathogenic clade B isolate of FIV (Webb et al., 2006). Therefore, we conducted a prospective study to assess the effects of acute FIV infection on oxidative stress in cats. Following experimental inoculation with a pathogenic strain of FIV, we assessed concentrations of antioxidant enzymes in cats for 16 weeks post-inoculation. We found that glutathione peroxidase and superoxide dismutase enzyme activity increased in blood of FIV-infected cats, as did intracellular $CD4^+$ GSH concentrations. These results suggest that infection with FIV leads to significant abnormalities in oxidative balance and that therapeutic correction of these abnormalities may be warranted.

2. Materials and methods

2.1. FIV infection of SPF cats

Six-, sixteen-week-old cats from a specific pathogen-free colony maintained at the Colorado State University were used for this study. Following pre-infection sample acquisition the cats were inoculated intraperitoneally with 1×10^8 viral copies in 500 μl of cell-free pooled plasma from cats infected with an FIV clade C virus (O'Neil et al., 1996; Dow et al., 1999). Infection was confirmed by PCR detection of FIV provirus within 1 month of inoculation (data not shown). Six, nine, twelve, and sixteen weeks following inoculation blood samples were obtained by jugular venipuncture from each of the infected cats to determine viral load, neutrophil number, and the assays of oxidative stress described below. All procedures were carried out with the approval of and in accordance with guidelines established by the Animal Care and Use Committee at Colorado State University.

2.2. Viral load

Proviral load from T cells was determined with real-time DNA PCR. Methods including primers and probes were adapted from those developed by Leutenegger et al. (1999). Cellular DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). PCR reactions were performed in a 25 μl volume containing 12.5 μl TaqMan Universal PCR Mastermix (Applied Biosystems), 400 nM of each primer (MWG Biotech), 80 nM of probe (MWG Biotech), and 5 μl of sample DNA or plasmid FIV DNA standard. Real-time PCR was performed on an iCycler iQ Real-time PCR Detection System (Bio-rad).

2.3. Preparation of blood samples for flow cytometry

Peripheral blood samples were preserved in EDTA tubes and stored at 4 °C and processed for analysis within 6 h of acquisition. Blood for analysis was processed to remove erythrocytes, as we reported previously (Webb et al., 2006). Briefly, 200 μl of EDTA-preserved blood was added to 15 ml of NH_4Cl erythrocyte lysis buffer and incubated for 15 min at room temperature. The sample was then washed twice in HBSS solution (Sigma-Aldrich, St. Louis, MO) and the leukocytes were resuspended in 500 μl of FACS buffer (PBS with 2% FBS and 0.1% sodium azide) and stored at 4 °C for less than 30 min prior to analysis. This procedure typically yielded a final cell concentration in the range of 4×10^6 cells/ml. Resuspending leukocyte samples in FACS buffer for <30 min did not affect intracellular GSH concentrations (data not shown).

2.4. Preparation of blood samples for spectrophotometric assays

Peripheral blood samples were preserved in heparinized saline and processed according to the manufacturer's instructions for spectrophotometric assay of whole blood reduced glutathione (GSH), glutathione peroxidase (GPx) enzyme, and erythrocyte superoxide dismutase (SOD) enzyme concentrations. Briefly, 250 μl and 50 μl of whole blood were placed in separate 1 ml eppendorf tubes and stored at −70 °C for GSH analysis and GPx analysis, respectively. For SOD analysis, 500 μl of heparinized whole blood was centrifuged at $3000 \times g$ for 10 min. The resulting

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