CONCISE COMMUNICATION

Cocaine Enhances Human Immunodeficiency Virus Replication in a Model of Severe Combined Immunodeficient Mice Implanted with Human Peripheral Blood Leukocytes

Michael D. Roth, 1 Donald P. Tashkin, 1 Ruth Choi, 2 Beth D. Jamieson, 2 Jerome A. Zack, 2 and Gayle Cocita Baldwin 2

Epidemiologic studies have identified cocaine as a cofactor for development of acquired immunodeficiency syndrome (AIDS). To evaluate this interaction, human peripheral blood leukocytes (PBL) were implanted into severe combined immunodeficient mice and infected with human immunodeficiency virus (HIV) in both the presence and absence of cocaine. Concurrent administration of cocaine resulted in significantly more PBL becoming infected with HIV in vivo (38.85% vs. 18.5%). The number of CD4 + cells recovered from HIV-infected, cocaine-treated animals was significantly lower than that from mice infected with HIV in the absence of cocaine (6.5 × 10^4 vs. 19 × 10^4) and was associated with a lower CD4:CD8 ratio and a dramatic increase in virus load. Exposure to cocaine alone did not affect the implantation of PBL, suggesting a specific interaction between cocaine and HIV. This report describes a model for evaluating HIV cofactors and supports cocaine’s role in the development and progression of AIDS.

A variety of cofactors may increase the risk for or progression of clinical disease associated with human immunodeficiency virus (HIV) [1, 2]. There is strong circumstantial evidence that exposure to drugs of abuse, specifically cocaine, fall into this category (reviewed in [3]). By multiple logistic regression analysis, 3084 persons attending a sexually transmitted disease clinic were studied for associations between HIV infectivity and individual subject characteristics [4]. Of the 1911 subjects who denied having high-risk behaviors (male homosexuality, intravenous drug use, or sexual contact with high-risk persons), crack cocaine and HIV infection were associated with an odds ratio of 10.6:1 for women and 3.3:1 for men. This relationship is supported by other epidemiologic studies [5, 6] and by reports suggesting that cocaine depresses the immune system both in vivo and in vitro [7–9]. If cocaine mediates clinically significant effects on host defense, the link between cocaine abuse and progression of AIDS could be related to an increased risk for secondary opportunistic infections. Alternatively, since cocaine modifies the distribution and activation of target lymphocytes, modifies cytokine production, and alters cell trafficking of immune cells, cocaine might affect HIV infectivity and/or replication by these means. Finally, and perhaps most significant, study results show that cocaine facilitates the replication of HIV in human peripheral blood leukocytes (PBL) in vitro [10–12].

Although compelling, the link between cocaine use and HIV is based largely on indirect evidence, and the exact mechanisms responsible for this link have not been clearly delineated. To more directly evaluate the interaction between cocaine and HIV in vivo, we adapted a hybrid human-murine model (huPBL-SCID mouse). This approach was initially developed by Mosier et al. [13] as a relevant animal model for in vivo assessment of HIV pathogenesis. Our goal was to determine whether systemic exposure of huPBL-SCID mice to cocaine would affect HIV burden and/or the number and distribution of T cell subsets in vivo.

Methods

Cell preparation. PBL were purified by density gradient centrifugation and resuspended at 1.5 × 10^8 cells/mL in 0.9% saline, and 0.2 mL of the solution was implanted into the peritoneal cavity of each mouse 12–15 days before exposure. PBL from Epstein-Barr virus (EBV)-seronegative donors were used to prevent the development of EBV-associated B cell lymphoproliferative disease and to eliminate EBV as another potential cofactor in the pathogenesis of HIV-1.

Monoclonal antibodies (MAbs) and flow cytometric analysis. Fluorochrome-conjugated MAbs, specific for human CD4, CD8,
CD45, B220, and Mac-1α, were obtained from BD Biosciences. Anti-murine heat-stable antigen (HSA) was obtained from Pharmingen. Conjugated isotype control MAbs were used to control for nonspecific binding. Cells (3 × 10^7) recovered from huPBL-SCID mice were labeled with MAbs, fixed, and analyzed by multiparameter flow cytometry with a FACStar™ flow cytometer (BD Biosciences). In all, 5000–10,000 events were acquired and analyzed with CELLQuest software (BD Biosciences). Forward–versus–side scatter analysis of mock-implanted implants was used for gating of live lymphocytes, and gating of human anti-CD45–stained cells was used to exclude contaminating murine cells.

**Preparation and testing of virus stock.** To construct the reporter HIV virus, NFN-SX-HSAS, the full-length gene encoding murine HSA (mCD24) was cloned into the partially deleted vpr gene region of the CXCR4-tropic strain, HIV-1NL4-3, as described elsewhere [14]. The resulting construct, NL-r-HSAS, was further digested with PstI and EcoRI to liberate the new vpr/HSA region. This 588-bp fragment was then ligated into HIV-1NSN-SX, which was previously digested with the same enzymes. Virus stocks were prepared by electroporation of 30 μg of reporter virus DNA into 5 × 10^7 mycoplasma-free HeLa-tat cells. HIV-1NL4-3 and Tris buffer were used as positive and negative controls, respectively. Supernatants were collected on postelectroporation days 2 and 3. Virus production was quantitated by ELISA for p24 Gag (Coulter), and expression of HSA on the cell surface of infected cells was determined by flow cytometry using anti-mCD24 MAb. PBL were infected with 18 ng/10^6 cells of NFN-SX-HSAS in RPMI 1640 medium (Life Technologies) containing 20% fetal bovine serum (Gemini Biologicals), phytomengagglutin (PHA; 2.5 μg/mL; Calbiochem), and interleukin-2 (5 U/mL; Roche Diagnostics). Virus absorption was done for 2 h at 37°C with intermittent shaking, followed by addition of fresh media (final cell concentration, 10^8 cells/mL). Cells were cultured for 7 days and then analyzed by flow cytometry for expression of HSA, CD3, and CD4. Infectious units (IU) were determined by limiting dilution on PHA-stimulated human peripheral blood mononuclear cells (PBMC) by using 2-fold dilutions of virus plated in duplicate. Alternatively, IU were approximated on the basis of p24 antigen. Viral supernatants were stored at −70°C.

**Construction, infection, and treatment of huPBL-SCID chimeras.** CB-17 scid/scid (SCID) mice were bred and maintained in the Mouse/Human Chimera Core Facility at UCLA under laminar flow conditions. All cages, food, water, and bedding were autoclaved before use.

SCID mice (8–12 weeks old) were implanted with 2 × 10^7 human PBL by a single intraperitoneal (ip) injection. Immune reconstitution was documented to be complete by 2 weeks after implantation and stable for up to 3 months, as described elsewhere [13]. Blood samples were obtained from the tail vein on days 7, 10, 14, 18, and 21 after PBL implantation, and human serum immunoglobulin levels were measured by isotype-specific ELISA. At postimplantation days 12–15, the mice were infected by an ip injection containing 300–400 IU of the reporter virus, NFN-SX-HSAS. Mock infections of huPBL-SCID mice were done with supernatants from mock-electroporated HeLa-tat cells, diluted in the same manner as virus stocks. Experimental groups consisted of 3 mice unless otherwise stated.

Cocaine hydrochloride (5 mg/mL in saline), obtained from the National Institute on Drug Abuse, was diluted in saline prior to use. Cocaine was delivered by ip injection beginning 4 or 5 days after infection, and animals were treated with 5 mg/kg cocaine daily for the ensuing 10–12 days. At postinfection day 14 or 15, a puncture of the retro-orbital venous plexus was made to collect serum. Mice were then euthanized, and PBL were recovered, by peritoneal lavage, for flow cytometry.

**Quantitative RNA polymerase chain reaction (PCR).** We used the Amplicor HIV-1 Monitor PCR assay (Roche Diagnostics) to measure RNA in the plasma obtained from the animals at the time of death, in accordance with the manufacturer’s instructions. The level of detection for the ultrasensitive assay is 100–24,000 HIV RNA copies/mL.

### Results

**In vivo exposure to cocaine did not affect B or T cell implantation in huPBL-SCID mice.** In the absence of HIV infection, huPBL-SCID mice were treated daily with either saline or cocaine (0.1, 5, or 10 mg/kg) 10–21 days after huPBL implantation. Levels of human IgG were followed as a measure of B cell engraftment and function. Cocaine had no effect on the spontaneous rise in human IgG titers that occurred during the first 2 weeks after implantation or on the plateau level that was maintained through day 21 (figure 1A). Cocaine- and saline-treated huPBL-SCID mice were sacrificed after 10 days of treatment, and peritoneal lavage was done to recover implanted human cells. Similarly, exposure to cocaine did not significantly affect either the number or viability of recovered human CD4+ or CD8+ cells (figure 1A).

**In vitro and in vivo confirmation of the HIV reporter construct.** HIV-1NSN-SX, the backbone for the reporter construct, was chosen because of its CCR5 tropism and its capacity for high-efficiency infection via a CD4 receptor mechanism. Murine HSA was selected as the reporter gene because of the small size of the cDNA (231 nt) and the availability of species-specific detection MAbs, as reported elsewhere [14]. Flow cytometry performed 7 days after in vitro infection routinely showed infection of 25%–35% of CD4+ cells (figure 1B). Infectivity and replication were independently corroborated by HIV-p24 ELISA of culture supernatants (mean ± SD, 220 ± 30 ng/mL of p24 at day 7 after infection). The reporter construct was also effective in vivo. Flow cytometry done 10 days after in vivo infection of huPBL-SCID animals demonstrated that 15%–25% of human CD45+ cells were infected (figure 1C).

**In vivo cocaine administration, in combination with HIV, significantly increased HIV replication and expression of the murine HSA reporter construct in huPBL-SCID mice.** In both cocaine-treated and untreated HIV-infected animals, CD45+ human cells represented 67.4% ± 9.5% (mean ± SD) of total recovered peritoneal cells. Although cocaine had no independent effect on PBL implantation, administration of cocaine, in combination with HIV, significantly increased the percentage of peritoneal cells expressing murine CD24 as a marker of HIV infection (figure 2A). This augmentation was associated with a significant increase in the number of infected cells (figure 2B).
with a marked loss of CD4+ cells (figure 2B) and a resulting decrease in the CD4:CD8 ratio (figure 2B). HIV infection resulted in a modest decrease in human CD8+ cells, which was not further altered by concurrent exposure to cocaine.Corroborating previous control experiments (figure 1A, right panel), cocaine treatment did not affect human cell recovery (CD45+ cells) or T cell subset distribution in mock-infected controls (CD4+ positivity [mean ± SD]: 20.6 ± 4.9 in mock-infected,
saline-treated animals (control-mock) and 22.8 ± 8.5 in mock-infected, cocaine-treated animals (cocaine-mock); CD8⁺ positivity: 5.9 ± 3.5 in control-mock and 6.7 ± 8.1 in cocaine-mock). These results suggest that the loss of CD4⁺ cells in HIV-infected, cocaine-treated animals was most likely caused by a cocaine-mediated increase in HIV infectivity and commensurate lysis of CD4⁺ target cells. As a confirmation of this hypothesis, we noted a 200- to 300-fold increase in HIV RNA copy numbers in the peripheral blood obtained from cocaine-treated, HIV-infected animals when compared with HIV-infected controls (figure 2C).

Discussion

The use of highly active antiretroviral therapy targeting HIV as the etiologic agent of AIDS has significantly reduced the development and progression of disease. However, a number of disease-modifying cofactors have been identified and provide additional targets for ongoing research and intervention [1–3]. Among these cofactors, cocaine warrants careful scrutiny. Epidemiologic studies have linked cocaine to the progression of AIDS and to the risk for opportunistic infections [3–6]. In vitro studies of human PBL have suggested a potential interaction between cocaine and HIV at several levels, including cocaine-related alterations in the expression of chemokines and immunoregulatory cytokines, activation and function of leukocytes, and infectivity and/or replication of HIV in human cells [7–12].

To directly examine cocaine exposure for its consequences on HIV infection in vivo, we developed an exposure model using the huPBL-SCID mouse. Our results clearly link cocaine exposure to accelerated HIV replication. Systemic cocaine administration resulted in accelerated infection of human PBL, a decrease in CD4 cells, a decrease in the CD4:CD8 ratio, and a dramatic rise in circulating virus load. These data suggest a causal relationship between cocaine exposure and enhanced HIV replication in vivo, supporting the role of cocaine as an important cofactor in the pathobiology of AIDS.

The xenotransplant model is a successful in vivo model for the study of HIV, both for testing antiviral agents and for understanding the pathogenicity of HIV infection [15]. By adapting this model to study the interaction of HIV with cocaine, we extended its use as a tool for examining interactions between HIV and potential disease cofactors. Similar studies examining the effects of other drugs, dietary factors, or concurrent opportunistic infections all appear feasible. The use of a functional HIV reporter construct, NFN-SX-HSAS, allowed for rapid and reproducible detection of infected human cells by flow cytometry [14, 15]. This provides a powerful tool that can be exploited in future studies to detect cocaine-related changes in cell surface receptor expression or intracellular cytokine production or to purify infected cells for specific molecular analysis. However, the levels of infected CD4⁺ cells may be somewhat underestimated when these reporter constructs are used [14]. After in vitro infection of human CD4⁺ T cells with NFN-SX-HSAS, cells expressing the highest levels of the viral reporter HSA were CD4⁺ (figure 1B). This could represent infection of contaminating myeloid cells or down-regulation of CD4 following NFN-SX-HSAS infection of target T cells, as previously reported for nef-competent HIV [14]. In the huPBL-SCID model, the latter could lead to an overestimation of the impact of cocaine on CD4⁺ cell death. However, this effect is likely to be minor; moreover, quantitative RNA PCR analysis provided an independent confirmation that cocaine resulted in extremely high levels of viral infection.
Although both in vitro analyses and epidemiologic studies suggest that cocaine increases susceptibility to HIV, our data provide direct evidence that cocaine can enhance HIV replication in vivo. In vitro studies are constrained by their inability to account for complex interactions that occur in vivo, such as the effects of cocaine on the hypothalamic-pituitary-adrenal axis, on neurotransmitter release, or on the production of chemokines and cytokines by other cell populations. Because cocaine is rapidly degraded in tissue culture medium, high concentrations must be added to in vitro cultures in order to observe effects. Use of the huPBL-SCID mouse model enabled us to examine the impact of cocaine under more physiologic conditions in vivo. The huPBL-SCID mouse model will also provide the opportunity to dissect different pathways involved in the enhancement of HIV infection, such as the roles of specific cytokines, cell subsets, and neuroimmune interactions.

In summary, we adapted the huPBL-SCID mouse model to examine a clinically important HIV cofactor for its effect on the replication and spread of HIV in vivo. Our findings confirm existing indirect evidence and provide a significant connection between cocaine exposure in vivo and the progression of HIV.

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References