Antibody-Dependent Cellular Cytotoxicity (ADCC)-Mediated Destruction of Human Immunodeficiency Virus (HIV)-Coated CD4⁺ T Lymphocytes by Acquired Immunodeficiency Syndrome (AIDS) Effector Cells

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Accepted: July 7, 1988

The acquired immunodeficiency syndrome (AIDS) is defined in clinical terms by the development of Kaposi's sarcoma and/or severe opportunistic infections in persons without predisposing conditions. A hallmark of the syndrome has been a decrease in the number of CD4⁺ T helper cells. The reduction in the frequency of the CD4⁺ lymphocytes has been postulated to be primarily the result of human immunodeficiency virus (HIV) tropism and cytopathogenicity for the T-cell subset. Yet only a small percentage of cells is actually infected with HIV. Recently, we provided evidence indicating that AIDS patients' natural killer cells can mediate normal levels of antibody-dependent cellular cytotoxicity (ADCC) despite exhibiting a defect in natural killer (NK) effector function (J Immunol 139:55, 1987). This finding prompted us to investigate whether AIDS patients' effector cells could mediate ADCC against circulating CD4⁺ T cells infected with or expressing HIV antigen. The findings reported herein demonstrate that AIDS effector cells can mediate lysis of CEM (CD4⁺ T-cell line) coated with HIV protein in the presence of HIV-specific antibody. Lysis was specific, as non-HIV-coated CEM or the addition of HIV-negative serum resulted in no lysis. We then examined HIV-coated peripheral blood-derived CD4⁺ T lymphocytes as targets in ADCC. We demonstrate that in the presence of HIV-specific antibody, HIV-coated CD4⁺ T lymphocytes serve as targets for ADCC by AIDS effector cells. The lytic activity obtained with AIDS effector cells was comparable to that obtained with normal effector cells. These results demonstrate that AIDS effector cells can mediate ADCC against HIV-coated CD4⁺ T lymphocytes and suggest that ADCC may play a role in vivo in the pathogenesis of AIDS.

KEY WORDS: Acquired immunodeficiency syndrome (AIDS); antibody-dependent cellular cytotoxicity (ADCC); CD4⁺ T lymphocytes; human immunodeficiency virus (HIV).

INTRODUCTION

AIDS³ and ARC patients suffer from severe cellular and humoral immune dysfunctions that are manifested by a reduced T4/T8 ratio and a decrease in the absolute number of CD4⁺ T lymphocytes (1,2). These reductions have been postulated to be due primarily to tropism and cytopathogenicity of the CD4-positive subset of T lymphocytes (3–5). However, other possible mechanisms may be envisaged in which the HIV-infected T cells may serve as targets in an autoimmune reaction such as destruction of HIV-infected targets by HIV-specific cytotoxic T cells, lysis in a complement-dependent cytotoxicity reaction by anti-HIV antibody, and target-cell lysis by antibody-dependent cellular cyto-
Toxicity (ADCC) by the NK/K cells. Several published reports demonstrated the presence of antibody-mediating ADCC in sera derived from HIV-infected patients (6,7). In a recent report from our laboratory, we have provided evidence indicating that AIDS patients' NK/K cells mediate normal ADCC function against normal target cells although defective in natural killer-cell function (8). Therefore, it is possible that in vivo, HIV-seropositive AIDS patients mediate the autologous ADCC reaction against HIV-infected cells or cells coated with HIV antigens. Although we have shown that AIDS effector cells can mediate ADCC against HIV-infected cell lines, it was important to determine whether AIDS effector cells can also mediate ADCC against HIV-coated peripheral CD4+ lymphocytes in the presence of HIV antibodies. We present evidence which demonstrates that AIDS peripheral blood effector cells can mediate the destruction of HIV-coated CD4+ T lymphocytes in the presence of HIV antibody.

MATERIALS AND METHODS

**Effector Cells**

Peripheral blood was obtained from healthy male donors (normal) or from AIDS patients as described (9). Mononuclear cells were separated by Ficoll-Hypaque buoyant density centrifugation. Nonadherent PBL were collected after incubation of mononuclear cells at 37°C for 1 hr in assay medium. PBL were washed in PBS or normal saline, resuspended at the required density, and used immediately. The cell preparations were ≥95% lymphocytes as determined by Wright/Giemsa staining.

**Patients with AIDS and KS**

Patients with AIDS were diagnosed according to criteria established by the Centers for Disease Control (10,11). All of the AIDS patients were not receiving chemotherapy, interferons (IFN), or interleukin 2 (IL 2) at the time of or prior to blood donation. Informed consent for blood donation was obtained in all instances.

**Media and Reagents**

RPMI 1640 supplemented with L-glutamine (2 mM), nonessential amino acids (0.1 mM), sodium pyruvate (0.11 g/liter), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml) supplemented with an additional 5% heat-inactivated fetal bovine serum (FBS) was used for all assays (assay medium). Target cells were cultured in the aforementioned medium without antibiotics or antifungals and were supplemented with an additional 10% FBS (culture medium). All of the above reagents were purchased from GIBCO, Grand Island, NY.

**Target Cells**

The CEM target cell line was maintained in culture in RPMI 1640 supplemented with 10% FBS, 1% nonessential amino acids, and 1% sodium pyruvate (culture medium). CEM linked overnight with 200 μCi of Na251CrO4 were used as target cells. CD4+ T cells used as target cells were isolated from PBL by passage over nylon wool to enrich for T cells, after which 10^7 cells were resuspended in 1.0 ml of 0.3% BSA/HBSS solution containing OKM1 (1:500, final) and OKT8 (1:100, final) and incubated at room temperature for 1 hr with gentle shaking. Then the cells were washed and resuspended in a 1:10 dilution of rabbit low-tox complement for 1 hr at 37°C. Viable cells were separated on Ficoll-Hypaque and were washed in HBSS. CD4+ cells were then cultured for 48 hr in culture medium supplemented with 10 μg/ml of Con A. CD4+ cells were then incubated overnight with 100 U/ml of rIL-2 and labeled with 200 μCi of Na251CrO4.

**Virus Preparation**

The prototype human immunodeficiency virus (HIV-1) strain HTLV-IIIb was obtained from R. C. Gallo, National Cancer Institute, Bethesda, MD. The virus was grown in Molt-4f and/or CEM cell lines in serum-free AT-IMDM medium (12). Virus was prepared from the supernatants of infected cell cultures by centrifugation first at 800 rpm for 5 min and thereafter at 2500 rpm for 30 min for removal of cell debris. Supernatants were filtered through a 0.45-μm filter (Gelman) and stored at 20°C. Highly concentrated virus was prepared by pelleting the virus-containing supernatant at 100,000 g for 2 hr at 4°C.

**Inactivation of HIV by β-Propiolactone (BPL)**

This was done according to the method of Nishanian et al. (13).