Interleukin-2 Enhances the Depressed Natural Killer and Cytomegalovirus-specific Cytotoxic Activities of Lymphocytes from Patients with the Acquired Immune Deficiency Syndrome

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ABSTRACT The recently described acquired immune deficiency syndrome (AIDS) is characterized by the occurrence of severe opportunistic infections and an aggressive form of Kaposi's sarcoma. A variety of profound defects in cell-mediated immunity have been reported in association with the AIDS, including deficiencies in natural killer (NK) cell activity and cytomegalovirus (CMV)-specific cytotoxicity. In the present study, the in vitro effects of interleukin-2 (IL-2) and interferon beta (IFN Beta) on these abnormalities were examined to assess the potential use of these lymphokines in the immunotherapeutic treatment of this syndrome. The peripheral blood lymphocytes (PBL) from six male homosexuals with AIDS and an active CMV infection exhibited markedly depressed NK cell and CMV-specific cytotoxic lymphocyte responses compared with uninfected, heterosexual control subjects. Incubation of PBL with IFN Beta enhanced the NK cell activity and the CMV-specific cytotoxicity of only one of six and neither of two AIDS patients, respectively, while enhancing the NK cell activity of all six control subjects. In contrast, IL-2 dramatically enhanced both the NK cell and the CMV-specific cytotoxic lymphocyte activities of all of the patients. These results indicate that IL-2 can substantially potentiate the depressed cytotoxic effector functions of PBL from AIDS patients, while IFN Beta has little effect.

INTRODUCTION

Since 1978, more than 1,800 individuals have developed the acquired immune deficiency syndrome (AIDS),[1] which is characterized by severe defects in cellular immunity, opportunistic infections with agents such as cytomegalovirus (CMV), Herpes-simplex virus, and Pneumocystis carinii, and an unusually aggressive form of Kaposi's sarcoma (1–3). None of the patients with AIDS has regained immune function. The current

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[1] *Abbreviations used in this paper:* AIDS, acquired immune deficiency syndrome; CMV, cytomegalovirus; CTL, cytotoxic T cell; IFN, IFN Alpha, IFN Beta, interferon; IL-2, interleukin-2; NK, natural killer; PEG, polyethylene glycol.

mortality rate for all diagnosed cases is 40%; however, with time, mortality may approach 100% (1). In a preliminary report, we described abnormalities in natural killer (NK) and cytomegalovirus-specific cytotoxic lymphocyte activities in AIDS patients (4). In other immunosuppressed individuals, and more specifically, in transplant patients, these cytotoxic lymphocyte activities are important determinants of recovery from CMV infection (5, 6). Since the lymphokines interferon (IFN) and interleukin-2 (IL-2) may enhance NK cell and cytotoxic T cell (CTL) activities (7–9), we performed studies to determine if they could augment the depressed cytotoxic activities of lymphocytes from patients with AIDS. We report here that patients with AIDS and CMV infection had a marked depression in their NK cell and CMV-specific cytotoxic lymphocyte activities which could be restored to normal levels by in vitro incubation of their lymphocytes with highly purified IL-2. IFN Beta did not enhance the NK cell or the CMV-specific cytotoxic lymphocyte activities of the AIDS patients, although it did produce a significant increase in the NK cell activity of normal control subjects.

METHODS

Patient population. The patient group consisted of six male homosexuals with a clinical diagnosis of AIDS. The diagnosis was based upon the Centers for Disease Control's definition of AIDS as the occurrence in a previously healthy individual of a disease that is at least moderately predictive of a defect in cell-mediated immunity (1). These men were 25 to 48 yr old (mean 39 yr); four were Caucasian and two were Black. All described having had at least 40 lifetime male sexual partners and having occasionally used recreational drugs, including nitrites. None of the patients had a prior indication of an immunodeficiency disorder or a prior history of immunosuppressive drug use. CMV was isolated from the urine of all six patients, from the blood of five, from the throat washings of four, and the lymph node of one. Three patients had previous cases of *Pneumocystis carinii* pneumonia and one had Kaposi's sarcoma. Other opportunistic infections included disseminated Mycobactrium avium intracellulare, Candida esophagitis, and cryptococcal meningitis. All had leukopenia (white blood cell counts 2,200–3,800/mm^3) and lymphopenia. All had normal or elevated serum levels of IgG, IgA, and IgM, and ratios of circulating OKT4 antigen positive (helper T cell) to OKT8 antigen positive (suppressor/cytotoxic T cell) lymphocytes of <0.25. Six healthy heterosexual subjects served as controls.

Lymphocyte suspensions. Peripheral blood lymphocytes (PBL) were obtained from whole blood containing preservative-free sodium heparin (Hynson, Westcott, & Dunning, Inc., Baltimore, MD) by Ficoll-Hypaque density gradient centrifugation. PBL were washed twice in RPMI 1640 prior to use in cytotoxicity assays and were suspended in RPMI 1640 supplemented with 10% fetal bovine serum (medium).

Assays for natural killer cell and cytomegalovirus-specific cytotoxic lymphocyte activity. Target cells that were used in assays for NK cell activity were prepared from suspension cultures of the K562 myeloid cell line (10) and those in assays for CMV-specific cytotoxic lymphocyte activity were prepared from cultures of human diploid skin fibroblasts derived from normal, HLA-typed donors, as previously described (11). CMV-infected and uninfected target cells were stored in aliquots in liquid nitrogen before use. Target cells were chromium-labeled for NK cell and CMV-specific cytotoxicity assays by suspension at a concentration of $1–2 \times 10^6$ cells/ml in medium containing 100 μCi/ml of [^{51}Cr]sodium chromate (New England Nuclear, Boston, MA) for 60 min at 37°C, washed twice, suspended in medium at a concentration of 5×10^4 cells/ml, and dispensed in 0.1 ml aliquots into wells of round-bottomed microtiter plates (Linbro, Hamden, CT). Lymphocytes were suspended at a concentration of 2.5×10^6 cells/ml and were added in 0.1 ml aliquots to wells containing target cells to yield an effector-to-target ratio of 50:1. The patients' lymphopenia prevented the use of multiple effector-to-target ratios. After a 16-h incubation at 37°C in 5% CO_2 in air at 100% humidity, supernatants were harvested and counted on a gamma counter. Percent lysis of each target cell was determined by the equation:

$$\% \text{ lysis} = \text{cpm}_{\text{test}} - \text{cpm}_{\text{control}}/\text{cpm}_{\text{max}} - \text{cpm}_{\text{control}} \times 100,$$

where cpm_{test} indicates chromium counts per minute released in the presence of lymphocytes, $\text{cpm}_{\text{control}}$ indicates release in the presence of medium alone, and cpm_{max} indicates release in the presence of BRIJ-35 detergent (Sigma Chemical Co., St. Louis, MO). Each test was performed in quadruplicate. Significance of percent lysis was determined by comparing mean cpm_{test} with mean $\text{cpm}_{\text{control}}$ by t test. The percent CMV-specific lysis was calculated by subtracting percent lysis of uninfected fibroblasts from percent lysis of CMV-infected fibroblasts. CMV-specific lysis was significant if the mean percent lysis for infected cells was statistically greater by t test than the mean percent lysis for uninfected cells. Each assay for CMV-specific lysis included one or more sets of target cells (infected and uninfected) that were matched to the patient for one or more HLA-A or B antigens and one or more sets of completely HLA-mismatched target cells to determine whether a CMV-specific response was HLA- or non-HLA-restricted.

Purification of human IL-2. A conditioned medium containing IL-2 was obtained from 2-d-old cultures containing peripheral blood nonadherent cells and 2.5 μg/ml of purified phytohemagglutinin (PHA, Wellcome Research Lab., Beckenham, England). IL-2 was purified by successive column chromatography on phenyl-Sepharose and DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by Kasahara et al. (12). After the DEAE-Sephacel passage, IL-2-containing fractions were supplemented by 0.1% of polyethylene glycol (PEG); 6,000 mol wt; Sigma Chemical Co. IL-2 activity was then eluted as a single peak at a molecular weight of 20,000–25,000 by Sephacryl S-200 gel filtration (2.5 × 90 cm). Fractions from successive runs were pooled, concentrated, and dialyzed extensively against Dulbecco modified phosphate-buffered saline and RPMI 1640 medium containing 0.1% PEG. IL-2 prepared in this way generally contained ~5,000 U/ml (2×10^4 U/mg protein) IL-2 activity and was completely devoid of lectin and IFN activity (12).

IFN and IL-2 treatment of lymphocytes. Some cytotoxicity assays were performed using lymphocytes pretreated with either purified fibroblast IFN Beta (HEM Research, Inc., Rockville, MD) or the purified IL-2 described above. After suspension at 2.5×10^6 cells/ml, lymphocytes were incubated for 60 min at 22°C or for 72 h at 37°C with either

400 U of IFN Beta or 16 U of IL-2/10^6 cells. In preliminary studies, these concentrations induced optimal responses in NK cell activity of normal lymphocytes. The cells were then washed, counted, and dispensed into the microtiter plates as described above. IFN-B and IL-2 in concentrations used for treatment of lymphocytes did not effect spontaneous release of ^{51}Cr from target cells.

RESULTS

The results of NK cell assays are shown in Fig. 1. The mean (±1 SEM) NK cell activity of lymphocytes from the patients with AIDS was 29.8±6.5%. This was significantly below (P = 0.007) the mean of 60.2±6.4% for the six normal volunteers tested in this study. In vitro incubation of lymphocytes with IFN Beta resulted in significant enhancement of NK cell activity in only one of six AIDS patients and all six normal volunteers (Fig. 1). However, exposure of these same lymphocytes to IL-2 for 1 h significantly enhanced NK cell activity in all six AIDS patients and five of five normal volunteers tested (Fig. 2). Exposure to IL-2 for 72 h resulted in a further significant enhancement in all patients tested. After pretreatment with IFN Beta, IL-2 for 1 h, and IL-2 for 72 h, the mean results were 78.0±4.4, 73.5±6.5, and 89.3±3.6% for normal subjects' lymphocytes and 35.0±7.0, 58.7±6.8, and 84.6±3.2% for AIDS patients' lymphocytes.

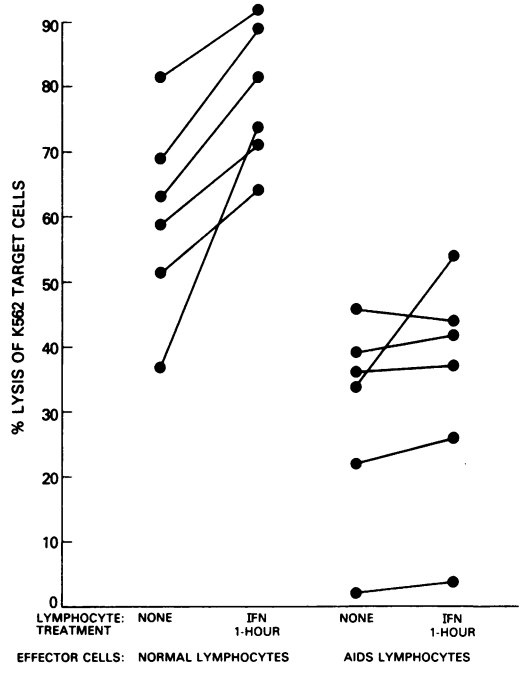

FIGURE 1 The effect of in vitro IFN Beta on the NK cell activity of lymphocytes from normal subjects and AIDS patients in a standard 16 h ^{51}Cr release assay using a 50:1 effector to target cell ratio and K562 cells as targets.

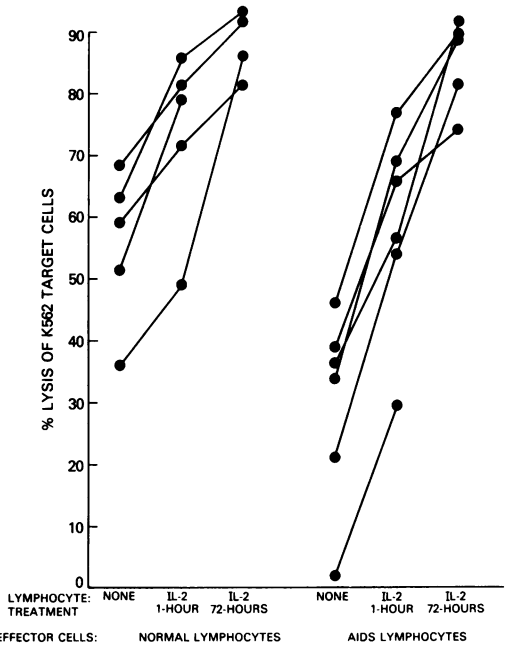

FIGURE 2 The effect of in vitro IL-2 on the NK cell activity of lymphocytes from normal subjects and AIDS patients. Data are shown for a 50:1 effector to target ratio. PBL were either untreated or were cultured in IL-2 for 1 or 72 h prior to assay.

The results of the CMV-specific cytotoxicity assays are shown in Fig. 3. Lymphocytes from the six normal, uninfected volunteers had significant CMV-specific cytotoxicity against both HLA-matched and mismatched target cells, as has been described previously (13). After exposure of the normal lymphocytes to IL-2 for 72 h, only one individual had significant enhancement of their activity. Four AIDS patients had no detectable CMV-specific cytotoxicity. The other two AIDS patients had low levels of CMV-specific cytotoxicity that did not exceed 3.5%. Two AIDS patients without detectable CMV-specific cytotoxicity had no increase in this activity after their lymphocytes were cultured in IFN Beta for 72 h (data not shown). In contrast, after AIDS lymphocytes were cultured for 72 h in IL-2, the mean CMV-specific cytotoxic activity against both HLA-matched and mismatched targets increased significantly from pretreatment values (P < 0.001). In five of six patients tested, lysis of HLA-matched and mismatched targets was comparable. Thus, the IL-2 enhanced CMV-specific cytotoxic activity was not HLA-restricted (significant lysis of HLA-matched target cells with no lysis of mismatched targets).

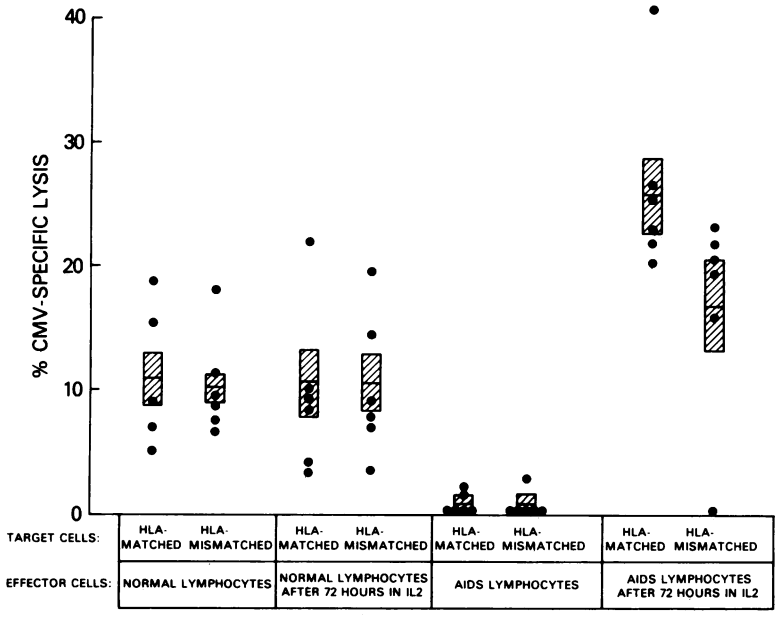

FIGURE 3 The effect of in vitro IL-2 on the CMV-specific cytotoxic lymphocyte activity of AIDS patients. Assays were 16 h ^{51}Cr release employing pairs of CMV-infected and uninfected human diploid skin fibroblasts as targets. Each experiment had at least one pair of partially HLA-matched and one pair of HLA-mismatched targets for each group of effector PBL. Data are expressed at a 50:1 effector to target ratio. The percent CMV-specific lysis was calculated by subtracting percent lysis of uninfected fibroblasts from percent lysis of CMV-infected fibroblasts. The shaded areas indicate the mean±1 SEM.

DISCUSSION

We studied homosexual men with Kaposi's sarcoma and/or opportunistic infections who had active CMV infections. In these patients, NK cell and CMV-specific cytotoxic lymphocyte activities were depressed. The cultivation of lymphocytes with IFN Beta enhanced NK cell activity and CMV-specific cytotoxicity in only one of six and neither of two patients, respectively, whereas both CMV-specific and NK cell cytotoxicity were significantly enhanced by IL-2. Furthermore, the level of cytotoxic lymphocyte activity measurable after treatment of their lymphocytes with IL-2 was comparable to levels measurable in normal subjects.

The importance of cytotoxic lymphocytes in recovery from CMV infection has been demonstrated in studies of bone marrow and renal transplant recipients (5, 6, 14). In uninfected healthy individuals, regardless of their CMV antibody status, circulating lymphocytes are found that kill CMV-infected target cells (13). These lymphocytes have properties of NK cells and are not HLA-restricted. During acute CMV infection, CMV-specific cytotoxicity increases (5). The responding cells may be HLA-restricted cytotoxic T cells (CTL) or nonrestricted, non-T lymphocytes (with properties of NK cells) or both (5). The failure of transplant patients to develop CMV-specific cytotoxic responses is uniformly associated with either severe and complicated or fatal infections. In these same transplant patients with absent CMV-specific cytotoxic responses, NK cell activity, measured using tumor cells as target cells, is also deficient.

The results of our study indicated that the AIDS patients had deficiencies in both CMV-specific and NK cell cytotoxic lymphocyte activities. The patients' were deficient in two respects. Their CMV-specific cytotoxicity was substantially lower than that measured in normal volunteers. In addition, despite having active CMV infections, they did not have HLA-restricted T cell-mediated cytotoxic responses. These deficiencies are probably related to their enhanced susceptibility to CMV infection.

The cytotoxic activities of lymphocytes from all the patients in this study were enhanced by exposure to IL-2. The effect of IL-2 can be understood in the context of the known effects of this lymphokine on various types of cytotoxic lymphocytes. IL-2 enhances NK cell activity (9). Similarly, the in vivo generation of CTL responses to alloantigens can be augmented by IL-2 (9). However, the effect of IL-2 on the in vitro production of HLA-restricted, virus-specific CTL has not been well studied. This latter response can be obtained

by cultivating the lymphocytes from a previously immune donor with syngeneic stimulator cells displaying viral antigens on their surface (15). Addition of IL-2 to cultures of lymphocytes that are responding to virus-infected stimulator cells is not ordinarily necessary to initiate the HLA-restricted, virus-specific cytotoxic response. However, both IFN and IL-2 are produced in these cultures and are probably necessary for development of virus-specific CTL (16). Exposure of lymphocytes in vitro to IL-2 in the absence of virus-infected stimulator cells, as performed in this study, resulted in the induction of nonspecific cytotoxicity mediated by cells with T cell surface antigens. These cells were described by Grimm et al. as "lymphokine-activated killer cells" and are not HLA-restricted (17). After the cultivation of lymphocytes from our patients in IL-2, comparable lysis of HLA-matched and mismatched target cells was observed in five of the six patients, which indicated that these responses were not HLA-restricted. Thus, in these five instances, NK cells or lymphocytes with the properties of lymphokine-activated killer cells were probably responsible for the killing of the CMV-infected target cells. In one case, however, the response was HLA-restricted. This response raised the possibility that some of this patient's mononuclear cells were infected with CMV and acted as CMV-specific stimulator cells, which induced a classical virus-specific CTL response.

The failure of IFN to enhance the depressed NK cell activity in five of six patients is particularly noteworthy. Normally, NK cell activity can be enhanced by IFN (7). Our observations in the healthy volunteers revealed a consistent augmentation of the NK cell activity by IFN in all six cases. The mechanism for the resistance to IFN among the AIDS patients is uncertain. DeStefano et al. (18) have noted that many patients with AIDS have significant levels of an acid-labile IFN Alpha in their serum. The presence of this IFN in the blood may contribute to the lack of in vitro responsiveness of their NK cells to exogenous IFN. Conversely, our study indicated that a defect in in vitro responsiveness of lymphocytes to IL-2 was not present in immunodeficient homosexual men.

The cause of AIDS is unknown and no specific treatment is available. Epidemiologic data suggest that a transmissible agent is involved (19) and that the infection that results causes a characteristic immune deficiency state. In this study, we demonstrated that cytotoxic lymphocyte activities, which are immune functions important for recovery from CMV infection, were deficient in this syndrome, but could be enhanced by in vitro treatment of lymphocytes with IL-2. These results focus attention on an abnormality that could be the primary immune defect responsible for AIDS. IL-2 is important in cytotoxic lymphocyte responses and can replace helper cell functions (20). Deficiencies in both these cell types could result from reduced IL-2 activity. Further study of the effects of infection on IL-2 could elucidate the pathogenesis of AIDS, while treatment of these patients with IL-2 might restore normal immune function.

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