Expression of a Functional p75 Interleukin-2 Receptor on Lung Lymphocytes from Patients with Human Immunodeficiency Virus Type 1 (HIV-1) Infection

RENATO ZAMBELLO, LIVIO TRENTIN, ROBERTO BENETTI, ANGIOLO CIPRIANI, CARLO CRIVELLARO, PAOLO CADROBBI, CARLO AGOSTINI, and GIANPIETRO SEMENZATO

Accepted: May 8, 1992

Interventions in patients infected by HIV-1 infection is characterized by an alveolitis sustained by the accumulation of CD8+ T lymphocytes. To investigate whether in situ T cell growth plays a relevant role in the pooling of CD8+ lymphocytes, we have analyzed the activity of two lymphokines involved in the mechanisms of T cell proliferation, i.e., interleukin-2 (IL-2) and interleukin-4. To this aim, following appropriate triggering and blocking, the expression and the functional role of IL-2 receptors (IL-2R) (both p55 and p75 chains) and IL-4 receptors have been analyzed on T lymphocytes obtained from the bronchoalveolar lavage (BAL) of 16 HIV-1+ patients. Molecular and phenotypic studies we performed demonstrated that CD8+ lymphocytes from the BAL of HIV-1+ patients strongly expressed the p75 chain of IL-2 receptor, while neither p55 mRNA nor its surface membrane product (Tac antigen) was detectable; in addition, there was no expression of IL-4 receptors. IL-2 stimulation was able to induce T cell growth in a dose-dependent manner, whereas IL-4 did not. Finally, using mAbs which specifically block the p55 or p75 IL-2R, we showed that both subunits of IL-2R were involved in the proliferative activity of lung lymphocytes. The results obtained in the present study directly demonstrate that BAL T lymphocytes of HIV-1+ patients express a fully functional IL-2 receptor apparatus, pointing to the role for this lymphokine in maintaining the alveolitis taking place in the lungs of AIDS patients.

KEY WORDS: Interleukin-2 receptors; pulmonary immunocompetent cells; human immunodeficiency virus type 1 (HIV-1) infection.

INTRODUCTION

Over the last few years, evidence has been accumulating that HIV-1 may affect the cellular immune system in the lung of patients with AIDS (1, 2). Major immunologic abnormalities reported to date include alveolitis, characterized mainly by cytotoxic/suppressor CD8+ lymphocytes, severe depletion of pulmonary helper-related CD4+ lymphocytes, and, in some cases, an increase in polymorphonuclear cells and alveolar macrophages (PAM) (3–7). Although many phenotypical and functional properties of the CD8+ lymphocytes accounting for the alveolitis in the lung of HIV-1+ patients have been characterized to some extent (3–5), the mechanisms responsible for the accumulation and/or local proliferation of CD8+ cells are still unknown. The comprehension of these mechanisms might help in understanding the pathogenesis of pulmonary involvement in AIDS patients since CD8+ cells have been claimed to represent the expression of the lung immune response against HIV-1-infected cells (8, 9).

IL-2 is a lymphokine which is central in the processes leading to T-cell proliferation (10, 11). IL-2 mediates its own activity by binding to specific surface receptors (IL-2R): a p55-kd glycoprotein (αIL-2R) with a low affinity ($K_d$, $10^{-8} M$) and a p75-kd glycoprotein (βIL-2R) with an intermediate affinity ($K_d$, $10^{-9} M$) for IL-2 binding (12). The noncovalent association of the p55 and p75 IL-2R chains leads to the generation of the high-affinity receptors ($K_d$, $10^{-12} M$). Several laboratories have recently produced monoclonal antibodies (mAbs) which specifically bind to the p55 and p75 IL-2R (13–16), thus allowing the detection of these anti-
gens on leukocytes and the evaluation of their functional roles. IL-4 is also able to promote the growth of T cells (17); it exerts a low proliferative effect on resting T cells but it is able to induce a strong proliferative signal on T cells preactivated in vitro with lectins (17, 18). In particular, in in vitro conditions IL-4 has been reported to enhance directly the immune responses independently from IL-2 (18, 19).

The present study was aimed at investigating the mechanisms accounting for the accumulation of CD8+ cells in the lung of HIV-1+ patients, with the ultimate goal of understanding the pathogenesis of pulmonary involvement in AIDS patients. In particular, we studied the expression, at both genotypic and phenotypic levels, and the functional role of p55 and p75 IL-2R and IL-4 receptors on T lymphocytes obtained from the bronchoalveolar lavage (BAL) of 16 HIV-1+ patients.

MATERIALS AND METHODS

Study Populations

Sixteen HIV-1-seropositive patients (average age, 33 ± 5 years; 15 males and 1 female) were studied. All patients belonged to the IV group of CDC classification (20), which includes patients with clinical symptoms and signs of HIV-1 infection other than, or in addition to, lymphoadenopathy. According to these criteria, five patients had AIDS-related complex (ARC) and belonged to the IV-A subgroup and 11 patients belonged to the IV-C1 subgroup. Bronchoalveolar lavage was performed on these patients to obtain a specific diagnosis of opportunistic pulmonary infections and/or AIDS-related tumors. Our study protocol of HIV-1 patients with pulmonary involvement includes the use of BAL and transbronchial lung biopsy (TLB) during fiberoptic bronchoscopy. According to the declaration of Helsinki, this invasive procedure was performed only for diagnostic purposes when a pulmonary complication had been suspected. Ten of the 16 patients were smokers. The risk factors for AIDS included intravenous drug abuse (n = 10) and homosexuality (n = 6). None of our patients had a pulmonary localization of Kaposi’s sarcoma.

Six non-smoking HIV-1 seronegative subjects not at risk for AIDS (4 men and 2 women, with an average age of 36 years) were used as controls. They had normal physical examination, chest radiographs, and pulmonary functions.

Preparation of Cell Suspensions and Purification of Lymphocytes from the Lung

Bronchoalveolar lavage was performed following local anesthesia according to the method described previously (21). The percentage of lavage recovery was 58.6 ± 4.7 and 51.5 ± 6.9% of the injected fluid in controls and HIV-1 patients, respectively. A differential count of macrophages, lymphocytes, neutrophils, and eosinophils (made from total counts of 300 cells) was accomplished by morphological criteria in cytocentrifuged smears stained with Wright-Giemsa. Cells recovered from the BAL were centrifuged on a Ficoll-Hypaque (F/H) density gradient, washed, and then resuspended in RPMI 1640 culture medium (Gibco Limited, Paisley, Scotland) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20% fetal calf serum (FCS; Flow Laboratories, UK). For functional studies, alveolar macrophages were removed from the entire mononuclear cell suspension by two sequential incubations at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 60 min in plastic petri dishes. The cell population recovered after the adherence procedure was further depleted of contaminating alveolar macrophages following a complement-mediated lysis using the LeuM5 (CD11c) mAb (Becton Dickinson) and complement, as previously reported in detail (22). The expression of CD11c by BAL lymphocytes was below 3%. More than 98% of the resulting population was represented by lymphocytes, as determined by nonspecific esterase staining (less than 2% of cells were esterase positive). More than 95% of cells were viable, as judged by the trypan blue exclusion test.

Monoclonal Antibodies

The following fluorescein (FITC) or phycoerythrin (PE)-conjugated mAbs were used: anti-CD3 (OKT3), anti-CD4 (OKT4), and anti-CD8 (OKT8) purchased from Ortho Diagnostic (Raritan, NJ); anti-CD56 (Leu19) and anti-CD69 (Leu23) purchased from Becton Dickinson (Sunnyvale, CA); B-ly-7 mAb, which defines an activation antigen on normal CD8+ T cells, kindly provided by Dr. S. Poppema (23); ascites fluid containing anti-CD25 mAbs (anti-Tac or B-BI0 mAbs), which recognize the p55 IL-2R and block IL-2 binding to this subunit (13, 14), a gift from Dr. T. Uchiyama (Kyoto, Japan) and Dr. J. Wijdenes (Besançon, France),