Dexamethasone Inhibits CD4 T Cell Deletion Mediated by Macrophages from Human Immunodeficiency Virus–Infected Persons

Thorsten W. Orlikowsky,1,2 Z. Q. Wang,1 Anita Dudhane,1 Günther E. Dannecker,3 Dietrich Niethammer,1 Gary P. Wormser,2 Michael K. Hoffmann,3 and Harold W. Horowitz2

Prednisolone slows the loss of CD4 T cells in individuals with human immunodeficiency virus (HIV) disease and inhibits antigen-induced apoptosis of recently HIV-infected CD4 cells in vitro. This study investigated whether dexamethasone inhibits the ability of macrophages to delete CD4 T cells via anti-CD4 antibody or immune-complexed HIV envelope protein gp120. Peripheral blood mononuclear cells from HIV-negative persons were incubated with CD4-reactive ch412 monoclonal antibody or with gp120/IgG immune complexes and resident macrophages, with and without dexamethasone. Dexamethasone inhibited CD4 cell deletion in a dose-dependent manner. The deletion of normal CD4 cells by macrophages from HIV-infected patients also was inhibited by dexamethasone. Furthermore, up-regulation of CD95 expression on T cells exposed to anti-CD4 and gp120/IgG, which predisposes T cells to CD95-mediated apoptosis, is inhibited by dexamethasone in a dose-dependent fashion. Dexamethasone inhibits the macrophage-mediated deletion of CD4 lymphocytes in HIV-infected persons.

Glucocorticosteroids are immunosuppressive agents that affect nonspecific inflammatory responses and specific immunologic interactions between lymphoid cells. T cell activation, T cell proliferative responses induced by antigens or mitogens, and secretion of a variety of proinflammatory cytokines by mononuclear cells are inhibited by glucocorticosteroids. The ability of glucocorticosteroids to modulate the capacity of the antibody-dependent cellular cytotoxicity (ADCC) reaction is not as well studied. Gudewicz [1] demonstrated that dexamethasone suppresses macrophage-mediated cytotoxicity against antibody-coated target cells [1]. However, few other data are available on whether glucocorticosteroids modulate ADCC.

Progressive loss of CD4 lymphocytes is a hallmark of the immune deficiency that occurs in human immunodeficiency virus (HIV) infection. Apoptosis, rather than direct viral cytoxicity, appears to be the major cause for T cell deletion [2]. In a series of experiments, we demonstrated that CD4 lymphocyte depletion in vitro is, at least in part, due to macrophage-mediated ADCC [3, 4]. Macrophages from HIV-infected donors express CD4-reactive gp120/IgG complexes on their surfaces that facilitate the destruction of CD4 lymphocytes in vivo and in vitro. Macrophages from healthy donors have the same effect when exogenously produced gp120/IgG is presented to them. Administration of glucocorticosteroids to patients with early HIV infection slows the loss of CD4 lymphocytes [5]. The mechanism for this effect is unknown. Some of the glucocorticoid effect has been attributed to inhibition of apoptotic cell death that occurs when CD4 T cells acutely infected by HIV-1 are activated by anti-CD3 monoclonal antibody (Mab)/mitogen stimulation [6]. Here we report the effects of dexamethasone on macrophage-dependent ADCC. We attempted to determine whether some of the observed effects of glucocorticosteroids on CD4 cell loss may result from the effects of such medications on macrophage-mediated deletion.

Materials and Methods

Study subjects. We studied adult patients at the New York Medical College (Valhalla, NY) who were at various stages of HIV-1 infection. Control human blood samples were obtained from HIV-negative employees of New York Medical College.

Cell cultures. Peripheral blood mononuclear cells (PBMC) were isolated from donor blood by Ficoll-Hypaque (Sigma) density sedimentation. Washed cells were resuspended in RPMI 1640 culture medium (Sigma) containing 10% fetal calf serum (Sigma), counted in an ultraplane improved Neubauer hemocytometer, and
placed at a level of $2 \times 10^7$ cells per $0.1 \text{ mL}$ in flat-bottom 96-well microtiter plates (Falcon; Becton Dickinson). To separate monocytes from lymphocytes, cultures were agitated 60 min later, and nonadherent cells were collected. Adherent cells were thoroughly washed twice and used as the macrophage source. Nonadherent cells were washed and plated at a level of $3 \times 10^6$ cells per 1.5 mL for 60 min in specially coated 60 $\times$ 15-mm culture vessels (NoK4-3802-4; Becton Dickinson). This procedure was repeated once. Usually, we found <1% monocytes after the first adherence cycle and <0.2% after 2 cycles. The cells were cultured alone or in mixtures, as described in figure 1, and contained $2 \times 10^4$ unseparated PBMC or $2 \times 10^4$ nonadherent cells with or without adherent cells from $2 \times 10^4$ PBMC. Dexamethasone was added at the start of culture at concentrations of $10^{-7}$ to $10^{-5}$ M. Cultures incubated in the absence of dexamethasone served as control cultures.

Flow cytometry. PBMC were phenotyped before and after tissue culture. Cells were stained, as described elsewhere [4], with fluorescein isothiocyanate–labeled or biotinylated MAb. The biotin label was revealed using streptavidin–phycoerythrin (Sigma). We used anti-CD4 and anti-CD8 antibodies (OKT4 and OKT8, respectively; Ortho Diagnostics) that were not cross-reactive with ch412 MAb [4]. All macrophage stainings were performed in the presence of 10% heat-inactivated human serum to block nonspecific Fc receptor–mediated binding of the test antibody. Macrophages were gated by high forward and side scatter, as described elsewhere [4]. More than 95% of cells passing this gate expressed CD14.

Harvested lymphocytes were counted in the hemocytometer. Live cells were assayed by flow cytometry (FACScan; Becton Dickinson) for expression of CD4 and CD8 coreceptors. The numbers of CD4 and CD8 T cells were calculated, and the percentage of deleted cells was determined by this formula: [(cells cultured with control macrophages − cells cultured with treated macrophages) × 100]/[cells cultured with control macrophages] × 100.

Reagents. Recombinant HIV-1 gp120s2f2 was provided by K. Steimer (Chiron Biocine, Emeryville, CA). Serum from a seropositive donor was used as the source of anti-gp120 antibody. Equivalent results were obtained with a gp120 V3-specific MAb (clone 447; provided by S. Koening [Medimmune, Gaithersburg, MD]). A humanized anti–human CD4 MAb (ch412) was a gift of G. Rethmuller (Institute of Immunology, Munich, Germany). Dexamethasone was purchased from Sigma. Fresh dilutions in PBS solution were prepared for each experiment. Anti-CD3 MAb (OKT3) and anti-CD4 MAb (OKT4) were gifts of P. Rao (Ortho Diagnostics, Raritan, NJ).

Results

Inhibition by dexamethasone of antibody-mediated and immune complex–mediated deletion of CD4 T cells by macrophages. PBMC from healthy adults were treated with CD4-reactive ch412 MAb in the presence or absence of dexamethasone. Resident macrophages destroyed a large proportion of antibody-reactive CD4 T cells. Dexamethasone did not affect macrophage survival but inhibited the deletion of T cells in a time-dependent (figure 1A) and dose-dependent (data not shown) fashion. Immune-complexed CD4-reactive HIV envelope molecules (gp120) mediated an ADCC-based deletion of CD4 T cells, much as does anti-CD4 MAb (figure 2A).

Inhibition by dexamethasone of macrophage-mediated deletion of CD4 lymphocytes. Other studies have shown that macrophages from HIV-infected persons express gp120/IgG complexes on their membranes that mediate spontaneous deletion of conjugated CD4 T cells in tissue culture [4]. Our results (figure 1B) confirm these findings and show that addition of dexamethasone inhibits this reaction. Spontaneous macrophage cytotoxicity for CD4 T cells in AIDS patients can be further enhanced by loading macrophages with exogenous gp120/IgG complexes. This enhancement is also subject to suppression by dexamethasone (figure 1B).

Discussion

Our data demonstrate that the ADCC-based destruction of CD4 cells by macrophages is inhibited by dexamethasone.
Addition of dexamethasone, gp120, or anti-gp120 MAb alone had no effect. The fraction of deleted CD4 T cells was determined in triplicate cultures counted and phenotyped for expression of CD4 and CD95 after 48 h. (MAb; 5 μg/mL), both alone and with dexamethasone. Cells were counted and phenotyped for expression of CD4 and CD95 after 48 h. The fraction of deleted CD4 T cells was determined in triplicate cultures (mean results of 3 experiments are shown). Error bars designate SD. Addition of dexamethasone, gp120, or anti-gp120 MAb alone had no effect (data not shown).

ADCC is an important immune effector mechanism against virus-infected, senescent, and malignant cells. These cells are destroyed by specific antibodies directed against cell surface-associated antigens via effector cells such as NK cells and macrophages. Recent studies that used a mouse model suggest that the macrophage may be an important ADCC effector cell [8]. ADCC directed by gp120 is a major determinant in the rate of decline of CD4 lymphocytes in HIV-1 disease [9]. This suggests that the magnitude of the ADCC reaction correlates with HIV disease progression. However, Baum et al. [10] reported that the ADCC response declines as HIV disease progresses. They attributed this to the loss of HIV-specific antibodies.

T cells targeted by macrophages in this ADCC reaction are deleted via apoptosis in a CD95-dependent fashion [3]. Interaction of macrophage-produced Fas ligand (CD95L) with its receptor (CD95) on T cells results in lymphocyte apoptosis [11]. Increased susceptibility to apoptosis has been demonstrated in lymphocytes from HIV-infected persons [12]. HIV infection causes up-regulation of CD95 expression on T cells [13] and increases CD95 ligand expression on macrophages [14]. In the experiments described here, we show that the up-regulation of CD95 on T cells, which predisposes T cells for Fas-mediated apoptosis, is inhibited by dexamethasone. This may account for the effects of dexamethasone on macrophage-mediated T cell depletion. Because glucocorticosteroids can induce monocyte apoptosis [15], it is possible that some of the glucocorticosteroid effects in our system are mediated by disturbance of macrophage function.

In summary, our data show that dexamethasone blocks the deletion of CD4 lymphocytes, which is dependent on conjugate formation with macrophages, and suggest a possible mechanism for the in vivo observations that dexamethasone can prevent the decline of CD4 lymphocytes [5]. Furthermore, our findings are in agreement with reports that glucocorticosteroids can diminish both macrophage-mediated apoptosis [1] and antigen-induced lymphocyte apoptosis [6]. Our experiments were undertaken in a virus-free in vitro system, and, therefore, the activity attributed to dexamethasone could not have been the result of inhibition of viral replication.

References