Polyspecific Self-Reactive Antibodies in Individuals Infected with Human Immunodeficiency Virus Facilitate T Cell Deletion and Inhibit Costimulatory Accessory Cell Function

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Self-reactive polyspecific IgG antibodies (PSAs) arise in human immunodeficiency virus (HIV)–seropositive subjects before they develop AIDS. Self-reactive PSA levels correlate with the destruction of CD8 T cells in HIV-infected individuals and mediate the antibody-dependent cellular toxicity–based destruction of human T cells in tissue culture. PSAs react across the species barrier and bind to T cell antigens in mice. Such reactivity with mouse lymphocytes was not detected in normal human serum. Injection of human PSA IgG causes massive T cell depletion in the spleen, lymph nodes, and thymus in mice: evidence that PSA IgG facilitates T cell destruction in vivo. In addition to facilitating macrophage cytotoxicity, self-reactive PSA IgG inhibits the macrophage-mediated activation of T cells with antigen receptor–specific monoclonal antibody or with antigen. Exogenous costimulatory stimuli or interleukin (IL)–12 can reverse the inhibition. In contrast, exogenous IL-10 mimics this inhibition. These data implicate PSA IgG as a pathogenic factor in the development of HIV disease.

Developing an adaptive immune system, B and T lymphocytes establish reactivity with a diverse universe of antigens by rearranging variable antigen receptor sequences. After leaving the bone marrow, the site of their early maturation, B cells complete their development by executing two major tasks, the immunoglobulin (Ig) class switch and somatic Ig mutation [1]. Ig class switching is induced by cytokines and proceeds independently of antigen receptor (B cell reactivity [BCR]) engagement, whereas somatic mutation is antigen-driven [2, 3].

The BCR rearrangement, which endows B cells with clonotypic receptors, is a random process that results in the formation of receptors reactive with either self-antigens or foreign antigens. Before reaching the stage of functional maturity, each B cell tests its newly made receptors for reactivity with self-antigen and dies when it succeeds [4]. This negative-selection mechanism assures that self-antigens that trigger the BCR into signal transduction destroy the B cell that generated the self-reactive receptor. Self-tolerance is thereby established at a particular trigger threshold of self-reactivity. Self-antigens that react with B cell Ig below that threshold level fail in the negative-selection process and may survive. This must not be cause for concern, because the low-affinity threshold of these self-antigens will not trigger an antigen receptor–mediated immune response of mature B cells. Apart from antigen receptor-mediated activation signals, B cells can be induced to secrete Ig by other stimuli, notably by microbial products [5]. Polyclonally stimulating microbial products react with B-cell surface components distinct from the BCR. They fail to activate the Ig-switching mechanism and do not induce antibody affinity maturation. The switching mechanism is mediated by cytokines, notably interleukin (IL)–4, which are released by helper T cells when they form antigen-specific cellular conjugates with antigen-reactive B cells [6].

It is thus conceivable that nonspecific B cell stimuli can induce B cells to secrete antibodies that may then react with self-antigens that themselves failed to trigger the immune response via the antigen receptor. Unable to engage the antigen receptor, these nonspecific stimuli will not engage B cells in the formation of cellular conjugates with helper T cells and thus neither procure the cytokines needed for Ig class switching nor facilitate antibody affinity maturation. Therefore, nonspecifically activated B cells are not capable of increasing antibody affinities to a given antigen. The antibodies retain low-affinity characteristics with a spectrum of antigens, including self-antigens. Such antibodies are known as polyspecific antibodies (PSAs) [7–9]. Initially secreting IgM, PSA-producing B cells can gen-
erate the whole spectrum of antibody effector functions when they activate the switching mechanism. In the absence of antigen-mediated cellular conjugates with helper T cells, the IgG switch may proceed in a bystander fashion if the production of switch cytokines occurs for other reasons [2]. These reasons include conditions of chronic pathological immune stimulation seen in long-term systemic exposure to auto-antigen, for example, systemic lupus erythematosus (SLE) [10], and long-term exposure to infectious agents, such as human immunodeficiency virus (HIV) [11,12]. These conditions are characterized by the generation of polyspecific IgG antibodies that react with self-antigen [10–12]. Typically, the T helper (Th) 1/Th2 balance tilts towards a Th2 predominance with enhanced production of the switch cytokines IL-4 and IL-10 [13,14]. CD5-positive B lymphocytes, which are considered to be major PSA producers, express IL-10 themselves [15]. Herein we investigate whether switching to the IgG class enables lymphocyte-reactive PSAs to disturb immune functions in the host.

Materials and Methods

Study subjects. Serum was prepared from 19 HIV-infected subjects with high levels of polyspecific autoreactive antibodies and from 3 healthy control donors. Six-week-old female Balb/C mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Animals were injected intravenously with human serum (0.1 mL, diluted 1:1 in PBS) or purified Ig fractions (600 μg). Lymphoid organs were removed 1–3 days later (as indicated in the data displays), and single-cell suspensions were prepared as described [16]. Lymphocytes were counted and phenotyped in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Experiments were repeated at least twice.

Cell culture. Human peripheral blood mononuclear cells (PBMC) were isolated from donor blood by ficoll-hypaque density sedimentation. Washed cells were resuspended in RPMI 1640 (Sigma, St. Louis, MO) containing 5% fetal calf serum (FCS; Sigma) and counted in an ultraplane-improved Neubauer hemocytometer. Cells at a concentration of 2.5×10⁷ were cultured in 0.1 mL RPMI (with 5% FCS) in 96-well, flat-bottom tissue culture plates. PBMC were stimulated with immobilized anti-CD3 monoclonal antibody (mAb) [17]. For immobilization, antibody was added to culture wells at a concentration of 100 μg/mL, incubated overnight at 4°C, and washed 3 times before PBMC was added. An identical immobilization procedure was done with B7-1 Ig and with CD28 mAb. When indicated, Staphylococcus aureus enterotoxin B (SEB; Sigma) was added at 1 μg/mL. Other additions are specified in the data displays.

Macrophage-depleted lymphoid cells were prepared by incubating PBMC in RPMI with 20% FCS for 60 min and subsequently gently collecting nonadherent cells. The process was repeated once. The nonadherent cells were cultured alone or were recombined with adherent cells at a ratio of 8:1 as described elsewhere [17].

Flow cytometry. Mouse and human lymphoid cells were phenotyped immediately after preparation and after culture by use of a FACScan flow cytometer (Becton Dickinson). Washed cells were stained as described elsewhere [17] with fluorescein-isothiocyanate (FITC)-labeled or biotinylated mAbs. The biotin label was revealed by streptavidin-phycocerythrin (Sigma). The following antibodies were used: anti-mouse CD4 [18], anti-mouse CD8 [19], anti-human CD4 and anti-human CD8 (OKT4 and OKT8, Ortho Diagnostics, Raritan, NJ), and anti-human CD28 (produced by R. Mittler, Bristol-Meyer Squibb, Princeton, NJ). Surface Ig binding was measured by incubating PBMC with PSA-containing human serum (1:10 dilution, 4°C, 30 min) followed by treatment with FITC-labeled goat-anti human IgG F(ab)₂ (Cappel, West Chester, PA).

Results

Human PSAs react with human and mouse lymphoid cells and facilitate massive deletion of lymphoid cells in vivo. Human PSAs in the serum of HIV-infected donors and of patients with lupus erythematosus have been shown to bind to human T cells and to enable human macrophages to destroy these T cells in tissue culture [17]. Figure 1 confirms this observation. Serum from an HIV-infected individual and serum from a healthy control

![Figure 1](image-url)
donor were added to freshly prepared human peripheral blood lymphocytes in the presence or absence of macrophages. The cells were harvested 3 days later, counted, and phenotyped for the expression of T cell markers. The experiment shows that macrophages added to lymphocytes in a physiologic ratio destroy lymphocytes in the presence of PSAs but not in their absence. Doubling the monocyte/lymphocyte ratio to that typical of patients with AIDS doubles the fraction of deleted lymphocytes [17]. The observation that self-reactive PSAs facilitate the deletion of T cells in tissue culture has led to the speculation [17] that these antibodies may destroy T cells in vivo and explains the loss of T cells in individuals who express these antibodies. Because these antibodies are self-reactive as a consequence of their polyspecificity, it is possible that they react with lymphocytes from other species. To test this hypothesis, we evaluated antibody-dependent cellular toxicity (ADCC) function of human PSA in mice. Results in figure 2 show that human PSAs cross-react readily with mouse lymphoid cells. Studies were performed injecting serum from HIV-infected subjects into mice. Preliminary results showed that such sera caused rapid and massive depletion of mouse lymphoid cells in blood, lymph nodes, the spleen, and the thymus; serum from healthy donors had no such effect (data not shown). IgG-positive and -negative fractions were prepared from serum of patients with AIDS and administered to mice in comparison to serum from healthy human individuals. Results are shown in figure 3. Approximately one-half of the T cells in spleen and lymph nodes disappear within 1 or 2 days after injection of patient serum IgG. When tested 5 days after injection of human IgG, patient IgG was still found to reduce the frequency of T cells in lymph nodes and spleen. Both single-positive, as well as double-positive, T cells in the thymus declined in frequency (figure 4) [17]. The data confirm the previous results from tissue culture studies and provide evidence that PSA facilitates the massive deletion of lymphocytes in individuals who exhibit them.

Polyspecific self-reactive human antibodies inhibit the receptor-mediated activation of human T cells. Patients with SLE and individuals infected with HIV exhibit both a reduction in T cell counts and diminished or absent T cell responses to mitogenic or antigenic stimuli, reflecting the fundamental immune deficiency in these diseases. To investigate a possible immune-suppressive role of PSAs, we examined whether PSAs affect the response to antigen receptor (T cell reactivity [TCR]) engagement by T cells from healthy donors. Human PBMC were stimulated with CD3 mAb in the presence of IgG from a healthy donor or IgG from an HIV-infected donor. CD3 mAb was immobilized in the culture dish to avoid competition between the mitogenic antibody and human serum IgG fractions for macrophage FcRs. Immobilization renders the TCR stimulus macrophage-FcR-independent [21]. Figure 5A shows that patient IgG supports only a fraction of the proliferative mitogen response seen in control cultures and in cultures given IgG from healthy individuals. A similar effect of IgG from patients with AIDS is seen in cultures stimulated with major histocompa-

Figure 2. Polyspecific antibodies in the serum of individuals infected with human immunodeficiency virus (HIV) react with autologous lymphoid cells and with mouse lymphocytes. Human peripheral blood mononuclear cells (PBMC) and mouse lymph node cells were incubated for 30 min at room temperature with the IgG fraction of serum from HIV-seronegative (normal IgG) or -seropositive (patient IgG) donors, washed, and counterstained with fluorescein isothiocyanate-labeled goat anti human IgG F(ab), antibody. Open-flow cytometer histograms depict anti-IgG reactivity of untreated lymphoid cells; shaded histograms represent treated cells.
Figure 3. IgG fraction of serum from a patient with AIDS facilitates the deletion of lymphoid cells in Balb/C mice. Balb/C mice were injected with 0.1 mL (1:1 diluted with PBS) of normal human serum or the IgG-depleted fraction of serum from patient with AIDS or received an intravenous inoculation with 600 μg IgG from patients with AIDS. Single-cell suspensions were prepared from the spleen or the inguinal lymph nodes 1 or 2 days later, and the cells were counted and phenotyped for coreceptor expression by flow cytometry. The absolute cell counts of several phenotypes were calculated and are shown as the averaged results from 3 animals with SD (error bars). LN, lymph node cells; SC, spleen cells.

Figure 4. IgG from a patient with AIDS induces a deficiency in the number of mature thymocytes, as well as of immature thymocytes, in the mouse. Balb/C mice were injected with 600 μg IgG from human immunodeficiency virus–seronegative (normal IgG) or –seropositive (patient IgG) donors. Lymphoid organs were recovered 5 days later and phenotyped and evaluated as described in figure 3. The data represent the averaged results of 5 animals ± SD. CD4s, CD8s, and CD4/CD8DP cells are double-positive immature thymocytes.

It has been suggested that the immune system in HIV-infected subjects lacks appropriate costimulatory capacity [23] and that the defective in vitro mitogen response of the T cells of patients with AIDS can be restored by substitution with exogenous costimulatory stimuli [24]. Costimulatory signals are mediated by CD28 molecules on the T cell surface and are induced by B7 family molecules expressed on the surface of accessory cells [25]. We immobilized in the culture vessel 1 of 2 exogenous costimulatory stimuli, soluble B7-1 molecules [20] or CD28 mAb. Both CD28 ligands abrogated the immune suppression induced by PSA IgG (figure 5). These results suggest that IgG from patients with AIDS inhibits the T cell immune response by generating a deficit in costimulatory signals.

Patients with SLE and patients with AIDS have been shown to have an altered cytokine balance, with a shift from a type 1 cytokine predominance (IL-2, interferon-γ, IL-12) to a type 2 cytokine predominance (IL-4, IL-10) [26]. IL-12 and IL-10 are macrophage cytokines whose release has been attributed to 2 distinct macrophage subsets, M1 and M2, respectively [27]. It has been suggested that the M1/M2 ratio declines in HIV-infected subjects [17], which would result in a deficiency of IL-12 production and an excess of IL-10 production. We examined the effects of IL-12 and IL-10 on the immune suppressive activity of IgG from patients with AIDS. We found (figure 6) that IL-12 reverses IgG-mediated immune suppression in vitro of patients with AIDS, whereas IL-10 mimics it. Figure 6 shows an additional assay of the mitogen-induced T cell response in vitro, namely the assessment of T cells that upregulate the expression of CD28 molecules beyond the highest level of resting cells. Fewer T cells respond in this assay in the presence of IgG from patients with AIDS compared with control T cells or T cells treated with normal human IgG. Again, the deficiency is...
Figure 5. IgG from patients with AIDS containing polyspecific autoreactive antibodies inhibits the receptor-mediated activation of T cells in vitro. Peripheral blood mononuclear cells (PBMC) prepared from a healthy individual were stimulated with a mitogenic T cell reactivity ligand, CD3 monoclonal antibody (A) or the superantigen Staphylococcus aureus enterotoxin B (B) in the absence of human IgG (○) or in the presence of IgG from a donor seronegative to human immunodeficiency virus (HIV; ○) or a donor seropositive to HIV (▲). The error bars represent the SD among 3 experiments. ▲, no treatment.

Corrected in the presence of IL-12 and mimicked in the presence of IL-10. Identical results were obtained with superantigen stimulation of T cells (figure 6B).

The concentration of lymphocyte-reactive IgG in serum of patients with AIDS correlates with ADCC and immune-suppressive activity. We wished to determine whether, and to what extent, T cell–reactive antibodies account for immune-suppressive activities of the serum of patients with AIDS. The question was examined in a 2-step procedure. First, we purified IgG from serum of patients with AIDS to isolate antibodies from potentially immune-suppressive serum elements other than antibody. We established the IgG T cell–binding reactivity in serial dilutions and determined for each concentration immune-suppressive and ADCC reactivity values. In a second step, we assayed IgG T cell binding by several unfractionated patient sera and again correlated T cell IgG reactivity with immune-suppressive and ADCC serum reactivity. The results of the experiment are consistent with the interpretation that the immune-suppressive capacity and the ADCC reactivity of unfractionated serum of patients with AIDS is mediated by T cell–reactive IgG and neither enhanced nor suppressed by other serum components.

IgG T cell reactivity was first assessed by incubating fresh PBMC with purified serum IgG and then determining IgG binding in an indirect immunofluorescence assay (figure 7A). PBMC were, in addition, treated with the same IgG doses and assayed for ADCC-based T cell depletion and for the ability to proliferate in response to CD3 mAb stimulation. T cell counts were plotted against IgG surface reactivity (anti-human Ig reactivity; figure 7B). The results show a good correlation between the amount of PSA IgG bound by the lymphocytes, the induced T cell deletion, and the suppression of the T cell mitogen response.

In the same experiment, we tested 12 unfractionated serum samples from patients with AIDS for levels of lymphocyte-reactive IgG and correlated the intensity of IgG T cell reactivity, measured by indirect fluorescence, with the degree of in vitro immune suppression and ADCC activity (figure 7C). Again, the depletion of T cells and the suppression of the mitogen response increased with the amount of IgG bound by the T cells. The regression curves, computer calculated for figures 7B and 7C, are superposed in figure 7C (dashed lines) over those calculated by the computer for the data plotted in figure 7C (solid line). The lines show a high degree of correlation and suggest that ADCC reactivity and lymphocyte-related polyreactive antibodies decisively mediate immune suppressive activity of the serum of patients with AIDS.

Discussion

AIDS is an immunologic disease caused by a virus. A hallmark of the disease is a dramatic and initially exclusive decline of CD4 T cells. CD4 T cells play a central role in the regulation of immune functions. Their loss has been regarded as a central cause of the ensuing immune deficiency [28]. This assumption remains popular, despite the fact that experimental depletion of CD4 T cells in humans and animals produced no immune defects comparable to those seen in HIV infection. Extremely low levels of functional CD4 T cells were found to suffice in carrying out basic immune functions [29]. By contrast, T lymphocytes in subjects infected with HIV are compromised in their capacity to carry out immune functions [24, 28, 30]. This is true for CD4 T cells, which are infected by HIV, and for CD8 T cells, which are not infected by HIV [30]. The possibility has been raised that the deficiency is caused by accessory cell dys-
function, namely by their inability to generate appropriate costimulatory signals [23]. It has been shown that macrophages in HIV-infected donors lose the capacity to present specific recall antigens [31].

Controversy persists regarding the mechanism of CD4 T cell depletion. The virologic view is that HIV infects CD4 T cells and accounts for their direct destruction. However, immunologic studies have revealed that among the masses of dying T cells, only a small fraction is HIV-infected [32]. As an alternative explanation, it has been suggested that CD4 T cells are destroyed by indirect means. It has been shown that the CD4-reactive HIV envelope molecule gp120 forms gp120/IgG immune complexes that mediate the ADCC-based destruction of CD4 T cells [17, 23, 33, 34]. Treatment of human PBMC cultures with gp120/IgG causes the destruction of CD4 T cells [17, 23, 33, 34], and injection of such immune complexes in CD4hu-transgenic mice deletes T cells that exhibit the human marker [35].

Recent studies emphasize a critical role of HIV-infected macrophages in the pathogenesis of AIDS [17]. It has been known for some time that chimpanzees replicate T-tropic HIV variants readily but not M-tropic variants and that chimpanzees infected with HIV do not develop AIDS [36]. Humans with a genetic deficiency in replicating M-tropic HIV variants are also resistant to HIV disease [37]. HIV infection alters macrophage functions fundamentally. It inhibits macrophage costimulatory activities [17, 23, 24, 26] and enhances macrophage cytotoxic activities in the ADCC reaction [17, 23, 33, 34]. Two distinct macrophage subsets have been proposed, a costimulatory set that disappears after HIV infection and a cytotoxic subset that expands after HIV infection [17, 23, 27]. The biologic function of lymphocyte-reactive PSAs should be considered in the context of macrophage functions, namely in the context of costimulation and cytotoxicity. We have previously shown, and confirm here, that lymphocyte-reactive PSAs facilitate the deletion of CD4 and CD8 T cells by cytotoxic macrophages [17]. Reacting with T cell surface components, PSAs target T cells for ADCC-based destruction by macrophages or other cytotoxic cells with ADCC activity. Furthermore, our data show that PSAs generate a deficit of macrophage costimulatory activity.
Figure 7. Concentration of lymphocyte-reactive IgG in the serum of patients with AIDS correlates with its antibody-dependent cellular toxicity activity and immune suppressive action. A, human peripheral blood mononuclear cells incubated with different concentrations of IgG from human immunodeficiency virus–seronegative (Δ) or –seropositive (○) donors. The self-reactivity was measured in the flow cytometer by indirect immunofluorescence by using fluorescein isothiocyanate–conjugated anti-human IgG F(ab)2. B, T cell reactivity (anti-IgG fluorescence, abscissa) of the same IgG doses correlated with the effect on T cell survival (open symbols) and proliferation in response to CD3 monoclonal antibody (filled symbols). Regression lines were drawn by the computer. Unfractionated sera from 3 healthy individuals and from 12 patients with AIDS were tested in (C) for T cell reactivity (anti-IgG fluorescence, x axis) and for their effects on T cell survival (open symbols) and mitogen responsiveness (filled symbols). Regression lines were drawn by the computer, but the regression lines from (B) were transferred by hand (dashed lines) to examine the fit of the 2 lines.
that can be reversed when exogenous costimulatory factors are administered. Self-reactive PSAs may thus contribute to both the depletion of T cells and to the impaired immune response in HIV-infected subjects.

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References

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