Defective T Suppressor-Inducer Cell Function in Human Immune Deficiency Virus-Seropositive Hemophilia Patients

By Liesbeth J.M. Sjamsoedin-Visser, Cobi J. Heijnen, Ben J.M. Zegers, and Jan W. Stoop

In human immune deficiency virus (HIV)-seropositive hemophilia patients, a low number of CD4+ lymphocytes is found, as well as a low CD4+/CD8+ ratio. In previous studies, it has been shown that antigen-specific T-helper cell (CD4+) function was present and no excessive antigen-specific T-suppressor cell (CD8+) function could be demonstrated. In this report, we studied another activity of CD4+ cells, namely the capacity to induce T-suppressor cell activity. The results clearly show a selective dysfunction of CD4+ suppressor-inducer (Tsi) cell function. Since these HIV-seropositive hemophilia patients showed the presence of activated B cells in the peripheral circulation refractory to antigen-specific T-helper cell signals and secreting specific antibodies spontaneously, we raised the hypothesis that the activated B cells in the patients activate the Tsi cells in vivo. This constant activation leads to a functional exhaustion of the Tsi cell pool.

PATIENTS

Ten severe hemophilia A or B patients, who were otherwise in good health, were investigated. All but one patient were treated intensively (>50,000 U/yr) with various clotting products. In four of ten patients, peripheral blood B cells could not generate an antibody response in vitro to the T-cell-dependent antigen OA, as demonstrated by a negative PFC response. These four patients showed anti-HIV antibodies; one patient showed detectable amounts of HIV antigen in the serum. As patient controls we selected two hemophilia carriers, one untreated hemophilia patient, one patient with a β-thalassemia major receiving erythrocyte concentrates every 5 weeks, and two IgG subclass (IgG2 and IgG4) deficiency patients treated with γ-globulin on a regular basis. Ten normal donor controls were included in the study.

MATERIALS AND METHODS

All materials and methods concerning the isolation of peripheral blood mononuclear cells (PBMC) as well as the technique for the generation and enumeration of PFC have been described in detail elsewhere. The purity of the T-cell subpopulations was measured by flow cytometry using the monoclonal antibodies Leu4 (CD3), Leu3 (CD4), and Leu2 (CD8) obtained from Becton & Dickinson, Mountain View, Calif. In addition, the antibody Leu8 (Becton & Dickinson) was used to assess the cells operative in the T-suppressor cell circuit. Stained cells were analyzed on a FACS analyzer (Becton & Dickinson).

Isolation of CD4+ cells. T cells were isolated by rosetting PBMC with sheep erythrocytes pretreated with 2-aminoethylisothiouronium bromide hydrobromide. CD4+ cells were isolated from the T-cell preparations by removal of CD8+ cells by means of the method described by de Gast and Platts-Mills. Briefly, purified T cells were incubated with the monoclonal antibody FK18 (a gift of Dr. F. UydeHaag, National Institute of Health and Environmental Hygiene [RIVM], Bilthoven, The Netherlands), which recognizes the CD8 determinant. After 30 minutes of incubation at 37°C, the cells were rosetted with ox erythrocytes that had been precoated with goat antimouse IgG (Tago, Burlingame, Calif) in an appropriate dilution. The rosetted suspension was layered at ficoll-isopaque (6 - 1.077 g/mL; Pharmacia, Uppsala, Sweden) and centrifuged at 1,000 x g for 20 minutes. Cells present in the interphase of the gradient will be referred to as CD4+ cells. The suspensions of the
patients and the controls contained <2% CD8+ cells and 92% ± 6% CD4+ cells, as determined with the monoclonal antibody Leu 3 (Becton & Dickinson).

Assay for Tsi cell activity. Analysis of Tsi cell activity was performed essentially as described earlier. In brief, purified CD4+ cells were cultured in the presence of 100 μg OA/mL in culture medium consisting of RPMI 1640, glutamine (2 mmol/L), penicillin (100 μg/mL), streptomycin (100 μIU/mL), and 10% human AB serum. After five to six days of culture, the cells were washed and added in graded numbers (0.5 and 1 x 10^6 cells), usually in the fixed amount of 0.5 x 10^6 CD4+ cells to 5 x 10^6 fresh target PBMC derived from normal donors. These mixtures were cultured again for a period of six days at 37°C in culture medium in the presence of 3 μg OA/mL, which is the optimal dose of antigen to generate PFCs in this system. Value are expressed as percentage of suppression:

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\text{number of PFCs in cultures containing OA activated CD4+ cells} \times 100\% \text{ without CD4+ cells}
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RESULTS

The generation of Tsi cell activity. When purified CD4+ T cells are cultured for five to six days in the presence of a high dose of antigen, they acquire the capacity to induce in donor PBMC the maturation of T-suppressor-precursor cells (Tsp) into T-suppressor-effector cells (Tse). The lymphocyte subset responsible for the induction of Tse cells is functionally defined as Tsi cells. The phenotypes of the lymphocyte subsets operative in the T-suppressor cell circuit have been described earlier. Tsp and Tse cells appeared to be CD8+ TQ1+, and Tsi cells CD4+/TQ1+. In contrast, CD4+ cells operative in T-helper function are TQ1−. Analysis of PBMC of the patients and the controls by use of Leu 8 (which identifies the same glycoprotein as TQ1) showed no differences between the HIV+ and HIV− hemophilia patients, as compared with the controls: controls 50% ± 8% (n = 10); HIV+ patients, 49% ± 8% (n = 6); and HIV− patients, 45% ± 11% (n = 5).

In order to test the functional capacity of the Tsi cells of hemophilia patients (n = 10), we purified CD4+ cells from the peripheral blood T cells of the patients and cultured these cells for five days at 37°C in the presence of 100 μg OA/mL. Similarly, we tested the Tsi cell activity in the two control groups. After the priming period with 100 μg OA/mL, the CD4+ cells were washed extensively and added in various dilutions (usually 0.5 and 1 x 10^6 cells) to target cell cultures consisting of 5 x 10^6 PBMC from normal donors. When normal PBMC are cultured for six days in the presence of an optimal dose of the antigen (3 μg OA/mL), we can observe the generation of antigen-specific IgM producing plaque-forming B cells. In normal donors, the addition of 10% to 20% of primed CD4+ cells to a culture of PBMC and 3 μg OA/mL causes a decrease of the PFC response to baseline values (Fig 1). However, the OA-primed CD4+ cells of four of ten hemophilia patients are incapable of inducing suppression of the PFC response of the normal target cell culture (Fig 1). Moreover, it appeared that there was a correlation between the presence of anti-HIV antibodies in the serum of these hemophilia patients and the absence of Tsi cell function (Fig 1). HIV-seronegative patients and all control patients showed the presence of Tsi function, since target cell PFC responses were suppressed by OA-primed CD4+ cells. The difference in Tsi function between HIV-seropositive patients and those of normal donors and HIV-seronegative patients is statistically significant (P < .01; Student’s t-test).

DISCUSSION

This report shows that HIV-seropositive hemophilia patients have a defective Tsi cell function. In a recent report, we showed that HIV-seropositive hemophilia patients also have a defective B-cell function. This conclusion was based on the fact that PBMC of the HIV-seropositive patients, when cultured with the optimal dose of OA, are not capable of generating an antibody response to OA. In addition, it appeared that the defect was not located in the CD4+ T-helper cell subset, but was due to the B cells that appeared to be unresponsive to T-cell–derived helper signals. Moreover, the absence of a normal PFC response coincided with the presence of activated B cells in the blood secreting antibodies of various specificities spontaneously. Therefore, we proposed the hypothesis that the resting B-cell population was no longer present to be activated by specific T-helper cells and antigen, as a consequence a negative PFC response. The T-helper cell function, when tested separately on B cells derived from normal donors, appeared to be intact. The low PFC response was not due to the presence of excessive Tse cell function either, since the Tse cell function of these patients was shown to be somewhat below the control level. HIV is especially infectious for CD4+ cells, and a low number of CD4+ cells is found in HIV-seropositive hemophilia patients. The latter might suggest the presence of a defect of this cell fraction; therefore, we were interested in
the function of a separate subset of CD4+ cells, the Tsi cell that resides specifically in the Leu 8+ CD4+ T cell subset. As previously mentioned, Leu 8− CD4+ cell-mediated antigen-specific T-helper cell function was shown to be normal in HIV-seropositive hemophilia patients. The results clearly show that HIV-seropositive patients cannot generate Tsi cell activity after activation by OA. The lack of Tsi cell function might be the result of selective infection of this subset of CD4+ cells with HIV. Nicholson et al have indeed described that HIV infects a subset of CD4+ cells that react with the TQI monoclonal antibody (which is similar to Leu 8). However, we found no evidence that Leu 8− cells were decreased in the HIV-seropositive hemophilia patients. However, double staining experiments were not performed systematically.

The defective Tsi function of the HIV-seropositive hemophilia patients can also be explained in another way. Recently, de Kruyff et al showed in the mouse that activated B cells in the G1 phase of the cell cycle could activate Ly 1+ T cells. Furthermore, L'Age Stehr et al previously discussed the role of B-cell blasts in the induction of T-suppressor cell function. Since our HIV-seropositive hemophilia patients have activated B cells in the peripheral circulation, we would like to propose the hypothesis that the activated B cells in these patients activate the Tsi cells in vivo in the absence of antigen. The constant activation of the Tsi cell subset in vivo consequently would impede reactivation in vitro. Preliminary data obtained in our laboratory show that activated human B cells cultured with purified CD4+ cells can induce Tsi activity in the absence of antigen (Heijnen et al, manuscript in preparation). Our hypothesis is further supported by the results obtained with patients suffering from chronic lymphatic leukemia; in this group of patients, we could not demonstrate a Tsi-cell activity of CD4+ PBMC after appropriate stimulation and most of the leukemic B cells were shown to be in an activated stage. We previously found one HIV-seropositive patient with a normal PFC response and no activated B cells in the peripheral blood. This patient showed a normal Tsi function (data not shown), which again supports the idea that the presence of activated B cells is linked to a decreased Tsi infection.

In spite of the incapability to induce Tsi function in CD4+ cells of HIV-seropositive patients, the Tse cells appeared to be present in vivo. These cells can be demonstrated separately when T cells or isolated CD8+ cells are cultured with a high dose of OA (100 μg/mL). After four days of culture, an antigen-specific T-suppressor (Ts) (effector) cell factor can be detected in the supernatant. When T cells of HIV-seropositive patients were cultured in this way for the secretion of Ts cell factor, we could show that the T (CD8+) cells could produce the factor, although the production was somewhat lower compared with that of the control donors. The finding that Tse cells from the circulation of the HIV-seropositive patients can be stimulated to produce suppressor factor seems to contradict the absence of Tsi cell function. One should bear in mind, however, that in vivo generation of Tse cells, which require reactivation in vitro by antigen without a need for other cell types, may be effected without Tsi cell involvement. Palacios et al have shown that the Tsi cell can be bypassed by a direct polyclonal stimulation of Ts precursor cells, and hence Tse cells are generated. Since these patients are constantly treated with blood products and exposed to viral antigens, one may hypothesize this bypass effect to play a role.

In conclusion, we have found that peripheral blood B lymphocytes of HIV-seropositive hemophilia patients are in an activated state and show refractoriness to antigen-specific T-cell–derived signals. This finding is in agreement with those of others who showed hyperactivity of B cells of patients with AIDS and AIDS-related complex on the basis of activation by HIV in a polyclonal way. As a result, one would conclude that B cells of these patients may not participate in the immunoregulatory circuit. We would like to hypothesize that the selective absence of functional Tsi cells, as evidenced in the present study, is related to the presence of activated B cells, possibly since Tsi cells interact with activated B cells. Support for this assumption is delivered by studies in mice. Further studies are required to elucidate the significance of this novel immunoregulatory circuit in humans.

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