Helper and Suppressor T-Cell Function in HIV-Infected Hemophilia Patients

By Rolf Weimer, Thomas Schweighoffer, Klaus Schimpf, and Gerhard Opelz

T-lymphocyte helper and suppressor functions were assessed in 61 hemophilia patients. Twenty one patients (Group 1), 27 were HIV-positive without having AIDS-related complex (ARC)/AIDS (Group 2), and 13 had ARC/AIDS (Group 3). T, CD4-positive, or CD8-positive T lymphocytes were cocultured with B lymphocytes and pokeweed mitogen for 6 days and immunoglobulin producing cells were assessed in a reverse hemolytic plaque assay. In HIV-infected patients, T cells as well as the CD4-positive T cell subset exhibited reduced helper (P < .01, Group 2; P < .0005, Group 3) and elevated suppressor activity (P < .02, Group 2; P < .005, Group 3). However, no significant difference was found between HIV-negative patients and controls. The number of CD4-positive cells was not correlated with CD4 cell function. CD4-positive cells showed no helper activity (<10% of control T cells) in 8/11 (73%), but an excessive suppressor activity (>80% suppression of plaque formation) in 6/11 (55%) Group 3 patients. Our results show that defective helper and elevated suppressor functions of T cells in HIV-infected patients are caused not only by a change in the CD4/CD8 cell counts but also by functional abnormalities of the CD4-positive T-cell subset. These abnormal helper and suppressor functions may play a role in the development of the immunodeficiency state of AIDS patients.

In patients with AIDS or AIDS-related complex (ARC), the number of CD4+ cells in peripheral blood is diminished and the proliferative capacity of peripheral blood lymphocytes (PBL) is impaired. Further evidence for functional T-cell defects in HIV-infected patients is a reduced Interleukin-2 production of CD4+ cells after stimulation with soluble antigen and a reduced antigen-stimulated gamma interferon production. Preliminary data on a reduced helper function of T cells with respect to PWM-driven B cell differentiation were published by Middvan et al, who found defective helper activity in one AIDS patient, and by Benveniste et al, who described T-cell mediated suppression of plaque formation in 2 AIDS patients. A diminished helper activity of CD4+ cells was found by Benveniste et al in two AIDS patients, by Lane et al in two AIDS patients, and by Seki et al in four hemophilia patients. To our knowledge, reports on a suppressor function of CD4+ cells have not been published.

We studied the helper and suppressor activities of “whole” T cells, and of CD4+ or CD8+ T-cell subsets in 61 hemophilia patients.

MATERIALS AND METHODS

Patient and control groups. Blood samples of 60 patients with hemophilia A or B and one patient with von Willebrand’s disease were investigated. Forty-three patients (70%) were severely affected (factor VIII or IX activity <1%), 14 (23%) were moderately affected (factor VIII or IX activity 1% to 5%) and four (7%) were mildly affected (factor VIII or IX activity >5%). The patients were cared for at the Heidelberg Hemophilia Center. All patients were treated with lyophilized commercial factor VIII or IX concentrates of intermediate or high purity. Virus inactivated products were used since 1984. Twenty-one of the patients were HIV negative at the time of investigation (Group 1), 27 were HIV positive without having ARC/AIDS (CDC IV according to the classification of the Centers for Disease Control; Group 2), and 13 had ARC/AIDS (Group 3). Nine Group 3 patients and three Group 2 patients were treated with IgG 100-200 mg/kg intravenously every two weeks, and seven Group 3 patients were treated with zidovudine 4 x 200 mg/day orally, twenty-eight healthy blood donors served as controls.

HIV antibodies. IgG antibodies to HIV Type 1 and 2 were determined in the laboratory of Professor Gürtl (Max-Pettenkofer-Institute, Munich, FRG) with ELISA, Western blot and immunofluorescence techniques. Antibodies to HIV Type 2 were not detected in any patient.

Flow cytometric analysis. Lymphocyte subsets were determined by indirect immunofluorescence and laser flow-cytometry using the following monoclonal antibodies: OKT3 (pan T lymphocytes), OKT1 (E rosette-positive cells), OKT4 (helper/inducer subset), OKT8 (suppressor/cytotoxic subset), OKB7 (B cells), and OKIa-1 (B cells, monocytes, activated T cells). All monoclonal antibodies were purchased from Ortho Pharmaceuticals, Raritan, NJ. Ten microliters of monoclonal antibody were added to 100 μl of whole blood and incubated for 30 min at 4°C. Erythrocytes were lysed with NH4Cl solution. The cells were washed and incubated with 50 μl FITC conjugated goat-anti-mouse Ig (Medac, Hamburg, FRG) for 30 minutes at 4°C. An additional wash the cells were analyzed with an Ortho Spectrum III flow cytometer.

Cell separations. Cell separations were performed as described. Briefly, peripheral blood mononuclear cells were separated into “whole” T and non-T cells (term B cells in the following) by rosetting with sheep red blood cells (SRBC) pretreated with 2-aminoethylisothiouronium bromide hydrobromide (Sigma, St Louis). Monocytes or other adherent accessory cells were not especially depleted. Whole T cells were separated further into CD4 cell-depleted and CD8 cell-depleted subpopulations (termed CD8+ and CD4+ subsets) by treatment with OKT4 or OKT8 monoclonal antibodies, respectively, and rabbit complement (Pel Freez, Rogers, AR). Unlike Witherspoon et al and Lum et al, we did not irradiate the T cells before they were used in the helper assay in order not to abrogate helper or suppressor functions of radiosensitive CD4+ cell subsets; Thomas et al found that irradiation of CD4+ cells resulted in enhanced helper activity especially of preactivated CD4+ cells. Our B-cell-enriched populations contained 3% to
6% OKT11+ cells, 21% to 36% OKB7+ cells and 43% to 64% OKT1-1+ cells. T-cell-enriched populations were 90% to 95% OKT11+ and no more than 2% OKB7+. T cells treated with OKT8 monoclonal antibody and complement contained 72% to 82% OKT4+ cells and no more than 7% OKT8+ cells, T cells treated with OKT4 monoclonal antibody and complement were 69% to 77% OKT8+ and no more than 12% OKT4+.

Coculture conditions. Cultures were performed in duplicate in round-bottom microtiter plates (Greiner, Solingen, FRG) at 37°C in a 5% CO2 humidified atmosphere. Each microwell contained a final volume of 250 μl RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (Boehringer, Mannheim, FRG), 4 mmol/L glutamine, and penicillin-streptomycin. For assessment of T-cell or T-subset helper activity, 5 x 10⁶ B cells and 10⁵ T cells or T cell subsets were cocultured with or without PWM (GIBCO). To assess suppressor activity, patient T cells or T-cell subsets (10⁷ cells) were cocultured with control T cells (5 x 10⁶) and B cells (5 x 10⁵). Due to limited cell numbers not every assay could be performed in each patient. B cells (10⁷) and T cells (10⁵) alone were cultured with pokeweed mitogen (PWM) in order to control the quality of cell separation. Their PFC responses did not exceed those of unstimulated control T- or B-cell cultures, indicating a functionally sufficient separation.¹⁰¹⁷

Plaque assay. After a culture period of 6 days the cells were washed and plated in a reverse hemolytic plaque forming cell (PFC) assay using Protein A (Pharmacia, Uppsala, Sweden) coupled SRBC.²⁵³ Plaques were developed by incubation with rabbit anti-human IgG, IgA, IgM (Dakopatts, Hamburg, FRG) coupled to 1:32 dilution of normal CD4+ cell helper activity, and in 7 of 11 (64%) we found no evidence for helper activity at all (Fig 1). Four of 11 (36%) Group 2 patients with normal CD4+ cell counts (>500/μL, based on results of 51 healthy controls) and 2 of 13 (15%) with reduced CD4+ cell counts showed a defective CD4+ cell helper activity. Thus, the helper function of CD4+ cells may be defective in spite of normal CD4+ cell counts in the peripheral blood.

Suppressor activity of T cells and T-cell subsets. A significantly enhanced T-suppressor function compared to controls was found in patients of Groups 2 and 3 compared to controls (P < .01 and P < .0001). Only 3 of 11 (27%) Group 3 patients exhibited a normal CD4+ cell helper activity, in the T cells from Group 3 patients 80% (36%) Group 2 patients with normal CD4+ cell counts showed a suppressive activity. CD4+ cell helper activity. Thus, the helper function of CD4+ cells may be defective in spite of normal CD4+ cell counts in the peripheral blood.

Statistics. Data are expressed as mean ± SEM; chi-square test and Wilcoxon rank-sum test were used as two-tailed tests for statistical analysis.

Table 1. CD4/CD8 Values of HIV-Negative Hemophilia Patients (Group 1). HIV-Positive Hemophilia Patients Without ARC/AIDS (Group 2), and Hemophilia Patients With ARC/AIDS (Group 3)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>T Cells per μL</th>
<th>CD4/CD8 Ratio</th>
<th>CD4+ Cells per μL</th>
<th>CD8+ Cells per μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>21</td>
<td>1322 ± 107</td>
<td>1.40 ± .12</td>
<td>849 ± 75</td>
<td>640 ± 66</td>
</tr>
<tr>
<td>Group 2</td>
<td>27</td>
<td>1104 ± 119</td>
<td>.71 ± .07</td>
<td>524 ± 75</td>
<td>764 ± 89</td>
</tr>
<tr>
<td>Group 3</td>
<td>13</td>
<td>567 ± 116</td>
<td>.34 ± .06</td>
<td>131 ± 28</td>
<td>478 ± 100</td>
</tr>
</tbody>
</table>

P Values*<.001,.0001,.001 NS

Abbreviation: NS, not significant.

*Wilcoxon rank-sum test was used for statistical comparison.
†Mean ± SEM.
Suppression of plaque formation was mediated by CD4+ T cells in 8% (2/25) of controls, 19% (3/16) of Group 1 patients, 50% (12/24) of Group 2, and 73% (8/11) of Group 3 patients (Fig 2). Groups 2 and 3 showed a significantly stronger suppressor activity of CD4+ cells than controls ($P < .01$ and $P < .0001$, respectively). Group 3 patients exhibited a stronger suppression than Group 2 patients ($P < .02$). It is a striking finding that the CD4+ cells in 6 of 11 Group 3 patients suppressed B-cell responses by more than 80%.

Fig 1. Helper activity of T cells, CD4+ T cells, and CD8+ T cells in controls (C) and patient groups 1 through 3 (Gr 1, Gr 2, Gr 3). Median values are indicated with bars. Dots beneath the horizontal lines represent defective helper function.

Fig 2. Suppressor activity of T cells, CD4+ T cells, and CD8+ T cells in controls (C) and patient groups 1 through 3 (Gr 1, Gr 2, Gr 3). Median values are indicated with bars. Dots beneath the three horizontal lines represent weak, moderate, or strong suppression of plaque formation by T cells. Negative values represent stimulation of plaque formation.
There was no significant difference in CD4 + cell counts of patients with or without CD4 + cell suppressor activity (Group 2: 542 ± 118 (n = 12) vs 551 ± 121 (n = 12) CD4 + cells/μL; Group 3: 138 ± 39 (n = 8) vs 140 ± 7 (n = 3) CD4 + cells/μL). Thus, our results demonstrate that CD4 + cells act as suppressor cells in a substantial proportion of activity of CD4 + cells was associated with patients with or without CD4 + cell suppressor activity in parallel. Cells fit L). Thus, our results demonstrate that CD4 + CD4 + enhances suppressor activity of CD8 + cells was responsible to discern whether CD4 + cells mediating suppression or an be considered, however, that suppressor function of contami-
tation, whereas suppressor activity may occur independent of a condition, whereas suppressor activity may occur independent of a infected patients (11/12) with defective helper activity. However, CD4 + cell mediated suppression was also seen in 11 of 33 (33%) patients (Group 1: 3/14; Group 2: 6/16; Group 3: 2/3) and 2 of 24 (8%) controls in spite of existing helper activity. These findings suggest that defective helper activity of CD4 + cells is associated with suppressor function, whereas suppressor activity may occur independent of a normal helper function even in a minority of healthy controls.

A strong suppressor activity of CD8 + cells (>80% sup-
pression) was found in 33% (8/24) of controls and 71% (22/31) of HIV-positive patients (P < .01; Fig 2). It has to be considered, however, that suppressor function of contaminating CD4 + cells—especially in Group 3 patients—might at least in part account for this result. Thus, we were unable to discern whether CD4 + cells mediating suppression or an enhanced suppressor activity of CD8 + cells was responsible for the stronger suppressor activity in HIV-positive patients.

DISCUSSION

The decreased helper function of T lymphocytes in HIV-
injected patients has been attributed to a loss of CD4 + T cells. The striking finding in this study is that the remaining CD4 + T cells show a defective helper and even suppressor function in a substantial part of HIV-infected patients without ARC/AIDS and in the majority of ARC/AIDS patients. Considering the central role of CD4 + cells in the immune response, this dysfunction may play a role in the development of the immunodeficiency state of ARC/AIDS patients. A reduction in CD4 cell counts, reduced helper activity of CD4 + cells, preponderance of CD8 + suppressor cells, and suppressor function of CD4 + cells all may contribute to the defective helper activity of "whole" T cells in HIV-infected patients shown in Fig 1. Interestingly, these functional defects of T cells and the CD4 + subset resemble the findings observed in the early phase after bone marrow transplantation.16,17,20 It is important, furthermore, that functional defects of CD4 + cells occur independent of CD4 + cell counts. Suppressor activity of CD4 + cells was observed in most HIV-infected patients (11/12) with defective CD4 + helper activity, but also in 11 in 33 patients and even 2 of 24 controls with normal helper activity. Thomas et al showed with cells from healthy individuals that PWM-preactivated CD4 + cells suppressed B-cell differentiation, but were capable of providing T-helper function in the PWM-stimulated system.18-21 This is supported by our finding that suppressor function of CD4 + cells occurs "physiologically" in a minority of healthy controls. The enhanced suppressor activity of CD4 + cells in HIV-infected patients may be an effect of HIV itself or of other concurring viral infections such as Cytomegalovirus, Epstein-Barr, or Herpes simplex infections. A depletion of certain subsets of the CD4 + cell population in HIV-infected patients as a possible cause of functional dysregulation could so far not be established.25,26

ACKNOWLEDGMENT

The excellent technical assistance of Marita Heilke and Sabine Helm is gratefully acknowledged. We thank R. Gutbrod and R. Braun, Institute of Medical Virology, University of Heidelberg, for performance of the HIV antigen test.

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