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 were HIV-negative (Group 1), 27 were HIV-positive without having AIDS-related complex (ARC)/AIDS (Group 2), and 13 had ARC/AIDS (Group 3). 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), whereas no significant difference was found between HIV-negative patients and controls. The number of CD4-

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 × 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 Gurtler (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 was added to 100 µl of whole blood and incubated for 30 min at 4°C. Erythrocytes were lysed by treatment 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. After a further wash the cells were analyzed with an Ortho Spectrum III flow cytometer.

Cell separations. Cell separations were performed as described.16-18 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 al16 and Lum et al,17 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.19 20 Our B-cell-enriched populations contained 3% to
6% OKT11+ cells, 21% to 36% OKB7+ cells and 43% to 64% OKLa-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+ . 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 × 10^5 B cells and 10^5 T cells or T cell subsets were cocultured with or without PWM (GIBCO). To assess suppressor activity, patient T cells or T-cell subsets (10^5 cells) were cocultured with control T (5 × 10^5) and B cells (5 × 10^5). Due to limited cell numbers not every assay could be performed in each patient. B cells (10^5) and T cells (10^5) 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.

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. Plaques were developed by incubation with rabbit anti-human IgG, IgA, IgM (Dakopatts, Hamburg, FRG) in a 1:80 dilution and guinea pig complement (Virion, Würzburg, FRG) in a 1:32 dilution. T-cell functions of patients were calculated from the results (PFC/10^6 B cells) of the following cocultures:

T-cell or T-subset helper activity:

\[ [B(C) + T(P) + PWM] - [B(C) + T(P) + M] \]

T-cell or T-subset suppressor activity:

\[ [B(C) + T(C) + T(P) + PWM] - [B(C) + T(C) + PWM] \]

B(C), T(C) = B and T cells of a control; T(P) = Patient T cells, CD4+ or CD8+ T cell subsets, respectively; M = Culture medium. Values under 10% of helper function were defined as defective helper activity, values above 10% suppression as suppressor activity. Ten percent to forty percent suppression was defined as weak, 40% to 80% as moderate and greater than 80% as strong suppression.

HIV antigen test. HIV antigen was determined in culture supernatants of unstimulated and PWM-stimulated cultures using the HIV I Antigen EIA (Abbott Laboratories, Wiesbaden, FRG).

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>

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%.
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 HIV-positive patients without ARC/AIDS and in the majority of ARC/AIDS patients. Importantly, the suppressor function of CD4+ cells appears to be independent of the reduction in peripheral blood CD4+ cells caused by HIV infection.

In 24 controls and 45 hemophilia patients (14 of Group 1, 21 of Group 2, and 10 of Group 3), suppressor and helper activity of CD4+ cells was investigated in parallel. We found CD4+ cells to cause suppression in nearly all HIV-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% suppression) 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 CD4+ cells was responsible for the stronger suppressor activity in HIV-positive patients.

DISCUSSION

The decreased helper function of T lymphocytes in HIV-infected 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 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 of 33 patients and even 2 of 24 controls with normal helper activity. Thomas et al showed with cells from healthy individuals that PWM-primed 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 concurrent viral infections such as Cytomegalo, 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

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