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). 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 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-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.

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6% OKT11 + cells, 21% to 36% OKB7 + cells and 43% to 64% OKT8 + 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 × 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 (10⁵ cells) were cocultured with control T (5 × 10⁶) and B cells (5 × 10⁵). Due to limited cell numbers not every assay could be performed in each patient. B cells (10⁵) 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.¹⁶,¹⁷

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.²⁵,²⁶ 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/10⁶ B cells) of the following cocultures:

T-cell or T-subset helper activity:

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

T-cell or T-subset suppressor activity:

\[
\frac{B(C) + T(C) + T(P) + PWM}{B(C) + T(C) + PWM}
\]

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) |
|---|---|---|---|---|
| No. of Patients | T Cells per μL | CD4/CD8 Ratio | CD4+ Cells per μL | CD8+ Cells per μL |
| Group 1 | 21 | 1322 ± 107 | 1.40 ± .12 | 849 ± 75 | 640 ± 66 |
| Group 2 | 27 | 1104 ± 119 | .71 ± .07 | 524 ± 75 | 764 ± 89 |
| Group 3 | 13 | 567 ± 116 | .34 ± .06 | 131 ± 28 | 478 ± 100 |
| P Values | <.001 | <.0001 | <.001 | NS |

Abbreviation: NS, not significant.

*Wilcoxon rank-sum test was used for statistical comparison.

†Mean ± SEM.

RESULTS

The CD4/CD8 values of the three groups of hemophilia patients are listed in Table 1. In agreement with published results we found a profound decrease in the CD4/CD8 ratio and CD4+ cell counts, and no significant increase of CD8+ cell counts in HIV-infected patients.

Helper function of T cells and T-cell subsets. The helper function of T cells was examined in PWM-stimulated allogeneic cocultures with B cells from healthy controls. Figure 1 shows that a defective T-helper function was found in none of the controls (n = 27) or Group 1 patients (n = 15), but in 30% (7/23) of Group 2 patients and in 83% (10/12) of Group 3 patients. T-helper activity was significantly reduced in HIV-positive patients compared to controls (P < .005, Group 2 and P < .0005, Group 3), whereas HIV-negative patients and controls were not significantly different. We tested for an increase in HIV antigen concentration in the supernatants of PWM-stimulated compared to unstimulated cultures. No significant increase was found in the cultures of 36 HIV-positive patients, which makes it very unlikely that T-cell function was influenced by an in vitro propagation of virus.

When we examined the helper activity of CD4+ T cells, we found a significantly reduced activity in HIV-positive 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, 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 (P < .02) and 3 (P < .005). The differences were also statistically significant between Group 1 and Group 2 patients (P < .02) and between Group 1 and Group 3 patients (P < .005). T cells exerted a suppression of plaque formation (>10% suppression) in 11% (3/27) of controls, 28% (5/18) of Group 1 patients, 44% (11/25) of Group 2 patients, and 64% (7/11) of Group 3 patients (Fig 2). Quantitatively, the strongest suppressive activity of T cells was found in Group 3 patients: 1/25 (4%) Group 2 patients, but 4/11 (36%) Group 3 patients showed strong (>80%) suppression.
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.

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 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 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 have been missed 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-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 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. 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-activated CD4 + cells suppressed B-cell differentiation, but were capable of providing T-helper function in the PWM-stimulated system. 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.

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