Immunological Abnormalities in Human Immunodeficiency Virus (HIV)-infected Asymptomatic Homosexual Men

HIV Affects the Immune System before CD4+ T Helper Cell Depletion Occurs


*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam; †Department of Internal Medicine, University of Amsterdam; ‡Department of Infectious Diseases, Municipal Health Service; and †Department of Virology, University of Amsterdam, Amsterdam, The Netherlands

Abstract

To investigate the effect of persistent HIV infection on the immune system, we studied leukocyte functions in 14 asymptomatic homosexual men (CDC group II/III) who were at least two years seropositive, but who still had normal numbers of circulating CD4+ T cells. Compared with age-matched heterosexual men and HIV-negative homosexual men, the CD4+ and CD8+ T cells from seropositive men showed decreased proliferation to anti-CD3 monoclonal antibody and decreased CD4+ T-helper activity on PWM-driven differentiation of normal donor B cells. Monocytes of HIV-infected homosexual men showed decreased accessory function on normal T cell proliferation induced by CD3 monoclonal antibody. The most striking defect in leukocyte functional activities was observed in the B cells of HIV-infected men. B cells of 13 out of 14 seropositive men failed to produce Ig in response to PWM in the presence of adequate allogeneic T-helper activity. These findings suggest that HIV induces severe immunological abnormalities in T cells, B cells, and antigen-presenting cells early in infection before CD4+ T cell numbers start to decline. Impaired immunological function in subclinically HIV-infected patients may have clinical implications for vaccination strategies, in particular the use of live vaccines in groups with a high prevalence of HIV seropositivity.

Introduction

The etiological agent of AIDS is a retrovirus designated HIV (1–3). HIV is toxic for human cells that express the human leukocyte-differentiation antigen CD4 (T4) (4), including T-helper cells (4), monocytes and macrophages (5–6), follicular dendritic cells (7), and EBV-transformed B cell lines (8, 9).

In patients with AIDS and AIDS-related complex (ARC), a wide variety of cellular and humoral immunologic abnormalities have been reported (10). T cell activation by soluble antigen (11), T-helper activity, B cell differentiation (12, 13), monocyte accessory function (14), and specific and nonspecific cytotoxic activities (14) are included in the defective immune functions. Although the induction of B cell activation in vitro is severely decreased (12) and specific antibody responses to neo-antigens are weak (10, 12, 13), serum Ig levels are usually elevated in AIDS patients (10, 12, 13). It has been suggested that the lack of inducible B cell activation in vitro might be caused by hyperactivation in vivo of B cells by HIV, reflected in vitro in a relatively high spontaneous Ig production (15, 16).

These findings may have implications for understanding the pathogenesis of HIV infection. HIV-induced immunodeficiency can no longer be considered to be caused by a mere depletion of CD4+ T-helper cells.

The present study was aimed at elucidating the early effects of HIV infection on the immune system. Immunological studies were performed with leukocytes of asymptomatic, HIV-infected homosexual men who had normal numbers of circulating CD4+ T cells. In contrast to normal leukocyte functional activities in HIV-negative homosexual men, T cell, monocyte, and B cell functions were severely decreased in HIV-positive asymptomatic homosexuals. Our results imply that HIV affects the immune system in an early, preliminary stage of HIV infection before CD4+ T-helper cell numbers are beginning to decline.

Methods

Subjects. 14 anti-HIV seropositive homosexual men and 20 HIV-seronegative homosexual men were studied. Individuals were selected who had normal absolute CD4+ T cell numbers (normal range 0.4–1.5 × 10^9/liter) and were clinically asymptomatic, classified as group II or III of the CDC classification (17). Extensive physical examination and hematological analysis were performed at the same time that blood samples were taken. Heterosexual male controls were laboratory personnel in the same age range as the homosexual men. Table I shows the laboratory findings of the three groups studied.

Isolation of leukocytes and cell separations. Peripheral blood mononuclear cells (MNC) were isolated from heparinized blood by Percoll density-gradient centrifugation. T and non-T cells (B cells) were separated by E-rosette sedimentation using neuraminidase-treated sheep red blood cells. CD4+ and CD8+ T cells were separated by a negative “panning” technique on sheep anti-mouse Ig-coated petri dishes as described previously (18).

Lymphocyte subpopulation determination. Cell surface marker analysis was performed on an Epics-C fluorometer (Coulter Electronics, Inc., Hialeah, FL). T cell subsets were determined with CD4 and CD8 MAb. B cells were detected by CD19 MAb (B4), and monocytes were enumerated with MO2 and MO1 (CD11b) MAb in combination with fluorescein-conjugated goat anti-mouse IgG (C.L.B., Amsterdam, The Netherlands) diluted in 10% human and goat serum.
Table I. Clinical and Laboratory Findings in Anti-HIV-positive and -negative Homosexual Men and Heterosexual Male Controls

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Anti-HIV ab*</th>
<th>CDC group</th>
<th>Leukocytes</th>
<th>Lymphocytes</th>
<th>CD4 cells</th>
<th>CD8 cells</th>
<th>B cells</th>
<th>Serum Ig levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual men (n = 14)</td>
<td>+</td>
<td>II/III</td>
<td>×10⁶/μl</td>
<td>%</td>
<td>×10⁶/μl</td>
<td>×10⁶/μl</td>
<td>IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.2±</td>
<td>35.5</td>
<td>0.7</td>
<td>1.1</td>
<td>0.13</td>
<td>113-555</td>
</tr>
<tr>
<td>Homosexual men (n = 20)</td>
<td>−</td>
<td>−</td>
<td>5.8±</td>
<td>31.1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.20</td>
<td>102-196</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.2±</td>
<td>31.5</td>
<td>0.8</td>
<td>0.5</td>
<td>0.15</td>
<td>65-200</td>
</tr>
<tr>
<td>Heterosexual men (n = 49)</td>
<td>−</td>
<td>−</td>
<td>5.2±</td>
<td>31.5</td>
<td>0.8</td>
<td>0.5</td>
<td>0.15</td>
<td>40-225</td>
</tr>
</tbody>
</table>

* Serum antibodies to HIV were detected by commercial ELISA (Organon, Oss, The Netherlands) and confirmed by immunoblot techniques. All individuals were seropositive for at least two years at the time of study. † Mean values (±SD) per study group are shown. ‡ Ig levels normal range; IgG was elevated in eight and IgA in three HIV-infected men.

Results

Clinical and laboratory findings in asymptomatic HIV-infected men. The clinical and laboratory findings of the individuals studied, seropositive and seronegative homosexual men and heterosexual male controls, are summarized in Table I. Among the three groups, no significant differences existed for the parameters shown, except the anti–HIV antibodies in the seropositive homosexuals, elevated CD8⁺ cell numbers (P < 0.01) and elevated serum IgM levels in eight persons in the anti-HIV-positive group (P < 0.01), and elevated IgA levels in three persons (not significant) in this group.

Proliferative responses of T cells. This study was undertaken to evaluate T cell, B cell, and monocyte/accessory cell functions in HIV-infected healthy individuals. The capacity of T cells to proliferate in response to triggering via the complex of T cell receptor and CD3 molecule was tested in an anti-CD3-induced culture system. To exclude a possible defect in accessory function of monocytes required for optimal T cell activation, cultures were performed in the presence of an excess number of normal allogeneic monocytes. T cells of anti-HIV seropositive men had a significantly decreased proliferative response to anti-CD3 MAb CLB-T3/3 (Fig. 1). Background proliferation in the absence of anti-CD3 antibodies induced in these cocultures of allogeneic cells by alloreactivity were negligible (< 200 cpm). This indicates that the observed differences were solely caused by differences in anti-CD3 reactivity of T cells. Anti-CD3 MABs of the IgG2a subclass induce proliferative responses in the T cells of all subjects; thus, the observed difference could not be ascribed to FcR polymorphism of the monocytes of the tested persons (22). To exclude the possibility that differences in T cell proliferation were due to variable CD4/CD8 ratios in the patients, experiments were performed with CD4⁺- and CD8⁺-enriched normal T cells. In 12 experiments the mean proliferative response of CD8⁺ cells was 83% of the response of CD4⁺ cells in this culture system. This shows that CD8⁺ cells have a somewhat lower response to anti-CD3 antibodies, which can clearly not account for the decreased response observed in the HIV-infected men. Moreover, the proliferative capacity of enriched CD8⁺ and CD4⁺ cells was compared with unfractionated CD3⁺ cells in seropositive asymptomatic individuals. Fig. 2 shows that both enriched CD8⁺ and CD4⁺ cells of the HIV-infected men exhibited a functional defect similar to that demonstrated in...
unfractionated CD3+ T cells. These data indicate that both CD4+ and CD8+ cells have a decreased capacity to proliferate in response to anti-CD3 MAb, and rule out possible suppression of CD4+ cell proliferation by CD8+ cells.

Accessory function of monocytes. T cell proliferation to soluble antigen and to anti-CD3 MAb is dependent on accessory-cell functions of monocytes. Accessory function of monocytes of anti-HIV seropositive men was studied in anti-CD3 MAb-induced proliferation of monocyte-depleted normal T cells. As shown in Fig. 3, accessory functions provided by monocytes of HIV-infected homosexuals were significantly decreased (P < 0.01) compared with HIV-negative homosexuals and heterosexual male controls. This observed abnormality in monocyte function is not a quantitative effect, because monocyte numbers in the isolated MNC fraction were not significantly different for HIV-positive or HIV-negative homosexual men.

Spontaneous Ig synthesis. Since it has been published that B cells from AIDS patients show spontaneous Ig synthesis (12), we first tested whether this phenomenon existed in asymptomatic HIV-infected men. Table II shows that increased spontaneous Ig secretion was not found in asymptomatic HIV-positive men. In these experiments both cryopreserved and freshly obtained lymphocyte fractions were used. Slightly elevated spontaneous IgM production was observed for HIV-negative homosexuals as compared with heterosexuals (P < 0.01).

B cell differentiation induced by polyclonal activators. We next examined the capacity of B cells of HIV-positive homosexual men to secrete IgM and IgG in response to the polyclonal activator PWM. Fig. 4 shows that, while MNC of heterosexual men and HIV-negative homosexuals produced Ig, lymphocytes of 13 out of 14 HIV-seropositive men could not be induced by PWM to secrete Ig. The lack of Ig synthesis in response to PWM can be caused by a defect in T-helper-cell function, B cell function, or by excessive suppressor activity. This was further investigated by cocultivation experiments. Mononuclear cells of HIV-seropositive subjects were cocultivated with allogeneic normal CD4+ T-helper cells and stimulated with PWM. To exclude the possibility of excessive CD8+ suppressor-cell activity, MNC of a restricted number of HIV-positive persons, depleted for CD8+ T cells, were cultured with PWM. Neither addition of T-helper cells nor suppressor cell depletion resulted in Ig production by B cells of HIV-positive individuals (Fig. 4). A significant decrease was also noted in the B cell response in seronegative homosexuals, compared with heterosexual controls. Previously we reported (18, 21) that IL-2 plays a key role in the T-helper activity required for B cell differentiation in the PWM-driven system and this was later confirmed by other investigators (23). B cells from HIV-positive men also did not secrete Ig when cultured with normal CD4+ T cells in the presence of 30 U/ml rIL-2 and Staphylococcus aureus Cowan I (data not shown).

Thus, B cells of HIV-infected subjects have an intrinsic defect in their capacity to produce IgM and IgG in response to PWM or IL-2 in the presence of adequate allogeneic CD4+ helper cells.

CD4+ T helper activity on polyclonal B cell differentiation. Finally, we investigated the functional capacities of CD4+ T-helper cells in HIV-infected homosexual men. Helper T cell

Figure 2. Anti-CD3–induced proliferation of unfractionated (c), CD8+ (e), and CD4+–enriched (a) T cells of a normal HIV− control (A) and five HIV-infected asymptomatic homosexuals (B–F) in the presence of 150,000 irradiated MNC as accessory cells. Percentage of CD3+ cells in CD8+ (CD4-depleted) and CD4+ cell fractions ranged from 60 to 74%; residual CD4+ cells in the CD8+ cell fraction were < 5%. CD4+–enriched fractions obtained by positive “panning” were 50–60% CD4+ and 9–18% CD8+. Anti-CD3–induced proliferation in normal CD4+ cells and CD8+ cells was 98 ± 22% and 81 ± 7%, respectively, of proliferation by unseparated T cells (n = 12).
A similar T-helper-cell defect was observed in the presence of exogenous rIL-2, which indicates that the T-helper defect is not at the level of IL-2 production induced by PWM required for B cell differentiation (18, 19). Since both the HIV-negative and -positive groups were selected for normal absolute CD4 numbers, these findings suggest a qualitative helper defect in T-helper cells from HIV-infected asymptomatic men.

It could be argued that the observed B and T cell defects were due to inhibitory factors produced by monocytes from HIV-infected individuals that are present in the culture system used to evaluate T cell-dependent B cell differentiation. To address this issue, MNC from five HIV-infected individuals, depleted for CD8+ cells to avoid CD8+ T-suppressor activity, were cocultivated with normal unseparated MNC in the presence of PWM. Although B and T cell functions of these five

Table II. Lack of Spontaneous Ig Production by B Cells from HIV-infected Homosexuals

<table>
<thead>
<tr>
<th>Group</th>
<th>HIV</th>
<th>n</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterosexuals</td>
<td>–</td>
<td>17</td>
<td>19±36</td>
<td>51±25</td>
</tr>
<tr>
<td>Homosexuals</td>
<td>–</td>
<td>20</td>
<td>102±123</td>
<td>78±77</td>
</tr>
<tr>
<td>Homosexuals</td>
<td>+</td>
<td>10</td>
<td>9±5</td>
<td>64±45</td>
</tr>
</tbody>
</table>

80,000 MNC were cultured for 7 d without stimuli. IgM and IgG synthesis was determined and expressed as nanograms IgM/IgG per well.

Figure 3. Accessory function of monocytes on CD3-induced normal monocyte-depleted T cell proliferation. [3H]Thymidine incorporation on day 3 by T cells cultured with irradiated MNC from the three groups as monocyte source is shown (cpm). Background proliferation in the absence of added monocytes or in the absence of anti-CD3 MAb was < 200 cpm. Percentage monocytes in the purified MNC fractions of HIV-infected and HIV+ homosexual controls used as accessory-cell source was 11.9 (±7.7) and 12.5 (±6.4), respectively.

Figure 4. Ig synthesis induced by PWM in MNC of heterosexuals aHIV− (A), and homosexuals aHIV+ (B) and aHIV− (C). MNC (80,000/well) were cultured with 50 μg/ml PWM. MNC (80,000/well) of aHIV+ homosexuals (C) were cultured with allogeneic CD4+ helper cells (20,000/well) or depleted for CD8+ cells (< 5% CD8+). Ig in culture supernatants was measured on day 7 by ELISA. Significant differences exist between A and B, A and C, and B and C (P < 0.001). Ig synthesis in the absence of PWM was negligible. a shows IgM synthesis; b shows IgG synthesis.

Figure 5. T-helper activity on normal B cell differentiation. Helper activity provided by 500-rad-irradiated MNC (40,000) on Ig production by normal 40,000 non-T cells of a single donor was tested. Ig production in culture supernatants on day 7 was measured. Helper activity is expressed as percentage of the response of normal controls.
Table III. Helper Activity of CD4+ Cells of HIV-infected Homosexuals

<table>
<thead>
<tr>
<th>CD4+ cells*</th>
<th>T-helper activitya</th>
<th>No. of T cells added (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

Exp. 1

1 392 630 1,000
2 630 559 811
3 730 1,207 875
4 283 247 414
5 351 242 337
Control 1,263 2,203 1,300

Exp. 2

6 275 <8.5 276
7 757 565 2,142
8 <8.5 73 235
9 <8.5 <8.5 NT
10 132 495 NT
Control 2,622 3,853 3,491

* CD4+-enriched cells were prepared by depletion of MNC for CD8+ cells (residual CD8+ cells: < 5%; CD4+ cells: 70–80%).
† CD4+ cells were added in graded numbers to 40,000 normal non-T cells in the presence of 50 ng/ml PWM. After 7 d of culture IgM (nanograms per well) was determined.

Table IV. Effect of Monocytes from HIV-infected Individuals on Normal T Cell-dependent B Cell Differentiation

<table>
<thead>
<tr>
<th>Normal MNC</th>
<th>Individual</th>
<th>CD8+-depleted MNC added*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM ng/well</td>
<td>20,000</td>
<td>40,000</td>
</tr>
</tbody>
</table>

6096 (±164) 1 478 268
2 691 786
3 839 686
4 1,311 1,195
5 570 924
Control 2,647 1,663

* Graded numbers of CD8+-depleted MNC from HIV-infected individuals were added to 40,000 unseparated normal MNC.
† IgM production (nanograms per well) after 7 d culture with 50 ng/ml PWM by 40,000 unseparated MNC of a normal donor.
‡ HIV-negative heterosexual laboratory control.

Discussion

Here we demonstrate immunological abnormalities, including T cell, B cell, and accessory cell functions, in healthy homosexual men infected with HIV. Although the men had been anti-HIV antibody-positive for at least two years, circulating CD4+ T cell numbers were normal. The most striking functional defect was observed in the B cell compartment. B cells of HIV-seropositive homosexual men failed to produce IgM and IgG in response to PWM or rIL-2. It is believed that in normal individuals, large, in vivo preactivated B cells are the responding cells to PWM (24, 25). The B cell defects and a slightly elevated spontaneous IgM synthesis observed in the HIV-negative homosexuals may be caused by immunosuppression due to lifestyle and sexual practices, or by immune activation as a result of more frequent viral infections (cytomegalovirus, EBV, etc. 26). The failure of the B cells from HIV-positive men to respond to PWM may be explained by a persistent stimulation in vivo by HIV, which hyperactivates B cells beyond the stage of susceptibility to PWM and immunoregulatory signals of T-helper cells.

Evidence for a direct or indirect stimulating effect of HIV on B cells has been obtained in vitro (15, 16), and similar B cell abnormalities have been reported in AIDS (12, 13) and lymphadenopathy patients (27). The lack of inducible B cell differentiation capacity in vitro was reflected in a decreased antibody response to primary immunization in vivo with keyhole limpet hemocyanin in the HIV-seropositive homosexual men (27a). However, a reduced response to immunization with keyhole limpet hemocyanin was also noted in the seronegative homosexual compared with the heterosexual men. Elevated spontaneous Ig production has been reported for B cells of AIDS patients (12). These authors used a reverse hemolytic plaque-forming cell assay. By measuring 7 d cumulative Ig synthesis in culture supernatants, Nicholson et al. (27) could not detect spontaneous Ig synthesis by B cells from lymphadenopathy patients. However, evidence for elevated spontaneous Ig synthesis after 1–2 d of culture was obtained (27). Others only showed marginally elevated spontaneous Ig synthesis by B cells of asymptomatic HIV-infected men after a 10-d culture period (28).

These results are in agreement with our findings in asymptomatic HIV-infected men. In 7-d cultures, elevated spontaneous Ig synthesis was not observed with B cells of asymptomatic individuals. However, when Ig-secreting B cells were enumerated in a spot-ELISA (29), AIDS patients showed significantly elevated numbers of spontaneous Ig-secreting B cells, but asymptomatic persons did not (data not shown). This indicates that detection of spontaneous Ig secretion is optimal in short-term cultures (27) and in plaque-forming cell assays enumerating individual B cells as shown previously by Lane et al. (12).

Both CD4+ and CD8+ T cell proliferation induced by anti-CD3 MAb was decreased in the seropositive homosexual men compared with seronegative homosexual and heterosexual controls. In this functional test system, T cells are specifically triggered via the membrane structure that comprises the T cell receptor for antigen and the CD3 molecule, which are intimately and functionally linked (30). Lane et al. reported that the capacity to respond to antigen-specific triggering via TCR/CD3 is decreased in T cells from AIDS/ARC patients (11). Here we demonstrate a similar defect in T cells from HIV-infected asymptomatic men.

T cell functional defects are also reflected in the severely decreased capacity to provide T-helper activity for B cell differentiation, observed in CD4+ cells from HIV-infected men. Helper activity is confined to the CD4+ T cell population and, although normal numbers of CD4+ T cells were present, we showed in addition that neither deletion of CD8+ suppressor
cells nor addition of IL-2 reversed the CD4+ T-helper cell defect. Apart from the quantitative depletion of CD4+ T cells observed in AIDS and ARC patients, qualitative CD4 functional defects have been demonstrated in these later stages of HIV-induced disease (10–12).

Thus, we provide evidence here that similar qualitative T cell defects already exist in asymptomatic HIV-infected men before CD4+-cell depletion is detectable.

Accessory cell defects in monocytes from seropositive homosexual men were observed in this study in a culture system identical to that used by Prince et al. (31) to demonstrate monocyte functional defects in AIDS and ARC patients. It has been shown that next to CD4+ T cells, monocytes, macrophages, and follicular dendritic cells can be infected with HIV (5–7) and may be important in the dissemination of HIV in the infected host (32–34). Recently, we showed that accessory function provided by monocytes in this assay system is diminished after infection with HIV (35). This HIV-induced defect was not at the level of Fc receptor expression but at the level of secondary signals, such as IL-1 release, required for CD3-induced T cell proliferation.

This study excluded the fact that defects in T and B cell functions of HIV-infected men were due to inhibitory factors produced by monocytes of HIV-infected men. Moreover, in a longitudinal study on leukocyte functions of seroconverted men, we observed B and T cell defects before accessory functions of monocytes were decreased, indicating that these defects are independent (Terpstra, F. G., B. J. M. Al, M. Roos, F. de Wolf, J. Goudsmid, P. H. Schellekens, and F. Miedema, manuscript submitted for publication).

In blood and lymph nodes of infected individuals, a very low proportion (0.001–0.01%) of the leukocytes was found to contain viral RNA (36). Since we found that few leukocytes are infected with HIV, a direct effect of HIV infection on functional properties of T-helper cells and monocyte cells is hard to envisage. Indirect effects such as those described for B cells (15, 16) have not yet been documented for T cells, but cannot be ruled out at this time. Direct effects of HIV-encoded proteins on T-cell proliferation have been reported using physiologically high amounts of protein (> 5 μg/ml) (37, 38). Lower concentrations of gp120 affected PHA-induced, but not PWM-, antigen- or alloantigen-induced T cell responses (39). Functional defects due to a preactivated state of monocytes in AIDS patients have been reported. This activated state leads to the paradoxical situation that the seemingly functionally competent monocyte cannot respond to activating stimuli (40).

These results suggest that HIV infection induces immunological defects not only by depletion of CD4+ cells, but that HIV affects the immune system in an early stage of infection, by interference with virtually all leukocyte functions crucial to cellular and humoral immunity. The precise mechanisms, cellular activation, or general effect on hemopoiesis (41) by which HIV infection induces leukocyte functional defects remains to be elucidated.

Our observations on impaired immunological function in apparently healthy HIV-infected individuals have far-reaching clinical implications. It has been reported that subclinically HIV-infected persons show impaired responsiveness to an otherwise efficacious hepatitis-B vaccine (42). Of special concern is the use of live vaccines in HIV seropositives. Redfield et al. (43) recently described a healthy HIV-seropositive man who developed disseminated vaccinia and AIDS after a primary smallpox vaccination.

Acknowledgments

This study was supported by the Netherlands Foundation for Preventive Medicine grants 28-1079 and 28-1026. We are indebted to Dr. W. Schaaaberg for performing statistical analyses.

F. Miedema is a senior fellow of the Royal Dutch Academy of Arts and Sciences.

References


