Inhibition of Normal B-Cell Function by Human Immunodeficiency Virus Envelope Glycoprotein, gp120

By Narendra Chirmule, Naoki Oyaizu, V.S. Kalyanaraman, and Savita Pahwa

Despite the occurrence of hypergammaglobulinemia in human immunodeficiency virus (HIV) infection, specific antibody production and in vitro B-cell differentiation responses are frequently impaired. In this study, we have examined the effects of HIV envelope glycoprotein gp120 on T-helper cell function for B cells. In the culture system used, B-cell functional responses were dependent on T-B-cell contact, since separation of T and B cells in double chambers by Transwell membranes rendered the B cells unresponsive in assays of antigen-induced B-cell proliferation and differentiation. Cytokines secreted by T cells were also essential, since anti-CD3 monoclonal antibody (mAb)-activated, paraformaldehyde-fixed T-cell clones failed to induce B-cell proliferation and differentiation. Pretreatment of the CD4+ antigen-specific T cells with gp120 was found to impair their ability to help autologous B cells, as determined by B-cell proliferation, polyclonal IgG secretion, and antigen-specific IgG secretion. The gp120-induced inhibition was specific in that it was blocked by soluble CD4. Furthermore, only fractionated small B cells (which are T-cell-dependent in their function) manifested impaired responses when cultured with gp120-treated T cells. Antigen-induced interleukin (IL)-2 and IL-4, but not IL-6, secretion were markedly reduced in gp120-treated T-cell clones. Addition of exogenous cytokines failed to compensate for defective helper function of gp120-treated T cells. The findings in this study indicate that gp120 impairs helper functions of CD4+ T cells by interfering with T-B-cell contact-dependent interaction; the inhibitory effects of soluble envelope proteins of HIV may contribute to the immunopathogenesis of the HIV-associated disease manifestations.

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MATERIALS AND METHODS

Envelope glycoproteins. Envelope glycoprotein of HIV-1, gp120, was purified from supernatants of HIV-1(AD)-infected clone of Hut 78 cells as described previously.18 The gp120 was greater than 98% pure and was stored at −80°C.

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pure as detected by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting. A control protein, mannosylated-bovine serum albumin (m-BSA), was made by diazotation using p-amino-a-D-mannopyranoside (Sigma, St Louis, MO) as described previously. The presence of mannose was confirmed by double-diffusion precipitation in 1% agarose using concanavalin A.

Isolation of lymphocytes. Purified B cells were obtained from peripheral blood lymphocytes (PBL) of healthy volunteers as described earlier. Briefly, PBL were isolated by Ficoll-Hypaque density gradient centrifugation. PBL were depleted of monocyte/macrophages by adherence to plastic Petri dishes for 45 minutes at 37°C. Purified B cells were isolated from the nonadherent fraction by double-rosetting with neuraminidase treated sheep red blood cells (n-SRBC) to remove T cells; the rosetting and nonrosetting fractions were separated on Ficoll-Hypaque density gradients. The contaminating T cells in the nonrosetting fraction in the interface were lysed by anti-CD2 monoclonal antibody (MoAb) (7E4) and newborn rabbit serum complement treatment. As analyzed by flow cytometry, using anti-CD19 and anti-CD3 MoAb (B1 and T3, Coulter Immunology, Hialeah, FL) for B and T cells, the resulting B cells were greater than 97% pure with less than 1% contaminating T cells. There were less than 2% monocytes/macrophages as determined by myeloperoxidase staining.

Density gradient separation of B cells. Purified B cells were further separated by centrifugation on discontinuous Percoll density gradients according to the method of Suzuki et al. Briefly, 1 x 10^7 B cells, suspended in 2.5 mL of 30% Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) were layered onto the gradients ranging from 45% to 60% Percoll in 5 increments of 2.5 mL in 15-mL centrifuge tubes. The gradients were centrifuged at 15,000 g for 15 minutes at 4°C. Cells in the interfaces were removed with a pasteur pipette. The layer between 45% and 50% Percoll consists of high-density fraction (small B cells); between 50% and 55%, intermediate-density fraction; and between 55 and 60%, low-density fraction (large B cells).

Antigen-specific T-cell clones. For the source of T-helper cells, tetanus antigen-specific T-cell clones, Tt 1.1, Tt 1.3, and Tt 4.2 were generated as described previously by intermitting antigenic stimulation and rest periods, in the absence of exogenous IL-2. These clones were of the CD4^+CD8^-CD45RO^+ phenotype as determined by staining with MoAbs T4, T8 (Coulter Immunology), and UCLH-1 (Dako, Carpinteria, CA), and secreted multiple cytokines (IL-2, IL-4, and IL-6) after stimulation with appropriate antigen and APC.

Model culture system. Antigen-specific T cells from the T-cell clones were irradiated and cultured with autologous unfractionated or Percoll density gradient-separated B cells in the presence of tetanus antigen (2 μg/mL). In this culture system, the autologous B cells served as APC, as well as responder cells for assessment of B-cell function. All cultures were performed in media consisting of RPMI 1640 (Whittaker, Walkersville, MD), supplemented with penicillin, streptomycin, 1-glutamine, and 10% fetal calf serum (FCS; Gibco, Grand Island, NY) in 96-well, round-bottomed plates (Sumilon, Sumitomo, Osaka, Japan). Proliferation of the B cells was analyzed by ^3H-thymidine incorporation, 3 days after antigenic stimulation in the presence of irradiated (3,000 rad) T cells. B-cell differentiation was analyzed on day 7 by measuring polyclonal IgG in the supernatants by enzyme-linked immunosorbent assay (ELISA) with anti-human IgG-coated plates as described previously. The amount of IgG secreted was calculated by the Softmax ELISA program (Molecular Devices, Menlo Park, CA). Tetanus antigen-specific IgG secretion was also measured by ELISA as described previously in some experiments with T-cell clone Tt 2.1 and autologous B cells.

To determine the molecules involved in T-B-cell contact-dependent interactions, T or B cells were pretreated with the following MoAbs at 4°C for 16 hours: OKT4A and OKT4 (R.W. Johnson Pharmaceuticals, Raritan, NJ); LFA-1α (TSI/22), LFA-1β (TSI/18), and ICAM-1 (RR1/1) (from Dr T. Springer); and anti-HLA-DR mAb (Becton Dickenson, Mountainview, CA). The cells were then washed, and cocultured in various experimental protocols as specified (see Results).

To determine the effect of gp120 on T-helper cell functions, T cells were adjusted to a concentration of 1 x 10^6/mL and cultured in the presence of medium or various concentrations of m-BSA or gp120 (range, 0.001 to 1 μg/mL) overnight at 4°C. Cells were washed, irradiated, and cultured with tetanus antigen and autologous B cells and evaluated for B-cell function. The specificity of the gp120-induced effects was determined by the ability of soluble CD4 (sCD4, American Biotechnologies, Cambridge, MA) to block the gp120 effects. For this purpose, 1 μg/mL gp120 was premixed with various concentrations of sCD4 (range, 0.01 to 10 μg/mL) before overnight preincubation with T cells at 4°C. Cells were washed, irradiated, and cultured with autologous B cells and tetanus antigen as before. In some experiments, gp120-treated T cells were compared with cyclosporine A (CsA)-treated T cells. For these studies, T cells were pretreated with CsA (1 μg/mL, Sigma), washed, irradiated, and cultured with B cells and tetanus antigen.

Fixation of T-cell clones with paraformaldehyde. To prevent active cytokine secretion, T cells were stimulated for 24 hours with immobilized anti-CD3 mAb (4 μg/mL, Leu-4) and fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS) as described previously. Fixation was stopped by addition of cold 0.6% glycol-glycine and washing. Leaching of any possible remaining paraformaldehyde was accomplished by incubation for 60 minutes at 37°C, after which the cells were washed with PBS and resuspended in fresh culture medium. Fixed-activated T cells were cultured with autologous B cells in the absence or presence of various combinations of recombinant cytokines IL-2 (100 U/mL), IL-4 (100 U/mL), and IL-6 (1,000 U/mL).

Transwell experiments. To analyze the effect of T-B-cell contact for B-cell functions, cultures were performed in double chambers separated from each other by a 0.4-μm polycarbonate membrane (Transwell plates, Costar, Cambridge, MA). As T-cell clones cultured in the absence of a source of APC have very poor survival, Epstein-Barr virus (EBV)-transformed autologous B cells were used in these experiments to serve as APC. T cells plus APC were placed in the upper chamber to separate them from the B cells, which were in the lower chamber. In other experiments, B cells were cultured alone in the lower chamber or together with irradiated T cells plus APC. Antigen-induced B-cell proliferation and differentiation of B cells were analyzed as described above.

Cytokine assays. Culture supernatants of medium/gp120/m-BSA-treated T cells cultured with APC and antigen were examined for cytokines IL-2, IL-4, and IL-6 by ELISA. Briefly, 2 x 10^6 cloned T cells were treated with medium or various concentrations of gp120 or m-BSA ranging from 0.001 to 1 μg/mL and cultured with tetanus antigen in the presence of 2 x 10^6 irradiated EBV-transformed autologous B cells for 24 hours. Supernatants collected were assessed for the presence of cytokines IL-2, IL-4, and IL-6 by ELISA (Intertest-2, Intertest-4, and Intertest-6, Genzyme) as described by the manufacturer’s protocols. Cytokine amounts were expressed as U/mL for IL-2, and ng/mL for IL-4 and IL-6.

RESULTS
Characterization of the cellular interactions required for functional B-cell responses in the model culture system. As
shown in Fig 1, when irradiated CD4+ T cells were cultured with autologous B cells in presence of tetanus antigen, B-cell proliferation was observed on day 3, and maximal polyclonal IgG secretion and tetanus antigen-specific IgG secretion was noted on day 7. Other clones from different individuals (Tt 1.3 and 4.2), which could help autologous B cells to secrete polyclonal IgG, failed to induce tetanus antibody secretion by these same B cells, presumably due to low frequency of antigen-specific B cells in these individuals.24 When irradiated T-cell clones were cocultured with allogeneic B cells, then, as expected based on MHC restriction requirements,25 B-cell proliferation and differentiation could not be induced by specific antigen (data not shown).

The induction of T-cell–dependent B-cell responses is a complex process requiring direct T-B-cell contact and soluble cytokines.26,27 To determine the requirement for contact-dependent T-B–cell interaction in tetanus antigen-specific B-cell responses in this system, experiments were performed in Transwell plates using B cells and irradiated tetanus-specific T-cell clones and APC. Table 1 shows that B cells failed to proliferate or differentiate in response to tetanus antigen, when T cells were separated from the B cells by the Transwell membrane. In wells containing T plus B cells, the B cells proliferated vigorously and secreted IgG in response to tetanus antigen. Addition of supernatants of activated T-cell clones failed to induce proliferation and differentiation of purified B cells (data not shown). Thus, we established in our culture system that T–B-cell contact was essential, and that soluble factors secreted by T cells were insufficient to support B-cell proliferation or differentiation.

We examined the requirement for certain cell surface molecules believed to be essential for T–B-cell contact-dependent interaction, chief among them being CD4–MHC class II interaction, which is known to play a major role.5,37 Several adhesion molecules have also been implicated on this interaction. Table 2 shows that T-cell clones treated with mAbs OKT4A (but not OKT4), LFA-1α, and LFA-1β were impaired in their ability to support T-cell–dependent B-cell proliferation and differentiation. B cells treated with anti–HLA-DR mAb or anti–ICAM-1 mAb failed to undergo antigen-induced proliferation and differentiation.

Next, we examined whether cytokines secreted by T cells were also required for optimal B cell function. Figure 2 shows that fixation of anti-CD3–activated T-cell clones with paraformaldehyde, abrogated B-cell proliferation and differentiation. Addition of combinations of exogenous IL-2, IL-4, and IL-6 restored B-cell functions. IL-2 and IL-4 alone could induce partial B-cell proliferation without differentiation, while IL-6 alone induced some proliferation and IgG secretion in the presence of fixed, activated T cells. Supernatants of activated T-cell clones also restored B-cell functions in these cultures. The above experiments demonstrate that the T–B-cell contact interaction involves cell-cell contact and secretion of cytokines by activated T cell clones for induction of antigen-induced B-cell proliferation and differentiation.

Table 1. Requirement of T–B–Cell Cognate Interaction for T-Cell–Dependent B-Cell Function

<table>
<thead>
<tr>
<th>Transwell Chamber</th>
<th>Tetanus Antigen</th>
<th>Proliferation TdR (cpm)</th>
<th>Differentiation IgG (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>Lower</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>B</td>
<td>–</td>
<td>36 ± 9</td>
</tr>
<tr>
<td>None</td>
<td>B</td>
<td>+</td>
<td>108 ± 12</td>
</tr>
<tr>
<td>T [+APC]</td>
<td>B</td>
<td>+</td>
<td>112 ± 34</td>
</tr>
<tr>
<td>None</td>
<td>T + B [+APC]</td>
<td>–</td>
<td>116 ± 21</td>
</tr>
<tr>
<td>None</td>
<td>T + B [+APC]</td>
<td>+</td>
<td>8,527 ± 164</td>
</tr>
</tbody>
</table>

Irradiated T-cell clones (1 × 10⁶) and APC (EBV-transformed B cells, 1 × 10⁶) were cultured with autologous B cells (1 × 10⁶) in upper/lower chambers of Transwell 24-well plates (membrane pore size, 0.4 μm) in the absence (–) or presence (+) of tetanus antigen. The results are a representative of three separate experiments.

Fig 1. Kinetics of B-cell proliferation, polyclonal and tetanus IgG secretion. Tetanus antigen-specific cloned T cells were cultured with autologous B cells in the presence of tetanus antigen for various time intervals (days). Proliferation was measured by ³H-thymidine (³H TdR) incorporation and differentiation by determination of polyclonal IgG secretion by ELISA. The data for tetanus-specific IgG are denoted as optical density at wavelength 492 nm.
viability of T cells was 97% at 16 hours after gp120.

The gp120 effect was dose-dependent, with a maximum effect occurring at a dose of 1 pg/mL gp120. washed, and cultured in the presence of specific antigen. Proliferative responses of the T-helper cells (clone Tt 2.1) were pretreated with gp120. As controls, T cells were precultured with medium or Gp120 was not toxic at the concentrations used, and all B-cell functions tested). A similar inhibitory pattern was secretion by ELISA. Numbers in parentheses denote percent inhibition.

1 x 10⁴ irradiated T-cell clones (Tt 4.2 or Tt 2.1) or 1 x 10⁴ autologous B cells (B-400) or (B-200) were treated with anti-CD4 or anti–HLA-DR mAbs, washed, and cultured in the presence of specific antigen. Proliferative responses of the B cells were analyzed by ¹⁴C-thymidine incorporation and IgG secretion by ELISA. Numbers in parentheses denote percent inhibition. Abbreviation: ND, not done.

*p < .001 by Student’s t test analysis.

secretion (Fig 3) of B lymphocytes were impaired when the T-helper cells (clone Tt 2.1) were pretreated with gp120. The gp120 effect was dose-dependent, with a maximum effect occurring at a dose of 1 µg/mL gp120 (P < .001 for all B-cell functions tested). A similar inhibitory pattern was observed with two other helper T-cell clones (Tt 1.3 and Tt 4.2) obtained from different individuals (data not shown). Gp120 was not toxic at the concentrations used, and viability of T cells was 97% at 16 hours after gp120 treatment, as determined by trypan blue dye exclusion test. As controls, T cells were preincubated with medium or m-BSA and could provide adequate help for tetanus antigen-induced B-cell function. The specificity of the gp120-induced effects was determined by pretreatment of gp120 with soluble CD4. Figure 4 shows that if gp120 was premixed with soluble CD4, its ability to impair the helper function of Tt 2.1 cells was no longer evident. At 1 µg/mL of sCD4, the inhibitory effect of gp120 (1 µg/mL) was almost completely abrogated (<5% inhibition). Soluble CD4 by itself did not induce any significant B-cell proliferation and differentiation (data not shown).

To rule out the possibility that gp120 was not directly inhibitory for B cells in this culture system, B cells were treated with gp120 as for T cells by overnight incubation followed by washing. When cultured with untreated T cells, these B cells were not inhibited in their ability to proliferate or differentiate in response to T-cell–independent stimuli, eg, Staphylococcus aureus Cowan strain (SAC) + IL-2 or EBV or to T-cell–dependent stimuli. Furthermore, gp120-treated T cells did not impair the ability of autologous B cells to proliferate in response to EBV, a T-cell–dependent B-cell stimulus (data not shown).

gp120 inhibits T-cell-dependent B-cell function by interfering with T-B-cell contact-dependent interaction and inhibition of cytokine secretion by T cells. Figure 5 shows the secretion of IL-2, IL-4, and IL-6 by T cells in the presence of APC.
HIV-gp120 INHIBITS B-CELL FUNCTION

Fig 3. gp120-treated T cells inhibit antigen-induced proliferation, polyclonal IgG, and tetanus antigen-specific IgG secretion by autologous B cells. Tetanus antigen-specific cloned T cells were pretreated with various concentrations of mBSA or gp120 overnight at 4°C, washed, and irradiated. Test T cells, $1 \times 10^5$, were cultured with $1 \times 10^6$ autologous B cells in the presence of tetanus antigen. Proliferation was measured by $^{3}H$-thymidine incorporation and differentiation by determination of polyclonal IgG secretion by ELISA. The data for tetanus-specific IgG is denoted as optical density at wavelength 492 nm.

and tetanus antigen. The source of the secreted cytokines was attributed predominantly to the T cells, rather than to APC, since their levels were unaffected if the antigen-pulsed APC were paraformaldehyde-fixed (data not shown).

Fig 4. Soluble CD4 (sCD4) abrogates inhibitory effect of gp120. Various concentrations of soluble CD4 were premixed with 1 µg/mL gp120, for 2 hours at 4°C. T-cell clones, $1 \times 10^5$, treated overnight with medium or sCD4-treated gp120 were added to cultures of $1 \times 10^6$ autologous B cells and tetanus antigen. Similar results were obtained for B-cell proliferation.

Fig 5. Gp120 treatment of the T-cell clone inhibits secretion of cytokines, IL-2 and IL-4, but not of IL-6. Cloned T cells, $2 \times 10^5$, were pretreated with medium or various concentrations of gp120 overnight at 4°C, washed, and cultured with $2 \times 10^6$ APC (EBV-transformed autologous B cells) and tetanus antigen for 24 hours. Cytokines IL-2, IL-4, and IL-6 were quantified in the culture supernatants by ELISA.

Preincubation of T cells with various concentrations of gp120 resulted in reduced amounts of tetanus antigen-induced secretion of IL-2 and IL-4, but the IL-6 secretion was unaffected. (We have recently reported the observation that in the absence of antigen, gp120 itself induces IL-6 secretion in helper T-cell clones.3) To examine the ability of exogenous cytokines to restore B-cell functions, various
concentrations of recombinant IL-2, IL-4, and IL-6 were added singly and in combination to cultures containing gp120-treated T cells, B cells, and tetanus antigen. Addition of these exogenous cytokines failed to restore T-cell-dependent proliferation and IgG secretion by B cells (Fig 6); supernatants of antigen-activated T-cell clones also failed to restore B-cell function. Gp120-treated T cells were compared with CsA-treated T cells in this assay system. As shown in Fig 6, CsA-treated T cells also manifested impaired helper function for B cells. In contrast to their effects on gp120-treated T-cell cultures, the combination of exogenous cytokines IL-2 + IL-4 + IL-6 (10 U/mL each) completely restored B-cell responses in cultures of CsA-treated T cells. The above observations suggest that gp120-mediated inhibition of cytokine secretion is by itself not the primary explanation for impaired T-cell helper function and that other mechanisms involved in T-B-cell contact-dependent interaction are affected.

The inhibition of contact-dependent T-B-cell interaction by gp120 was investigated further using Percoll gradient-fractionated B cells. Table 3 shows the cell surface markers expressed on fractionated B cells. The cell size determination was based on mean channel number of the forward light scatter on the flow cytometer. All three fractions of B cells expressed equal percentages of CD19, CD20, and CD21 antigens. The large B-cell fraction manifested an increased percentage of cells staining positive for B6 (CD23), CD25 (IL-2R alpha chain), mAb 4F2,38 and transferrin receptor, all of which are expressed on activated B cells.39,40 The small and intermediate B-cell subpopulations failed to proliferate or secrete IgG in the absence of T cells; addition of exogenous cytokines in the absence of T cells was insufficient for their function. As has been previously demonstrated,28 large B cells incorporated low levels of 14C-thymidine and secreted IgG in the presence of IL-6 alone. These results indicated that the low-density, large B cells were enriched in activated B cells, while the small B cells consisted predominantly of resting B cells. Results of proliferation and IgG secretion by subpopulations of B cells cultured with Tt 2.1 T cell clones in the presence of tetanus antigen are shown in Fig 7. When Tt 2.1 T cells were pretreated with gp120, proliferation and polyclonal IgG production by the small B cells was markedly reduced (92% and 83% reduction) and that of intermediate B cells to a lesser degree (75% and 53% reduction). Immunoglobulin production by large B cells, which function independently of T-cell help, was unaffected when cultured with gp120-treated T cells.

**Table 3. Phenotypic Expression of Surface Antigens in Percoll Density Gradient-Separated B-Cell Fractions**

<table>
<thead>
<tr>
<th>Percent Positive Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Mean channel no.</td>
<td>70</td>
</tr>
<tr>
<td>B1 (CD20)</td>
<td>94.8</td>
</tr>
<tr>
<td>B2 (CD21)</td>
<td>99.6</td>
</tr>
<tr>
<td>B4 (CD19)</td>
<td>98.4</td>
</tr>
<tr>
<td>B6 (CD23)</td>
<td>1.6</td>
</tr>
<tr>
<td>IL-2R (CD25)</td>
<td>17.4</td>
</tr>
<tr>
<td>B6 (CD23)</td>
<td>2.6</td>
</tr>
<tr>
<td>Transferrin R</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Immunofluorescence staining of cell surface markers on subpopulations of B cells separated on Percoll density gradients. The results are for a mean of three experiments.

In this study we have examined the effect of gp120 on T-helper cell functions. We have demonstrated that HIV-gp120-treated T cells fail to provide adequate help to B cells; the effect was specific since pretreating gp120 with soluble CD4 abrogated the inhibitory effect. The impairment of T-helper function by gp120 was attributed to interference of T-B-cell contact-dependent interaction and inhibition of cytokine secretion by T cells.

**DISCUSSION**

B-cell dysfunction is one of the earliest features accompanying HIV infection. Patients manifest polyclonal hypergammaglobulinemia in association with spontaneous immunoglobulin-secreting and HIV-specific activated B cells29,30 in peripheral circulation. A direct stimulatory effect of HIV20 and its soluble proteins22,23,24 on B-cell differentiation responses has been demonstrated. Despite evidence for heightened B-cell activity in HIV infection, peripheral blood B cells of patients respond poorly to T-dependent and T-independent antigens13,14 and antibody responses to primary vaccinations are decreased.15,17 The impairment of
B-cell function has been attributed partially to depletion of circulating CD4+ T-cell numbers. However, even before the quantitative decline in CD4+ T-cell numbers, patients with HIV infection manifest qualitative defects in a variety of CD4+ T-cell functions. In this context, we and others have demonstrated that HIV envelope proteins can profoundly inhibit antigen-specific lymphoproliferation of CD4+ T cells. In the present study, we have investigated the effect of gp120 on T-helper cell function for B-cell proliferation and differentiation.

Antigen-specific, MHC-restricted T-cell activation by APC is followed by antigen-nonspecific, MHC-unrestricted polyclonal B-cell activation, requiring cytokines secreted by T cells and direct contact between T and B cells. Various cytokines secreted by T cells have been shown to induce B-cell proliferation and differentiation. IL-2, which primarily acts on T cells, has been shown to induce proliferation and differentiation in B cells as well. IL-4 has been associated with entry of activated B cells into the S-phase of the cell cycle. IL-4 also induces increase in the density of MHC class II antigens on B cells and thereby assists antigen-specific B cells to form conjugates with T cells. IL-6 plays an essential role in the terminal differentiation of activated B cells. The requirement for T-cell-derived lymphokines for B-cell responses was established in our culture system by parafomaldehyde fixation of anti-CD3-activated T cells. Addition of exogenous cytokines IL-2 and IL-4 partially restored B-cell proliferation, while IL-6 alone partially restored IgG secretion. Maximal restoration of proliferation and differentiation responses was achieved with a combination of IL-2, IL-4, and IL-6. In the present study, T-cell clones, pretreated with gp120, were inhibited in their ability to secrete IL-2 and IL-4 in response to specific antigen, but induction of IL-6 was unaffected (see also Chirmule et al). However, addition of exogenous cytokines failed to restore B-cell function in these cultures. Because recombinant cytokines have been shown to influence in vitro lymphocytic responses by complex mechanisms, the concentration of exogenous cytokines to be used in this study was determined by the ability of recombinant IL-2, IL-4, and IL-6 to restore T-cell-dependent B-cell function in the presence of CsA-treated T cells. CsA inhibits cytokine secretion in T cells by inhibiting induction of nuclear binding proteins, NFAT-1 and NF-kB, but does not affect cell-cell interactions directly. The inhibitory effects of CsA-treated T cells could be effectively overcome by the addition of exogenous cytokines at concentrations that had no effect on the gp120-treated T cells containing cultures. These results indicated that inhibition of cytokine secretion by T cells was not the primary mechanism by which gp120 impaired T-cell-dependent B-cell functions and that other mechanisms, eg, interference with T-B-cell contact-dependent B-cell interactions were most likely involved.

We confirmed in our culture system that gp120 treatment of T cells preferentially impaired function of T-helper cells, and that the impaired B-cell responses were not due to the direct effect of gp120 on B cells. First, B cells pretreated with gp120 and washed were not impaired in their responses. Second, the gp120 treatment of T cells did not adversely influence B-cell responses to T-independent stimuli such as EBV. Last, if B cells were fractionated on Percoll density gradients, responses only of small- and intermediate-size B cells were impaired by gp120 treatment of T-helper cells, while that of large B cells was spared. These findings are in agreement with the notion that large B cells are representative of activated B cells, and are not dependent on T-B-cell contact, rather they require B-cell differentiation factor (IL-6) for terminal differentiation. Function of large B cells was thus spared, because IL-6 secretion by the T-cell clones was unaffected with gp120. Differentiation requirements of small B cells are more complex and include cytokines, as well as a direct interaction with T cells. The observation that IL-2 and IL-4 secretion were impaired in gp120-treated T cells and that exogenous cytokines failed to overcome the deficiency in helper function of gp120-treated T cells also argues strongly in favor of impaired contact-dependent interaction as the major mechanism of gp120-mediated inhibition of T-helper cell function.

Contact-dependent T-B-cell interaction has been shown to be essential for T-cell-dependent B-cell function. In our experimental system, contact-dependent T-B-cell interaction was found to be necessary, since the functions of B cells were markedly impaired when their physical contact was prevented by culturing the T and B cells in separate compartments of Transwell chambers. The interaction of CD4 molecules on T cells and MHC class II molecules on B cells has been shown to play a major role in T-B-cell contact. Pretreatment of B cells with anti-HLA-DR mAb impaired antigen-induced T-cell-dependent B-cell proliferative responses and IgG secretion. Pretreatment of T cells with MoAb OKT4A (which maps to the V-1 domain of CD4), but not OKT4 (which maps outside the V-1 domain of CD4), was able to inhibit T-cell-dependent B-cell proliferation and differentiation. Several investigators have also demonstrated that addition of anti-CD4 mAb (Leu-3a) blocks physical interaction between CD4 and MHC class II. In this context, the observation of Tohma
and Lipsky that CD4-MHC class II interaction is not involved in T-B-cell interactions is most likely due to their use of anti-CD4 MoAb OKT4 in their experiments. Our experiments have clearly demonstrated that T-B-cell contact involves CD4-MHC class II interaction and that the latter is essential for optimal B-cell functional responses. Thus, CD4-MHC class II interaction, shown to be important for T-cell activation, also plays an important role in B-cell activation; MHC class II-mediated responses have in fact been shown to be critical for expression of B7. Recent evidence has indicated that LFA-1 molecules on T cells and ICAM-1 on B cells are also involved in the cellular adhesion. In the culture system in this study, addition of mAbs to LFA-1 and ICAM-1 also inhibited T-B-cell interaction. Based on existing information on gp120-CD4 interaction, it can be hypothesized that a major mechanism of interference between T-B-cell contact in gp120-treated T cells involves the prevention of CD4-MHC class II interaction due to steric hindrance. Our recent studies with anti-CD4 MoAb indicate that OKT4a or Leu-3a MoAb closely mimic gp120 in its effects on CD4 T-cell function (Oyaizu N, Chirmule N, Pahwa S, submitted for publication). Whether negative signaling by CD4+ T cells is also involved in the inhibitory effect of gp120 on T-helper cell function is an attractive possibility, but needs to be proven.

Our data show that gp120 is inhibitory for T-cell-dependent B-cell responses at concentrations of 1 μg/mL, at which concentration the envelope preparation was nontoxic. The biologic relevance of these observations merits consideration, since soluble gp120 is readily shed from the surface of HIV-infected cells. Thus, there exists the possibility that gp120 can bind to CD4+ T cells and impair T-cell function in vivo. Because gp120 has a high affinity for CD4, it is presumed that majority of the shed gp120 is bound to CD4+ cells. Amadori et al have recently shown that CD4+ cells from acquired immunodeficiency syndrome (AIDS) patients are covered with gp120/anti-gp120 complexes, with consequent downregulation of CD4 expression and T-cell function. Recently, using a sandwich-type enzyme immunosassay and mAb to gp120 IIIB, circulating gp120 in the range of 25 to 1,000 ng/mL has also been shown in plasma of HIV-seropositive individuals. These findings suggest that gp120 could bind to uninfected CD4+ T cells in vivo and impair a variety of functions of CD4+ T cells, including helper function for B cells. These findings have important implications for the use of envelope proteins as vaccines.

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