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Deleterious Effect of HIV-1 Plasma Viremia on B Cell Costimulatory Function

Angela Malaspina,1 Susan Moir,1,2 Shyamasundaran Kottilil, Claire W. Hallahan, Linda A. Ehler, Shuying Liu, Marie A. Planta, Tae-Wook Chun, and Anthony S. Fauci

HIV infection leads to numerous immunologic defects, including impaired B cell function. An effective humoral response requires bidirectional interactions between B cells and CD4+ T cells, critical of which are interactions between CD80/CD86 expressed on activated B cells and CD28 expressed on responder CD4+ T cells. In the present study, we examined the effect of active HIV replication on B cell costimulatory function. Induction of CD80/CD86 on B cells following B cell receptor and CD40 triggering and responsiveness of CD4+ T cells to activated B cells were investigated in a system where B cells of HIV-infected patients were compared concurrently to B cells of HIV-negative donors. In contrast to HIV-aviremic patients, B cells of HIV-viremic patients were ineffective at stimulating CD4+ T cells, as measured by the induction of activation markers and proliferation. The importance of interactions of CD80/CD86 and CD28 in activating CD4+ T cells was clear; the ablation of a normal response following the addition of neutralizing anti-CD86/CD80 Abs mirrored the response of CD4+ T cells to B cells of HIV-infected patients, while the addition of exogenous CD28 ligands partially restored the poor CD4+ T cell response to the B cells of HIV-viremic patients. Ineffective B cell costimulatory function in HIV-viremic patients was associated with low induction of CD80/CD86 expression on B cells. Our findings further delineate the scope of defects associated with cognate B cell-CD4+ T cell interactions in HIV infection and suggest that therapeutic interventions designed to enhance CD28-dependent costimulatory pathways may help restore immune functions. The Journal of Immunology, 2003, 170: 5965–5972.
effects of HIV-induced immune activation (28), similar to what occurs in other inflammatory diseases (29, 30). Increased levels of CD80 and CD86 on B cells have also been described in inflammatory diseases such as systemic lupus erythematosus and Behcet’s disease (31, 32). Little is known regarding these costimulatory molecules in the setting of HIV-induced B cell hyperactivation, however, there are indications from histological studies that CD80 expression but not CD86 is reduced on germinal center B cells of HIV-infected patients (33).

In the present study, we have investigated the effect of ongoing viral replication on the expression of CD80 and CD86 on B cells of HIV-infected individuals both in cross-sectional and longitudinal analyses before and after administration of antiretroviral therapy. We also investigated B cell effecter function mediated by CD80 and CD86 following B cell activation in vitro. Our findings indicate that whereas B cells of HIV-infected viremic patients tend to express increased levels of CD80 and CD86 in vivo, the inducibility of these markers in vitro is impaired, resulting in reduced capacity to deliver appropriate stimulatory signals to CD4⁺ T cells. These results shed new light on the dysregulation of cognate B-T cell interactions associated with HIV infection and active viral replication.

Materials and Methods

Study subjects

Study subjects included 30 HIV-infected patients (Table I) and 31 HIV-negative donors. Of the HIV-infected patients, 25 were studied cross-sectionally; 12 had detectable levels of plasma HIV viremia (viremic group) and 13 had undetectable levels of plasma HIV viremia (aviremic group). The HIV-negative donors were studied longitudinally before and after receiving antiretroviral therapy. Levels of plasma HIV RNA were measured by branched chain DNA amplification (limit of detection of 50 copies/ml; Bayer, Tarrytown, NY). Leukapheresis and standard blood draws were conducted in accordance with protocols approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD).

Cell preparation and culture conditions

PBMC isolated from blood-derived products were used to isolate B cells and CD4⁺ T cells by a column-based negative-selection technique (Stem-Cell Technologies, Vancouver, Canada) as previously described (34). The purity of each cell population was generally >95%, as measured by flow cytometry. B cells were cultured at 2 × 10⁶/ml in 24-well plates in RPMI 1640 medium supplemented with 10% FCS, 500 ng/ml CD40 ligand (CD154) trimer, and 20 μg/ml goat anti-human IgM (Jackson ImmunoResearch, West Grove, PA). At 48 h poststimulation, B cells were recovered, washed, irradiated at 5000 rad, and cocultured at 5 × 10⁵ cells/well in 96-well plates with freshly isolated 1 × 10⁶ CD4⁺ T cells. After 5 days of coinoculation, proliferation of CD4⁺ T cells was measured by [¹³C]thymidine uptake during an additional 16 h. Presence of HIV in day 5 coculture supernatants was evaluated by p24 HIV-1 ELISA (Beckman Coulter, Brea, CA, except anti-CD86, which was from BD PharMingen). CD4⁺ T cell surface activation markers were measured at day 5 poststimulation with allophycocyanin-conjugated anti-human CD4, PE-conjugated anti-human CD154, FITC-conjugated anti-human CD25, and corresponding mouse IgG isotype controls (all from BD Biosciences, San Diego, CA, except anti-CD86, which was from BD PharMingen). CD4⁺ T cell surface activation markers were measured at day 5 poststimulation with allophycocyanin-conjugated anti-human CD4, PE-conjugated anti-human CD154, FITC-conjugated anti-human CD25, and corresponding mouse IgG isotype controls (BD Biosciences). Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences).

Flow cytometry

B cell surface markers were analyzed by flow cytometry at day 0 and after 48 h of activation using allophycocyanin-conjugated anti-human CD39, PE-conjugated anti-human CD80, PE-conjugated anti-human CD86, PE-conjugated anti-human CD21, FITC-conjugated anti-human CD25, and corresponding mouse IgG isotype controls (all from BD Biosciences, San Diego, CA, except anti-CD86, which was from BD PharMingen). CD4⁺ T cell surface activation markers were measured at day 5 poststimulation with allophycocyanin-conjugated anti-human CD4, PE-conjugated anti-human CD154, FITC-conjugated anti-human CD25, and corresponding mouse IgG isotype controls (BD Biosciences). Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences).

Statistical analysis

Baseline means and means of other cross-sectional data were compared by ANOVA with Tukey’s multiple comparison test. Paired mean differences in values from baseline to day 2 were tested for significance by the one-sample Student’s t test. Two group comparisons of median CD4⁺ T cell proliferations and ratio was done by the Wilcoxon two-sample test; CD4⁺ T cell proliferation ratios of viremic and aviremic patients to paired normal volunteers were tested for significance using the Wilcoxon signed rank test. The Spearman rank method was used to test for correlation. Adjustment of p values for multiple testing was done with the Bonferroni method.

Results

Identification of defective interactions involved in the stimulation of CD4⁺ T cells by B cells of HIV-infected viremic patients

Ag-driven activation of CD4⁺ T cells generally requires two signals, the first signal delivered following recognition of Ag by the TCR and the second involving costimulatory molecules. In the setting of cognate bidirectional interactions between B cells and CD4⁺ T cells, the most important costimulatory interactions involve CD80/CD86 expressed on activated B cells and CD28 expressed on responder CD4⁺ T cells. To study the effect of HIV

![Image](http://www.jimmunol.org/Downloaded-from-ht...)

FIGURE 1. Scheme of study protocol. B cells were stimulated through BCR/CD40 ligation for 48 h, measured for cell surface expression of CD80, CD86, and CD25, and irradiated before cocultivation with CD4⁺ T cells isolated from PBMCs of a healthy HIV-negative individual. After an additional 20 h in culture, the activated CD4⁺ T cells were analyzed for expression of activation markers CD154 and CD25 and assayed for proliferation by thymidine incorporation.
infection on the efficacy of B cells to deliver the proper costimulatory signals to CD4+ T cells, B cells isolated from PBMC of HIV-infected patients were compared concurrently to B cells isolated from PBMC of HIV-negative healthy donors in a series of functional and phenotypic assays schematized in Fig. 1. The B cells were stimulated via the B cell receptor (BCR) and CD40 for 48 h and then analyzed for up-regulation of activation and costimulatory markers. The B cells were then irradiated and cocultured with allogenic CD4+ T cells isolated from PBMCs of a healthy HIV-negative individual. After an additional 5 days of coculture, the stimulated CD4+ T cells were analyzed for expression of activation markers and assayed for proliferation by thymidine incorporation. The rationale for the use of allogenic CD4+ T cells in this system is explained in Materials and Methods.

The molecular interactions involved in the responsiveness of CD4+ T cells to activated B cells were first established for HIV-negative healthy donors. As shown in the left panels of Fig. 2A, effector B cells induced the appearance of a distinct population of activated CD4+ T cells expressing CD154 and CD25 that represented 12.3% of the total CD4+ T cell population. In the presence of anti-CD80 and anti-CD86 neutralizing mAbs, this activated CD4+ T cell population was reduced to 0.57% (Fig. 2A, middle panel); furthermore, the addition of either neutralizing mAb against CD80 or CD86 alone at the same concentration as the total of the combined mAbs resulted in a partial block of activation of CD4+ T cells (data not shown). These data indicate that the expression of both CD80 and CD86 on activated B cells is required for optimal activation of CD4+ T cells and confirm the importance of costimulatory signals between CD80/CD86 and CD28 on CD4+ T cells in this activation process.

When B cells from an HIV-infected patient at high plasma viremia were analyzed concurrently with B cells from an HIV-negative healthy donor, a striking defect in the capacity to activate CD4+ T cells was observed in the HIV-infected patient (Fig. 2A, right panel). The inability of B cells from the HIV-infected viremic patient to induce responder CD4+ T cells to express CD25 and CD154 was similar to the effect of adding blocking anti-CD80/CD86 mAbs to cultures containing B cells from an HIV-negative healthy donor (Fig. 2A, middle panel). Although the unresponsiveness of CD4+ T cells to effector B cells from the HIV-viremic patients could have been due to the presence of virus in the cultures (34), this was found not to be the case since no measurable HIV could be detected in the coculture supernatant (data not shown). This was not surprising considering that, in contrast to the current assay conditions (Fig. 1), we had previously demonstrated that HIV virions associated with B cells of viremic patients were only propagated if activated CD4+ T cells were added to the cultures at day 0 (34). In another series of concurrent experiments, the low response of CD4+ T cells to effector B cells from the HIV-infected viremic patient was restored by the addition of an exogenous CD28 ligand (anti-CD28) (Fig. 2B), whereas the response of CD4+ T cells to effector B cells from the HIV-negative healthy donor was not.

**FIGURE 2.** Receptor-ligand interactions involved in the responsiveness of CD4+ T cells to activated B cells. A, CD4+ T cells were analyzed for surface expression of CD4, CD25, and CD154 following stimulation with BCR/CD40-activated B cells from a representative HIV-negative donor in the absence (left panel) or presence (middle panel) of neutralizing anti-CD80 and anti-CD86 mAbs, or stimulated with BCR/CD40-activated B cells from a representative HIV-viremic patient (right panel). B and C, CD4+ T cells were analyzed for surface expression of CD4, CD25, and CD154 following stimulation with BCR/CD40-activated B cells from a representative HIV-viremic patient (B) or HIV-negative donor (C) in the absence (left panel) or presence (right panel) of agonistic anti-CD28 mAb.
B cell surface expression of CD80, CD86, and CD25 before and after BCR/CD40-mediated activation was measured longitudinally for donors (right panels). Of the HIV-infected patients compared with their aviremic counterparts and the HIV-negative healthy donors; however, the differences between baseline levels of CD80 and CD86 and levels induced following activation were significantly lower in HIV-viremic patients when compared with both of the other groups. The expression of CD80 on B cells of HIV-viremic patients rose by 19.0% compared with 32.4% for the HIV-aviremic group and 36.1% for the HIV-negative healthy donor group (p = 0.01, Fig. 3A). For CD86, expression rose 43.9% in the HIV-viremic group compared with 62.8% for the HIV-aviremic group and 68.5% for the HIV-negative healthy donor group (p = 0.001, Fig. 3A). To further evaluate the phenotypic perturbations of the B cells of HIV-infected patients, we also measured the induction of the activation marker CD25. In contrast to CD80/CD86 patterns of expression, levels of CD25 on freshly isolated B cells of HIV-viremic patients as well as HIV-aviremic patients were found to be significantly depressed compared with the HIV-negative healthy donor group (p = 0.001). In addition, the levels of induction of CD25 following B cell stimulation were significantly lower in the HIV-viremic group compared with the two other groups, with expression rising a mere 13.4% compared with 26.9% for the HIV-aviremic group and 31.1% for the HIV-negative healthy donor group (p = 0.005, Fig. 3A). These findings indicating a defect in the induction of CD25 expression on B cells of HIV-viremic patients are consistent with previous findings showing a similar defect following stimulation with apparently normal CD4+ T cells (35) and suggest an intrinsic B cell defect.

We then sought to determine whether a correlation existed between the levels of plasma viremia in HIV-infected individuals and the ability to induce activation markers on their B cells. As shown in Fig. 3B, a strong inverse correlation was observed between expression of CD80/CD86 following B cell activation and levels of plasma viremia. For CD80, expression rose 53.2% in the HIV-viremic group compared with 50.8% for the HIV-aviremic group and 43.9% for the HIV-negative healthy donor group (p = 0.001). In addition, levels of CD86 expression rose 50.8% in the HIV-viremic group compared with 43.9% for the HIV-aviremic group and 36.1% for the HIV-negative healthy donor group (p = 0.001). These findings, indicating a defect in the induction of CD25 expression on B cells of HIV-viremic patients, are consistent with previous findings showing a similar defect following stimulation with apparently normal CD4+ T cells (35).

FIGURE 3. Effect of HIV infection and plasma viremia on activation of B cells. A, B cell surface expression of CD80, CD86, and CD25 before and after BCR/CD40-mediated activation was measured for 12 HIV-infected viremic patients (left panels), 13 aviremic patients (middle panels), and 16 HIV-negative donors (right panels). B, Correlation between levels of plasma viremia and levels of CD80/CD25 on BCR/CD40-activated B cells of HIV-viremic patients (C, B cell surface expression of CD80, CD86, and CD25 before and after BCR/CD40-mediated activation was measured longitudinally for five HIV-infected patients before (left panels) and after (right panels) reduction of plasma viremia by antiretroviral therapy. Horizontal bars and accompanying numbers represent the mean (A) or median (C) percent expression and bold numbers represent the difference between mean or median values at day 0 and day 2.
FIGURE 3.
Reduced induction of CD80/CD86 on activated B cells of HIV-infected patients translates into low CD4\(^+\) T cell responsiveness

Having established a deleterious effect of ongoing HIV replication on the induction of several cell surface markers associated with B cell function, we then investigated the consequence of these deficiencies on CD4\(^+\) T cell responses. Accordingly, the approach described in Fig. 1 was applied to determine the costimulatory component of the B cell function for all viremic and aviremic patients described in Table I and analyzed phenotypically in Fig. 3. As described earlier, the responsiveness of CD4\(^+\) T cells to activated B cells from each HIV-infected patient was assessed concurrently with the responses of activated B cells from an HIV-negative healthy donor to evaluate deviation from a normal response. In the 12 cases where HIV-viremic patients were compared with HIV-negative healthy donors, the proliferation of CD4\(^+\) T cells was significantly lower in response to BCR/CD40-activated B cells of the viremic patients, with B cells of HIV-negative donors inducing a median 2.2-fold greater response than those of viremic patients (\(p < 0.01\), Fig. 4A). When HIV-aviremic patients were compared with HIV-negative healthy donors, the proliferation of CD4\(^+\) T cells was again significantly lower in response to BCR/CD40-activated B cells of the aviremic patients, with B cells of HIV-negative donors inducing a median 1.3-fold greater response than those of aviremic patients (\(p < 0.01\), Fig. 4B). However, a significant normalization of B cell costimulatory function was observed with decreasing viral load in that the ratios of 2.2 and 1.3 corresponding to viremic and aviremic groups, respectively, were significantly different (\(p < 0.01\)).

Finally, consistent with the data presented in Fig. 2, a direct correlation was found between proliferation of CD4\(^+\) T cells and their capacity to become activated, as measured by cell surface expression of CD25/CD154 (Fig. 4C). Taken together, these findings indicate that B cells of HIV-infected viremic patients show defective costimulatory effector function, as evidenced by the poor proliferative and activation responses of responder CD4\(^+\) T cells, that is significantly restored upon reduction of plasma viremia.

The effect of plasma viremia on the capacity of activated B cells to induce CD4\(^+\) T cells to proliferate was also confirmed through longitudinal analyses with the same B cells used in describing the phenotypic defects of activated B cells in Fig. 3C. For this set of data, the CD4\(^+\) T cell proliferation at the time of high and low plasma viremia was reported as fold enhancement of proliferation induced by the B cells of the reference HIV-negative healthy donor over the proliferation induced by B cells of the HIV-infected patient. Consistent with the cross-sectional data reported in Fig. 4, A and B, the fold enhancement of CD4\(^+\) T cell proliferation was much higher when effector B cells of HIV-negative healthy donors were paired to B cells of patients at a time of high plasma viremia (3.1-fold enhancement) than when effector B cells of HIV-negative healthy donors were paired to B cells of the same patients at a time of low plasma viremia (1.1-fold enhancement; Fig. 4D). Taken together, these data indicate that reduction of plasma viremia by effective antiretroviral therapy leads to a normalization of B cell effector function.
Discussion

The ligation of CD28 on CD4+ T cells by CD80/CD86 represents a dominant costimulatory pathway that plays a critical role in CD4+ T cell activation, proliferation, and capacity to drive both humoral and cellular immune responses. In the present study, we investigated the efficiency of B cells of HIV-infected patients to deliver CD80/CD86-mediated costimulatory signals to CD4+ T cells by focusing on the effect of active HIV replication on B cell costimulatory potential following BCR/CD40-mediated activation in vitro. This question was addressed in cross-sectional analyses by comparing HIV-viremic and HIV-aviremic patients as well as in longitudinal analyses before and after the reduction of plasma viremia in individual patients by antiretroviral therapy. We found that B cells of HIV-viremic patients expressed significantly increased in vivo levels of CD86, and a trend toward increased levels of CD80, when compared with B cells of aviremic patients and HIV-negative healthy donors. Despite these elevated baseline levels of expression, induction of CD80/CD86 on B cells following in vitro stimulation was impaired in HIV-viremic patients and this translated into a poor capacity to induce CD4+ T cell activation and proliferation. These findings are consistent with and extend into the arena of costimulatory functions of previous studies describing the paradoxical effects of active HIV replication in vivo on B cells in that hyperactivation drives increased in vivo expression of activation markers (7, 8) and depressed inducible B cell function in vitro (6, 9). HIV-mediated immune activation is also associated with poor Ab responses following immunization to both T cell-dependent and -independent Ags (10–12), further suggesting that manifestations of B cell hyperactivity, including increased expression of activation and costimulatory molecules described herein, translate into impaired antigenic responses.

The observation that B cells of HIV-viremic patients express increased levels of CD86 and a similar trend for CD80 is consistent with other studies describing enhanced levels of CD80/CD86 on T cells of HIV-infected patients (28) and on B cells of patients suffering from autoimmune-driven inflammatory diseases (31, 32). On the other hand, there are indications that CD80 expression is decreased on germinal center B cells of HIV-infected patients, suggesting that B cells residing in lymphoid tissues and B cells circulating in peripheral blood may be perturbed by different mechanisms (33). Of note, we also observed that CD25, another marker of cellular activation, was depressed on B cells of HIV-viremic patients, suggesting that HIV-induced hyperactivation is selective rather than generalized. The data showing reduced levels of CD25 are consistent with several studies reporting decreased levels of CD25 on CD4+ T cells as well as B cells of HIV-infected patients (4, 36–39). Whether HIV-induced hyperactivation triggers pathways that exclude CD25 or whether CD25-expressing cells are selectively inhibited or killed remains to be determined. It is also possible that reduced levels of CD25 expression on CD4+ T cells of HIV-infected patients are due to ineffective APC-mediated costimulatory signals, as suggested by our current finding that activated B cells of HIV-viremic patients induced low levels of CD25 on responder CD4+ T cells (Fig. 2).

Numerous studies have shown that B cells of HIV-infected and particularly HIV-viremic patients function very poorly when stimulated in vitro. In this study, we further describe this phenomenon by demonstrating that B cells of viremic patients show poor induction of CD80 and CD86 following stimulation in vitro, hence confirming the paradox that a hyperactive state in vivo translates into a hypoinactive state in response to ex vivo stimulation. Furthermore, we show that the consequence of depressed CD80/CD86 induction on B cells results in poor costimulatory function toward CD4+ T cells. When activated B cells of HIV-viremic patients were used to stimulate CD4+ T cells, the response was identical to that observed when CD4+ T cell activation was blunted by the presence of anti-CD80/CD86 blocking Abs. Furthermore, activation of CD4+ T cells by B cells of HIV-viremic patients could be partially restored by the addition of an agonistic anti-CD28 mAb, suggesting that B cell costimulatory deficiencies could be reversed with exogenous activators. Although our findings clearly identify a defect in the CD80/CD86-CD28 pathway, it remains to be seen whether other costimulatory pathways are impaired in HIV-viremic patients, including those involved in the homing mechanisms that allow CD4+ T cells to migrate to B cell areas of lymphoid tissues, such as CXCR5, and lymphoid tissue-associated signaling pathways, such as ICOS-B7h and OX40-OX40 ligand (14, 16). In this regard, CD28 has been shown to be critical in events that lead to migration of T cells to B cell areas (40). Finally, costimulatory pathways may offer potential therapeutic avenues, especially considering numerous other studies that have shown similar APC deficiencies with monocytes and dendritic cells of HIV-infected patients (23–27).

Few studies have shown a direct link between HIV plasma viremia and a phenotypic or functional perturbation of immune competent cells. A likely explanation for the paucity of data related to this question is the constant fluctuation of HIV plasma viremia, especially in patients who are viremic because of low compliance, as was the case in the current study. It was thus quite remarkable to see a significant correlation between plasma viremia and induction of costimulatory and activation markers following BCR/CD40-mediated triggering of B cells (Fig. 3B). This observation suggests that B cell perturbations are closely linked to ongoing HIV replication. There may also be a link between the current findings on CD80/CD86 and our previously reported findings showing that an increase in plasma viremia leads to loss of CD21 expression on B cells (41) in that CD80 and CD86 regulation is thought to be regulated by CD21-CD35-CD19 complex formation following antigenic stimulation (42). Decreased levels of CD21 at baseline and following BCR activation, as confirmed here (data not shown), may prevent adequate complex formation required for induction of CD80 and CD86 expression.

In summary, our findings indicate a clear dysregulation of CD80 and CD86 expression following B cell activation in HIV-infected viremic patients. Such an impaired induction of CD80 and CD86 on B cells consequently results in poor delivery of costimulatory signals, as evidenced by low CD4+ T cell activation and proliferation in response to B cells of HIV-viremic patients. Considering the bidirectional nature of cognate cell-cell interactions following antigenic stimulation, the poor induction of CD4+ T cells by B cells and other APCs may in turn lead to poor CD4+ T cell helper function (43). Hence, our findings add new insight to the scope of immunologic defects associated with HIV disease and underscore the deleterious effects of ongoing viral replication on immune function.

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References

B CELL COSTIMULATORY FUNCTION IN HIV INFECTION


