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HIV-1 infection is accompanied by qualitative and quantitative defects in CD4+ T lymphocytes. Loss of immune function in HIV patients is usually associated with a profound dysregulation of cytokine production. To investigate whether cytokine signaling defects occur during HIV infection, PHA blasts from healthy human donors were infected with two strains of HIV-1 and screened for the expression of STAT proteins used in cytokine signaling. A selective decrease in STAT5B was seen 8 days after infection with the BZ167 dual-tropic HIV isolate, but not with the Ba-L, M-tropic strain. Based on these findings, purified T cells from HIV-infected patients in different stages of disease were also tested for STAT expression; decreases in STAT5A, STAT5B, and STAT1α were observed in all patients. The reduction in STATs seen in vivo and in vitro after HIV infection may contribute to the loss of T cell function in HIV disease. The Journal of Immunology, 1998, 160:28–31.

Cutting Edge: HIV-1 Infection Induces a Selective Reduction in STAT5 Protein Expression

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HIV infection is associated with a progressive immune suppression, characterized by an early impairment of CD4+ T cell function and a later decline in absolute CD4+ T cell number (1, 2). Several recent studies have focused on the disruption of signaling pathways in T cells as possible causes for HIV-induced immune dysfunction; HIV-related alterations in src family protein tyrosine kinases (3–7), CD45 phosphatase (8), protein kinase C (9), intracellular Ca2+ (9), and ERK-2 and JNK kinases (10) have all been observed. Although it is not known whether these defects relate to the syndrome of HIV disease, it is likely that disruption of some of these signaling pathways would lead to losses in T cell functions.

A second factor that could account for the impairment of immune function in HIV-infected individuals is the profound alteration in cytokine secretion patterns seen in vivo and in vitro after HIV infection (11–13). The cytokines that a cell secretes typically depend upon the signals it receives from other cytokines in the medium. Most cytokines transduce biologic signals from cell surface receptors to the nucleus through the JAK-STAT signaling pathway (14–16). JAK kinases constitutively associate with the intracellular domains of cytokine receptors, and ligation with cytokine leads to JAK activation. STAT transcription factors are recruited to the activated cytokine receptor-JAK kinase complex where they are tyrosine phosphorylated. The phosphorylated STATs then translocate to the nucleus where they promote transcription of genes that control cell growth, differentiation, and maintenance of cellular homeostasis. Seven STAT genes, STATs 1–4, two closely related isoforms of STAT5, A and B, and STAT6 have been identified to date (16, 17). Of all the STATs, STAT5 is used most extensively in the signaling pathways of immunologically relevant cytokines, including IL-2, -3, -5, -7, -9, -15, and granulocyte-macrophage (GM)-CSF (18). Recently, we demonstrated that STAT5A and STAT5B protein and message were strongly depressed in tumor-bearing mice, while the expression of other members of the STAT family were unaltered (19). The selective reduction of STAT5 was associated with the loss in T and B cell function, suggesting that the STAT5 signaling pathway might play a crucial role in the tumor-dependent immunodeficiency. These observations raised the possibility that STAT down-regulation might also occur in other immunosuppressive conditions.

Therefore, in the current report we studied the expression of STAT proteins in T cells from normal donors following in vitro infection with HIV-1 and in T cells from HIV-infected patients. We show that in vitro HIV-1 infection can directly induce a selective down-modulation of STAT5B and that T cells from HIV-infected individuals have decreased expression of both STAT5A and -B.

Materials and Methods

Cells

PBMC were prepared fromuffy coats from normal National Institutes of Health Blood Bank donors by Ficoll/Hypaque separation (20). PBL from HIV-infected patients were isolated by Ficoll-Hypaque separation and plastic adherence (21). CD3+ T cells were enriched from PBL by negative selection using a mAb mixture plus complement (Lymphokwik, One...
Proteins were detected by enhanced chemiluminescence followed by Western blotting with the same Ab used for immunoprecipitation. Adsorbed proteins were analyzed by SDS-PAGE under (1%) lysing buffer and spun. Supernatants were incubated with anti-STAT antibodies and probed for the indicated STAT using the same Ab for both precipitation and Western blotting. Results are representative of three experiments.

Lambda Inc., Los Angeles, CA). Patients were classified according to the Walter Reed guidelines for the staging of HIV-infected individuals (22). The use of human blood samples in these studies was approved by the Institutional Review Boards of both institutions.

Viral stocks

Two HIV-1 isolates were used in this study. HIV-1BZ167 was grown in human PHA blasts, as previously described (23). HIV-1Bz167 was grown in monocyte-derived macrophages. Cell-free supernatants were titered for p24 core Ag by ELISA (AIDS Vaccine Program, NCI, Frederick, MD).

HIV infection

PBMC (2 × 10⁶/ml) were stimulated with 2 μg/ml PHA (Sigma, St. Louis, MO) for 3 days at 37°C, and washed twice. PHA blasts (10³/sample) were incubated for 2 h with HIV-1Bz167 or HIV-1Ba-L (172 and 570 tissue culture ID₅₀, respectively) and washed three times. Infected cells (1 × 10⁶/ml) were incubated 3 or 8 days in medium supplemented with 10 U/ml of recombinant IL-2 (Boehringer Mannheim, Indianapolis, IN). Medium in the 8-day cultures was replaced 4 days after infection. HIV-1 infection was followed by ELISA for HIV-1 p24.

Immunoprecipitations

The immunoprecipitation procedure has been described previously (19). Briefly, 5 × 10⁶ cells/group were incubated for 1 h at 4°C in Triton X-100 (1%) lysis buffer and spun. Supernatants were incubated with anti-STAT polyclonal Ab and adsorbed with protein A-Sepharose beads (Pharmacia, Piscataway, NJ). Adsorbed proteins were analyzed by SDS-PAGE under reducing conditions on a 7.5% homogeneous PhastGel (Pharmacia) followed by Western blotting with the same Ab used for immunoprecipitation. Proteins were detected by enhanced chemiluminescence.

Results

In vitro HIV-1 infection reduces STAT5 expression

We previously showed that immunosuppression in tumor bearing mice was accompanied by a loss in STAT5 expression (19). To determine whether HIV infection would elicit a similar effect, PHA blasts from healthy, HIV-negative donors were infected with the primary HIV-1 isolate, BZ167, and STAT expression was assessed by immunoprecipitation and Western blotting 3 and 8 days after infection (Fig. 1). By day 8, when viral replication was well advanced (Table I), STAT5B expression was strikingly reduced. By contrast, STATs 1, 2, 3 and 5A were not significantly altered.

In the current study we demonstrated that in vitro infection of PBMC or in vivo infection of patients with HIV-1 is accompanied by a loss in STAT5 expression. Cell lysates from 3- and 8-day cultures of uninfected and HIV-1BZ167-infected PHA blasts were immunoprecipitated and probed for the indicated STAT using the same Ab for both precipitation and Western blotting. Results are representative of three experiments.

No loss in STAT5B was observed 3 days after infection when viral replication was significantly less, indicating that STAT5B modulation occurred late in the infection cycle. Because gp120 and tat proteins have been found to affect immune function (24–27), we tested whether culturing PHA blasts with either of these HIV-1 proteins would alter STAT5A or B expression. A mixture of recombinant gp120 and tat had no effect on STAT5A or B expression, and similar negative results were obtained when the cells were cultured for 3 or 8 days with either tat or gp120 alone (data not shown).

Preferential down-regulation of STAT5B by HIV-1BZ167

The capacity of the dual-tropic HIV-1 strain BZ167 to down-modulate STAT5B was compared with that of the M-tropic strain Ba-L. HIV-1BZ167 isolate (Fig. 2A), which uses predominantly CXCR4 and CCR3 coreceptors (D. Cohen, unpublished observation), markedly decreased STAT5B expression but had no effect on STAT1 or STAT5A, consistent with the results presented above. By contrast, the Ba-L M-tropic HIV-1 strain (Fig. 2B), which uses primarily CCR5 as coreceptor (28), had little effect on the expression of any of the three STATs examined. HIV-1BZ167 was significantly more virulent than HIV-1Ba-L (Table I), suggesting that the difference between the two strains in blocking STAT5B expression was due to differences in their abilities to infect the cells.

STAT5A and -B are reduced in T cells from HIV-1-infected individuals

The capacity of HIV-1BZ167 to decrease STAT5B expression in infected PHA blasts raised the possibility that STAT levels might also be reduced in the T cells of HIV-1-infected donors. Purified T cells from 5 HIV-positive patients exhibiting a range of CD4 counts (Table II) were examined for the levels of expression of STAT5 proteins. As seen in Figure 3, both STAT5A and STAT5B were markedly reduced in all 5 of the HIV-positive patients (P1 through P5), as compared with 2 HIV-negative (N1 and N2) control donors studied simultaneously. By contrast, STAT3, STAT6, and the lower m.w. splice variant of STAT1 (STAT1β) showed near normal levels of protein expression in all donors. Interestingly, the higher m.w. variant, STAT1α, appeared to be absent in the cells from the infected patients. No quantitative correlation between the reduction in STAT protein and either the stage of the disease or therapy was observed in this small group of patients. Further studies in an extended group of patients will be necessary to elucidate the relationship between clinical characteristics and alteration in STAT expression. Reduced expression of STAT5A, STAT5B, and STAT1α in patients’ T cells differed from in vitro HIV-1-infected cultures in which the reduction was limited to STAT5B.

Discussion

In the current study we demonstrated that in vitro infection of PBMC or in vivo infection of patients with HIV-1 is accompanied by a loss in STAT5 expression.
by a marked decrease in the expression of STAT5, a transcription factor used in the transduction of intracellular signals by a wide variety of cytokine receptors (14). In vitro infection of PHA blasts from HIV-negative donors with the dual-tropic HIV-1 strain BZ167 induced a selective loss of STAT5B protein. The specificity of this response was striking because it was not observed with any of the other STAT proteins examined, including the highly homologous STAT5A isoform (Fig. 1A). The loss in STAT5B protein may have been due to a decrease in its rate of synthesis or an increase in its degradation rate, but it was not due to its translocation to the nucleus since the lysis conditions used in our experiments released both cytoplasmic and nuclear STATs. In addition, the down-regulation of STAT5B was observed only late after infection with the BZ167 strain, and in cultures that were producing a high amount of virus, as measured by p24 release (Fig. 1, Table I). Infection of PHA blasts with the M-tropic strain HIV-1m4-BZ167 failed to decrease STAT5B (Fig. 2B), possibly because the level of infection was much lower with this isolate than with HIV-1BZ167 (Table I). Most sexual transmissions of HIV-1 are caused by M-tropic viruses (29, 30); therefore, the down-regulation of STAT5B associated with the direct infection of T cells would likely occur in infected individuals only after the virus evolved in vivo into a T-tropic form.

The decrease in STAT5 and STAT1α expression in purified T cells from HIV infected patients was remarkable because it occurred in all five patients examined (Fig. 3), although these patients differed with respect to their disease stages and CD4+ T cell counts (Table II). In contrast to in vitro infection of PHA blasts, STAT5A, STAT5B and STAT1α were all reduced in patients' T cells. The loss of these STATs was selective because no consistent change was detected in the expression of STAT1, -3, -5A, -5B, and -6 (Table II). Shown are Western blots of immunoprecipitates using Abs against the indicated STATs.

**References**


![FIGURE 2. Decreased STAT5B expression in HIV-1BZ167- but not Bα-L-infected PHA blasts. PHA blasts from four separate donors were infected, cultured for 8 days, and tested for expression of the indicated STAT. Lanes 1 and 3, uninfected cells; lanes 2 and 4, cells infected with HIV-1BZ167 (A) or with HIV-1m4-BZ167 (B). These data have been confirmed in three separate experiments.](http://www.jimmunol.org/)

![FIGURE 3. Reduced expression of STAT1α, STAT5A and STAT5B in T cells from HIV+ patients. Purified T cells from two healthy, HIV-negative (N) donors and five HIV-infected patients (P1-P5) were analyzed for STAT1, -3, -5A, -5B, and -6 expression. Patients’ disease status are indicated in Table II. Shown are Western blots of immunoprecipitates using Abs against the indicated STATs.](http://www.jimmunol.org/)


