Cutting Edge: JAK3 Activation and Rescue of T Cells from HIV gp120-Induced Unresponsiveness

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In early HIV disease, immunodeficiency is characterized by the inability of CD4⁺ T cells to produce a critical cytokine, IL-2, and to express the receptor for IL-2 (IL-2R) in response to antigenic or mitogenic stimulation. The shared common γ-chain (γc) of IL-2R and its associated Janus kinase, JAK3, are indispensable for normal T cell function. Here, we show that the inhibition of IL-2R expression and proliferation induced by ligation of CD4 by HIV envelope glycoprotein, gp120, is correlated with inhibition of expression and activation of JAK3. Stimulation through the γc-related cytokine receptors restores JAK3 expression and activation and rescues CD4-mediated T cell unresponsiveness. Collectively, these data argue that inhibition of JAK3 expression and activation may, in part, explain the T cell dysfunction seen in early HIV disease. In addition, rescue from gp120-mediated T cell unresponsiveness by activation of JAK3 suggests a novel therapeutic approach for enhancing immune function in HIV disease. *The Journal of Immunology, 1998, 160: 5697–5701.*

**Materials and Methods**

**Isolation of CD4⁺ T cells**

Heparinized venous blood obtained from healthy adult human donors was separated on a Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) gradient to obtain lymphocytes. CD4⁺ T cells were isolated by incubation with anti-CD8 mAb (OKT8; American Type Culture Collection (ATCC), Manassas, VA), followed by negative selection on goat anti-mouse IgG-coated Immulon beads (Biotex Laboratories, Houston, TX). Isolated cells were 80 to 95% CD4⁺ by flow cytometric analysis (data not shown).

**T cell proliferation assay and analysis of IL-2R expression**

Purified CD4⁺ T cells in balanced salts solution were incubated with or without HIV surface glycoprotein gp120 (gp120SF2, 20 µg/ml) and cross-linked with anti-gp120 Ab (1:250 dilution) or anti-CD4 mAb (Leu-3a, 20 µg/ml) for 1 h at 37°C. Cells were washed, resuspended in RPMI 1640 culture media (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Gemini BioProducts, Calabas, CA) and 1 × 10⁶ cells were added to triplicate wells of a 96-well plate (Becton Dickinson, Lincoln Park, NJ). In some experiments 20 U/ml IL-2, IL-4, IL-6, IL-12 (R&D Systems, Minneapolis, MN), or IL-7 (Genzyme, Cambridge, MA) were added to the culture and incubated for 3 days at 37°C with 1 µCi/well of [³H]thymidine (NEN, Boston, MA) present during the final 5 h of culture. The cells were harvested and processed to determine [³H]thymidine incorporation.

To determine the expression of IL-2R (α-chain, CD25), the culture plates were set up as described above and incubated at 37°C for 24 h. Cells (2 × 10⁶) were stained with FITC-conjugated anti-CD25 mAb (PharMingen, San Diego, CA) and analyzed flow cytometrically (Coulter XL).

**Immunoprecipitation and Western blotting of JAK1 and JAK3**

Purified CD4⁺ T cells were incubated with or without gp120 and anti-gp120 Ab or anti-CD4 mAb for 1 h at 37°C. 2.5 × 10⁶ cells per well were tyrosine phosphorylation and consequent activation of JAK3. Latent cytoplasmic transcription factors termed STATs are recruited to the cytokine receptor and are phosphorylated by JAK3. The phosphorylated STATs then enter the nucleus to regulate transcription of many different genes (8). Studies of genetically deficient mice and humans show that γc and JAK3 are critical for the development and function of the immune system (6–10). In addition, cross-linking of the γc-chain of the γc-related cytokine receptors, IL-2R, IL-4R or IL-7R, prevents induction of anergy in murine T cell lines activated in the absence of costimulation (11). The effect of CD4 ligation on human T cells by HIV gp120 is phenotypically similar to the anergic state. Thus, aberrant regulation of the γc/JAK3-STAT signaling pathway could explain the defective T cell function and loss of CD4⁺ T cells in HIV-infected individuals. Here we show that prior CD4 ligation markedly inhibits TCR-induced JAK3 expression and activation. Furthermore, we show that engagement of γc-related cytokine receptors rescues TCR-induced IL-2R expression and proliferation and that this rescue correlates with JAK3 activation.
incubated at 37°C in an anti-CD3 mAb-coated (OKT3, ATCC) 12-well plate. In some experiments, 20 U/ml IL-2, IL-4, IL-7, or IL-12 were added. Cells were harvested after various times and lysed in Tris-buffered saline (TBS) containing 1% Nonidet P-40, phosphatase inhibitors, and protease inhibitors. Postnuclear lysates were used for immunoprecipitation, first with anti-JAK3 Ab (Santa Cruz Biotechnology, Santa Cruz, CA), and then, in some experiments, with anti-JAK1 Ab (PharMingen). The Ab-protein complex was pelleted using Sepharose-conjugated Protein A (Sigma, St. Louis, MO) boiled in sample buffer (0.4% SDS, 3% glycerol and 1% 2-ME), and the proteins were separated by 7.5% SDS-PAGE. The proteins expressed low levels of JAK3, and TCR/CD3 stimulation increased JAK3 expression, when normalized to the actin expression. Interestingly, prior CD4 ligation with gp120 or anti-CD4 mAb inhibited the TCR/CD3-induced expression and phosphorylation of JAK3 (Fig. 3A). This was confirmed by Western blotting of whole cell lysates with anti-JAK3 Ab and anti-actin mAb. Resting T cells expressed low levels of JAK3, and TCR/CD3 stimulation induced increased JAK3 expression, when normalized to the actin control. Prior CD4 ligation with gp120 or anti-CD4 inhibited the TCR/CD3-induced expression of JAK3 (Fig. 3B). These data show that gp120 or anti-CD4 mAb-mediated T cell unresponsiveness is correlated with inhibition of JAK3 expression and activation.

While JAK3 activation was significantly inhibited in CD4+ cells after 24 and 48 h of stimulation, expression and activation of JAK3 were noted after 72 h. This was correlated with an increase in IL-2R expression (data not shown), although these cells did not proliferate in response to anti-TCR mAb (Fig. 1). At late time points (72 h) following T cell activation, inhibition of JAK3 activation reversed spontaneously. However, inhibition of T cell proliferation was sustained even at late time points, possibly because critical downstream targets of activated JAK3 were still present. These observations were confirmed by unpublished data showing that addition of exogenous IL-2 after 24 h of anti-TCR/CD3 stimulation did not reverse CD4-mediated inhibition of proliferation. Reversal of CD4-mediated inhibition was possible only within 24 h of activation. These data suggest that, although the effects of CD4-mediated inhibition on JAK3 activation are short-term, they result in long-term effects on late events of T cell activation. Reversal of these long-term inhibitory effects cannot be achieved without addition of exogenous cytokines within 24 h of T cell activation. These data suggest that an early window of opportunity exists for rescue of T cell function by γc-related cytokines.

Activation of JAK3, but not JAK1, correlates with rescue of CD4-mediated T cell unresponsiveness

As shown above, engagement of γc-related cytokine receptors restored CD4-mediated inhibition of T cell activation. We therefore determined the activation status of JAK3 in these rescued cells.
FIGURE 2. Stimulation through γc-related cytokine receptors rescues CD4-mediated inhibition of TCR/CD3-induced IL-2R expression. CD4+ T cells were incubated with gp120 or anti-CD4 mAb (α-CD4) and then plated on wells coated with anti-TCR mAb (α-TCR). CD25 expression was assayed flow cytometrically after 24 h of incubation in the presence or absence of 20 U/ml IL-2, IL-4, IL-7, or IL-12. A, Histogram from a representative experiment of five performed is shown. B, Data are represented as the percentage of CD4+ T cells positive for CD25 in the presence or absence of 20 U/ml IL-2 or IL-12 and are the average ± SEM of five experiments.

FIGURE 3. CD4 priming inhibits TCR/CD3-induced JAK3 expression and activation. A, Purified CD4+ T cells were incubated with or without gp120 or anti-CD4 mAb (α-CD4) and plated on anti-CD3 mAb (α-CD3)-coated plates. Cells were harvested after the indicated time period, lysed, and immunoprecipitated (IP) with anti-JAK3 Ab. Nitrocellulose membrane was immunoblotted (IB) with anti-phosphotyrosine Ab (P-Tyr, top) and then stripped and immunoblotted with anti-JAK3 Ab (bottom). A representative experiment of four performed is shown. B, Cells treated as in A were incubated for 48 h. Cell lysates were analyzed by immunoblotting with anti-JAK3 Ab (top) and then stripped and immunoblotted with anti-actin mAb (bottom). The OD of each band was determined, and the ratio of JAK3/actin was plotted. A representative experiment of four performed is shown.
CD4-primed T cells. CD4 cytokine receptors rescues JAK3 activation in the presence or absence of IL-2, IL-4, IL-7, or IL-12. Nitrocellulose membrane was immunoblotted with anti-P-Tyr mAb (top) and then stripped and immunoblotted with anti-JAK3 Ab (bottom). A representative experiment of four performed is shown.

Addition of exogenous IL-2, IL-4, or IL-7, but not IL-12, completely reversed the gp120- or anti-CD4 mAb-induced inhibition of JAK3 expression and activation (Fig. 4, and data not shown). These data show that rescue of CD4-mediated inhibition of T cell activation correlates with activation of JAK3.

Another Janus family kinase, JAK1, associates with the β-chain of IL-2R, and with the α-chains of IL-4R and IL-7R, and is autophosphorylated upon activation (12, 13). We analyzed the activation of JAK1 in T cells stimulated through TCR/CD3 with or without prior CD4 ligation. As shown in Figure 5, JAK1 is expressed constitutively, and a low level of phosphorylation is seen in resting T cells. Stimulation through TCR/CD3 increased the phosphorylation of JAK1. However, prior CD4 ligation with gp120 or anti-CD4 mAb did not significantly change the activation status of JAK1 (Fig. 5). Collectively, these data suggest that activation of JAK3, and not JAK1, plays a role in cytokine rescue of CD4-mediated T cell unresponsiveness.

Discussion

Ligation of the CD4 coreceptor on human primary T cells leads to significant inhibition of Ag- and mitogen-induced T cell proliferation (3–5). The mechanism(s) of this inhibition are not understood. In this paper, we show that increases in JAK3 expression and activation induced by Ag receptor ligation are inhibited by prior CD4 ligation. These data suggest that, at least in this in vitro system, binding of CD4 by the HIV envelope glycoprotein down-regulates activation-induced transcriptional regulation through JAK3 and its target STATs. In vivo evidence for inhibition of the JAK-STAT pathway in HIV disease comes from recent data of Pericle et al. (14). The authors observed a selective reduction of STAT5B expression in HIV-infected PBMC and reduced expression of STAT1α, STAT5A, and STAT5B in T cells from HIV-seropositive individuals. These data suggest that the T cell dysfunction seen in HIV infection could result from defective JAK-STAT signal transduction. It is also intriguing to speculate as to the role of JAK3-STAT pathways in the multiplicity of T cell defects seen in HIV disease, which include T cell dysfunction, death, and failure of regeneration of the T cell repertoire (15). Mutations in the γ- and JAK3 genes can result in X-linked SCID in humans, which is characterized by a severe impairment of T lymphopoiesis (6, 7). Genetic deletion of the γ-chain in mice causes a severe reduction in numbers of T cells (9), and deletion of STAT1 produces impaired innate immunity to viral infections (16). Finally, recent data from γ-deficient mice suggest that a primary function of γ-mediated signals in the T cell lineage is to maintain cell survival (17). Thus, aberrant JAK3-STAT signaling might contribute to defective T cell development, survival, and function in HIV disease.

Our data show that, remarkably, stimulation with the cytokines IL-2, IL-4, or IL-7, but not IL-6 or IL-12, restored expression of IL-2R on CD4-primed cells and rescued the CD4-induced proliferative block. To address the mechanism of cytokine rescue of T cell anergy in murine T cell lines, Boussiotis et al. (11) showed that stimulation through the γ-chain of IL-2R, IL-4R, or IL-7R restored T cell activation and that this was correlated with activation of JAK3. In preliminary experiments, we have found that specific ligation of the γ-chain, but not the IL-2Rβ-chain, corrects CD4-mediated T cell unresponsiveness (data not shown). Collectively, these data suggest that the rescue of gp120-energized human T cells by IL-2, IL-4, or IL-7 is the result of signaling through γ.

The polypeptide chains of the cytokine receptors have no intrinsic tyrosine kinase activity but are noncovalently associated with Janus kinases (8). JAK1 is associated with the β-chain of IL-2R and with the α-chain of IL-4R and IL-7R (12, 13). JAK3 is associated with the γ-chain of IL-2R, IL-4R, IL-7R, IL-9R, and IL-15R (12, 13, 18). Resting T cells express very low levels of JAK3, and stimulation through TCR/CD3 up-regulates both the expression and activation of JAK3 (Fig. 3 and Ref. 12). We have shown here that inhibition of T cell activation by prior CD4 ligation and its rescue by specific cytokines are correlated with JAK3 expression and activation. Specifically, CD4 ligation markedly inhibited the up-regulation and activation of JAK3 with subsequent

![Figure 4](http://www.jimmunol.org/)

![Figure 5](http://www.jimmunol.org/)
Ag receptor ligation, and addition of IL-2, IL-4, or IL-7 fully restored Jak3 expression and activation. The association of Jak1 with alternate chains of the Jak3-associated cytokine receptors suggested that IL-2, IL-4, or IL-7 might also activate Jak1 (12, 13). In our system, a basal level of Jak1 activation was observed in resting T cells. But while stimulation through the TCR/CD3 increased the activation of Jak1, prior CD4 ligation had no significant effect on activation of Jak1. Thus, activation of Jak3, but not Jak1, is correlated with the rescue of CD4-mediated T cell unresponsiveness. We propose that the activation of Jak3 in response to γc ligation by IL-2, IL-4, or IL-7 rescues T cells from gp120-induced unresponsiveness.

The γc-related cytokine receptors and Jak3 might also play a critical role in maintaining T cell survival and restoring T cell maturation in HIV disease. IL-2 prevents apoptosis of CD4+ T cells from HIV-seropositive individuals in vitro, and this is correlated with Bcl-2 expression (19). Interestingly, forced expression of Bcl-2 has been shown to restore all stages of T lymphopoiesis in γc-deficient mice, suggesting a role for γc-mediated signals in maintenance of T cell survival (17). Thus, signaling through γc may protect cells from gp120-mediated apoptosis and facilitate reconstitution of the T cell immune system.

These data suggest a novel therapeutic approach to the early immunodeficiency seen in HIV-infected individuals. IL-2 has been used in therapeutic trials to enhance immune function and to increase T cell numbers in HIV disease (20). Our data suggest that related, less toxic γc cytokines, or selective activation of Jak3, may provide valuable therapeutic tools. In combination with aggressive anti-retroviral therapy, therapies that prevent loss of immune surveillance could significantly delay progression of HIV disease.

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