Interleukin 23 Produced by Myeloid Dendritic Cells Contributes to T-Cell Dysfunction in HIV Type 1 Infection by Inducing SOCS1 Expression

Ankita Garg,1 Pratima Rawat,1 and Stephen A. Spector1,2

1Department of Pediatrics, Division of Infectious Diseases, University of California–San Diego, La Jolla, and 2Rady Children’s Hospital, San Diego, California

The mechanism of myeloid dendritic cell (mDC)–mediated impaired T-cell function was investigated during human immunodeficiency virus type 1 (HIV-1) infection. HIV or gp120 were found to inhibit lipopolysaccharide-induced mDC maturation and cause defects in allogeneic T-cell proliferation, interleukin 2 and interferon γ (IFN-γ) production, and phosphorylated STAT1 expression. gp120-treated mDCs downregulated autologous T-cell proliferation and IFN-γ production against a peptide pool consisting of cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF). These T-cell defects were associated with a decrease in production of the T-helper type 1–polarizing cytokine interleukin 12p70 and an increase in interleukin 23 (IL-23) production by gp120-treated mDCs. gp120-induced IL-23 upregulated suppressor of cytokine signaling 1 (SOCS1) protein in T cells, which inhibited IFN-γ production and killing of CEF-pulsed monocytes. These effector functions were recovered by silencing SOCS1 in T cells. Furthermore, we observed IL-23–induced SOCS1 binding to the IFN-γ transcription complex. These results identify SOCS1 as a novel target to improve the immune function in HIV-infected persons.

Keywords. HIV-1; gp120; myeloid dendritic cells; IL-23; SOCS1.

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Correspondence: Stephen A. Spector, MD, University of California–San Diego, 9500 Gilman Dr, La Jolla, CA 92037-0672 (saspector@ucsd.edu).

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Human immunodeficiency virus type 1 (HIV)-infected persons frequently lose T-helper type 1 (Th1) responses [1–4], are unable to mount protective immunity, and exhibit diminished responses to viral and bacterial vaccines [5–8]. The progressive loss in maintenance of T-cell responses is attributed to the decline in numbers and function of T cells and dendritic cells (DCs) [9–12]. Nevertheless, despite restoration of T cells and DC numbers following successful combination antiretroviral therapy (cART), abnormalities in myeloid DCs (mDCs) persist and are likely a major cause of abnormal T-cell stimulatory capacity and function [13, 14].

Cytokines play an important role in HIV pathogenesis [15, 16]. Recently, interleukin 23 (IL-23), a heterodimeric cytokine composed of a unique p19 and a p40 subunit that is shared with interleukin 12 (IL-12) [17, 18], was shown to be produced by DCs and macrophages in response to microbial pathogens. The limited information available suggests that IL-23 contributes to HIV-associated disease [19, 20]; however, how IL-23 impacts immune function during HIV infection remains unclear.

Cytokine signaling induces expression of suppressor of cytokine signaling (SOCS) genes [21, 22]. Of the 8 SOCS proteins, SOCS1 and SOCS3 have been shown to control many aspects of lymphocyte function [21, 23, 24], and SOCS1 is critical for T-cell function [25, 26]. A defective interferon γ (IFN-γ) response is a characteristic feature of HIV infection, and the upregulation of SOCS1 in infected rhesus macaques and humans [27] is associated with decreased IFN-γ production and T-cell functions [28]. However, the mechanism by which SOCS1 and mDCs mediate the decline in T-cell effector functions is not well understood. In the present study, we demonstrate that HIV or its envelope protein gp120 inhibit lipopolysaccharide (LPS)-induced mDC maturation and increase IL-23 production. We further show that...
SOCS1 plays a central role in regulating T-cell effector functions.

**METHODS**

**Patient Population**

This study was approved by the Institutional Review Board of the University of California, San Diego. The donors were healthy, HIV-seronegative, or HIV-seropositive persons aged >18 years who provided written informed consent before donating blood.

**Cell Isolation and Culture**

Peripheral blood mononuclear cells (PBMCs) isolated from freshly obtained blood by Ficoll density centrifugation (GE Healthcare) were used for isolation of CD14+, CD3+, and CD4+ cell populations, using magnetic beads (Miltenyi Biotec), and cultured in Roswell Park Memorial Institute 1640 medium (Gibco) and 10% AB-human serum (MP Biomedicals).

**Generation of mDCs**

mDCs were generated from CD14+ monocytes [29]. Immature mDCs (DCs) were cultured for an additional 24 hours in the presence of 1 µg/mL LPS (Sigma), baculovirus-derived gp120 (1 µg/mL; Abcam catalog no. ab69717) supplied in 30 mM Tris, 150 mM sodium chloride, and 0.01% Tergitol. The controls were prepared in the identical manner and resuspended in the identical buffer.

Alternatively, DCs were cultured with LPS in the presence of infectious or inactivated HIVBaL (AIDS Research and Reference Reagent Program).

**HIVBaL Inactivation**

HIVBaL was heat inactivated at 56°C for 50 minutes with intermittent shaking. Binding to CD4 was evaluated by flow cytometry (Supplementary Figure 1).

**T-Cell Proliferation**

Proliferation of CD3+ T cells in response to allogeneic and autologous stimulation was measured by a carboxyfluorescein succinimidyl ester (CFSE) dilution assay [30] (Supplementary Materials) and analyzed by flow cytometry.

**Cytokine Determination**

CD3+ T cells were cultured with autologous or allogeneic mDCs in a 96-well plate. Cell-free supernatants were collected and concentrations of interleukin 2 (IL-2) and IFN-γ quantified. In some experiments, CD3+ T cells were isolated from cocultures and cultured for an additional 72 hours in the presence or absence of anti-CD3/anti-CD28; concentrations of IFN-γ were quantified by enzyme-linked immunosorbent assay (ELISA; Biolegend).

In some experiments, DCs were stimulated with LPS (1 µg/mL) for 24 hours in the presence or absence of gp120 (1 µg/mL), and concentrations of IL12-p70, interleukin 6 (IL-6), interleukin 15 (IL-15), and IL-23 in culture supernatants were measured by ELISA (Biolegend).

**Flow Cytometry**

Expression of CD80, CD83, and HLA-DR was measured by incubating mDCs with allophycocyanin (APC)–anti-CD83, phycocerythrin–anti-HLA-DR, and AF488–anti-CD80 (all Biolegend) on ice for 30 minutes. Intracellular phosphorylated STAT1 (pSTAT1) was detected using cytofix/Phosphaflow Perm Buffer III and AF647-mouse anti-phospho-STAT1 or isotype according to the manufacturer’s protocol (BD Biosciences). For intracellular SOCS1 and SOCS3, blood was surface stained with APC–anti-CD3, APC–eF780-CD4, and eF450–anti-CD8 (all from eBiosciences); treated with RBC lysis buffer; and fixed and permeabilized with buffers according to the manufacturers’ protocols (Biolegend). Cells were incubated with isotype or rabbit anti-SOCS1 and mouse anti-SOCS3, stained with RPE-sheep anti-rabbit immunoglobulin G (IgG) and fluorescein isothiocyanate–goat anti-mouse IgG (all from AbDSerotec), and resuspended in phosphate-buffered saline for flow cytometry. Data were analyzed using CellQuest Pro or FlowJo software.

**gp120-Conditioned mDC Medium and Culture of T Cells**

DCs were stimulated with LPS in the presence or absence of gp120, and cell-free supernatant was stored. CD3+ T cells were isolated from healthy donors and cultured with control or gp120-treated conditioned mDC medium or with 10 ng/mL recombinant IL-23 (rIL-23; R&D Systems) for 72 hours. Cell lysates were prepared using radioimmunoprecipitation assay buffer supplemented with protease inhibitor cocktail (both Thermo Scientifics) according to the manufacturer’s protocol, and immunoblotting was performed for SOCS1 and SOCS3 proteins (Supplementary Materials). In some experiments, polyclonal anti-IL23p19 or isotype control (10 µg/mL; R&D Systems) was added to neutralize IL-23 in conditioned medium.

**SOCS1 Silencing and T-Cell Cytotoxicity**

SOCS1 was knocked down in CD3+ T cells by transfecting 100 nm of control or SOCS1 small interfering RNA (siRNA; Smartpool, Dharmacon), using the Human T-cell Nucleofector kit (Lonza) according to the manufacturer’s protocol. These were used as effectors to determine lysis of cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF) peptide pool-stimulated monocytes, using the lactate dehydrogenase (LDH) release assay (Cytotox 96 nonradioactive cytotoxicity assay kit; Promega) according to the manufacturer’s protocol (Supplementary Materials).

**Chromatin Immunoprecipitation (ChiP) Assay**

CD3+ T cells cultured with rIL-23 or conditioned medium in the presence or absence of anti–IL-23 antibody were treated...
with formaldehyde followed by glycine neutralization, DNA shearing, and preparation of chromatin supernatants. Chromatin was immunoprecipitated using antibodies to SOCS1 and cAMP response element binding protein (CREB; both from Abcam), from which DNA was purified and amplified by real-time PCR to determine binding to the IFN-γ proximal promoter (Supplementary Materials).

**Communoprecipitation**

CD3+ T-cell lysate was incubated with anti-CREB antibody, and the antigen-antibody complex was immunoprecipitated by protein A/G magnetic beads (Pierce, Thermo Scientific). The eluates were analyzed by immunoblotting with anti-SOCS1 antibody.

**Statistical Analysis**

The paired Student t test was used to determine the statistical significance for in vitro experiments. SOCS analysis in whole blood was performed with the nonparametric Wilcoxon rank test, using Prism 5 (La Jolla, California). A P value of <.05 was considered statistically significant.

**RESULTS**

**HIV and gp120 Inhibit mDC Maturation**

To test whether the interaction between HIV and mDCs alters the mDC phenotype [11, 31], DCs were stimulated with LPS in the presence or absence of infectious or heat-inactivated HIV (multiplicity of infection, 0.1). The treatment of DCs with LPS resulted in increased expression of costimulatory CD80, HLA-DR, and DC maturation marker CD83; the presence of HIV inhibited their expression (Figure 1Ai and 1Aii). To determine whether these phenotypic changes could be replicated following exposure to gp120, DCs were treated with LPS and gp120 (1 µg/mL), and markers of maturation were determined as described above. The decrease in expression of costimulatory and mDC maturation markers following gp120 exposure were similar to those observed with infectious and noninfectious HIV (Figure 1Bi and 1Bii). These findings suggest that infectious and noninfectious HIV or gp120 downregulate mDC maturation.

**HIVgp120 Inhibits mDC-Induced Th1 Responses**

Next we analyzed the allostimulatory capacity of mDCs exposed to infectious or noninfectious HIV or to gp120 in a coculture system of mDCs and CFSE-labeled allogenic T cells. Exposure to HIV or gp120 significantly reduced the mDC-induced T-cell proliferation (infectious HIV, P = .01; noninfectious HIV, P = .02; and gp120, P = .01; Figure 2Ai and 2Aii). Culture of gp120-treated mDCs with allogenic T cells resulted in an approximately 1.5-fold decrease in IL-2 production (mean level [± SD], 264 ± 53 vs 169 ± 31 pg/mL; P = .02; Figure 2B) and a >2-fold decrease in IFN-γ production (1827 ± 759 vs 748 ± 521 pg/mL; P = .02; Figure 2C) compared to LPS treated mDCs.

To determine whether this defect in IFN-γ production can be recovered, CD3+ cells were isolated from mDC–T-cell cocultures and cultured in wells coated with or without anti-CD3/anti-CD28. After 72 hours, increased IFN-γ production was observed for T cells cultured with LPS-matured mDCs (mean level [± SD], 2735 ± 965 pg/mL), compared with T cells cultured with control DCs (1009 ± 11.5; P = .05) and gp120-treated mature mDCs (1447 ± 918 pg/mL; P = .05). Similarly, T cells cultured with mature mDCs produced more IFN-γ in the presence of anti-CD3/anti-CD28 antibodies, compared with T cells cultured with control (mean level [± SD], 10 677 ± 318 vs 5318 ± 1616 pg/mL; P = .04) or gp120-treated mDCs (10 677 ± 187 vs 7428 ± 260; P = .02; Figure 2D) and stimulated identically. The defect in IFN-γ production was associated with a decrease in intracellular pSTAT1 (Figure 2E). These findings collectively suggest that, during HIV infection, bystander T cells fail to mount a Th1 response through the TcR/CD3 pathway.

**gp120-Treated mDCs Inhibit Recall to Viral Antigens**

To investigate whether mDCs exposed to HIV could inhibit recall responses to a broad range of viral pathogens [4, 7], mDCs were cultured with LPS and noninfectious HIV or gp120 and then were cocultured with CFSE-labeled autologous T cells in the presence or absence of CEF peptide pool. After 5 days, mean fold-change in proliferation of T cells cultured with LPS-treated mDCs in the presence of the CEF peptide pool was 2.1 ± 0.13, which was significantly greater than that for control mDCs (1.3 ± 0.26; P = .04), mDCs treated with noninfectious HIV (1.4 ± 0.25; P = .01), and mDCs treated with gp120 (1.41 ± 0.17; P = .05; Figure 3Ai and 3Aii). Additionally, compared with untreated mDCs (mean level [± SD], 365 ± 82 pg/mL), there was a decrease in IFN-γ production by T cells cultured with noninfectious HIV (210 ± 53 pg/mL; P = .02) and by gp120-treated mDCs (100 ± 10.2 pg/mL; P = .03; Figure 3B). These findings suggest that HIV- or gp120-exposed mDCs not only inhibit alloresponses but also downregulate T-cell function against intracellular viruses.

**gp120 Modulates mDC Cytokine Production**

Cytokines produced by mDCs strongly affect T-cell differentiation and function [32, 33]. To examine whether gp120 alters cytokine production from mDCs, LPS-treated mDCs were cultured with or without gp120 for 24 hours. IL-12p70, IL-6, IL-15, and IL-23p19 levels were assayed in the culture supernatants. LPS-treated mDCs produced more IL-12p70 than control mDCs (mean level [± SD], 1014 ± 233 vs 141.3 ± 105 pg/mL; P = .01), with production among LPS-treated mDCs decreasing in the presence of gp120 (mean level [± SD], 384 ± 266 pg/mL; P = .002, compared with LPS-treated mDCs; Figure 4A). Similarly, LPS-treated mDCs produced more IL-15 and IL-6 than control mDCs (mean level [± SD], 1577.5 ± 459 vs 157.5 ± 118 pg/mL
for IL-15 \(P = .01\) and 2579 ± 734 vs 100 ± 5 pg/mL for IL-6 \(P = .002\)); the presence of gp120 did not cause a significant difference in production by LPS-treated mDCs (Figure 4B and 4C). Consistent with these findings, compared with control mDCs (mean level \[± SD\], 11 ± 1 pg/mL), LPS treatment yielded increased IL-23 production from mDCs (404 ± 351 pg/mL; \(P = .04\)), which was further enhanced in the presence of gp120 (962 ± 688 pg/mL; \(P = .04\); Figure 4D). These findings
Figure 2. Human immunodeficiency virus (HIV) gp120–treated myeloid dendritic cells (mDCs) inhibit the T-helper type 1 response. mDCs were generated in vitro and stimulated with 1 µg/mL lipopolysaccharide (LPS) in the presence or absence of infectious or heat-inactive HIV (multiplicity of infection, 0.1) or gp120 at 1 µg/mL for 24 hours. These were cultured with carboxyfluorescein succinimidyl ester–labeled allogeneic CD3+ T cells at a DC to CD3 ratio of 1:10 in round-bottomed 96-well plates for 5 days and stained with allophycocyanin–anti-CD3. The percentage of proliferating CD3+ cells was determined by flow cytometry and analyzed using CellQuest Pro. A, Representative dot plot is shown. Aii, Data are mean percentages (± SD) of proliferating CD3+ cells (n = 3). B and C. The supernatants from cocultures of control mDCs or mDCs stimulated with LPS in the presence or absence of gp120 and allogeneic CD3+ T cells were collected after 24 hours for quantitation of interleukin 2 (IL-2; B) and, after 120 hours, for quantification of interferon gamma (IFN-γ; C) by an enzyme-linked immunosorbent assay (ELISA). Data are mean ± SD (n = 4). D and E, In vitro–generated mDCs were untreated (control) or treated with LPS at 1 µg/mL in the presence or absence of gp120 (1 µg/mL) for 24 hours and then cultured with allogeneic CD3+ T cells for 120 hours. CD3+ cells were subsequently isolated by positive selection using magnetic beads and cultured in a 96-well plate ex vivo or in the presence of anti-CD3/anti-CD28 for 72 hours. D, IFN-γ expression was measured in culture supernatants by an ELISA. Data are mean ± SD (n = 3). E, Intracellular expression of phosphorylated STAT1 was determined by flow cytometry, and data were analyzed using CellQuest Pro. A representative histogram from 3 independent experiments is shown. *P < .05. Abbreviations: APC, allophycocyanin; SD, standard deviation.
suggest that HIV gp120 confers phenotypic alterations in mDCs and also mitigates their cytokine responses, such that Th1 cell differentiation is inhibited.

**Differential Expression of SOCS Proteins in T Cells by IL-23**

SOCS proteins are inhibitors of IFN-γ signaling and regulators of T-cell development [21, 23, 34]. We next examined whether SOCS proteins are involved in the negative regulation of IFN-γ during HIV infection. LPS-treated mDCs were cultured with or without gp120 for 24 hours, and conditioned medium from the cultures was added to freshly isolated T cells. Total cell protein was immunoblotted to detect SOCS1 and SOCS3 expression. Increased SOCS1 expression in T cells cultured in LPS-matured mDC medium was observed, compared with T cells cultured in control mDC medium, with further increases in T cells cultured in gp120-treated mDC conditioned medium (Figure 5Ai). No difference was found in SOCS3 expression in T cells cultured with control or LPS-conditioned medium with or without gp120 (Figure 5Aii).

To test whether gp120-induced IL-23 could be responsible for increased SOCS1 expression, T cells were cultured in gp120-treated or untreated mDC-conditioned medium in the
presence or absence of neutralizing antibody to IL-23; SOCS1 protein expression was assessed by immunoblotting. T cells cultured in the presence of rIL-23 were used as controls. Consistent with our previous findings, SOCS1 protein expression was increased in T cells cultured with gp120-treated mDC medium but was significantly inhibited in the presence of anti-IL-23 antibody (Figure 5B). Additionally, T cells cultured with rIL-23 also exhibited increased expression of SOCS1 protein (Figure 5B) but not of SOCS3 protein (data not shown). Importantly, increased SOCS1 expression in T cells cultured with LPS-treated mDC medium without gp120 was not affected by the addition of anti-IL-23 antibody (Figure 5B). Thus, SOCS1 induced by LPS-treated mDCs is not dependent on IL-23 and may be due to increased IL-6 expression [34].

**HIV-Infected Persons Express Increased SOCS1 Levels**

Our in vitro findings demonstrated that HIV gp120 enhances the expression of SOCS1 in CD3+ T cells. To determine SOCS1 expression in HIV-infected persons, we performed whole-blood staining of samples obtained from HIV-infected persons and observed that levels of SOCS1-expressing CD4+ and CD8+ T cells were increased in HIV-infected persons, compared with healthy controls (Figure 5Ci and 5Cii). The number of SOCS1-expressing CD8+ cells was consistently greater than the number of CD4+ T cells; no difference in SOCS3 expression was observed.

**gp120-Induced SOCS1 Inhibits T-Cell Effector Functions**

IFN-γ controls viral infections by enhancing CD8+ T-cell lytic activity and inhibiting viral replication. Therefore, we examined...
Figure 5. Human immunodeficiency virus (HIV) gp120–induced interleukin 23 (IL-23) modulates suppressor of cytokine signaling (SOCS) expression in T cells. In vitro–generated myeloid dendritic cells (mDCs) were unstimulated (control) or stimulated with 1 µg/mL lipopolysaccharide (LPS) in the presence or absence of gp120 at 1 µg/mL. After 24 hours, culture supernatants were used to culture CD3+ cells from different healthy donors. Whole-cell lysates of CD3+ cells were prepared, and immunoblots were performed with antibodies to β-actin as a loading control, SOCS1, and SOCS3. A-i, CD3+ cells were cultured in control DC medium (lane 1), LPS DC medium (lane 2), and LPS plus gp120 DC medium (lane 3). SOCS1 (Ai) and SOCS3 (Aii) expression was analyzed by immunoblotting. Data are representative of 3 donors. Bi–ii, IL-23–neutralizing antibody was added to CD3+ cell cultures to determine the effect of blocking IL-23 on SOCS1 expression. CD3+ cells were cultured in control medium (lane 1), LPS DC medium (mDC medium) with isotype or anti–IL-23 antibody (lanes 3 and 4), and LPS plus gp120 DC medium (gp120 medium) with isotype or anti–IL-23 antibody (lanes 5 and 6). As an additional control, CD3+ cells were cultured in the presence of 10 ng/mL recombinant IL-23 (lane 2). Cells were lysed, and immunoblots were performed for β-actin as a loading control and SOCS1. Bi, Data shown are representative of 4 donors. Bii, The histogram shows mean results (± SD) of the densitometric analysis for SOCS1 (n = 4). C-i, Intracellular SOCS1 and SOCS3 expression was determined in CD4+ and CD8+ cells from HIV type 1–infected persons. Whole-blood specimens from healthy controls and HIV-infected persons were surface stained with antibody to CD3, CD4, and CD8; fixed; permeabilized; and incubated with nonimmune sera (isotype) or rabbit anti-SOCS1 and mouse anti-SOCS3, followed by staining with RPE sheep anti-rabbit immunoglobulin G and fluorescein isothiocyanate goat anti-mouse secondary antibody. The percentage of cells expressing SOCS1 and the mean fluorescence intensity (MFI) of SOCS1 were determined by flow cytometry, and data were analyzed using FlowJo. Cia–b, A representative histogram is shown. Cii, The percentages SOCS1-expressing CD3+CD4+ and CD3+CD8+ cells (upper panel) and mean fluorescence intensity of SOCS1 in CD3+CD4+ and CD3+CD8+ cells (lower panel) were calculated after normalization using an isotype control. Data are median (center horizontal line) with interquartile range (25% lower and 75% upper horizontal line, respectively) (n = 5 for healthy controls and n = 11 for HIV-infected patients). *P < .05, **P < .005, and ***P < .0005. Abbreviations: FITC, fluorescein isothiocyanate; PE, phycoerythrin; SD, standard deviation.
Interleukin 23 (IL-23)–induced suppressor of cytokine signaling 1 (SOCS1) inhibits T-cell effector functions. The effect of SOCS1 in CD3+ T-cell–mediated killing of cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF)–stimulated monocytes and interferon γ (IFN-γ) production was determined by partial knockdown of SOCS1, using small interfering RNA (siRNA). Ai–ii, CD3+ T cells from healthy donors were transfected with control or SOCS1 siRNA (100 nM), using the T-cell Nucleofection kit, and cells were subsequently cultured in the presence of anti-CD3 (5 µg/mL)/anti-CD28 (2 µg/mL; both from eBioscience). After 48 hours, control or SOCS1 siRNA–transfected CD3+ cells (effectors) were cultured with autologous monocytes unstimulated or stimulated with CEF peptide pool (targets) in triplicate at an effector to target ratio of 10:1 for 4 hours. CD3+ cell–mediated killing was determined by a lactate dehydrogenase (LDH) release assay, and the percentage of specific lysis was calculated as 100 × (experimental – effector spontaneous – target spontaneous)/(target maximum – target spontaneous) (Supplementary Materials). Cell lysates were made from CD3+ cells, and immunoblots were performed with antibodies to β-actin as a loading control and SOCS1 to determine knockdown of SOCS1 protein. Ai, A representative blot of 3 independent experiments is shown in the inset. The histogram shows the mean percentage (± SD) of specific lysis by CD3+ cells (n = 3). Ai, Levels of IFN-γ were measured by an enzyme-linked immunosorbent assay (ELISA) in culture supernatants of control and SOCS1 siRNA–transfected CD3+ cells subsequently cultured in the presence of anti-CD3/anti-CD28 for 48 hours. Data are mean ± SD (n = 3). Bi–ii, To determine whether SOCS1 upregulated by IL-23 or human immunodeficiency virus (HIV) gp120–induced soluble factors inhibits T-helper type 1 effector cell functions, CD3+ cells from healthy donors were cultured in the presence of recombinant IL-23 (10 ng/mL) or gp120-conditioned mature DC medium as previously described, transfected with control or SOCS1 siRNA, and cultured with autologous monocytes unstimulated or stimulated with CEF peptide pool (1 µg/mL) in triplicate at an effector to target ratio of 10:1 for 18 hours. CD3+ cell–mediated killing was determined by a LDH release assay, and data denote the percentage of specific lysis. Bi, A representative blot of 3 independent experiments is shown in inset. The histogram shows mean percentage of specific lysis (± SD) by CD3+ cells (n = 3). Bi, Levels of IFN-γ were measured by ELISA in culture supernatants of control and SOCS1 siRNA–transfected CD3+ cells after culturing in the presence of anti-CD3/anti-CD28 for 48 hours. Data are mean ± SD (n = 3). *P < .05. Abbreviation: SD, standard deviation.
Figure 7. Suppressor of cytokine signaling 1 (SOCS1) upregulated by human immunodeficiency virus (HIV) gp120–induced interleukin 23 (IL-23) binds to the interferon γ (IFN-γ) transcriptional complex. Binding of SOCS1 to the IFN-γ proximal promoter was determined by a chromatin immunoprecipitation (ChIP) assay. Aia–b, CD3+ T cells from healthy donors cultured with recombinant IL-23 or gp120-conditioned DC medium were fixed using 1% formaldehyde followed by neutralization with glycine. Chromatin immunoprecipitation was performed using the Imprint Ultra Chromatin Immunoprecipitation Kit (Sigma) according to manufacturer’s instructions. Antibodies to SOCS1, antibodies to cAMP response element binding protein (CREB), or isotype control antibody were used to immunoprecipitate chromatin-bound supernatants, from which DNA was purified. The binding of SOCS1 and CREB on the IFN-γ promoter was measured by real-time polymerase chain reaction (PCR), using primers specific for the IFN-γ proximal promoter sequence (Supplementary Materials) and the LightCycler fast Start DNA Master SYBR Green I (Roche Applied Sciences). As a specificity control, PCR was performed using primers for the β-actin promoter supplied with the ChIP kit. The positive control was PCR amplification with input chromatin before immunoprecipitation. Melting curves were generated, and Ct values were calculated by the equations $\Delta Ct = Ct_{target} - Ct_{input}$ and $\Delta\Delta Ct = \Delta Ct_{target} - \Delta Ct_{IgG}$. The fold-difference was calculated as $2^{-\Delta\Delta Ct}$. Aia, A representative gel from 3 independent experiments is shown. Aib, Data are mean fold-differences (± SD; n = 3). Aia–b, IL-23 induced by gp120 modulates SOCS1 binding to the IFN-γ proximal promoter. Aia, CD3+ T cells from healthy donors were cultured in gp120-conditioned DC medium (CM) with (lanes 2 and 4) or without (lanes 1 and 3) anti–IL-23 antibody; chromatin immunoprecipitation using antibodies to SOCS1 and IgG was performed as described above. A representative gel from 3 independent experiments is shown. Aib, Data are mean fold-difference (± SD; n = 3). B, Binding of SOCS1 to CREB was determined by coimmunoprecipitation. CD3+ cells were cultured with control or gp120-conditioned DC medium with or without anti–IL-23. Cell lysates were prepared, pulled with anti-CREB or IgG, and immunoblotted using a β-actin loading control and anti-SOCS1 antibody. CD3+ cells cultured with gp120-conditioned medium without anti–IL-23 and immunoprecipitated with anti-CREB exhibited more binding when immunoblotted with anti-SOCS1.
whether SOCS1 affects IFN-γ production and T-cell–mediated lysis of monocytes. T cells isolated from PBMCs from healthy donors were transfected with control siRNA or siRNA for SOCS1 and cultured for 48 hours in wells coated with anti-CD3/anti-CD28. IFN-γ production was determined in supernatants, and cells were used to evaluate killing of unstimulated or CEF peptide pool–stimulated autologous monocytes. Partial knockdown of SOCS1 (approximately 40%) in T cells augmented their capacity to produce IFN-γ (mean level [± SD], 16 418 ± 6241 vs 6910 ± 3453 pg/mL; P = .04) and also lysis of CEF peptide pool–stimulated monocytes (mean percentage of cells lysed [± SD], 23.1% ± 8% vs 13.3% ± 6.1%; P = .03; Figure 6Ai and 6Aii).

To further investigate whether HIV gp120–induced SOCS1 modulates T-cell effector functions, CD3+ T cells from healthy donors were cultured in the presence of rIL-23 or gp120-conditioned mDC medium for 72 hours and then transfected with control or SOCS1 siRNA. T-cell–mediated killing of unstimulated and CEF-stimulated monocytes was assessed without anti-CD3/anti-CD28 stimulation. Transfection of SOCS1 siRNA as compared to control siRNA in T cells cultured with gp120-conditioned medium increased the killing of CEF-stimulated monocytes (mean percentage of cells killed [± SD], 29% ± 5.4% vs 7.3% ± 1.8%; P = .01). Similarly increased killing was observed for SOCS1–transfected T cells cultured with rIL-23 (mean percentage [± SD], 25.7% ± 10% vs 5% ± 4%; P = .05). Additionally, T cells cultured with either rIL-23 or gp120-conditioned mDC medium did not kill monocytes as efficiently as T cells cultured in control medium, possibly because of the increased amount of SOCS1 protein in these cells.

To determine IFN-γ induction, SOCS1 siRNA–transfected T cells were cultured in anti-CD3/anti-CD28–coated wells for 48 hours, and IFN-γ was quantified by ELISA. Cells cultured in gp120-conditioned medium and transfected with SOCS1 siRNA produced more IFN-γ than cells cultured identically but transfected with control siRNA (mean level [± SD], 16 075 ± 2475 vs 9248 ± 251 pg/mL; P = .04). Similarly, increased IFN-γ was produced by cells cultured with rIL-23 and silenced for SOCS1, compared with cells transfected with control siRNA (mean level [± SD], 8267 ± 113 vs 13 400 ± 829 pg/mL; P = .01; Figure 6Bii). Concomitantly, T cells cultured with rIL-23 produced less IFN-γ, compared with cells cultured without rIL-23 (mean level [± SD], 8267 ± 113 vs 9720 ± 120 pg/mL; P = .01), indicating that IL-23 decreases the IFN-γ–producing capacity of T cells. Taken together, these results support the importance of SOCS1 in regulating T-cell function during HIV infection.

SOCS1 Binds to the IFN-γ Promoter and Regulates Its Activity

We hypothesized that the SOCS1-mediated decrease in IFN-γ production is due to its interaction with the IFN-γ transcriptional complex. Since CREB binds to the IFN-γ proximal promoter and positively regulates IFN-γ transcription in primary human T cells [35, 36], we examined ex vivo the interaction of SOCS1 with the IFN-γ proximal promoter and compared these findings with data on CREB binding to the IFN-γ proximal promoter. For these studies, ChIP was performed on chromatin supernatants from CD3+ T cells cultured in the presence of rIL-23 or gp120-conditioned mDC medium. We observed that SOCS1 and CREB bind to the proximal promoter of IFN-γ in these cells. As a specificity control, PCR for the β-actin promoter, which lacks the IFN-γ site, yielded negative results after immunoprecipitation with SOCS1 (Figure 7Aii). Next, to determine whether SOCS1 binding to the IFN-γ proximal promoter is modulated by gp120-induced IL-23, chromatin supernatants were prepared from CD3+ T cells cultured in gp120-conditioned mDC medium with anti–IL-23 and immunoprecipitated with antibody against SOCS1 or isotype. PCR for the IFN-γ proximal promoter on eluted DNA showed that SOCS1 binds to the IFN-γ proximal promoter in T cells cultured with gp120-conditioned DC medium and was inhibited in the presence of anti–IL-23 (Figure 7Aii). Since our quantitative PCR showed comparable binding of SOCS1 and CREB to the IFN-γ proximal promoter (Figure 7Aii), we investigated the SOCS1-CREB interaction by coimmunoprecipitation. Cell lysates of T cells cultured in control or gp120-conditioned mDC medium with or without anti–IL-23 were immunoprecipitated with anti-CREB or IgG antibody and immunoblotted with anti-SOCS1 antibody. In these experiments, increased binding of SOCS1 to CREB was observed in lysates cultured with gp120-conditioned mDC medium without anti–IL-23, compared with control medium or medium with anti–IL-23 (Figure 7B). These findings suggest that IL-23–induced SOCS1 interacts with the IFN-γ transcriptional complex and may inhibit its synthesis.

Figure 7 continued. (lane 2), compared with lysates of cells cultured with anti–IL-23 or control medium immunoprecipitated with anti-CREB and immunoblotted using anti-SOCS1 (lanes 1 and 3). A representative gel from 3 independent experiments is shown. C, Simplified model of SOCS1-mediated regulation of IFN-γ synthesis. C, IFN-γ gene expression is activated by the binding of the transcription factor T-box expressed in T cells to the proximal promoter, facilitated by chromatin remodeling. Additional transcriptional factors (CREB, ATF2, and AP1 [c-Fos/c-Jun]) recruit coactivators and histone acetyltransferases (CREB binding protein [p300]), which enhances the opening of the chromatin structure and maximizes IFN-γ gene transcription. Of these, CREB is the positive regulator of IFN-γ transcription in primary human T cells in response to microbial pathogens. Cii, Based on our results we propose that HIV-induced IL-23 upregulates SOCS1 in T cells; SOCS1 interacts with CREB and negatively regulates IFN-γ production. Additionally, SOCS1 might also downregulate pSTAT1, further dampening the IFN-γ activity. *P < .05.
DISCUSSION

In this study, we examined the effect of mDC-induced IL-23 on T-cell effector functions against viral pathogens during HIV infection. Consistent with others, we observed that HIV or gp120 blocks LPS-induced mDC maturation; gp120 impairs IL-12p70 and increases IL-23 production from mDCs. These aberrant mDCs inhibited proliferation and IFN-γ production of alloreactive T cells and recall response to viral pathogens in a CEF peptide pool. Further, we showed that gp120-induced IL-23 upregulated SOCS1 protein expression in T cells. Partial knockdown of SOCS1 in T cells enhanced IFN-γ production and lysis of CEF peptide pool–stimulated monocytes. Finally, we showed that IL-23–induced SOCS1 binds to the IFN-γ transcription complex. Importantly, higher levels of SOCS1–expressing CD3+ T cells were present in untreated HIV-infected persons. Thus, to our knowledge, we are the first to show the mechanism by which HIV–associated dysregulation of mDCs may cause T-cell dysfunction.

Our findings demonstrate that inactivated HIV or gp120 at concentrations found within lymphoid tissues of HIV-infected persons inhibits LPS-induced mDCs maturation and function [37, 38], which is evidence that our in vitro findings likely occur in HIV-infected persons. Our studies also suggest that gp120–treated mDCs induce T-cell hyporesponsiveness [39, 40], which could not be restored by anti-CD3/anti-CD28 stimulation. This may be of considerable clinical importance for the subset of HIV-infected persons with reconstituted normal T-cell numbers who, despite successful cART, are unable to generate efficient T-cell responses to viruses and viral vaccines.

Because of limited numbers of mDCs present in the peripheral blood of healthy or HIV–infected persons, we performed our studies by using in vitro–generated mDCs from CD14+ cells. These were phenotypically identical to the in vivo mDCs, were CD123+CD11c+DC SIGN”HLA-DR” (data not shown) [14, 19, 29]. Thus, we believe that our findings are representative of mDCs present in vivo. To obtain functionally active mDCs, we used LPS because of its higher plasma levels [41] and association with HIV disease progression [42]. Consistent with other reports, we also found that LPS-matured mDCs produce higher levels of IL-12p70, IL-6, and IL-15; however, only IL-12p70 was significantly inhibited in the presence of gp120. Our in vitro findings are supported by clinical studies in which low frequencies of IL-12p70–producing mDCs and similar frequencies of IL-6–producing mDCs were observed in HIV-infected patients, compared with healthy controls [9, 43].

Our finding that gp120 increases IL-23 production is consistent with that of Louis et al, who observed increased production of IL-23 but not IL-12p70 by LPS-stimulated PBMCs from HIV-infected persons [19]. In another study, LPS-activated mDCs from HIV–exposed but uninfected infants born to mothers with uncontrolled viremia exhibited increased IL-23 levels, compared with DCs from neonates born to mothers with undetectable viral loads [20]. Here, we established that gp120–induced IL-23 inhibits T-cell functions by inducing SOCS1. SOCS1 targets JAKs and the IFN-γRα chain and inhibits IFN-γ signaling [21, 22, 25, 26]. Our ChIP assay showed that SOCS1 binds to the IFN-γ transcription complex within the nucleus. The lower expression of intracellular pSTAT1 in T cells (Figure 2E) suggests that SOCS1 acts both at the cytoplasmic level, by interfering with STAT1 signaling, and at the nuclear level, by modulating IFN-γ synthesis. The presence of a nuclear localization sequence [44, 45] and nuclear translocation of SOCS1 under various conditions in cells of human origin have been reported [46]. To our knowledge, our ChIP experiment provides the first evidence of nuclear localization of SOCS1 in primary human T cells and its direct role in regulating T-cell activity. IFN-γ gene regulation is controlled by a complex consisting of CREB and other regulatory elements [47–50]; we speculate that SOCS1 binds to CREB and negatively regulates IFN-γ transcription (Figure 7C).

Collectively, our results demonstrate that IL-23 produced by mDCs during HIV infection induces SOCS1 expression in T cells and plays a critical role in the downregulation of T-cell effector functions. Our work highlights the contribution of dysregulated innate immune responses in the failure of HIV–infected persons to achieve full immune reconstitution despite apparent virologic suppression. Moreover, our findings that establish a link between high SOCS1 expression and defective T-effector responses suggest that SOCS1 antagonists might improve immune reconstitution of HIV–infected persons receiving cART with sustained viral suppression.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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### References


