



Interleukin-27 Promotes Divergent Effects on HIV-1 Infection in Peripheral Blood Mononuclear Cells through BST-2/Tetherin

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ABSTRACT Interleukin-27 (IL-27) is able to inhibit HIV-1 replication in peripheral blood mononuclear cells (PBMCs), macrophages, and dendritic cells. Here, we identify that IL-27 can produce opposing effects on HIV-1 replication in PBMCs and that the HIV-1 restriction factor BST-2/Tetherin is involved in both inhibitory and enhancing effects on HIV-1 infection induced by IL-27. IL-27 inhibited HIV-1 replication when added to cells 2 h after infection, promoting the prototypical BST-2/Tetherin-induced virion accumulation at the cell membrane of HIV-1-infected PBMCs. BST-2/Tetherin gene expression was significantly up-regulated in the IL-27-treated PBMCs, with a simultaneous increase in the number of BST-2/Tetherin⁺ cells. The silencing of BST-2/Tetherin diminished the anti-HIV-1 effect of IL-27. In contrast, IL-27 increased HIV-1 production when added to infected cells 4 days after infection. This enhancing effect was prevented by BST-2/Tetherin gene knockdown, which also permitted IL-27 to function again as an HIV-1 inhibitory factor. These contrasting roles of IL-27 were associated with the dynamic of viral production, since the IL-27-mediated enhancement of virus replication was prevented by antiretroviral treatment of infected cells, as well as by keeping cells under agitation to avoid cell-to-cell contact. Likewise, inhibition of CD11a, an integrin associated with HIV-1 cell-to-cell transmission, abrogated the IL-27 enhancement of HIV-1 production. Our findings illustrate the complexity of the HIV-1–host interactions and may impact the potential therapeutic use of IL-27 and other soluble mediators that induce BST-2/Tetherin expression for HIV-1 infection.

IMPORTANCE Here, we describe new findings related to the ability of the cytokine IL-27 to regulate the growth of HIV-1 in CD4⁺ T lymphocytes. IL-27 has long been considered a potent inhibitor of HIV-1 replication, a notion based on several reports showing that this cytokine controls HIV-1 infection in peripheral blood mononuclear cells (PBMCs), monocyte-derived macrophages, and dendritic cells. However, our present results are contrary to the current knowledge that IL-27 acts only as a powerful downregulator of HIV-1 replication. We observed that IL-27 can either prevent or enhance viral growth in PBMCs, an outcome dependent on when this cytokine is added to the infected cells. We detected that the increase of HIV-1 dissemination is due to enhanced cell-to-cell transmission with the involvement of the interferon-induced HIV-1 restriction factor BST-2/Tetherin and CD11a (LFA-1), an integrin that participates in formation of virological synapse.

KEYWORDS HIV-1, IL-27, BST-2/Tetherin, PBMCs

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Interleukin-27 (IL-27), a member of the cytokine family of IL-6, IL-12, and IL-23, is produced by antigen-presenting cells during the initial phase of the immune response and plays critical regulatory roles in the functioning of the immune system (1). IL-27 is a heterodimer comprising the protein subunits p28 and EB13 (Epstein-Barr virus-induced gene 3) (1, 2), and its receptor is a heterodimer formed from the cytokine type 1 common receptor WSX-1 and gp130, which is shared with the IL-6 receptor. Many cells, such as monocytes, macrophages, dendritic cells, T and B lymphocytes, and NK and mast cells, express the IL-27 receptor (3). IL-27 presents a variety of regulatory functions, such as the development of the Th1 and Th2 immune responses through inducing gamma interferon (IFN- γ) production by T cells (1) and inhibiting the expression of the transcription factor GATA-3 (4), production of pro- and anti-inflammatory mediators by activating STAT and NF- κ B transcription factors (5–7), and priming naive T cells for IL-12 action, thus favoring cell polarization toward the Th1 phenotype (1).

Infection with human immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS, causes a substantial loss of CD4⁺ T cells in gastrointestinal-associated lymphoid tissues (8) and leads to chronic immune activation (9) and a proinflammatory condition in the sites of viral growth (10), thus favoring HIV-1 propagation due to the influx of new target cells. The increased expression of several inflammatory cytokines able to regulate HIV-1 replication in the lymphoid tissues of HIV-1-infected patients (11) likely also facilitates HIV-1 dissemination. It is possible that HIV-1 propagation in the lymphoid tissues occurs mainly through cell-to-cell transmission, with dendritic cells and macrophages playing relevant roles in the process of viral particle transmission to CD4⁺ T lymphocytes (12, 13).

Works reporting whether HIV-1 infection impacts circulating levels of IL-27 are rare and found conflicting results. While He et al. (14) showed that IL-27 titer was significantly upregulated in HIV-1-infected individuals and that HIV-1 viral load negatively correlated with IL-27 titers, Swaminathan et al. (15) reported that IL-27 levels were not significantly altered in patients with HIV-1. On the other hand, several *in vitro* studies have shown that IL-27 reduces HIV-1 infection in all types of HIV-1-target cells, such as peripheral blood mononuclear cells (PBMCs), CD4⁺ T cells, monocyte-derived macrophages, and dendritic cells (16–20). It has also been shown that IL-27 generates an anti-HIV-1 setting by activating a number of interferon (IFN)-stimulated genes (ISGs), including MX1, OAS, PKR, APOBEC3G (20), and BST-2/Tetherin (21), in human macrophages, monocytes, and T cells in an IFN-independent manner. Accordingly, the IL-27-induced inhibition of HIV-1 replication can occur by either type 1 IFN-dependent (19) or -independent (17, 20) pathways.

The BST-2/Tetherin protein is an IFN-induced virus restriction factor that prevents the release of HIV-1 particles by anchoring the budding virions to the membrane of infected cells (22–24). Although it is believed that the BST-2/Tetherin ability to impede virus release from the cell membrane, thus restricting HIV-1 cell-free propagation, contributes for a lower virus burden, studies have shown that the BST-2/Tetherin-mediated virion accumulation at the cell surface may actually increase viral production by favoring HIV-1 cell-to-cell dissemination (25). Given that the expression of BST-2/Tetherin may be regulated by IL-27, investigations are needed to elucidate whether this cytokine may also present contradictory roles during HIV-1 infection. Here, we describe novel findings divergent of the current knowledge that IL-27 acts solely as an inhibitor of HIV-1 replication, since we observed that it is capable of either preventing or enhancing viral growth in PBMCs and that the BST-2/Tetherin protein is implicated in both outcomes.

RESULTS

IL-27 increases the expression of BST-2/Tetherin and promotes virion accumulation at the cell membrane of HIV-1-infected PBMCs. Considering that IL-27 has been considered a potential therapeutic agent for HIV-1 infection (26), we aimed to provide additional findings on the ability of IL-27 to regulate HIV-1 infection in PBMCs. Like other authors (16, 20), we initially found that IL-27 reduced HIV-1 replication by 60 to 70% when added to infected PBMCs immediately after infection (see Fig. S1 in the supplemental material). Next, based on reports showing that IL-27 activates several ISGs in uninfected cells (20, 21), we

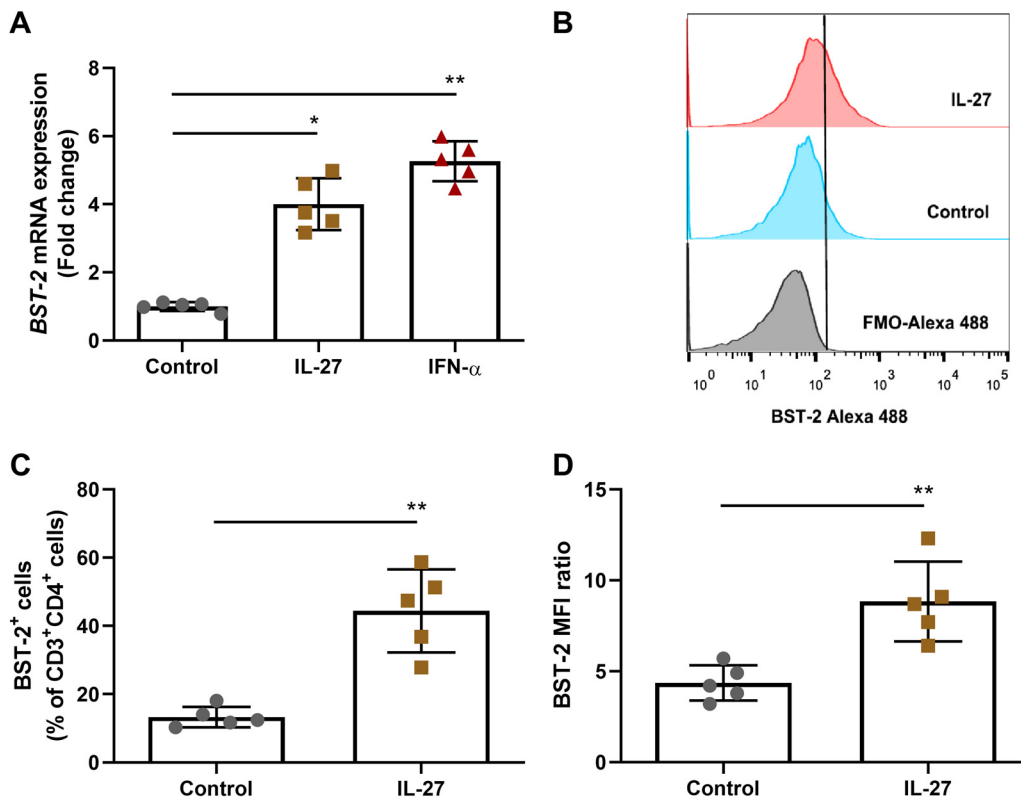


FIG 1 IL-27 promotes the expression of BST-2/Tetherin in PBMCs. (A) PBMCs were exposed to IL-27 (100 ng/mL) or IFN- α (10 ng/mL) and, after 18 h, the expression of *BST2* (Tetherin) was analyzed by real-time PCR. (B to D) HIV-1-infected PBMCs were treated with IL-27 (100 ng/mL) and, after 24 h, BST-2/Tetherin expression in cells within the CD3⁺ CD4⁺ gate was analyzed by flow cytometry. (B) Representative histogram of BST-2/Tetherin expression in the CD3⁺ CD4⁺ populations. (C) Percentage of BST-2/Tetherin⁺ cells in the CD3⁺ CD4⁺ population. (D) Median fluorescence intensity (MFI) of BST-2/Tetherin in positive cells, calculated as the MFI ratio = specific MFI/FMO MFI. *, $P < 0.05$; **, $P < 0.01$ (one-way ANOVA with Tukey's multiple-comparison test, $n = 5$ [A], and Student t test, $n = 5$ [B to D]). Error bars represent \pm the SD.

assessed whether this cytokine regulates the expression of the BST-2/Tetherin gene and the activity of the corresponding protein in HIV-1-infected PBMCs. It is well known that BST-2/Tetherin prevents the release of budding HIV-1 virions, thus inducing viral particle accumulation at the infected cell membrane and virion internalization, which eventually inhibits HIV-1 production (24). We detected that BST-2/Tetherin gene expression was significantly upregulated in the infected PBMCs treated with IL-27 (Fig. 1A), and this upregulation was accompanied by an augmentation in the number of CD3⁺ CD4⁺ BST-2/Tetherin⁺ cells in culture (Fig. 1B to E). Of note, IL-27 did not change the expression of the ISGs APOBEC3F and APOBEC3G in the PBMCs (see Fig. S2). We also observed, using transmission electron microscopy (TEM), that few virions were present near the infected cells maintained only with culture medium (Fig. 2A and B), whereas IL-27 promoted accumulation of HIV-1 particles adjacent to the infected PBMCs (Fig. 2C and D). The micrographs show the typical formations of virions attached to each other by BST-2/Tetherin molecules (Fig. 2C and D), according to the model previously proposed by other authors (23, 27). The accumulated virions were reinternalized by infected cells treated with IL-27 (Fig. 2E and F), a consequence of the inability of the virus to completely bud from the infected cell. Our results suggest that BST-2/Tetherin is an essential component of the ability of IL-27 to reduce HIV-1 production in PBMCs.

BST-2/Tetherin contributes to the IL-27-induced suppression of HIV-1 production when this cytokine is added to infected cells 2 h after infection. To further support our findings that BST-2/Tetherin participates in this IL-27-mediated anti-HIV-1 effect, we silenced the expression of the *BST2* gene in HIV-1-infected PBMCs. We found that *BST2* silencing 2 h after infection abolished the IL-27-mediated increase in the expression of BST-2/Tetherin (Fig. 3A to C) and, in addition, weakened the anti-HIV-1 effect of IL-27 (Fig. 3D). Altogether, our results show that IL-27 is able to inhibit HIV-1 production when

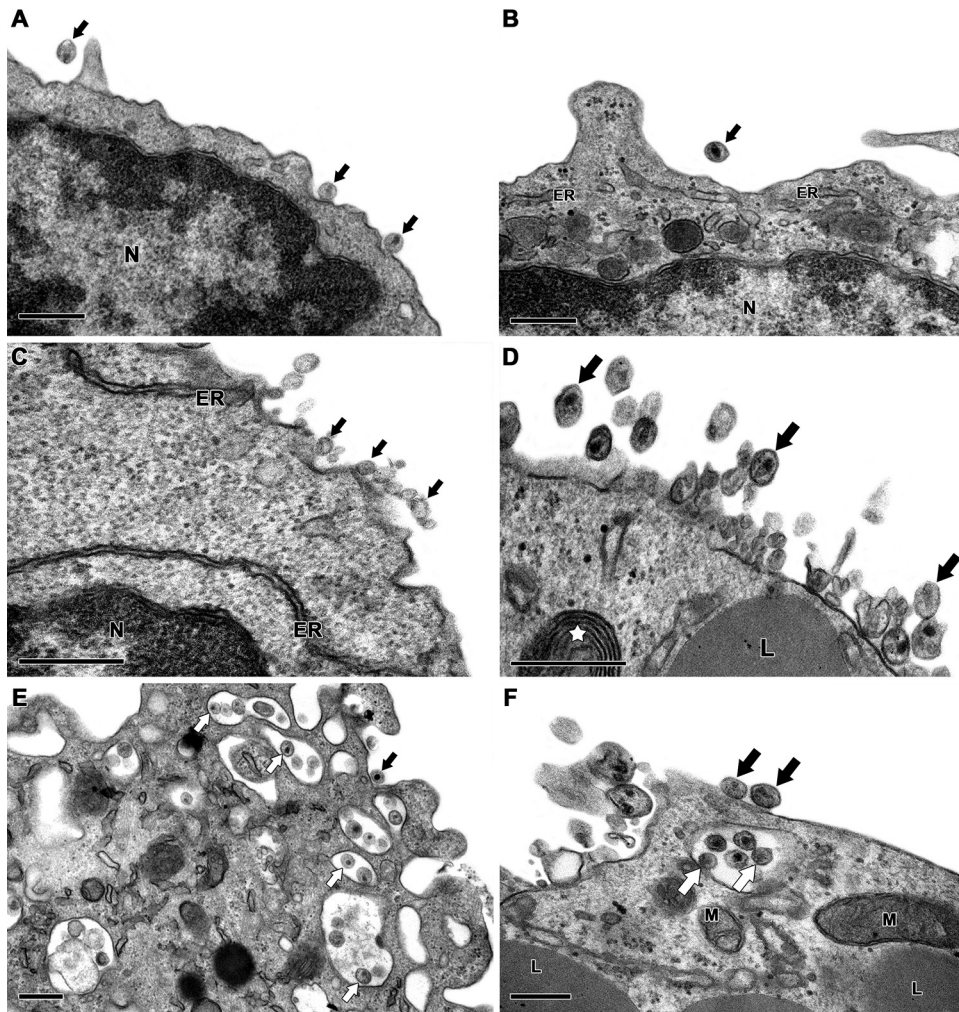


FIG 2 Ultrastructural evaluation of the effect of IL-27 on HIV-1-infected cells. (A and B) HIV-1-infected PBMCs show virus particles at the cell surface (black arrows). (C to F) Addition of IL-27 to HIV-1-infected PBMCs lead to a remarkable accumulation of viral particles adjacent to the infected PBMCs and viral distribution at the cell surface (black arrows) and inside cytosolic vesicles (white arrows). The nucleus (N), endoplasmic reticulum (ER), mitochondria (M), lipid droplets (L), and cytosolic concentric membrane structure (white star) are indicated. Cells were prepared for TEM 48 h after infection, and panels show representative images from one donor out of three. Bars, 0.25 μm ($n = 3$).

added to PBMCs just after infection and that the innate restriction factor BST-2/Tetherin is a critical mediator of this anti-HIV-1 effect.

IL-27 favors HIV-1 infection when added 4 days later to infected cells and this effect involves BST-2/Tetherin. Because HIV-1-infected cells are continuously exposed to a number of inflammatory mediators in the tissue microenvironment, regardless of how long they have been infected, we investigated whether IL-27 would keep its anti-HIV-1 effect if added later during the course of infection. We found that IL-27 enhanced HIV-1 growth when added on the fourth day after infection (Fig. 4A) as observed from a time course assay (see Fig. S3), contrasting with the HIV-1 inhibition when added to infected PBMCs 2 h after infection. We evaluated the role of BST-2/Tetherin in this enhancing effect by silencing the *BST2* gene in PBMCs infected with HIV-1 for 3 days that were then exposed to IL-27 the next day. As before, *BST2* silencing prevented the IL-27-mediated increase of BST-2/Tetherin expression (Fig. 4B to D) and, notably, abolished the positive effect of IL-27 on HIV-1 growth (Fig. 4E). In fact, silencing the BST-2/Tetherin gene permitted IL-27 to function again as a potent HIV-1 inhibitory factor, similar to when *BST2*-sufficient infected PBMCs were exposed to this cytokine shortly after infection. In conjunction, our results show that IL-27 can promote opposing effects on HIV-1 production in PBMCs and that the molecule BST-2/Tetherin is implicated in these contrasting actions.

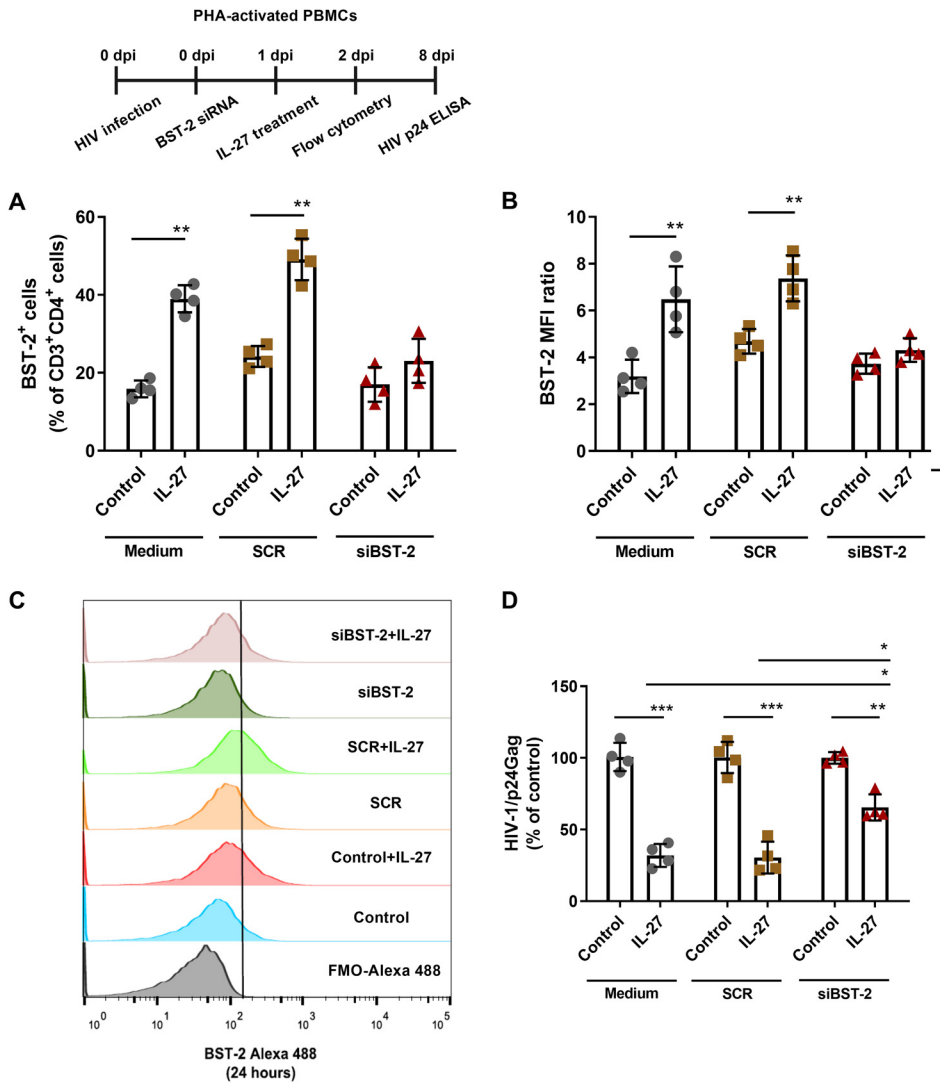


FIG 3 BST-2/Tetherin contributes to the IL-27-induced suppression of HIV-1 production when the cytokine is added to infected cells just after infection. (A to D) HIV-1-infected PBMCs underwent electroporation with a BST-2/Tetherin siRNA (siBST-2) or a scrambled siRNA (SCR) 2 h after infection and, after 24 h, were treated with IL-27 (100 ng/mL). After 24 h of treatment (A and B), BST-2/Tetherin expression was analyzed by flow cytometry within the CD3⁺CD4⁺ gate. (C) Representative histogram of the BST-2/Tetherin expression in cells exposed to IL-27 for 24 h. (D) After 8 days of infection, cell supernatants were collected, and HIV-1 replication was evaluated by measuring the concentration of HIV-1 p24 antigen by enzyme-linked immunosorbent assay (ELISA). The median fluorescence intensity (MFI) ratio was calculated as follows: MFI ratio = specific MFI/FMO MFI. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001 (two-way ANOVA with Tukey's multiple-comparison test; *n* = 4; error bars represent ± the SD).

The divergent effects of IL-27 on HIV-1 infection are associated with the dynamic of viral production by infected cells and dependent on cell-to-cell contact. Taking into account the distinct effects of IL-27 on HIV-1 replication when added immediately after infection (day 0) or 4 days postinfection (dpi) and that up to 60% of the HIV-1 infection in culture is derived from cell-to-cell transmission (28), we pondered whether such divergence could be due to the fact that, when added at 4 dpi, IL-27 would act in a culture containing a larger number of infected cells and, consequently, in an enlarged area of contact between infected and uninfected cells. Moreover, the induction of BST-2/Tetherin expression in this context would result in increased HIV-1 spreading and production, since the inhibition of cell-free virus transmission would be overcome by an enhanced cell-to-cell transmission.

To evaluate the impact of the infection levels on the divergent effects of IL-27 on HIV-1 production, infected PBMCs were maintained in culture for 4 days in the presence of the

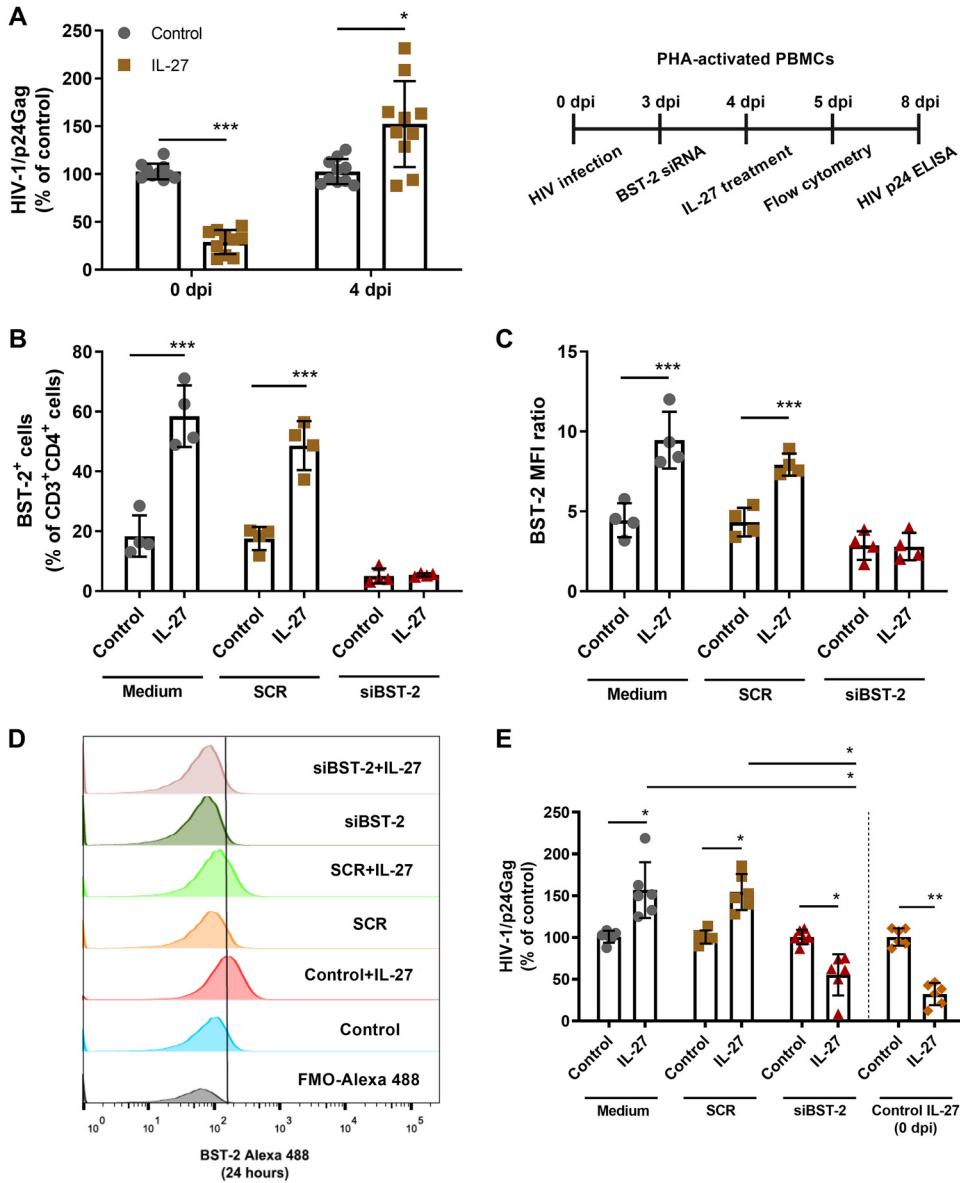


FIG 4 IL-27 favors HIV-1 infection when added later to infected cells, and this effect involves BST-2/Tetherin. (A) HIV-1-infected PBMCs were treated with IL-27 (100 ng/mL) immediately after infection (0 dpi) or 4 days after infection (4 dpi). After 8 days of infection, the cell supernatants were collected, and viral replication was measured by ELISA. (B to E) HIV-1-infected PBMCs underwent electroporation with a BST-2/Tetherin siRNA (siBST-2) or a scrambled siRNA (SCR) 3 days after infection and, after 24 h, were treated with IL-27 (100 ng/mL). After 24 h of treatment (B and C), BST-2/Tetherin expression was analyzed by flow cytometry within the CD3⁺CD4⁺ gate. (D) Representative histogram of BST-2/Tetherin expression in cells exposed to IL-27 for 24 h. (E) After 8 days of infection, cell supernatants were collected, and HIV-1 replication was evaluated by measuring the concentration of HIV-1 p24 antigen by ELISA. The MFI ratio was calculated as follows: MFI ratio = specific MFI/FMO MFI. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two-way ANOVA with Tukey's multiple-comparison test; $n = 10$ [A], $n = 4$ [B to G], and $n = 6$ [H]; error bars represent \pm the SD).

reverse transcriptase inhibitor zidovudine (AZT) to reduce HIV-1 replication and viral transmission between cells (29–31). IL-27 was then added, and virus production was measured at 8 dpi. We found that IL-27 did not favor HIV-1 production by the cells maintained with AZT during the first 4 days of culture, whereas addition of IL-27 enhanced HIV-1 production by the cells maintained in culture medium only, as seen before (Fig. 5, two bars on the right). This result strengthened the suggestion that the contrasting action of IL-27 on HIV-1 replication could be dependent on the level of infection dissemination in the culture, which is consequent to cell-free and cell-to-cell viral transmission events.

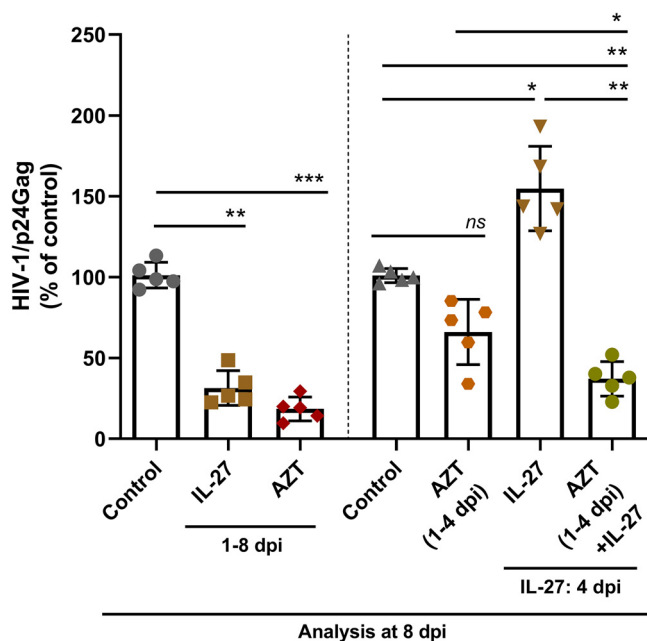


FIG 5 BST-2/Tetherin-mediated contrasting effects of IL-27 on HIV-1 infection are associated with the dynamic of viral production by target cells. PBMCs were infected, treated with IL-27 (100 ng/mL) immediately after infection or at 4 dpi, and maintained in culture for 8 days. HIV-infected PBMCs were maintained in culture the absence (Control) or presence of the reverse transcriptase inhibitor zidovudine (AZT; 1 μ M) as indicated, and IL-27 was then added. Infected cells were cultured for more 4 days in the absence of AZT. At the end of the culture period, supernatants were collected, and viral replication was evaluated by measuring the concentration of HIV-1 p24 antigen by ELISA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two-way ANOVA with Sidak's multiple-comparison test; $n = 5$; error bars represent \pm the SD).

Therefore, to define the influence of these events of transmission on the outcome of IL-27 treatment, we analyzed whether maintaining the culture under agitation, a condition that inhibits cell-to-cell transmission (32), could prevent the enhancement of HIV-1 replication observed when IL-27 is added at day 4 postinfection. In the static condition, IL-27 treatment at 4 dpi increased the number of HIV-1-infected CD4⁺ cells relative to control and IL-27 treatment at 0 dpi (Fig. 6A, static), but this augmentation did not occur when cells were maintained in constant agitation (Fig. 6A, shaking). At 8 dpi, we found that IL-27 lost its ability to favor HIV-1 growth when added later (4 dpi) to infected cells kept in agitation, recovering its inhibitory effect on HIV-1 replication (Fig. 6B, shaking 0 dpi). We also detected that when both agitation and IL-27 treatment were initiated on 4 dpi, IL-27 did not enhance HIV-1 replication, and the recovering of its inhibitory effect was less pronounced compared to shaking at 0 dpi (Fig. 6B, shaking 4 dpi).

Upon identifying that cell-to-cell contact was determinant for the IL-27-induced enhancement of HIV-1 at 4 dpi, we evaluated whether CD11a could be involved in this phenomenon. CD11a is an integrin widely expressed in lymphocytes (33) that contributes to cell-to-cell spread through the formation of virological synapses (34–37). We initially observed that neither HIV-1 infection nor IL-27 exposure modulated the expression of CD11a (see Fig. S5). Then, when cells were maintained in the presence of a CD11a-neutralizing antibody, IL-27 recovered its inhibitory effect when added at 4 dpi, reducing HIV-1 replication to the same level as that found when cells were treated at 0 dpi (Fig. 7). Overall, results from the assays that disturbed the cell-to-cell transmission of HIV-1 show that the dynamic of viral propagation in cultured PBMCs may influence the resultant vector of IL-27 modulation of HIV-1 replication, with participation of BST-2/Tetherin, since either effect, inhibition or increase of HIV-1 replication induced by IL-27, was impaired when this protein was silenced (Fig. 3 and Fig. 4).

DISCUSSION

Our present results add new evidence that IL-27 does not function solely as an HIV-1 restriction factor and, instead, can also act to favor viral propagation, findings that may

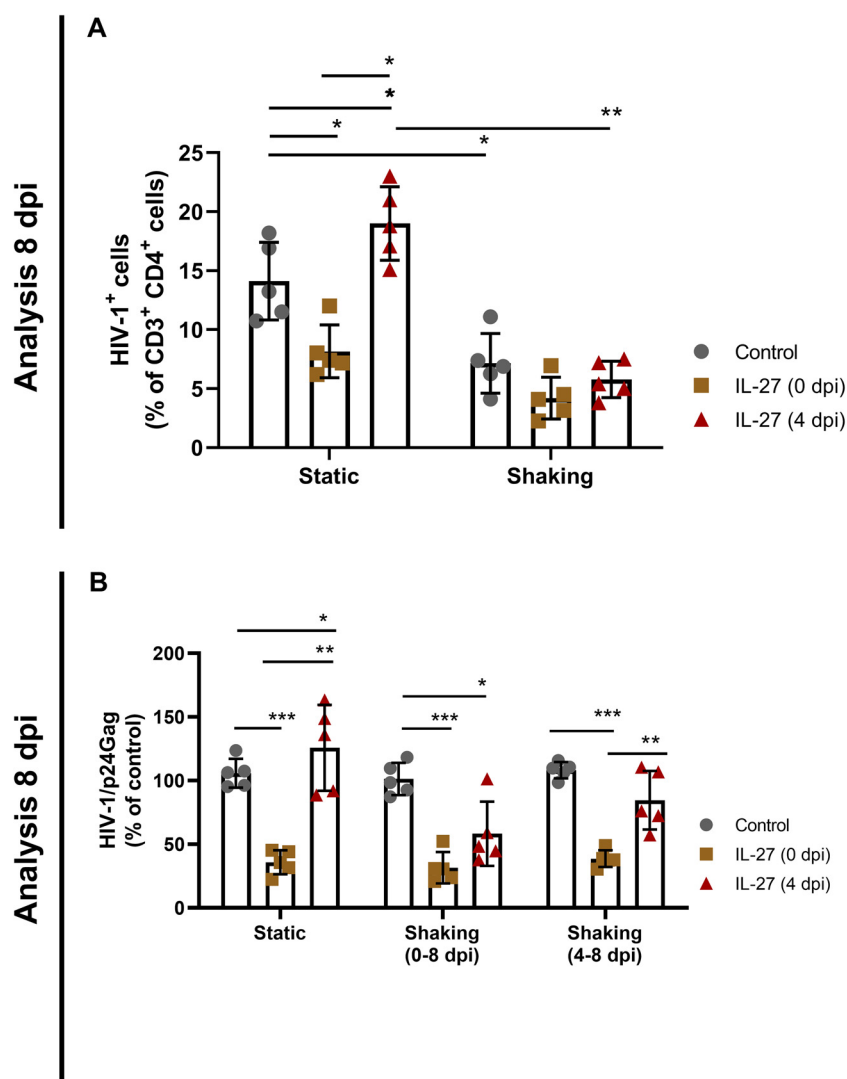


FIG 6 BST-2/Tetherin-mediated contrasting effects of IL-27 on HIV-1 infection are dependent on cell-to-cell contact. PBMCs were infected with HIV-1 and treated with IL-27 (100 ng/mL) immediately after infection (0 dpi) or 4 days later (4 dpi) and maintained under static or shaking culture conditions 8 days (A and B); also, in panel B, cells were maintained under static culture conditions for the first 4 days, followed by shaking for the next 4 days (right group of bars). HIV-1 infection was analyzed by flow cytometry and by ELISA at 8 days postinfection (8 dpi [A and B]). Data show the positive cell population for HIV-1 (A). Viral replication was evaluated by measuring the concentration of HIV-1 p24 antigen by ELISA (B). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two-way ANOVA with Tukey's multiple-comparison test; $n = 5$; error bars represent \pm the SD).

impact the perspectives of using this cytokine to treat HIV-1 infection. Further studies are needed to elucidate whether IL-27 exhibits similar divergent roles in other HIV-1 target cells, such as macrophages and dendritic cells, and if so, to search for the contribution of BST-2/Tetherin in those infection settings. Remarkably, the contrasting effects of IL-27 on HIV-1 growth in PBMCs are mediated by the same molecule, the IFN-induced protein BST-2/Tetherin. Our findings are summarized in the model presented in Fig. 8.

Initially, BST-2/Tetherin was described as an HIV-1 restriction factor that interferes with the budding and clearance of the virus at the membrane of infected cells (23). However, the consequence of this action is still debated, since several studies have noted differences in the BST-2 viral restraint capacity between HIV-1-infected macrophages, dendritic cells, and lymphocytes (25, 38–41). The most conflicting data show that, in lymphocytes, the “tethering” action of BST-2/Tetherin potentially limits the transmission of cell-free virus in culture but can

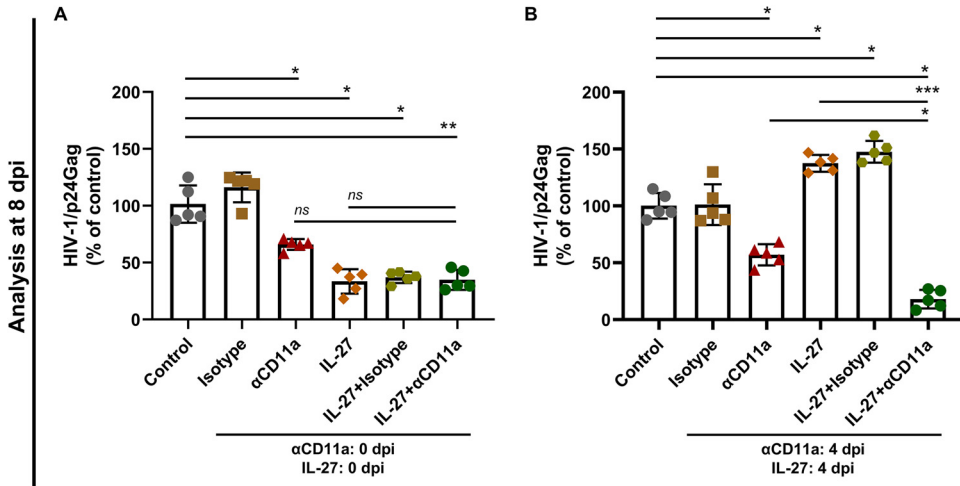


FIG 7 Involvement of CD11a in the effect of IL-27 on HIV-1 infection. (A and B) PBMCs were infected with HIV-1 and treated with IL-27 (100 ng/mL) and a CD11a neutralizing antibody (5 μg/mL) immediately after infection (0 dpi) (A) or 4 days later (4 dpi) (B). Viral replication was quantified 8 days later by measuring the concentration of HIV-1 p24 antigen in the cell culture supernatants by ELISA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (one-way ANOVA with Tukey’s multiple-comparison test, $n = 5$; error bars represent \pm the SD).

facilitate cell-to-cell transmission through the accumulation of viral particles at the infected cell membrane, thus leading to the formation of virological synapses (25).

We believe that the recovering of the anti-HIV-1 effect of IL-27 when BST-2/Tetherin is silenced at 4 dpi treatment can be accounted to other HIV-1-inhibitory mechanisms induced by IL-27, which, before surpassed by the pro-HIV-1 action of BST-2/Tetherin, now can act to inhibiting HIV-1 replication. Among them, we quote the cytokine IL-10, which expression is induced by IL-27 in CD4⁺ T lymphocytes (42–45) and can function as an HIV-1 inhibitory molecule, as reported by us and other authors (46–50). In fact, we found that IL-27 upregulated the IL-10 secretion in PBMCs and that neutralization of IL-10 signaling diminished the HIV-1 inhibitory effect of IL-27 (see Fig. S6). The participation of other viral restriction factors, such as MX-1, OAS, and APOBEC3 family members, in IL-27-induced HIV-1 inhibition could be expected; however, their induction by IL-27 seems to be restricted to macrophages, as reported by Imamichi’s group (17, 20), whose findings agree with our results concerning absence of APOBEC3F and APOBEC3G modulation by IL-27 in lymphocytes (see Fig. S2). Interestingly,

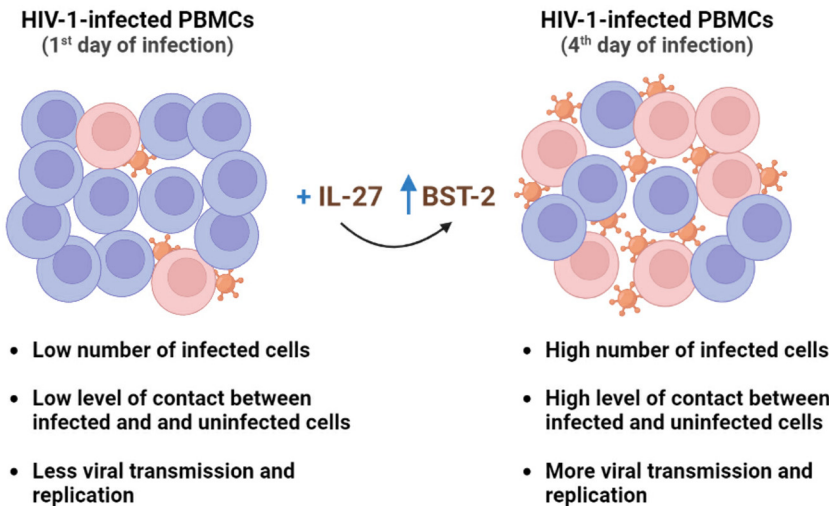


FIG 8 Graphical summary of study data. Induction of BST-2/Tetherin by IL-27 in HIV-1-infected PBMCs leads to divergent outcomes depending on the time of addition of this cytokine. The image was created with [BioRender.com](https://www.biorender.com).

Wahl et al. (19) showed that HIV-1-infected CD4⁺ T cells can express APOBEC3A upon IL-27 exposure, which has been, until now, the only APOBEC3 family protein known to be modulated in T lymphocytes by IL-27. Finally, macrophages differentiated in the presence of IL-27 present, upon maturation, elevated levels of antiviral microRNAs and reduction of β -spectrin, a cytoskeletal peptide that functions as an HIV-1 dependency factor (18, 51). Thus, it is up to question if CD4⁺ T cells exposed to or activated in the presence of IL-27 can provide the same results.

The difference in the effects of IL-27 observed between shaking and static conditions can be explained by the reduction of cell-to-cell contact in shaking condition. Thus, we hypothesize that most tethered virions are kept in the infected cells, and eventually recaptured (23), as opposed to a possible viral transference to new target cells when culture is maintained in static condition. Therefore, we propose that to function as an HIV-1 inhibitory factor in PBMCs, IL-27 requires that the number of infected cells should be small at the moment of its addition to infected cells, thus permitting BST-2/Tetherin to act as a restriction factor during the very first rounds of virus production. Also, we verified that the neutralization of CD11a, a key component of HIV-1 virological synapse (34–37), promoted the rescue of the HIV-1 inhibitory potential of IL-27, strengthening cell-to-cell transmission as the main mechanism involved in the HIV-1 propagation in cell culture. More studies are required to explore the possible involvement of IL-27 in HIV-1 transmission through virological synapses since, in the lymphoid tissue, HIV-1 propagation through cell-to-cell contact is expected to be predominant (52, 53).

Our findings that BST-2/Tetherin mediates the opposing effects of IL-27 on HIV-1 production by PBMCs are in agreement with those described by Jolly et al. (25), who found that the cell-to-cell spread of HIV-1 between primary CD4⁺ T cells is more efficient after type 1 IFN treatment, and that this type of HIV-1 dissemination is reduced following the knockdown of BST-2/Tetherin gene expression. In addition, our results are somewhat similar to those reported by Vendrame et al. (54), who observed that IFN- α is less efficient in countering HIV-1 cell-to-cell transmission when added at later times postinfection, or when a higher number of infected cells are present in the culture. IL-27 and IFN- α are intimately connected, as reported by Wahl et al. (19) and, together with BST-2/Tetherin and several other restriction factors, are the core of the innate immune response to HIV-1 (55). However, in more recent years, the canon regarding the beneficial effects of type 1 IFNs during HIV-1 infection has been questioned (56–60). The same scenario could be possible for IL-27, since Ruiz-Riol et al. (61) reported that elevated plasma levels of IL-27 and high expression of the subunit alpha of its receptor in PBMCs are correlated with increased viremia, high loads of HIV-1 provirus and lower HIV-1-specific T-cell response in patients. Because it is not clear whether HIV-1 infection impacts the plasma levels of IL-27 (14, 15), we may ponder that the potential beneficial effects of IL-27 on controlling viral loads in the initial course of an established infection could be jeopardized during the chronic phase, when an elevated number of infected cells are exposed to IL-27 and thus producing more viruses.

Our present work, together with previous data, shed light on the complexity of the HIV-1–host interactions, since the same factors that promote the innate control of HIV-1 could, in the course of infection, become detrimental to the host, through hindering the immune response and favoring viral dissemination. The identification of new interactions between components of the innate immunity, including the type 1 IFN response, could help to elucidate the dynamics of HIV-1 infection, thus contributing to improve the strategies to control viral propagation and disease progression.

MATERIALS AND METHODS

Cells, HIV-1 isolates, reagents, and ELISA kits. PBMCs from healthy donors were obtained by density gradient centrifugation (Ficoll-Paque Premium; GE Healthcare Life Sciences) from buffy coat preparations. These cells were resuspended in RPMI 1640 medium (LGC Bio, São Paulo, Brazil) supplemented with 10% heat-inactivated fetal bovine serum (Cultilab, Brazil), 100 U/mL penicillin, 100 mg/mL streptomycin, 2 mM glutamine, and 10 mM HEPES, stimulated with 5 μ g/mL phytohemagglutinin (PHA; Sigma-Aldrich) for 2 to 3 days, and further maintained in culture medium containing 5 U/mL recombinant human Interleukin-2 (Sigma-Aldrich). PBMC infection assays were performed with the HIV-1 R5-isolate Ba-L (donated by the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH,

Bethesda, MD). The virus isolates were expanded in phytohemagglutinin-activated PBMCs from normal donors, as described elsewhere (62). Recombinant human IL-27 and human IL-10 ELISA were purchased from R&D Systems, the antiretroviral drug zidovudine (AZT) was purchased from Sigma-Aldrich, HIV-1 p24 ELISA kits were acquired from Sino Biological, Human IL-10 ELISA was acquired from R&D, and neutralizing antibodies for CD11a and IL-10R, as well their respective isotypes, were purchased from Thermo Fisher and Abcam, respectively. Of note, cellular viability was evaluated throughout the study by trypan blue exclusion assay and by the XTT method, as described previously (50; data not shown).

HIV-1 infection and the effects of IL-27 on HIV-1 replication. PBMCs were infected by exposing them to cell-free virus suspensions containing 5 to 10 ng/mL p24 antigen as we described previously (63). After 2 h, the cells were washed to remove excess virus, and culture medium was added to the infected PBMCs. HIV-1 replication was evaluated by measuring the concentration of HIV-1 p24 antigen in the cell culture supernatants by a commercial ELISA kit (Sino Biological), according to the manufacturer's instructions. To address the role of IL-27 in HIV-1 replication, the HIV-1-infected PBMCs were distributed in 96-well culture plates (2×10^5 /well/200 μ L) and treated with recombinant human IL-27 at 0 or 4 dpi, without subsequent additions. In some experiments, IL-27 was added to PBMCs over 24 h, and the cells were washed and then infected with HIV-1 as described above. The cells were maintained in culture for different lengths of time, and HIV-1 replication was measured as described above. In specific experiments, the cells were distributed in 6-well culture plates (2×10^6 /well/2 mL), placed on an agitator (Bonther, Brazil) in a 37°C/5% CO₂ incubator, and gently shaken at 40 movements per min for different lengths of time.

Evaluation of APOBEC and BST-2/Tetherin gene expression by qPCR. PBMCs were left untreated or treated with IL-27 (100 ng/mL) or IFN- α (10 ng/mL) and, after 18 h, the cells underwent RNA extraction using the RNeasy minikit (Qiagen) according to the manufacturer's instructions. Total RNA was reverse transcribed with Superscript II according to the manufacturer's instructions (Invitrogen) in a 25- μ L reaction volume, and the resulting cDNA was amplified using SYBR green Master Mix with the following primers: BST-2/Tetherin, F-5'-CTGCAACCACACTGTGAT-3' and R-5'-ACGCGTCCTGAAGCTTAT-3'; APOBEC-3G, F-5'-GGCTCCACATAAACACGGTTT-3' and R-5'-AAGGAATCAGTCCAGGAA-3'; APOBEC-3F, F-5'-TGGAAAGTGTAAAGCACCACCTCA-3' and R-5'-AGCACCTTTCTGCATGACAATG-3'; and HPRT-1, F-5'-CCTGGCGTCGTGATTAGTG3' and R-5'-TCGAGCAAGACGTTTCAGTCC-3'. The HPRT gene was used as housekeeping control. Reactions were run in triplicate using a StepOne Plus instrument (Applied Biosystems) for all independent experiments.

Analysis of cell markers by flow cytometry. At the indicated times, cells were removed from culture flasks, incubated with AB+ human serum and mouse serum for 15 min (blocking solution: phosphate-buffered saline plus 2 mM EDTA, 1% bovine serum albumin, 25% human serum, and 25% mouse serum), labeled with specific antibodies for 40 min, and permeabilized for intracellular staining (when using an anti-HIV-1 antibody). All staining incubations were performed using the blocking solution as the diluent. The following antibodies, as well as the corresponding isotype controls, were used: mouse anti-human CD3-APC (BD Biosciences), mouse anti-human CD4-APC-Cy7 (BD Biosciences), mouse anti-human CD317 (BST2/PDCA-1)-Alexa Fluor 488 (eBioscience), anti-HIV-1 gag (RD1, KC57 clone; Beckman Coulter), and mouse anti-human CD11a-Pe-Cy5 (BD Biosciences). After a washing step, the cells were fixed, and the staining was evaluated with a FACSCelesta flow cytometer (BD Biosciences) equipped with FACSDiva software (BD Biosciences). All analyses were performed after doublet and debris exclusion using forward versus side scatter parameters, and live/dead exclusion was performed (Live/Dead Fixable Scarlet 723 stain kit; Thermo Fisher, USA). HIV-1, BST-2/Tetherin, and CD11a expression were analyzed based on FMO (fluorescence minus one) controls. The FMO controls and the HIV-1 negative control are depicted in Fig. S7. Figures S8 and S9 in the supplemental material show the representative histograms of experiments. The median fluorescence intensity (MFI) ratio was calculated as follows: MFI ratio = specific MFI/FMO MFI. The data were analyzed by using FlowJo software (TreeStar).

Ultrastructural analysis of the effects of IL-27 on virus release from infected cells. For TEM analysis, HIV-1-infected PBMCs treated or not with 100 ng/mL of IL-27 during 48 h were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 h at 25°C and postfixed with a solution of 1% OsO₄ containing 0.8% potassium ferricyanide and 2.5 mM CaCl₂ in the same buffer for 20 min at 25°C. Then, the samples were dehydrated in an ascending acetone series and embedded in Polybed 812 resin (Polysciences, USA). Ultrathin sections were obtained, stained with uranyl acetate and lead citrate, and examined with the transmission electron microscope JEM 1011 (Jeol, Tokyo, Japan) at the Focruz Electronic Microscopy Platform.

BST-2/Tetherin gene silencing. HIV-1-infected PBMCs underwent BST-2/Tetherin siRNA electroporation 2 h (protocol 1) or 3 days (protocol 2) after infection. A total of 10⁷ PBMCs were pelleted and resuspended in 100 μ L of prewarmed electroporation buffer [in-house prepared buffer solution 3P containing 5 mM KCl, 15 mM MgCl₂, 90 mM NaCl, 10 mM glucose, 0.4 mM Ca(NO₃)₂, and 40 mM Na₂HPO₄/NaH₂PO₄ (pH 7.2) (64)] premixed with BST-2/siRNA (5'-AAGCGTGAGAATCGCGGACAA; NM_004335; Hs_BST2_5 FlexiTube siRNA; Qiagen, catalog number SI02777054) or scramble/siRNA (Ctrl_AllStar_1; Qiagen, catalog number SI03650318) to a final concentration of 200 nM. Then, the cells were transferred to sterile 0.2-cm generic cuvettes (Mirus Biotech, catalog number MIR 50121) and electroporated with a Nucleofector 2b device (T-020 program; Lonza). After electroporation, the cells were immediately and gently resuspended in 1 mL of prewarmed RPMI medium supplemented with 5 U/mL rIL-2 (Sigma-Aldrich) and 20% fetal bovine serum, transferred to cell culture flasks, and incubated at 37°C in 5% CO₂ for 24 h. The day after electroporation, the cells were centrifuged and divided into two groups, one with and one without rIL-27. The cells were maintained in culture for an additional 24 or 48 h for flow cytometry analysis or for an additional 5 days (protocol 1) or 8 days (protocol 2) to measure HIV-1 replication.

Statistical analysis. All results presented in this study were prepared using Prism 9.0 software (GraphPad, San Diego, CA). Statistical analysis was performed using a Student *t* test or one-way analysis of variance (ANOVA) or two-way ANOVA, followed by a Sidak's or Tukey multiple-comparison test when appropriated. The results are shown as means \pm the standard deviations (SD), and comparisons between

values were considered significantly different when the *P* value was <0.05 (*, *P* ≤ 0.05; **, *P* ≤ 0.01; ***, *P* ≤ 0.001).

Statement of ethics. Experimental procedures involving human cells from healthy donors were performed with samples obtained after we obtained written informed consent and were approved by the Institutional Review Board of the Oswaldo Cruz Institute/Fiocruz (Rio de Janeiro, Brazil) under number 397-07.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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We declare there are no competing financial interests.

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