Interleukin-2 Inhibits HIV-1 Replication in Some Human T Cell Lymphotropic Virus-1-infected Cell Lines via the Induction and Incorporation of APOBEC3G into the Virion*

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Background: Some cytokines exhibit dichotomous effects on HIV replication in different cell types.

Results: IL-2 inhibits HIV-1 replication in MT-2 cells via intracellular induction and virion incorporation of the host restriction factor, APOBEC3G.

Conclusion: Stimulation of MT-2 cell with IL-2 produces viruses with impaired replication competency.

Significance: This new insight could open the door to additional host-based strategies to suppress HIV-1 replication.

IL-2 has been used in culture of primary T cells to maintain cell proliferation. We have previously reported that IL-27 inhibits HIV-1 replication in primary T cells in the presence of IL-2. To gain a better understanding of the mechanisms involved in this inhibitory effect, we attempted to investigate in detail the effects of IL-27 and IL-2 using several cell lines. Unexpectedly, IL-27 did not inhibit HIV-1 in T cell lines, whereas IL-2 inhibited HIV-1 replication in the human T cell lymphotrophic virus (HTLV)-1-transformed T cell lines, MT-2, MT-4, SLB-1, and ATL-2. No effects were seen in HTLV-1-negative cell lines. Utilizing MT-2 cells, we demonstrated that IL-2 treatment inhibited HIV-1 syncytia-inducing ability and dose-dependently decreased supernatant p24 antigen levels by >90%. Using real time PCR and Western blot analysis, we observed that IL-2 treatment induced the host restriction factor, APOBEC3G with production of incompetent viruses (6, 7). In addition to the antiviral restriction factors (17, 18), and some are capable of either inhibiting or enhancing replication depending upon the culture system and conditions. In this regard, IL-2 has been shown to be capable of suppressing HIV-1 replication through the enhancement of A3G (22, 26–29).

APOBEC3 (A3) proteins have been reported as potent host-antiviral restriction factors that encode DNA-editing deaminase enzymes. Of the seven A3 genes (A3A, A3B, A3C, A3D/E, A3F, A3G, and A3H) encoded by human genome, A3G and A3F are the most studied (22). A3G has been shown to be packaged into virions in Vif-deleted HIV. It confers resistance to retroviral infection through the deamination of deoxycytidines to deoxouridines during reverse transcription. This enzymatic activity results in G to A hypermutation of the plus strand DNA with production of incompetent viruses (6, 7). In addition to the enzymatic editing of HIV reverse transcripts, nonenzymatic mechanisms for A3G activity have also been suggested (23–25). HIV-1 Vif (virus infectivity factor) counteracts the antiviral activity of A3G by inducing the 26 S proteasome-mediated degradation of A3G (22, 26–29).

In previous studies, IL-27 was shown to have potent anti-HIV effects in primary CD4+ T cells, monocyte-derived macrophages, and dendritic cells (11, 30–33). In those studies, using...
primary CD4 T cells, IL-2 was supplemented in the culture medium to maintain T cell proliferation. Our lab also recently found that IL-27 induces potent antiviral microRNAs (34), thus suggesting that IL-27 could be a potential therapeutic cytokine for viral infection. The present study initially sought to extend the IL-27-mediated anti-HIV effect by looking at other cell types including the HTLV-1<sup>2</sup> transformed cell line, MT-2. Unexpectedly, we found that IL-2 alone had a profound suppressive effect on HIV-1 replication in this system. The study was then modified to understand this observation by examining the relationships between IL-2 and host factors known to be involved in the regulation of HIV-1 infection.

Here, we demonstrate that IL-2 inhibits HIV-1 replication in certain HTLV-1-infected cell lines by enhancing A3G expression and facilitating its incorporation into the virion of a Vif-positive virus. This new insight could open the door to additional host-based strategies to suppress HIV replication.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents—**The MT-2 (35, 36), MT-4 (37, 38), and H-9MN (39) cell lines were obtained through the AIDS Research and Reference Program, Division of AIDS, NIAID, National Institutes of Health (Rockville, MD). HTLV-1-transformed cell lines, SLB-1, TLMO-1, ED, and ATL-2, were kind gifts from Prof. Masao Matsuoka (Institute for Virus Research, Kyoto University, Kyoto, Japan). Rabbit polyclonal antibody to APOBEC3G and mouse monoclonal antibody to HIV-1 Vif were obtained from Abcam (Cambridge, MA). Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors by Ficoll-Hypaque centrifugation, as previously described (11). CD4<sup>+</sup> T cells were purified from PBMCs using CD4 microimmunomagnetic beads (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. CD4<sup>+</sup> T cells were stimulated with phytohemagglutinin (Sigma-Aldrich) plus IL-2 (Roche Molecular Biochemical) or with phytohemagglutinin alone. All cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FBS (HyClone Laboratories, Logan, UT), 10 mM of l-glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Quality Biologic, Gaithersburg, MD). IL-4, -7, -9, and -15 were purchased from R & D Systems (Minneapolis, MN); IL-21 was obtained from Drs. B. Matija Peterlin and Yong-Hui Zheng (WI). Briefly, DNA and the lipid reagent were diluted in serum-free RPM1 and mixed together at a ratio of 1 μg of DNA to 12 μl of lipid. The DNA-lipid complex was incubated at room temperature for at least 20 min and added dropwise to MT-2 cells at a ratio of 1 μg of DNA to 1 × 10<sup>6</sup> cells. MT-2 cells were then seeded at 4 × 10<sup>6</sup> cells/ml and cultured overnight at 37 °C. Transfected cells were cultured in the presence or absence of IL-2 for 48 h. The reporter assay was performed as previously described (43). Luciferase activity was normalized using total cellular protein measured with BCA protein assay kit (Pierce). Western Immunoblot Assay—Western blotting was performed as previously described (43). Briefly, HIV-infected MT-2 cells were cultured in the presence or absence of IL-2 (20 units/ml) for 2, 4, or 7 days at 37 °C. Total cell lysates were obtained using radioimmune precipitation assay buffer containing protease inhibitor cocktails (Sigma-Aldrich) and phos-

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2 The abbreviations used are: HTLV, human T cell lymphotrophic virus; PBMC, peripheral blood mononuclear cell; LMM, lower molecular mass.
phatase inhibitors (Thermo Scientific, Rockford, IL). Total protein was measured with a BCA protein assay kit (Pierce). An anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) served as an internal control (43). Fold changes were quantitated using the image processing and analysis in Java software (ImageJ software). For Western blotting using HIV virions, HIV-1-infected MT-2 cells were cultured for 4 days at 37 °C in the presence or absence of IL-2. The culture supernatants were filtered through a 0.22-μm filter, followed by ultracentrifugation, using the SW41 swing rotor at 10,000 × g for 1 h at 4 °C. Pelleted virions were washed with PBS and then lysed in radio-immune precipitation assay buffer, and the p24 amount was quantitated by a p24 capture antigen ELISA. A total of 2 ng of p24 was used for each Western blot analysis. The membranes were probed with patient plasma, anti-p24 monoclonal antibody (Abcam), anti-APOBEC3G antibody, or anti-Vif monoclonal antibody (Abcam).

Quantitation of HIV-1 Copy Number—MT-2 cells were infected with DNase I-treated HIVNL4.3 virus for 2 h at 37 °C in the presence or absence of 1 μM azidothymidine. The cells were washed and cultured for 1, 2, 4, or 7 days in the presence or absence of IL-2 and/or azidothymidine. HIV binding assays and proviral DNA copy numbers were determined by real time PCR. In the HIV binding assay, MT-2 cells were pretreated with or without 20 units/ml of IL-2 for 4 days, and the cells were incubated with DNase-treated HIV-1 at 4 °C for 2 h, followed by washing with ice-cold PBS. HIV-1 infection or binding was evaluated by measuring the products of viral cDNA synthesis at 24 and 48 h postinfection, as described previously (45). Copy numbers were normalized to total cell numbers determined by real time PCR using CCR5-specific primers (11, 46). Absolute numbers of copies of proviral DNA copy numbers were determined at days 1, 2, 4, and 7 postinfection. To obtain absolute proviral DNA copy numbers, a standard curve was generated using serial dilutions of a plasmid encoding HIV gag region (pHIV-gag) and RNaseP gene (pRNaseP) as described (47).

Viral Infectivity—HIVNL4.3-infected MT-2 cells were cultured for 4 days at 37 °C in the presence or absence of IL-2 (20 units/ml). The cells were washed and infected with HIVNL4.3 and then cultured for an additional 4 days. Culture supernatants from treated, pretreated, or untreated samples were filtered through a 0.22-μm filter, and virions were pelleted by ultracentrifugation. The 50% tissue culture infectious dose (TCID50) of each virion was determined as previously described (44).

RT-PCR and Real Time Quantitative PCR—Total cellular RNAs were isolated from MT-2 cells using an RNeasy kit (Qiagen). Reverse transcription was performed using SuperScript first strand synthesis system for RT-PCR (Invitrogen) as previously described (43). Real time quantitative PCR was performed as previously described (48). All probes were obtained from Applied Biosystems.

Analysis of APOBEC3G Molecular Size by FPLC—APOBEC3G molecular size was characterized by FPLC (GE Healthcare). Briefly, MT-2 cells (10 × 10^6 cells) were cultured at 0.2 × 10^6 cells/ml in the presence or absence of 20 units/ml of IL-2 for 48 h at 37 °C. After treatment, the cells were washed with ice-cold PBS. The cells were lysed using 2 ml of A3G lysis buffer (50 mM HEPES, 125 mM NaCl, and 0.2% Nonidet P-40 (Roche Applied Science), pH 7.4) containing a mixture of phosphatase (Thermo Scientific, Rockford, IL) and protease inhibitors (Sigma-Aldrich). To remove cellular debris, the cell lysate was centrifuged at 3000 × g for 10 min at 4 °C, and then, using a 0.45-mm membrane (Millipore, Billerica, MA), supernatants were filtered and stored at −20 °C until used. Protein concentrations of the whole cell lysates were determined using a BCA protein assay kit. A total of 2 mg of the lysate was applied to a calibrated Sephacryl S-300HR 6HR 16/60 column equilibrated with A3G buffer with 10% glycerol (Sigma-Aldrich), 1 mM DTT (Sigma-Aldrich), and 0.05% NaN₃. One column volume (120 ml) was collected in a total of 24 fractions (5 ml/fraction) at a 0.9 ml/min flow rate. All fractions were concentrated using the Ultracel-10K (Amicon Ultra; Millipore) and subjected to SDS-PAGE analysis in a Centricon Cell system (Bio-Rad), followed by Western blot using anti-A3G antibody.

siRNA-mediated Knockdown of APOBEC3G—MT-2 cells were transfected with siRNAs using an Amaxa nucleofection program (X-005). 200 nm of siRNAs targeting APOBEC3G messenger RNA at residues 726–746 (siA3G726) or 883–901 (siA3G883) in the coding sequence for A3G were used per 1 × 10^6 cells. The siRNAs were chemically synthesized by Qiagen as previously reported (49). siRNA targeting the GFP was chemically synthesized by Applied Biosystems and used as a control siRNA. After transfection, the cells were incubated at 37 °C for 48 h. They were then washed, infected with HIV-1NL4.3, and monitored for A3G protein expression or HIV infection by Western blotting or a p24 antigen capture ELISA, respectively.

Sequence of Virion RNA—HIV-1-infected MT-2 cells were cultured for 7 days in the presence or absence of IL-2. Virions were pelleted by ultracentrifugation and used to infect fresh MT-2 cells. Genomic RNAs were isolated 48 h after infection and used as templates for PCR of the env and pol (protease, reverse transcriptase, and integrase) regions using specific primers. Primers used for the amplification of the env and pol regions were: forward F208env, 5′-GCCACACATGCCTGTACCCACAAG-3′; reverse R709env, 5′-CTGCTAGACTGTGCGATTTAAGCAGCA-3′; forward HIV1996F, 5′-TTCATGCTGCGAAAGAAGGGCACC-3′; and reverse HIV5154R, 5′-CCAGTCCCTATGCTTTCCCTGA-3′, respectively. The PCR products obtained were cloned into the pCR2.1 TOPO TA cloning vector. Sequencing was done using an ABI Prism genetic analyzer 3130x.

Statistical Analysis—Statistical analysis was performed using the unpaired t test of the StatView program (Abacus Concepts, Berkeley, CA).

RESULTS

IL-2 Inhibits HIV-1 Replication in MT-2 Cells—We have previously demonstrated that IL-27 inhibits HIV-1 replication in primary T cells (11). In that study, IL-2 was supplemented in culture medium to maintain cell proliferation. To define the molecular mechanism by which IL-27 suppresses viral replication, the first set of experiments was designed to look at the individual and combined effects of IL-2 and IL-27 on HIV-1 replication in a variety of cell types. As shown in Fig. 1A, in the absence of IL-2 (IL-27 alone in the figure), IL-27 had no significant impact on HIV replication in all of the cell types studied.
IL-2 alone enhanced HIV replication in primary CD4(+) T cells, and this enhancement was inhibited by ~90% in the presence of IL-27. Surprisingly, IL-2 alone was associated with ~90 and 60% inhibition of HIV-1 replication in HTLV-1-infected MT-2 and MT-4 cell lines, respectively. This inhibition was not modified by the addition of IL-27. Viral replication in the chronically HIV-1-infected T cell line (H9MN) was not changed by either a cytokine or a combination of cytokines. To define the role of HTLV-1 in the IL-2-mediated effect on HIV replication, we next examined five other HTLV-1-transformed cell lines: SLB-1, TLOM-1, ED, ATL-2, and MT-1 (Fig. 1B). Of these cells, IL-2 significantly \( p < 0.01 \) inhibited HIV-1 replication in SLB-1 and ATL-2 cells, whereas it enhanced HIV-1 replication in ED cells. Because the inhibition was stronger in MT-2 than in MT-4, SLB-1, or ATL-2 cells, we used MT-2 cells in subsequent experiments and focused our attention on the mechanisms underlying IL-2-mediated inhibition of HIV-1 replication in this cell line.
We next sought to confirm this observation by examining the effects of IL-2 on HIV-1 replication in MT-2 cells under phase contrast microscopy and performing a series of dose-response experiments. IL-2 had an IC50 of 0.6 unit/ml (Fig. 1C) and at a concentration of 20 units/ml led to >85% reduction in syncytia formation (Fig. 1D). IL-2 from another source (R & D Systems) also showed the same anti-HIV effect (data not shown), indicating that the IL-2 effect is due to IL-2 and not to contaminated materials. MT-2 produces HTLV-1, and it is reported that HTLV-1 replication modulates HIV-1 replication; thus we analyzed the impact of IL-2 on the production of HTLV-1. Of interest, the IL-2-mediated inhibition was not associated with changes in HTLV-1 production (Fig. 1E). IL-2 is a member of common gamma cytokine chain receptor family (50–52), so we also assessed the anti-viral effect of the other member of this family. IL-4, -7, and -15 inhibited HIV; however, the anti-HIV effect was not seen with IL-9 or IL-21 (Fig. 1F).

IL2-mediated Inhibition Occurs Post-transcriptionally—To determine which stage of the HIV-1 replication cycle is interrupted by IL-2, we first examined whether or not there were reductions in the cell surface expression of molecules known to be involved in HIV-1 entry/infection. There was no change in CD4 expression and an increase in expression of the HIV-1 co-receptor CXCR4 (Fig. 2A). On the host cellular machinery, we show that IL-2 stimulation had no effect on the cell proliferation (Fig. 2B). These data indicate that IL-2 had no impact on either viral entry or cell growth. Next, we looked at whether or not IL-2 had an impact on the reverse transcription of the viral genome as evidenced by intracellular levels of proviral DNA. As can be seen in Fig. 2C, proviral DNA copy numbers were not different at days 1 and 2 between IL-2-treated and control cells, whereas at days 4 and 7, cells treated with IL-2 had significantly fewer copies of HIV-1 proviral DNA, indicating that IL-2 may not interfere with the initial round of reverse transcription but may have an effect on viral spread, leading to lower total levels of HIV proviral DNA in later time points. To determine whether or not IL-2 also had any effect on HIV transcription, we performed a reporter assay using luciferase reporter gene downstream of the HIV LTR. In these experiments, IL-2 was found to have no effect on HIV-LTR promoter activity (Fig. 2D), indicating that IL-2 treatment had no impact on HIV replication at the induction of HIV transcription.

To elucidate the impact of IL-2 on the expression level of HIV proteins in MT-2 cells, Western blotting was performed using total cellular proteins collected from HIV-infected cells at days 4 and 7 postinfection. As shown in Fig. 2E, in the presence of IL-2, there was a decrease in all HIV-1 proteins at day 7. These data correspond to the results in Fig. 2C and suggest that in HTLV-1-transformed MT-2 cells, IL-2 may suppress secondary infection by nascent viruses from primary infected cells. To more precisely determine whether or not IL-2 inhibits second round infection, we utilized vesicular stomatitis virus-G pseudotyped virus, which is known to be incapable of establishing a spreading infection but efficient in single round infection. As can be seen in Fig. 2E, IL-2 had no impact on the copy number of vesicular stomatitis virus-G pseudotyped virus on day 6 after infection. These data indicate that although the single round of infection was not affected by IL-2 treatment, there was interruption in subsequent rounds of infection.

IL-2 Induces and Leads to the Accumulation of Active Forms of A3G—To more precisely determine the infectivity of the viruses produced in the setting of IL-2-stimulated cells and to test the hypothesis that IL-2 treatment inhibits second and subsequent rounds of infection, we performed a series of viral infectivity assays. As shown in Fig. 3A, IL-2 treatment led to the production of virions with an 80% decrease in infectivity (p < 0.01, n = 3), demonstrating that IL-2 treatment of MT-2 cells leads to production of viruses with impaired replication in MT-2 cell lines. To define whether or not the impaired replication is only seen in MT-2 cells, PBMC and primary CD4+ T cells were infected with viruses from control or IL-2-treated MT-2 cells and cultured for 7 days. As shown in Fig. 3B, viruses derived from IL-2-treated MT-2 cells were less infection competent in both cell types. These results indicate that the nascent virus is impaired for infection regardless of T cell type. Given the known associations between some host restriction factors (for example, A3G, A3F, and tetherin (BST-2)) and HIV-1 replication, we next looked to see whether or not the expression levels of tetherin, A3F, or A3G were modulated by IL-2 treatment of MT-2 cells. IL-2 did not lead to any significant changes in the mRNA expression levels of tetherin but was associated with a 1.8-fold increase (+, p < 0.01, n = 3) in the mRNA levels of A3G as determined by relative quantitative real time PCR (Fig. 3C). In addition, the intracellular levels of A3G protein level were increased by ~6-fold in the presence of IL-2 (Fig. 3D). No increases were observed in A3F or BST-2 (Fig. 3E). Kinetic study indicated that the increase in A3G protein expression and inhibition of HIV-1 replication was evident from day 2 of stimulation (Fig. 3F).

A3G is known to restrict HIV infection by producing replication-incompetent forms of HIV-1 through hypermutation. This would then be predicted to be associated with a decrease in secondary rounds of infection and, as demonstrated in Fig. 2 (C and E), lower levels of proviral DNA copy number and cellular HIV-1 proteins at day 7 of culture. It is reported that overexpression of A3G overcomes the activity of the HIV-1 Vif protein and that A3G can be incorporated into virions (53). If the mechanism underlying the observed IL-2-mediated inhibition of HIV replication in MT-2 cells is due to A3G, one might expect to see increased incorporation of A3G in the virions. As shown in Fig. 3G, Western blot analysis of HIV-1 virions revealed that IL-2 stimulation led to a >10-fold increase (n = 3) in the levels of virion-associated A3G.

Because several other gamma chain cytokines (IL-4, IL-7, and IL-15) inhibited HIV replication in MT-2 cells as well (Fig. 1F), we evaluated by Western blotting whether or not these cytokines also induced A3G packaging into the virion. As can be seen in Fig. 3H, IL-2 and IL-15 substantially induced A3G packaging, whereas IL-4, IL-7, and IL-21 did not.

A3G Is a Key Factor in the IL-2-mediated Inhibition of HIV Replication—It is reported that A3G exists in two different molecular weight forms: an active low molecular mass (LMM) form and an inactive high molecular weight form (54–56). To determine the molecular size of the induced A3G in IL-2-treated MT-2 cells, size exclusion assay using FPLC was per-
formed. Of note was the fact that the IL-2 increases in the total levels of A3G were associated with increases in the active, LMM form of A3G (Fig. 4A), indicating that IL-2 induces A3G and probably maintains the newly synthesized A3G in the LMM form. Two other gamma chain cytokines that either inhibited (IL-7) or did not inhibit (IL-21) HIV replication in MT-2 cells were used as controls. None of these led to increases in LMM A3G.

To demonstrate that the phenotype of A3 that is responsible for the observed inhibition is truly due to A3G, we performed a series of A3G knockdown experiments using two siRNAs targeted at A3G as previously described (49). As shown in Fig. 4B, siGFP as a control siRNA has no effect on IL-2-mediated inhibition of HIV-1 replication or induction of A3G. Interestingly, knockdown of A3G with either of two siA3Gs (siA3G726 and siA3G883) resulted in an abrogation of the effect of IL-2, suggesting that A3G is responsible for a substantial amount of the observed effect.

If increased A3G activity is the mechanism underlying the observed decrease in HIV-1 production associated with IL-2 stimulation of MT-2 cells, one would also expect an increase in

FIGURE 2. The effect of IL-2 on HIV-1 replication cycle. A, the effect of IL-2 on the expression of HIV-1 entry molecules on cell surface. Uninfected MT-2 cells were cultured in the presence or absence of IL-2 (20 units/ml) for 4 days. Expression of CD4 and CXCR4 were measured by FACS using FITC-conjugated anti-CD4 (left panel) and PE-conjugated anti-CXCR4 antibodies (right panel), respectively. B, IL-2 did not change proliferation of MT-2 cells. The data represent the means ± S.E. from three independent experiments. C, MT-2 cells were infected with HIV-1 for 2 h at 37 °C and cultured for 1, 2, 4, or 7 days, and the HIV-1 proviral copy numbers were determined by real-time PCR and normalized using RNaseP probe. *, p < 0.01. The data represent the means ± S.E. from three independent experiments. D, IL-2 treatment does not alter HIV-1 LTR promoter function. MT-2 cells were transiently transfected with plasmid DNA containing an HIV-1-LTR-luciferase reporter gene (pLTR-Luc) with our without a full-length Tat expression plasmid (pTat). Transfected cells were cultured in the presence or absence of IL-2 (20 units/ml) for 48 h followed by luciferase reporter assay. Luciferase activity was normalized to total cellular protein obtained by BCA assay. The data represent the means ± S.E. from three independent experiments. E, IL-2 decreases the expression of HIV-1 proteins in MT-2 cells. HIV-infected MT-2 cells were cultured for 4 or 7 days in the presence or absence of IL-2 (20 units/ml). A total of 20 μg of cellular protein was separated on a 4–12% Bis-Tris gel and immunoblotted using HIV-infected patient plasma. F, IL-2 had no impact on the first cycle of HIV-1 infection as determined by the efficacy of pseudotyped HIV to produce viral particles. MT-2 cells were infected with vesicular stomatitis virus-G pseudotyped HIVNL4.3 virus at a concentration of 250 ng p24 virus per 1 × 10^6 cells for 2 h at 37 °C. The cells were washed and cultured for an additional 6 days in the presence or absence of IL-2 (20 units/ml). The genomic DNA was then isolated and used in a real-time PCR for quantification of proviral copy number. To obtain absolute proviral DNA copy numbers, a standard curve was generated using serial dilutions of plasmids encoding HIVgag or RNaseP gene. The results represent DNA copy numbers per 1 × 10^6 cells. The data show the means ± S.E. from three independent experiments. As a negative control, infection was also repeated in the presence or absence of 1 μM azidothymidine or with heat-inactivated virus (data not shown).
G to A mutations in the progeny viruses. An analysis of 3775-nucleotide bases from viruses expressed from control cells and 3775-nucleotide bases from viruses expressed from IL-2-treated cells revealed 8 G to A mutations (0.2%) in controls and 60 G to A mutations (1.6%) in viruses from IL-2-treated cells. dG to dA hypermutations (defined as ≥ 3 G to A mutations) were detected in three clones from IL-2-treated samples. In contrast, no G to A hypermutation was detected from the untreated control clones (Table 1).

Given that MT-2 cells treated with IL-2 for 48 h accumulated LMM A3G, we were interested in determining whether pretreatment of MT-2 with IL-2 prior to HIV infection could lead to MT-2 resistance to HIV infection. As shown in Fig. 4C, pretreatment of MT-2 cells with IL-2 made the MT-2 cells relatively resistant to HIV-1 replication, consistent with the IL-2 effect being mediated via induction of a host factor such as A3G.

**DISCUSSION**

IL-2 is a member of the common cytokine receptor gamma chain family (50–52) and is commonly used in cultures of primary CD4 (+) T cells to maintain cell proliferation. In the present study we have demonstrated that IL-2 and three other com-
mon gamma chain cytokines (IL-4, IL-7, and IL-15) inhibit HIV-1 replication in several HTLV-1-transformed cell lines (MT-2, MT-4, SLB1, and ATL2). Using the MT-2 cell line, we were able to demonstrate that part of the mechanism of IL-2-mediated inhibition involves enhancement of cellular expression and virion incorporation of A3G protein.

To determine the mechanism underlying the inhibition of HIV-1 by IL-2, we examined the effects of IL-2 on each stage of the HIV-1 life cycle. IL-2 had no effect on CD4 expression, initial proviral DNA synthesis by HIV reverse transcriptase, and transcriptional activation by the HIV-1 promoter. It did cause a profound decrease in the infectivity of the progeny virions.

Further study demonstrated that IL-2 treatment increased the total intracellular amount of A3G by 6-fold along with a 1.8-fold increase in A3G mRNA level, suggesting that IL-2 treatment may lead to an increase in intracellular A3G protein.

**FIGURE 4. Characterization of A3G as a key factor in IL-2-mediated inhibition of HIV replication.** A, IL-2 increases the production of LMM A3G. A3G molecular size was analyzed using FPLC, as detailed under "Experimental Procedures." A total of 24 fractions were collected from IL-2-, IL-7-, or IL-21-treated or untreated MT-2 cells and concentrated with centrifugal filter units. The samples were resolved with 4–12% SDS-PAGE, followed by Western blot, using anti-A3G antibody. B, knockdown of A3G protein suppresses the IL-2 inhibitory effect. MT-2 cells were transfected with mock siRNA, a control siRNA (siGFP), or one of two siRNAs targeted at A3G (siA3G726 and siA3G883) for 24 h. The cells were washed, infected with HIV-1, and cultured for an additional 7 days in the presence or absence of IL-2. HIV-1 replication in MT-2 cells was measured from the culture supernatants by p24 ELISA (top panel). The data represent the means ± S.D. from three independent experiments. *, p < 0.05. The cells were lysed with radioimmune precipitation assay buffer, and Western blotting was performed using anti-A3G antibody (bottom panel). Anti-β-actin was used as an internal control for equal loading of total protein. C, pretreatment of MT-2 cells with IL-2 inhibits HIV-1 replication. MT-2 cells were pretreated with IL-2 (20 units/ml) for 48 h at 37 °C (Pre-IL-2). The cells were washed, infected with HIVNL4.3 for 2 h, and cultured for 7 days in the absence of IL-2, whereas the untreated cells were cultured in the presence (IL-2) or absence (Control) of IL-2. HIV-1 replication was measured by p24 antigen ELISA.

**TABLE 1** Summary of IL-2-mediated APOBEC3G-induced mutations

HIV-1-infected MT-2 cells were cultured for 7 days in the presence or absence of IL-2. The virions were pelleted by ultracentrifugation and used to infect fresh MT-2 cells. Genomic DNA was isolated for 48 h after infection and used as templates in PCR of the env and pol (protease, reverse transcriptase, and integrase) regions using specific primers. PCR products were TA-cloned into the pCR2.1 TOPO cloning vector. DNA from resultant colonies was sequenced and analyzed for G to A mutations.

| Treatment | HIV replication | Total clones sequenced | Total bases sequenced | Total mutations vs. HIVNL4.3 | Total G to A mutations | Total GG to GA mutations | Total GA to AA mutations | Total GT to AT mutations | Other mutations | Number of clones with G to A hypermutations |
|-----------|-----------------|------------------------|----------------------|-----------------------------|------------------------|-------------------------|-------------------------|-------------------------|-----------------|------------------------------------------|
| Control   | 100             | 31                     | 3775                 | 19                         | 8                     | 0                      | 0                      | 11                      | 0               | 0                                        |
| IL-2      | 8               | 33                     | 3775                 | 81                         | 60                    | 36                     | 4                      | 21                      | 3               | 3                                        |
IL-2 Induces APOBEC3G Expression and Anti-HIV Activity

by decreasing its rate of degradation in MT-2 cells. Size exclusion column chromatography illustrated that IL-2-treated cells accumulated the LMM form of A3G. It has been reported that newly synthesized LMM A3G is rapidly recruited into HMM complexes in the presence or absence of HIV RNA (56). Thus it appears that IL-2 treatment suppresses the process of formation of HMM complexes from LMM forms of A3G in MT-2 cells. Because IL-2 treatment induced and led to accumulation of HMM in primary and non-HTLV-1-infected cells (Ref. 26 and data not shown), the suppression of LMM to HMM by IL-2 may be a mechanism unique to MT-2 cells and involve an interaction with HTLV-1 proteins.

A3G is a host cell cytidine deaminase that interferes with viral replication by inducing mutations, particularly G to A mutations (6, 7). The LMM forms are more active than the HMM (54–56), and the virions containing A3G are compromised in their ability to replicate because of poorer fidelity of reverse transcription, inhibition of reverse transcription, uracil-DNA glycosylase and apurinic-apyrimidinid endonuclease activities, and possibly other mechanisms yet to be described (6, 23, 25).

Previous studies have delineated the nucleotide substitution preferences of A3F and A3G, the two most widely studied A3 proteins with potent antiviral activity (57, 58). A3F and the other five APOBEC3 proteins (A3A, A3B, A3C, A3D, and A3H) prefer to deaminate GA to AA (57, 59–61), whereas A3G strongly prefers to deaminate GG dinucleotide motif resulting in AG hypermutations (6, 62, 63). According to Hultquist et al. (60), of the seven A3 proteins, A3A, A3B, and A3C appear irrelevant to HIV restriction, whereas A3D, A3F, A3G, and A3H are directly relevant to HIV restriction. Of note, IL-2 treatment induced the expression level of A3G but not A3F (Fig. 3D), suggesting that A3G may be the key factor in the IL-2 effect.

To determine whether or not the A3G in HIV virions derived from IL-2-treated MT-2 cells is functional, an extensive sequence analysis was performed on proviral DNA derived from MT-2 cells treated infected with virions derived from IL-2-treated MT-2 cells (Table 1). A higher number of G to A and GG to AG mutations were noted in the virions from the IL-2-treated MT-2 cells compared with virions from untreated cells.

Of note was the fact that the IL-2 effect was only observed in some HTLV-1 cells, and pretreatment of MT-2 cells with IL-2 was enough to suppress HIV replication (Fig. 4C). From these data we suspect that the IL-2-mediated inhibition of HIV replication may involve some direct interaction between HTLV-1 and the host that is modified by IL-2 in such a way as to make the cell less capable of producing infectious virions.

Three other members of the common cytokine receptor gamma chain family (IL-4, IL-7, and IL-15) were found to inhibit HIV-1 replication in MT-2 cells (Fig. 1F). Western blot analysis showed that only IL-2 and IL-15 led to pronounced incorporation of A3G into the virion, thus suggesting that IL-4 and IL-7 may utilize different mechanisms to inhibit HIV-1 replication. FPLC separations demonstrated that IL-4 and IL-7 did not induce accumulation of LMM A3G (Fig. 4A). Given that IL-2 and IL-15, but not IL-4 and IL-7, signal through a complex of the shared IL-2Rβ and common gamma chains (β/γc) (64). It is plausible that a signal mediated by the β/γc plays a role in the accumulation of the LMM form of A3G in MT-2 cells.

The effect of HIV/HTLV-1 co-infection on HIV-1 pathogenesis is a matter of debate. Soluble factors produced by HTLV-1-infected cells have been reported to be capable of either enhancing or suppressing HIV-1 infection (65). In addition HTLV-1 tax has been reported to up-regulate HIV-1 expression (66, 67). To evaluate the IL-2 effect on HIV-1 replication in the setting of HTLV-1 co-infection, we examined six HTLV-1-infected cells and one non-HTLV-1-infected cell line. Four (MT-2, MT-4, SLB1, and ATL2) of the six HTLV-1 cells lines tested showed some IL-2 effect, with no effect on the non-HTLV-1 cell lines. The reasons underlying this dichotomy are currently unclear. Further studies are warranted to define the relationships between IL-2, HIV, and HTLV-1.

Although HIV and HTLV-1 co-infection is rare worldwide, our finding reveals a potential new role for IL-2 and other common gamma chain cytokines on viral infection. This would provide a new insight for additional host-based strategies to suppress HIV replication.

In summary, we have identified a novel activity of IL-2 to inhibit HIV replication in some HTLV-1-transformed cell lines through the induction, modification, and incorporation of A3G. This activity is capable of overriding the effects of the Vif protein of HIV-1. However, this effect may not be a general effect on all T cell lines nor apply to primary CD4+ T cells. Given the rarity of HIV/HTLV-1 co-infection worldwide, this finding is unlikely to be of clinical significance but does provide some insights into basic mechanisms of host control of retroviral infections. The IL-2 effect reported in this study could be exploited to better understand the relationship between cytokines and HIV restriction through A3G-mediated mechanisms.

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