Human APOBEC3G (A3G), a deoxycytidine deaminase, is a broadly acting antiretroviral factor expressed in a variety of cells. Mitogen activation of CD4 T cells enhances A3G expression and leads to recruitment of low molecular mass (LMM) A3G, which functions as a post-entry human immunodeficiency virus (HIV) restriction factor, into enzymatically inactive, high molecular mass (HMM) RNA-protein complexes that include Stauфер RNA-transporting granules. We now report that interleukin-2 (IL-2), IL-15 and, to a lesser extent, IL-7 enhance the expression of A3G in peripheral blood lymphocytes and that this effect is blocked by inhibitors of the JAK and MAPK signaling pathways. In mixed cultures of CD4+ T cells containing either HMM or LMM A3G, HIV preferentially infected cells containing HMM A3G. A3G shifted into a HMM complex when IL-2, IL-7, or IL-15 was added to resting T cells, likely explaining how cytokine treatment renders resting CD4 T cells, likely explaining how cyto-

Infection. Similarly, poly(I:C)/tumor necrosis factor-

Human APOBEC3G (A3G),3 a powerful antiretroviral factor, is a deoxycytidine deaminase that induces dc-to-du mutations in plus-stranded DNA formed during reverse transcription. A3G employs this enzymatic activity, along with a possible non-

enzymatic activity, to disable a broad range of retroviruses, including human immunodeficiency virus (HIV), simian immunodeficiency virus, equine infectious anemia virus, murine leukemia virus, foamy virus, and human T-lymphotropic virus, as well as hepatitis B virus, a pararetrovirus (1–13).

A3G can exist in two different forms in cells: a low molecular mass (LMM) form, which restricts HIV spread, and an enzymatically inactive, high molecular mass (HMM) complex, which contains one or more inhibitory RNAs and lacks HIV-

restricting activity (14). HMM A3G complexes are found in mitogen-activated CD4+ T cells and in macrophages, whereas LMM A3G is present in blood-derived resting CD4+ T cells and monocytes, which are poorly permissive for HIV replication (14). In activated CD4+ T cells infected with HIV lacking Vif, A3G is incorporated into budding virions and exerts its potent antiviral activity in the subsequent target cell either by causing lethal hypermutation in nascent viral DNA transcripts formed during reverse transcription (1, 7, 9, 15) or by a non-
editing mechanism (16–18). A major function of HIV Vif is to deplete A3G from the cytoplasm of virus-producing cells, thereby preventing A3G from being incorporated into virions (19–23). Vif is thus absolutely required for the production of infectious virus emanating from A3G-expressing cells (24–29). Vif binds to A3G and promotes its polyubiquitication and sub-
sequent degradation by the 26 S proteasome (19–23).

A3G is a member of larger family of cytidine deaminases that includes APOBEC1, 2, 3A–H, and activation-induced cytidine deaminase. The APOBEC3A–H family is expressed in a tandem array on chromosome 12 and plays a critical role in class-switch recom-

bination and somatic hypermutation in B cells, is induced by phorbol esters and consequently were originally termed phorbolin-1 and -2, respectively (30). Another cytidine deaminase family member, activation-induced cytidine deaminase, which is present on chromosome 12 and plays a critical role in class-switch recom-

bination and somatic hypermutation in B cells, is induced by IL-4 (31) as well as by cell division (32).

The A3G gene is also inducible. Treatment of resting peripheral blood lymphocytes (PBLs) with phyoheammaglutin (PHA) and IL-2 increases expression of the A3G protein (19). Similarly, treatment of the H9 T cell line with phorbol myristate acetate (PMA) induces expression of A3G mRNA and protein (33), a result that correlates with the effects of phorbol esters on A3A and A3B. Further, PMA activation of A3G gene expression

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Cytokine-mediated Regulation of APOBEC3G

is dependent on activation of the mitogen-activated protein kinase (MAPK) signaling pathway in H9 leukemic T cells (33).

Mitogenic activation relieves the A3G-mediated post-entry restriction block in CD4+ T cells and causes these cells to become permissive to HIV infection (14). The mitogen effect is caused by shifting A3G from its active LMM form into inactive HMM complexes that include Staufen RNA-transporting granules and Ro ribonucleoprotein complexes (34). Certain cytokines, including IL-2, IL-7, and IL-15, have been reported to render resting CD4+ T cells permissive for HIV infection (35–38). We have explored the possibility that these cytokines activate A3G gene expression and recruit the LMM A3G present in primary resting CD4+ T cells into HMM A3G complexes. In addition, we have examined A3G expression in interferon-treated monocyte-derived macrophages and in dendritic cells (DCs).

EXPERIMENTAL PROCEDURES

Isolation and Culture of Primary Cells— Buffy coats from individual healthy donors were processed on Ficoll-Hypaque density gradients to isolate peripheral blood mononuclear cells. CD14+ PBLs were prepared by negative selection with anti-CD14 microbeads (Miltenyi Biotec) according to the manufacturer’s instructions using Automacs technology (Miltenyi Biotec). Residual CD14+ cells were further depleted by adherence to plastic for 3 h. To enrich CD4+ cells, PBLs preincubated with anti-CD4 microbeads were positively selected with the Automacs. The purity of these populations was >95% when analyzed by flow cytometry. Cells were routinely cultured (2 × 10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/streptomycin.

Immature DCs were derived by culturing CD14+ monocytes for 6 days in complete medium supplemented with 25 ng/ml IL-4 (R&D Systems) and 50 ng/ml granulocyte/macrophage colony-stimulating factor (BIOSOURCE) (39). DC maturation was induced by incubating the immature DCs for 24 h with 25 μg/ml poly(I:C) (Amersham Biosciences) and 5 ng/ml tumor necrosis factor-α (BIOSOURCE) (40). The phenotype of these DCs was verified by immunostaining with antibodies specific for immature and mature DCs. Macrophages were derived from CD14+ blood monocytes by culturing these cells for 7–12 days in medium supplemented with 5% human AB serum (Omega).

Cytokine Stimulation—To study potential changes in A3G expression over time, freshly isolated CD14+ PBLs were preincubated overnight at an approximate density of 2 × 10^6 cells/ml in medium and then stimulated with IL-2 (50 ng/ml), IL-6 (50 ng/ml), IL-7 (62.5 ng/ml), IL-9 (50 ng/ml), IL-15 (12.5 ng/ml), or PHA (5 μg/ml). All cytokines were purchased from R&D Systems. Samples were collected 0, 6, 12, 18, 24, 48, 120, and 168 h following treatment and analyzed by quantitative real-time PCR to assess changes in A3G mRNA levels or by immunoblotting with a rabbit anti-human A3G-specific antibody to detect changes in protein expression (19). Dose-response experiments with these cytokines as well as with IL-4, IL-12, and IL-13 (R&D Systems) were performed similarly with samples collected at 48 h for real-time PCR analysis and at 120 h for immunoblotting studies. The potential effects of interferon on A3G expression were studied in macrophages treated with interferon-α (1000 units/ml; Schering-plough, intron a); interferon-β (1000 units/ml; R&D Systems), or interferon-γ (1000 units/ml; R&D Systems). For the kinase inhibitor experiments, CD14+ PBLs were pretreated for 1 h with 12.5, 50, or 100 μM AG490 (Calbiochem) or 2.5, 10, or 20 μM U0126 (Cell Signaling) before being stimulated with the indicated cytokine or PMA. To analyze A3G complexes in cytokine-treated cells, freshly isolated CD4+ T cells were treated with the indicated cytokine or mitogen for 5 days, washed with ice-cold 1× phosphate-buffered saline, and lysed before analysis by fast protein liquid chromatography (FPLC) as described below.

Immunoblotting—Harvested cells were washed in ice-cold phosphate-buffered saline and then lysed in lysis buffer (LB; containing 50 mm Heps, pH 7.4, 125 mm NaCl, 0.2% Nonidet P-40, 0.1 mm phenylmethylsulfonil fluoride, and 1× EDTA-free protease inhibitor mixture (Calbiochem)) or, for the phosphorylation studies, radioimmune precipitation assay buffer (containing 50 mm Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.1% sodium deoxycholate, 150 mm NaCl, 2 mm NaVO₃, 25 mM NaF, 0.1 mM phenylmethylsulfonil fluoride, and 1× EDTA-free protease inhibitor mixture (Calbiochem)). After clarification of the lysates, protein concentrations were determined using the BCA protein assay kit (Pierce). An equal amount of lysate protein from different samples was loaded in individual lanes of 12.5% SDS-polyacrylamide gels followed by electrophoretic separation of proteins. Proteins were then transferred to Immobilon P polyvinylidene difluoride (Millipore) membranes followed by immunoblotting with anti-A3G antibodies (19), anti-ERK1/2 (Santa Cruz Biotechnology), anti-phospho-ERK1/2 (Cell Signaling), or anti-PKR (Santa Cruz Biotechnology). The specificity of the anti-A3G antibody has been described previously (41). Membranes were subsequently stripped and re-probed with anti-β-actin antibodies (Sigma) to compare protein loading.

Quantitative Real-time PCR—A3G mRNA transcripts were assayed using quantitative kinetic reverse transcription PCR methodology (Applied Biosystems) and Taqman chemistry (Roche Applied Science). Total RNA was extracted from pelleted cells with RNeasy (Qiagen) and subjected to on-column DNase prior to elution of the purified RNA. A portion of the RNA was reverse-transcribed and subjected to real-time PCR with an ABI Prism 7700 sequence detector (Applied Biosystems). A quantification standard prepared by run-off in vitro transcription of a molecular clone containing the A3G open reading frame was assayed along with the cellular RNAs and used to construct a standard curve. Total RNA concentrations in the extracts were measured on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), and A3G mRNA was reported as copies per nanogram of total RNA.

Infection and Sorting of CD4+ T Cells—Reporter pPT-GFP viruses were produced by calcium phosphate transfection of HEK 293T cells with VSV-G envelope expression plasmid (provided by Dr. J. Burns, University of California, San Diego, La Jolla, CA), the packaging construct CMVR8.2 (provided by Dr. D. Trono, University of Geneva, Geneva, Switzerland and described in Ref. 42), and the lentiviral vector pRRL.cPPT.
Cytokine-mediated Regulation of APOBEC3G

A3G Is Induced by IL-2, IL-7, and IL-15—A range of individual cytokines was first tested to assess whether any altered the expression of A3G in primary CD14^- PBLs. Cells were cultured for up to 120 h with four doses of each cytokine. Lysates were then prepared and subjected to SDS-PAGE and immunoblottting with anti-human A3G antibodies. IL-2 (5, 12.5, 25, 50 ng/ml) and IL-15 (1, 5, 12.5, 25 ng/ml) consistently induced moderate up-regulation of A3G protein expression (Fig. 1A). Treatment with IL-7 (1, 12.5, 25, 62.5 ng/ml) also produced an increase in A3G protein expression at this time point (Fig. 1A). Immunoblotting with anti-β-actin antibodies confirmed comparable protein loading (Fig. 1A). Stimulation of the cells with PHA (5 μg/ml) induced up-regulation of A3G protein expression (Fig. 1A).

RESULTS

Analysis of A3G HMM and LMM Complexes by Fast Protein Liquid Chromatography—FPLC analysis was performed as described previously (14). Briefly, CD4^+ T cells or DCs were washed in 1× phosphate-buffered saline and lysed in LB. Lysates were applied to a calibrated Superose 6 HR 10/30 gel filtration column attached to an FPLC apparatus (AKTA, Amersham Biosciences). The FPLC running buffer consisted of 50 mM Hepes, pH 7.4, 125 mM NaCl, 0.1% Nonidet P-40, 1 mM dithiothreitol, and 10% glycerol. One column volume (24 ml) was collected in 1-ml fractions.

A3G Is Induced by IL-2, IL-7, and IL-15—The effects of cytokines on expression of A3G protein were quantitated and normalized to the β-actin loading control bands and graphed as shown. Error bars representing standard error of the mean for the three donors were also included. Cytokines that up-regulated A3G were selected for further studies. The cytokines that consistently induced moderate up-regulation of A3G were selected for further studies. The cytokines that consistently induced moderate up-regulation of A3G were selected for further studies. The cytokines that consistently induced moderate up-regulation of A3G were selected for further studies.

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RESULTS

A3G Is Induced by IL-2, IL-7, and IL-15—A range of individual cytokines was first tested to assess whether any altered the expression of A3G in primary CD14^- PBLs. Cells were cultured for up to 120 h with four doses of each cytokine. Lysates were then prepared and subjected to SDS-PAGE and immunoblottting with anti-human A3G antibodies. IL-2 (5, 12.5, 25, 50 ng/ml) and IL-15 (1, 5, 12.5, 25 ng/ml) consistently induced moderate up-regulation of A3G protein expression (Fig. 1A). Treatment with IL-7 (1, 12.5, 25, 62.5 ng/ml) also produced an increase in A3G protein expression at this time point (Fig. 1A). Immunoblotting with anti-β-actin antibodies confirmed comparable protein loading (Fig. 1A). Stimulation of the cells with PHA (5 μg/ml) induced up-regulation of A3G protein expression (Fig. 1A).

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Three independent experiments were performed with three different donors, and the protein bands were quantitated with Scion Image 1.63e software. A3G protein was enhanced in PBLs treated with IL-2 (~3.5-fold) or IL-15 (~4-fold), whereas a lesser increase (~2-fold) was observed in PBLs treated with IL-7 (Fig. 1B).

When changes in A3G protein expression were monitored over time, IL-2 and IL-15 enhanced expression as early as 12–18 h (Fig. 1C). The response to these cytokines peaked after 48 h and persisted for 120–168 h (Fig. 1C). The time course following PHA stimulation was similar (Fig. 1C). In contrast, IL-7 required more than 48 h of stimulation to enhance A3G expression, and this response did not peak until 7 days after initial stimulation (Fig. 1C). A3G protein levels in the mock- and IL-6-treated samples remained constant over the entire time course (Fig. 1C). Together, these findings demonstrate that IL-2 and IL-15 enhance A3G expression in primary PBLs as early as 12 h after stimulation, with a peak response occurring ~48 h after stimulation. IL-7 also increased A3G expression, but induction by this cytokine occurred with much slower kinetics and could be the result of an indirect pathway.

Interferon Treatment of Monocyte-derived Macrophages, but Not PBLs, Results in Enhanced A3G Expression—We next assessed the regulation of A3G protein levels in monocyte-derived macrophages. A3G is recruited into a HMM complex when monocytes differentiate into macrophages, likely contributing to the enhanced permissivity of these cells to HIV infection (14). Macrophages contain significantly less A3G protein than do monocytes relative to total levels of protein and β-actin (Fig. 1D). However, when macrophages were treated with interferon-α, -β, or -γ, A3G protein levels markedly increased, almost achieving the level found in monocytes (Fig. 1D). These results suggest that, at least in macrophages, A3G may form part of the interferon response to viral infection. Similar induction of A3G expression by interferon-α and -γ has recently been described by Peng et al. (43). When PBLs were treated with interferon-α, -β, -γ, or both -α and -γ, no effect on A3G protein expression was observed, despite an increase in PKR expression (Fig. 1E). These results highlight differences in the inducible A3G response in macrophages and PBLs.

Enhanced A3G Protein Expression in PBLs Correlates with Higher Levels of A3G mRNA and Is Not the Result of Enhanced mRNA Stability—A3G mRNA levels in the same PBLs treated with the same doses of cytokine shown in Fig. 1A were assessed by quantitative real-time PCR. At 48 h, IL-2 induced an ~8-fold increase in A3G mRNA at the highest dose (50 ng/ml), whereas the highest dose of IL-15 (25 ng/ml) stimulated an ~4-fold increase in A3G mRNA (Fig. 2A). IL-7 caused an increase in A3G mRNA but displayed greater donor-to-donor variability at this time point, likely because IL-7 effects require more than 48 h to become manifest in most donors (Fig. 2A). When A3G mRNA levels were analyzed over time, IL-2 and IL-15 induced up-regulation of A3G mRNA as early as 6 h after treatment, whereas the effects of IL-7 were slower and less pronounced (Fig. 2, B–D). A3G mRNA levels in the mock-, IL-6-, and IL-9-treated samples remained relatively unchanged over the entire time course (Fig. 2E). To assess whether the observed increase in A3G mRNA was due to increased synthesis of mRNA or increased stability of mRNA transcripts, PBLs were stimulated with either IL-2 or IL-15 to enhance A3G mRNA levels and then tracked over time for the persistence of these transcripts after inhibiting new mRNA synthesis with actinomycin D. A3G mRNA transcripts in the IL-2- and IL-15-treated samples
Regulation of A3G Expression Is Sensitive to Inhibitors of the Janus Kinase (JAK) and MAPK Signaling Pathways—Although IL-2 and IL-15 play distinct roles in immunologic responses, they also share some overlapping biological properties and have nearly identical effects on T-cell proliferation (44–46). The IL-2 and IL-15 receptors share common β and γc chains but contain different α chains (IL-2Rα and IL-15Rα) (47). Interaction of IL-2 with its intermediate (IL2Rβγc) or high (IL2Rαβγc) affinity receptor or of IL-15 with its equivalent intermediate or high affinity receptor leads to the initiation of a variety of signaling cascades beginning with a rapid activation of JAK-1 and JAK-3 bound, respectively, to the β and γc receptor chains (47). Activated JAK-1 and JAK-3 phosphorylate select tyrosines on both the IL-2 receptor β and γc cytoplasmic tails, thus forming docking sites for various signaling intermediates including Stat5A/B. JAK-mediated phosphorylation of tyrosine residues on the cytokine receptor also initiates other signaling cascades, including the MAPK cascade. Specifically, JAK-induced phosphorylation of the cytokine receptor recruits the adaptor protein Shc, which in turn associates with Grb2, another adaptor protein, and mSos, a guanine nucleotide exchange factor (47). These serial binding events culminate in activation of the Ras/Raf/MEK/MAPK pathway.

To assess whether IL-2 or IL-15 up-regulation of A3G gene expression involves signaling through the JAK/MAPK signaling pathway, PBLs were pretreated with either AG490, a pan-JAK inhibitor, or U0126, a MEK inhibitor that blocks downstream activation of MAPK. Addition of either of these small molecule inhibitors effectively inhibited IL-2- and IL-15-mediated up-regulation of A3G protein (Fig. 3A) and mRNA expression (Fig. 3C). IL-7 had only a slight effect on A3G protein and mRNA levels at this time point, and therefore the action of the inhibitors could not be evaluated (Fig. 3, A and C). PMA activates the MAPK pathway but not through the JAK pathway. As such, U0126, but not AG490, blocked PMA-mediated up-regulation of A3G protein expression (Fig. 3A). When U0126 and AG490 were added in increasing amounts, a dose-related inhibition of IL-2 and IL-15-mediated up-regulation of A3G protein expression was detected (Fig. 3B). Similarly, both U0126 and AG490 blocked IL-2 and IL-15-mediated down-regulation of A3G mRNA, but only U0126 blocked the effects of PMA (Fig. 3C). We confirmed that IL-2 and IL-15 activate the MAPK pathway by analyzing the expression of phospho-ERK1/2, a known intermediate in the pathway. Enhanced phospho-ERK1/2 was observed in PBLs treated with IL-2 or IL-15 (Fig. 3D). Phospho-ERK1/2 was also elevated in PBLs treated with PMA, which was employed as a positive control (Fig. 3D). In addition, both AG490 and U0126 inhibited the induction of phospho-ERK1/2 in the cytokine-treated cells (Fig. 3D). However, because PMA does not act through a JAK pathway, only the MEK inhibitor U0126, and not the JAK inhibitor AG490, impaired ERK1/2 phosphorylation decayed at a rate similar to that of the mock-treated sample, indicating that increased mRNA synthesis, and not enhanced mRNA stability, likely underlies the observed increase in A3G expression (Fig. 2, F and G). Rose et al. (33) similarly found that PMA stimulation of H9 leukemic T cells stimulates increased A3G mRNA synthesis with no effect on mRNA stability. Our findings highlight the ability of IL-2 and IL-15 to activate de novo A3G gene expression in primary PBLs.

Cytokine-mediated Regulation of APOBEC3G

![Cytokine-mediated Regulation of APOBEC3G](image)
kinetics than that observed with IL-2 and IL-15, suggesting that either IL-7 triggers the JAK/MAPK kinase pathway less potently (36) or that A3G up-regulation by IL-7 involves a different, or possibly indirect, pathway.

A3G Is Recruited into HMM Complexes after Cytokine Stimulation of CD4+ T Cells—Because treatment of resting CD4+ T cells with cytokines or mitogens relieves a post-entry block and renders these cells permissive to HIV infection, we hypothesized that, like mitogens, cytokines may inactivate LMM A3G by triggering its recruitment into HMM RNA-protein complexes. We used gel filtration to assess the size of A3G complexes in resting CD4+ T cells that were either mock-treated or treated with IL-2, IL-7, or IL-15. Samples were harvested 5–7 days later depending on the time required for at least 50% of the cells to express the activation marker CD25 (data not shown). We observed that all three cytokines (IL-2, IL-7, and IL-15) promoted HMM A3G formation reflected by a shift of a portion of A3G from fractions 14–17 (LMM) to fractions 7–8 (HMM) (Fig. 4A). The fact that a significant fraction of A3G remained in the LMM form is consistent with the prior finding that cytokine treatment of resting T cells results in only 5–22% of cells acquiring permissiveness to HIV infection (37).

HIV-1 Preferentially Infects Cells Containing HMM A3G—PHA/IL-2-activated CD4+ T cells contain inactive, HMM A3G and are therefore more susceptible to infection by HIV-1 than resting CD4+ T cells, which contain an active, LMM form of the enzyme that restricts HIV infection. Because the cytokine-treated cells in our system contained a mixture of activated and less activated cells, we sought to determine whether HIV-1 preferentially infects the cells containing HMM A3G over those with LMM A3G in such mixed population. To emulate the cytokine-treated conditions, CD4+ T cells were treated with PHA (24 h) and IL-2 (72 h). Cells were then infected with vsv-g pseudotyped ppt-GFP lentiviral vectors. When the cells were harvested 2 days later, 57% of the cells were CD25+, indicating that the culture contained a mixed population of activated and less activated cells (Fig. 4B). The cells were then sorted based on GFP expression and assessed for purity. The GFP-negative cells were nearly 100% pure, whereas the GFP-positive cells were 90% pure, indicating a 10% contamination of this population by uninfected cells (Fig. 4C). The samples were then lysed in LB, and A3G complex formation was assessed using FPLC. In the GFP-positive, or infected, cells, A3G was primarily localized in a HMM complex (Fig. 4C). Although a small percentage of the A3G appeared to be LMM, this could stem from the 10% contamination of this population by non-GFP expressing cells or other experimental irregularities. Conversely, the vast majority of the A3G in the GFP-negative populations was LMM (Fig. 4C).

**FIGURE 4.** Cytokine-induced formation of A3G HMM complexes in primary human T cells. A, purified CD4+ T cells were cultured 5 to 7 days with the indicated cytokine or with PHA/IL2 as a positive control. Cell lysates were loaded on a gel-exclusion column for FPLC analysis. Twenty-four 1-ml fractions were collected and analyzed by immunoblotting for expression of the A3G protein. Two different donors are shown. B, separation of infected CD4+ T cells. Purified CD4+ T cells were infected with vsv-g pseudotyped ppt-GFP lentiviral vectors. Infected (GFP+) cells were separated from uninfected (GFP−) cells using a FACS DiVa instrument. Cells were stained for the CD25 or CD69 activation markers, or for CD3/CD4, and analyzed by flow cytometry. C, FPLC analysis of A3G complexes in infected versus uninfected cells. The sorted cells from B were lysed, and the samples were then subjected to FPLC analysis and analyzed by anti-A3G immunoblotting. The experiment presented is representative of four independent experiments performed with cells from four different donors.
A3G Expression Increases during the Maturation of DCs, and LMM A3G Appears in Mature DCs—Potential changes in A3G expression during DC maturation were also evaluated. A3G protein levels were much higher in mature DCs than in immature DCs (Fig. 5A). The presence of LMM versus HMM complexes was evaluated next in these two populations of DCs. In immature DCs, A3G was primarily localized to HMM complexes (Fig. 5B). This finding is consistent with the known permissivity of immature DCs to R5-tropic HIV infection, which reflects high levels of expression of the CCR5 chemokine receptor on these cells. In contrast, as reported earlier, A3G in monocytes was distinctly LMM, eluting in fractions 14–17, likely corresponding to a monomeric or dimeric form of A3G (Fig. 5B) (14). Interestingly, in mature DCs, which are less permissive to HIV infection than immature DCs, a higher proportion of A3G appeared in a LMM form. Further, intermediate-sized complexes present in fractions 13–15, roughly 155 kDa in size, were also more abundant. These findings reveal that DC maturation is associated with an up-regulation of A3G expression and the appearance of LMM A3G, perhaps reflecting saturation of a limiting host factor(s) required for HMM A3G complex formation in mature DCs.

DISCUSSION

Our studies indicate that IL-2, IL-7, and IL-15 induce up-regulation of A3G protein expression in PBLs. In contrast, other cytokines, including IL-4, -6, -9, -12, and -13, interferon-α, -β, and -γ, and tumor necrosis factor-α, did not alter A3G expression in PBLs. Because the IL-2 and IL-15 receptors share the same β-chain and γc subunits, it is perhaps not surprising that IL-2 and IL-15 exert similar inducing effects on A3G protein expression. These cytokines increased A3G expression even when they were administered in the absence of prior mitogen stimulation. Quantitative real-time PCR studies indicate that the increase in A3G protein expression is due to increased synthesis of A3G mRNA. IL-2 and -15 did not appear to alter the overall stability of A3G mRNA transcripts. In addition, because inhibiting both the activation of JAK and MAPK impaired IL-2 and IL-15 up-regulation of A3G mRNA and protein in PBLs, the observed up-regulation was likely the result of cytokine triggering of the JAK/MAPK signaling pathway. The IL-7-stimulated up-regulation of A3G occurred with much slower kinetics than the response triggered by IL-2 or IL-15, perhaps implicating a different signaling pathway or an indirect pathway. A3G expression was also enhanced during DC maturation and in macrophages treated with interferon-α, -β, or -γ, indicating that A3G expression can be influenced by Toll-like receptor signaling and is a component of the interferon response in certain cell types.

A curious feature of our T cell findings is that although IL-2, -7, and -15 increase the expression of A3G, which is a potent antiviral factor, these same agents render T cells permissive to HIV infection (37, 38). Like cytokines, mitogens also enhance A3G protein expression in T cells (19, 33), and mitogen-treated T cells also become permissive to HIV infection. The mechanism by which mitogens act is now known; they inactivate LMM A3G by promoting its recruitment into enzymatically inactive HMM complexes that are unable to interfere with reverse transcription (14). Similarly, treatment of T cells with IL-2, -7, or -15 led to the formation of HMM A3G complexes, likely explaining how these cytokines act to render resting T cells permissive to infection by HIV-1 in the absence of full-fledged cellular activation. Thus, although these cytokines induce higher levels of A3G expression, the post-entry restrictivity of A3G is forfeited because of cytokine-induced recruitment of A3G into enzymatically inactive HMM A3G complexes. In addition, we provide additional evidence indicating that HIV-1 preferentially infects activated cells containing HMM A3G complexes in mixed populations of activated and less-activated cells.

Our recent studies demonstrate that Staufen-containing RNA-transporting granules are an important component of the HMM A3G complex (34). Of note, endogenous Alu and hY retroelement RNAs are recruited to these granules in an A3G-specific manner. This recruitment interdicts Alu retrotransposition, apparently by sequestering the retroelement RNA away from the nuclear LINE enzymes required for the retrotransposition of non-autonomous Alu retroelements. These findings suggest that HMM A3G complexes form to protect activated T cells from the threat posed by select mobile genetic elements. Unfortunately, this response removes the post-entry restriction block provided by LMM A3G and thus renders these cells permissive for HIV infection.

In related studies, we found that the local action of much lower concentrations of IL-2, IL-15, and one or more additional cytokines present in lymphatic tissues underlies the permissive state of naive CD4+ T cells to HIV infection, which contrasts sharply with the nonpermissiveness of these same cells circulating in the blood stream (48). In the setting of A3G induction during DC maturation, LMM and intermediate-sized forms of A3G appear. Whether A3G plays a role in the replicative block observed in mature DCs seems likely but is unproven. Certainly, other explanations for the differences in permissivity between immature and mature DCs are also possible, including variations in receptor expression. Nevertheless, our findings suggest that the high levels of A3G protein occurring as a result of DC maturation may saturate one or more of the cellular factors...
required for HMM A3G complex formation. Alternatively, the differentiation signals underlying DC maturation may actively promote HMM A3G complex disassembly and give rise to LMM and intermediate-sized forms of A3G.

Together, our findings highlight the action of distinct cytokines as inducers of A3G gene expression in different cellular targets of HIV infection. IL-2 and IL-15 play an inductive role in T cells, as do the interferons in macrophages and poly(I:C)/tumor necrosis factor-α in DCs. Our findings also reveal distinct differences in the regulation of A3G activity in these different cell populations. In cytokine-stimulated CD4 T cells, induction of A3G gene expression correlated with HMM (inactive) A3G complex formation, whereas in DCs the increase in A3G levels associated with maturation appeared to result in the increased appearance of intermediate and LMM (active) forms of A3G. Cytokine-induced recruitment of A3G into HMM complexes correlated with the acquisition of permissiveness to HIV infection in CD4 T cells, whereas the appearance of LMM A3G in mature DCs correlated with the decreased permissivity of these cells to HIV infection. Whether it will be possible to manipulate the form of A3G expressed in HIV target cells for therapeutic benefit remains unclear. However, this possibility merits investigation.

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