HIV-1 Replication in Monocyte-derived Dendritic Cells is Stimulated by Melarsoprol, One of the Main Drugs Against Human African Trypanosomiasis

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Introduction

Human African trypanosomiasis (HAT), better known as sleeping sickness, is a potentially fatal disease caused by a flagellated protozoan. This unicellular parasitic organism exists in two morphologically identical subspecies, Trypanosoma brucei gambiense and T. brucei rhodesiense, and it continues to pose a major threat to 60 million people in 36 countries of sub-Saharan Africa. Accurate statistics for HAT are not available, but it is estimated that there are currently 300,000–500,000 cases, with 50,000 deaths annually.¹ HAT re-emerged through the 1990s in countries where disease control had been hampered by strife and civil wars. Following a substantial investment by public and private donors, the disease was successfully controlled once more, with 10,000–15,000 cases now reported each year to the World Health Organization, but its complete elimination will be difficult given the existence of a stable animal reservoir.² HAT and human immunodeficiency virus type-1 (HIV-1) infection are both
endemic in sub-Saharan Africa and co-infections are not uncommon. Although it is unclear whether HIV-1 influences the epidemiology and/or clinical course of HAT, a few studies indicate that HIV-1-positive subjects might be at higher risk for treatment failure and unfavourable outcome than seronegative patients.3,4

Until recently, the chemotherapy of HAT centred on three key drugs; pentamidine for early-stage T. brucei gambiense HAT, suramin for early-stage T. brucei rhodesiense HAT, and melarsoprol for late-stage disease when trypanosomes are present in the central nervous system.3,5,6 A recent randomized trial showed that the combination of oral nifurtimox and eflornithine was also effective, and this might eventually replace the parenteral treatments.7 Tryparsamide, a pentavalent arsenical, was widely used in the 1930s and 1940s for the treatment of Gambian HAT, before being replaced by melarsoprol in the early 1950s. Melarsoprol is a trivalent organoarsenical compound and it has been the drug of choice for late-stage trypanosomiasis for nearly 60 years. It is, however, highly toxic and kills 5% of patients who receive it.8 The potential targets of the drug in the parasite are thiol-containing enzymes, such as glycerol-3-phosphate dehydrogenase and trypanothione.9 Trivalent arsenicals are inhibitors of many enzymes that contain neighbouring thiol groups, and could conceivably inhibit several metabolic and transport functions within the cell.

Of more relevance here, arsenic trioxide (As2O3), another trivalent arsenical structurally related to melarsoprol, has been shown to affect retroviral infectivity through ill-defined mechanisms.10–12 Arsenic counteracts TRIM5α- or Ref1-mediated HIV-1 restriction in some specific cell lines;10,11 however, it can also enhance virus infectivity in primary human immature dendritic cells, but not in CD4+ T cells, in an APOBEC3G- and TRIM5α-independent manner.13 Interestingly, it has been shown that the pentavalent antimony compound sodium stibogluconate, another organometallic trypanocidal drug, can promote HIV-1 replication in primary CD4+ T cells.14

Given the structural similarity between melarsoprol and As2O3, as well as the reported poor clinical outcome in HAT patients co-infected with HIV-1, we analysed the effect of melarsoprol treatment on HIV-1 replication in various primary human cells. We demonstrate here that melarsoprol specifically increases HIV-1 infection in both immature and mature monocyte-derived dendritic cells (iDCs and mDCs, respectively), but not in other restricted (i.e. monocytes and resting CD4+ T lymphocytes) or non-restricted cell types (i.e. activated CD4+ T cells and monocyte-derived macrophages (MDMs)). Moreover, we report that melarsoprol renders iDCs sensitive to productive infection with HIV-2. Because DCs are thought to be crucial factors in HIV-1 propagation and mucosal transmission, these observations raise concern about the potential harmful effect that melarsoprol might exert on the natural history of HIV-1 infection in co-infected patients and/or HIV-1 transmission to HAT patients.

**Results**

**Melarsoprol promotes HIV-1 replication in DCs**

As2O3, the precursor of various arsenic compounds, has been shown to enhance the susceptibility of iDCs to infection with HIV-1.13 We decided to analyse the effect on HIV-1 replication in various target cells of two organic arsenical drugs used in the treatment of HAT; i.e. the trivalent arsenical melarsoprol and the pentavalent arsenical tryparsamide. When iDCs were treated with therapeutic concentrations of either drug and then infected with VSV-G-pseudotyped luciferase-encoding HIV-1, a dose-dependent increase in virus gene expression was observed upon treatment with melarsoprol (reaching about 10-fold increase at 4 μg/ml), whereas tryparsamide displayed no such positive effect (Fig. 1a). Hence, the subsequent experiments were conducted with melarsoprol only. Concentrations above 4 μg/ml were found to be toxic for iDCs (data not shown). The effect of melarsoprol was assessed in other primary human cell populations permisive to HIV-1, i.e. activated CD4+ T cells and MDMs. Pretreatment for 4 h was chosen for MDMs because of the higher toxicity of the drug in these cells. No increase in HIV-1 expression was observed in either cell type and concentrations of melarsoprol >2 μg/ml were too toxic to be assessed (Fig. 1b and c). Shorter exposures were less toxic but gave similar results; i.e. no positive effect on virus gene expression (data not shown). These data suggest that melarsoprol increases HIV-1 gene expression specifically in iDCs.

Next, we asked whether the stimulating effect of melarsoprol on virus gene expression was reflected in viral production. Using a replication-competent virus, the R5-tropic NL4-3Bam, we observed a dose-dependent increase in virus production (Fig. 2a). In addition, when similar viruses were used to infect mDCs, which are known to be refractory to productive HIV-1 infection,15,16 melarsoprol promoted weak but reproducible virus replication (Fig. 2b).

**Melarsoprol stimulates the replication of several strains of HIV-1 and HIV-2**

We next examined the ability of melarsoprol to facilitate infection by viral isolates displaying a limited capacity to grow in iDCs. For example, infection of this cell population by X4-tropic viruses
has been shown to be much less efficient than that of R5-using strains. A significant variability in susceptibility of iDCs to X4-virus infection was seen between donors, from a complete block to a weak but steady replication (data not shown). However, pretreatment with melarsoprol was able to promote infection by the X4-tropic NL4-3 strain in both iDCs and mDCs. (a) iDCs were treated with the diluent alone (Ctrl) or the given concentration of melarsoprol for 16 h and then infected with fully infectious NL4-3Balenv. Virus production was evaluated at 7 days post infection by measuring the p24 content in the cell-free supernatant. (b) iDCs and mDCs were pretreated with the diluent alone (Ctrl) or with 2 μg/ml melarsoprol (Mel) for 16 h and then infected with replication-competent NL4-3Balenv. Virus production was assessed by measuring the cell-free p24 content at the indicated time points. Numbers above the bars represent fold increase over untreated cells. The data shown are the mean±standard deviation of triplicate samples and are representative of at least three independent experiments with different donors.

**Fig. 1.** Melarsoprol up-regulates HIV-1 gene expression specifically in DCs. (a) iDCs, (b) PHA-activated CD4⁺ T cells, or (c) MDMs were pretreated with melarsoprol or tryparsamide at the concentrations indicated for (a and b) 16 h or (c) 4 h, and then infected for 1 h with VSVG-pseudotyped luciferase-encoding HIV-1 viruses. Some samples were treated with the diluent only (Ctrl). Luciferase activity was evaluated at 5 days post infection. The data shown are the mean±standard deviation of triplicate samples and are representative of at least (a) 10 independent experiments with different donors and (b and c) three independent experiments with different donors. Statistical analysis was done with the results from all donors: *, P<0.05; **, P<0.01; ***, P<0.001.

**Fig. 2.** Melarsoprol induces a dose-dependent increase of HIV-1 replication in both iDCs and mDCs. (a) iDCs were treated with the diluent alone (Ctrl) or the given concentration of melarsoprol for 16 h and then infected with fully infectious NL4-3Balenv. Virus production was evaluated at 7 days post infection by measuring the p24 content in the cell-free supernatant. (b) iDCs and mDCs were pretreated with the diluent alone (Ctrl) or with 2 μg/ml melarsoprol (Mel) for 16 h and then infected with replication-competent NL4-3Balenv. Virus production was assessed by measuring the cell-free p24 content at the indicated time points. Numbers above the bars represent fold increase over untreated cells. The data shown are the mean±standard deviation of triplicate samples and are representative of at least three independent experiments with different donors.
Fig. 3. Melarsoprol promotes replication of several strains of HIV-1 and HIV-2. iDCs were treated with the diluent alone (Ctrl) or 2 μg/ml melarsoprol for 16 h and then infected with (a and b) the HIV-1 laboratory strain NL4-3, (c) HIV-1 clinical isolate 92HT599 or (d) HIV-2 strains ACR23 and ROD/B. Production of HIV-1 particles was assessed by measuring the cell-free p24 content at the indicated time points. HIV-2 replication was estimated by assessing RT activity after 7 days. (a and b) NL4-3 results obtained with two typical donors (out of seven). (e) A summary of the results obtained with all tested strains and various donors (including data from Figs. 1 and 2), showing individual data as well as the median melarsoprol-induced increase in virus production. The number of donors tested for each viral strain is listed above each virus preparation tested. Also shown are the P-values obtained with a one-sample t-test.
infection by both ACR-23 and ROD/B strains (Fig. 3d). Altogether, our analysis using iDC preparations from multiple healthy donors that were infected with different HIV-1 and HIV-2 strains revealed that melarsoprol exerts a variable but constant stimulating activity in the range two- to 80-fold, with a median increase around threefold for most fully competent viruses and almost 10-fold for VSV-G pseudotyped HIV-1 particles (Fig. 3e).

**Melarsoprol exerts a higher stimulating effect when using low virus inputs**

The marked difference in the drug-stimulating potential is presumably due to the well established donor variability in HIV-1 susceptibility of iDCs. Interestingly, the most impressive melarsoprol-induced increases in HIV-1 replication were observed in samples where the extent of virus replication was very low in cells treated with the diluent alone (control). This observation suggests that the drug probably acts by removing or attenuating a block to HIV-1 infection rather than by increasing the overall virus gene expression level. Thus, we next monitored whether the melarsoprol-mediated enhancing activity was influenced by the initial virus input. When iDCs were infected with increasing concentrations of virus, the melarsoprol-mediated enhancing effect was highest at the lowest dose and diminished with increased virus inputs, both with fully competent NL4-3Balenv particles (Fig. 4) and single-cycle VSV-G-pseudotyped viruses (data not shown). These data indicate that treatment with melarsoprol and a high multiplicity of infection are not additive, suggesting that bypassing the partial blockade to productive infection of iDCs by HIV-1 can be achieved either by treatment with melarsoprol or a high virus inoculum. It should be noted that melarsoprol treatment of other restrictive cells types such as monocytes and quiescent CD4+ T cells had no effect (data not shown), which indicates that melarsoprol counteracts a block to HIV-1 infection that is specific to DCs.

**Melarsoprol facilitates an early step of the virus life cycle**

To gain a better understanding of the mode of action of melarsoprol, experiments were done to define which step(s) of the virus replicative cycle was affected by melarsoprol. First, we gained evidence that the drug does not modulate the amount of viral particles captured by iDCs, or the fusion efficiency of those particles, as assessed by a fusion assay based on virions carrying β-lactamase–Vpr chimeric proteins (data not shown). By contrast, when melarsoprol was added 24 h after virus exposure rather than before infection, the drug-enhancing effect was completely lost (Fig. 5a), thus suggesting that melarsoprol acts at an early step. This postulate is supported by experiments showing that treatment with melarsoprol resulted in an increase in the amount of integrated proviral DNA (Fig. 5b), confirming that a truly new infection cycle was analysed. The effect of melarsoprol on viral reverse transcription was also assessed using PCR primers that can detect early or late reverse transcribed products. The results illustrated by Fig. 5c suggest that melarsoprol increases HIV-1 reverse transcription in iDCs, slightly more so for the completion of reverse transcription products (right-hand panel) than for their initiation (left-hand panel). These data demonstrate that pretreatment of iDCs with melarsoprol augments HIV-1 replication by acting at a post entry step that results in an increased efficacy of reverse transcription events.

**Melarsoprol acts partly through an inhibition of APOBEC3G protein expression**

The natural low permissiveness of iDCs to productive HIV-1 infection has been shown to be mediated, at least partly, by the cellular cytidine deaminase APOBEC3G (A3G). To shed light on the possible implication of this cellular restriction factor in the melarsoprol-induced superior virus production in iDCs, cells were transfected with a small interfering RNA (siRNA) specific for A3G. Indeed, if melarsoprol acts through a reduction of APOBEC3G activity, removing the protein should
prevent the enhancing effect of the drug. As expected, treatment of iDCs with melarsoprol in the mock-transfected samples (called mock) resulted in higher virus production for the three donors studied (i.e. 2.6–10-fold increase; Fig. 6). These experiments indicated also that A3G depletion in untreated cells that were transfected with the siRNA specific for A3G (called A3G) increases HIV-1 replication in all donor samples, when compared to cells transfected with the control siRNA (called control), thus confirming the involvement of A3G in the constitutive low susceptibility of iDCs to HIV-1 infection. As expected, inter-donor variability was seen among iDC preparations with respect to the effect on virus production of the siRNAs used to silence A3G expression. Interestingly, the stimulating effect of pretreatment with melarsoprol was significantly diminished but not completely eliminated in iDCs transfected with the siRNA specific for A3G. For example, a 3.6–4.4-fold increase was seen in cells transfected with the siRNA specific for A3G as compared to a 9.1–15-fold increase in cells transfected with the control siRNA. These data suggest that the melarsoprol-dependent enhancing effect with respect to HIV-1 replication in iDCs is due, at least partly, to a downregulation in A3G expression.

**Melarsoprol inhibits IFN-α-mediated expression of the small A3G proteins in iDCs**

The potential contribution of A3G in the melarsoprol-directed enhancement of the permissiveness of iDCs to productive HIV-1 infection was further addressed by monitoring A3G expression by western blotting. The data shown in Fig. 6 suggested that, in the absence of melarsoprol, virus replication
was reduced in iDCs transfected with the control siRNA when compared to mock-transfected cells (compare donors 1 and 2). The results shown in Fig. 7 suggest that this is probably due to a higher expression of A3G in iDCs transfected with the control siRNA (compare lanes 1 and 2). Western blotting analysis also showed that melarsoprol reduced expression of the 38–40 kDa A3G monomer in(119,422),(833,492) samples transfected with the control siRNA and the siRNA specific for A3G (compare lanes 2 and 5, and lanes 3 and 6). Of equal interest here, RNA transfection provoked the apparition or increased expression of smaller A3G protein bands (i.e. 15–20 kDa), and treatment with melarsoprol seemed to inhibit the appearance of such small molecular mass A3G fragments. In some cell samples, these small forms represented a very large proportion of the detected A3G-specific bands (Fig. 7b) and those cells could not be productively infected by HIV-1 (data not shown), suggesting that these smaller forms of A3G are involved in the antiviral capacity of this restriction factor. Those A3G fragments were sometimes present even in untransfected cells, and disappeared upon treatment with melarsoprol (Fig. 7c). Importantly, such small A3G forms have been detected in iDCs upon treatment with IFN-α or poly(I:C). Therefore, we assessed whether melarsoprol can modulate the capacity of IFN-α and the double-stranded RNA analogue poly(I:C) to induce the smaller A3G bands. As expected, treatment of iDCs with IFN-α or with poly(I:C) induced the A3G-associated low molecular mass bands (Fig. 7d). The effect of poly(I:C) was blocked completely by addition of the soluble type I IFN receptor B18R, confirming the IFN-mediated effect of poly(I:C) on A3G expression. Interestingly, treatment with melarsoprol had only a modest modulatory effect on expression of the 40 kDa A3G monomer. However, the IFN-α-mediated appearance of the small A3G bands was strongly inhibited by melarsoprol. These observations suggest that siRNA transfection, like poly(I:C), induces type I IFN secretion in iDCs, a process leading to expression of smaller A3G forms and inhibition of HIV-1 replication. Treatment with melarsoprol appears to counteract this effect, thus inducing an increased HIV-1 replication in iDCs.

**Discussion**

Melarsoprol, a trypanocidal drug, is still widely used for the treatment of the second stage of sleeping sickness. We show for the first time that this medicinal drug can cause an increased replication of HIV-1 in DCs. The melarsoprol-mediated enhancement in virus production was seen with every HIV-1 strain tested and even with HIV-2. The effect is specific to melarsoprol, it was not observed with other trypanocidal drugs, such as pentamidine (data not shown) or the pentavalent arsenical tryparsamide. Hence, the drug mode of action is probably related to the trivalent form of arsenic, a hypothesis reinforced by the similar HIV-1-stimulating capacity displayed by As2O3, another trivalent arsenical.

The melarsoprol-induced augmentation in virus replication is cell type-specific; i.e. it was observed only in DCs. In fact, the drug was very toxic when used in activated CD4+ T cells and MDMs, causing cell death at low concentrations. Such a high toxicity could hide a possible effect on these cell types, but lower, less toxic concentrations did not affect HIV-1 replication. In addition, the drug had no effect on HIV-1 infection in other restrictive cell types, such as monocytes or resting CD4+ T cells. These observations suggest that the drug acts by neutralizing a replication block specific to DCs. Furthermore, the effect was observed in both iDCs and mDCs. However, the replication in the latter did not reach
the level detected in iDCs. We propose that the melarsoprol-mediated increase in viral production seen in mDCs could even reflect the action of the drug on the small proportion of iDCs still present. This suggests that the drug does not relieve the blocks to HIV-1 infection specific to mDCs; i.e. an inefficient fusion process and a post-integration inhibition at the transcriptional level.\(^{15,16}\)

Several observations allow us to hypothesise on the putative mode of action of melarsoprol. First, many lines of evidence indicate that the drug acts at a post entry level. Indeed, the effect is independent of the external envelope glycoprotein because it is seen with both X4- and R5-tropic laboratory-adapted as well as clinical isolates of HIV-1, with HIV-2 and even with VSV-G pseudotyped viruses. In addition, no effect on the amount of viral particles entering DCs was noted. By contrast, no impact on post integration events such as viral gene expression was detected. The drug had no effect when added 24 h after infection; i.e. after integration. In addition, integrated proviral DNA was increased upon treatment with melarsoprol, suggesting an effect of the drug on steps of the viral cycle between fusion and integration.

The proteasome has been found to be involved in antiviral activity in some cell lines, such as HeLa, where TRIM5\(\alpha\) targets the reverse transcription products to the proteasome.\(^{21,22}\) However, no effect of melarsoprol on degradation via the proteasome could be demonstrated, as the inhibitor of proteasome MG132 did not modify the drug activity (data not shown). An increased efficacy of reverse transcription is probably the main effect of the drug on the HIV-1 life cycle, especially the elongation, as a more potent stimulation was observed on complete reverse transcripts compared to early reverse transcript products. This effect is mediated, at least partly, by an inhibition of A3G expression, as shown by both western blotting and A3G knock-

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**Fig. 7.** Melarsoprol inhibits IFN-\(\alpha\)-mediated expression of the small A3G proteins in iDCs. (a and b) iDCs from two distinct donors were either mock-transfected (mock) or transfected with a control siRNA (ctrl) or an siRNA specific for A3G. After 48 h, cells were treated with the diluent alone (Ctrl) or with 2 \(\mu\)g/ml melarsoprol for 16 h. Proteins were extracted from the samples and were subjected to SDS-PAGE, then blotted with a polyclonal anti-A3G antibody. Equal loading in each lane was controlled by reblotting the same membrane with an anti-actin antibody. Donor 2 is the same as in Fig. 6. iDCs from donor 4 were not susceptible to productive HIV-1 infection (data not shown). (c) iDCs were either left untreated or treated with 2 \(\mu\)g/ml melarsoprol for 16 h. Detection of A3G was achieved by western blotting as described above. (d) iDCs were either left untreated or treated for 24 h with IFN-\(\alpha\) (1000 U/ml) or 25 \(\mu\)g/ml poly(I:C) in the absence or in the presence of B18R (100 ng/ml). Cells were treated with the diluent or with 2 \(\mu\)g/ml melarsoprol for the last 18 h. Cells were then lysed and total protein extracts were analysed for A3G expression by western blotting. The A3G proteins (i.e. \(\sim\)40 kDa monomer band and the small 15–20 kDa bands) and \(\beta\)-actin are shown in each western blot.
down using siRNA-mediated gene silencing. Because we could not achieve a complete inhibition of A3G expression, it is difficult to ascertain whether the melarsoprol-directed stimulating potential on HIV-1 replication is mediated solely through this antiviral protein. A3G has been shown to inhibit elongation of HIV-1 reverse transcripts, and has been described as responsible for the low susceptibility of iDCs to HIV-1 infection. In fact, differences in HIV-1 susceptibility of monocytes, macrophages and DCs could be explained, at least partly, by disparities in A3G expression between these cell types. Moreover, A3G levels in DCs has been shown to vary between donors, which could explain the high level of variability of melarsoprol activity observed among our preparations of iDCs.

IFN-α has been reported to increase A3G expression and inhibit HIV-1 replication in both myeloid and plasmacytoid DCs. Melarsoprol has only a marginal effect on expression of the classic 38–40 kDa A3G forms, whereas the drug strongly inhibits the expression of the small 15–20 kDa forms that are induced by type I IFN. These small bands originate from A3G complexes because they were detected only with the anti A3G antibodies. They probably correspond to cleaved A3G monomers; however, as stated by Trapp and colleagues, they were not induced by other stimuli, ruling out the possibility that these small fragments are simply degradation products of proteins from maturing DCs. We have observed that, among donors, the expression of these small A3G forms is inversely correlated with DC susceptibility to HIV-1 infection. For example, one DC preparation contained a very large amount of the low molecular mass form of A3G and these cells could not be productively infected with HIV-1. Our observations are quite consistent with published work showing that the low molecular mass A3G complexes display a significant antiviral activity. It is possible that some DC preparations contain a very small amount of plasmacytoid DCs, which are very strong producers of type I IFN. This type I IFN would, in turn, induce the expression of the small A3G forms in MDDCs. In conclusion, melarsoprol probably acts through an inhibition of IFN-α-induced expression of the small forms of A3G, leading to a more efficient reverse transcription and hence, a more efficient virus infection process.

The other trivalent arsenical, As$_2$O$_3$, has also been found to increase HIV-1 replication in DCs through a more efficient reverse transcription, but in an A3G-independent way. It is of interest that a recent study suggested that As$_2$O$_3$ induces an A3G-mediated restriction of HIV-1 infection in myeloid but not in monocyte-derived DCs. These results could be explained by the fact that expression of the small A3G forms was not analysed in those two studies, and by structural differences between the two compounds. Indeed, studies on the apoptosis-inducing properties of both compounds suggested that the two arsenicals could have different modes of action. For example, As$_2$O$_3$ has been shown to provoke alterations in several signal transduction pathways involving mitogen-activated protein kinases, phosphatidylinositol 3-kinase and Syk kinase, and to induce apoptosis by oxidative stress-mediated pathways in primary myeloid cells.

Our observations are highly relevant for HIV pathogenesis, especially in West and Central Africa, where HAT remains endemic. The concentrations of melarsoprol stimulating virus replication in our experiments (0.25–2 μg/ml) are similar to those observed in patients. Indeed, pharmacokinetic studies have shown that serum levels vary between 2 μg/ml and 4 μg/ml at 24 h following administration, with a maximum concentration of 6 μg/ml. Moreover, a concentration of 0.22 μg/ml, which is sufficient to enhance HIV-1 infection, is still present at 120 h after the last of four daily injections. These data demonstrate the physiological significance of our observations, suggesting that indeed, treatment with melarsoprol might aggravate HIV pathogenesis in co-infected patients and increase the risk of virus transmission. The high level of toxicity observed in many cell types at concentrations above 1 μg/ml could explain the very strong adverse effects reported for this drug, including fatal encephalopathy in 5% of cases.

The fact that melarsoprol potentiates HIV-1 infection in DCs, a cell type known to have an active role in mucosal transmission, raises concerns about an increased risk of virus transmission in HAT patients. In addition, DCs are usually refractory to HIV-2 infection. We show here that treatment with melarsoprol makes DCs susceptible to HIV-2. Fortunately, African trypanosomiasis has disappeared from most countries endemic for HIV-2, such as Senegal and Guinea-Bissau. Although this is highly hypothetical, we speculate that the massive use of melarsoprol in Guinea Bissau in the late 1940s and the 1950s might have contributed to the emergence of HIV-2 in this country, through iatrogenic transmission. Use of melarsoprol peaked in the 1950s, coinciding with an exponential expansion of the number of HIV-2-infected individuals. Among elderly inhabitants of Guinea Bissau, HIV-2 was associated with past treatment for trypanosomiasis. This hypothesis deserves further study.

**Materials and Methods**

**Cells and tissue culture**

Human embryonic kidney 293T cells, which express the simian virus 40 large T antigen, were maintained in
Production of virus stocks

Virions were produced by transient transfection in 293T cells as described. The full-length infectious molecular clone NL4-3 (X-tropic) was obtained from the NIH AIDS Research and Reference Reagent Program. The NL4-3ΔBaenv vector was generated by replacing the env gene of NL4-3 with that of the macrophage-tropic HIV-1 Bal strain, resulting in an infectious molecular clone with R5-tropic properties (provided by R. Pomerantz, Thomas Jefferson University, Philadelphia, PA). The ACR-23 and ROD/B molecular clones of HIV-2 were kindly given by P. Clapham (University of Massachusetts Medical School, Worcester, MA). The luciferase-encoding NL4-3LucE'R' and R5-tropic envelope-encoding cDNA-1/Amp-based JR-FL vectors were kindly provided by N.R. Landau (The Salk Institute for Biological Studies, San Diego, CA) and were used to generate single-cycle luciferase reporter viruses. The NL4-3Δenv vector encodes a complete HIV-1 genome containing a –1 frameshift in the envelope (Env) precursor and was used to produce ENV-deficient viruses (kindly provided by D.E. Ott from the National Cancer Institute, Frederick, MD). The HCMV-G vector codes for the broad host-range vesicular stomatitis virus Env glycoprotein-G (VSV G) and is placed under the control of the human cytomegalovirus promoter. HIV-1-based reporter viruses pseudotyped with JR-FL or VSV-G were produced upon cotransfection of 293T cells with pNL4-3LucE'R' and pcDNA-1-JRFL or pHCMV-G, respectively. A similar method was used to produce NL4-3Δenv pseudotyped with VSV-G. Progeny viruses were also produced upon acute infection of peripheral blood mononuclear cells and purified CD4+ T cells for 7 days with the X4-tropic clinical isolate 92HT599 (obtained through the NIH AIDS Research and Reference Reagent Program). The virus-containing supernatants were filtered through a 0.22 μm pore size cellulose acetate syringe filter and normalized for virion content using an in-house sensitive double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) specific for the viral p24 core protein.

Virus infection assays

Primary human cells (iDCs, 5 × 10^4; mDCs, 5 × 10^4; MDMs, 5 × 10^4; or activated CD4+ T cells, 1 × 10^5) were either left untreated or treated with melarsoprol or tryparsamide at the indicated concentrations for 16 h (4 h in the case of MDMs because of toxicity) before infection. Appropriate dilutions of propylene glycol (the diluent) corresponding to those used to prepare the drug solutions (i.e. melarsoprol) were used as controls. Cells were then washed extensively, and incubated with the virus preparation (10 ng of p24/10^5 cells unless indicated otherwise) for 60 min at 37 °C. After three washes with phosphate-buffered (PBS), the cells were maintained in complete culture medium. HIV-1 production was estimated by measuring the p24 content in the cell-free culture supernatant. HIV-2 replication was evaluated at 3 days and at 7 days post transfection by measuring reverse transcriptase activity in the cell-free supernatant using the RetroSys™ RT activity assay (Innovagen AB, Sweden). Infection with luciferase-encoding viruses was assessed 3–5 days post infection by measuring reporter gene activity (expressed as relative light units (RLU)).

Quantification of integrated proviral DNA and reverse transcribed products

iDCs (1 × 10^6) were either left untreated or treated with melarsoprol for 16 h, after which they were pulsed with DNasel-treated VSV-G-pseudotyped NL4-3Δenv (50 ng of p24/10^6 cells) for 1 h then washed twice. Cells were cultured for 6, 24 or 48 h. Then, genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN Inc., Mississauga, ON). Integrated proviral DNA copies were quantified using a combined Alu-HIV-1 PCR and real-time PCR assay. Briefly, genomic DNA (100 ng) was

Chemicals and antibodies

Melarsoprol was obtained as a solution for I.V. injection containing 3.6% (m/V) in propylene glycol (Arsobal®, Aventis). This solution was diluted to 1 mg/ml in propylene glycol and then to the working concentration in complete culture medium. Tryparsamide was obtained from V. Yardeley (London School of Hygiene and Tropical Medicine, London, UK). The following reagent was obtained through the NIH AIDS Research and Reference Reagent Program (Germantown, MD): anti-APOBEC3G from W.C. Greene. Interferon-α (IFN-α) was purchased from R&D Systems (Minneapolis, MN), B18R from ebiosciences (San Diego, CA), and poly(I:C) from InvivoGen (San Diego, CA).
first amplified with an Alu-sequence-specific sense primer and the HIV-1-specific antisense primer M661. Next, diluted PCR products were subjected to a real-time PCR assay in a 25 µl reaction volume containing 2× TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 2 µM sense primer M667, 2 µM antisense primer AA55 and 0.3 µM TaqMan probe HIV-5′-carboxyfluorescein (Biosearch Technologies, Novato, CA). The cycling conditions included a hot start (50 °C for 2 min and 95 °C for 10 min), followed by 40 cycles of denaturation (95 °C for 1 min) and extension (63 °C for 1 min) with end-point acquisition. NL4-3Bal DNA was used for the standard curve (i.e. 469–30,000 copies). For quantification of early and late reverse transcripts, 25 ng of DNA was subjected to a real-time PCR assay in a 25 µl reaction volume containing 2× TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 1 µM sense primer M667, 1 µM antisense primer AA55 or M661, and 0.3 µM TaqMan probe HIV-5′-carboxyfluorescein (Biosearch Technologies). The primer pair AA55/M667 amplifies the R-U5 region of the long terminal repeat and was used to detect the early reverse transcription products. The primer pair M661/M667 flanks the primer-binding site and specifically detects HIV-1 DNA within completed or nearly completed reverse transcripts. The HIV-1-based primers utilized to detect early and late reverse transcription products have been described.  

A3G gene silencing

siRNA targeting A3G, as well as a control siRNA (containing scrambled sequences), were obtained from QIAGEN Inc. and dissolved in RNase-free water. The A3G target sequence (A3G-1) was:

5′-CCAGGTTGATTCCGAACCTTA-3′

Gene silencing was achieved by first washing iDCs with OptiMEM (Invitrogen Life Technologies, Burlington, ON) without serum or antibiotics. Next, the tested siRNAs were transfected in six-well plates at 200 pmol/well using Oligofectamine according to the manufacturer’s instructions (Invitrogen Life Technologies). Controls consisted of cells treated with Oligofectamine alone (called mock) or with Oligofectamine plus scrambled sequences (called control). Transfection efficiency of 80% – 90% was measured using a fluorescent siRNA (i.e.siGlo®, Dharmacon). At 36 h following transfection, cells were treated with diluent alone or with melarsoprol for 16 h, and infected with HIV-1 as described above. Thereafter, A3G protein expression was analysed by western blot assays. Briefly, cells were washed with cold PBS and resuspended in lysis buffer (50 mM Hepes, pH7.4, 125 mM NaCl, 0.2% Triton X-100) to a density of 10⁶ cells/ml. Proteins were separated by SDS-PAGE in 12% acrylamide gels and transferred to Immobilon PVDF membranes (Millipore, Bedford, MA). Immunoblotting was done with the appropriate antibodies. Rabbit polyclonal anti-human A3G (1:1000) and monoclonal anti-actin (1:1000) were detected using a horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody, and visualised with a Western Lightning Plus-ECL kit (Perkin Elmer, Waltham, MA).

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