Identification of an Arsenic-Sensitive Block to Primate Lentiviral Infection of Human Dendritic Cells

Marjorie Pion, Romaine Stalder, Rafael Correa, Bastien Mangeat, Greg J. Towers, and Vincent Piguet

Department of Dermatology and Venereology and of Microbiology and Molecular Medicine, University Hospital and Medical School of Geneva, Geneva, Switzerland, and MRC Centre for Medical Molecular Virology, Department of Infection, Royal Free and University College Medical School, UCL, London W1T4JF, United Kingdom

Received 13 April 2007/Accepted 18 August 2007

Dendritic cells are central to the early events of human immunodeficiency virus type 1 (HIV-1) transmission, but HIV-1 infects dendritic cells inefficiently in vitro compared to activated CD4+ T cells. There is a strong postentry restriction of HIV-1 infection in dendritic cells, partly mediated by the cellular restriction factor APOBEC3G. Here, we reveal that arsenic trioxide markedly increases HIV infection of immature and mature dendritic cells as well as blood-derived myeloid dendritic cells in an APOBEC3G- and TRIM5α-independent way. Our data suggest the presence of powerful, arsenic-sensitive antiviral activities in primary human immune cells of the dendritic cell lineage.

Several model systems have indicated that a key event in the early transmission of human immunodeficiency virus (HIV) is the transfer of virus from dendritic cells (DC) to CD4+ T cells, as reviewed in references 19, 33, and 38. However, high doses of HIV are required to infect DC in vitro (9, 16), as reviewed in reference 24. In the absence of viral replication, DC, including Langerhans cells, can capture and transfer HIV type 1 (HIV-1) to CD4+ T cells via an infectious synapse, which results in high levels of infection (1, 13, 20, 37). There is an early block to HIV-1 entry into DC (6, 9, 25), as well as a strong postentry block to infection (26). The TRIM5 and APOBEC families have recently been shown to be able to block retroviral infection in primate cells (31, 35), as reviewed in references 5, 23, and 32. Specifically, TRIM5α or APOBEC3G (A3G) can operate, at least in some circumstances, after viral entry and prior to viral integration (7, 35). Human TRIM5α restricts HIV-1 only weakly (12, 35) and is not thought to have a strong impact on HIV-1 infection or pathogenesis in vivo (15, 28). The restriction factors A3G and, to a lesser extent, APOBEC3F can restrict HIV-1 infection in DC (26). The mechanism of A3G-mediated restriction correlates with the presence of an active form of A3G in low-molecular-mass (LMM) complexes in resting CD4+ T cells (7) and in DC (26, 34).

Arsenic trioxide (As2O3) has been shown to increase retroviral infectivity in some cases of restricted infection, but the mechanisms by which it does this are not well understood. In some cases, arsenic has been shown to have an inhibitory effect on TRIM5 (2, 4, 17, 18, 27, 30). Here, we show that As2O3 treatment restores HIV-1 infectivity in immature DC (iDC) but not in the more permissive CD4+ T cells. The effect of arsenic on DC is specific to primate lentiviruses HIV-1 and HIV-2 and simian immunodeficiency virus from rhesus macaques (SIVmac) but not to the equine lentivirus equine infectious anemia virus (EIAV). Furthermore, it occurs independently of TRIM5α and A3G.

First, we assayed whether As2O3 affects HIV-1 replication in human iDC. iDC were generated by 6-day culture of monocytes in a mixture of granulocyte-macrophage colony-stimulating factor and interleukin-4 as described previously (8, 26). The iDC were then pretreated with As2O3 (2 μM) for 18 h, and groups of iDC were infected in parallel with a variety of HIV-1 strains using either CXCR4 or CCR5 coreceptors, as described previously (25) (Fig. 1). Remarkably, As2O3 treatment increased the percentage of HIV-infected DC by up to 50-fold, depending on the virus strain. As iDC express a relatively low quantity of HIV coreceptors in comparison to CD4+ T cells (25), we verified that As2O3 treatment did not result in the alteration of HIV receptor or coreceptor levels by quantitative fluorescence-activated cell sorter (FACS) analysis of iDC as described previously (25). Modest changes in receptor and coreceptor levels induced by As2O3 could not explain the strong stimulation of HIV replication (data not shown). Furthermore, 1 or 2 μM As2O3 did not affect iDC viability, as determined by 7-amino-actinomycin D staining, and As2O3 did not affect the maturation state of iDC, as tested by FACS analysis of DC maturation markers CD83 and major histocompatibility complex class II (data not shown).

In order to test whether the route of viral entry influences sensitivity to As2O3, we employed a single-round assay using green fluorescent protein (GFP)-encoding HIV-1-derived lentivirus vectors (lentivectors). We pseudotyped the vectors with HIV-1 envelope glycoproteins that use CCR5 (JRXL) or CXCR4 (HXB2), as well as the G protein from vesicular stomatitis virus (VSV-G). In this single-round assay, As2O3 (1 μM) stimulated HIV infection of iDC very strongly, up to...
150-fold compared to the infection of untreated iDC, regardless of the viral envelope or coreceptor usage (Fig. 2A and C). As$_2$O$_3$ (2 μM) also stimulated the infection of lipopolysaccharide-matured DC with HIV pseudotyped with VSV-G (Fig. 2C), as well as the infection of blood-derived myeloid DC (MyDC) (Fig. 2B and C). MyDC were extracted from buffy coats by using a CD1c DC isolation kit according to the instructions of the manufacturer (Miltenyi Biotec). Cells were maintained in Iscove modified Dulbecco’s Eagle medium supplemented with 10% fetal calf serum and granulocyte-macrophage colony-stimulating factor (250 U/ml). Cells were >95% CD11c$^+$ and HLA-DR$^+$. Contamination with CD3$^+$ T cells was less than 5% (data not shown). Treatment with arsenic (2 μM) also led to a modest but reproducible stimulation.
of HIV infection, by two- to threefold, in CD4+ Jurkat cells, in agreement with previous observations (4). HIV infection of activated primary blood lymphocytes was stimulated by As2O3 (2 μM) only at doses that were close to toxicity (data not shown). Together, these results show that As2O3 stimulates a postentry aspect of HIV infection in primary human cells in a cell type-dependent manner.

Next, we assayed whether As2O3 generally enhances lentiviral infection of iDC. We employed four different lentivectors encoding GFP, HIV-1 (21), HIV-2 (10), SIVmac (22), and EIAV (14), each pseudotyped with VSV-G, as described previously (11). In a single-round infection assay, each of these lentiviruses infected iDC with very low efficiency, despite the high dose used. However, treatment with As2O3 (2 μM) induced strong infection of iDC specifically with the primate lentiviruses, HIV-1, HIV-2, and SIVmac (Fig. 3). In contrast, very low levels of EIAV infection were detected, and these levels were not affected by As2O3 treatment (Fig. 3). This result may be because EIAV is insensitive to the factor affected by arsenic but may also be because EIAV encounters a further block after the first is relieved by arsenic treatment.

Previous studies have demonstrated that As2O3 increases the reverse transcription of HIV-1 in cell lines. We therefore tested whether As2O3 increases HIV-1 reverse transcription in iDC by measuring early and late reverse transcription products by quantitative PCR analysis of incoming HIV-1 pseudotyped with VSV-G. The results demonstrated a strong stimulation of HIV reverse transcription by As2O3 (2 μM) (Fig. 4A). We amplified reverse transcription products using Power SYBR green master mix (Applied Biosystems), forward primer 5'-GGAGTTCTTGCCCTGGAGCTG-3' and reverse primer 5'-GGAGCAGAGAAGCCTATG-3' for early reverse transcription products, and forward primer 5'-TGTGTCGCCCAG-3' and reverse primer 5'-CGAGTCTCTGCAC-3' and reverse primer 5'-CGAGTCTCTGCAG-3' for late reverse transcription products. The β-actin gene was amplified to measure the DNA concentration. Each reaction was performed in triplicate, results were
normalized to those for the β-actin gene, and changes after arsenic treatment were calculated. The analysis of integration of HIV-1 X4 strains showed a corresponding increase after As2O3 treatment (data not shown). Thus, As2O3 acts at an early postentry step in the infection of iDC with HIV to increase the efficiency of reverse transcription and therefore of integration and infection. The block to reverse transcription that is eliminated by arsenic treatment may be due to an effect on the uncoating or rearrangement of the virion that precedes reverse transcription or a factor that blocks reverse transcription, such as TRIM5α or A3G.

We previously reported that HIV-1 restriction in iDC is, at least in part, due to the cellular restriction factors A3G and APOBEC3F (26). The LMM active form of A3G has been shown to be active against HIV-1 in resting CD4+ T cells (7) and DC, whereas the higher-molecular-mass (HMM) form is not (26). Using an assay that we described previously (26), we therefore tested whether the ratio between the HMM and LMM forms of A3G present in DC was modulated by treatment with As2O3 (2 µM). We separated the A3G LMM fraction in the supernatant (SN) from the HMM form of A3G, which remains in the pellet (P) fraction, and measured A3G protein levels by Western blotting as described previously (26). We showed that A3G was present exclusively in the P fractions from cells highly sensitive to HIV infection, such as H9 CD4+ T cells, and no A3G was detected in the SN fractions (Fig. 4Bi). In contrast, the A3G distribution in monocytic restrictive HIV infection showed a strong proportion of A3G in the SN, as previously reported (26). Treatment with As2O3 did not significantly change the distribution of A3G in the SN and P fractions from immature and mature DC (Fig. 4B).

Several reports suggest that As2O3 inhibits TRIM5α-mediated restriction, at least in some cell types (3, 4, 18, 27, 29, 30, 36). To seek a contribution of TRIM5α to the restriction of HIV infection of iDC and its suppression by As2O3, we measured the levels of TRIM5α in iDC by Western blotting. As2O3 treatment did not affect the levels of TRIM5α (Fig. 4C, panel i) but led to a significant enhancement of the infection of iDC with HIV-1 pseudotyped with VSV-G (Fig. 4C, panel ii). Furthermore, the reduction of TRIM5α expression by approximately 80% using RNA interference did not lead to the stimulation of HIV infection (Fig. 4C). We therefore conclude that As2O3 inhibits a very potent postentry restriction in iDC that is likely to be independent from A3G and TRIM5α.

In conclusion, we have shown that arsenic trioxide eliminates a potent postentry restriction of HIV infection of iDC, lipopolysaccharide-matured DC, and MyDC. The effect of As2O3 on iDC is independent of the viral envelope used and operates independently of the restriction factors A3G and TRIM5α. The stimulation of infectivity in DC by arsenic occurs postentry, at the level of reverse transcription. The mechanisms of stimulation remain unclear, but it is possible that cell type-specific antiviral factors act to limit viral infection of DC and that these factors are inhibited by arsenic treatment. Understanding the molecular details of the mechanism of restriction and sensitivity to arsenic is likely to be important for the development of novel therapeutic strategies and the improvement of animal models for HIV sexual transmission.

We thank L. Ylinen, T. Schaller, D. Littman, D. Trono, J. Sodroski, F. L. Cosset, J. Stoye, K. Mitrophanous, and A. Lever for reagents and F. Leuba for excellent technical help. We thank the Genomics Platform of the NCCR program Frontiers in Genomics, University of Geneva, for help with PCR experiments.

This work was supported by the Geneva Cancer League and Swiss National Science Foundation, and by grants from the Human Science Frontier Program to V.P. and from the Wellcome Trust to G.J.T.

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