Measles virus inhibits human immunodeficiency virus type 1 reverse transcription and replication by blocking cell-cycle progression of CD4+ T lymphocytes

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Acute measles virus (MV) infection results in a decrease in plasma human immunodeficiency virus type 1 (HIV-1) RNA levels in co-infected children. An in vitro peripheral blood mononuclear cell (PBMC) culture system was used to assess the mechanisms by which MV blocks HIV-1 replication. MV inhibited proliferation of CD4+ T lymphocytes, the target cell for HIV-1 replication. In the presence of MV, cells did not progress to G1b and S phases, steps critical for the completion of HIV-1 reverse transcription and productive replication. This block in cell-cycle progression was characterized by an increased proportion of CD4+ and HIV-1-infected cells retained in the parental generation in PBMCs co-cultured with MV and HIV-1, and decreased levels of cyclins and RNA synthesis. Early HIV-1 replication was also inhibited in the presence of MV, as measured by reduced expression of a luciferase reporter gene and lower levels of both early (LTR) and late (LTR–gag) DNA intermediates of HIV-1 reverse transcription in the presence of CCR5-tropic HIV-1. The effects of MV on lymphoproliferation and p24 antigen production were reproduced by n-butyrate and hydroxyurea, drugs that block the cell cycle in G1a and G1/S, respectively. It was concluded that MV inhibits HIV-1 productive replication in part by blocking the proliferation of CD4+ T lymphocytes.

INTRODUCTION

Acute measles virus (MV) infection results in a transient but profound decrease in plasma human immunodeficiency virus type 1 (HIV-1) RNA levels. Despite immune activation, plasma HIV-1 RNA levels were markedly reduced in Zambian children hospitalized with measles, returning to expected levels approximately 1 month after hospital discharge (Moss et al., 2002). Ugandan children were observed to have a mean 1.4 log decline from baseline levels in HIV-1 viral load during acute measles (Ruel et al., 2007). Decreased plasma HIV-1 RNA levels also have been reported following acute infection with Orientia tsutsugamushi (Watt et al., 2000) and dengue virus (Watt et al., 2003), and during chronic infection with GB virus C (Williams et al., 2004). However, the mechanisms responsible for the reduction in plasma HIV-1 RNA levels are incompletely understood and may differ for each co-infecting pathogen (Kannangara et al., 2005).

Multiple mechanisms could account for the suppression of HIV-1 replication by MV. Both viruses replicate in lymphoid tissue, providing extensive opportunities for interaction (Kilby, 2001; Yamanouchi et al., 1973). In an ex vivo model using human lymphoid tissues, MV inhibited the replication of both CXCR4-tropic and CCR5-tropic HIV-1, but the inhibitory effect was greater for CCR5-tropic HIV-1 (Grivel et al., 2005). MV upregulated RANTES in tissues co-infected with CCR5-tropic HIV-1 (Grivel et al., 2005), suggesting that increased levels of β-chemokines may in part account for the suppressive effect of MV on replication of CCR5-tropic HIV-1. However, upregulation of β-chemokines does not explain the inhibition of CXCR4-tropic HIV-1 replication, and the effects of MV on lymphoproliferation could not be assessed in this system.

To understand better the mechanisms underlying the reduction in plasma HIV-1 RNA levels in children with measles, we used an in vitro co-infection system in which MV suppresses HIV-1 replication (Garcia et al., 2005). We showed that MV suppresses HIV-1 replication in part by blocking the proliferation of CD4+ T lymphocytes.
METHODS

Cells. Peripheral blood was obtained from healthy, anonymous donors through the blood bank of the Johns Hopkins Hospital, MD, USA. Peripheral blood mononuclear cells (PBMCs) were separated and cultured as described previously (Garcia et al., 2005). Vero cells (ATCC CCL-81) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 μg gentamicin ml⁻¹. Cells were assessed for viability by trypan blue exclusion.

Viruses and controls. The Chicago-1 and Edmonston strains of MV were grown in and assayed by plaque formation on Vero cells as described previously (Garcia et al., 2005). The Chicago-1 strain was isolated in 1989 during a measles outbreak in Chicago and is in genotype group D3 (World Health Organization, 2001). Edmonston wild-type (ATCC VR-24) is a minimally passaged derivative of the original genotype A Edmonston strain of MV isolated by Enders & Peebles (1954). The concentration of lipopolysaccharide was estimated using a Limulus amoebocyte lysate assay (QCL-1000 Chromogenic LAL; BioWhittaker) to be 0.01–0.04 ng ml⁻¹ for Chicago-1 MV, 0.005 ng ml⁻¹ for Edmonston MV and 0.008 ng ml⁻¹ for Vero cell lysate, and all were negative for mycoplasma (MycoAlert; Cambrex BioScience Rockland). Stocks of HIV-1_BaL and HIV-1_HIV were prepared as described previously (Garcia et al., 2005) and titrated using TZM cells (Derdyn et al., 2000).

HIV-1 and MV infection of PBMCs. For most experiments, cells were first infected with HIV-1. HIV-1_BaL was incubated with 10⁷ PBMCs ml⁻¹ in RPMI 1640 containing 20% FBS and 10 IU interleukin (IL)-2 ml⁻¹ at an m.o.i. of 0.0005 for 48 h. For HIV-1_HIV, PBMCs were stimulated with 2 μg phytohaemagglutinin (PHA); Sigma) ml⁻¹ for 24 h in RPMI 1640 containing 20% FBS, 10 IU IL-2 ml⁻¹, 2 mM glutamine, 100 U penicillin ml⁻¹ and 100 μg streptomycin ml⁻¹ before the addition of HIV-1_HIV at an m.o.i. of 0.0004 for 24 h. MV was added (m.o.i. of 0.15) to 2 × 10⁶ HIV-1_BaL-infected or HIV-1_HIV-infected PMBCs per well. Lysates of uninfected Vero cells and medium alone served as controls. HIV-1-uninfected cells, in combination with MV, uninfected Vero cell lysates or medium, served as additional controls for some experiments. p24 antigen production was measured by enzyme immunoassay (EIA; NEN Life Science Products) on days 4, 6, 9 and 14 of culture, and expressed as a percentage of the p24 antigen concentration of HIV-1-infected PBMCs with medium alone. The percentage inhibition was calculated as: 100 – (p24 test sample x 100/p24 positive-control sample).

Lymphoproliferation assays. HIV-1-infected PBMCs were plated in triplicate in 96-well, round-bottomed plates at 10⁶ cells per well in 100 μl RPMI 1640. One hundred microlitres of each of the following was added: (i) Chicago-1 MV at an m.o.i. of 0.15, (ii) Edmonston MV at an m.o.i. of 0.05, (iii) lysates of uninfected Vero cells at the same dilution as MV and (iv) medium alone. Cells were pulsed at 311 °C and cultured as described previously (Garcia et al., 2005) and titrated using TZM cells (Derdyn et al., 2000).

Flow cytometry. To monitor cell proliferation by cell type, HIV-1-infected cells were stained using the Vybrant CFDA SE Cell Tracer kit (V-12883) (CFSE; Molecular Probes). Briefly, 2 × 10⁶ PBMCs ml⁻¹ were washed twice and resuspended in 40 ml PBS. CFSE was added at a final concentration of 0.25 μM and the cells were incubated for 7 min at 37 °C. The cells were pelleted at 311 g, washed three times and plated in triplicate in 96-well, round-bottomed plates at 2 × 10⁶ cells per well in 100 μl RPMI 1640. MV, Vero cell lysate or medium (100 μl) was added to each well. HIV-1-uninfected cells were used as controls. The cells were harvested at 4, 7 and 12 days after the addition of MV (6, 9 and 14 days in culture), incubated with a 1:5 dilution of human serum, washed with 2% FBS in PBS and stained with peridinin–chlorophyll–protein complex-conjugated mouse monoclonal antibodies (mAb) against human CD4 (BD BioSciences Pharmingen). After staining, the cells were washed and fixed with BD Cytofix/Cytoperm (BD BioSciences Pharmingen). Intracellular staining for HIV-1 used phycocerythrin (PE)-conjugated mouse mAb against HIV-1 core antigens (K57-91D1; Coulter), and for MV used unconjugated mAb against the MV nucleoprotein or an isotype control (Chemicon International), followed by aliphosphocytocyanin (APC)-conjugated rat mAb against mouse IgG1 (BD BioSciences Pharmingen).

In other experiments, after HIV-1 infection and prior to the addition of MV, 2 × 10⁶ cells incubated with 10 μl bromodeoxyuridine (BrDU) solution (1 mM BrDU in PBS) ml⁻¹ were stained using an APC BrDU Flow kit (BD Biosciences). Cells were plated in triplicate in 96-well, round-bottomed plates at a concentration of 2 × 10⁵ cells per well in 100 μl RPMI 1640, and 100 μl MV or control sample was added. Four days after the addition of MV, the cells were harvested and incubated with a 1:5 dilution of human serum, washed with PBS supplemented with 2% FBS and stained with fluorescein isothiocyanate (FITC)-conjugated mouse mAb against human CD4 or isotype control antibody (BD BioSciences Pharmingen). After staining, the cells were washed and fixed with BD Cytofix/Cytoperm. After DNase treatment according to the manufacturer’s protocol, BrDU and intracellular staining for HIV-1 and MV was performed with PE-conjugated mAb KC57-91D1 against HIV-1 core antigens and FITC-conjugated mAb against MV. 7-Aminoactinomycin D (7AAD, diluted 1:100; BD Biosciences) was used to stain total cellular DNA. The cells were analysed on a FACSCalibur using Cell Quest Pro software version 5.2 (Becton Dickinson) and FlowJo version 6.3 (Tri Star).

Western blot analysis. PBMCs were infected with MV, HIV-1 or both as described above. Medium and Vero cell lysates served as controls, with or without the addition of HIV-1. After 7 days (9 days in culture), the cells were pelleted, washed three times and frozen at −80 °C. After at least 12 h, the cells were warmed to room temperature and lysed on ice with 70–100 μl cell extraction buffer (BioSource International) supplemented with 4-(2-aminoethyl)benzenesulphonyl fluoride, HCl and a 1 × mix of protease inhibitor cocktails (ICN). The protein concentration was quantified using the Bradford method (Bradford, 1976).

Protein (10–30 μg) was loaded onto 10% polyacrylamide Criterion pre-cast gels (Bio-Rad) under denaturing conditions. The protein was transferred to nitrocellulose Hybond-C Extra (Amersham Bioscienes), air dried and incubated for 1 h at room temperature in 1× TBS/0.1% Tween 20 (TBST) plus 5% non-fat dried milk supplemented with 10% SuperBlock blocking buffer (Pierce Biotechnology). Blots were incubated with primary antibodies in 10 ml TBST plus 5% non-fat dried milk with gentle agitation overnight at 4 °C. The primary antibodies were 1:2000-diluted mouse anti-cyclin D1 and mouse anti-cyclin D3, mouse anti-cyclin E (1 μg ml⁻¹) and 1:10000-diluted mouse anti-actin (Chemicon). After 24 h, the blots were washed three times with TBST at room temperature for 5 min. The membranes were incubated with the horseradish peroxidase-conjugated secondary antibodies diluted 1:2000 for sheep anti-mouse or 1:4000 for actin, or 1:2000-diluted donkey anti-rabbit (Amersham Biosciences). Secondary antibodies were added to 10 ml TBST plus 5% (w/v) non-fat dried milk with gentle agitation for 1 h at room temperature. Blots were washed three
times with TBST at room temperature for 10 min and developed in 12 ml SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology) for 1 min. The blots were wrapped in plastic and exposed to X-ray films (Amersham Biosciences).

**RNA synthesis.** HIV-1-infected PBMCs were plated in triplicate in 96-well, round-bottomed plates at a concentration of 10^5 cells per well in 100 μl RPMI 1640 containing 20% FBS and 10 IU IL-2 ml⁻¹. MV at an m.o.i. of 0.15, Vero cell lysate or medium was added and the cells were incubated at 37 °C for 8 h before or 16, 40, 64, 88, 160 and 280 h after the addition of MV.[^H]Uridine [10 μl; 1 μCi (37 kBq) per well] was added for an additional 8–10 h. RNA was harvested onto glass-fibre filters and the radioactivity read in a scintillation counter.

**Luciferase assay.** TZM cells expressing a luciferase reporter gene under the control of an LTR promoter (Derdeyn et al., 2000) were plated in 24-well plates at a concentration of 5 x 10^4 cells per well in 1 ml DMEM supplemented with 10% FBS, 100 U penicillin ml⁻¹ and 100 μg streptomycin ml⁻¹. The medium was removed and the cells were exposed to MV (m.o.i. of 3, 0.3 and 0.03), lysates of uninfected Vero cells or medium alone for 3 h, before 20 μg diethylaminoethyl ml⁻¹ and 200 IU HIV-1 per well were added for 2 h. Two days after infection, the cells were lysed in 100 μl cell culture lysis reagent (Promega) and pelleted, and supernatant fluids were added to 50 μl luciferase assay substrate (Promega) and the volume brought up to 100 μl with lysis buffer. Luciferase activity was measured using a TD-20/20 luminometer (Turner Designs). Results were normalized by the protein concentration present in lysates. The percentage luciferase activity reduction was calculated as 100 – (luciferase activity in the test sample x 100/luciferase activity in the positive control sample). Positive controls consisted of TZM cells infected with HIV-1.

**TaqMan assays for LTR and LTR–gag.** Cells were activated for 3 days with 0.5 μg of purified PHA (Remel) ml⁻¹ in RPMI 1640 containing 20% FBS, 10 IU IL-2 ml⁻¹, 2 mM glutamine, 100 U penicillin ml⁻¹ and 100 μg streptomycin ml⁻¹. Cells (2 x 10^5 per well) were infected with 500 IU HIV-1 per well (m.o.i. of 0.025) and MV at an m.o.i. of 0.15. Lysates of uninfected Vero cells and medium alone served as controls. Reverse transcription products of early (LTR) and late (LTR–gag) cellular DNA intermediates of HIV-1 reverse transcription were quantified by TaqMan PCR at 8, 12, 24 and 40 h post-infection. Cellular DNA was purified using a FastDNA kit (Bio 101). Published primer pairs (M667/AA55; BioSource International) and a specific TAMRA-labelled probe (HIV-FAM; Applied Biosystems) were used to detect full-length, double-stranded viral DNA (Suzuki et al., 2003). U5/gag primers (LG564/LG699; BioSource International) and a specific TAMRA-labelled probe (LG-FAM; Applied Biosystems) were used to detect linear DNA (Suzuki et al., 2003). The number of copies was determined using a YU-2 plasmid as standard and as controls for linear amplification in the reaction (Li et al., 1992). Primers specific for

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**Fig. 1.** Effect of MV on cell proliferation and p24 antigen production. (a, b) Numbers of viable cells in HIV-1_{Bal}-infected (a) and HIV-1_{BIB}-infected (b) PBMCs co-infected with Chicago-1 or Edmonston MV (m.o.i. of 0.15). Controls were a 1 : 10 dilution of Vero cell lysate. Viability was determined by trypan blue exclusion on days 2, 6, 9 and 14 of culture. Data are presented as means ± SEM of duplicate wells with PBMCs from seven different donors. (c–f) Incorporation of[^H]thymidine by PBMCs infected with HIV-1_{Bal} (c) or HIV-1_{BIB} (d) and co-infected on day 2 with MV (m.o.i. of 0.15). Controls were a 1 : 10 dilution of Vero cell lysate. Data are presented as means ± SEM of triplicate wells with PBMCs from 10 different donors. The reduction in p24 antigen production after culture of HIV-1-infected PBMCs with MV is indicated (e, f), expressed as a percentage of the p24 antigen concentration of HIV-1-infected PBMCs with medium alone. *P<0.05 between Chicago-1 MV and Vero cell lysates; †P<0.05 between Edmonston MV and Vero cell lysates.
the human β-actin gene were used to monitor the amount of cellular DNA loaded and to normalize the results. Thermal cycling conditions used during the experiments were 10 min at 95 °C and 45 cycles of 15 s at 95 °C and 1 min at 55 °C.

Cell-cycle inhibition. After the addition of HIV-1, cells were incubated with 1:10 serial dilutions of thapsigargin (0.01–10 nM; Calbiochem), hydroxyurea (10, 50 or 100 μM; Calbiochem) or n-butyrate (0.1, 1 or 2 mM; Sigma). Supernatant fluid was harvested 4 days after the addition of the drug (corresponding to 6 days in culture) and assayed for HIV-1 p24 antigen by EIA. Control samples consisted of supernatant fluids from PBMCs infected with HIV-1 alone. Lymphoproliferation was measured by [3H]thymidine incorporation by HIV-1-infected and uninfected cells 4 days after the addition of drug.

Cell stimulation and addition of deoxynucleosides. Mouse mAbs anti-CD3 and anti-CD28 (BD Biosciences) were added at 1 μg ml⁻¹ with Chicago-1 MV at an m.o.i. of 0.15 to HIV-1BaL-infected cells. After 96 h, p24 antigen production was measured by EIA and DNA synthesis by [3H]thymidine incorporation. 2'-Deoxynucleosides (ICN) were added at 10 μM to IL-2-stimulated cells 2 h before the addition of Chicago-1 MV (m.o.i. of 0.15) and HIV-1BaL (m.o.i. of 0.0005) (Korin & Zack, 1999). Lysates from uninfected Vero cells or medium alone were used as controls. After 18 h, cells were pelleted and medium was added supplemented with 10 μM 2’-deoxynucleosides. After 96 h, p24 antigen was measured by EIA and proliferation by [3H]thymidine incorporation.

**Statistical analysis.** Results are shown as means ± SEM. Paired comparisons were made with the Wilcoxon signed-ranks test. Unpaired comparisons were made with Student’s t-test. Statistical analyses were conducted using Stata Statistical Software release 7.0 (Stata Corp.).

**RESULTS**

**Effect of MV on the viability of HIV-1-infected PBMCs**

Previous observations have shown that only 10–20% of cells are non-viable after 4 days of culture with MV (Garcia et al., 2005). To measure the number of cells capable of supporting HIV-1 replication in our in vitro co-infection system, viable cells were counted 4, 7 and 12 days after the addition of MV (corresponding to 6, 9 and 14 total days in culture) to cells infected with CCR5-tropic HIV-1BaL.
Fig. 1a) or CXCR4-tropic HIV-1IIIB (Fig. 1b). The number of viable cells remained almost constant for at least 7 days after the addition of Chicago-1 or Edmonston MV, whereas the number of viable cells increased in the absence of MV. During the second week in culture, the number of viable cells decreased in the presence of MV. For cells infected with HIV-1Bal, the number of viable cells on day 9 in culture was approximately 60% lower with MV than without MV ($P<0.01$). For cells infected with HIV-1IIIB, the number of viable cells on day 14 was approximately 30% lower with MV than without MV ($P=0.04$ for Edmonston MV).

**Effect of MV on lymphoproliferation**

The number of viable cells in culture is a function of both cell proliferation and cell death. We have previously shown that MV did not induce cell death in this co-infection system (Garcia et al., 2005), suggesting that MV inhibited the expansion of PBMCs during the first week in coculture. To determine whether there was inhibition of proliferation, incorporation of [3H]thymidine was measured. [3H]Thymidine incorporation into cellular DNA was decreased by MV throughout the period of culture (Fig. 1c, d). This inhibition of proliferation was greater with the Chicago-1 strain than with the Edmonston MV strain. Consequently, subsequent experiments are reported for the Chicago-1 MV strain only.

The kinetics of PBMC proliferation differed in the presence of different HIV-1 strains, with peak proliferation at 9 days in HIV-1Bal-infected PBMCs and 6 days in HIV-1IIIB-infected PBMCs, consistent with the addition of PHA and IL-2 to the HIV-1IIIB cultures and only IL-2 to the HIV-1Bal culture. Consequently, the maximal inhibitory effect on lymphoproliferation was observed 7 days after the addition of MV to HIV-1Bal-infected PBMCs (total of 9 days in culture) ($P=0.003$ for Chicago MV and $P=0.01$ for Edmonston MV) and 4 days after the addition of MV to HIV-1IIIB-infected PBMCs (6 days in culture) ($P=0.003$ for Chicago MV and $P=0.004$ for Edmonston MV). The decrease in lymphoproliferation induced by MV overlapped with the reduction in p24 antigen concentration (Fig. 1e, f) (Garcia et al., 2005).
MV co-infects HIV-1-infected CD4⁺ T lymphocytes and blocks their proliferation

To understand the mechanisms by which MV inhibits HIV-1 replication, we next determined by flow cytometry whether MV infects CD4⁺ cells when co-cultured with HIV-1BaL to establish whether the two viruses could co-infect CD4⁺ T lymphocytes. Four days after the addition of Chicago-1 MV to PBMCs co-cultured with HIV-1BaL, 38% of cells infected by MV were CD4⁺. Importantly, 40% of HIV-1-infected PBMCs and 35% of HIV-1-infected CD4⁺ T lymphocytes were co-infected with Chicago-1 MV.

MV blocks the proliferation of lymphocytes (Hirsch et al., 1984; Ward et al., 1991). To determine whether MV inhibited proliferation of CD4⁺ T lymphocytes in the presence of HIV-1, PBMCs were labelled with CFSE and co-stained for CD4 or HIV-1 core antigens. CFSE enters cells, where esterases convert it to a fluorescent dye incapable of crossing cell membranes. As cells divide, the relative intensity of the dye is decreased by half, permitting identification of successive cell generations. MV inhibited the proliferation of CD4⁺ cells in both the absence and presence of HIV-1 (Fig. 2a), as demonstrated by the greater percentage of CD4⁺ cells retained in the parental generation after addition of MV than after addition of medium or lysates of uninfected Vero cells (Fig. 2a, b). Importantly, a greater percentage of HIV-1-infected cells also remained in the parental generation after addition of MV than after addition of medium or lysates of uninfected Vero cells (Fig. 2b), confirming that MV blocks proliferation of HIV-1-infected cells.

MV impairs cell-cycle progression of HIV-1-infected CD4⁺ T lymphocytes into the S phase

To characterize further the effect of MV on cell-cycle progression of CD4⁺ T lymphocytes and cells co-infected with HIV-1, we measured the percentage of cells that stained with BrdU, a thymidine analogue that is incorporated into newly synthesized DNA during the S phase of the cell cycle. DNA synthesis was impaired in CD4⁺ T lymphocytes co-cultured with MV, as indicated by a reduction in the proportion of BrdU-positive CD4⁺ T lymphocytes in the presence of Chicago-1 MV compared with cells cultured with medium or lysates of uninfected Vero cells (Fig. 3a). Importantly, the percentage of BrdU-staining HIV-1BaL-infected cells was reduced in the presence of Chicago-1 MV compared with medium or lysates of uninfected Vero cells (Fig. 3a).

Flow cytometric analysis of cellular DNA content provides additional information on the proportion of cells in different phases of the cell cycle. Co-staining with BrdU and 7AAD, which binds total DNA, allows simultaneous assessment of DNA content and newly synthesized DNA. The percentage of HIV-1BaL-infected CD4⁺ T lymphocytes in S and G₂/M was lower when co-cultured with Chicago-1 MV than with medium or lysates of uninfected Vero cells (Fig. 3b), further demonstrating the block in cell-cycle progression of HIV-1-infected cells.

MV suppression of HIV-1 replication
progression of HIV-1-infected CD4⁺ T lymphocytes induced by MV.

**MV decreases levels of cyclins critical to cell-cycle progression and HIV-1 replication**

Cell-cycle progression into the S phase is characterized by increased levels of cell-cycle regulatory proteins that are also critical to productive HIV-1 replication (Clark et al., 2000; Kashanchi et al., 2000). Cyclin levels were measured by Western blotting to determine whether these regulatory proteins were decreased in PBMCs co-cultured with MV and HIV-1. Decreased levels of the cyclins D1, D3 and E were observed after co-culture of PBMCs with Chicago-1 MV or co-culture of PBMCs with Chicago-1 MV and HIV-1Bal (Fig. 4a), consistent with a block in cell-cycle progression into the S phase induced by MV (Engelking et al., 1999; Heaney et al., 2002; Naniche et al., 1999).

**MV decreases RNA synthesis in HIV-1-infected PBMCs**

The G₁b phase of the cell cycle involves an increase in RNA synthesis in preparation for entry into the S phase (Darzynkiewicz et al., 1980; Toba et al., 1995). To determine whether MV blocked progression into G₁b, RNA synthesis was measured by [³H]uridine uptake. RNA synthesis was low in uninfected PBMCs during the 14 days in culture (Fig. 4b). PBMCs co-cultured with HIV-1 or HIV-1 plus Vero cell lysates responded to infection with increased RNA synthesis (Fig. 4b). However, RNA synthesis decreased within 4 days in PBMCs co-cultured with Chicago-1 MV and HIV-1Bal or HIV-1IIIb compared with PBMCs cultured with HIV-1 alone or with HIV-1 and Vero cell lysate. Levels of RNA synthesis remained low in PBMCs co-cultured with MV and HIV-1 for 12 days in culture (Fig. 4b), consistent with a block in cell-cycle progression prior to the increase in RNA synthesis during G₁b.

**MV blocks HIV-1 reverse transcription in HIV-1-infected PBMCs**

Transition from G₁a to G₁b of the cell cycle is necessary for completion of HIV-1 reverse transcription (Kootstra et al., 2000; Korin & Zack, 1998). To determine whether the MV-induced block in cell-cycle progression was associated with decreased HIV-1 reverse transcription, the effect of MV on early steps in HIV-1 replication was measured using TZM cells. TZM cells are engineered to express CD4 and CCR5 and contain integrated reporter genes for firefly luciferase and *Escherichia coli* β-galactosidase under the control of an HIV-1 LTR (Derdeyn et al., 2000). HIV-1 Tat acts through the transactivating response region to augment LTR-directed expression of luciferase. Consistent with the effect on cell-cycle progression, the reduction in luciferase activity was greater after the addition of Chicago-1 MV to PBMCs infected with HIV-1Bal or HIV-1IIIb than after the addition of lysates of uninfected Vero cells at different m.o.i. for MV (Fig. 5a).

To determine further the effect of MV-induced cell-cycle arrest on HIV-1 reverse transcription, early (LTR) and late (LTR–gag) DNA intermediates of HIV-1 reverse transcription were measured by quantitative RT-PCR. Decreased levels of both LTR (R/U5; 84.7% reduction) and LTR–gag (U5/gag; 89.7% reduction) were observed for HIV-1Bal when co-infected with Chicago-1 MV (Fig. 5b). However, for HIV-1IIIb, the decrease in LTR–gag was observed only 24 h post-infection (78.8% reduction) (Fig. 5b).
Cell-cycle inhibitors mimic the effect of MV on lymphoproliferation and p24 antigen production

Cell-cycle blocking agents were used to induce phase-specific inhibition of the cell cycle and simulate the effect of MV on lymphoproliferation and p24 antigen production. Thapsigargin induces a block in G0 (Furuya et al., 1994), n-butyrate at G1a (Darzynkiewicz et al., 1981; Korin & Zack, 1998) and hydroxyurea in G1/S (Maurer-Schultze et al., 1988). Thapsigargin failed to produce consistent dose-dependent reductions in either lymphoproliferation or p24 antigen production (Fig. 6). n-Butyrate and hydroxyurea, however, produced dose-dependent blocks in proliferation of similar magnitude to the reduction in p24 antigen production (Fig. 6). Thus, a block in late G1/S by hydroxyurea and in G1a by n-butyrate mimicked the inhibitory effects of MV on lymphoproliferation and p24 antigen production.

Effects of MV on lymphoproliferation and p24 antigen production are not reversed by cellular activation or deoxyribonucleosides

To assess whether cellular activation could overcome the inhibitory effect of Chicago-1 MV on lymphoproliferation and p24 antigen production, cells were either stimulated with PHA prior to infection with Chicago-1 MV and HIV-1 Bal or stimulated with anti-CD3 and anti-CD28 mAbs at the time of MV infection. Lymphoproliferation and p24 antigen production were not substantially enhanced by stimulation with either PHA or anti-CD3 and anti-CD28 antibodies (Table 1). Similarly, the addition of deoxyribonucleosides, which can partially alleviate the block in HIV-1 reverse transcription (Korin & Zack, 1999), did not reverse the effects of MV on lymphoproliferation or p24 antigen production (Table 1).

Table 1. Effect of cell activation and addition of nucleosides on p24 antigen production and lymphoproliferation 96 h after the addition of Chicago-1 MV and HIV-1 Bal to PBMCs

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Reduction in p24 Ag (%)</th>
<th>Reduction in lymphoproliferation (%)</th>
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<tbody>
<tr>
<td></td>
<td>Without stimulus</td>
<td>With stimulus</td>
</tr>
<tr>
<td>PHA</td>
<td>83.2 ± 3.8</td>
<td>67.2 ± 12.8</td>
</tr>
<tr>
<td>Anti-CD3/CD28 mAbs</td>
<td>87.6 ± 3.9</td>
<td>88.9 ± 5.3</td>
</tr>
<tr>
<td>Nucleosides</td>
<td>85.2 ± 13.3</td>
<td>83.6 ± 6.6</td>
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Fig. 6. Simulation of the effects of MV by cell-cycle blocking agents. (a) Proliferation was measured by incorporation of [3H]thymidine by PBMCs cultured in the presence of different concentrations of thapsigargin, n-butyrate and hydroxyurea with 10 U IL-2 ml⁻¹ (control) or with 10 U IL-2 ml⁻¹ and HIV-1 Bal. Drugs were added on day 2 of culture. Data are presented as means ± SEM of triplicate wells with PBMCs from five donors. (b) HIV-1 p24 antigen production was measured by EIA in the supernatant fluid of PBMCs cultured in the presence of different concentrations of thapsigargin, n-butyrate and hydroxyurea with 10 U IL-2 ml⁻¹ and HIV-1 Bal. Drugs were added on day 2 of culture. Data are presented as means ± SEM of duplicate wells with PBMCs from five to eight donors. *P < 0.05, comparing drugs at different concentrations with medium alone.
DISCUSSION

Our findings suggest that MV inhibits HIV-1 replication in part by blocking proliferation of CD4+ T lymphocytes. In vivo, more than one mechanism may contribute to altered HIV-1 replication. HIV-1 cell entry requires binding of the virion gp120 to CD4 on host cells and the subsequent engagement of chemokine co-receptor CCR5 or CXCR4 (Berger et al., 1999). Downregulation of these receptors or increases in their natural ligands (e.g. chemokines) could inhibit HIV-1 replication by blocking fusion. Consequently, co-infection of human lymphoid tissues with MV and HIV-1 results in inhibition of HIV-1 replication, in part through production of β-chemokines (Grivel et al., 2005).

Productive HIV-1 replication requires target-cell activation and proliferation (Stevenson et al., 1990; Zack, 1995). HIV-1 infection of quiescent CD4+ T lymphocytes results in incomplete, labile reverse transcripts (Korin & Zack, 1999). Progression to the G1b phase of the cell cycle is necessary for nuclear importation, integration of the viral genome and production of viral progeny (Korin & Zack, 1998; Zack, 1995). Transcription of HIV-1 is regulated by cyclin-dependent kinases and cyclins involved in the cell cycle (Clark et al., 2000; Kashanchi et al., 2000). Entry of host cells into the S phase is necessary for RNA splicing, transport, translation and packaging of virion-specific genes (Clark et al., 2000), and a reduction in the number of target cells capable of entering the S phase can inhibit productive HIV-1 replication. After cellular DNA synthesis is completed, the Vpr protein inhibits cell-cycle progression at G2, a process also important for efficient HIV-1 replication (Yoshizuka et al., 2005).

MV can inhibit the proliferation of lymphocytes through multiple mechanisms. PBMCs isolated from people with measles have suppressed lymphoproliferative responses to mitogens (Hirsch et al., 1984; Ward et al., 1991). Co-expression of MV haemagglutinin and fusion glycoproteins on non-lymphoid cells inhibits the proliferation of lymphocytes through a mechanism that requires cleavage of the fusion protein (Weidmann et al., 2000). MV nucleoprotein arrests cell proliferation through interaction of N-TAIL with an unknown nucleocapsid receptor on the cell surface (Laine et al., 2005). Inhibition of lymphoproliferation by MV in the cotton rat model is associated with retardation of the cell cycle in G0/G1 (Niewiesk et al., 1999). Arrested lymphoproliferation in the G0/G1 phase of the cell cycle after infection with MV or contact with MV-infected cells is associated with reduced expression and activity of G1 cyclin-dependent kinase complexes and delayed degradation of p27kip (Engelking et al., 1999). Cyclin D1 regulates progression of cells through G1/S phase (Stacey, 2003) and downregulation of cyclin D1 by MV has not been described previously.

The effects of MV on lymphoproliferation and p24 antigen production were simulated by hydroxyurea, a drug that blocks the cell cycle in G1/S phase (Maurer-Sultzte et al., 1988), and by n-butyrate, which blocks the cell cycle at G1a (Darzynkiewicz et al., 1981; Korin & Zack, 1998). Thapsigargin, which blocks the cell cycle at G0, did not consistently reduce lymphoproliferation or p24 antigen production. Hydroxyurea inhibits HIV-1 replication (Lori et al., 1994) and has been used in combination with dideoxynucleoside reverse transcriptase inhibitors for the treatment of HIV-1 infection (Lori & Lisziewicz, 2000).

We identified a novel mechanism by which MV co-infection inhibits HIV-1 replication by blocking proliferation of CD4+ T lymphocytes. Understanding the mechanisms by which MV suppresses HIV-1 replication provides insights into HIV-1 pathogenesis and the complex virological interactions that can occur within a single host.

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REFERENCES


