

# Human immunodeficiency virus types 1 and 2 have different replication kinetics in human primary macrophage culture

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Received 28 July 2005  
Accepted 25 October 2005

This study compares the replication of primary isolates of human immunodeficiency virus type 2 (HIV-2) and type 1 (HIV-1) in monocyte-derived macrophages (MDMs). Eleven HIV-2 and five HIV-1 primary isolates that use CCR5, CXCR4 or both coreceptors to enter cells were included. Regardless of coreceptor preference, 10 of 11 HIV-2 viruses could enter, reverse transcribe and produce fully infectious virus in MDMs with efficiency equal to that in peripheral blood mononuclear cells. However, the kinetics of replication of HIV-2 compared with HIV-1 over time were distinct. HIV-2 had a burst of virus replication 2 days after infection that resolved into an apparent 'latent state' at day 3. HIV-1, however, continued to produce infectious virions at a lower, but steady, rate throughout the course of infection. These results may have implications for the lower pathogenesis and viral-load characteristics of HIV-2 infection.

## INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) both cause AIDS. Compared with HIV-1, HIV-2 is less pathogenic, less transmissible (Marlink *et al.*, 1994) and limited mainly to West Africa (Clavel *et al.*, 1987). In contrast, HIV-1 is the cause of the current worldwide pandemic.

Both viruses use CD4 and a seven-transmembrane coreceptor, usually CCR5 or CXCR4, to gain entry into cells. For the majority of infections by HIV-1, CCR5-using isolates (R5) predominate in primary infection. Later, in about 40–50% of patients, CXCR4-using viruses (X4) or viruses using both coreceptors (X4R5) develop (Clapham & McKnight, 2002; Gorry *et al.*, 2005). The emergence of X4 viruses is associated with decline of CD4 counts and faster disease progression (Connor *et al.*, 1997). A similar association between X4 variants and disease has been seen with HIV-2 (Blaak *et al.*, 2005; Kulkarni *et al.*, 2005; Mörner *et al.*, 1999; Sol *et al.*, 1997). It has been postulated that both X4 and R5 viruses are transmitted, but the viruses that infect macrophages flourish (Schuitemaker, 1994; Schutten *et al.*, 2001). The success of R5 viruses may be due to a greater availability of CCR5-expressing cells in initial infection. R5 HIV-1 strains isolated from AIDS patients are more efficient at macrophage infection than R5 strains isolated from asymptomatics (Li *et al.*, 1999; Tuttle *et al.*, 2002).

Unlike HIV-1, HIV-2 can use a broader spectrum of coreceptors *in vitro*, including CCR1, CCR2b, CCR3, CCR8, BOB, BONZO, CXCR1, GPRI, APJ, US28 and CXCR4 (Clapham & McKnight, 2002; Deng *et al.*, 1996; Feng *et al.*,

1996; Maddon *et al.*, 1986; McKnight *et al.*, 1998; Mörner *et al.*, 1999, 2002; Sattentau *et al.*, 1988; Sol *et al.*, 1997). AIDS still develops in individuals who maintain CCR5-only-using viruses. Broader coreceptor use has not so far been associated with pathogenesis (Blaak *et al.*, 2005) or with a more widened tropism *in vivo* (Mörner *et al.*, 2003).

All lentiviruses, including maedi-visna virus (MVV), *Caprine arthritis encephalitis virus* and *Equine infectious anemia virus* (EIAV), have a propensity to replicate in macrophages. Indeed, EIAV and MVV apparently replicate solely in macrophages *in vivo*; therefore, the pathogenesis of these infections can be attributed to macrophage tropism (Dawson, 1987; Sellon *et al.*, 1992).

Compared with HIV-1, HIV-2 infection is associated with a longer period of latency, slower disease progression, low viral loads and reduced transmission rates (Berry *et al.*, 2002; Kanki *et al.*, 1994; Marlink *et al.*, 1994). As infection of macrophages may be a determinant of pathogenesis, we compared their susceptibility to HIV-1 with that to HIV-2, to possibly gain insight into why HIV-1 is significantly more pathogenic than HIV-2.

Numerous studies have addressed the broad coreceptor use of HIV-2, but not tropism for primary monocyte-derived macrophages (MDMs). Here, we characterize the replication of 11 HIV-2 primary isolates in MDMs and T cells from peripheral blood mononuclear cells (PBMCs) and compare it with the replication of five primary HIV-1 isolates. Our study shows that MDMs accommodate entry, reverse transcription, assembly and virus production with equal efficiency for both HIV-1 and HIV-2. However, unlike HIV-1,

HIV-2 has an initial burst of viral production followed by an apparent latency phase.

## METHODS

**Cells.** MDMs were isolated from peripheral blood monocytes by adherence to plastic as described previously (Simmons *et al.*, 1995), except that washes were performed with serum-free RPMI 1640, penicillin (100 U ml<sup>-1</sup>) and streptomycin (100 µg ml<sup>-1</sup>). Blood was layered onto Lymphoprep (Axis-Shield) and centrifuged for 30 min at 700 g. The white-blood-cell layer was harvested, washed with PBS and suspended in RPMI 1640. Cells were counted and then plated at 1.0 × 10<sup>8</sup> cells per 140 mm bacterial dish in 5% heat-inactivated human serum RPMI 1640. After 2 h, the plates were washed three times in RPMI 1640 and then incubated at 37 °C overnight. The cells were left to differentiate into MDMs for 7 days, then washed with PBS, treated with 5 mM PBS/EDTA at 37 °C for 20 min, harvested gently with a cell scraper, counted and replated on 96- or six-well trays at 10<sup>4</sup> and 10<sup>6</sup> cells per well, respectively, as described previously (McKnight *et al.*, 2001). MDMs were normally >99% pure by staining for CD14 and flow-cytometric analysis.

PBMCs were prepared from the same donor. The white-blood-cell layer was harvested and cells were resuspended at 1.0 × 10<sup>6</sup> cells ml<sup>-1</sup> in 10% heat-inactivated fetal calf serum (FCS) and 0.5 µg phytohaemagglutinin (PHA) ml<sup>-1</sup>. Two days later, the PBMCs were washed twice in serum-free RPMI 1640 and then suspended in 10% FCS, 20 U interleukin 2 (IL-2) ml<sup>-1</sup> for 2 days.

NP2/CD4/CXCR4 and NP2/CD4/CCR5 cells were used for titrating virus and were maintained in Dulbecco's modified Eagle's medium (DMEM), 5% FCS (Soda *et al.*, 1999).

**Viruses and preparation of virus stocks.** Primary HIV-2 and HIV-1 strains used (Table 1) were prepared in PHA- and IL-2-stimulated PBMCs from the peripheral blood of infected individuals as described previously (McKnight *et al.*, 1998; Simmons *et al.*, 1996; Reeves *et al.*, 1999).

**Quantification of reverse transcriptase (RT) activity.** RT activity from harvested PBMCs or MDMs was measured by using a Caviditech Lenti-RT Activity RT-ELISA kit (as per the manufacturer's instructions).

**Virus infectivity assays.** TCID<sub>50</sub> values of HIV-1 and HIV-2 were estimated for both MDMs and PBMCs. MDMs and PBMCs were plated on 96-well trays at 10<sup>4</sup> and 10<sup>5</sup> cells per well, respectively. Virus was diluted serially in half-logs and 50 µl aliquots were incubated with target cells. Supernatant was harvested at days 14 and 21 from PBMCs and MDMs, respectively. Virus production was detected by RT-ELISA (Caviditech) according to the manufacturer's instructions.

Time courses of virus infectivity of MDMs were done in six-well trays. Cells were plated at 1.5 × 10<sup>6</sup> cells per well. The following day, 1 ml 10<sup>4</sup> TCID<sub>50</sub> ml<sup>-1</sup> (as determined on PBMCs) of virus in 10% heat-inactivated human serum RPMI 1640 was added to its corresponding well. After 3 h infection, the virus was removed and the cells were washed with serum-free RPMI 1640. Fresh 10% heat-inactivated human serum RPMI 1640 was added and the first aliquot (200 µl), time 0, was taken. Every 2 days, a 200 µl aliquot was removed and stored at

**Table 1.** Coreceptor use of HIV-1 and -2 primary isolates

Isolate	Coreceptor(s)*	Preferred coreceptor*	Titre on NP2/CD4/CXCR4 (f.f.u. ml <sup>-1</sup> )	Titre on NP2/CD4/CCR5 (f.f.u. ml <sup>-1</sup> )
HIV-2				
MLC	<b>R5</b>	R5	0	1.0 × 10 <sup>4</sup>
TER	<b>R5</b> + R1, R3, CXCR6	↓	0	4.5 × 10 <sup>4</sup>
ALI	<b>R5</b> + R1		0	4.0 × 10 <sup>4</sup>
JAU	<b>R5/X4</b> + R1, R2b, R3, R8		5.0 × 10 <sup>3</sup>	5.0 × 10 <sup>3</sup>
ST	<b>R5/X4</b> + R1, R2b, R3		NT†	NT†
ETP	<b>R5/X4</b> + R1, R2b, R3		1.0 × 10 <sup>5</sup>	5.0 × 10 <sup>2</sup>
MIR	<b>R5/X4</b> + R1, R2b, R3		5.5 × 10 <sup>4</sup>	5.0 × 10 <sup>2</sup>
prCBL23	<b>R5/X4</b> + R1, R2b, R3		4.0 × 10 <sup>3</sup>	5.0 × 10 <sup>2</sup>
MIL	<b>X4</b>		2.3 × 10 <sup>4</sup>	0
SAB	<b>X4</b> + R3		4.0 × 10 <sup>4</sup>	0
AND	<b>X4</b> + R1, R2b, R3		X4	1.0 × 10 <sup>5</sup>
HIV-1				
SF162	<b>R5</b>	R5	0	2.5 × 10 <sup>5</sup>
SL2	<b>R5</b>	↓	0	1.0 × 10 <sup>5</sup>
2076	<b>R5/X4</b> + R3, R8, STRL-33, GPR-15		2.0 × 10 <sup>4</sup>	1.8 × 10 <sup>4</sup>
2028	<b>R5/X4</b> + R3, R8, STRL-33, GPR-15		7.5 × 10 <sup>4</sup>	7.3 × 10 <sup>4</sup>
2044	<b>X4</b>		X4	7.8 × 10 <sup>4</sup>

\*R5, CCR5; X4, CXCR4. +, Additional coreceptor use as determined previously (McKnight *et al.*, 1998; Neil *et al.*, 2005; Reeves *et al.*, 1999). Coreceptors shown in bold denote predominant use of that coreceptor. Arrows indicate increasing CXCR4 usage from highest CCR5 usage (top). Therefore, the isolates listed in the middle of the table will use both CCR5 and CXCR4 equally as well on NP2/CD4/CCR5 and NP2/CD4/CXCR4 indicator cells, respectively.

†NT, Not tested.

-40 °C for determination of RT activity and focus-forming units (f.f.u.). After the removal of each time-point aliquot, the MDMs were washed once with 10% heat-inactivated human serum RPMI 1640 to eliminate the possibility of measuring 'carry-over' virus in the following time point.

**Immunostaining of HIV-infected cells.** HIV-2 primary isolates were titrated on NP2/CD4/CXCR4 or NP2/CD4/CCR5 cells, depending on coreceptor usage (Table 1). Methanol:acetone (1:1)-fixed cells infected with HIV-2 were immunostained with HIV-2 serum as described previously (McKnight *et al.*, 1998).

**PCR amplification of infected cells.** For full-length *gagLTR* and *env-3* PCR, MDMs were set up as for time courses, except that the viruses were treated with 25 U DNase per 0.5 ml vial of virus before dilution and incubation with cells. MDMs were incubated with  $1.0 \times 10^3$  TCID<sub>50</sub> virus (determined in PBMCs) and DNA was extracted at various time points with a QIAamp DNA blood mini kit (Qiagen). Half-log serial dilutions were made of the DNA and *gagLTR* PCR was conducted as described previously (Schmitz *et al.*, 2004).

**Measurement of viral mRNA transcripts by RT-PCR.** Cells for RT-PCR were dissolved *in situ* with 1 ml TRIzol RNA-extraction reagent (Gibco-BRL). RNA was extracted as per the manufacturer's instructions. Extracted RNA was quantified spectrophotometrically by measuring  $A_{260}$  ( $1 A_{260} = 50$  ng RNA). One microgram of total RNA was DNase-treated for 30 min at 37 °C. To remove RNA secondary structure and to inactivate the DNase, two-fifths of the DNase-treated RNA was heated at 75 °C for 10 min in the presence of DNase stop solution and random primers before putting on ice. The RNA was added to reactions that were either RT enzyme-positive or -negative (to control for contaminating DNA). RT reactions, plus or minus RT, were incubated at the reaction temperature of 42 °C for 1 h. A 2 µl aliquot of each reaction was added to a 50 µl GAPDH and *gagLTR* PCR and subjected to the PCR conditions described in the section 'PCR amplification of infected cells'.

## RESULTS

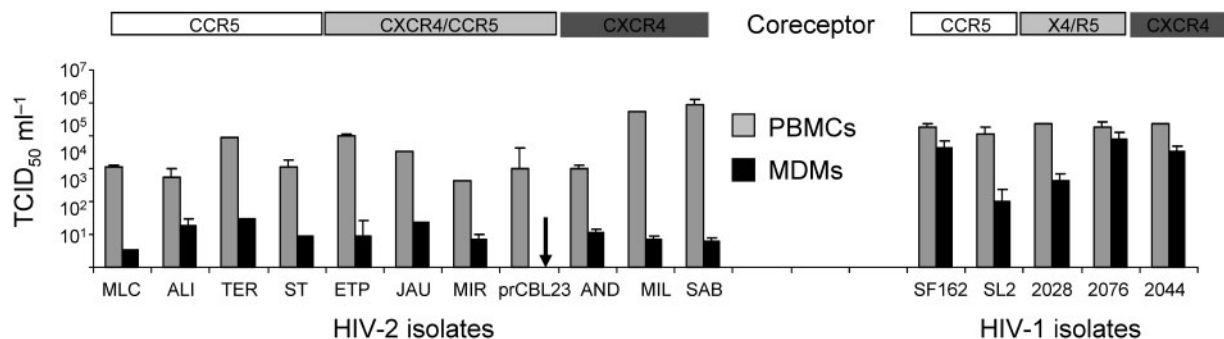
### Tropism of HIV-2 for primary MDMs

We first determined the tropism of a panel of 11 HIV-2 primary isolates for infection of MDMs and compared it with that of five HIV-1 isolates. The HIV-2 isolates chosen had a range of coreceptor usage, such as CCR5 or CXCR4

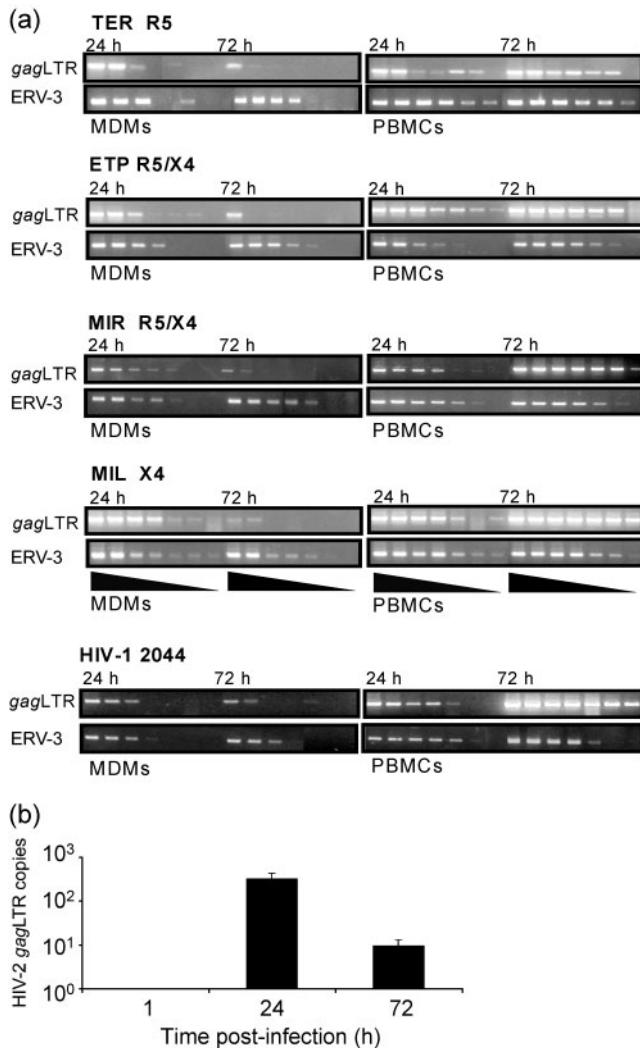
alone, dual-tropic CXCR4/CCR5 and broader usage: CCR1, CCR2b and/or CCR3 (see Table 1). All virus stocks were produced in PBMCs. Viral supernatants were titrated on PBMCs and MDMs, infection was monitored for RT activity and TCID<sub>50</sub> values ml<sup>-1</sup> were calculated. For comparison, we chose a panel of HIV-1 isolates with a known range of efficiencies of MDM infection, either using CCR5 or CXCR4 (or both) as coreceptor: SF162 (R5), SL2 (R5), 2028 (R5/X4), 2076 (R5/X4) and 2044. 2044 is unusual because, unlike most HIV-1 X4 viruses, it can use CXCR4 on MDMs (Simmons *et al.*, 1998). Fig. 1 shows that the results for HIV-1 were as expected: SF162, 2076 and 2044 were almost as efficient at infection of MDMs as of PBMCs. SL2 and 2028 were less efficient at MDM infection. In contrast, none of the HIV-2 isolates demonstrated efficient infection of MDMs compared with 'macrophage-tropic' HIV-1 isolates. All of the HIV-2 isolates were between 2 and 4 logs less efficient at infection of MDMs. Of the HIV-2 isolates, ALI was the most efficient, but was still almost two logs less efficient than its efficiency in PBMCs.

### Early post-entry events for HIV-2 replication are accommodated in primary MDMs

Rhesus tripartite motif protein 5α (TRIM 5α) has recently been identified to restrict HIV-1 replication, resulting in abortive infection prior to completion of reverse transcription (Stremlau *et al.*, 2004). Others have reported pre- and post-reverse transcription restrictions of some R5 HIV-1 viruses in monocytes (Eisert *et al.*, 2001; Neil *et al.*, 2001; Sonza *et al.*, 1996; Triques & Stevenson, 2004). We sought to determine whether the limited replication of HIV-2 isolates in MDMs could be attributed to inhibition at an early post-entry step. We thus determined whether the entry and early-replication events, such as reverse transcription of HIV-2, are accommodated efficiently in MDMs. To do this, we PCR-amplified full-length reverse transcripts (*gagLTR*) by using specific primers. The *gagLTR* PCR will detect full-length proviral and viral DNA transcripts. PCR amplification of ERV-3, an endogenous retrovirus that is present in



**Fig. 1.** HIV-2 is less efficient at infection of MDMs than HIV-1. TCID<sub>50</sub> ml<sup>-1</sup> values of HIV-2 and HIV-1 primary isolates on MDMs and PBMCs, harvested at 21 days (MDMs) and 14 days (PBMCs) post-infection. Coreceptor use is indicated (see Table 1). HIV replication was detected by RT-ELISA.



**Fig. 2.** Limiting-dilution *gagLTR* PCR and *erv-3* PCR analysis of HIV-2 time courses on MDMs and PBMCs. (a) To determine the extent of HIV-2 reverse transcription in PBMCs and MDMs, the final stages of reverse transcription were measured by *gagLTR* PCR. 10<sup>6</sup> PBMCs and MDMs were infected with 10<sup>3</sup> TCID<sub>50</sub> HIV-2 (HIV-2 titres were determined on PBMCs). Cells were infected with HIV-2 inoculum for 3 h and then washed twice with RPMI/10% human serum. Serial half-log dilutions were made of the infected PBMC or MDM DNA, starting with neat and ending with a dilution of 10<sup>-3</sup>. Macrophage-tropic HIV-1 2044 is shown for comparison. (b) qPCR was conducted on DNA from MDMs infected with HIV-2 MIR in (a) above, to verify the serial-dilution method in Fig. 2(a).

the human genome as a single copy, was used as a positive/input control. The results for HIV-2 TER, ETP, MIR and MIL are shown in Fig. 2(a). Comparable levels of MIR *gagLTR* were produced at 24 h post-infection in both MDMs and PBMCs. However, at 72 h, although the levels of *gagLTR* transcripts continued to increase in PBMCs, in MDMs, a decrease was observed (1.5–2 logs). A similar pattern was seen for TER, ETP and MIL. For comparison,

HIV-1 isolate 2044 was tested alongside these HIV-2 isolates. There were apparently threefold (0.5 log) fewer *gagLTR* transcripts at the 72 h time point compared with 24 h, indicating comparatively little loss of RT activity for HIV-1.

Quantitative PCR (qPCR) analysis of *gagLTR* transcripts confirmed the accuracy of the titration PCR data in Fig. 2(a). Fig. 2(b) confirms by qPCR that there is a 1.5-log loss in MIR *gagLTR* transcripts in MDMs from 24 to 72 h.

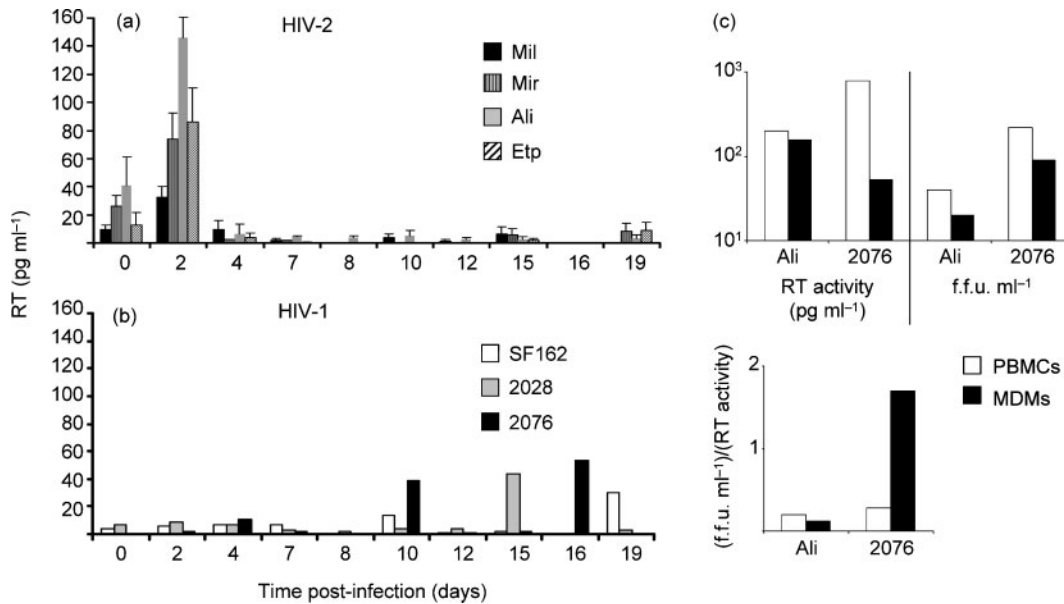
### Kinetics of HIV-2 replication compared with those of HIV-1 are different in MDMs

To further characterize MDM tropism by HIV-2, a time course of viral infection (measured by RT activity) was followed up to 19 days and compared with that of HIV-1. Samples were taken every 2–3 days. Cells were washed after each sampling point so that only newly produced virus was measured at each subsequent sampling, unlike previously (Fig. 1), where RT activity was allowed to accumulate (21 days). Fig. 3(a, b) shows that HIV-2 and HIV-1 had different kinetics of viral production in primary MDMs. For HIV-2, there was typically a burst of RT activity at day 2 that diminished at later time points (Fig. 3a). HIV-1 production was also detected 2 days post-infection but, in contrast to HIV-2, was lower but generally continued throughout the time course (Fig. 3b). HIV-1 2076 was most productive, with RT activity continuing to rise up to day 16 (50 pg RT activity ml<sup>-1</sup>), but to much lower levels than HIV-2 ALI (140 pg RT activity ml<sup>-1</sup>).

### Transient replication of HIV-2 is not due to lack of production of infectious virus

We investigated whether the apparent inefficient replication of HIV-2 in MDMs after day 2 could be due to lack of production of infectious virus. This, for example, could occur if these HIV-2 viruses were susceptible to Lv2, a previously described post-entry restriction in MDMs (McKnight *et al.*, 2001; Schmitz *et al.*, 2004). Lv2 inhibits early replication at a stage after reverse transcription, but prior to nuclear entry. Thus, Lv2 may become even more apparent in MDMs after multiple rounds of infection. Alternatively, MDMs might fail to produce infectious virions of HIV-2. To examine these possibilities, we harvested virus (HIV-2, ALI) produced from either MDMs or PBMCs and compared RT activity and titre (f.f.u. ml<sup>-1</sup>). If MDMs were inefficient at events after reverse transcription (including assembly and release of virus) or produced defective viral particles, we would expect the ratio of f.f.u. ml<sup>-1</sup> to RT activity to be lower for MDMs than for PBMCs. Fig. 3(c) shows that the ratios of f.f.u. ml<sup>-1</sup>: RT on MDMs and PBMCs were equivalent, demonstrating that the entire replication cycle of HIV-2 is as efficient in MDMs as in PBMCs.

We also compared the infectiousness of HIV-1 2076 harvested from MDMs and PBMCs. Interestingly, HIV-1 2076



**Fig. 3.** HIV-2 replication in MDMs is early and transient. (a) Time courses of HIV-2 RT production and (b) HIV-1 RT production in MDMs. Aliquots were taken at 2-day intervals for RT-ELISA. Time 0 was measured after the inoculum was left on MDMs for 3 h followed by two washes. (c) Particle infectivity. The ratio of infectivity (f.f.u. ml<sup>-1</sup>) to virus particles [RT activity (pg ml<sup>-1</sup>)] of output HIV-2 ALI from PBMCs and MDMs was used to determine that HIV-2 production in MDMs resulted in infectious particles. One time point from ALI in PBMCs (day 4) is compared with a time point from ALI in MDMs (day 2), and HIV-1 2076 (day 16).

harvested from MDMs was more infectious than MDM-harvested HIV-2 ALI (Fig. 3c).

### HIV-2 mRNA production is suppressed, but can be stimulated in HIV-2-infected MDMs

We next tested whether the HIV-2-infected MDMs produced viral mRNA transcripts. We measured for all spliced and unspliced RNA species by using PCR primers that are upstream of the first splice-donor site. The results show that mRNA transcript production ceased after 48 h for HIV-2 (Fig. 4a). In contrast, a constant level of HIV-1 SF162 mRNA production continued to day 5.

To determine the presence of functional provirus, we reasoned that we should be able to ‘reactivate’ viral production. We treated MDMs with lipopolysaccharide (LPS) at 4 days post-infection and harvested supernatant from MDMs for RT-PCR analysis the following day. In Fig. 4(b), HIV-1 SF162 shows continuous viral RNA production through to 5 days post-infection; however, HIV-2 JAU demonstrates no viral RNA production after 3 days infection (Fig. 4a). LPS-stimulated replication of JAU RNA at day 5 can be titrated 100-fold in an end-point PCR titration of cDNA.

We determined whether LPS stimulation of HIV-2 viral RNA production in Fig. 4(a) translated to production of virus particles. We detected virus particles in harvested supernatants by RT-ELISA (Fig. 4c) and showed that HIV-2

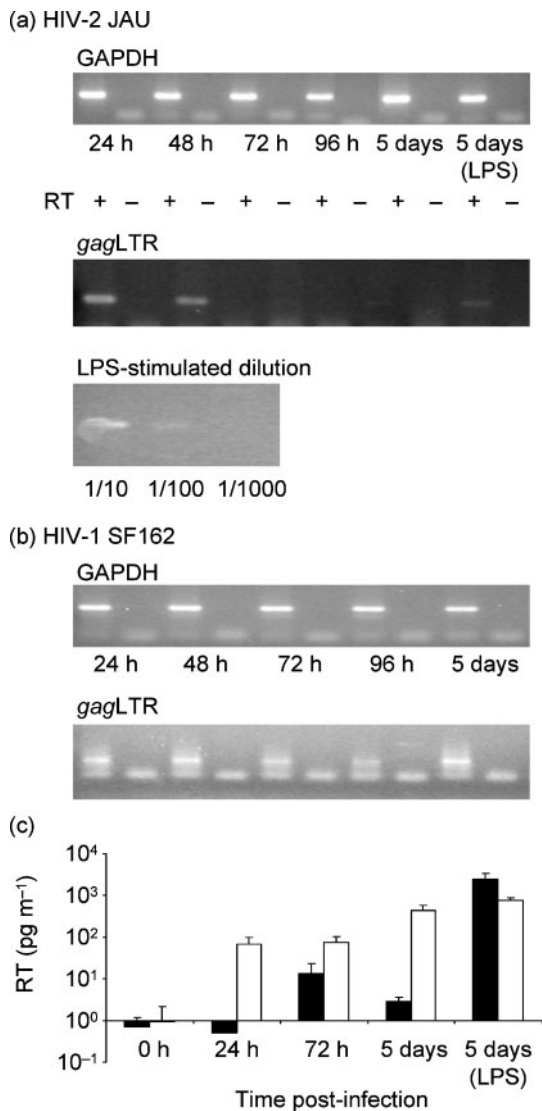
JAU can be stimulated to higher levels than HIV-1 SF162 at day 5 by LPS. SF162 was apparently unaffected by LPS treatment.

## DISCUSSION

The data presented here show that, apart from a single burst of virus replication, continuous replication of HIV-2 in MDMs is largely absent or inefficient. However, the presence of HIV-2 provirus was demonstrated by stimulation of viral production with LPS. In contrast, HIV-1 showed a lower level of replication that was, however, constant throughout the time course.

We initially observed that HIV-1 and HIV-2 had equivalent infectious titres on PBMCs, but HIV-2 showed a significantly lower titre on MDMs than did HIV-1 (21 days post-infection). Further studies showed that HIV-2 could enter and reverse transcribe in MDMs as efficiently as HIV-1, but there was a difference in the kinetics of replication over time. Unlike HIV-1, HIV-2 infection of MDMs was followed by a burst in viral production on day 2, coincident with a peak in synthesis of HIV-2 *gagLTR* product. Again, unlike HIV-1, there was a considerable loss of viral *gagLTR* product on day 3, associated with loss of particle production and *gagLTR*.

Subsequent to the containment of this burst in replication, a small quantity of HIV-2 DNA was detected in MDMs in



**Fig. 4.** HIV-2 infection of MDMs is transient and can be restimulated with LPS treatment. RT-PCR of (a) HIV-2 JAU- and (b) HIV-1 SF162-infected MDMs harvested at indicated time points post-infection. MDMs were infected and then incubated for the time points indicated. LPS (50 ng ml<sup>-1</sup>) was added to the HIV-2 JAU culture 96 h post-infection and harvested on day 5 to restimulate virus replication. RNA was extracted at the time points indicated and cDNA was made for GAPDH and *gagLTR* PCR. (c) RT-ELISA-determined RT activity from the experiment in (a) and (b) above. Filled bars, JAU; empty bars, SF162.

the absence of detectable virion production. Addition of LPS to the MDM culture resulted in restimulated HIV-2 replication, as measured by release of RT activity and production of fully infectious virions. LPS is a stimulator of the NF- $\kappa$ B transcription factor, a potent activator of HIV LTR (reviewed by Muller *et al.*, 1993). Thus, HIV-2 appears to revert to a latent form in MDMs. Latency may be one mechanism that results in the restricted replication of HIV-2 observed in MDMs. Second, we show that HIV-1

produced in MDMs is more infectious than HIV-2 and may result in more spread in the infected culture. Third, we cannot exclude the possibility that HIV-2 may target a minor resting population of MDMs, as suggested previously for HIV-1 (Schuitemaker *et al.*, 1994).

MDMs are a steady source of HIV-1 because they can survive virus replication, whereas HIV-1-infected PBMCs readily undergo apoptosis and necrosis (Meylan *et al.*, 1998; Wang *et al.*, 2001; Zhang *et al.*, 2001; reviewed by Alimonti *et al.*, 2003). Gartner *et al.* (1986) demonstrated that, *ex vivo*, MDMs can be infected with HIV-1 and may continue producing virus for more than 40 days. Certain factors have been shown to promote the survival of MDMs infected with HIV-1. Garaci *et al.* (1999) have shown that nerve growth factor (NGF) is an autocrine factor that inhibits apoptosis and is upregulated by HIV-1-infected MDMs.

The natural course of HIV-2 infection *in vivo* is different from that of HIV-1. Proviral loads in the PBMCs (which include monocytes) are similar between HIV-1 and HIV-2 patients (Ariyoshi *et al.*, 1996; Damond *et al.*, 2001; Gomes *et al.*, 1999; Norrgren *et al.*, 1997; Popper *et al.*, 2000). However, plasma RNA levels (viral production) are much lower (Damond *et al.*, 2002; Loussert-Ajaka *et al.*, 1995; Popper *et al.*, 1999). It has previously been postulated that this could be due to a more efficient neutralizing-antibody response (Lizeng *et al.*, 2003; Thomas *et al.*, 2003; Weiss *et al.*, 1988). Alternatively, lower activation states in HIV-2 infection may account for lower viral loads (Sousa *et al.*, 2002). Our results suggest another model, where lower viral loads may be due to a latent state of HIV-2 in MDMs. However, there are two obvious caveats to this. First, it is not known what contribution infected macrophages make (compared with T cells) to the overall viral load in either HIV-1 or HIV-2 infection. Second, infection of MDMs *in vitro* may not reflect the situation *in vivo*.

## ACKNOWLEDGEMENTS

We wish to thank Keith Aubin for technical assistance and Gemma Carter for critically reading the manuscript. Á.M. is supported by a Wellcome Trust Research Career Development Fellowship (no. 060758).

## REFERENCES

- Alimonti, J. B., Ball, T. B. & Fowke, K. R. (2003). Mechanisms of CD4<sup>+</sup> T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol* **84**, 1649–1661.
- Ariyoshi, K., Berry, N., Wilkins, A. & 9 other authors (1996). A community-based study of human immunodeficiency virus type 2 provirus load in rural village in West Africa. *J Infect Dis* **173**, 245–248.
- Berry, N., Jaffar, S., van der Loeff, M. S. & 9 other authors (2002). Low level viremia and high CD4% predict normal survival in a cohort of HIV type-2-infected villagers. *AIDS Res Hum Retroviruses* **18**, 1167–1173.

- Blaak, H., Boers, P. H. M., Gruters, R. A., Schuitemaker, H., van der Ende, M. E. & Osterhaus, A. D. M. E. (2005). CCR5, GPR15, and CXCR6 are major coreceptors of human immunodeficiency virus type 2 variants isolated from individuals with and without plasma viremia. *J Virol* **79**, 1686–1700.
- Clapham, P. R. & McKnight, Á. (2002). Cell surface receptors, virus entry and tropism of primate lentiviruses. *J Gen Virol* **83**, 1809–1829.
- Clavel, F., Mansinho, K., Chamaret, S., Guetard, D., Favier, V., Nina, J., Santos-Ferreira, M. O., Champalimaud, J. L. & Montagnier, L. (1987). Human immunodeficiency virus type 2 infection associated with AIDS in West Africa. *N Engl J Med* **316**, 1180–1185.
- Connor, R. I., Sheridan, K. E., Ceradini, D., Choe, S. & Landau, N. R. (1997). Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. *J Exp Med* **185**, 621–628.
- Damond, F., Descamps, D., Farfara, I. & 7 other authors (2001). Quantification of proviral load of human immunodeficiency virus type 2 subtypes A and B using real-time PCR. *J Clin Microbiol* **39**, 4264–4268.
- Damond, F., Gueudin, M., Pueyo, S. & 8 other authors (2002). Plasma RNA viral load in human immunodeficiency virus type 2 subtype A and subtype B infections. *J Clin Microbiol* **40**, 3654–3659.
- Dawson, M. (1987). Pathogenesis of maedi-visna. *Vet Rec* **120**, 451–454.
- Deng, H., Liu, R., Ellmeier, W. & 12 other authors (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661–666.
- Eisert, V., Kreutz, M., Becker, K., Königs, C., Alex, U., Rübsamen-Waigmann, H., Andreesen, R. & von Briesen, H. (2001). Analysis of cellular factors influencing the replication of human immunodeficiency virus type 1 in human macrophages derived from blood of different healthy donors. *Virology* **286**, 31–44.
- Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**, 872–877.
- Garaci, E., Caroleo, M. C., Aloe, L. & 8 other authors (1999). Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. *Proc Natl Acad Sci U S A* **96**, 14013–14018.
- Gartner, S., Markovits, P., Markovitz, D. M., Kaplan, M. H., Gallo, R. C. & Popovic, M. (1986). The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**, 215–219.
- Gomes, P., Taveira, N. C., Pereira, J. M., Antunes, F., Ferreira, M. O. S. & Lourenço, M. H. (1999). Quantitation of human immunodeficiency virus type 2 DNA in peripheral blood mononuclear cells by using a quantitative-competitive PCR assay. *J Clin Microbiol* **37**, 453–456.
- Gorry, P. R., Churchill, M., Crowe, S. M., Cunningham, A. L. & Gabuzda, D. (2005). Pathogenesis of macrophage tropic HIV-1. *Curr HIV Res* **3**, 53–60.
- Kanki, P. J., Travers, K. U., Mboup, S. & 9 other authors (1994). Slower heterosexual spread of HIV-2 than HIV-1. *Lancet* **343**, 943–946.
- Kulkarni, S., Tripathy, S., Agnihotri, K. & 7 other authors (2005). Indian primary HIV-2 isolates and relationship between V3 genotype, biological phenotype and coreceptor usage. *Virology* **337**, 68–75.
- Li, S., Juarez, J., Alali, M., Dwyer, D., Collman, R., Cunningham, A. & Naif, H. M. (1999). Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates. *J Virol* **73**, 9741–9755.
- Lizeng, Q., Skott, P., Sourial, S., Nilsson, C., Andersson, S., Ehnlund, M., Taveira, N. & Björling, E. (2003). Serum immunoglobulin A (IgA)-mediated immunity in human immunodeficiency virus type 2 (HIV-2) infection. *Virology* **308**, 225–232.
- Loussert-Ajaka, I., Simon, F., Farfara, I., Descamps, D., Collin, G. & Brun-Vezinet, F. (1995). Detection of circulating human immunodeficiency virus type 2 in plasma by reverse transcription polymerase chain reaction. *Res Virol* **146**, 409–414.
- Maddon, P. J., Dagleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A. & Axel, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**, 333–348.
- Marlink, R., Kanki, P., Thior, I. & 13 other authors (1994). Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* **265**, 1587–1590.
- McKnight, Á., Dittmar, M. T., Moniz-Periera, J. & 8 other authors (1998). A broad range of chemokine receptors are used by primary isolates of human immunodeficiency virus type 2 as coreceptors with CD4. *J Virol* **72**, 4065–4071.
- McKnight, Á., Griffiths, D. J., Dittmar, M., Clapham, P. & Thomas, E. (2001). Characterization of a late entry event in the replication cycle of human immunodeficiency virus type 2. *J Virol* **75**, 6914–6922.
- Meylan, P. R., Baumgartner, M., Ciuffi, A., Munoz, M. & Sahli, R. (1998). The *nef* gene controls syncytium formation in primary human lymphocytes and macrophages infected by HIV type 1. *AIDS Res Hum Retroviruses* **14**, 1531–1542.
- Mörner, A., Björndal, Å., Albert, J., KewalRamani, V. N., Littman, D. R., Inoue, R., Thorstensson, R., Fenyö, E. M. & Björling, E. (1999). Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* **73**, 2343–2349.
- Mörner, A., Björndal, Å., Leandersson, A.-C., Albert, J., Björling, E. & Jansson, M. (2002). CCR5 or CXCR4 is required for efficient infection of peripheral blood mononuclear cells by promiscuous human immunodeficiency virus type 2 primary isolates. *AIDS Res Hum Retroviruses* **18**, 193–200.
- Mörner, A., Thomas, J. A., Björling, E., Munson, P. J., Lucas, S. B. & McKnight, Á. (2003). Productive HIV-2 infection in the brain is restricted to macrophages/microglia. *AIDS* **17**, 1451–1455.
- Muller, J. M., Ziegler-Heitbrock, H. W. & Baeuerle, P. A. (1993). Nuclear factor kappa B, a mediator of lipopolysaccharide effects. *Immunobiology* **187**, 233–256.
- Neil, S., Martin, F., Ikeda, Y. & Collins, M. (2001). Postentry restriction to human immunodeficiency virus-based vector transduction in human monocytes. *J Virol* **75**, 5448–5456.
- Neil, S. J. D., Aasa-Chapman, M. M. I., Clapham, P. R., Nibbs, R. J., McKnight, Á. & Weiss, R. A. (2005). The promiscuous CC chemokine receptor D6 is a functional coreceptor for primary isolates of human immunodeficiency virus type 1 (HIV-1) and HIV-2 on astrocytes. *J Virol* **79**, 9618–9624.
- Norrgrén, H., Cardoso, A. N., da Silva, Z. J., Andersson, S., Dias, F., Biberfeld, G. & Naucner, A. (1997). Increased prevalence of HIV-2 infection in hospitalized patients with severe bacterial diseases in Guinea-Bissau. *Scand J Infect Dis* **29**, 453–459.
- Popper, S. J., Sarr, A. D., Travers, K. U., Guèye-Ndiaye, A., Mboup, S., Essex, M. E. & Kanki, P. J. (1999). Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. *J Infect Dis* **180**, 1116–1121.
- Popper, S. J., Sarr, A. D., Guèye-Ndiaye, A., Mboup, S., Essex, M. E. & Kanki, P. J. (2000). Low plasma human immunodeficiency virus type 2 viral load is independent of proviral load: low virus production in vivo. *J Virol* **74**, 1554–1557.
- Reeves, J. D., Hibbitts, S., Simmons, G., McKnight, Á., Azevedo-Pereira, J. M., Moniz-Pereira, J. & Clapham, P. R. (1999). Primary human immunodeficiency virus type 2 (HIV-2) isolates infect CD4-negative cells via CCR5 and CXCR4: comparison with HIV-1 and

- simian immunodeficiency virus and relevance to cell tropism in vivo. *J Virol* **73**, 7795–7804.
- Sattentau, Q. J., Clapham, P. R., Weiss, R. A., Beverley, P. C., Montagnier, L., Alhalabi, M. F., Gluckmann, J. C. & Klatzmann, D. (1988).** The human and simian immunodeficiency viruses HIV-1, HIV-2 and SIV interact with similar epitopes on their cellular receptor, the CD4 molecule. *AIDS* **2**, 101–105.
- Schmitz, C., Marchant, D., Neil, S. J. D., Aubin, K., Reuter, S., Dittmar, M. T. & McKnight, Á. (2004).** Lv2, a novel postentry restriction, is mediated by both capsid and envelope. *J Virol* **78**, 2006–2016.
- Schuitemaker, H. (1994).** Macrophage-tropic HIV-1 variants: initiators of infection and AIDS pathogenesis? *J Leukoc Biol* **56**, 218–224.
- Schuitemaker, H., Kootstra, N. A., Fouchier, R. A. M., Hooibrink, B. & Miedema, F. (1994).** Productive HIV-1 infection of macrophages restricted to the cell fraction with proliferative capacity. *EMBO J* **13**, 5929–5936.
- Schutten, M., van Baalen, C. A., Guillon, C., Huisman, R. C., Boers, P. H. M., Sintnicolaas, K., Gruters, R. A. & Osterhaus, A. D. M. E. (2001).** Macrophage tropism of human immunodeficiency virus type 1 facilitates in vivo escape from cytotoxic T-lymphocyte pressure. *J Virol* **75**, 2706–2709.
- Sellon, D. C., Perry, S. T., Coggins, L. & Fuller, F. J. (1992).** Wild-type equine infectious anemia virus replicates in vivo predominantly in tissue macrophages, not in peripheral blood monocytes. *J Virol* **66**, 5906–5913.
- Simmons, G., McKnight, Á., Takeuchi, Y., Hoshino, H. & Clapham, P. R. (1995).** Cell-to-cell fusion, but not virus entry in macrophages by T-cell line tropic HIV-1 strains: a V3 loop-determined restriction. *Virology* **209**, 696–700.
- Simmons, G., Wilkinson, D., Reeves, J. D. & 8 other authors (1996).** Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J Virol* **70**, 8355–8360.
- Simmons, G., Reeves, J. D., McKnight, Á. & 8 other authors (1998).** CXCR4 as a functional coreceptor for human immunodeficiency virus type 1 infection of primary macrophages. *J Virol* **72**, 8453–8457.
- Soda, Y., Shimizu, N., Jinno, A., Liu, H.-Y., Kanbe, K., Kitamura, T. & Hoshino, H. (1999).** Establishment of a new system for determination of coreceptor usages of HIV based on the human glioma NP-2 cell line. *Biochem Biophys Res Commun* **258**, 313–321.
- Sol, N., Ferchal, F., Braun, J., Pleskoff, O., Trébouté, C., Ansart, I. & Alizon, M. (1997).** Usage of the coreceptors CCR-5, CCR-3, and CXCR-4 by primary and cell line-adapted human immunodeficiency virus type 2. *J Virol* **71**, 8237–8244.
- Sonza, S., Maerz, A., Deacon, N., Meanger, J., Mills, J. & Crowe, S. (1996).** Human immunodeficiency virus type 1 replication is blocked prior to reverse transcription and integration in freshly isolated peripheral blood monocytes. *J Virol* **70**, 3863–3869.
- Sousa, A. E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z. & Victorino, R. M. M. (2002).** CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol* **169**, 3400–3406.
- Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P. & Sodroski, J. (2004).** The cytoplasmic body component TRIM5 $\alpha$  restricts HIV-1 infection in Old World monkeys. *Nature* **427**, 848–853.
- Thomas, E. R., Shotton, C., Weiss, R. A., Clapham, P. R. & McKnight, Á. (2003).** CD4-dependent and CD4-independent HIV-2: consequences for neutralization. *AIDS* **17**, 291–300.
- Triques, K. & Stevenson, M. (2004).** Characterization of restrictions to human immunodeficiency virus type 1 infection of monocytes. *J Virol* **78**, 5523–5527.
- Tuttle, D. L., Anders, C. B., Aquino-De Jesus, M. J. & 9 other authors (2002).** Increased replication of non-syncytium-inducing HIV type 1 isolates in monocyte-derived macrophages is linked to advanced disease in infected children. *AIDS Res Hum Retroviruses* **18**, 353–362.
- Wang, J., Guan, E., Roderiquez, G. & Norcross, M. A. (2001).** Synergistic induction of apoptosis in primary CD4<sup>+</sup> T cells by macrophage-tropic HIV-1 and TGF- $\beta$ 1. *J Immunol* **167**, 3360–3366.
- Weiss, R. A., Clapham, P. R., Weber, J. N., Whitby, D., Tedder, R. S., O'Connor, T., Chamaret, S. & Montagnier, L. (1988).** HIV-2 antisera cross-neutralize HIV-1. *AIDS* **2**, 95–100.
- Zhang, M., Li, X., Pang, X., Ding, L., Wood, O., Clouse, K., Hewlett, I. & Dayton, A. I. (2001).** Identification of a potential HIV-induced source of bystander-mediated apoptosis in T cells: upregulation of TRAIL in primary human macrophages by HIV-1 Tat. *J Biomed Sci* **8**, 290–296.