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, GPR1, AP1, 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\(^{-1}\)) and streptomycin (100 µg ml\(^{-1}\)). 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 10 % 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 plated at 1 \( \times 10^6 \) cells per well. Cells were plated at 1 \( \times 10^6 \) cells per well. Cells were plated at 1 \( \times 10^6 \) cells per well. 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).

**Viruses infectivity assays.** TCID\(_{50}\) 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\(^4\) and 10\(^5\) 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 \( \times 10^6 \) cells per well. The following day, 1 ml 10\(^4\) TCID\(_{50}\) (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

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

*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 err-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 x 10^4 TCID50/ml of 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 A260 (A260 = 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 TCID50 values ml⁻¹ 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 5a (TRIM 5a) 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...
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 transcripts 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⁻¹), but to much lower levels than HIV-2 ALI (140 pg RT activity ml⁻¹).

### 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⁻¹). 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⁻¹ to RT activity to be lower for MDMs than for PBMCs. Fig. 3(c) shows that the ratios of f.f.u. ml⁻¹: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
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
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-κB 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; Lousset-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.

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