JOURNAL OF VIROLOGY, Nov. 1997, p. 8237–8244
0022-538X/97/$04.00+0
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Vol. 71, No. 11

Usage of the Coreceptors CCR-5, CCR-3, and CXCR-4 by Primary and Cell Line-Adapted Human Immunodeficiency Virus Type 2

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Received 27 May 1997/Accepted 1 August 1997

The chemokine receptors CCR-5 and CXCR-4, and possibly CCR-3, are the principal human immunodeficiency virus type 1 (HIV-1) coreceptors, apparently interacting with HIV-1 envelope, in association with CD4. Cell lines coexpressing CD4 and these chemokine receptors were infected with a panel of seven primary HIV-2 isolates passaged in peripheral blood mononuclear cells (PBMC) and three laboratory HIV-2 strains passaged in T-cell lines. The CCR-5, CCR-3, and CXCR-4 coreceptors could all be used by HIV-2. The ability to use CXCR-4 represents a major difference between HIV-2 and the closely related simian immunodeficiency viruses. Most HIV-2 strains using CCR-5 could also use CCR-3, sometimes with similar efficiencies. As observed for HIV-1, the usage of CCR-5 or CCR-3 was observed principally for HIV-2 strains derived from asymptomatic individuals, while HIV-2 strains derived from AIDS patients used CXCR-4. However, there were several exceptions, and the patterns of coreceptor usage seemed more complex for HIV-2 than for HIV-1. The two T-tropic HIV-2 strains tested used CXCR-4 and not CCR-5, while T-tropic HIV-1 can generally use both. Moreover, among five primary HIV-2 strains all unable to use CXCR-4, three could replicate in CCR-5-negative PBMC, which has not been reported for HIV-1. These observations suggest that the CCR-5 coreceptor is less important for HIV-2 than for HIV-1 and indicate that HIV-2 can use other cell entry pathways and probably other coreceptors. One HIV-2 isolate replicating in normal or CCR-5-negative PBMC failed to infect CXCR-4$^+$ cells or the U87MG-CD4 and sMAGI cell lines, which are permissive to infection by HIV-2 but not by HIV-1. This suggests the existence of several HIV-2-specific coreceptors, which are differentially expressed in cell lines and PBMC.

The process of human immunodeficiency virus type 1 (HIV-1) entry is triggered by the interaction of the viral envelope surface glycoprotein (gp120) with two cell surface molecules, CD4 and a coreceptor belonging to the family of G-protein-coupled receptors with seven membrane-spanning domains (reviewed in reference 32). The principal HIV-1 coreceptors seem to be the β (or CC) chemokine receptor CCR-5, which is expressed in peripheral blood mononuclear cells (PBMC) and macrophages (2), and the α (or CXC) chemokine receptor CXCR-4, which is also expressed in PBMC and macrophages and in a great number of tissues and immortalized cell lines (2, 20). Cells coexpressing CD4 and CCR-5 can be infected by most primary HIV-1 isolates, whether they have a non-syncytium-inducing (NSI) phenotype and replicate in PBMC and macrophages (M-tropic strains) or have a syncytium-inducing (SI) phenotype and replicate in PBMC and T-cell lines (T-tropic strains). The transition from M tropism to T tropism is usually observed at late stages of HIV disease and seems to be associated with acquisition of CXCR-4 coreceptor usage (13, 17, 45). Some T-tropic HIV-1 isolates and the majority of strains passaged in T-cell lines (cell line-adapted or laboratory strains) have lost the ability to use the CCR-5 coreceptor and seem to use CXCR-4 only (17, 45, 51). The importance of the CCR-5 pathway for HIV-1 entry in vivo was suggested by the resistance of cells from individuals bearing mutations inactivating both alleles of the *CCR5* gene (*CCR5* −/−) to infection by M-tropic and NSI HIV-1 strains, which represent the majority of clinical isolates (28, 42). In the initial surveys, all *CCR5* −/− individuals tested were HIV-1 seronegative, suggesting that this genetic defect conferred resistance to HIV-1 infection (14, 24). However, exceptions were recently reported (1, 33, 48), indicating that HIV-1 infection can be established in vivo in the absence of CCR-5 expression. This could be related to the permissivity of *CCR5* −/− cells to infection by T-tropic and cell line-adapted HIV-1 strains.

A subset of M-tropic HIV-1 strains was found to infect CD4$^+$ cells expressing CCR-3 (8, 17). This β-chemokine receptor is naturally expressed in eosinophils but is also expressed in microglial cells and could therefore play a role in the infection of the central nervous system (23). The HIV-1 coreceptor activity of CCR-3 was not observed by other investigators (15, 18, 51), possibly due to a relatively inefficient expression of CCR-3 in transfected cells (8). Differences in cell surface expression may also explain why the coreceptor activity of US28, a β-chemokine receptor encoded by the human cytomegalovirus (CMV), was observed only under certain experimental conditions (37).

HIV-2 is prevalent in West Africa and more closely related genetically to the simian immunodeficiency viruses (SIVs) from rhesus macaque (SIVmac) and from sooty mangabey (SIVsm) than to HIV-1 (reviewed in reference 29). The CD4 molecule also behaves as a receptor for HIV-2 (43). However, a number of laboratory HIV-2 strains were found to infect CD4-negative cell lines after treatment with soluble CD4, and sometimes constitutively (10, 30). We have only limited information on the requirement of chemokine receptors for cell entry by cell line-adapted HIV-2 and no information for pri-

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TABLE 1. Origins of HIV-2 strains used in this study

Strain	Origin	Clinical condition of subject	CD4$^+$ cells/ml	Reference
Cell line-adapted strains				
ROD	Cape Verde	AIDS patient		12
MIR	Guinea Bissau	AIDS patient		12
EHO	Côte d'Ivoire	AIDS patient		40
Primary isolates				
A (BATI)	Guinea	Asymptomatic	362	
B (BAJE)	Sénégal	Asymptomatic	300	
C (BAPA)	Sénégal	Asymptomatic	30	
D (VEGE)	Martinique	Asymptomatic	186	
E (DESY)	Cape Verde	Asymptomatic	272	
F (SYLLA)	Côte d'Ivoire	AIDS patient	52	
G (BAYO)	Mali	AIDS patient	27	

mary HIV-2 isolates. The expression of CXCR-4, and also CCR-5 or US28, could render CD4$^+$ cells permissive to infection by HIV-2 ROD (36, 37), while its ROD/B derivative could infect CD4-negative cells upon expression of CXCR-4, CCR-3, or the orphan receptor V28 (39). The permissivity of CD4$^+$ cell lines to infection by HIV-2, but not to HIV-1 entry (9, 30), suggests the existence of HIV-2-specific coreceptors. Here, we have addressed the ability of the HIV-1 coreceptors CXCR-4, CCR-5, and CCR-3 to mediate infection by a panel of primary and cell line-adapted HIV-2 strains.

MATERIALS AND METHODS

Origin and production of HIV strains. Primary HIV-2 strains (designated A to G [Table 1]) and primary HIV-1 strains (H to J) were isolated from patients' PBMC by coculture with healthy-donor PBMC. All the HIV-2-seropositive subjects were seronegative for HIV-1 in two independent tests. The PBMC were grown in RPMI medium supplemented with 15% fetal calf serum and 10% interleukin-2 (Lymphocult; Biotest, Dreicich, Germany). They were activated for 3 days before infection in medium containing 3 μg of phytohemagglutinin (Sigma, St. Louis, Mo.) per ml. Reverse transcriptase (RT) activity was assayed in culture supernatants as described previously (44), and viral stocks were harvested at the peak of production.

The cell line-adapted HIV-2 strains ROD, MIR, and EHO, all previously characterized (Table 1), were propagated in the T-cell line MT-4 (50). Only ROD was derived from a molecularly cloned provirus (41). The cell line-adapted HIV-1 strain LAI (35) and M-tropic HIV-1 strains ADA (49) and YU-2 (26) were produced by transfection of recombinant proviruses in HeLa cells. ADA is a recombinant LAI provirus with the ADA *env* gene (37).

Cell lines. Cell lines growing in suspension (MT-2 and MT-4) were propagated in RPMI medium, and adherent cell lines (HeLa derivatives, sMAGI, U87MG-CD4, and U373MG-CD4) were propagated in Dulbecco's modified Eagle's medium. The culture media were supplemented with 10% fetal calf serum, antibiotics (penicillin and streptomycin), and 2 mM glutamine. The HeLa-P4 (11), U373MG-CD4 (22), U87MG-CD4 (36), and sMAGI (4) cell lines express human CD4 and are stably transfected with an HIV-1 long terminal repeat (LTR)-*lacZ* construct, allowing detection of infected cells by assaying β-galactosidase activity in situ. The HeLa-P5 cell line, which stably expresses CCR-5, was derived from HeLa-P4 cells (37). The HeLa-P3 cell line, which stably expresses CCR-3, was obtained by the same strategy. HeLa-P4 cells were cotransfected with a CCR-3 expression vector (see below) and a hygromycin B resistance vector. Drug-resistant cell clones were tested for their ability to fuse with the HeLa-Env/ADA cell line (37), which stably expresses Env from the M-tropic strain ADA.

Expression of chemokine receptors. Chemokine receptor cDNAs were subcloned in the Rc/CMV vector (InVitrogen, La Jolla, Calif.), allowing their expression from the CMV immediate-early promoter. The CXCR-4 and CCR-5 vectors have been described elsewhere (36, 37). The CCR-3 open reading frame was PCR amplified from HeLa cell DNA with the primers 5′-GGCTTAAGCTTCTATCACAGGGAGAAGTG (plus strand; *Hin*dIII site underlined) and 5′-CTTCATCTCCTTGGGCCCTCCTCTTTAGG (minus strand; *Apa*I site underlined) and cloned as a *Hin*dIII-*Apa*I fragment in Rc/CMV. The CCR-3 open reading frame was completely sequenced and found to be identical to that previously reported by Ponath et al. (38).

Infectivity assays. Infections of PBMC and MT-2 cells (2×10^6 and 5×10^5 cells per well, respectively) were performed in 24-well trays, using an inoculum corresponding to approximately 100,000 cpm of RT, except for experiments comparing normal and *CCR5* −/− PBMC, which were performed with 5×10^5 cells per well and 30,000 cpm of RT. Virus was left in contact with cells for 90 min and removed by washing the cells twice in RPMI medium. The MT-2 cells were infected in the presence of 2 μg of Polybrene (Sigma) per ml. The cells were then resuspended and grown in 1.5 ml of complete medium. Half of the medium was replaced twice weekly. Cultures were monitored for production of RT and the presence of syncytia. The infections of the LTR-*lacZ* cell lines were performed in 12-well trays. The inoculum (approximately 20,000 cpm of RT activity for primary isolates and 10 to 25 ng of p24 for other strains) was added to subconfluent monolayers and left in contact with the cells for 2 h. The U373MG-CD4 cells transiently expressing chemokine receptors were infected 24 h after transfection, as described previously (36). The cells were washed with phosphate-buffered saline, fixed with 0.5% glutaraldehyde, and stained with the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) 48 h after infection, or 60 h for U373MG-CD4 cells. Extending the infection from 24 to 48 h markedly increased virus titers, due to the accumulation of β-galactosidase in infected cells, rather than to a second cycle of infection. Indeed, the same increase in virus titer was observed when an RT inhibitor was added 20 h after infection (data not shown).

RESULTS

Infection of cell lines. Primary HIV-2 strains were isolated from the PBMC of five asymptomatic individuals (isolates A to E) and from two AIDS patients (isolates F and G) (Table 1). Their replication in PBMC, estimated by RT activity in the culture supernatants at day 7, was comparable to that of three primary HIV-1 isolates (H to J) tested in parallel (Fig. 1). Four CD4$^+$ cell lines of different tissue origins were infected by using supernatant from PBMC infected with primary HIV-1 and HIV-2 or from MT-4 cells infected with three previously described cell line-adapted HIV-2 strains (ROD, MIR, and EHO [12, 40]). The results of these experiments are summarized in Table 2.

The human T-cell line MT-2, which endogenously expresses CD4 and CXCR-4 (data not shown), is routinely used to characterize HIV-1 strains. The ability to replicate in these cells is seen principally for HIV-1 strains isolated from AIDS patients, almost always with an SI phenotype (25), as is the case for isolate J here. The HIV-2 strains derived from AIDS patients (F, G, and ROD) and also isolate E could replicate in MT-2

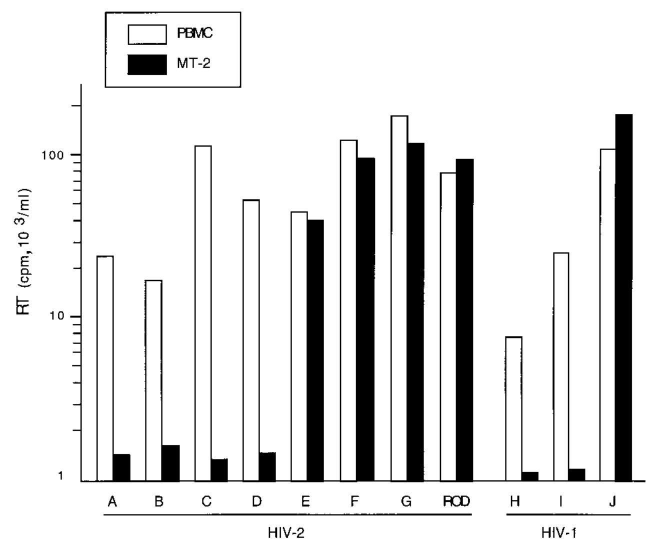

FIG. 1. Replication of primary HIV-1 and HIV-2 isolates in PBMC and in the T-cell line MT-2. The two cell types were infected in parallel with all the HIV-1 and HIV-2 strains, using approximately 100,000 cpm of RT activity for 2×10^6 cells (PBMC) or 5×10^5 cells (MT-2). Bars represent RT activity in supernatants at day 7 (log scale, means of duplicate wells). Syncytia were detected in MT-2 cells for the E, G, and ROD HIV-2 strains and for the J HIV-1 strain.

TABLE 2. Replication of HIV-2 and HIV-1 strains in CD4$^+$ cell lines

Virus and strain	MT-2[a]		Replication in LTR-*lacZ* cell line[b]:			
	Replication	Syncytium formation	HeLa-P4	HeLa-P5	sMAGI	U87MG-CD4
HIV-2						
A	−	−	−	+	+	+
B	−	−	−	+	+	+
C	−	−	−	+	+	+
D	−	−	−	−	−	−
E	+	+	−	+	+	+
F	+	+	+	+	−	+
G	+	−	+	+	+	±
ROD	+	+	+	+	+	+
MIR	NT[c]	NT	+	+	−	−
EHO	NT	NT	+	+	+	+
HIV-1						
H	−	−	−	+	−	−
I	−	−	−	+	−	−
J	+	+	+	+	−	−
LAI	NT	NT	+	+	−	−
ADA	NT	NT	−	+	−	−
YU-2	NT	NT	−	+	−	−

[a] Results from Fig. 1. +, positive result; −, negative result.
[b] Results from Fig. 2 and 3. +, ratio of blue-stained cells in infected cells versus uninfected cells > 3; ±, ratio between 2 and 3; −, ratio < 2.
[c] NT, not tested.

cells (Fig. 1). Syncytium formation was observed for the E, F, and ROD strains but not for the G strain (Table 2). The latter was highly cytopathic, apparently due to single-cell killing, as previously reported for strain EHO (40).

The HeLa-P4 cell line is stably transfected with CD4 and naturally expresses CXCR-4 (20). Accordingly, HeLa-P4 cells could be infected by cell-line adapted HIV-1 strains, such as LAI, and by primary SI HIV-1 isolates, such as isolate J in this study (Fig. 2A). They were efficiently infected by all HIV-2 strains derived from AIDS patients and not by HIV-2 isolates derived from asymptomatic individuals, including isolate E, which replicated in MT-2 cells (Fig. 2A). This discrepancy could suggest that isolate E infects MT-2 cells by a pathway other than CXCR-4 or that the cellular context can influence the usage of this coreceptor.

The human glioma cell line U87MG-CD4 (7, 9) and the epithelial rhesus macaque sMAGI cell line (4), both transfected with human CD4, are considered to be permissive to infection by HIV-2 but not by HIV-1. These cell lines do not express CXCR-4 (20, 46). We found that they could be infected by most, but not all, primary and laboratory HIV-2 strains (Fig. 2B and C). Indeed, the MIR strain showed a high titer in HeLa-P4 cells but did not infect sMAGI or U87MG-CD4 cells, while primary isolate F showed a low titer in U87MG-CD4 cells and apparently did not infect sMAGI cells. Overall, it seemed that primary HIV-2 isolates able to infect HeLa-P4 cells had lower titers in sMAGI and U87MG-CD4 cells. Conversely, isolate E had a high titer in sMAGI and U87MG-CD4 cells but did not infect HeLa-P4 cells. These experiments showed that these four CD4$^+$ cell lines were infected by different subsets of HIV-2 strains, suggesting that different entry pathways were used.

Role of chemokine receptors. The role of chemokine receptors in HIV-2 entry was addressed by using the HeLa-P5 and HeLa-P3 cell lines, which stably express CCR-5 and CCR-3, respectively, and a human glioma cell line (U373MG-CD4) transiently expressing CCR-5, CCR-3, or CXCR-4. The HeLa-P5 and HeLa-P3 cell lines could be infected by M-tropic HIV-1 strains (ADA and YU-2) and by primary HIV-1 isolates H and I (Fig. 3A), all of which are unable to infect the parental cell line HeLa-P4. The HeLa-P5 and HeLa-P3 cells could be infected by primary HIV-2 isolates from asymptomatic individuals, with the exception of isolate D (Fig. 3). Since these HIV-2 isolates did not infect the parental HeLa-P4 cells, they were apparently able to use the CCR-5 or CCR-3 coreceptor. The HIV-2 strains that were previously found to infect HeLa-P4 cells (isolates F and G and laboratory strains) showed similar titers in HeLa-P4, HeLa-P5, and HeLa-P3 cells (data not shown), indicating that the expression of CCR-5 or CCR-3 did not increase the permissivity of parental cells.

The primary HIV-1 and HIV-2 isolates had markedly lower titers in HeLa-P3 cells compared with those in HeLa-P5 cells (Fig. 3B). However, the level of CCR-3 transcripts in HeLa-P3 cells was low in comparison with the level of CCR-5 transcripts in HeLa-P5 cells (46). This probably limited the efficiency of infection of HeLa-P3 cells. Accordingly, the number of infected cells was markedly increased by treating HeLa-P3 cells with 5 mM sodium butyrate, a compound increasing the rate of transcription from the CMV and other viral promoters (8, 34), before infection (Fig. 3B). This treatment also increased the background level of β-galactosidase activity in LTR-*lacZ* cell lines, and therefore the number of HeLa-P3 cells stained with X-Gal in uninfected controls (Fig. 3B). However, it did not appear to allow infection of HeLa-P3 cells by a pathway independent of CCR-3. Indeed, this treatment did not allow detectable infection of the parental HeLa-P4 cells by M-tropic HIV-1 strains, since the number of blue-stained cells was the same in the absence of virus (data not shown).

The human glioma cell line U373MG-CD4 (also bearing an LTR-*lacZ* reporter gene) was previously shown to be resistant to HIV-1 infection (22) but could be rendered permissive after transient transfection of CXCR-4 or CCR-5 (36, 37). In our experience, this cell line was not fully resistant to infection by HIV-2 and some primary HIV-1 isolates, but the number of infected cells was increased when U373MG-CD4 cells were transfected with chemokine receptor expression vectors (Fig. 4). As expected, expression of CXCR-4 allowed infection of U373MG-CD4 cells by LAI and not by ADA, while the opposite was seen upon expression of CCR-5. The number of ADA-infected cells was lower when cells expressed CCR-3. However, there was less difference in the infectious titers of primary HIV-1 isolates in U373MG-CD4 cells expressing CCR-5 or CCR-3, while ROD apparently infected CCR-5$^+$ and CCR-3$^+$ cells with the same efficiency (Fig. 4). Therefore, a level of CCR-3 expression sufficient for coreceptor activity was apparently achieved in this system. The primary HIV-2 isolates previously found to infect HeLa-P5 cells could infect U373MG-CD4 cells expressing CCR-5, but the number of infected cells was modestly increased (two- to threefold) compared to that of mock-transfected cells or cells expressing CXCR-4 (Fig. 4). Isolate E, which was able to infect HeLa-P3 cells, was apparently unable to use CCR-3 in U373MG-CD4 cells. The reason for this discrepancy is not known. The primary HIV-2 isolates infecting HeLa-P4 cells (F and G) were unable to infect U373MG-CD4 cells expressing CCR-5 (or CCR-3), in contrast to most primary T-tropic HIV-1 strains, exemplified by isolate J in the present study, using CXCR-4 or CCR-5. Both isolates F and G could infect U373MG-CD4 cells expressing CXCR-4, as expected, but with markedly different efficiencies, contrasting with their similar titers in HeLa-P4 cells.

Discrepancies in the efficiency of infection of U373MG-CD4 cells expressing CXCR-4 were also observed among laboratory

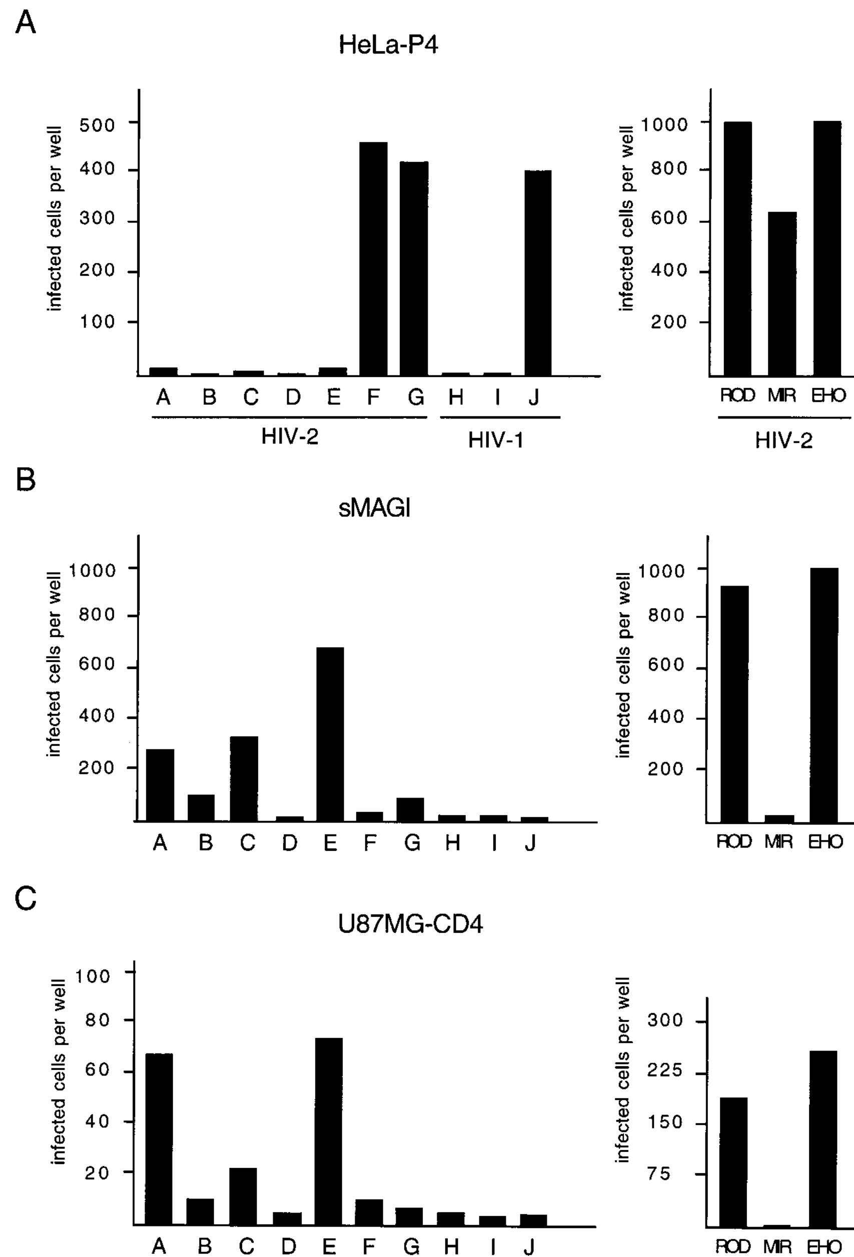

FIG. 2. Infection of $CD4^+$ cell lines by HIV-1 and HIV-2. HeLa-P4 (A), sMAGI (B), and U87MG-CD4 (C) cells are all stably transfected with an LTR-*lacZ* construct, allowing detection of HIV-infected cells by their high β-galactosidase activity (blue staining with X-Gal). Subconfluent cell monolayers in 12-well plates were infected with the same viral stocks and stained with X-Gal 48 h later. Results are the means of two parallel infections. Numbers >200 were extrapolated from randomly selected fields.

HIV-2 strains, which all displayed a high infectious titer in HeLa-P4 cells. Cells expressing CXCR-4 were efficiently infected by ROD and MIR and much less efficiently by EHO (Fig. 4). These strains also had very different titers in U373MG-CD4 cells expressing CCR-5 or CCR-3. Both coreceptors allowed efficient infection by ROD, whereas they modestly increased EHO infection and were apparently not used by MIR. Therefore, three laboratory HIV-2 strains passaged in the same T-cell line had totally different preferences for coreceptors. Only the MIR strain seemed to be specialized for CXCR-4, like cell line-adapted HIV-1 strains.

Infection of CCR-5-negative PBMC. We have tested the ability of primary HIV-2 isolates to replicate in PBMC from an individual homozygous for a 32-nucleotide deletion in the *CCR-5* gene, which results in a truncated form of CCR-5 that is not expressed at the cell surface and is devoid of HIV-1 coreceptor activity (28, 42). This individual has remained uninfected despite multiple exposures to HIV-1. As expected, a primary NSI HIV-1 isolate (isolate H) did not replicate in CCR-5-negative (ΔCCR-5) PBMC, while these cells supported the replication of a cell line-adapted HIV-1 strain (LAI) and of primary HIV-2 isolates F and G, previously found to use the

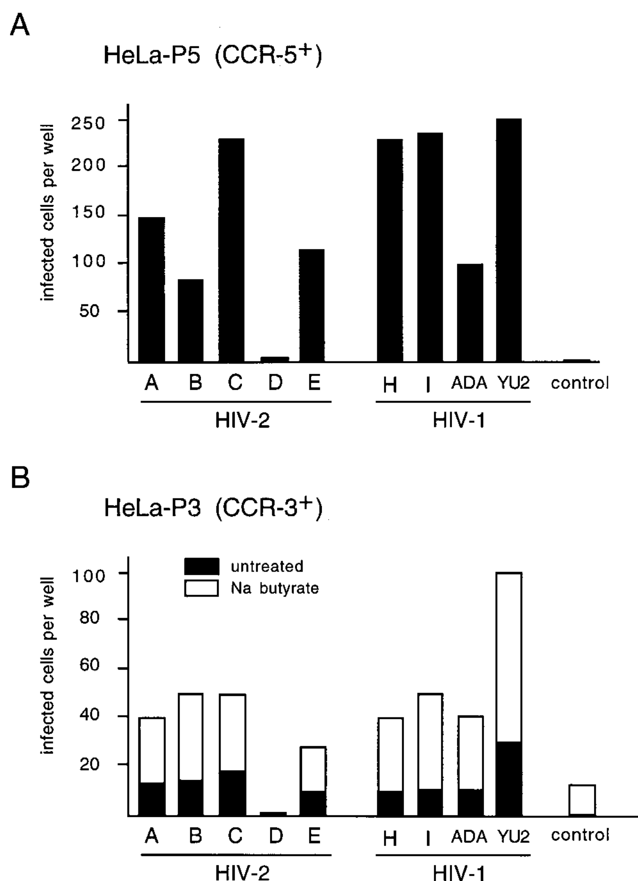

FIG. 3. Infection of CD4$^+$ HeLa cells stably expressing CCR-5 (HeLa-P5) (A) or CCR-3 (HeLa-P3) (B). Where indicated, HeLa-P3 cells were treated with 5 mM sodium butyrate for 24 h before infection in order to increase expression of CCR-3. The experiment was performed as described for Fig. 2.

CXCR-4 coreceptor (Fig. 5). Among the primary HIV-2 isolates that did not infect CXCR-4$^+$ cells, isolates A and B failed to replicate in ΔCCR-5 PBMC. Like the majority of primary HIV-1 isolates, these HIV-2 isolates seemed to rely upon CCR-5 to infect PBMC. In contrast, isolates C, D, and E could replicate in ΔCCR-5 PBMC, which was observed only for HIV-1 isolates able to use CXCR-4 as their coreceptor (Fig. 5). The level of production of isolates C, D, and E was lower in ΔCCR-5 PBMC than in control PBMC. This does not seem to be significant in terms of virus entry efficiency, since the level of virus production in ΔCCR-5 PBMC was also lower for LAI and for HIV-2 isolates F and G. The fact that HIV-2 isolates C and E, but not D, could infect U87MG-CD4 and sMAGI cells suggests the existence of several HIV-2 coreceptors which are not functional with HIV-1 and which are differentially expressed in PBMC and cell lines.

DISCUSSION

In this series of experiments, we have observed that the chemokine receptors CCR-5, CCR-3, and CXCR-4, the principal HIV-1 coreceptors, could also be used by primary and cell line-adapted HIV-2 strains. However, there were marked differences between HIV-1 and HIV-2 and among HIV-2 strains in their ability to use these coreceptors. The usage of a given type of coreceptor by HIV-2 could not be simply correlated to phenotypical traits, such as the ability to induce syncytium formation or tropism for T-cell lines. Also, our experiments showed that HIV-2 used cell entry pathways independent from the HIV-1 coreceptors.

CCR-5 and CCR-3. The CCR-5 coreceptor seems to be essential to the HIV-1 life cycle. It can be used by most primary HIV-1 strains, whatever their genetic subtype and tropism (51). Only a fraction of primary T-tropic HIV-1 isolates, usually isolated from immunodeficient individuals, and cell line-adapted HIV-1 strains were apparently unable to use CCR-5 and strictly dependent upon the CXCR-4 coreceptor (45, 51). The incapacity to use CCR-5 was relatively frequent in our HIV-2 panel, since it was observed for one strain derived from an AIDS patients (MIR), but also for primary isolate D derived from an asymptomatic individual. This isolate and two other primary HIV-2 isolates (C and E) could replicate in PBMC genetically deficient for CCR-5 expression (ΔCCR-5). Interestingly, isolates C and E were able to use CCR-5 to infect the HeLa-P5 cell line. Either they do not rely upon CCR-5 to infect PBMC, or they can easily shift to another pathway. To our knowledge, this type of observation has not been made for HIV-1. Also, two primary HIV-2 isolates derived from AIDS patients used CXCR-4 and not CCR-5, while most T-tropic HIV-1 strains seem to use both types of coreceptors (17, 45, 51). Overall, the role of CCR-5 seems less important for HIV-2 than for HIV-1.

In our experiments, there was a great extent of overlap between the subset of HIV-1 and HIV-2 strains using CCR-5 and the subset using CCR-3. This confirms the potential importance of CCR-3 as an HIV coreceptor, even if its usage in vivo remains hypothetical. The lower level of expression of CCR-3 in HeLa-P3 cells probably explains the lower infectious titers of HIV-1 and HIV-2 in this cell line than in HeLa-P5, which stably expresses CCR-5 (46). Indeed, similar infectious titers could be observed for several HIV-1 and HIV-2 strains, in particular ROD, in U373MG-CD4 cells transiently expressing CCR-3 or CCR-5. By contrast, we and others observed that M-tropic HIV-1 strains, such as ADA, apparently used CCR-5 more efficiently than CCR-3 (8, 23). These strains might be particularly well adapted to CCR-5, adaptation being detrimental to the usage of other coreceptors.

CXCR-4. Laboratory HIV-1 strains, such as LAI, and primary T-tropic HIV-1 strains can use the CXCR-4 coreceptor. Hence, they replicate in T-cell lines, such as MT-2, or in other CXCR-4$^+$ cell lines, such as HeLa, provided that CD4 is expressed. Also, their ability to use CXCR-4 generally parallels an SI phenotype and is usually observed for strains derived from AIDS patients (17, 45). In the case of HIV-2, the ability to use CXCR-4 was also seen for strains derived from AIDS patients and not from asymptomatic individuals. However, the ability to use CXCR-4 did not correlate strictly with replication in the MT-2 cell line and SI phenotype (isolate E). The HIV-2 strains infecting HeLa-P4 cells could also infect CXCR-4$^+$ U373MG-CD4 cells but with marked differences in efficiency. For example, strains EHO and F showed high titers in HeLa-P4 cells but infected CXCR-4$^+$ U373MG-CD4 cells with only modest efficiency. The cellular context might influence the usage of the CXCR-4 coreceptor by certain viral strains, but it may be simpler to envision that HIV-2 strains can infect MT-2 cells, and possibly HeLa-P4 cells, by a CXCR-4-independent pathway. Accordingly, SDF-1, the CXCR-4 ligand, blocked almost completely the infection of HeLa-P4 cells by a cell line-adapted HIV-1 strain (LAI) but had a limited antiviral activity, or no effect, for HIV-2 infection (data not shown). However, the HIV-1 and HIV-2 envelope proteins seem to have different requirements for their interaction with CXCR-4 (3), and it cannot be ruled out that HIV-1 and HIV-2 strains

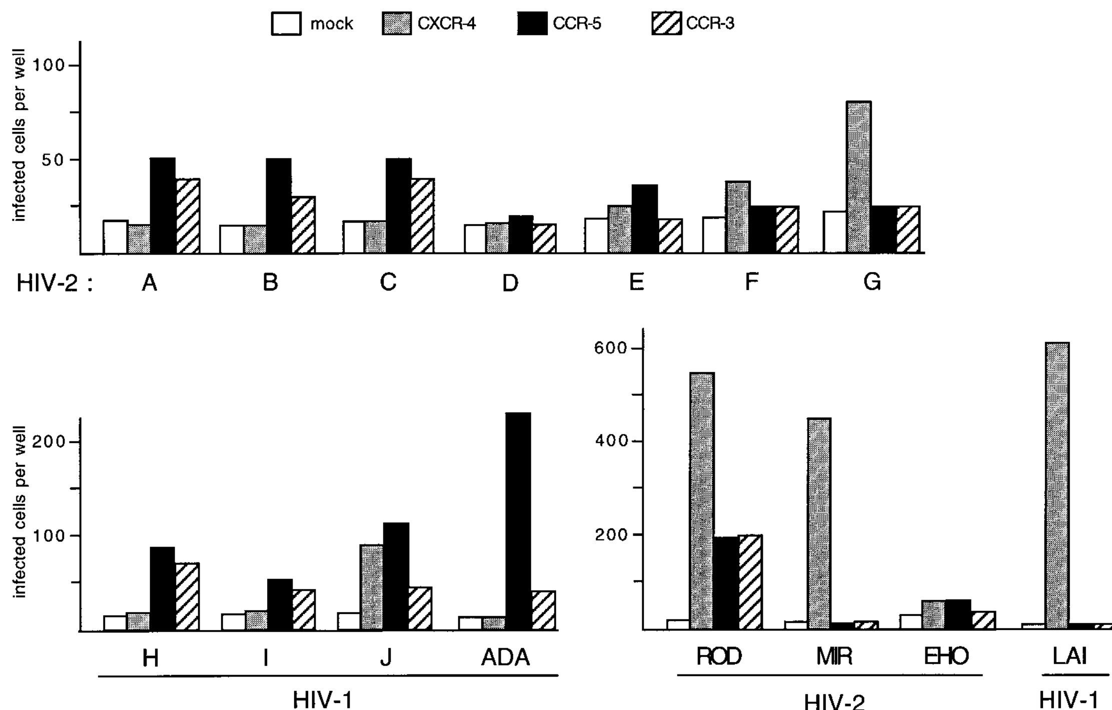

FIG. 4. Infection of U373MG-CD4 cells expressing CXCR-4, CCR-5, or CCR-3. The experiment was performed as for Fig. 2 and 3 except that U373MG-CD4 cells were previously transfected with Rc/CMV vectors expressing the different chemokine receptors or with Rc/CMV only (mock). Staining with X-Gal was performed 60 h after infection.

using CXCR-4 are affected differently by SDF-1. There were marked differences among HIV-1 strains in their sensitivity to the antiviral activity of the 12G5 anti-CXCR-4 antibody (31, 47).

Role of other coreceptors. The permissivity of a number of $CD4^+$ cell lines to infection by HIV-2 and not by HIV-1, first observed by Clapham et al. for the U87MG-CD4 cell line (9), led to postulation that HIV-1 and HIV-2 require different cellular components to infect target cells. Selective permissivity to HIV-2 infection was observed for other $CD4^+$ cell lines of nonhuman origin (30), for example, sMAGI from rhesus macaque (4). In previous studies, the sMAGI and U87MG-CD4 cell lines were infected by all HIV-2 strains tested, although with variable efficacy (4, 9). Their complete resistance to a cell line-adapted HIV-2 strain (MIR) was therefore unexpected. This strain appeared to use CXCR-4 with high efficiency, like cell line-adapted HIV-1. Adaptation of MIR to CXCR-4 might have prevented usage of other coreceptors, as is the case for cell line-adapted HIV-1 strains. One primary HIV-2 isolate (D) replicated in normal or ΔCCR-5 PBMC but not in the $CD4^+$ cell lines we have tested, in particular U87MG-CD4 and sMAGI. This suggests that PBMC express an HIV-2 coreceptor which is not present in U87MG-CD4 and sMAGI cells and which also cannot be used by the majority of primary HIV-1 strains, since they do not replicate in ΔCCR-5 PBMC.

After this study was completed, Deng et al. reported isolation of two chemokine receptor-like proteins behaving as coreceptors for SIV and HIV-2, designated Bonzo and BOB (16). Bonzo is actually identical to STRL33, previously reported to be a coreceptor for certain M-tropic and T-tropic HIV-1 strains (27). Transcripts for Bonzo and BOB were detected in PBMC, while only Bonzo/STRL33 appeared to be expressed in U87MG-CD4 cells. These entry cofactors could apparently be used by several, but not all, HIV-2 strains, and further studies are clearly needed to assess their exact role in the infection of cell lines resistant to HIV-1 and their usage by primary HIV-2 isolates.

Differences between human and simian retroviruses. Phylogenetic studies of primate lentiviruses show that HIV-2, SIVsm, and SIVmac isolates are closely clustered, while HIV-1 represents a distinct branch (21). SIVsm is apparently nonpathogenic for its natural hosts (sooty mangabeys) but pathogenic for macaques. Since the area of endemicity of HIV-2 corresponds to the natural habitat of sooty mangabeys, HIV-2 was postulated to have evolved from SIVsm after cross-species transmission (21). Both SIVsm and SIVmac infected $CD4^+$ cells expressing CCR-5, either from human or from rhesus macaque (5, 6, 19), but they can probably use additional coreceptors in vivo, since they could replicate in ΔCCR-5 PBMC (6, 19). Although they replicated in T-cell lines, the SIV strains tested were unable to use CXCR-4 of either human or macaque origin, while HIV-1 strains could use both types (6, 19). The adaptation to CXCR-4 might have occurred after the divergence of HIV-2 and SIV from their common ancestor. However, a chimpanzee virus (SIVcpz) apparently more closely related to HIV-1 than to HIV-2 or SIVsm also failed to replicate in CXCR-4-expressing cells (6). The study of a larger number of SIV isolates is required before it can be asserted that only human lentiviruses can use CXCR-4.

Although the clinical manifestations of HIV-1 and HIV-2 infection are similar, individuals infected with HIV-2 generally exhibit longer clinical latency periods and progress more slowly toward immune deficiency (29). This seems in apparent contradiction of the ability of HIV-2 to use additional coreceptors and hence to have access, at least in theory, to a larger number of target cells. In the case of HIV-1, the CCR-5 pathway was first thought to be absolutely required to establish infection, but exceptions are now reported (1, 33, 48). The use

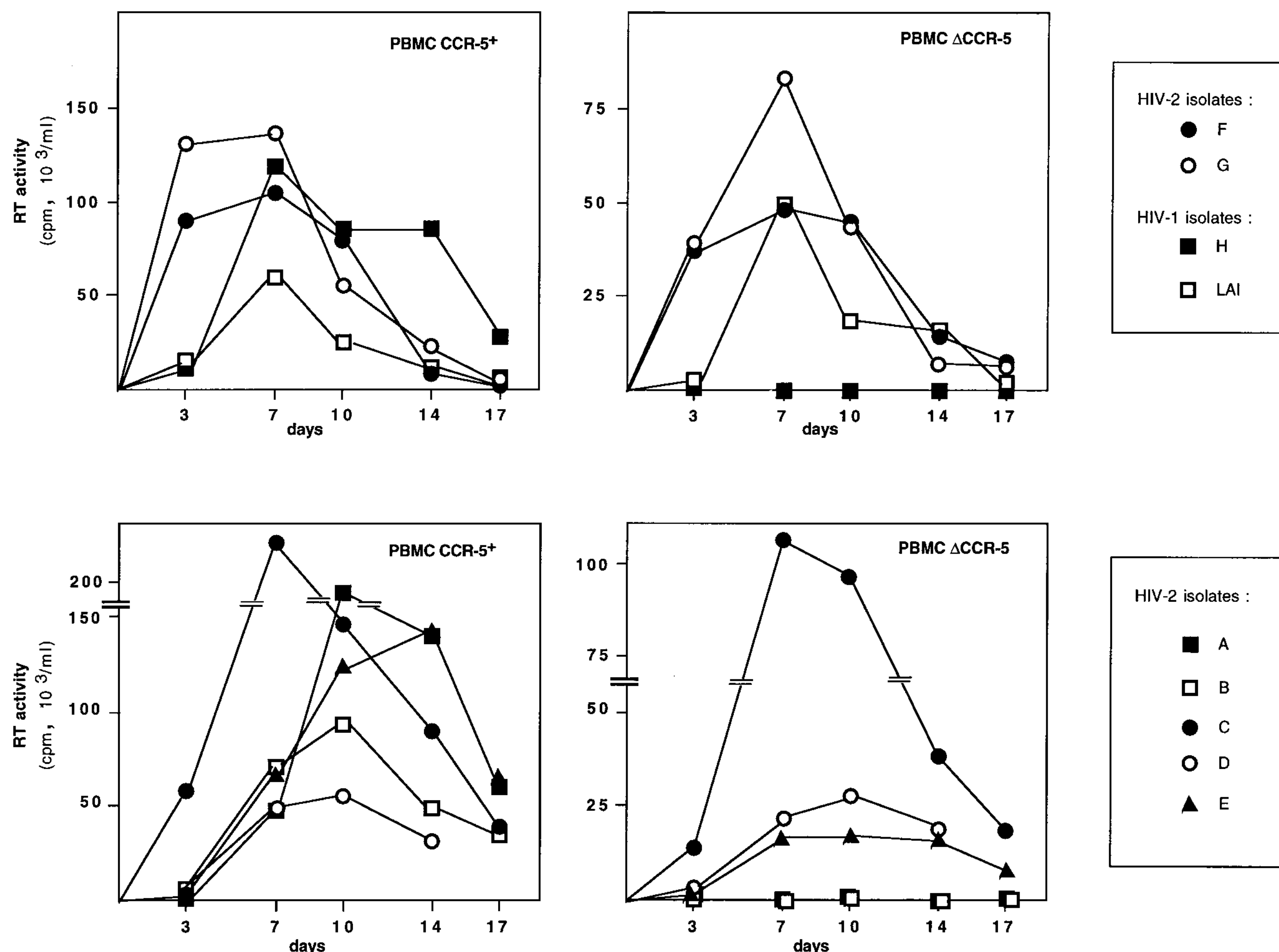

FIG. 5. Replication of HIV-1 and HIV-2 in normal and CCR5-negative (ΔCCR-5) PBMC. The two types of PBMC were infected in parallel, with an inoculum containing approximately 30,000 cpm of RT activity (or 10 ng of p24 for LAI) for 5×10^5 cells.

of CXCR-4 by HIV-1 strains isolated at late stages of the disease might suggest a role of this pathway in pathogenicity. However, pathogenic strains of SIV use CCR-5 and other coreceptors but not CXCR-4. While the coexpression of CD4 and chemokine receptors at the cell surface seems essential to HIV entry, the pathogenicity of lentiviruses might not be related to the use of a particular type of coreceptor.

ACKNOWLEDGMENTS

We thank F. Morinet (Hôpital St.-Louis) for his interest in this work, G. Gonzales-Canali (CIRBS) for providing CCR5-negative cells, F. Letourneur and E. Gomas (ICGM) for assistance with sequencing, and L. Picard for comments on the manuscript.

This work was supported by the Agence Nationale de Recherches sur le SIDA and by fellowships from Ensemble contre le SIDA to N.S. and O.P.

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