A rodent cell line permissive for entry and reverse transcription of human immunodeficiency virus type 1 has a pre-integration block to productive infection

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Replication of human immunodeficiency virus type 1 (HIV-1) is restricted to CD4-expressing primate cells. This tropism may be due partly to the absence from non-primate cells of a species-specific factor which has an accessory role to CD4 during virus penetration. In this study we describe a rat B lymphocyte cell line in which there is efficient CD4-dependent entry of HIV-1. However, this cell line has a block to productive infection of HIV-1 at a stage between reverse transcription and integration. Our results demonstrate that the putative accessory factor for HIV-1 penetration is not restricted to primate cells and that there is a novel, uncharacterized cell–virus interaction at a stage between penetration and integration.

Introduction

Human immunodeficiency viruses (HIV) are the aetiological agents of AIDS, a slow degenerative disease which leads to severe dysfunction of the immune system. During the course of infection, HIV-1 is found primarily in leukocytes, notably in macrophages and a subset of T lymphocytes known as T helper cells. This tropism is associated with expression of CD4, which is the receptor for HIV (Dalgleish et al., 1984; Klatzmann et al., 1984b).

Expression of CD4 is not, however, sufficient for cellular susceptibility to infection by HIV. In general, HIV cannot infect non-primate cells and some human cells, even though they express human CD4 (Maddon et al., 1986; Chesebro et al., 1990a; Clapham et al., 1991). The block to infection is at the level of membrane fusion (Maddon et al., 1986; Chesebro et al., 1990a) and is unidirectional, with the composition of the CD4-expressing membrane rather than that of the HIV Env-expressing membrane being critical (Ashorn et al., 1990; Aoki et al., 1991). However, there are a few exceptions in which CD4-expressing non-human cells are able to form syncytia with cells expressing high levels of HIV-1 Env (Ashorn et al., 1990). In addition, HIV-2 infection of CD4-expressing cell lines derived from mink and cat, and HIV-2-mediated cell fusion of a rabbit cell line and amphotericin B-treated rat cells have been observed (McKnight et al., 1994).

HIV-1 can infect CD4-expressing hybrid cells of murine origin that contain just five human chromosomes (Weiner et al., 1991). This observation suggests that in addition to CD4, susceptible human cells express a factor which plays a role during virus penetration. This idea is reinforced by reports in which the block to syncytium formation in CD4-expressing animal cells was overcome by forming heterokaryons with human cells (Dragic et al., 1992; Broder et al., 1993). In these reports, neither the CD4-expressing non-primate nor the CD4-negative human parental lines were competent for syncytium formation. Fusion of these cells resulted in heterokaryons that were able to form syncytia with HIV Env-expressing cells suggesting that the human cells provide a factor which, in addition to CD4, is required for fusion competence of the non-primate cells (Dragic et al., 1992; Broder et al., 1993).

In addition to the block at fusion, many murine cells have restrictions to other stages of the HIV life cycle. Studies in which HIV DNA was transfected into mouse cells reveal that many of these are poor producers of HIV and that some do not produce virions at all (Adachi et al., 1986; Levy et al., 1986; Mizrachi et al., 1992). This restriction is due to poor transactivation by Tat in some...
cell lines (Hart et al., 1989; Newstein et al., 1990) and inefficient Rev action in others (Trono & Baltimore, 1990).

In this paper we describe the first rodent cell line in which there is efficient, CD4-dependent penetration by cell-free HIV-1 showing that expression of a putative Rev action in others (Trono & Baltimore, 1990). In this paper we describe the first rodent cell line in which there is efficient, CD4-dependent penetration by cell-free HIV-1 showing that expression of a putative accessory factor is not restricted to primates. We also describe a novel block to replication of HIV-1 at a stage between penetration and integration. This restriction suggests the existence of uncharacterized interactions between the cell and HIV which could potentially be exploited for therapeutic intervention.

Methods

Cell lines: The human carcinoma cell line HeLa (Gey et al., 1952), the mouse fibroblast cell line L929 (Earle et al., 1943) and the baby hamster kidney cell line BHK-21 (MacPherson & Stoker, 1962) were obtained from Flow Laboratories. The HIV-1 Env-expressing HeLa cell line H32 was obtained from Dr Lee Bacheler (DuPont, Wilmington, Del., U.S.A.). The human CD4-expressing cat kidney cell line CCC S+L- (Clapham et al., 1991) and the packaging cell line PA317 hCD4 (Cheesbro et al., 1990a), were kind gifts from Paul Clapham (Chester-Beatty Laboratories). The rat myeloma Y3 was obtained from Dr C. Milstein (MRC, Cambridge, U.K.) (Galfré et al., 1979). These cell lines were cultured in DMEM (Gibco BRL) containing 10% (v/v) fetal bovine serum (FBS; Gibco BRL), 2 mM-L-glutamine (Sigma) and antibiotics (50 U/ml penicillin, 50 μg/ml streptomycin and 100 μg/ml neomycin). In addition, H32, CCC S-L- hCD4 and PA317 hCD4 cells were maintained in 0.6 mg/ml Geneticin G418 (Sigma). The mouse macrophage cell line P388D1 (Karen et al., 1975) was cultured in L15 Leibovitz medium, supplemented with 10% FBS, 15% tryptose phosphate broth, L-glutamine and antibiotics. The mouse macrophage cell line PU5-1.8 (Ralph et al., 1976) was obtained from ECACC (Porton Down, Wilts., U.K.) and grown in MEM supplemented with 10% FBS, L-glutamine and antibiotics. The rat natural killer cell line RNK-16 was a gift from Dr J. Imboden (UCSF, Calif., U.S.A.) (Seaman et al., 1987). ACH-2 cells (Clouse et al., 1989) containing a single copy of HIV-1 proviral DNA, the human T lymphotrophic virus type 1-transformed T cell line C8166 (Salahuddin et al., 1983) and the T lymphoblastoid cell line H9 (Popovic et al., 1984) were obtained from the NIH AIDS Research and Reference Reagent Program. The rat T lymphocyte cell line C58 was obtained from Dr A. Conzelmann (Conzelmann et al., 1982). These cells were maintained in RPMI 1640 medium (Gibco BRL) with 10% FBS, L-glutamine, antibiotics, 25 μM 2-mercaptoethanol and 1 mM-sodium pyruvate.

Generation of human CD4-expressing cell lines: Cells were either infected with virus-containing supernatant from PA317 hCD4 cells or transfected by the calcium phosphate precipitation method with pKG5 vector (Gould et al., 1987) containing the cDNA of human CD4. Cells were fed twice a week with fresh medium containing 0.8 ng/ml Geneticin G418 (Sigma) until resistant colonies emerged. Resistant colonies were pooled and analysed for CD4 expression by indirect flow cytometry as previously described (Simon et al., 1993). Cells were either sorted as previously described (Simon et al., 1993) to select sub-lines expressing similar levels of CD4 (Y3 hCD4 and HeLa hCD4) or used as a polyclonal population if CD4-expression was evident (all other cell lines).

Viruses. HIV-1ΔΝ (Ratner et al., 1985) was obtained from the NIH AIDS Research and Reference Reagent Program and propagated by acute infection of C8166 cells. The culture supernatants were clarified, filtered through 0.45 μm filters, treated with 40 U/ml DNase I (Sigma) for 30 min at room temperature to remove contaminating proviral DNA and stored in 1 ml aliquots at −80 °C. Detection of core antigen was by ELISA as described (Simon et al., 1993).

Cocal virus (COC) (Jonkers et al., 1964) was obtained from Robert Shope (Yale Arbovirus Unit) and propagated in BHK-21 cells. COC(HIV) pseudotype virus was made by super-infecting acutely HIV-1ΔΝ-infected H9 cells with COC and pseudotype plaque assays were performed essentially as described (Gregory et al., 1993; Simon et al., 1993) except that non-monolayer forming cells were overlain with BHK-21 indicator cells.

Mink lung cells (Mv-i-Lu) chronically infected with amphotropic murine leukemia virus (MLV-A) were obtained from Paul Clapham (Chester-Beatty Laboratories). HUT-78 cells (Gootenberg et al., 1981) were infected with MLV-A-containing supernatant in the presence of 8 μg/ml polybrene. When a chronic infection was established, the cells were super-infected with HIV-1ΔΝ and maintained until cytopathic effects were seen. The supernatant was harvested and treated as described for HIV-1ΔΝ above.

Table 1. Sequences of primers used for PCR analysis

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>Mouse leukosialin 1 (ML1)*</td>
<td>AGA GGA TGA AGG AAT GTA CCT A</td>
</tr>
<tr>
<td>Mouse leukosialin 2 (ML2)*</td>
<td>GCA GGA GCA GGA TGT GGG AC</td>
</tr>
<tr>
<td>Rat leukosialin 1 (RL1)†</td>
<td>GGT AGC ACT AGA AGA GCT GAA G</td>
</tr>
<tr>
<td>Rat leukosialin 2 (RL2)†</td>
<td>GGG GAG GTG GAA AAC AGC GG</td>
</tr>
<tr>
<td>Feline 1 (CF1)‡</td>
<td>TTT GAC CCC CTG TCA TAA TAT GC</td>
</tr>
<tr>
<td>Feline 2 (CF2)‡</td>
<td>TAT CGG GGT GGA GTC AAG TAC</td>
</tr>
<tr>
<td>Human β-globin 1 (CCG)§</td>
<td>CCT TGT TTCCT AAG TCC AA</td>
</tr>
<tr>
<td>Human β-globin 2 (CCG)§</td>
<td>CAC ACC TTC TCT TCA TGG AG</td>
</tr>
<tr>
<td>R-</td>
<td>CAA GCT TTA TGG AGT C</td>
</tr>
<tr>
<td>Gag-</td>
<td>ACT GAC GCT TCT GCA CCC AT</td>
</tr>
<tr>
<td>U5-</td>
<td>GAT CTC TAG TTA CCC GAG TCA C</td>
</tr>
<tr>
<td>U5§</td>
<td>GTG ACT CTC GTA ACT AAG CAT G</td>
</tr>
<tr>
<td>U3§</td>
<td>CAC ACA CAA GGC TAC TCC G</td>
</tr>
</tbody>
</table>

* Mouse specific primers amplify a region of mouse leukosialin (Cyster et al., 1990).
† Rat specific primers amplify a region of rat leukosialin (Killeen et al., 1987).
‡ Cat specific primers amplify a region of the LTR of the endogenous feline leukaemia virus CF-14 (Berry et al., 1988).
§ HIV-specific primers and human β-globin primers were obtained from M. Collin (Sir William Dunn School of Pathology, Oxford, U.K.).
A pre-integration block to HIV replication

Transfection of HIV DNA into cells. Plasmid pSVIIIIB contains the XbaI fragment of HXB2-D subcloned into the pBluescriptII SK + vector. This vector was transfected into cells by the calcium phosphate precipitation method (Davis et al., 1990). One week after transfection, $5 \times 10^6$ cells were co-cultivated with $10^6$ C8166 cells, and samples of cell culture supernatant were taken for analysis by p24 ELISA twice weekly for 2 to 3 weeks.

PCR. The products of reverse transcription were analysed by PCR, essentially as described previously (Collin et al., 1991). Briefly, cells were challenged with HIV-1$_{111B}$ and $10^6$ cells were lysed at various time points by addition of 200 µl Tris-HCl buffer (20 mM, pH 8.4) containing 100 mM-KCl, 0.1% NP40 and proteinase K (5 mg/ml). The lysates were incubated at 60 °C for 2 h to digest cellular protein and then at 95 °C for 15 min to inactivate proteinase K. PCR amplifications were performed as previously described (Collin et al., 1991) using an MJ PTC-100 programmable thermal controller. Table 1 lists the sequences of primers used.

In order to detect production of 2LTR (long terminal repeat) circular forms of HIV DNA, cell lysates were subjected to 35 rounds of amplification using the U5+/R- primer pair. The amplification products were electrophoresed on a 1% agarose gel and then blotted onto a positively charged nylon membrane (Hybond N+, Amersham) using a 0.4 M-NaOH transfer solution. The membrane was then washed in 3x SSC/0.1% SDS and incubated at 37 °C for 1 h in pre-hybridization solution (6x SSC, 1x Denhardt’s solution, 0.05% sodium pyrophosphate, 100 µg/ml boiled salmon sperm DNA and 0.5% SDS). The U3+ primer was radiolabelled with $^{32}$P and hybridized to the filter overnight at 46 °C (1 ng probe in 6 x SSC, 1 x Denhardt’s solution and 0.05 % sodium pyrophosphate).

Results

Entry of HIV-1 into a CD4-expressing rat myeloma

A number of mouse, rat, cat and human cell lines expressing similar levels of human CD4 were challenged with HIV-1$_{111B}$ and incubated for 20 h at 37 °C. To detect virus entry, the cells were then lysed and analysed by PCR for the presence of viral DNA using the LTR specific U3+/U5- primer pair (Table 1). No HIV-1 DNA was detected in any of the non-human cell lines except in the rat B lymphocyte cell line Y3 hCD4 (Fig. 1a). In addition, HIV-1$_{111B}$ entry was also detected in HeLa hCD4 cells but not in CD4-negative HeLa cells using this technique. It has previously been shown that HIV virions contain a low level of viral DNA derived from partial reverse transcription prior to cellular penetration (Lori et al., 1992; Trono, 1992). This DNA is below the detectable limit of our assay (Collin et al., 1991) and the amplified viral DNA detected in Y3 hCD4 and HeLa hCD4 cannot be derived from virions trapped on the surface of the cells, as no such DNA is detected in any of seven other CD4 expressing cell lines (Fig. 1a). However, to confirm that the PCR signal in Y3 hCD4 cells is as a result of genuine HIV entry followed by de novo DNA synthesis, cells were incubated for 1 h with either fresh...
Fig. 2. Effect of AZT and the anti-CD4 MAb Q425 on synthesis of viral DNA in Y3 hCD4 cells. Y3 hCD4 cells were incubated at 37 °C for 1 h with growth medium, 10 μM-AZT or 5 μg/ml anti-CD4 MAb Q425. The cells were then challenged with HIV-1_IIIb for 1 h at 37 °C, washed and incubated with fresh medium or with medium containing 10 μM-AZT or 5 μg/ml Q425. Samples of cells were collected and lysed for PCR analysis immediately after challenge (time 0), immediately after washing (1 h) and at various time points over 2 days. PCR amplification products (using the U3+/U5- primer pair) of the lysates of cells incubated in growth medium, AZT and Q425 are shown. Each lane of the agarose gel has been loaded three times to show amplification products from cells treated with medium, AZT or Q425 at the various time points. As a positive control, the lysate of ACH-2 cells were amplified (C) and loaded once at the same time as each of the three sets of samples.

Fig. 3. Production of HIV DNA intermediates. HeLa hCD4 and Y3 hCD4 cells were challenged with HIV-1_IIIb and samples were collected and lysed for PCR analysis over 48 h. Cell lysates were amplified using the U3+/U5-, U5+/gag- and U5+/R- primer pairs which detect DNA after the first template switch, second template switch and 2LTR circle formation, respectively. Circular DNA was detected by blotting and probing PCR products with a radiolabelled oligonucleotide as described in Methods.

Table 2. Analysis of virion production after either challenge with cell-free HIV or transfection with proviral DNA

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time (days)</th>
<th>Cell-free virus infection*</th>
<th>Transfection with pSVIIIB†</th>
</tr>
</thead>
<tbody>
<tr>
<td>L929 hCD4</td>
<td>0 10 17 24</td>
<td>- - - -</td>
<td>0 2 15 &gt; 300</td>
</tr>
<tr>
<td>P388(D1) hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>12 &gt; 300</td>
</tr>
<tr>
<td>PU5-1.8 hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>C58 hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>R2(D) hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>RNK16 hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Y3 hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>CCC S′L- hCD4</td>
<td>- - - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>HeLa</td>
<td>- 0.2 22 &gt;300</td>
<td>- 0.4 &gt;300</td>
<td>- 0.9 &gt;300 p§</td>
</tr>
<tr>
<td>HeLa hCD4</td>
<td>&gt;300 &gt;300 &gt;300</td>
<td>&gt;300 &gt;300 &gt;300 d</td>
<td>&gt;300 &gt;300 &gt;300 d</td>
</tr>
</tbody>
</table>

*+ CD4-expressing cell lines were either challenged with HIV (+), or transfected with the vector pSVIIIB (+) (day 0). After 1 week, the cells were co-cultivated with C8166 cells, and samples of tissue culture supernatant were collected for analysis by p24 ELISA. The table shows the concentration of p24 (ng/ml) in the supernatants at various times after challenge or transfection.

p, p24 concentrations below the detectable limit of the assay (0.1 ng/ml).

§ p, No sample taken as the cells had died.

This was confirmed by the finding that HIV DNA was not amplified from CD4-negative Y3 cells after challenge with virus (data not shown).

Cocal virus (COC) is closely related to vesicular stomatitis virus (VSV). Like VSV, it has a lytic life cycle and can form pseudotypes with HIV. These pseudotypes have the genome of COC and the spike glycoproteins of HIV, permitting HIV Env-mediated penetration of cells by COC. Such an infection leads to formation of plaques, making COC(HIV) pseudotypes a useful tool to investigate HIV entry. The amount of gp120-mediated entry is calculated as the difference between the number of plaques produced when cells are challenged with virus neutralized with anti-COC antiserum and the number of background plaques when cells are challenged with growth medium, growth medium containing 10 μM-AZT (3′-azido-3′-deoxythymidine) or growth medium containing 5 μg/ml of the anti-CD4 MAb Q425 (a gift from Q. J. Sattentau, CIML, Marseille, France), which inhibits entry of HIV without inhibiting virus binding (Healey et al., 1990). These cells were then challenged with HIV-1_IIIb, incubated for 1 h at 37 °C, washed and incubated with fresh medium for a further 47 h. Samples of cells were collected at various time points and lysed for PCR analysis. Fig. 2 shows that the level of HIV DNA increases with time in Y3 hCD4 cells, and that synthesis of DNA is inhibited by incubation of cells with either AZT or Q425. Inhibition of HIV entry by the anti-CD4 MAb Q425 demonstrates that virus penetration into these cells is dependent upon expression of human CD4. This was confirmed by the finding that HIV DNA was
COC(HIV) neutralized with both anti-COC antiserum and anti-gp120 polyclonal antibody. Significant HIV Env-mediated entry of COC(HIV) pseudotypes was observed in HeLa hCD4 and Y3 hCD4 cells but not in any of the other non-human cells (Fig. 1b). A low level of pseudotype entry (above background) was detected in CD4-negative HeLa cells and this is in accordance with earlier results (Chesebro et al., 1990). Taken together, the above evidence conclusively demonstrates that cell-free HIV-1 is able to penetrate Y3 hCD4 cells.

As an additional method of examining fusion-competence, each of the CD4-expressing cell lines was mixed with an equal number of HIV Env-expressing HeLa H32 cells and incubated overnight at 37°C. Syncytia were seen only for Y3 hCD4 and HeLa hCD4 cells and not for any of the other cells.

To confirm that the various cell lines were not contaminated by other cells, lysate from each cell line was analysed by PCR using species-specific primers to amplify regions of mouse leukosialin, rat leukosialin, a feline endogenous retrovirus and human β-globin (Fig. 1a).

**Y3 cells have a block to HIV replication which is overcome by transfection with HIV DNA**

The CD4-expressing cells were challenged with HIV-1_{11HR} (10⁶ TCID₅₀ as titrated on C8166 cells) and, after incubation for 1 week (with daily trypsinization to remove input virus), were co-cultivated with C8166 cells. Samples of tissue culture supernatant were taken over the next 3 weeks for analysis by p24 ELISA. Core antigen was detected in the supernatant of HeLa hCD4 cells within a few days but was not detected in the supernatants of the mouse, cat or rat cells (Table 2). Notably, no virus was recovered from Y3 hCD4 cells despite efficient penetration of these cells by HIV. Virus was also recovered from CD4-negative HeLa cells by cocultivation with C8166 cells (Table 2) confirming a low level of CD4-independent infection (Chesebro et al., 1990).

When these cell lines were transected with a proviral clone of HIV-1_{11HR}, virus was recovered from all cultures after co-cultivation with C8166 cells except the rat C58 hCD4 and mouse PU5-1.8 hCD4 cell lines (Table 2). These two cell lines are not resistant to transfection as colonies were obtained after transfection with the pBABE hygro vector and selection with hygromycin. The results demonstrate that with the exception of C58 hCD4 and PU5-1.8 hCD4 cells all of the cell lines tested are able to undergo all post-integration events necessary for production of infectious virus.

**Reverse transcription is retarded in Y3 cells**

The data presented above suggest that there is a restriction to HIV-1 replication at a stage before integration in Y3 cells. In order to identify the stage at which the life cycle is interrupted and to investigate the fate of proviral DNA, HeLa hCD4 and Y3 hCD4 cells were challenged with a low titre of HIV-1_{11HR} (to ensure being in the linear range of the PCR) and 10⁶ cells were harvested and lysed at various time points over a period of 48 h. Cell lysates were analysed by PCR using the U3+/U5−, U5+/gag (which amplify proviral DNA after the first and second template switches of reverse transcription, respectively) and the U5+/R (2LTR circles) primer pairs. Fig. 3 shows that the product of the first template jump is evident between 2 and 4 h after challenge in both HeLa hCD4 and Y3 hCD4 cells. However, as can be seen in Fig. 3, the product of the second template switch is first observed after 4 h in HeLa hCD4 cells, but is not seen until the 24 h time point in Y3 hCD4 cells.

Several nuclear forms of unintegrated retroviral DNA have been described (Varmus & Swanstrom, 1982). Among these is a circular form with two LTRs which is only found in the nucleus, presumably because the host enzymes that mediate ligation of the termini of double stranded linear DNA are located there. Production of such circles can consequently be used as a marker for entry of the pre-integration complex into the nucleus (Bukrinsky et al., 1992). Formation of 2LTR circles in HeLa hCD4 and Y3 hCD4 cells challenged with HIV-1_{11HR} was investigated as described in Methods. Circles are first seen at the 24 h time point in HeLa hCD4 cells (Fig. 3), but, consistent with the delay in the kinetics of the second template switch, circles are not seen until the 48 h time point in Y3 hCD4 cells.

To investigate the fate of viral DNA over a longer period, HeLa hCD4 and Y3 hCD4 cells were challenged with high-titre HIV-1_{11HR} and 10⁶ cells were harvested and lysed each day for 8 days until the HeLa hCD4 cells were no longer viable. No cytopathic effect was seen in Y3 hCD4 cultures. Cell lysates were analysed by PCR using the U5+/gag primer pair. Fig. 4 shows that the level of proviral DNA decreased in Y3 hCD4 cells. Due to the delay in the kinetics of reverse transcription, circles were not seen until the 48 h time point in Y3 hCD4 cells.
Fig. 5. Challenge of cells with HIV(MLV-A) pseudotype virus. Y3, CCC S’T’ hCD4 and HeLa cells were challenged with HIV(MLV-A) pseudotype virus. They were incubated for 1 h, washed twice with PBS, and incubated in fresh growth medium at 37 °C for a further 19 h. The cells were then lysed and analysed by PCR for the presence of HIV DNA. As a negative control, HeLa hCD4 cells were challenged with an aliquot of HIV(MLV-A) which had been heat-inactivated for 10 min at 90 °C.

Fig. 6. Production of virions by cells infected with HIV(MLV-A) pseudotype virus. One week after cells were challenged with HIV(MLV-A), 5 x 106 cells were co-cultured with 106 C8166 cells and samples were taken twice weekly for 3 weeks for analysis by p24 ELISA. The figure shows the concentration of p24 core antigen at various time intervals in the supernatants of C8166 cells co-cultivated with HeLa (○), CCC S’T’ hCD4 (●) and Y3 cells (□).

to the high titre stock of virus used, the PCR was out of the linear range for HeLa hCD4 cells; however, productive infection was evident in these cells as judged by cytopathic effects.

The block to infection is not overcome by pseudotyping HIV with amphotropic murine leukaemia virus

The receptor for amphotropic murine leukaemia virus (MLV-A) is expressed by cells from many species leading to the use of this virus in retroviral packaging cell lines. Budding HIV virions are able to incorporate the Env protein from MLV-A to form HIV(MLV-A) pseudotypes capable of delivering HIV capsids to cells (Canivet et al., 1990; Chesebro et al., 1990a; Spector et al., 1990). To confirm that the block to HIV replication in Y3 cells was not at the level of entry, Y3, CCC S’T’ hCD4 and HeLa cells were challenged with HIV(MLV-A) pseudotype virus in the presence of polybrene (8 μg/ml). After a 20 h incubation at 37 °C, a sample was lysed for PCR analysis and the remaining cells were maintained for subsequent co-cultivation with C8166 cells as described above.

Viral DNA was amplified from all cells challenged with HIV(MLV-A) but not from a control in which a sample of virus was heat-inactivated for 10 min at 90 °C and used to mock-infect HeLa hCD4 cells (Fig. 5), demonstrating that amplified viral DNA was not derived from input virions. After co-cultivation of HIV(MLV-A)-infected cells with C8166 cells, HIV-1H1B was rescued from HeLa and CCC S’T’ hCD4 cells but not from Y3 cells (Fig. 6).

Discussion

Numerous cell lines of mouse, rat, mink, rabbit and monkey origin that express human CD4 are resistant to HIV penetration and syncytium formation (Maddon et al., 1986; Chesebro et al., 1990a; Clapham et al., 1991). The results presented here are in agreement with those of others, demonstrating that the block to infection in most non-primate cells is at the stage of membrane fusion. However, using two independent techniques [PCR amplification of newly synthesized viral DNA and a COC(HIV) pseudotype assay], we have conclusively shown that there is efficient, human CD4-dependent entry of cell-free HIV-1H1B in the rat B-lymphocyte cell line Y3.

The block to fusion in non-primate cells has been attributed to these cells not expressing a factor(s) which has an accessory role to CD4 during penetration (Weiner et al., 1991; Dragic et al., 1992; Broder et al., 1993). Our results show that this putative accessory factor is expressed in Y3 cells. In addition, some mink, cat and rabbit cells as well as some amphotericin B-treated rat cells are permissive for HIV-2-mediated fusion and consequently are likely to express an accessory factor employed by this virus (McKnight et al., 1994). Therefore such accessory factors cannot be exclusively primate variants of a surface molecule such as human CD26, which has recently been proposed to be the accessory factor (Callebaut et al., 1993).

Despite barely detectable entry of HIV in CD4-negative HeLa cells, progeny virions are rescued after co-cultivation with C8166 cells. This is in contrast to Y3 hCD4 cells from which virions cannot be rescued despite having a level of virus penetration comparable to that of HeLa hCD4 cells. In addition, the block to productive infection in Y3 cells could not be overcome by pseudotyping HIV with MLV-A. Consequently, there must be a block at some post-entry stage of the life cycle
of HIV-1 in Y3 hCD4 cells. Such a block is not due to cell lineage as HIV-1 infection has been reported in human B lymphocytes (Maddon et al., 1986; Chung-Mayer et al., 1991; Clapham et al., 1991). It has recently been shown that a rabbit cell line and other rat cells have post-penetration blocks to HIV-2 (McKnight et al., 1994).

There are several reports describing poor, or even no production of HIV from murine cells transfected with proviral DNA (Adachi et al., 1986; Levy et al., 1986; Mizrachi et al., 1992), which may be accounted for by inefficient Tat or Rev action (Hart et al., 1989; Newstein et al., 1990; Trono & Baltimore, 1990). Two of the cell lines tested here, C58 and PU5-1.8, fit into one of these categories as they do not produce progeny virions after transfection with an infectious molecular clone of HIV-1<sub>HIV</sub>. However, Y3 cells produce virions after transfection with infectious proviral DNA and, consequently, the block to replication in this cell line is unlikely to be at a stage following integration. Consistent with this, the level of viral DNA declines during the week following infection of Y3 hCD4 cells. If integration were achieved but the infection was non-productive, the proviral copy number would remain constant unless the host cells were killed. As no cytopathic effect is seen after challenge of Y3 hCD4 cells with HIV, we conclude that infection is probably aborted before integration.

It is possible that there is inefficient reverse transcription, nuclear import or integration of the proviral genome in Y3 hCD4 cells. Reverse transcription is a complex process involving two steps in which elongation of the nascent DNA switches from using one template to using another (Varmus & Swanstrom, 1982). The most likely sites for stalling of reverse transcription are during these template switches (Varmus & Swanstrom, 1982). Indeed, we have shown that although both of these jumps are achieved in Y3 cells, there is a significant delay between the first and second template switches in Y3 hCD4 cells compared to HeLa hCD4 cells. Similarly, nuclear import of the pre-integration complex, as assessed by formation of 2LTR circles, is slower in Y3 hCD4 cells.

It seems unlikely that the slower kinetics of reverse transcription on their own can account for the absolute block to productive infection by HIV in the Y3 hCD4 cells. An alternative explanation is that a combination of inefficient steps (such as reverse transcription together with inefficient nuclear import and/or integration) is to blame. However, if this was the case, we would expect there to be a very low level of virus production which could be detected after co-cultivation with a highly permissive cell line. As no such replication was found, it appears more likely that there is an absolute block at or prior to integration.

There have been several recent reports describing certain strains of HIV (Schmidtmaierova et al., 1992; Huang et al., 1993) or simian immunodeficiency virus (Mori et al., 1993) for which replication is restricted at a stage after virus penetration in macrophages. These restrictions have similarities to the one described here, although it should be noted that in the present study, we have used a dividing immortalized cell line as opposed to non-dividing primary cells.

In addition, the block to replication of HIV in Y3 hCD4 has similarities to Fv-1 restriction of MLV in mouse cells (Jolicoeur, 1979). In both cases the block is after penetration but before or during integration and can be overcome by transfection of proviral DNA (Huang et al., 1973; Jolicoeur & Baltimore, 1976; Sveda & Soeiro, 1976; Jolicoeur & Rassart, 1980; Yang et al., 1980; Chinsky & Soeiro, 1981; Jolicoeur & Rassart, 1981). Important differences between the two blocks are firstly that Fv-1 restriction is not an absolute block and can be overcome by challenge with a high m.o.i. whereas no progeny are produced by Y3 cells and, secondly, that the kinetics of reverse transcription of MLV are not delayed in Fv-1 restricted cells.

In summary, we have described a rat myeloma in which there is efficient, CD4-dependent entry of cell-free HIV-1, and consequently have shown that expression of a putative accessory factor necessary for penetration of HIV-1 is not restricted to primates. In addition, we have identified a block to HIV-1 replication at a stage between penetration and prior to or during integration of viral DNA. Further investigation of the mechanism of this block may lead to a clearer understanding of cell–virus interactions that occur between penetration and integration of HIV-1.

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References


A pre-integration block to HIV replication


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