Characteristics of primary infection of a European human immunodeficiency virus type 1 clade B isolate in chimpanzees

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The aim of the study was to select, from a panel of candidate European human immunodeficiency virus type 1 (HIV-1) clade B primary virus isolates, one isolate based on replication properties in chimpanzee peripheral blood mononuclear cells (PBMC). Second, to evaluate the in vivo kinetics of primary infection of the selected isolate at two different doses in two mature, outbred chimpanzees (Pan troglodytes). Four different low passage, human PBMC-cultured ‘primary’ HIV-1 isolates with European clade B consensus sequence were compared for their ability to replicate in vitro in chimpanzee versus human PBMC. The isolate which yielded the highest titre and most vigorous cytopathic effect in chimpanzee PBMC was evaluated for coreceptor usage and chosen for evaluation in vivo. Only the HIV-1Han2 isolate replicated in chimpanzee PBMC in vitro at detectable levels. This isolate was demonstrated to utilize CCR4, CCR5 and CXCR4 co-receptors and could be inhibited by β-chemokines. Infection of chimpanzees was demonstrated by viral RNA and DNA PCR analysis, both in plasma as well as in PBMC and lymph node cells as early as 3 weeks after inoculation. Antibodies developed within 6 weeks and continued to increase to a maximum titre of approximately 12,800, thereafter remaining in this range over the follow-up period of 2 years. Compared to cell line-adapted HIV-1 isolates there were slight but no dramatic differences in the kinetics of infection of chimpanzees with this particular primary isolate.

Introduction

The chimpanzee–human immunodeficiency virus type 1 (HIV-1) model has been used successfully to demonstrate the efficacy of several experimental HIV-1 vaccines in protection from infection (Arthur et al., 1989; Berman et al., 1988; Bruck et al., 1994; Girard et al., 1991) with common laboratory strains of HIV-1 such as Lai/IIIB (Arthur et al., 1989) and SF2 (Murthy et al., 1996). There is substantial concern that the characteristics of these laboratory strains are not representative of primary clade B clinical isolates of HIV-1 that are circulating in Europe and the USA. HIV-1SF2 stock grown in human peripheral blood mononuclear cells (PBMC) to avoid problems with human cell lines induced a relatively weak infection in chimpanzees with only transient and inconsistent PCR and virus isolation, and subsequently a relatively poor immune response (Girard et al., 1995; Murthy et al., 1996). In an effort to provide a more clinically relevant virus stock for HIV-1 vaccine efficacy trials in chimpanzees, an initiative was launched by Programme EVA (European Vaccine against AIDS) sponsored by the EC Biomedicine and Health programme, and undertaken together with the European Centralised Facility for preclinical HIV-1 vaccine development.
Iscove's medium with 10% heat-inactivated (56 °C) sera and donor PBMC should be available; (4) the clinical history of the donor should be known; (5) an infectious molecular clone should be available; (6) the complete full-length sequence should be known; and finally, (7) the virus should be capable of replicating in chimpanzee PBMC. Only four candidate viruses – W6, Q17, 4803 and Han2 – fulfilled the first six selection criteria. The Han2 strain, isolated in Germany in 1986, proved to be capable of growing in vitro in PBMC of at least seven different outbred chimpanzees. A large low passage, PBMC-grown stock of this strain was propagated (Programme EVA). Direct sequencing of the C2–V3 domain of 10 clones derived from this stock confirmed the uniformity of these sequences and identity with the published sequence of Han2 (Sauermann et al., 1990). This report documents the characteristics of in vitro culture and the kinetics of in vivo infection of chimpanzees with HIV-1<sub>Han2</sub>.

**Methods**

### Viruses

Four HIV-1 clade B virus strains were selected for their ability to infect chimpanzee PBMC from outbred animals at the Biomedical Primate Research Centre (BPRC) in Rijswijk, The Netherlands. The isolates selected were Q17 (ACH 168.7) (Schuitemaker et al., 1992) and W6 (ACH 320.3) (Groenink et al., 1991) from the CLB, The Netherlands, Han2 from the DPZ in Germany (Sauermann et al., 1990) and isolate 4803 from the Karolinska Institute in Sweden. From all four virus isolates, stocks were generated by short-term culture in human PBMC for evaluation in vitro. Virus stocks were identified as EVA160/Q17BC, EVA161/WoBC, EVA158/Han2 and EVA159/4803. The Han2 and 4803 stocks were obtained after four passages in human PBMC, WoBC and Q17BC after three human PBMC passages. The in vitro analyses of the virus stocks in human and chimpanzee PBMC as well as in MT2 cells were performed at the CLB (Amsterdam) and at the BPRC (Rijswijk).

### Animals

Outbred HIV-seronegative chimpanzees (Pan troglodytes) housed at the BPRC were used as PBMC donors for in vitro infectivity and titration assays. The in vivo study was carried out in two unrelated mature chimpanzees: ch-Su.A (23 years) and ch-On (14 years). They were confirmed to be negative for HIV-1 infection by ELISA, Western blot and PCR. Blood sample collections and inoculations were done under sedation induced by ketamine hydrochloride (10 mg/kg intramuscular). All protocols were approved by independent Scientific and Ethical Committees and were performed under strict international guidelines. For lymphocyte subset analysis, freshly isolated PBMC were analysed for several surface antigens including CD3, CD4, CD8 and CD20 on a FACSort flow cytometer using Cell Quest software (Becton and Dickinson).

### In vitro evaluation of primary virus isolates

Each HIV-1 isolate received from the donor institutes was tested for its infectivity in MT2 cells and CD8-depleted chimpanzee PBMC. MT2 cells were incubated for 1 h at 37 °C/5% CO<sub>2</sub> with 5 µg/ml polybrene (Aldrich) in Iscove’s medium with 10% heat-inactivated (56 °C, 30 min) foetal calf serum (FCS). Thereafter, cells were plated in 96-well flat-bottom microtitre plates (2 x 10<sup>4</sup> cells/100 µl per well). Viruses were diluted in octuple tenfold serial dilutions in microtitre plates. Subsequently, 100 µl samples of the dilutions were added to the MT2 microtitre plates. Cells were cultured for 4 weeks, refreshed every 3–5 days and evaluated every 3–5 days for cytopathic effect (CPE). Supernatant taken on the last day of culture was tested for the presence of p24 with a Coulter HIV-1 p24 Antigen Capture ELISA.

Chimpanzee PBMC were isolated from heparin-treated blood by ficoll separation. Depletion of CD8 cells was performed by incubation with Dynabeads with anti-CD8 monoclonal antibody according to the manufacturer’s recommendations. Subsequently, cells were cultured for 3 days at 1 x 10<sup>6</sup> cells/ml in RPMI medium supplemented with 20% FCS and 5 µg/ml phytohaemagglutinin (PHA) (Pharmacia) in 24-well plates. Prior to infection, cells were harvested and incubated with 5 µg/ml polybrene in RPMI medium with 10% FCS for 1 h. Tenfold serial virus dilutions were made in tubes; 0.5 ml dilutions of virus were added to 0.5 ml (1 x 10<sup>6</sup> cells) of cell suspensions. Cell–virus suspensions were incubated for 1 h at 37 °C and subsequently washed four times with medium. Final cell pellets were resuspended in 1 ml RPMI–20% FCS supplemented with 100 U/ml IL-2 (Biogen) and cells were cultured in 24-well plates. Cultures were evaluated every 2–4 days for CPE and 200 µl of supernatant was frozen for analysis of p24 production at intervals of several days up to 30 days post-infection. As a control, HIV-1<sub>HIV393</sub> (kindly provided by P. Nara, NCI Frederick, MD, USA) was titrated in parallel. The TCID<sub>50</sub>/ml was calculated using the Kärber formula (Kärber, 1931).

### In vitro evaluation of virus stocks

From all four candidate viruses (Programme EVA), low passage stocks were made in human PBMC. For determination of the virus titres, the TCID<sub>50</sub>/ml of each virus stock was tested in parallel in human and chimpanzee PBMC, and as a control in the MT2 assay. Primary PBMC were obtained from buffycoats prepared at CLB from volunteer blood donors, and from EDTA blood from HIV-seronegative chimpanzees. PBMC were cultured for 2 days in PHA-containing medium (1 µg/ml) to obtain PHA-stimulated T-cell blasts. Virus stocks were diluted in quadruplicate in five- or tenfold serial dilutions in microtitre plates. Subsequently, the dilutions of virus were added to wells of further microtitre plates containing 1 x 10<sup>5</sup>/ml PHA-stimulated PBMC (human or chimpanzee) or 1 x 10<sup>5</sup>/ml M12 cells as controls. The cells were incubated at 37 °C for 10 days. After this culture period a small volume of the supernatant was withdrawn and the p24 concentration assayed. Supernatants were subsequently added to a new microtitre plate containing 1 x 10<sup>6</sup>/ml PHA-stimulated PBMC (human or chimpanzee) or 1 x 10<sup>5</sup>/ml MT2 cells as controls. The TCID<sub>50</sub>/ml was calculated using the Kärber formula (Kärber, 1931).

### HIV-1<sub>Han2</sub> challenge stock

Based on the results for the four candidate viruses obtained in vitro, one virus strain was selected for evaluation in vivo in chimpanzees. The virus was propagated for large-scale in vitro production in human PBMC. At the peak of virus production, the culture supernatants were harvested, clarified by centrifugation, aliquoted into 500 x 1 ml amounts, labelled (EVA 158/Han2 challenge stock (supernatant from Hu PBL) 28.11.93 CAMR) and batch-frozen at −70 °C. Vials were then stored under liquid-nitrogen vapour phase.
Chemokine receptor usage and sensitivity to β-chemokines of HIV-1\textsubscript{Han}. To determine the coreceptor use of the HIV-1\textsubscript{Han} isolate, CD4-positive human osteosarcoma HOS cell lines expressing the chemokine receptors CCR1, CCR2b, CCR3, CCR4, CCR5, CXCR4, BOB/GPR15 and BONZO/STRL33 (K. Ramani and others, unpublished), and the astrogial cell line U87 stably expressing human CD4 and separately each of the chemokine receptors CCR2b, CCR5 or CXCR4 were used. The CD4-positive HOS cells containing HIV-2 LTR driving green-fluorescence protein (GFP), introduced via cotransfection with CMV, were maintained in Dulbecco’s modified Eagle’s Medium (DMEM; Gibco) supplemented with 10% FCS under G418 (1 mg/ml; Calbiochem) and hygromycin (100 µg/ml) selection. For cell-free infection experiments HOS cells expressing the different coreceptors were seeded at 2 × 10⁴ cells per well (1 ml) in 12-well plates. The infection was performed in the presence of polybrene (20 µg/ml) overnight at 37 °C with a tenfold dilution of the HIV-1\textsubscript{Han} stock. After infection the cultures were washed three times and cultured for another day. Forty-eight hours after infection cells were analysed for GFP fluorescence. U87.CD4 cells were maintained in DMEM supplemented with 10% FCS under G418 neomycin (0.4 mg/ml; Gibco) and puromycin (1 µg/ml; Calbiochem) and hygromycin (100 µg/ml) selection. For cell-free infection experiments with the Han2 virus, U87.CD4 cells expressing the different coreceptors were seeded in 24-well plates at 2 × 10⁴ cells per well in 1 ml of medium. The infection was performed overnight at 37 °C with tenfold serial dilutions of virus (1 ml final volume) beginning with a 1:8 dilution of the HIV-1\textsubscript{Han} stock. After infection, the cultures were washed three times with DMEM and cultured for 13 days. Medium was refreshed twice a week. Cultures were examined microscopically for CPE. Supernatants, collected at several time-points after infection, were tested for p24 antigen production.

Chemokine inhibition was performed in parallel with the infection experiments. U87.CD4 cells expressing the CCR5 coreceptor were first preincubated with 0.5 µl of a known HIV-1 inhibitory concentration of 10 ng/ml of the β-chemokines RANTES, MIP-1α and MIP-1β (R&D Systems) for 2 h at 37 °C. Thereafter, infection of the cells was performed overnight by adding 0.5 ml of tenfold serial dilutions of the Han2 virus. Medium was refreshed twice a week in the presence of the β-chemokine mixture (final chemokine concentrations: 5 ng/ml). Inhibition by β-chemokines was evaluated by measuring p24 antigen production.

In vivo HIV-1 inoculum. To evaluate the characteristics of HIV-1 infection in vivo, three vials of the frozen virus stock were thawed in a 37 °C water bath (a standard operation procedure used by programme EVA), allowed to stand for 60 min at 37 °C with brief whisk mixing, and then pooled. Based on the TCID₅₀/ml in chimpanzee PBMC of 10⁶ (see Table 1), pooled virus was diluted in RPMI to a titre of 100 or 10 TCID₅₀/ml. Two adult female chimpanzees were inoculated intravenously with 1 ml of diluted virus stock. One chimpanzee, ch.Su.A, received 100 TCID₅₀ of the virus; the other, ch.On, received 10 TCID₅₀. The total 1 ml inoculum was followed by a 5 ml flush of saline. Blood samples were collected on the day of virus inoculation, weekly for 8 weeks, at weeks 10 and 12 post-infection and at monthly intervals thereafter. The concentration of p24 antigen in chimpanzee plasma and/or serum was measured at several time-points after infection. After inoculation of the chimpanzees, the remaining material was used in vitro for confirmatory titrations on human and chimpanzee PBMC and on MT2 to verify the TCID₅₀/ml. MT2 cells were cultured in RPMI–10% FCS and 5 µg/ml polybrene for 1 h at 37 °C before virus infection. Tenfold serial dilutions of the two virus inocula (100 and 10 TCID₅₀/ml) were prepared, starting from a 1:2 dilution. The virus dilutions were then added to 2 × 10⁴ MT2 cells per well of a 96-well microtitre plate and cultured for 3 weeks. Syncytia were scored and supernatants were tested for p24 antigen on days 14 and 21 using the p24 antigen capture ELISA. Human and chimpanzee PBMC were stimulated for 2 days with 5 µg/ml PHA and 5 µg/ml polybrene in RPMI–10% FCS. Fivefold serial dilutions of the two virus inocula were prepared, starting from a 1:25 virus dilution. The virus was added to 10⁵ PBMC in 150 µl of RPMI medium per well in 96-well round-bottom microtitre plates. After incubation for 1 h at 37 °C, the PBMC were washed twice every 24 h for 3 days and cultured in RPMI + 10% FCS + 50 U IL-2 (Biogen) for 1 week. On day 7 supernatants were tested for p24 antigen production.

Detection and quantification of viral DNA/RNA. At regular time-points the load of viral RNA in plasma, proviral DNA in PBMC and in lymph node-derived mononuclear cells (LNMC) was determined. RNA load in plasma was measured using a PCR-based kit (Ambicor HIV-1 Monitor, Roche Diagnostic systems). Provirial DNA load in PBMC and LNMC was assessed by real-time PCR. Infections in cell cultures were performed either by cocultivation with MT2 cells or cultivation of autologous PBMC with blood samples taken weekly after infection. To assay for syncytium-inducing isolates, 10⁴ MT2 cells (in 0.5 ml) were cocultivated with twofold dilutions of freshly isolated chimpanzee PBMC starting with 10⁵ cells in 0.5 ml culture medium, or plasma starting with 40% in 0.5 ml, cocultivated in 24-well plates for 3 weeks and observed for development of CPE with regular replenishment of medium. In addition, virus isolation was performed with chimpanzee PBMC. Approximately 10⁵ chimpanzee PBMC were depleted for CD8 cells as described above. CD8-depleted PBMC were then seeded in a volume of 200 µl (10⁴ cells) per well in 96-well microtitre plates containing feeder cells (Heeney et al., 1994). Cultures were incubated for up to 10–12 days. Then cells and supernatant from each well were mixed; 30 µl per well was then transferred to fresh feeder plates (containing 170 µl medium) and cultured for another 10–12 days. From each well 50 µl of supernatant was tested for the presence of HIV p24 antigen. The number of infected cells per 10⁶ was calculated using the formula for Poisson distribution.

HIV-1 antibody assay. Serum or plasma samples collected prior to virus inoculation and at various time-points post-inoculation were tested for the presence of HIV-1-specific antibodies using a commercial Western blot test (Cambridge Biotech) and gp160 ELISA. Microtitre plates (96-well, TiterTek) were coated with 1 µg/ml of recombinant gp160 (Centralized Facility for AIDS Reagents, NBSC, UK) overnight at 4 °C followed by a saturation step with PBS containing 0.1% Tween 20 (Merck), 1% BSA (Sigma) and 4% newborn calf serum (Gibco) for 1 h at 37 °C. Twofold serial dilutions starting at 1:50 were incubated for 1 h followed by incubation with a biotinylated sheep anti-human Ig for 1 h (Amersham). Finally, streptavidin-horseradish peroxidase conjugate (Amersham) was added and incubated for 0.5 h followed by
addition of o-phenylenediamine substrate (Sigma). Absorbance was measured at 490 nm.

Results

In vitro characterization

The prescreening of the various HIV-1 clade B isolates provided by the different donor laboratories confirmed infection and replication in the control cell line MT2 (Table 1a). Of the four candidate viruses, only the Han2 isolate was able to infect and replicate in CD8-depleted chimpanzee PBMC (Table 1a), including PBMC from seven different chimpanzees (H. Schuitemaker, unpublished results). Low passage human PBMC stocks were prepared from all four strains and the in vitro titrations were again carried out in MT2 cells as well as in human and chimpanzee PBMC (Table 1b). Again, only the Han2 virus stock was able to infect chimpanzee PBMC. The TCID₅₀/ml of this stock was 10⁵⁻²³, 10⁵⁻¹² and 10²⁻⁹⁹ in MT2, human and chimpanzee PBMC, respectively.

Table 1. In vitro infectivity of HIV-1 clade B isolates and human (hu)PBMC-derived stocks for MT2, human and chimpanzee (ch)PBMC

(a) Infectivity of low passage primary isolates for MT2 cells and CD8-depleted chimpanzee PBMC. Data are expressed as log₁₀ TCID₅₀/ml. Neg., no p24 production; pos., p24 production. HXB3: positive control virus proven to infect human and chimpanzee PBMC. *Han2 also infects total chimpanzee PBMC populations as has been found in PBMC isolated from seven different chimpanzees. (b) Infectivity of low passage human PBMC-derived virus stocks for MT2 cells and for human and chimpanzee PBMC. Data are expressed as log₁₀ TCID₅₀/ml. Neg., no p24 production; pos., p24 production. * Mean value of six different vials tested. ** Mean of six different vials tested with three different PBMC populations. (c) TCID₅₀/ml values of both inocula in MT2 cells, when diluted and measured 18 months apart, were almost identical – the inoculum of ch-Su.A was 10⁻⁸⁻⁵, the inoculum of ch-On was 10⁻⁹⁻⁸ – which indicated that no loss of infectivity of the stock had occurred within this time-frame. Variation in methodology between the two laboratories (BPRC and CLB) accounted for slight differences in TCID₅₀ values obtained.

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>MT2</th>
<th>CD8-depleted Ch PBMC</th>
<th>MT2</th>
<th>HuPBMC</th>
<th>ChPBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q17</td>
<td>4.8</td>
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<td>4.1</td>
<td>4.1</td>
<td>Neg.</td>
</tr>
<tr>
<td>W6</td>
<td>4.2</td>
<td>Neg.</td>
<td>3.8</td>
<td>4.1</td>
<td>Neg.</td>
</tr>
<tr>
<td>Han2</td>
<td>3.2</td>
<td>Pos.*</td>
<td>5.23*</td>
<td>5.12**</td>
<td>2.99*</td>
</tr>
<tr>
<td>4803</td>
<td>3.8</td>
<td>Neg.</td>
<td>4.1</td>
<td>5.2</td>
<td>Neg.</td>
</tr>
<tr>
<td>HXB3</td>
<td>4.3</td>
<td>Pos.</td>
<td>6.7</td>
<td>5.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

(c) TCID₅₀/ml determination of the in vivo inocula

<table>
<thead>
<tr>
<th>Inoculation date/chimps to infect</th>
<th>Virus</th>
<th>MT2</th>
<th>HuPBMC</th>
<th>ChPBMC</th>
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<td>Han2</td>
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<td>ND</td>
<td>No calculation</td>
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<tr>
<td></td>
<td>HXB3</td>
<td>4.75</td>
<td>ND</td>
<td>3.0</td>
</tr>
<tr>
<td>7 August 1996/(ch-On)</td>
<td>Han2</td>
<td>3.8</td>
<td>4.8</td>
<td>3.1</td>
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<tr>
<td></td>
<td>HXB3</td>
<td>4.75</td>
<td>3.9</td>
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</table>
Coreceptor use and inhibition by β-chemokines

The ability of these isolates to grow on MT2 cells confirmed their syncytium-inducing capacity and suggested use of the CXCR4 coreceptor. To determine the coreceptor characteristics of the selected primary isolate, HOS cells expressing various coreceptors were infected with dilutions of Han2. Also, for quantification of p24 production, U87.CD4 cells expressing CCR2b, CXCR4 or CCR5 were infected with serial tenfold dilutions of Han2. In addition, β-chemokines were added at an inhibitory concentration (10 ng/ml) to the CCR5-expressing cells. The Han2 isolate was able to infect HOS cells expressing CCR4, CXCR4 and CCR5 coreceptors, but could not infect HOS cells expressing the CCR1, CCR2b, CCR3, BOB/GPR15 or BONZO/STRL33 coreceptors (data not shown). As shown in Fig. 1 the ability of Han2 to infect and replicate in U87.CD4 cells expressing different coreceptors was confirmed both by syncytium induction and by p24 production (Fig. 1). From both assays it became apparent that Han2 had the ability to use CCR5 in addition to CCR4 but not CCR2b. Furthermore, infection was clearly blocked by the addition of β-chemokines. This indicated that the HIV-1 Han2 inoculum used to infect chimpanzees had retained the characteristics of primary isolates.

Detection and quantification of viral RNA/DNA in vivo

After inoculation of chimpanzees, the quantity of circulating viral RNA copies as well as the number of provirus-infected cells were measured. In ch-Su.A, viral RNA in plasma reached a maximum peak at week 4.5 (4 x 10^6 RNA copies/ml plasma). This decreased to 220 copies/ml at week 24 but increased again and remained persistent at levels around 4-5 x 10^5 RNA copies/ml plasma up to 105 weeks post-infection (Fig. 2a). Measuring proviral DNA, we first detected infected cells in the peripheral blood 3 weeks post-infection in ch-Su.A (Fig. 2b). The number of infected PBMC reached a peak at week 12 post-infection (192 infected PBMC/10^6 PBMC), and then decreased gradually to low levels at week 24 [plasma samples analysed at 18 months post-infection still showed a low number of virus-infected cells (10 provirus-containing cells/10^6 PBMC; data not shown)]. Interestingly, in the animal which received a tenfold lower dose of virus (ch-On), the plasma RNA levels reached a 7.5-fold higher peak at week 4 (3 x 10^5 RNA copies/ml plasma; Fig. 2d). Also in ch-On, the level of plasma RNA copies decreased gradually with time. In ch-On, the number of proviral DNA-containing cells in circulation was at a maximum level 6 weeks post-infection with 288 infected cells/10^6 PBMC, with a second peak following at 22 weeks (Fig. 2e). The kinetics of the infection in LN differed in the early stages of infection. The sampling of LN coincided on weeks 4 and 12 in both animals. By 4 weeks post-infection, 48 provirus-containing cells/10^6 LNMC were detected in ch-Su.A (Fig. 2b) while the number of infected LNMC in ch-On was approximately five times higher (240 provirus-containing LNMC; Fig. 2e). By week 12 both chimpanzees had reached similar levels. A 22 week follow-up in ch-On revealed that this level of provirus-infected cells in LN persisted. The numbers of provirus-infected cells in LN for the most part followed the same general pattern of infection as in peripheral blood but remained at higher levels.

Virus-producing cells

Virus-producing PBMC were detected as early as 3 weeks post-infection (ch-Su.A) in the MT2 coculture assay (Fig. 2c). The number of infected cells decreased gradually thereafter, and they could no longer be detected from week 12 post-infection. Samples taken at week 76 post-infection were also shown not to produce virus (data not shown). Quantitative virus isolation was also performed with CD8-depleted PBMC from ch-Su.A at several time-points after infection (data not shown). With this assay two peaks of virus-producing PBMC were observed at two different time-points. At both 4-5 weeks and 10 weeks, peaks of 5-8 virus-producing cells/10^6 CD8-depleted PBMC were detected. By 16 weeks and as late as 18 months post-infection virus-producing cells were no longer detected in our assays. This two-phase kinetics was somewhat similar to that observed in the MT2 assay (Fig. 2c). The kinetics of appearance of virus-producing cells differed slightly in the two animals. The time of maximum number of virus-producing cells occurred at week 5 in ch-On as compared to week 3 in ch-Su.A while, as already mentioned, the peak number of virus-producing cells detected in ch-On was lower than that detected in ch-Su.A. The number of virus-producing LNMC reached a higher peak of 9 virus-producing cells/10^6 PBMC in ch-On but none could be detected from week 12 onwards (Fig. 2f). Cell-free antigen was measured both in plasma and in serum from ch-Su.A at several time-points after infection. p24 concentration in all samples (both plasma and serum) never exceeded twofold background values in the Coulter ELISA. Thus, cell-free p24 antigen could not be detected in peripheral blood (data not shown).
Antibody responses

Antibodies were detected at week 6 post-infection by Western blot against gp160 and p24 (Fig. 3). Western blot reactivity increased progressively with gp120 and p17 bands clearly visible from week 10, and gp41 reactivity apparent from week 16 in serum from ch-Su.A. Antibody titres as determined by ELISA could be detected from week 6 (ch-On) and week 8 (ch-Su.A) post-infection (Fig. 4). The antibody levels continued to increase reaching maximum titres of 6400 by week 22 (ch-On, last time-point determined) and 12800 after 25 months (ch-Su.A).

Flow cytometric analysis

FACS parameters were measured at various time-points after infection for both ch-On and ch-Su.A. There were no remarkable changes over time either in the absolute number or percentage of CD4-, CD8- and CD3-positive cells. Long-term follow up of these animals is ongoing to determine if changes
HIV-1 clade B infection in chimpanzees

Fig. 3. Western blot analysis of serum samples of ch-On (a) and ch-Su.A (b). Lane A represents positive control, B represents negative control. Serum samples collected at different weeks post-infection are indicated.

Fig. 4. Antibody end-point titres to gp160 at various time-points after infection in ch-Su.A (○) and ch-On (●).

Discussion

The objective of this study was to evaluate well-defined European HIV-1 clade B primary isolates for potential use as challenge virus in the testing of HIV-1 vaccine efficacy in chimpanzees. Of the four primary isolates selected which fulfilled the criteria of EU programme EVA, only one virus (Han2) was able to routinely and reproducibly infect chimpanzee PBMC in a number of outbred animals (Table 1). The selected isolate, HIV-1_{Han2}, was shown to use CCR4 (data not shown), CXCR4 and CCR5 coreceptors and infection could be inhibited by β-chemokines (Fig. 1). Primary syncytium-inducing HIV-1 variants can use both CXCR4 and CCR5 as coreceptors for entry (Simmons et al., 1996; Zhang et al., 1996). However, once adapted to grow in T-cell lines, these viruses lose their capacity to use CCR5 (Kozak et al., 1997), indicating that the HIV-1_{Han2} inoculum used to infect chimpanzees had retained primary isolate characteristics. The fact that only the Han2 isolate is able to infect and replicate in chimpanzee PBMC might be explained by its ability to enter PBMC via different receptors. Whether the specific coreceptor use of Han2 is unique as compared with the other primary isolates which infect chimpanzee cells is a matter for further investigation.

Two chimpanzees were inoculated intravenously with 100 or 10 TCID_{50} of the Han2 virus stock. With both doses successful infection of the chimpanzees was achieved. Animals became PCR-positive for provirus as early as 3 weeks post-inoculation. Viral RNA plasma levels for both chimpanzees reached a maximum at approximately week 5 post-infection and then decreased gradually to steady-state levels after 24 weeks. Since ch-On was infected 18 months later than ch-Su.A that follow-up is still in progress. Despite a tenfold lower HIV-1_{Han2} inoculum used for ch-On as compared to ch-Su.A, the RNA peak levels in plasma were 7-5 times higher. This result indicates that a higher virus inoculum does not necessarily result in higher viraemia, confirming our earlier observations in the SIV/SHIV rhesus monkey model (Bogers et al., 1997a).

The numbers of proviral DNA-containing cells in both PBMC and LNMC from ch-Su.A and ch-On were similar despite a tenfold difference in virus inoculum dose and this level remained relatively persistent. The numbers of virus-producing cells in the two chimpanzees were also of the same order of magnitude, with a maximum at weeks 3–5 and a second peak in CD4 cell numbers or other parameters occur after chronic infection.
occurring at weeks 7 and 10 (ch-On and ch-Su.A respectively). This observation was confirmed with an additional assay using freshly cultured CD8-depleted chimpanzee PBMC. It is possible that mutations leading to escape variants of the virus population occurred after the first peak resulting in a second peak of virus-producing cells. This hypothesis needs further investigation. Despite the relatively high levels of viral RNA in plasma from ch-Su.A and virus-producing cells in both peripheral blood and LN, we could not detect significant amounts of cell-free p24 antigen during the study, indicating the predominantly cell-associated nature of this virus infection in chimpanzees. Interestingly, both chimpanzees developed a high-titre antibody response (Fig. 4), which might be responsible for the clearance of plasma antigen, as well as lower levels of viral RNA in plasma. Furthermore, in addition to the antibody titres observed, HIV-1\textsubscript{Hanz}-specific neutralizing antibodies were detected 6 months post-infection (unpublished). Another possible reason for the absence of plasma antigen might be the presence of suppressor factors able to suppress HIV-1 replication in provirus-containing cells (Bogers et al., 1997b; Heeney et al., 1996).

Infection of chimpanzees with an HIV-1\textsubscript{SF2} stock was described by Murthy et al. (1996). Of the (non-Lai/IIIb) challenge virus stocks described so far for chimpanzees, only HIV-1\textsubscript{SF2} is highly similar to the North American clade B consensus sequence (Myers et al., 1994). The titre of the HIV-1\textsubscript{SF2} stock as compared to the HIV-1\textsubscript{Hanz} stock was similar when titrated on human PBMC (10^4 \times 10^5 of SF2 versus 10^6 of Han2) but not when titrated on chimpanzee PBMC (10^4 versus 10^6 of SF2 versus 10^8 of Han2). Thus, the in vitro infectivity of the SF2 stock for chimpanzee PBMC was 10 times higher than that of the Han2 stock. However, we have shown recently that the in vitro TCID\textsubscript{50} values do not necessarily predict in vivo infectivity. It is possible to achieve high virus loads in vivo with virus inocula of low TCID\textsubscript{50}/ml values (Bogers et al., 1997a). Chimpanzees inoculated with various TCID\textsubscript{50} of SF2 showed relatively low viral RNA plasma levels which were cleared faster from circulation than observed for Han2 virus infection in this study. HIV-1\textsubscript{SF2} brought about only a weak infection with transient virus isolation and virus loads which declined beyond DNA PCR detection. Another HIV-1 clade B strain (HIV-1\textsubscript{D1112}) which could infect chimpanzees was described by Shibata et al. (1995). Infection with DH2 virus resulted in lower and inconsistent plasma RNA levels, as compared with Han2 infection, while no differences in CD4- and CD8-positive cells were observed during the first 32 weeks post-infection. The antibody titres to DH2 were considerably lower (Shibata et al., 1995) as compared with antibody titres induced by the Han2 isolate, taking into account possible differences in methodology.

In conclusion, HIV-1\textsubscript{Hanz} readily infected chimpanzees from which virus could be easily isolated and resulted in persistent viral RNA and provirus loads in both PBMC and LNMC. Infection induced high antibody titres able to neutralize the Han2 virus (as well as specific cellular immune responses; unpublished). Although evidence of disease progression has not yet been observed, this virus is suitable for evaluating HIV-1 vaccines for their ability to prevent infection from primary virus isolates and may be of use for evaluating the effect of vaccination on acute phase infection.

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References


HIV-1 clade B infection in chimpanzees


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