Efficient replication of human immunodeficiency virus type 1 and measles virus in a human-to-mouse graft versus host disease model permits immunization research

Wim Huppes, Klara Tenner-Racz and Georg Kraal

1 Health Research-TNO, 2280 HV Rijswijk, The Netherlands, 2 Knochenmark Labor, St Georg Krankenkasse, 2299 Hamburg, Germany and 3 Department of Cell Biology, Vrije Universiteit, 1081 BT, Amsterdam, The Netherlands

An acute graft versus host disease (GvHD) murine model was developed to study the pathogenic and protective mechanisms against viruses that replicate in cells of the human immune system. The model allowed efficient replication of lymphotropic, macrophage and amphitropic strains of human immunodeficiency virus type 1 (HIV-1) and measles virus (MV). Cytopathic lymphotropic strains of HIV-1 and a wild-type MV strain replicated in a 'burst'-like manner, whereas a non-cytopathic lymphotropic HIV-1 strain and all macrophage-tropic HIV-1 strains caused persistent infection of the graft. The replication kinetics of infection with these viruses were highly reproducible and were very similar to those observed in natural infection of humans. Infection with these viruses, with the exception of HIV-1sF2, led to a significant delay and abrogation of the GvHD, indicating a direct immunosuppressive effect. Interestingly, infection with the lymphotropic HIV-1sF2 strain was rapidly and spontaneously abrogated. The model was also shown to be suitable for the evaluation of passive immunization strategies. Administration of a combination of antibodies against the HIV-1 V3 loop and the HIV-1 CD4 binding sites prevented subsequent infection with HIV-1IIIb. In contrast, administration of CD4 binding site specific human monoclonal antibody at a concentration that would neutralize the virus in vitro enhanced in vivo infection with HIV-1IIIb. The model also allowed evaluation of in vivo immunization studies. Immunization with a live attenuated measles vaccine resulted in protection from a wild-type MV challenge, whereas immunization with a subunit candidate vaccine appeared to give partial protection.

Introduction

One of the major problems in studying the pathophysiology of human immunodeficiency virus type 1 (HIV-1) infection is the lack of a suitable animal model. Although HIV-1 has been shown to replicate in some other primates, like the chimpanzee, the susceptibility to the various phenotypes of the virus is different and these animals do not appear to develop the acquired immunodeficiency syndrome (AIDS) (Nara et al., 1989; Saxinger et al., 1987).

So far, two murine models for HIV-1 have been developed based on transplanted human cells. The first model, as described by D. E. Mosier and co-workers is based on human peripheral blood leukocytes (PBL) injected intraperitoneally (i.p.) into non-conditioned severe combined immunodeficient (SCID) mice. These human PBL SCID mice do not develop signs of graft versus host disease (GvHD) due to the limited numbers of transplanted cells (Torbett et al., 1991). It was shown that T cell line adapted lymphotropic HIV-1 strains replicated in these human PBL SCID mice (Mosier et al., 1991, 1993a), enabling passive immunization studies to be done (Vittecoq et al., 1992). However, the human graft was shown to have defective immunological properties, because the T cells, which are mostly activated CD8+ cells, are anergic to T cell receptor activation (Simpson et al., 1991; Tary-Lehmann & Saxon, 1992).

The other model, developed by McCune et al. (1991) is based on human fetal thymus grafts in the subcapsular space of the kidney of non-conditioned SCID mice. HIV-1 primary isolates were shown to infect grafted lymphocytes and histiocytes (Namikawa et al., 1988; Bonyhadi et al., 1993; Aldrovani et al., 1993). This method is not only very laborious, but the human grafts are also immunologically deficient, as they do not significantly populate the murine tissues (Krowka et al., 1991).
In search of a more satisfactory rodent model for the study of the pathogenesis of HIV-1 we explored a human-to-mouse acute GvHD model (Huppes et al., 1992). This model is characterized by the development of human lymphocyte CD4/CD8 ratios of \( \geq 1 \) (Huppes et al., 1994) and the generation of donor-type immune function (W. Huppes & G. Hoffmann-Fezer, unpublished results). We speculated that in this model the abundant presence of human CD4+ lymphocytes in the murine lympho-haemopoietic tissues would serve as an efficient substrate for the replication of lymhotropic virus. In addition, the presence of large numbers of activated macrophages would provide an optimal substrate for macrophage-tropic viruses (Hoffmann-Fezer et al., 1992, 1993). Infection of these cells is thought to be important for the persistence of HIV-1 after primary infection (Schuitemaker et al., 1993a, b). Here we present data on the kinetics of HIV-1 and measles virus (MV) replication in this model and the effects of immunological interventions.

**Methods**

**Production of virus stocks.** Virus stocks of the primary HIV-1 isolates HIV-1$_{1992}$ (Schuitemaker et al., 1993a, b) and HIV-1$_{320.2.4}$ (AIDS Research Reference Reagent Program) and the molecular clone HIV-1$_{1A}$ (Cheng-Majer et al., 1990) were propagated on stimulated human PBL. The stimulation was carried out with phytohaemagglutinin (PHA) and the Epstein–Barr virus transformed B cell lines APD and BSM (VanDeGriend et al., 1984). Stocks of the primary isolates HIV-1$_{PB}$ and HIV-1$_{1992}$ and of the PHA-PBL culture adapted HIV-1$_{HP}$ strain (Popovic et al., 1984) called HIV-1$_{PB}$ were produced in PHA-stimulated PBL. The HIV-1$_{PB}$ strain was obtained from an asymptomatic patient (gift of J. C. C. Borleffs, Academical Hospital Utrecht, the Netherlands). Stocks of the HIV-1 molecular clones HIV-1$_{1992.2.1A.2}$ and HIV-1$_{1992.3.1}$ (Groenink et al., 1991) were prepared in the CB15 T cell line, within 3 weeks of culture (Nick et al., 1993). The CB15 cell line was infected with the supernatant of transfected HeLa cells. The supernatant obtained after the transfection of HeLa cells with the HIV-1$_{1992.2.1A.2}$ molecular clone (Groenink et al., 1991) was used directly. The stocks of the T cell line adapted HIV-1 strains MN, RF and H1B (Gallo et al., 1984) were produced on H9 cells. Characteristics of the tropism and the ability of the virus strains to form syncyta are given in Table 1. Stocks of the wild-type MV-BIL strain were produced in the Epstein–Barr virus transformed B cell line JF (VanBinnendijk et al., 1990, 1992, 1994).

**Conditioning of mice.** Inbred BALB/c-SCID/Rij and CBA/N/Rij mice were kept and bred under specified pathogen-free (SPF) conditions in our own breeding colony (Huppes et al., 1992). The CBA/N mice were used for experiments 4 weeks old. The SCID mice were either newborn or 8 weeks old.

Total body irradiation (TBI) was carried out with a \(^{137}\)caesium source, type Gamma-Cell 20 (Atomic Energy of Canada, Ottawa, Canada), at a dose rate of 0.87 Gy/min. The conditioning scheme was 9 Gy for the CBA/N mice, with supportive care of 5 \( \times 10^8 \) syngeneic bone marrow cells intravenously (Huppes et al., 1992). The newborn and 8-week-old SCID mice received 1 Gy and 3 Gy TBI, respectively.

**Transplantation procedure.** Human PBL for xenografting were isolated from freshuffy coat cells of healthy blood donors using lymphocyte separation medium (LSM; Organon Teknika) (Huppes et al., 1992). CBA/N mice grafted intraperitoneally (i.p.) with 2 \( \times 10^7 \) human PBL/g (Huppes et al., 1992) were used for survival studies. The remaining experiments were carried out with i.p. PBL grafts of 4 \( \times 10^7 \) lymphocytes/g (xeno-GvHD mice) (Huppes et al., 1994). In each experiment four CBA/N mice conditioned with 9 Gy and intravenously transplanted with 5 \( \times 10^7 \) syngeneic bone marrow cells served as transplantation procedure controls. These mice invariably died at day 11–16 after transplantation. Also, in each experiment, four CBA/N mice conditioned with 9 Gy and intravenously transplanted with 5 \( \times 10^7 \) syngeneic bone marrow cells served as transplantation procedure controls. These mice invariably lived throughout the observation period of 30 days.

**Immunization procedures.** Mice were immunized passively 1 h after the cell graft by i.p. injection with 250 µg antibodies or with 300 µg serum. The monoclonal antibodies were F8H3 to the HIV-1$_{1992}$ V3 loop, 257-D to the HIV-1$_{1992}$-like V3 loop (Gorny et al., 1993) and GP13 to the HIV-1 CD4 binding site (Schutten et al., 1993). F8H3 neutralizes HIV-1$_{1992}$ specifically (VanBinnendijk et al., 1990, 1992, 1994; Devash et al., 1990). The mice that served as negative controls were injected with the K14 monoclonal antibody, which has the same isotype as F8H4 and 257-D. This antibody does not recognize the glycoprotein complex of HIV-1, nor HIV-1 epitopes on virus infected cells (Teuwen et al., 1990). The antisera used was the anti-HIV-1 immunoglobulin (HIVIG) (Schuitemaker et al., 1993a, b). This contains polyclonal specific anti-HIV-1 antibodies (Schuitemaker et al., 1993a, b; Prince et al., 1988, 1991).

Active immunization studies are described for the MV vaccines because HIV-1 vaccines have not been shown to give effective protection. Xeno-GvHD mice were immunized 1 h and 3 days after the xenograft by i.p. injection with the vaccine. One thousand infectious units (i.u.) of live virus Schwarz vaccine, i.e. the MV-Edmonton B strain (VanBinnendijk et al., 1990, 1992, 1994), or 5 µg of non-live immunostimulating complexes (ISCOM) vaccine, containing the F and H proteins (VanBinnendijk et al., 1990, 1992, 1994) were used. The mice that served as negative controls received empty ISCOMs. After 8 days, ascitic fluid cells of the xeno-GvHD mice were harvested in culture medium containing 100 i.u. of Thrombolyticin/ml (heparin; Organon Teknika). The culture medium was RPMI 1640 (Gibco) with 10% decomplemented bovine calf serum (Hyclone). Subsequently, these ascitic fluid cells were serially transplanted at a dose of 4 \( \times 10^6 \) cells/g recipient body weight into heavily conditioned CBA/N mice. The mice received a challenge i.p. of 1000 TCID$_{50}$ (50% tissue culture infective dose) of MV-BIL 1 h after all the previous procedures. In addition, 3 days later, the infected centre (IC) was determined.

**Detection of virus.** The mice were sacrificed and the ascitic fluid cells were collected in culture medium to which 100 i.u. IL-2/ml had been added. Subsequently, IC assays were done to determine the number of these cells needed to demonstrate virus infection. The cells were titrated in 5-fold dilutions from 2 \( \times 10^8 \) to 0 cells, using 96-well plates with 2 \( \times 10^5 \) CB15 cells (Nick et al., 1993), SUPT1 cells (AIDS Research and Reference Reagent Program) or JP cells (VanBinnendijk et al., 1990, 1992, 1994) per well. The IC read-out was done 5–7 days after plating, respectively, either with a p24 HIV-1 ELISA (a kind gift of Organon Teknika) or, when analysing MV infection, by analysis of syncytia using a microscope (VanBinnendijk et al., 1990, 1992, 1994). The IC was determined as the lowest dilution at which virus could still be detected. Flow cytometry of the harvested cells was done after labelling them with the FITC labelled anti-human CD4 antibody (Becton and Dickinson) (Huppes et al., 1994).

**Culture and transplantation of CB15 cells.** Before transplantation, the human CD4+ cell line CB15, derived from herpesvirus saimiri transformed cord blood (Nick et al., 1993), was propagated in culture medium, with the addition of 100 i.u. IL-2/ml. To obtain mice without
Table 1. Characterization of 12 HIV-1 stocks

<table>
<thead>
<tr>
<th>HIV-1 strain</th>
<th>Passages*</th>
<th>Composition†</th>
<th>Syncytia</th>
<th>T cell tropism</th>
<th>Macrophage Tropism‡</th>
<th>TCID₅₀/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>&lt; 10</td>
<td>Pr</td>
<td>+ / −</td>
<td>+</td>
<td>−</td>
<td>200</td>
</tr>
<tr>
<td>Q17</td>
<td>&lt; 10</td>
<td>Pr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1000</td>
</tr>
<tr>
<td>PR</td>
<td>&lt; 10</td>
<td>Pr</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>320.2A.1.2</td>
<td>&lt; 10</td>
<td>Cl</td>
<td>+ / −</td>
<td>+</td>
<td>−</td>
<td>40</td>
</tr>
<tr>
<td>320.2A.2.1</td>
<td>&lt; 10</td>
<td>Cl</td>
<td>−</td>
<td>+ / −</td>
<td>+</td>
<td>40</td>
</tr>
<tr>
<td>320.3.1</td>
<td>&lt; 10</td>
<td>Cl</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>200</td>
</tr>
<tr>
<td>SF2</td>
<td>&lt; 10</td>
<td>Ol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>IIIB</td>
<td>&lt; 10</td>
<td>Ol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>RF</td>
<td>&lt; 10</td>
<td>Ol</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>60</td>
</tr>
<tr>
<td>MN</td>
<td>&lt; 10</td>
<td>Ol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>600</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 10</td>
<td>Ol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Number of in vitro passages of the virus.
† Composition of the stock, either as mono- (Cl), oligo- (Ol) or polyclonal (Pr) virus.
‡ Tropism of the virus for histiocytic macrophage cells.

Immune capacities the CB15 cell line was transplanted i.p. into heavily conditioned SCID mice at a dose of 1 × 10⁶ cells/g (CB15-SCID mice) (Huppes et al., 1994).

Histopathology. Gross necropsy was carried out on mice in the various experiments. Also, at each time point three mice which had received neither human cells nor virus and of three mice that had received human cells but no virus (negative controls) were examined. Tissue samples were fixed in phosphate-buffered 4% formaldehyde and were then paraffin embedded. Tissue slides were stained with haematoxylin (Fluka Chemika) and eosin-yellow (HE) (Merck). HE slides were prepared from alternating tissue sections. The intervening slides were double labelled in situ for HIV-1 Gag RNA and human CD4, respectively with in situ hybridization and immunohistochemistry (Embretson et al., 1993).

Results

Replication and dissemination of HIV-1 and MV in the xeno-GvHD mice

Primary isolates and molecular clones of lymphotropic, macrophage and amphitropic HIV-1, T cell line adapted HIV-1,IIIB and the wild-type MV-BIL were shown to replicate efficiently in the xeno-GvHD mouse model (Fig. 1). The kinetics of the infectivity patterns were not significantly influenced by the use of different human donors. Between day 5 and day 8 post-infection with the primary HIV-1 isolates a 10–100-fold increase in the percentage of infected cells was observed. The percentages of infected cells after infection with molecular clones of HIV-1 were largely identical to those of the primary isolates at day 5 post-infection. However, infection with these molecular clones gave no significant increase in the virus load after that day (Fig. 1).

Histological analysis revealed that the peritoneum of infected xeno-GvHD mice was invariably heavily infiltrated with lymphoblastic cells which showed strong staining with anti-human CD4 (Table 2). The percentage of these cells also showing an HIV-1 Gag signal largely coincided with the results obtained with the IC test (Fig. 1, Table 2). The ability to form syncytia coincided with the in vitro abilities of the virus strains. In contrast to earlier reports (Schuitemaker et al., 1993b), small syncytia were observed after infection with HIV-1,BAL, both in vitro (Table 1) and in vivo (Table 2). The peritoneum also contained histiocytic cells with weak staining for human CD4. Although the slides from some of the mice showed few histiocytic cells, it was generally observed that HIV-1 with macrophage tropism had infected most of these cells (Table 2). Interestingly, the lymphotropic molecular HIV-1 clone 320.2A.1.2 did not show hybridization with human macrophages, whereas the macrophage-tropic strains 320.2A.2.1 and 320.3.1 did (Table 2). The mice that were not grafted with human cells (negative controls) showed no human CD4 or Gag signal. The xeno-GvHD mice that were not infected with virus were positive for human CD4, but negative for HIV-1 Gag.

The high level of infection with the primary isolates at day 11 led to the dissemination of virus to various tissues. The peritoneum showed many double positive lymphoblastic cells. Many of these cells were also seen at perivascular sites in the lung, the periportal sinuses of the liver and in lymph nodes. Few double positive lymphoblastic cells were observed in the thymus. Human CD4⁺ histiocytic cells were not observed outside the peritoneum.

Virus infection results in delay and abrogation of the GvHD

Consistent with the dissemination of HIV-1, it was shown that HIV-1 infection of the 2 × 10⁷ PBL/g grafts suppressed the GvHD significantly. It appeared that
Fig. 1. Virus production with xeno-GvHD mice. Heavily conditioned CBA/N mice (see Methods) received 4 × 10⁸ human PBL and subsequently 10 TCID₅₀ of virus. The results show the number of ascitic fluid cells from an individual mouse needed to give an IC in the in vitro read-out test. The mice analysed at day 5 post-infection had blood cells from different human blood donors. The same donors were used for the mice analysed at day 8 post-infection. * The 2 × 10⁶ ascitic fluid cells of the four mice analysed gave no IC. The ascitic fluid cells contained > 20% human CD4⁺ cells by flow cytometry.

Table 2. Histological analysis of HIV-infected xeno-GvHD mice*

<table>
<thead>
<tr>
<th>HIV-1 strain</th>
<th>Time post-infection (days)</th>
<th>HIV-Gag⁺ Hu-CD4⁺ cells⁺ (%)</th>
<th>HIV-Gag⁺ Hu-Macrophage cells⁺ (%)</th>
<th>Syncytia</th>
</tr>
</thead>
<tbody>
<tr>
<td>320.2A.1.2</td>
<td>6</td>
<td>0–1–1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0–1–1</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>320.2A.2.1</td>
<td>6</td>
<td>0–1–1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0–1–1</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>320.3.1</td>
<td>6</td>
<td>0–1–1</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0–1–1</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>BAL</td>
<td>6</td>
<td>0–1–1</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>10–100</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>Q17</td>
<td>6</td>
<td>0–1–1</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>10–100</td>
<td>100</td>
<td>+</td>
</tr>
</tbody>
</table>

* Necropsies of the CBA/N mice were done on day 6 or 11 post-infection with HIV-1. Before the infection these mice had been conditioned heavily and had subsequently been transplanted with 4 × 10⁸ human PBL/g of recipient body weight (see Methods).
⁺ The immunohistochemistry of human CD4 and HIV-1 Gag (p24) RNA was done on alternating slides of the peritoneum. The lymphoblastic cells and their syncytia were high in human CD4⁺ expression.
⁺⁺ The histiocytic macrophage cells were low in human CD4⁺ expression.

HIV-1_{IIIb} delayed the GvHD for 1–3 days and abrogated it completely in about 10% of the mice (Fig. 2). Statistical analysis of data presented in Fig. 2 showed that the results are significant (Table 3). A comparable effect on survival after infection with HIV-1_{p} and primary isolates HIV-1_{Q17} and HIV-1_{pR} was observed (Table 3).

It was also shown that MV (MV-BIL) infection delayed and abrogated the GvHD. Finally, the GvHD was also suppressed by MV-BIL infection of newborn SCID mice transplanted with rhesus monkey PBL (Table 3).

Abrogation and enhancement of HIV-1 infection with HIV-1 antibodies

It was observed that the lymphotropic HIV-1_{IIIb} strain infected many human PBL by day 5, but at day 8 no infected PBL were observed. This pattern was also observed with MV-BIL infection (Fig. 1). HIV-1_{IIIb} infection was enhanced by treatment of the xeno-GvHD mice with GP13, the passively transferred monoclonal...
**Replication of HIV-1 and MV in GvHD model**

**HIV-1\textsubscript{SP2} infection is spontaneously abrogated in vivo**

While validating the model, a surprising phenomenon was found. The *in vivo* i.p. injection of as many as 100 TCID\textsubscript{50} of HIV-1\textsubscript{SP2} did not cause infection of PBL grafts of the xeno-GvHD CBA/N chimeras (Table 4). To distinguish between a failure of virus replication *per se* and immunological control of the infection, we modified our model to one without any immunological response, using heavily conditioned SCID mice with CB15 cell grafts (CB15-SCID mice). Interestingly, *in vivo* infection of these mice with HIV-1\textsubscript{SP2} could not be established either (Table 4), although replication of HIV-1\textsubscript{SP2} on the CB15 cell line readily occurs *in vitro*. To investigate whether replication of HIV-1\textsubscript{SP2} could occur at all *in vitro*, CB15 cells or PBL were infected *in vitro* before engraftment. It appeared that CB15 grafts supported HIV-1\textsubscript{SP2} infection for at least 8 days. In contrast, PBL grafts became negative for HIV-1\textsubscript{SP2} 3 days after transplantation (Table 4). Consistently, no abrogation of the GvHD was observed in a large group of 23 xeno-GvHD mice, with PBL grafts that had been infected *in vitro* with HIV-1\textsubscript{SP2} (Table 3).

**Prevention of MV-BIL infection by pre-exposure to MV vaccines**

The effects of pre-exposure of the xeno-GvHD mice to MV vaccines were also studied. Fig. 4 shows that MV-BIL infection of PBL grafts in CBA/N mice was prevented by pre-exposure to the Schwarz live vaccine. The MV ISCOM subunit vaccine appeared to induce partial protection. However, the empty ISCOMs may also have had some protective effect, as the number of infected ascitic fluid cells was one-quarter (one dilution step) less with these empty ISCOMs when compared to control mice without ISCOMs. The challenge MV-BIL was found in large quantities in all these sham immunized mice (Fig. 4).

**Discussion**

Quantitative analysis showed efficient replication of primary isolates of lymphotropic, macrophage and amphitropic HIV-1 strains and of the MV-BIL strain in the acute xenogeneic GvHD model. Consistent with the fact that human CD4\textsuperscript{+} lymphocytes are the main initiator of the GvHD (Huppes *et al.*, 1992, 1994), direct cytopathic effects of HIV-1 and MV-BIL are implied by the significant delay and complete abrogation of the GvHD (Fig. 2, Table 3).

The *in situ* hybridization data for HIV-1 RNA indicate that the peritoneum of the acute xeno-GvHD mice contains many HIV-1 infected human T cells and macrophages. As each chimeric mouse was made with

---

**Fig. 2. Suppression of human-to-mouse GvHD with HIV-1\textsubscript{IIIB}.** Heavily conditioned CBA/N mice received 2 x 10\textsuperscript{7} human PBL grafts/g of recipient body weight and were subsequently infected with HIV-1\textsubscript{IIIB} (see Methods). Survival of these mice was plotted against time. Line (a), sham treatment of mice with i.p. injection of 250 \textmu l of culture medium (n = 8). Line (b), i.p. injection of 10 TCID\textsubscript{50} of cell-free supernatant of the HIV-IH\textsubscript{B} stock in 250 \textmu l of culture medium (n = 8).

**Table 3. Virus infection significantly enhances the survival of mice with human-to-mouse GvHD**

| Virus strain | No. of Mice per group | P value | Significance |t |
|--------------|----------------------|--------|--------------|
| HIV-IIHB     | 8                    | 0.0493 | +            |
| HIV-IMN      | 7                    | 0.1778 | -            |
| HIV-1\textsuperscript{P} | 6              | 0.0447 | +            |
| HIV-1\textsuperscript{RF} | 6              | 0.8719 | -            |
| HIV-1\textsuperscript{MA} | 5              | 0.1885 | -            |
| HIV-1\textsuperscript{PR} | 6              | 0.0209 | +            |
| HIV-1\textsuperscript{Q1} | 12             | 0.0484 | -            |
| HIV-1\textsuperscript{SP2} | 23             | 0.3974 | -            |
| MV-BIL       | 6                    | 0.0048 | +            |
| MV-BIL\textsubscript{G} | 6               | 0.0384 | -            |

* Human PBL were transplanted into heavily conditioned CBA mice at a dose of 2 x 10\textsuperscript{7}/g of recipient body weight. Two groups of mice were identified. One individual human donated PBL to one mouse of each group. Subsequently, the mice of one group were injected with 10 TCID\textsubscript{50} of virus. The other group had sham injections with culture medium. The survival of these mice was plotted against time and was subsequently analysed using the Kaplan-Meier method. Each row represents a different experiment.

† Statistical significance is P < 0.05.

‡ The P and PR strains were injected as 10\textsuperscript{5} PBL of cell-bound virus.

§ In this experiment, instead of 4-week-old CBA/N mice and human PBL, newborn SCID mice and rhesus monkey PBL were used.

¶ HIV-1\textsubscript{SP2} was dosed at 100 TCID\textsubscript{50}.

**antibody to the CD4 binding site (Fig. 3).** HIV-1\textsubscript{IIHB} infection was abrogated completely by the combination of monoclonal antibodies to the V3 loop and the CD4 binding site (Fig. 3). Infection with the primary isolate HIV-1\textsubscript{Q1} was not prevented by these monoclonal antibodies or the HIVIG.
the PBL of a different human donor, it was remarkable that the number of cells needed to induce an IC after a particular virus infection showed little variation. As it is easy to generate these chimeras, the model provides a convenient tool for the study of host-virus interaction. Interestingly, human macrophages in mice infected with the lymphotropic HIV-1320.2A.1.2 did not show staining for HIV-1 RNA, whereas they did show staining in mice infected with the macrophage-tropic strains 320.2A.2.1 and 320.31. This, in addition to the highly cytopathic lymphotropic HIV-1 strain IIIB showing 'burst'-like replication, indicates that during the initial infection macrophage-tropic HIV-1 strains play an important role (Fig. 1, Table 2). This is similar to human HIV-1 infection, as it was found that lymphotropic syncytia inducing strains are cleared soon after infection [J. Goudsmit (Academical Centre, Amsterdam), personal communication]. The fact that infection with the primary isolates resulted in a 1–2 log higher number of HIV-1 infected cells as compared to the HIV-1 strains and clones also indicates synergism of infection through polyclonality of the virus in the initial phase of infection (Figs 1 and 3; Table 2).

In contrast to all the other viruses used, it appeared that the highly cytopathogenic full-length molecular clone HIV-1spo is rapidly eliminated in vivo (Table 4). Consistently, HIV-1spo is highly sensitive to neutralization by complement and antibodies in vitro [S. Zolla-Pazner (Neuviém Colloque de Cents Gards, Marne la Coquette, France) personal communication]. The fact

Fig. 3. Passive vaccination for HIV-1 infection in xeno-GvHD mice. Heavily conditioned CBA/N mice (see Methods) received $4 \times 10^6$ human PBL/g. The mice analysed at day 5 post-infection had blood from different human blood donors. The same donors were used for the mice analysed at day 8 post-infection. Passive vaccination was with 250 µg of anti-HIV-1 CD4 binding site GP13 and/or anti-HIV-1 V3 loop F58H3 or 257-D monoclonal antibody or with 500 µg of HIVIG. The controls had 250 µg of K14, a non-neutralizing anti-HIV-1 GP41 monoclonal antibody. Subsequently, the xeno-GvHD mice were infected with 10 TCID$_{50}$ of HIV-1. The results represent the number of ascitic fluid cells from an individual mouse needed to give an IC in the in vitro read-out test. * The $2 \times 10^6$ ascitic fluid cells of the mice analysed gave no IC. The ascitic fluid cells contained > 20% human CD4$^+$ cells by flow cytometry. † The xeno-GvHD mouse produced an IC centre with $5 \times 10^5$ ascitic fluid cells.
CB15 cell line with HIV-1sF2 prior to transplantation, these CB15-SCID chimeric mice showed persistent HIV-1 infection. Interestingly, human PBL grafted SCID mice eliminated the HIV-1sF2 infection under these conditions (Table 4). This shows that human PBL grafts are able to participate in the elimination of HIV-1sF2. Together, this indicates that HIV-1sF2 may be useful for intervention strategies. In addition, the reproducible replication of the HIV-1sF2 strain in these CB15-SCID mice indicates that these chimeras are a valuable extension of the model.

In agreement with the findings using human PBL SCID mice (Mosier et al., 1988; Vittecoq et al., 1992; Safrit et al., 1993), our results with xeno-GvHD mice demonstrate the feasibility of passive immunization studies with lymphotropic HIV-1 strains (Fig. 3). However, it can be speculated that enhancement through Fc-mediated macrophage infection contributes to prolonging the ·burst'-like in vivo replication of the lymphotropic HIV-1sIIB strain by in vitro neutralizing concentrations of the HIV-1 CD4 binding site specific antibody GP13 (Fig. 3). Furthermore, neither the combination of V3 loop specific and CD4 binding site specific human monoclonal antibodies nor the HIVIG prevented infection by the primary isolate HIV-1sQ17 (Fig. 3). This is consistent with studies of patients, where HIVIG did not clear HIV either (Prince et al., 1991). However, some beneficial effect cannot be excluded, as in our murine model and in patients these antibodies appear to reduce the number of infected cells significantly (Figs 1 and 3; Prince et al., 1991). It is concluded that the study of passive immunization for post-exposure treatment should include in vivo infection with primary isolates.

With the human PBL SCID mouse, secondary antibody recall responses of recently vaccinated PBL donors were detected (Mosier et al., 1993b). However, aspecific antibody responses and naturally occurring red blood cell xeno-reactive antibodies could not be excluded (Hesselton et al., 1993; Williams et al., 1992; Nonoyama et al., 1993). In contrast, we recently found, using distantly located allogeneic skin grafts, that induction of cellular immunity in the xeno-GvHD mice was indeed a primary cellular immune response (W. Huppes & G. Hoffmann-Fezer, unpublished results). In this paper, active protection by immunization with live attenuated MV vaccine has been demonstrated. Partial protection may have been conferred by immunization with subunit MV vaccine (Fig. 4). The complete protection conferred by the live attenuated MV vaccine is not likely to be caused by natural killer cells, as these cells could not be found in the model (Huppes et al., 1994). Neither did it appear to be caused by anti-MV antibodies, as none of the sera from the mice were positive using an MV ELISA (VanBinnendijk et al., 1990, 1992, 1994; results not

### Table 4. HIV-1sF2 is eliminated in vivo

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>No. of mice</th>
<th>Human graft</th>
<th>Method of infection</th>
<th>HIV-1 strain</th>
<th>Day of sacrifice†</th>
<th>HIV test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID 1</td>
<td>20</td>
<td>CB15</td>
<td>in vivo</td>
<td>SF2</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>SCID 2</td>
<td>5</td>
<td>CB15</td>
<td>in vivo</td>
<td>Q17</td>
<td>8</td>
<td>Positive</td>
</tr>
<tr>
<td>SCID 3</td>
<td>6</td>
<td>CB15</td>
<td>in vivo</td>
<td>SF2</td>
<td>8</td>
<td>Positive</td>
</tr>
<tr>
<td>SCID 4</td>
<td>3</td>
<td>PBL</td>
<td>in vitro</td>
<td>SF2</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>SCID 5</td>
<td>3</td>
<td>PBL</td>
<td>in vitro</td>
<td>SF2</td>
<td>4</td>
<td>Negative</td>
</tr>
<tr>
<td>SCID 6</td>
<td>8</td>
<td>PBL</td>
<td>in vitro</td>
<td>SF2</td>
<td>8</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* The human CD4+ CB15 cell line (1·3 × 10⁶ cells/g) or the human PBL (4 × 10⁶ cells/g) were transplanted into heavily conditioned SCID mice. Subsequently, the mice were infected in vivo with 100 TCID₅₀ of HIV-1sF2 or with 10 TCID₅₀ of HIV-1sQ17. The in vitro infection was by co-culture of the graft with the virus for 1 h with 100 TCID₅₀ of HIV-1sF2.

† At the indicated time after cell transfer, the mice were sacrificed and 5 × 10⁶ ascitic fluid cells were cultured with the same number of CB15 cells. One week later the Gag (p24) HIV-1 ELISA test was performed.

Fig. 4. Vaccination against MV infection. Heavily conditioned CBA/N mice received 4 × 10⁶ human PBL/g and were subsequently immunized with either 5 μg of subunit vaccine (MV ISCOM) or with 100 i.u. of live virus vaccine (Schwartz; see Methods). After 8 days the ascitic fluid cells were transferred into heavily conditioned CBA/N mice at a dose of 4 × 10⁶ cells/g. Subsequently, these mice were challenged with 1000 TCID₅₀ of MV-BIL. Three days later an IC test was done to determine the number of ascitic fluid cells needed to give an IC in the in vitro read-out test. * The 2 × 10⁶ ascitic fluid cells analysed gave no IC. The ascitic fluid cells contained > 20% human CD4+ cells by flow cytometry.
published). Protection by plasma factors like gamma-interferon, is not likely either, because empty ISCOMS did not significantly protect against MV infection. As the protection was obtained by transfer of a cell fraction and within 2 weeks of the transplantation procedure (Fig. 4), it is ascribed to T cell immune mechanisms.

In contrast to earlier SCID mouse models (Mosier et al., 1991; McCune et al., 1991), the technologically simple and economic xeno-GvHD murine model allowed replication of HIV-1 and MV. Modification of the model to one without any immunological capability (CB15-SCID mice) allowed virus replication and immunological interaction to be differentiated. The model also enabled the study of cytopathological effects of virus on T cells and macrophages of the human immune system. In addition, these xeno-GvHD chimeras enabled both passive and active immunization studies.

The authors acknowledge G. H. Schuitemaker (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) for support, H. M. J. P. M. Brok (Health Research-TNO, Rijswijk, The Netherlands) for biotechnical assistance and the Ministry of Public Health Care, The Hague, for financial support.

References


SAFBIT, J. T., FENG, M. S., ANDREWS, C. A., BRAUN, D. G., SUN, W. N., CHANG, T. W. & KOP, R. A. (1993). Hu-PBL-SCID mice can be protected from HIV-1 infection by passive transfer of mono-
clonal antibody to the principal neutralizing determinant of envelope gp120. *AIDS* 7, 15–21.


VANBENNDIJK, R. S., POELEN, M. C. M., KUIJPERS, K. C., OSTERHAUS, A. D. M. E. & UYTDEHAAG, F. G. C. M. (1990). The predominance of CD8+ T cells after infection with measles virus suggests a role for CD8+ class I MHC-restricted cytotoxic T lymphocytes (CTL) in recovery from measles. Clonal analyses of human CD8+ class I MHC-restricted CTL. *Journal of Immunology* 144, 2394–2399.


(Received 6 December 1994; Accepted 20 June 1995)