Virus load in chimpanzees infected with human immunodeficiency virus type 1: effect of pre-exposure vaccination

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Many reports indicate that a long-term asymptomatic state following human immunodeficiency virus type 1 (HIV-1) infection is associated with a low amount of circulating virus. To evaluate the possible effect of stabilizing a low virus load by non-sterilizing pre-exposure vaccination, a quantitative virus isolation method was developed and evaluated in four chronically infected chimpanzees infected with a variety of HIV-1 related isolates. This assay was then used to monitor a group of chimpanzees (n = 6) challenged with HIV-1 following vaccination with gp120 or gp160. Data indicated that of the three vaccinated animals which became infected after challenge, the animal with the lowest neutralizing titre at the time of challenge acquired a virus load similar to the control animals, whereas the two other chimpanzees had reduced numbers of virus producing cells in their peripheral circulation. One animal became virus isolation negative, developed an indeterminant PCR signal on lymph node DNA and subsequently became negative for HIV-1 DNA as determined by PCR on PBMC (peripheral blood mononuclear cells) and bone marrow DNA. Recently, the second animal has also become PCR negative. To confirm observations from quantitative virus isolations, quantification of HIV-1 DNA in PBMC and virus RNA in serum was performed by PCR on serially diluted samples at two different time points. Comparison of virus load as determined by these three methods confirmed that there was an effect of vaccination in reducing virus load and demonstrated a correlation between decreased numbers of virus producing cells, HIV-1 DNA containing cells and virus RNA molecules in serum.

Currently, true human immunodeficiency virus type 1 (HIV-1) vaccine efficacy studies are feasible only in the HIV-1 chimpanzee model. The humoral response to HIV-1 in chimpanzees mimics the human situation (Nara et al., 1987; Goudsmit et al., 1987). HIV-1 infection in chimpanzees is of particular relevance since wild caught chimpanzees have been identified which are naturally infected with lentiviruses closely related to HIV-1 (Peeters et al., 1989, 1992). The close immunological similarities between chimpanzees and humans make this model the most appropriate model for HIV-1 vaccine studies. Several chimpanzee trials have been reported (Berman et al., 1988, 1990; Girard et al., 1991; Fultz et al., 1992; Arthur et al., 1989); in these trials whole inactivated virus, purified (recombinant) viral proteins or synthetic peptides have been used for vaccination, followed by homologous intravenous challenge. Recently, we reported on an envelope subunit vaccine study in chimpanzees in which antibody titres on the day of challenge correlated with protection from HIV-1 infection (Bruck et al., 1994).

The aim of this study was to assess the effect of vaccination on virus load, specifically with regard to enumerating the number of virus infected cells, virus producing cells and virions in serum. To monitor virus load in peripheral blood it is crucial to have both sensitive and accurate methods of measurement. Towards this goal, we developed a quantitative virus isolation assay based on propagating chimpanzee peripheral blood mononuclear cells (PBMC) depleted of CD8+ cells in a primary T cell culture system. This method was evaluated on samples of a small cohort of chronically infected chimpanzees (n = 4) that was monitored annually over a 3 year period. To further assess the value of this method for determining virus load in the period directly after HIV-1 infection, longitudinal
samples from two experimentally infected chimpanzees were also analysed for the number of virus producing cells present during the post-infection period.

To corroborate the data and to estimate the relative accuracy of the quantitative virus isolation as well as to evaluate its applicability in vaccine efficacy trials, a quantitative DNA PCR assay and a quantitative RNA PCR assay were developed and used retrospectively to estimate virus load in an HIV-1 vaccine efficacy study in chimpanzees. As practical vaccines are not always capable of inducing sterilizing immunity, we set out to determine if non-sterilizing vaccination would be able to reduce virus load in immunized animals which became infected after challenge and to determine the relationship between virus infected and producing cells as well as virions.

To estimate the number of virus producing cells in circulation by quantitative virus isolation, cryopreserved chimpanzee PBMC were obtained from HIV-1 infected chimpanzees and seronegative controls. Two of the animals, ch-Bu and ch-Ma were infected with HIV-1 (data not shown); ch-Co was infected in 1982 with blood from an AIDS patient and ch-No was naturally infected with the HIV-1-related chimpanzee immunodeficiency virus, CIV-ANT. The PBMC were depleted for CD8+ cells using monoclonal antibody and magnetic beads, in order to enrich for CD4+ cells and to remove possible suppressive effects of CD8+ cells. A total of 1 x 10⁴ depleted PBMC were seeded per well in 96-well plates. The cells were grown in a feeder system consisting of irradiated (25 Gray) human PBMC (2 x 10⁴/well), APD and BSM (two human B cell lines, 5 x 10⁹/well each) and 1 µg/ml leucoagglutinin and 1 µg/ml indomethacin. Primary chimpanzee cells were grown for 15 days and then transferred to fresh feederplates. After another 15 days, the supernatant of sets of four wells was pooled and tested for the presence of HIV-1 using a highly sensitive commercial p24-antigen capture assay (Coulter). The number of virus producing cells per pool was calculated by following the formula (Levkovits et al., 1984) 
n = − ln F₀ (Poisson distribution), where F₀ is the number of negative pools divided by the total number of pools tested. The resulting titre was corrected for the CD8 depletion using the percentage of CD8+ cells as estimated by fluorescence activated cell scan (FACS). The detection level was established at between 1 and 800 positive cells/10⁶ depleted PBMC.

The quantitative RNA PCR method was performed as follows using the primers shown in Table 1. RNA was purified from 50 µl of serum as described elsewhere (Boom et al., 1990); MS2 RNA (2 x 10⁷ molecules) was added to the lysis buffer to serve as a carrier for low amounts of HIV-1 RNA and as a control for the efficiency of the RNA isolation. The amount of MS2 RNA was chosen empirically so that only with an efficient RNA isolation without degradation a positive MS2 PCR signal could be obtained. In all cases where the HIV-1 PCR was negative, the MS2 PCR was positive, indicating that the HIV-1 PCR result was not a false negative due to loss of RNA. The RNA was converted to cDNA using avian myeloblastosis virus reverse transcriptase (AMV RT). The RT reaction mix contained 75 mM-KCl, 50 mM-Tris-HCl (pH 8.3), 3 mM-MgCl₂, 10 mM-DTT, 1 mM-dNTPs, 20 units RNase inhibitor, 100 ng of 3’ primer (SK 39 or MS-2), 5 U AMV-RT and 10 µl of RNA. For quantification (described in detail by Brinchmann et al., 1991), a dilution series was prepared by twofold dilutions. A nested gag PCR was performed using SK 39 and SK 145 as outer primers and SK 431 and SK 102 as inner primers. The reaction mixture contained 20 mM-Tris-HCl (pH 8.3), 50 mM-KCl, 1 mg/ml BSA, 2.5 mM-MgCl₂, 100 ng of each primer, 200 µM of each dNTP and 2 units of Taq polymerase. After an initial denaturation of 3 min at 95 °C, 35 cycles of 1 min at 95 °C, 1 min at 55 °C and 2 min at 72 °C were applied in the outer PCR, followed by an 8 min final extension at 72 °C. The same programme using 25 cycles was applied in the inner PCR. All quantifications were performed in duplicate. The detection level of the RNA PCR procedure was determined by performing PCR on 12 replicates of a twofold dilution series from a known quantity of gag RNA, transcribed in vitro from plasmid DNA. The detection level was established at 20 RNA molecules (data not shown). This procedure was validated by comparing the results obtained with a virus preparation.

### Table 1. RT and PCR primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'–3')</th>
<th>Location*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' MS2</td>
<td>CCC CTC TCC GTA TCC ACX GGC GGC GTT AAG</td>
<td>297–326a</td>
</tr>
<tr>
<td>3' MS2</td>
<td>GGT CCA CGG CGG GTT CGC AAC GTT CTG CGG</td>
<td>478–507b</td>
</tr>
<tr>
<td>5' SK145</td>
<td>AGT GGG GGG ACA TCA AGC AGC CAT GCA AAT</td>
<td>1359–1387b</td>
</tr>
<tr>
<td>3' SK39</td>
<td>TTT GGT CCT TGT CTT ATG TCC AGA ATG C</td>
<td>1630–1657b</td>
</tr>
<tr>
<td>5' SK102</td>
<td>GAG ACC ATC AAT GAG GAA GCT GCA GAA TGG GAT</td>
<td>1395–1427b</td>
</tr>
<tr>
<td>3' SK431</td>
<td>TGC TAT GTC AGT TCC CCT TGG TTC TCT</td>
<td>1473–1499b</td>
</tr>
</tbody>
</table>

* a, Location on bacteriophage MS2 RNA; b, location on HIV-1 isolate HXB2.
to the results obtained from direct quantification with an
electron microscope particle count (Layne et al., 1992).
The average direct particle count was 3.7 × 10^7/ml.
Assuming two RNA molecules per virion, this is
equivalent to 7.4 × 10^10 molecules/ml. Our PCR pro-
cedure gave a count of 9.6 × 10^10 molecules/ml. This
difference is within the limits of experimental variation.

To estimate the concentration of virus containing
PBMC by quantitative DNA PCR, DNA was extracted
from 5 × 10^6 PBMC in duplicate for each time point.
DNA was isolated as previously described (Boom et al.,
1990), with two minor modifications: a 15 min incu-
bation with NP40 and RNase A at 56 °C prior to
nuclear lysis to prevent the co-purification of RNA, and
an extra wash with 100 % ethanol to result in a cleaner
DNA isolate. The DNA concentration was measured
spectrophotometrically and standardized at 50 ng/ml. A
serial twofold dilution series was prepared in duplicate in
50 ng/ml PBMC-DNA from a non-HIV/HTLV infected
chimpanzee. A 1 µg amount of DNA was used as
template in each PCR reaction. PCR and quantification
conditions were the same as in the quantitative RNA
PCR. All quantifications were performed in duplicate.
The detection level of the DNA PCR procedure was
determined by performing PCR on a twofold dilution
series from a plasmid containing HIV-1
gag
expression is active in the lymphoid tissue (Pantaleo
et al., 1993). This phenomenon was also
noticed with the infected controls ch-La and ch-Th (Fig.
1). In humans, during the period of clinical latency there
are low numbers of infected cells in the blood; however,
expression is active in the lymphoid tissue (Pantaleo
et al., 1991, 1993). Estimates of the frequency of cells
capable of producing virus in human peripheral blood
range from 20 to 26/10^6 PBMC in asymptomatic humans
up to a range of 2.5 × 10^5 to 3.3 × 10^6/10^6 PBMC in
AIDS patients (Ho et al., 1989; Lu et al., 1992). Our
findings indicate that chimpanzees have approximately
20 times fewer infected PBMC capable of producing
HIV-1 than asymptomatic humans, and approximately
2000 times fewer than human AIDS patients. The ability
of chronic infected chimpanzees to suppress virus
replication and to maintain a low virus load may be
related to their relative resistance to AIDS (Heeney
et al., 1993).

In the early post-infection period (between 6 and 12
weeks), the number of virus producing cells in circulation
in chimpanzees reaches a peak between 30 to 50 virus
producing cells/10^6 PBMC (Fig. 1), similar to levels
reported in asymptomatic humans. After 12 weeks the
peak drops to below five virus producing cells/10^6
PBMC. Due to the limited sensitivity of this assay alone,
we utilized quantitative DNA PCR and RNA PCR
techniques to support our assessment of virus load by
quantitative virus isolation and to retrospectively analyse
a chimpanzee vaccine efficacy trial (Bruck et al., 1994).

Table 2. Virus load of chronically infected chimpanzees
as determined by quantitative virus isolation

<table>
<thead>
<tr>
<th>Chimpanzee*</th>
<th>Date...</th>
<th>06/90</th>
<th>08/91</th>
<th>07/92</th>
<th>06/93</th>
</tr>
</thead>
<tbody>
<tr>
<td>ch-Bu</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 4</td>
<td></td>
</tr>
<tr>
<td>ch-Co</td>
<td>&lt; 4</td>
<td>10</td>
<td>18</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ch-Ma</td>
<td>4</td>
<td>&lt; 6</td>
<td>14</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ch-No</td>
<td>Not</td>
<td>68</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

* Year of infection: ch-Co, 1982; ch-Bu, 1984; ch-Ma, 1984; ch-No, unknown (natural infection).

Fig. 1. Early post-challenge virus load of vaccinated and naïve chimpanzees determined by quantitative virus isolation. The peak load of the infected vaccinated animals ranges from 3 to 38 HIV-1 producing cells/10^6 PBMC; the mean was 18.7 with a standard deviation of 14.5. The peak virus load of the control animals ranged from 33 to 54 HIV-1 producing cells/10^6 PBMC; the mean was 43.5 with a standard deviation of 10.5.
Table 3. Comparison of the numbers of virus producing cells, provirus containing cells and cell-free virus particles in sera of naive and vaccinated chimpanzees

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Animal</th>
<th>Time post-challenge (weeks)...</th>
<th>HIV-1 (virus neutralization titre)</th>
<th>Q-VI (producing PBMC/10⁶ PBMC)</th>
<th>Q-DNA-PCR (infected PBMC/10⁶ PBMC)</th>
<th>Q-RNA-PCR (×10⁴ HIV-1 RNA molecules/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive controls gpD</td>
<td>ch-La</td>
<td>&lt; 1/50</td>
<td>10</td>
<td>100</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Vaccine HIV-1 gp120</td>
<td>ch-Ze</td>
<td>1/400</td>
<td>3</td>
<td>24</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Vaccine HIV-1 gp160</td>
<td>ch-Os</td>
<td>1/1200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Summary of diagnostic DNA PCR data post-HIV-1 exposure from naive and vaccinated chimpanzees

<table>
<thead>
<tr>
<th>Immune</th>
<th>Animal</th>
<th>Virus neutralization titre at time 0</th>
<th>Time post-challenge (weeks)...</th>
<th>DNA PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive controls gpD</td>
<td>ch-La</td>
<td>&lt; 1/50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vaccine HIV-1 gp120</td>
<td>ch-Ze</td>
<td>1/400</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vaccine HIV-1 gp160</td>
<td>ch-Os</td>
<td>1/1200</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The quantitative DNA PCR on the vaccine group (Table 3) revealed that at 6 weeks post-challenge, the first week that vaccinated animals became PCR positive, the number of HIV-1 DNA containing cells was approximately ten times higher than the number of virus producing cells (Fig. 1). As was found in the virus isolation assay, in the majority of cases the number of HIV-1 DNA containing cells in circulation was found to be highest at 6 weeks, and lower at 24 weeks, suggesting a clearance of infected cells from circulation after sufficient development of the host immune response. The vaccinated animal (ch-Ze) which had the highest antibody titre of the animals which became infected, had the lowest number of HIV-1 DNA containing cells in circulation at both time points. This animal became virus isolation negative at week 16 and PCR negative at week 32, with transient positive PCR signals at later time points until week 72 when both PBMC and bone marrow samples were negative (Table 4), also suggesting clearance of infected cells from circulation. At week 24, all vaccinated chimpanzees had fewer HIV-1 DNA containing cells in circulation than unvaccinated controls. This was in contrast to the virus isolation results which showed exceptions to this trend. The number of HIV-1 DNA containing cells/10⁶ PBMC ranged from 20 to 120 in vaccinated chimpanzees versus 100 to 400 in naive chimpanzees 6 to 12 weeks post-infection as animals seroconverted. This can be compared to estimates of virus load in humans reported at the time of seroconversion when the number of HIV-1 DNA containing cells/10⁶ PBMC ranged between 600 and 1000 (Jurriaans et al., 1992), between eight and thirty times greater than in chimpanzees.

The number of virus RNA molecules in serum as determined by quantitative RNA PCR (Table 3) was estimated to range from less than 10⁴ up to 20 × 10⁴/ml. These low virus titres are in the same range as found in asymptomatic humans (Piatak et al., 1993). In all except one animal which had similar values, the number of virus particles/ml serum declined from the higher levels at week 6 post-challenge to lower levels at week 24 post-challenge. This observation confirmed the results from both quantitative virus isolation and quantitative DNA PCR. Furthermore, the observation that ch-Os was protected post-exposure was confirmed by this assay. The trend that the virus load in the peripheral circulation
decreased from levels detected at 6 weeks to lower levels after seroconversion at 24 weeks was also confirmed by assessing the number of viral RNA molecules/ml. It is of interest to note that one animal, who had fewer viral RNA molecules/ml at 24 weeks than could be detected by this assay, was the same animal that became virus isolation negative, and had the lowest copy number of HIV-1 DNA containing cells detectable in PBMC on this occasion. Furthermore, this animal (ch-Ze) was virus isolation negative from week 32 and was at the level of detection with PCR on DNA from lymph nodes and negative with PCR on DNA from bone marrow biopsies at weeks 48 and 72 post-challenge (Table 4). Importantly, this vaccinated animal was the animal which had the highest virus neutralizing titre (1/400) of those that became infected following challenge. Similarly, ch-Er, which had the second highest virus neutralizing titre after vaccination but which also became infected, showed a large decline in the number of HIV-1 producing cells/10⁶ PBMC as well. At the last time point measured for ch-Er, 96 weeks post-challenge, PCR on PBMC DNA was negative for HIV-1 (Table 4).

Several groups (Connor et al., 1993; Lu et al., 1993; Sheppard et al., 1993; F. J. M. Hogervorst, personal communication) have shown that long-term HIV-1 infected, asymptomatic individuals have low virus loads. These observations suggest that a stable low virus load during the asymptomatic phase of HIV infection may be related to the delay of the onset of AIDS. Our results strongly suggest that HIV-1 vaccination may not only limit initial virus infection, but may also lower the amount of free circulating virus after the early infection period. These findings have been recently supported by a somewhat similar observation in the rhesus/simian immunodeficiency virus model (Israel et al., 1994). Unfortunately, the contribution of HIV-1 vaccination in preventing the development of AIDS can only be truly evaluated in humans. These results suggest the importance of studies to evaluate the prevention of disease progression in addition to prevention of infection in future trials of candidate HIV-1 vaccines.

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References


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