DNA Vaccination as Anti–Human Immunodeficiency Virus Immunotherapy in Infected Chimpanzees


The role of the immune response in controlling human immunodeficiency virus type 1 (HIV-1) replication is controversial. Immunotherapeutic strategies that have the ability to broaden immune responses might play a role in slowing disease progression. DNA immunization was studied as immunotherapy in infected chimpanzees. Two HIV-1–infected chimpanzees were vaccinated with DNA plasmid vaccines, one with plasmid pCMN160, which expresses the envelope glycoprotein of HIV-1menv and rev, and the other with a control plasmid. The chimpanzee immunized with pCMN160 demonstrated enhanced humoral responses. Virus load was monitored. Virus load in the chimpanzee receiving pCMN160 decreased at week 20 and has remained at background levels. The control chimpanzee was subsequently vaccinated with pCMN160. After immunization, the antibody responses increased and, as in the first animal, the virus load decreased. These results indicate the potential of the immune response to have a direct impact on HIV-1 replication in chimpanzees.

The immune responses that the majority of human immunodeficiency virus (HIV)-1–infected persons develop following infection do not in general prevent the eventual onset of AIDS. However, a more potent and broader immune response has been associated with presumed delay of progression to disease [1]. Significant cytotoxic T lymphocyte (CTL) activity [2, 3], as well as high concentrations of antibodies, most notably neutralizing and cross-reactive neutralizing antibodies against HIV-1, have been observed in populations of long-term survivors [2]. In HIV-infected pregnant mothers, stronger immune responses have been correlated with lower virus loads and a decreased rate of transmission of HIV to their infants [4, 5]. However, the suppression of virus load during infection has previously been associated solely with the use of antiretroviral agents, and efforts to demonstrate similar effects through immunomodulation remain subject to controversy. Recent efforts have demonstrated the safety and immunogenicity of several recombinant protein products, including recombinant gp160 [6, 7], strain LAI gp120 [8], and strain MN gp120 [9], as well as recombinant vaccinia [10], in immunotherapy as well as vaccination protocols. In addition, some evidence suggests that vaccination with preparations of recombinant proteins can expand anti–HIV-1 gp120 antibody and perhaps cellular responses in infected persons [11–15]. However, there is little evidence that such vaccination strategies can have an impact on viral replication and result in decreases in virus load.

In these studies, we analyze the use of nucleic acid (DNA)–based vaccines as an immunotherapeutic regimen in HIV-1–infected chimpanzees. DNA vaccination (also known as genetic immunization and nucleic acid immunization) is dependent on the injection of a DNA plasmid containing a specific nucleic acid sequence directly into a host target tissue (e.g., muscle, skin). The synthesis of specific foreign proteins from these constructs occurs in the host and results in customized antigen production, with host-biased glycosylation patterns and natural processing and immune presentation. In vivo production of such gene-delivered proteins is conceptually similar to that occurring during either wild type or attenuated viral infection. These host-synthesized viral proteins are then subject to natural immune surveillance by the exogenous and endogenous antigen pathways, resulting in both major histocompatibility complex (MHC) class I and class II responses. Our laboratory and others [16–23] have previously demonstrated that DNA inoculation technology can be used to develop antigen-specific immune response against HIV-1 in mice and primates.

Chimpanzees (Pan troglodytes) provide a useful model for evaluation of the role of immune responses for controlling HIV-1 replication in vivo. Importantly, the chimpanzee and
Materials and Methods

Antibody binding analysis. Sera from chimpanzees 116 and 118 were diluted and assayed for the ability to bind to gp120 protein as well as 70 overlapping peptides spanning the HIV-1envelope, gp160. ELISA-based analysis was done according to modifications as previously described [25, 26]. In addition, serologic binding analysis was done against a gp41 peptide spanning aa 601–620 at the following dilutions: 1:100, 1:500, and 1:2500. ELISA-based analysis was done according to modifications as previously described [25, 26]. In addition, serologic binding analysis was done against a gp41 peptide spanning aa 601–620 at the following dilutions: 1:100, 1:500, and 1:2500. The recombinant gp120 from MN was purchased from Immunodiagnostic (Bedford, MA), and the peptides were obtained from the AIDS Research and Reference Reagent Program (Rockville, MD).

Viral coculture. Virus load was assessed by a viral coculture technique. PBMC were suspended in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (R10) to a concentration of 10⁶ cells/mL. Five milliliters of the cell suspension was placed in T-25 tissue culture flasks with phytohemagglutinin-P at a concentration of 2.5 µg/mL. After a 24-h incubation in a 37°C, 5% CO₂ humidified incubator, interleukin-2 was added at a final concentration of 1000 U/mL. The cultures were incubated for an additional 3 days, at which point the cells were reseeded at a concentration of 10⁶/mL. An equal quantity of naive PBMC and an additional 1000 U/mL interleukin-2 were aliquoted to each flask. Cultures were returned to the incubator for an additional 7 days. Supernatant from tissue culture flasks was collected by centrifugation at 1600 rpm (Sorvall RT6000B; DuPont, Wilmington, DE) for 10 min. The supernatant was precipitated with polyethylene glycol (molecular weight, 8000). The precipitate was pelleted by centrifugation for 30 min at 2800 rpm. The pellet was then resuspended in 100 µL of virus-solubilizing buffer containing 0.8 M NaCl, 20% glycerol, 0.05 M TRIS (pH 7.8), 0.5% Triton-X100, 1 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride. Twenty micro liters of the virus suspension was added to 75 µL of the reaction mixture containing 0.06 M TRIS, 1 mM DTT, 0.1 mM ATP, 2.25 U adenosine ribonucleotide deoxyribosylhydridine, 12.5 mM MgCl₂, and 110 µCi of [³H]TTP. The mixture was incubated for 1.5 h at 37°C. To stop the reaction, 10 µL of trichloroacetic acid and three washes with 5% trichloroacetic acid through glass fiber filters. The dried filters were placed in scintillation vials and ³H incorporation was counted on a liquid scintillation counter.

Reverse transcriptase–polymerase chain reaction (RT-PCR). Virus load was also assessed by isolating RNA from plasma samples and using an RT-PCR technique as previously described [27].

Branched-chain DNA. The quantiplex HIV RNA 2.0 assay kit (Chiron, Emeryville, CA) was used to assess virus load.

Cell cytometric analysis. Cells were isolated by ficoll-hypaque centrifugation and washed two times in buffer (1% bovine serum albumin and 0.1% NaN₃ in PBS). The cells were incubated at a concentration of 3 x 10⁶ cells with anti-CD28 conjugated antibody (Pharmingen, San Diego) for 1 h at 4°C. The cells were washed two times in buffer and fixed in 0.5 mL of 2% paraformaldehyde. The cells were analyzed on a flow cytometer (EPICS XL; Coulter, Hialeah, FL).

Results

Serum binding reactivity to gp120 and envelope glycoprotein peptides. Two chimpanzees that had been infected with HIV-1 for >4 years were inoculated in the quadriceps muscle on day 0, week 6, and week 12 with 100 µg of the DNA constructs. One chimpanzee (no. 118) received the construct designated pCMN160, which drives expression of the HIV-1 envelope protein from the MN isolate as well as the HIV-1rev gene product. The other chimpanzee (no. 116) was inoculated with the control vector, which did not contain the specific neutralizing antibodies and possibly CTL. In addition, serum binding reactivity to gp120 (figure 1A) as well as to several regions in gp41 spanning aa 551–570 and 571–590 and region 781–800 (figure 2A–C). In addition, enhanced humoral immune responses were noted, after specific DNA plasmid immunization, to a linear gp120 V3 MN peptide (aa 301–320; figure 2D). Figure 2 shows the percentage change in optical density values at 450 nm after immunization with the pCMN160 or control construct. Examination of binding after immunization with the control plasmid failed to demonstrate any nonspecific enhancement of binding to any of the recombinant proteins or peptides. The V3MN peptide represents part of the principal neutralizing determinant of gp120 [28, 29]. The peptide spanning aa 781–800 is part of the cytoplasmic domain of gp41 and has not conventionally been thought to be exposed at the cell surface [30]. However, HIV-1–infected patients have been reported to develop neutralizing antibodies that bind to this region of the envelope [31], and recently we have shown that high binding of maternal serum antibodies to this epitope occurs in women who do not transmit infection to their babies [26].

In binding studies to gp120 as well as the other peptides (except the peptide spanning aa 551–570), a persistent enhancement of binding was noted compared with prevaccination antibody values. This enhancement persisted through day 168 of the study. There was also an indication of a boosting effect after additional immunizations. The enhancement of binding...
Figure 1. Enhancement of binding of serum antibodies to envelope glycoprotein gp120MN in HIV-1–infected chimpanzees vaccinated with (A) pCMN160 construct or (B) control plasmid. Standard ELISA with recombinant gp120 was used.
Figure 2. % increase in binding (optical density at 450 nm [OD450]) after vaccination of HIV-1–infected chimpanzees with DNA constructs. Peptides spanned aa 551–570 (QQQNLLLRAIEAQHQMLQLT; A), 571–590 (WVGKQLQARVL-AVERYLKD; B), 781–800 (IVE-LLLGRGWEVLKYYWNNLL; C), 301–320 (CTRPNYNKRKRIHG-PGRAF; D). Solid bars, values for chimpanzee 116 (vaccinated with control plasmid); hatched bars, values for chimpanzee 118 (vaccinated with experimental pCMN160 plasmid). SDs for optical density readings were within 10%.

to the peptide spanning aa 551–570 demonstrates an effect on binding after the primary immunization (binding increase up to 80%–100%). This is followed by a fall in percentage of enhancement, with subsequent increases occurring after the two remaining booster immunizations. The relevance of such boosting requires further investigation.

Figure 3 demonstrates binding of serum samples to a peptide from gp41 (aa 601–620). The data indicate that a significant enhancement of binding of serum antibodies occurs after immunization with the pCMN160 plasmid (figure 3A) but not after immunization with the control plasmid (figure 3B). Reactivity to this peptide is of interest, since it contains an epitope (GCSGKLIC) to which a human monoclonal antibody possessing neutralizing activity against several divergent HIV-1 isolates has recently been described [32].

RT-PCR and viral coculture analysis. Virus load in these studies was monitored by viral coculture (table 1), by RT-PCR (figure 4), and by DNA PCR (data not shown). Plasma from chimpanzee 118 demonstrated a decrease in virus load to below the detectable limits of all three assays by week 21 after vaccination. The chimpanzee has subsequently been followed long-term by RT-PCR (sensitivity of the RT-PCR assay, 50 copies/mL of plasma), and the suppression of virus load has been persistent, remaining below detectable limits for >1 year after immunization (figure 4). In contrast, control vaccinated chimpanzee 116 exhibited continued plasma viremia in all three assay systems during the analysis period. Some variability in RT levels was noted in this control chimpanzee, but this variability appears unrelated to vaccination. Furthermore, banked plasma samples were assessed for preimmune virus load by RT-PCR, and both animals 116 and 118 were positive. We next evaluated the variability of infection in a retrospective fashion in 10 animals infected with HIV-1 for a period from 4 to >8 years. During the course of this study, all displayed continued plasma viremia by RT as well as DNA PCR (table 2). This finding suggests that plasma viremia is persistent in HIV-1–infected chimpanzees and further supports that
the decrease in viremia in chimpanzee 118 was related to vaccination with the pCMN160 plasmid.

Cell cytometric analysis of CD28 expression in PBMC of vaccinated chimpanzees. Chimpanzees express immunologically important costimulatory surface antigens that are cross-reactive with human monoclonal reagents. Changes in T cell functions have been described as a consequence of HIV-1 infection [33, 34]. In particular, down-regulation of CD28 on CD8 T cells has been associated with progressive infection [35]. These studies suggest that CD28 cell decline inversely correlates with infection. In contrast, there is a CD4 subset decline in some chimpanzees following HIV-1 infection. However, when we examined the CD28 subset of infected and noninfected animals, we observed that the CD28 subset declines following HIV-1 infection. We examined the effects of DNA immunization on the CD28 subset in this study. Cell cytometric analysis of PBMC isolated from these animals revealed a CD28 cell profile in HIV-1–positive chimpanzee 116 similar to that reported in humans infected with HIV-1: significantly lower levels of CD28 expression than in uninfected animals. In contrast, the CD28 expression pattern of chimpanzee 118 following vaccination was analogous to patterns of persons seronegative for HIV-1 [36] (table 3). These data demonstrate the effects of this type of immune stimulus (DNA plasmid vaccination) on this important T cell population and further support the results observed in the virus load studies.

Table 1. Virus load studies on HIV-1–positive chimpanzees by HIV-1 coculture.

<table>
<thead>
<tr>
<th>Study week</th>
<th>Chimpanzee 116</th>
<th>Chimpanzee 118</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>583</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>235</td>
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<td></td>
<td>17</td>
<td>12,858</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1105</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>11,419</td>
</tr>
</tbody>
</table>

NOTE. Data are cpm.

Figure 4. Reverse transcriptase–polymerase chain reaction (RT-PCR) for detection of HIV-1 from peripheral blood mononuclear cells. Lane 1 = negative control; lane 2 = reagent control; lanes 3 and 9 = PhiX-174 standards; lanes 5–8 = chimpanzee 116 at weeks 56, 64, 68, and 72; lane 10 = blank; lanes 11–15 = chimpanzee 118 at weeks 56, 64, 68, and 72. In addition, plasma has been analyzed past 82 weeks, and chimpanzee 116 remains positive and 118 remains negative by RT-PCR.
Table 2. Historical chimpanzee virus load status.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Years infected</th>
<th>Viral DNA coculture</th>
<th>DNA PCR</th>
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<tbody>
<tr>
<td>86-a</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-b</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-c</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-d</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-e</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-f</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-g</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-h</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>86-i</td>
<td>8</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>88-a</td>
<td>5</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Negative control</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

NOTE. PCR, polymerase chain reaction. Bands measured on a gel can vary in brightness from – to +++.

In an effort to further explore the relationship between DNA vaccination and virus load in this model system, we next vaccinated chimpanzee 116 according to the same protocol originally investigated for chimpanzee 118 with the pCMN160 plasmid. Immunization with pCMN160 was demonstrated to augment the antibody response to gp120 (figure 5). The boost to the antibody responses was seen as early as 2 weeks after immunization. Virus load was monitored by RT-PCR as well as by the newer quantitative technique of branched-chain DNA. Over the course of 1 year this animal has remained positive by RT-PCR; however, the virus load as measured by branched-chain DNA decreased from ~50,000 copies/mL to <5000 copies/mL, a 90% decrease (figure 6). While the previous animals seemed to have decreases in virus load within 21 weeks, this animal’s virus load cleared over the course of 1 year. In addition, the level of CD28 expression as described above (table 3) increased as virus load decreased.

Clinical parameters of vaccinated chimpanzees. The clinical health of the animals following DNA vaccination was assessed throughout the experiment by monitoring hematologic and serum chemistry values and by repeated physical examinations. The standard hematologic and serum chemistry parameters monitored during the study period remained within normal limits. Other clinical monitoring also appeared within normal limits. No significant changes were noted in muscle-specific enzyme release into blood. There was no increase in anti-DNA or in anti-nuclear antibodies in either animal during the course of the studies. CD4 and CD8 lymphocyte counts remained constant throughout the study in both HIV-1–infected chimpanzees. The immunization procedure appeared well-tolerated.

Discussion

We have investigated the ability of immunization with DNA plasmid expressing particular HIV-1 genes to boost the immune response.
responses in the context of HIV infection in a chimpanzee model, and we analyzed the effect of this procedure on virus load. The suppression of virus load has previously been associated solely with the use of antiretroviral agents [37], with attempts to demonstrate similar effects through immunomodulation being uncertain. While there appeared to be boosting to humoral responses, analysis of the cellular responses has not demonstrated significant changes from baseline levels (data not shown). The effects of this immunization procedure on modulation of cellular immune responses in this animal model are under further examination. Interestingly, in concert with the suppression of virus load, the return of CD28 expression on the lymphocytes was observed, which illustrates the effects of this immunization regimen on the immune status. However, understanding the contribution that cellular and humoral responses make on affecting viral replication in vivo will need additional investigation.

All animals appeared to be in good health throughout the studies, as assessed by body weights and clinical observations. Abnormal clinical observations were consistent with those noted sporadically in captive nonhuman primates. These included soft feces, focal alopecia, abrasions or scabs, and bruising at the sites of blood collection. We have previously reported a lack of histopathology observed in the muscle at the site of DNA injection [23]; in the present study there was a lack of redness or tenderness observed in any of the animals at the site of injection throughout the studies. In addition, CD4 and CD8 lymphocyte counts remained constant throughout the study in both HIV-1-infected animals. These measurements may be particularly important for the HIV-1-infected chimpanzees, since several in vitro studies have supported the hypothesis that activated CD4 lymphocytes are more likely to become infected. Consequently, it has been postulated that CD4 lymphocyte activation in vivo may increase virus load, leading ultimately to CD4 lymphocyte depletion and progression to AIDS. This was not observed in the present study and supports the lack of acute toxicity associated with this novel vaccination approach as well as a clear lack of increase of virus load following vaccination.

These results are not necessarily directly transferable to the human infection situation. Accordingly, vaccination in this

Table 3. % of peripheral blood mononuclear cells expressing CD28 on cell surface.

<table>
<thead>
<tr>
<th>Week</th>
<th>Chimpanzee 116</th>
<th>Chimpanzee 118</th>
<th>Controls*</th>
<th>HIV-positive</th>
<th>HIV-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>35.7</td>
<td>65.5</td>
<td>HIV-positive</td>
<td>26.6</td>
<td>61.0</td>
</tr>
<tr>
<td>128</td>
<td>26.6</td>
<td>53.6</td>
<td>HIV-positive</td>
<td>31.1</td>
<td>53.0</td>
</tr>
<tr>
<td>51</td>
<td>12.1</td>
<td></td>
<td>HIV-negative</td>
<td>44.2</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td></td>
<td></td>
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</table>

NOTE. Chimpanzee 118 was vaccinated with plasmid expressing HIV-1 env and rev (pCMN160), while chimpanzee 116 received control plasmid (data for postinoculation weeks 104 and 128). Later, chimpanzee 116 was vaccinated with pCMN160 (data for post-pCMN160 weeks 51 and 52). * Represent 4 different animals.
model is likely to mimic the situation early in the course of HIV-1 infection in some humans, when CD4 cell counts are high and there is considerable control of virus replication. In this light, the immunization procedure described is possibly similar to immunization in the context of controlled viral infection, such as in the presence of combination drug therapy in immunologically competent persons, who may represent an ideal target for such an immunotherapeutic regime. However, possible differences in the nature of HIV-1 infection in the chimpanzee model versus human infection might require a more potent engineered immunogen to achieve additional effectiveness. We are currently evaluating such a multicomponent DNA vaccine/immune therapeutic approach in an effort to further enhance immune induction in the presence of viral replication [38, 39]. Overall, these results support the ability of the immune system to affect viral replication in an important primate model and suggest that further investigation of DNA immunization, ultimately for treatment or control of human infectious disease, is warranted.

Acknowledgments

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References