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Nucleic acid immunization of chimpanzees as a prophylactic/ immunotherapeutic vaccination model for HIV-1: prelude to a clinical trial

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Vaccine development strategies have often utilized recombinant envelope glycoproteins which usually generate strong humoral immune responses but which do not generate strong cytotoxic T lymphyocytes (CTL). A recent novel experimental vaccination approach involves the technology known as nucleic acid immunization in which DNA plasmids expressing a gene of interest is injected intramuscularly in experimental animals. These expressed proteins then are presented to the immune system with the subsequent development of strong antibody and cellular (particularly CTL) immune responses. These types of immune responses have been elicited in rodents as well as nonhuman primates including chimpanzees. Results from studies on nucleic acid immunization of HIV-1 infected chimpanzees with envelope glycoprotein expressing constructs indicated that this method was able to decrease substantially HIV-1 viral load in these chimpanzees. These data are useful for the development and implementation of human phase 1 clinical trials with HIV constructs expressing various genes from the HIV-1 genome. © 1997 Elsevier Science Ltd

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The development of effective vaccination strategies against human retroviral infections such as HIV-1 has proven to be a particularly difficult problem. The inability of the candidate vaccines to elicit relevant humoral and cellular immune responses, particularly cytotoxic T lymphocytes (CTL) is, presumably, at least partly responsible for the failure to develop an effective vaccine. Historically the majority of successful antiviral vaccines demonstrating both humoral and CTL responses have often been formulated as live attenuated viruses. The use of live attenuated HIV-1 as a vaccinogen in the uninfected population should be viewed as an important advance in HIV vaccine development[1]. However, this approach has associate safety concerns with HIV because of the invariably fatal nature of AIDS. Vaccination strategies such as a live recombinant poxvirus vector expressing the HIV-1 envelope has lead to HIV-1 specific CD8+ cytotoxic activity. However, the ability of such vectors to develop relevant antibody responses may be limited.

Another approach is direct nucleic acid vaccination (i.e. DNA inoculation/genetic vaccination) technique[2–5]. We have reported on this novel vaccination approach using facilitated (i.e. with bupivacaine–HCl) DNA injection with plasmid constructs that drive expression of retroviral genes[6,7]. The expression of these genes generates proteins *in vivo* which are presented to the immune system with the resultant development of both humoral and cellular immune responses. We developed a multigene vaccine delivery strategy utilizing structural and regulatory (*gag/pol* and *env/rev* genes) in order to produce a broad immune response against HIV-1 in our effort to limit immunologic escape. We then studied the ability of individual gene expression cassettes to generate

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Table 1 Humoral immune response in HIV-1 infected chimpanzees after *env* DNA plasmid vaccination compared to responses in nonvaccinated HIV-1 seropositive humans

	Humoral immune responses			
	Pre-DNA plasmid vaccination ($O.D._{450}$ nm)	Boosting after DNA plasmid vaccination ($O.D._{450}$ nm) (% change)	Pre-DNA plasmid vaccination ($O.D._{450}$ nm)	Boosting after DNA plasmid vaccination ($O.D._{450}$ nm) (% change)
Sera samples	Peptide 1	Peptide 1	Peptide 2	Peptide 2
Chimpanzee A	0.065	0.049 (−21.6%)	0.227	0.177 (−22%)
Chimpanzee B	0.0325	0.315 (+870%)	0.085	0.192 (+125%)
Human HIV-1$^+$	[a]	Not applicable	[a]	Not applicable

Chimpanzee A was vaccinated with a vector control plasmid. Chimpanzee B was vaccinated with an MN *env* glycoprotein expressing plasmid. Human sera samples were from HIV-1 seropositive patients. Peptide 1, amino acids 601–620 (MN based): GKLICTTVPWNASWSNKSL; peptide 2, amino acids 781–800 (MN based): IVELLGRRGWEVLKYWWNLL

[a]Positive reactivity in >50% of all human sera samples

cross reactive immune responses. Humoral responses induced in vaccinated animals recognized several different isolates of HIV-1. Furthermore, we observed the induction of cross reactive proliferative responses as well as divergent isolate and cross clade CTL activity. Specific cytokine measurements as well as an analysis of immunoglobulin isotype induction further demonstrated that a TH_1 response profile is produced by this vaccination methodology. Novel sets of expression cassettes were then engineered to encode the canonical *gag/pol/ env/rev*. These expression cassettes were characterized *in vitro* and *in vivo* for their ability to drive expression. The immunogenicity of the *gag/pol* and *env/rev* expression cassettes have now been evaluated in nonhuman primates including chimpanzees with our findings described below.

Preclinical studies of facilitated nucleic acid vaccination in chimpanzees

We studied the ability of nonhuman primates in this investigation to develop immune responses after inoculation with a DNA plasmid which expresses a foreign gene. Specifically, we evaluated the effectiveness of facilitated HIV-1 specific DNA vaccines (i.e. mixed with bupivacaine–HCl) in both HIV-1 infected and naive (noninfected) chimpanzees.

HIV-1 negative chimpanzees

Three naive chimpanzees were immunized intramuscularly with 100 μg of an HIV-1 envelope expressing construct (pM160-MN) six times at 3 week intervals. A fourth chimpanzee was vaccinated with a vector control at the same dose.

HIV-1 positive chimpanzees

Two HIV-1 infected chimpanzees were vaccinated by an intramuscular route with DNA plasmids. One (No. 18) was immunized with 100 μg of pM160-MN and the second (No. 16) with a vector control (i.e. lacking the $HIV\text{-}1_{MN}$ envelope glycoprotein gene) three times at 6 week intervals. Intramuscular DNA plasmid inoculation were all made in the same general site on the muscle.

Cytotoxic T cell lymphocyte assay

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll–Hypaque separation gradient and infected with Epstein–Barr virus (EBV) to establish immortalized lymphoblastoid cell (LBCs) lines. These cell lines were used as stimulators and targets for the determination of CTL responses. The freshly isolated PBMCs were cultured in the presence of Concanavalin A (2.5 μg ml^{-1}) for 3 days. The LBCs were infected overnight with a recombinant vaccinia virus (vPE16) that expresses gp160 from $HIV\text{-}1_{IIIB}$, fixed with glutaraldehyde as described previously and mixed with effectors at a ratio of 1:20 effectors and incubated for an additional 3 days. A second set of vaccinia infected LBCs were prepared and used as targets in a standard 5 h chromium release assay.

ELISA assays

Chimpanzee serum samples were diluted and analyzed by ELISA assays by standard methods. ELISA analysis was performed with recombinant proteins gp120 and gp41 (Intracel, Inc.). $HIV\text{-}1_{MN}$ envelope based peptides were obtained from the AIDS Research and Reference Reagent Program. $O.D._{450}$ values as well as the level of boosting was analyzed on sera samples taken 2 weeks after the third inoculation.

Reverse transcriptase (RT) assay

The presence of retrovirus and particle associated RT in the culture supernatant was determined by assaying for RT activity. Virus suspensions were prepared by standard technologies and 20 μl volumes were added to a reaction mixture containing 0.1 MATP, 2.25 U rAdT, 12.5 mM $MgCl_2$ and 110 μCi [^{3}H]TTP (Thymidine triphosphate). The mixture was incubated for 1.5 h at 37°C. The reaction was stopped and precipitated by standard techniques. For the assay of the sera samples analyzed in this report a control standard curve was performed to establish that the viral load RT values from the samples were in the linear range of detection of the assay.

RESULTS AND DISCUSSION

An indication of the potential therapeutic efficacy of the nucleic acid vaccination technique was observed in the increased humoral immune responses to several epitopes of the HIV-1 envelope glycoprotein. An HIV-1 infected chimpanzee vaccinated with the *env* expressing construct showed a boosting effect to several regions (i.e. epitopes of the envelope glycoprotein) while the chimpanzee vaccinated with a control vector did not (*Table 1*).

Table 1 summarizes the humoral responses to two HIV-1_{MN} gp41 envelope glycoprotein peptides, spanning amino acids 601–620 and 781–800. Boosting responses to peptide 601–620 is of interest since we have recently determined that this peptide contains a neutralizing epitope identified by a human monoclonal antibody[8]. This human monoclonal antibody was demonstrated to neutralize diverse laboratory and clinical isolates. At least 50% of all sera samples from HIV-1 infected individuals significantly bind to this peptide as indicated in *Table 1*. The second peptide shown (aa 781–800) is of potential significance since we have demonstrated a correlation between the presence of antibodies to this region and a lack of perinatal transmission from HIV-1 infected pregnant women to their offspring[9].

While anti-HIV-1 humoral immune responses may be important for immunotherapy it is likely that effective CTL responses will be an important element of an efficacious vaccine for HIV 1. The ability of HIV-1 to spread by fusion of infected with uninfected cells as well as the establishment by the virus of high levels of replication in the cell dictates that a cell mediated immune response will be important in achieving full protection. It has been demonstrated that patients who mount a strong gp160-specific CTL response show a rapid reduction of acute viremia and antigenemia, while in contrast, primary viremia and antigenemia are poorly controlled in patients with low or undetectable virus-specific CTL activity. In addition, a few % repeatedly exposed sexual workers remain seronegative and PCR negative in the presence of CTL activity against HIV-1. Therefore, measurable CTL responses seems to hinder infection and certainly disease progression.

The HIV-1 positive chimpanzee in our study did not show clear changes in cellular responses after DNA immunization. It is possible that in this study the background CTL levels masks further effects on cellular immune responses. However, the cellular responses noted in the vaccinated HIV-1 naive chimpanzees (*Figure 1a*) were comparable to results obtained in people early in the course of infection with HIV-1 (*Figure 1b*). The background level of lysis was *ca* 10%. This value was subtracted from total lysis noted in the vaccinated chimp and HIV-1 infected patients. Therefore the values shown in *Figure 1* are specific lysis (i.e. with background level subtracted). The level of specific lysis in pre-immune samples was 0%. The level of lysis in the previously infected chimpanzee is not shown since a retrospective analysis of these samples was not available.

Perhaps most importantly, nucleic acid vaccination also demonstrated the ability to control viral load in challenge experiments of macaques[10]. In addition, a decrease in viral load was observed in an infected chimpanzee participating in a therapeutic DNA vaccination protocol (*Table 2*).

Human phase I clinical studies of DNA vaccination as an immunotherapeutic as well as prophylactic strategy for HIV-1 have recently been initiated. No significant safety related issues have developed in the studies to date. Further evaluation of immune responses induced by DNA immunization as well as the utility of this approach in different experimental settings as a potential immunotherapeutic or vaccine approach for HIV-1 is ongoing.

In summary, our initial analysis indicates that both humoral and cellular responses are induced and may be relevant to the control of viral replication. These responses include site directed humoral immune responses against regions of gp41 in an HIV-1 infected chimpanzee vaccinated with an HIV-1 *env* expressing DNA construct. In addition, HIV-1 naive chimpanzees developed specific CTL responses after DNA vaccination. The demonstration of these HIV specific responses in both arms of the immune system and the corresponding control of viral replication suggest that a DNA plasmid based vaccine may be a useful immunological weapon for use in the control of HIV-1 infection[10].

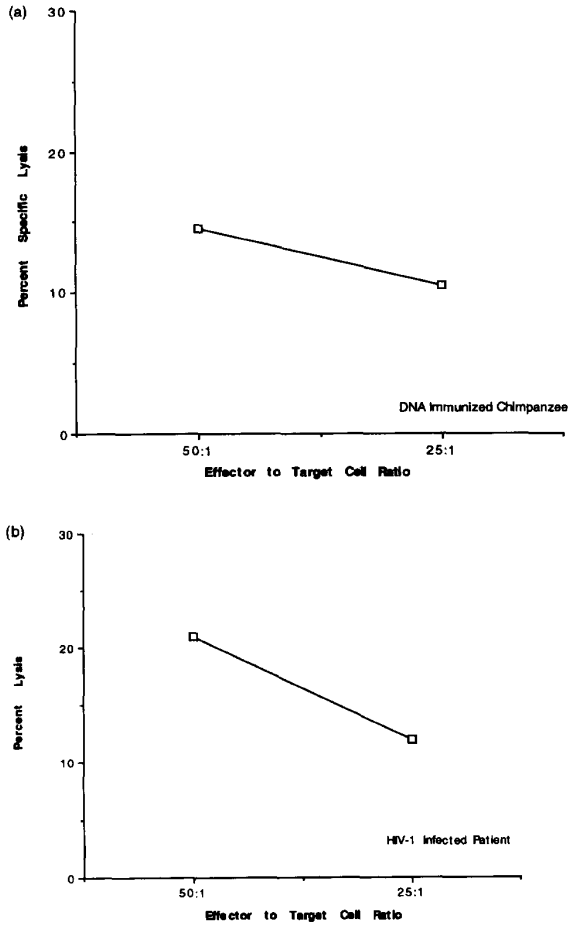

Figure 1 The CTL assay was performed with cells from a nonHIV-1 infected chimpanzee vaccinated with an *env* DNA expressing plasmid (a) or a nonvaccinated HIV-1 infected human (b) by methods as described in the text. The percent specific lysis was graphed vs effector to target cell ratio (50:1 and 25:1)

Table 2 HIV-1 viral load analysis on infected chimpanzees before and after DNA plasmid vaccination with a construct expressing the MN envelope glycoprotein

	RT activity[a]	
Chimpanzee	Pre-DNA vaccination	Week 21[b]
A	5000	1105
B	8000	24

Chimpanzee A was vaccinated with a vector control plasmid. Chimpanzee B was vaccinated with an MN *env* expressing plasmid. See text for more extensive details of the assay; [a]RT activity was in counts per minute; [b]Time after DNA plasmid vaccination

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