Systemic and Mucosal Immunity Is Elicited after Both Intramuscular and Intravaginal Delivery of Human Immunodeficiency Virus Type 1 DNA Plasmid Vaccines to Pregnant Chimpanzees


DNA vaccines encoding human immunodeficiency virus type 1 (HIV-1) env/rev and gag/pol were delivered intravaginally (IVAG) and intramuscularly (IM) to 2 pregnant chimpanzees. Vaccination was well tolerated and each chimpanzee developed antibodies (up to 1 year later) to both vaccines. Placental transfer of anti-Env and anti-Gag IgG was demonstrated in both maternal/infant pairs. Specific IgG was also demonstrated in saliva, vaginal, and rectal washes after IVAG immunization. Predominantly anti-HIV-1 IgA was detected in the milk of both mothers after both IM and IVAG immunization. Cellular responses included Gag-specific proliferation of lymphocytes and cytotoxic T lymphocytes against both antigens. These data suggest a strategy for induction of mucosal and systemic responses after both IM and IVAG delivery of DNA vaccines in a primate model and could ultimately be useful in lowering maternal-to-fetal transmission of HIV-1, perinatally and through breastfeeding.

A campaign to immunize infants and children with an effective vaccine would provide the best opportunity to control the worldwide human immunodeficiency type 1 (HIV-1) epidemic. Of the traditional vaccines, only live attenuated preparations consistently stimulate both arms of the immune system. However, the safety of these agents is a major concern in designing a vaccine for HIV-1. Vaccines based on nucleic acids stimulate both humoral and cellular immunity through transient production of multiple immunogens and avoid the dangers of a live vaccine in potentially immunosuppressed subjects. Thus, DNA vaccines provide a promising alternative to traditional vaccines for the prevention and/or control of HIV-1 infection and its progression to AIDS. We have previously reported on protection of chimpanzees from HIV-1 challenge [1], reduction in viral load in HIV-infected animals [2], and enhancement of immunity in HIV-infected humans [3] after immunization with HIV-1 DNA vaccines.

Maternal-to-fetal transmission rates have been substantially reduced in developed nations, mainly through the use of perpartum antiretroviral agents. A strategy in which patients are given a short course of zidovudine was recently shown to significantly reduce transmission rates in a developing nation. This is very encouraging, but an effective mass immunization strategy would not require immunizing women during pregnancy only. We immunized 2 pregnant chimpanzees with HIV-1 DNA constructs to assess their safety and immunogenicity in this important animal subset.

Methods

Two pregnant chimpanzees, Pan troglodytes, seronegative for HIV-1, received their first immunization at 60 ± 10 days into the
period of gestation (normal, ~220 days). The env/rev and gag/pol plasmids were injected intramuscularly (IM) with 100 μg of the HIV-1 gag/pol DNA construct (pCgag/pol) at weeks 0 and 4. Injections were repeated at weeks 9 and 13 with 200 μg of pCgag/pol (600 μg total). Subsequent injections were administered to the contralateral leg to maximize the ability to differentiate adverse reactions. Intravaginal (IVAG) immunization was performed by injecting 50 μg (at 1 mg/mL) of an HIV-1MN Env(gp160)/Rev construct (pCMN160) into the vagina with a needleless syringe at weeks 0 and 4. The dose was doubled to 100 μg at week 9 and again to 200 μg at week 13 (400 μg total).

HIV-1 Env and Gag proteins (i.e., gp120, p55, p24 protein) were obtained from the NIH AIDS Research and Reference Reagent Program (ARRRP) and Immunodiagnostics (Bedford, MA). The specific ELISA methodology has been described elsewhere [6]. Different isotypes were measured by using the appropriate peroxidase-conjugated antihuman secondary antibodies (Sigma, St. Louis; IgA-catalyst A0295, IgG-catalyst A6029). End-point titers were determined by comparing OD readings from samples taken post-vaccination (PV) to naïve samples. The titer is defined as the highest dilution at which the mean of duplicate OD readings from PV samples is greater than the mean of duplicate naïve OD readings by at least 3 SD. SD is calculated for both the PV and naïve readings, and the higher of the 2 is used in the preceding calculation.

Peripheral blood mononuclear cells (PBMC) separated on a Ficoll density gradient were assayed for β-thymidine incorporation to determine lymphoproliferative responses (LPRs) as described elsewhere [6]. Stimulation index (SI) is defined as the mean of triplicate wells with media alone. Mitogen (phytohemagglutinin; PHA) responses were used to assess the relative cellular immunocompetence of each subject.

Cytotoxic T lymphocyte (CTL) activity was measured by use of vaccinia/HIV-1 recombinant virus (vSC8, β-galactosidase; vPE16, gp160; VV:gag, gag/pol) from the NIH ARRRP to infect lymphoblastoid cells (LBC). Chimpanzee PBMC were nonspecifically stimulated with PHA and IL-2 (Collaborative Biomedical Products, Bedford, MA) for 3 days, then specifically stimulated with auto-logous LBC (glutaraldehyde-fixed) infected with the same vaccinia used for targets. Cells were fed with fresh media every 4 days for a total of 21 days. Percentage lysis is calculated as (experimental – spontaneous)/(maximum – spontaneous) × 100%. Percentage-specific lysis is calculated by subtracting background lysis of vSC8-infected targets from specific lysis of HIV-1 gene product-infected targets. Maximum effector : target ratio was 100 : 1 with 3 or 4 ratios used per assay, depending on the number of viable cells. The remaining chromium release assay has been described elsewhere [6].

Results

The chimpanzees were monitored closely and showed no sign of toxicity as a result of DNA vaccination. Biweekly hematological, chemistry, and physical parameters remained normal throughout the study periods of the pregnant and newborn animals. Most importantly, 2 healthy (no congenital abnor-

Discussion

The immunogenicity of DNA vaccines directed against HIV-1 has been documented in primates by a number of investigators [1, 2, 5, 7, 8]. Results include the protection of chim-

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Humoral and cellular responses were generated by pCgag/pol and pCMN160 as in previous primate experiments. However, several unique results were observed in this study. Anti-Env and anti-Gag IgG was not only detected in the serum of both pregnant animals but also in the serum of their offspring. These antibodies likely represent passive placental transfer of IgG. The functional capacity of these placentally transferred antibodies may be important because trends toward nontransmitting status have been demonstrated in relation to the ability to neutralize autologous virus [9]. Another unique observation is the generation of systemic immunity after IVAG pCMN160 and antibodies detected in BM after IM pCgag/pol. We previously reported the generation of mucosal IgG after IVAG delivery of HIV-1 DNA in mice [10]. Others have reported similar findings after IVAG administration of live attenuated strains of simian immunodeficiency virus (SIV) to macaques [11]. This study shows that systemic immunization leads to responses at mucosal sites in the HIV-1/chimpanzee model.

The further observation that HIV-specific IgA was detected in BM was expected because it is the primary Ig found in milk, although it is interesting to note that BM IgA was detected after both IM pCgag/pol and IVAG pCMN160. It is unlikely that these antibodies merely represent the appearance of transudated antibodies from extracellular fluid into BM, because only IgA and not IgG was detected. Specific IgA was more...
Figure 2. Cellular immune responses of pregnant chimpanzees in response to vaccination (Vax, ▲). Lymphocyte proliferative response (LPR) to human immunodeficiency virus type 1 (HIV-1) A. Rev and B. Gag protein at 5 μg/mL in animal P3 (striped box) and P6 (□). Cytotoxic T lymphocyte (CTL) activity against HIV-1 C. T-tropic (MN) envelope (effector : target ratio, 25 : 1) and D. Gag/Pol (effector : target ratio, 40 : 1) targets in animals P3 (striped box) and P6 (□).

difficult to demonstrate in serum and mucosal specimens because these responses were primarily IgG and persisted up to at least 1 year after the initial vaccination. The low-titer IgG measured at mucosal surfaces may merely represent serum transudate but could still play a role in viral neutralization at these surfaces.

We have also previously reported LPR after IM delivery of HIV-1 plasmids to mice and macaques [6]. Further studies in mice and primates will be required to investigate the lack of anti–Env LPR in either animal after IVAG pCMN160 delivery.

The generation of specific CTL responses to Env and Gag/Pol targets in these animals is consistent with results obtained in previous primate experiments with DNA vaccines [1, 2]. This is more encouraging than results obtained after vaccination of chimpanzees with avipox vectors [12]. The level of CTL response is more consistent with results obtained after vaccination of humans with vaccinia vectors [13] and vaccination of chimpanzees with adenovirus vectors [14]. Of note, IVAG pCMN160 generated persistent anti–Env CTLs in 1 animal. This has been previously reported after IVAG administration of attenuated SIV to macaques [11].

Conservative IVAG dosing of 50 μg was chosen because this dose was immunogenic in our previous murine studies and is just above the lowest dose administered to human volunteers already infected with HIV-1 (30 μg with no adverse effects). Once safety was observed at weeks 0 and 4, doses of both pCgag/pol and PCMN160 were doubled to 100 and 200 μg, respectively, for the immunization at week 9. The fourth dose of pCMN160 was doubled again to 200 μg. Both cellular and humoral responses seem to have improved with the later doses, although this could also be a boosting effect. The optimal doses of these DNA vaccines have not been established, although 200 μg is a relatively conservative dose in comparison with doses of 1 and 2 mg in other primate studies of DNA vaccines [8].
These results may be enhanced by the codeelivery of immunomodulatory DNA vectors. Vectors found to be useful in murine studies of DNA vaccines, such as IL-12, granulocyte macrophage colony stimulating factor, and CD86 (B7.2), may prove useful in future primate experiments [15].

These observations support the assertion that both mucosal and systemic DNA administration are capable of inducing immune responses in large primates. The further characterization of this approach for vaccination as well as immunotherapy appears to be warranted because it may increase our understanding of not only the utility but also the mechanism(s) of DNA vaccination.

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References