Evaluation in macaques of HIV-1 DNA vaccines containing primate CpG motifs and fowlpoxvirus vaccines co-expressing IFNγ or IL-12

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Abstract

Induction of HIV-specific T-cell responses by vaccines may facilitate efficient control of HIV. Plasmid DNA vaccines and recombinant fowlpoxvirus (rFPV) vaccines are promising HIV-1 vaccine candidates, although either vaccine alone may be insufficient to protect against HIV-1. A consecutive immunisation strategy involving priming with DNA and boosting with rFPV vaccines encoding multiple common HIV-1 antigens was further evaluated in 30 macaques. The DNA vaccine vector included CpG immunostimulatory molecules, and rFPV vaccines were compared with rFPV vaccines co-expressing the pro-T cell cytokines IFNγ or IL-12. Vaccines expressed multiple HIV-1 genes, mutated to remove active sites of the HIV proteins. The vaccines were well tolerated, and a significant enhancement of DNA-vaccine primed HIV-1 specific T lymphocyte responses was observed following rFPV boosting. Co-expression of IFNγ or IL-12 by the rFPV vaccines did not further enhance immune responses. Non-sterilising protection from a non-pathogenic HIV-1 challenge was observed. This study provides evidence of a safe, optimised, strategy for the generation of T-cell mediated immunity to HIV-1.

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Keywords: Vaccine; DNA; Recombinant fowlpoxvirus; Prime/boost; Macaque; Cytokine co-expression

1. Introduction

A vaccine against HIV-1 is urgently needed. The induction of broadly reactive neutralising antibodies to HIV-1 is desirable, but has not been achievable with any viable vacc-
Consecutive immunisation strategies to generate T cell immunity involving priming by DNA vaccination and boosting with recombinant fowlpoxvirus (rFPV) vectors encoding common HIV and other antigens have been described [4,5]. The DNA immunisation elicits low-level but persistent high-avidity immunity and primes for greatly enhanced T cell responsiveness following boosting with viral vaccine vectors (such as FPV or vaccinia). This approach has been confirmed by several research groups in primates [6–10] and for malaria antigens, recently in humans [11].

HIV-1-specific cellular immunity alone can only limit infection by blunting the acute and chronic infection, rather than prevent it altogether. This is a concern for HIV-1 vaccines since (a) the virus integrates into the host genome and can remain viable and latent, and (b) high rates of viral mutation select for vaccine-escape mutants. Long-term protection from lentiviral disease may be difficult to achieve with purely T cell-based vaccines [12,13]. However, studies during acute lentiviral infection have suggested that the magnitude, phenotype, and breadth of the vaccine-induced T cell response may correlate with the control of the initial viremia [3,14]. Enhancing cellular immunity by improving vaccines may further limit the degree of late escape from immune control.

DNA vaccines, on their own, have limited immunogenicity in human clinical trials [15]. DNA vaccines may be improved by including multiple primate-specific CpG oligodeoxynucleotides [16]. CpG motifs enhance T cell immunity by binding to Toll-like receptor 9 [17]. Co-delivery of cytokines that promote T cell immunity together with DNA vaccines can improve their immunogenicity [3]. The co-expression of cytokines from viral vectors such as vaccinia and FPV can also dramatically modulate viral immunogenicity [18,19]. The benefits of encoding CpG motifs within the DNA vaccine, or co-expressing cytokines such as interferon gamma (IFNγ) or interleukin (IL)-12, to further enhance the immunogenicity of prime/boost vaccines is not known, and is the subject of this study. The mutation of active enzymes or binding sites within the HIV genes, while maintaining the immunogenicity to multiple HIV antigens from a single vector, also presents a challenge to vaccine design. We assessed an immunisation strategy that employed priming the immune system with CpG optimised DNA encoding HIV-1 antigens and boosting with rFPV encoding shared HIV-1 antigens with or without co-expression of IFNγ or IL-12.

2. Materials and methods

2.1. Vaccines

The DNA and rFPV vaccines and in vitro protein expression is described elsewhere (Purcell et al., manuscript in preparation, [37]). Briefly, the DNA vaccine (pHIS-HIV-B) contained approximately 65% of the B subtype pNL(AD8) provirus, with sequences expressing modified Gag, modified RT, Protease, Rev, Tat, Vpu, truncated Nef (the first 31 aa) and truncated Env (the first 275 aa only). HIV-1 genes whose function posed a theoretical risk were either deleted (Integrase, LTRs, Vif, Vpr) or mutated (RT, Zn2+ fingers of Gag). The modified HIV-1 genome was inserted into the plasmid DNA vaccine vector pHIS-HIV-B DNA vaccine described above. Pol antigens in the vaccines (RT, protease) are expressed via a frameshift mutation. Primers were designed to add the FPV early/late promoter and an early transcription terminator to the genes during the PCR. The promoter-gagpol PCR product was cloned into plasmid pkG10 for insertion into FPV M3 at the F6, 7, 9 site. Human IFNγ or IL-12 genes (known to be biologically active in macaques [20–23]) were inserted into rFPV under the control of the FPV IE1/L promoter immediately downstream of the FPV TK gene to construct separate rFPV co-expressing these pro-T cell cytokines. Early poxvirus transcription terminators, TSNT, in IL-12 were removed by PCR mutagenesis. Recombinants were selected on the basis of co-expression of the E. coli gpt gene and plaque purified on the basis of co-expression of the E. coli beta-galactosidase gene.

2.2. Macaques and vaccinations

Juvenile Macaca nusestrina were free from HIV-1/SIV/Simian retrovirus infection, housed under physical containment level 3 conditions and anaesthetised with ketamine (10 mg/kg intramuscular (IM)) prior to procedures. All experiments were performed according to National Institutes of Health guidelines on the care and use of laboratory animals, and were approved by the University of Melbourne and CSIRO Animal Ethics Committees. Vaccine regimens using groups of four to six macaques are shown in Table 1.

2.3. Power and statistical considerations

The study was primarily powered to detect two-fold differences in IFNγ ELISPOT responses between regimens. We have observed a standard deviation in ELISPOT assays of 16% between similarly immunised macaques, predicting that a six versus six macaque comparison has 80% power to detect a 1.26-fold difference in HIV-specific IFNγ ELISPOT responses, and a four versus four comparison was powered to detect a 1.32-fold difference. Statistical comparisons of secondary endpoints of immune responses and viral levels between vaccine groups utilised a t-test of pair-wise comparisons without compensation for the multiple analyses performed. Comparison of viral concentrations across groups utilised a time-weighted area-under-the-curve analysis.
Table 1

<table>
<thead>
<tr>
<th>Vaccine regimen</th>
<th>N</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>17 (challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA/rFPV</td>
<td>6</td>
<td>DNA</td>
<td>DNA</td>
<td>rFPV</td>
<td>rFPV</td>
<td>HIV-1</td>
</tr>
<tr>
<td>DNA/rFPV-IFNγ</td>
<td>6</td>
<td>DNA</td>
<td>DNA</td>
<td>rFPV-IFNγ</td>
<td>rFPV-IFNγ</td>
<td>HIV-1</td>
</tr>
<tr>
<td>DNA/rFPV-IL-12</td>
<td>6</td>
<td>DNA</td>
<td>DNA</td>
<td>rFPV-IL-12</td>
<td>rFPV-IL-12</td>
<td>HIV-1</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>Placebo</td>
<td>Placebo</td>
<td>Placebo</td>
<td>Placebo</td>
<td>HIV-1</td>
</tr>
<tr>
<td>rFPV-IFNγ</td>
<td>4</td>
<td>None</td>
<td>rFPV-IFNγ</td>
<td>rFPV-IFNγ</td>
<td>rFPV-IFNγ</td>
<td>HIV-1</td>
</tr>
</tbody>
</table>
rFPV-IL-12      | 4 | None | rFPV-IL-12 | rFPV-IL-12 | rFPV-IL-12 | HIV-1 |

a DNA vaccine: 1 mg (as 1 mg/mL IM).

b rFPV vaccines: $5 \times 10^7$ plaque forming units (pfu) (as $5 \times 10^7$ pfu/mL).
c M. nemestrina-passaged HIV-1 LAI isolate K98227/W35 was given as $10^6$/mL TCID50 IV.

2.4. ELISPOT and lymphoproliferative responses

Fresh macaque peripheral blood mononuclear cells (PBMC) were stimulated with whole aldrithiol-2 inactivated HIV-1$_{1838}$ (10 μg/mL, kindly provided by Dr. J. Lifson, National Cancer Institute (NCI), Frederick, MD), a single concentrated pool of 122 15mer HIV-1HXB2 Gag peptides overlapping by 11 aa spanning the entire Gag protein (3 μg/mL; National Institutes of Health (NIH) AIDS Reagent Program) dissolved in dimethyl sulfoxide (DMSO), and the appropriate negative control for each antigen (microvesicles from the same cell line used to grow the inactivated HIV-1, and DMSO used to dissolve the peptides). Whole inactivated HIV-1 has proved to be a useful antigen for quantifying T cell responses in vitro as it is taken up and processed for class I and II presentation efficiently[24,25]. The samples were assayed using the monkey IFNγ ELISPOT kit (U-CyTech BV, Utrecht, The Netherlands), according to the manufacturer’s instructions and as previously described[23]. ‘Spots’ were counted using an automated counter (Autoimmun Diagnostika GmbH, Strassberg, Germany).

Lymphoproliferative responses were assessed by standard 3H-thymidine incorporation assay to 10 μg/mL recombinant HIV-1 P55, control antigen (supplied by Chiron Corporation to Quality Biological, Inc., Gaithersburg, MD), whole inactivated HIV-1$_{1838}$ and Staphylococcal enterotoxin B as described [26].

2.5. HIV-1 antibody responses

Antibodies to HIV-1 were tested by a competitive enzyme immunoassay (EIA, Wellcozyme HIV Recombinant, UK) and by western blot using 200 μg standardised HIV-1 viral lysate [26].

2.6. HIV-1 challenge of the macaques

Stocks of HIV-1$_{1838}$, passaged through neonatal M. nemestrina, were kindly provided by Drs. M. Agy and M. Bosch (U. Washington, Seattle, WA) [27–29]. The HIV-1 isolate K98227/W35 was selected from an infected neonatal macaque, with high HIV-1 plasma RNA 35 weeks post-infection. As it was impractical to expand this stock separately in autologous PBMC from each of the 30 macaques studies, the challenge stock was derived from infecting phytohaemagglutinin (PHA)-stimulated human PBMC with HIV-1$_{1838}$ isolate K98227/W35 and maintained in RF10 media supplemented with HUMAN IL-2 (50 U/mL, Hoffmann-La Roche, NJ) for 14 days. Infected PBMC were pelleted and added to PHA-stimulated, CD8-depleted PBMC from a further three donors and maintained for 7 days. Supernatants were harvested and assayed for reverse transcriptase (RT) activity as previously described [25]. The tissue culture infectious dose in 50% (TCID50, on C8166 human T cell line) of $10^6$/mL was administered via the femoral vein initially to two naïve juvenile pigtailed macaques (to confirm infectivity—data not shown), and then to vaccinated and control macaques (Table 1).

2.7. Detection of HIV-1 infection

Plasma HIV-1 RNA was assessed by RT-PCR (AmpliCor HIV Monitor™, Roche Diagnostic Systems, Branchburg, NJ) according to the manufacturer’s instructions for human plasma. PBMC HIV-1 DNA was quantified by real-time PCR as described [30,31]. HIV copies were quantified per million PBMC using conserved CCR5 primers and PCR to amplify cellular CCR5. Quantitative PCR was performed using the 7700 Sequence Detector (Applied BioSystems, Foster City, CA).

2.8. FPV-specific antibody responses

To assess the potential for anti-FPV immune responses to modulate responses to the vaccines, an FPV-specific western blot was developed. A band at approximately 36 kDa was found to be specific for animals immunised with FPV by comparing baseline profiles to serial samples obtained from both macaques and humans who had been immunised with FPV based vaccines (K. Wilson, unpublished data). This 36 kDa band was quantified by densitometry.
3. Results

3.1. Reactogenicity of the DNA and rFPV vaccines in macaques

Six groups of four to six pigtailed macaques received either two DNA priming vaccinations followed by two rFPV booster vaccinations, three rFPV vaccinations alone, or control vaccinations with placebo DNA and/or FPV (Table 1). All macaques remained healthy following the vaccinations and gained weight normally. No large swelling or erythema was observed at the injection sites. Serial analyses of blood cell counts, plasma electrolytes, urea, total protein, albumin, bilirubin, liver enzymes, and creatine kinase did not reveal any adverse effects of vaccination (data not shown). Similarly, detailed pre-clinical toxicology experiments performed on 36 cynomolgus macaques did not reveal any adverse effects of the vaccines (data not shown).

3.2. T cell Immunogenicity

HIV-specific IFNγ production from T cells was quantified by ELISPOT 5 weeks following the final rFPV boost using whole inactivated HIV-1, and on the day of challenge using both the whole inactivated HIV-1 antigen and a single pool of HIV-1 Gag 15mer overlapping peptides (Fig. 1a). Macaques in the three groups receiving DNA prime and rFPV boost vaccinations had elevated DNA/rFPV-IL-12 following all vaccinations (Fig. 1b; P = 0.008, Fig. 1b and c). However, in the two groups receiving rFPV vaccines alone, only low levels of HIV-specific IFNγ producing cells were detected compared to control macaques (P > 0.05). There was no further enhancement of the HIV-specific IFNγ response by ELISPOT in the groups administered the rFPV vaccine co-expressing either IFNγ or IL-12 compared to the prime/boost group not co-expressing cytokines. Indeed, the response following vaccination with the DNA/rFPV regimen was not significantly greater than control-vaccinated macaques (P > 0.05), observed (Fig. 1b).

There was no further enhancement of the HIV-specific lymphoproliferative responses detected between the DNA/rFPV-IL-12 regimen was 0.04 to whole inactivated HIV-1 and 0.01 to P55 Gag. Following the second rFPV boost (week 11), the P-values were 0.04 to whole inactivated HIV-1 and 0.01 to P55 Gag. There was no significant difference in the lymphoproliferative responses detected between the DNA/rFPV-IL-12 arm and the DNA/rFPV arm, except to P55 Gag at week seven (P = 0.03). Two rFPV boosts were administered in this study to ascertain whether further rFPV boosts were required to enhance T cell immunity. However, there was no significant increase in HIV-specific lymphoproliferative activity 1 week following the second rFPV boost (week 11) compared to one week following the first rFPV boost across any of the three prime/boost regimens either to the P55 Gag protein antigen or to whole inactivated HIV-1 antigen (P > 0.05).

3.3. HIV-1 antibodies

Although the vaccine regimens were primarily designed to induce HIV-specific T cells, HIV-1 antibodies were also assessed, both by a commercial EIA to quantify responses, and by western blot to assess responses to various HIV-1 proteins (Fig. 3). HIV-1 antibody responses by EIA were only detected in the three groups of macaques immunised with the prime/boost regimens and were similar across these groups. The responses were weak, with a mean sample/cut-off ratio in the 18 prime/boost vaccinated macaques of ~1.0 in the competitive EIA, which represents a borderline positive result in this sensitive assay (Fig. 3a). By western blot, weak HIV-1 P24 Gag-specific responses were detectable following the DNA vaccine prime in only 6 of the 18 macaques receiving the DNA prime (example shown in Fig. 3b, week 7). Gag-specific bands were readily detected following the rFPV boosts in all 18 macaques in the three DNA prime/rFPV boost regimens (Fig. 3b, weeks 7 and 15). HIV antibody responses

significant T cell proliferative activity was detected to either whole inactivated HIV-1 or P55 Gag protein (Fig. 2). In macaques receiving rFPV vaccines alone, no significant proliferative response was detected above control macaques (P > 0.05). Macaques in the three groups receiving DNA prime and rFPV boost vaccinations generated enhanced T cell proliferative activity to both whole inactivated HIV-1 (Fig. 2a) and P55 Gag protein (Fig. 2b). The second rFPV boost vaccination did not detectably enhance HIV-specific T cell proliferative activity compared to the response after a single rFPV boost (week seven versus week 11, P > 0.05). There was no enhancement of the T cell proliferative response in the DNA vaccine primed macaques boosted with the rFPV-IFNγ or rFPV-IL-12 vaccines compared to responses from macaques in the DNA/rFPV regimen. Furthermore, the lymphoproliferative responses following boosting with the rFPV-IFNγ vaccine were significantly inferior to the responses observed in macaques immunised with DNA/rFPV not co-expressing cytokines. After the first rFPV boost (week 7), the t-test P-value comparing the lymphoproliferative activity of the DNA/rFPV and the DNA/rFPV-IFNγ arms was 0.04 to whole inactivated HIV-1 and 0.01 to P55 Gag. Following the second rFPV boost (week 11), the P-values were 0.04 to whole inactivated HIV-1 and 0.01 to P55 Gag. There was no significant difference in the lymphoproliferative responses detected between the DNA/rFPV-IL-12 arm and the DNA/rFPV arm, except to P55 Gag at week seven (P = 0.03). Two rFPV boosts were administered in this study to ascertain whether further rFPV boosts were required to enhance T cell immunity. However, there was no significant increase in HIV-specific lymphoproliferative activity 1 week following the second rFPV boost (week 11) compared to one week following the first rFPV boost across any of the three prime/boost regimens either to the P55 Gag protein antigen or to whole inactivated HIV-1 antigen (P > 0.05).
Fig. 1. (a) Example of HIV-specific IFNγ ELISPOT result to both whole inactivated HIV-1 and a single pool of HIV-1 Gag 15mer peptides in PBMC (week 17) from a macaque vaccinated with control empty DNA and rFPV vaccines (Control) and a macaque vaccinated with HIV-1 expressing DNA and rFPV vaccines (DNA/rFPV). Control wells have minimal responses and a strong response is seen in the wells stimulated with Staphylococcal enterotoxin B (SEB). (b) Individual ELISPOT responses to whole inactivated HIV-1 in outbred macaques vaccinated with the six DNA and rFPV vaccine regimens noted in Table 1. The PBMC were obtained at week 15, after the last vaccination. The DNA/rFPV group had significantly superior responses as noted (t-test by pairwise comparison). Mean and standard error of the mean ELISPOT responses across vaccine regimens to whole inactivated HIV-1 and a single pool of HIV-1 Gag peptides at week 17, the day of challenge.
Fig. 2. Lymphoproliferative responses in PBMC of macaques to: (a) whole inactivated HIV-1; and (b) HIV-1 P55 Gag protein. Mean and standard error of the mean proliferative responses across vaccine regimens at week 4 (1 week after the second DNA vaccination in the prime/boost groups—the rFPV only groups were not analysed at this time), week 7, after the first rFPV boost, and week 11, after the second rFPV boost are shown.

Fig. 3. (a) HIV-1 antibody responses to vaccination by EIA. Mean and standard error of the mean across vaccine regimens are shown. (b) HIV-1 antibody responses following HIV-1 challenge by EIA. (c) Example of HIV-1 Western blot in a control macaque and a DNA/rFPV vaccinated macaque prior to vaccination (week 0), following vaccination (weeks 6, 7 and 15) and following HIV-1 challenge (week +3 and +11 post-challenge). Negative (−) and positive (+) controls are human HIV-1 positive sera.
were not induced in the 12 macaques administered either rFPV vaccines alone or control vaccines.

3.4. HIV-1 challenge

To assess protection from HIV-1, macaques were challenged intravenously (IV) with a non-pathogenic HIV-1 challenge (Fig. 4). The mean HIV-1 viral load in control macaques two weeks post-challenge was 3.3 \( \log_{10} \) copies/mL and all four control macaques still had detectable plasma HIV-1 RNA 5 weeks post-challenge. The group vaccinated with DNA/rFPV had a mean viral load at week 2 of 2.7 \( \log_{10} \) copies/mL and was undetectable (<1.3 \( \log_{10} \) copies/mL) in all six DNA/rFPV vaccinated macaques at 5 weeks post-challenge (Fig. 4b). A time-weighted area-under-the-curve analysis demonstrated significant protection from challenge in the DNA/rFPV immunized animals compared to controls (\( P = 0.01 \)) and the DNA/rFPV/eIFN\(_\gamma\) (\( P < 0.01 \)) arms.

A quantitative analysis of cellular HIV-1 DNA in PBMC was used to further analyse protective efficacy. A non-significant trend of reduced HIV-1 in macaques in the DNA/rFPV prime/boost group in comparison to the control group (\( P = 0.17 \)) and the DNA/rFPV-eIFN\(_\gamma\) group (\( P = 0.13 \)) was observed (Fig. 4f). The continued presence of HIV-1 DNA 8 weeks following challenge, even in macaques with controlled HIV-1 plasma RNA, was notable and consistent with seeding of cellular reservoirs following challenge.

Fig. 4. Plasma HIV-1 RNA levels by RT-PCR following HIV-1 challenge. Individual time-courses are noted in (a)-(d) in four groups, and the mean and standard error of the mean for each group is shown in (e). Panel (f) shows the cellular HIV-1 DNA detected by real-time PCR (mean and standard error of the mean for each group). Symbols used in panel (e) are the same as defined in panel (f).
prime/boost arms. If similar safety and immunogenicity is ob-
specific T cell responses were induced in the DNA and rFPV
The vaccinations were well tolerated and high levels of HIV -
prime/rFPV boost regimens intended for clinical evaluation.

4. Discussion

Anamnestic antibody responses to HIV-1 challenge
showed more rapid rises in antibody titers in macaques rec-
cieving the three prime/boost regimens compared to con-
trols at three weeks post-challenge, consistent with vaccine-
induced priming of the immune response (Fig. 3c). A lower,
late antibody response at 11 weeks post-challenge was ob-
served in the DNA/rFPV group in comparison to controls
(P = 0.02), consistent with the control of viremia observed.
Western blots also demonstrated the rapid development of
reactivity against multiple viral proteins by 3 weeks post-
challenge in macaques receiving the prime/boost regimens
(Fig. 3b, 3 and 11 weeks post-challenge).

3.5. Anti-FPV responses

FPV-specific western blot bands were observed follow-
ing rFPV vaccinations (data not shown) and antibody re-
sponses were quantitated by densitometry (Fig. 5). Anti-FPV
responses were readily detected in plasma samples from all
eight macaques studied receiving the rFPV-IFNγ or rFPV-IL-
12 vaccinations alone. Antibodies to FPV were also detected
following the two rFPV vaccinations delivered after DNA
priming. Interestingly, the anti-FPV responses quantitated by
densitometry of the FPV-specific 36 kDa band after all vacci-
nations (week 15) were suppressed in the DNA/rFPV-IL-12
in comparison to the DNA/rFPV (P = 0.04) and DNA/rFPV-
IFNγ groups (P = 0.02).

4. Discussion

This large outbred macaque study evaluated DNA
prime/rFPV boost regimens intended for clinical evaluation.
The vaccinations were well tolerated and high levels of HIV-
specific T cell responses were induced in the DNA and rFPV
prime/boost arms. If similar safety and immunogenicity is ob-
 served in humans, this regimen could evaluate whether prior
induction of HIV-specific T cell responses can ameliorate
HIV-1 infection or disease in humans.

DNA priming together with attenuated pox-virus boost-
ing for HIV vaccination has been described previously by
our group [4,19,32] and others [6–8,33–35]. Several aspects
differentiated this study from previous DNA prime/poxvirus
boost studies. First, a series of mutations were introduced into
the HIV-1 proteins expressed by the DNA and rFPV vaccines
to address potential safety concerns. Second, an abbreviated
vaccination schedule was studied and found effective. Third,
we demonstrated no further enhanced T cell immunogenic-
ity following a second rFPV boost suggesting the regimen
might be able to be abbreviated still further. Fourth, rFPV was
used as the boosting vector which is unlikely to have signifi-
cant cross-reactive immunity against vaccinia viruses. Fifth,
primate-optimised CpG motifs were included in the DNA
priming vaccination, potentially improving priming ability
of the DNA vaccination, although further analysis with more
definitive comparative primate studies is required. Lastly, in-
corporating multiple HIV-1 genes (gag, pol, tat, rev, vpu, env,
nef) in the DNA vaccine may have facilitated a broad-based
immune response.

This study confirms previous findings of non-sterilising
immunity associated with the cellular immunity induced by
the DNA prime/poxvirus boost approach. The non-
pathogenic nature of the challenge stock does not allow a
rigorous analysis of protective immunity. However, the study
did demonstrate lower and more transient plasma HIV-1 RNA
concentrations following challenge, and a brisk but aborted
rise in anamnestic antibodies following challenge. The detect-
ing of persisting levels of cell-associated HIV-1 DNA, even
in this non-pathogenic model, is of concern given the propen-
sity of HIV-1 to mutate and escape immune responses. The
degree of protection from pathogenic SHIV challenge (with
much higher levels of viremia) by DNA and rFPV vaccines
is currently being assessed.

The lack of enhancement of T cell immune responses by
the cytokines in the rFPV boost was surprising [18,19]. Both
the IFNγ and IL-12 co-expressing prime/boost regimens in-
duced significantly inferior HIV-specific T cell responses,
suggesting a negative immunomodulatory affect. The find-
ing in both ELISPOT and lymphoproliferation assays was
corroborated using multiple, separate HIV-1 antigens.

Possible explanations for the reduced HIV-specific T cell
immunogenicity in the DNA/rFPV/IFNγ and DNA/rFPV/IL-
12 regimens were explored by analysing anti-FPV humoral
immunity. Greater FPV antibody responses in the DNA/rFPV
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The vaccinations were well tolerated and high levels of HIV-
specific T cell responses were induced in the DNA and rFPV
prime/boost arms. If similar safety and immunogenicity is ob-
FPV-specific T cell immunity may also have been modulated by the cytokine co-expression in the rFPV boost. Preliminary attempts to examine FPV-specific T cell immunity by ELISPOT using whole live FPV as an antigen were hampered by large background T cell responses to contaminating chicken embryo dermal cells used to grow the rFPV (data not shown). The large FPV genome size makes it difficult to identify FPV-specific immunodominant T cell responses in outbred macaques. Anti-FPV immunity most likely also resulted in the lack of enhancement of T cell immunity to HIV following a second rFPV boost.

Planned studies to further evaluate these promising vaccines will address several outstanding issues. First, we will assess a similarly expanded antigen content in the rFPV boosting vaccine. This strategy should further broaden immune responses [12,13]. Second, we will assess protection against a pathogenic SHIV challenge in macaques. Only pathogenic models with high levels of viremia will provide further pre-clinical information on the limitations of this HIV vaccine approach and assist in evolving strategies to improve them. Third, we will consider assessing co-delivery of a pro-T cell cytokine gene in the DNA priming vaccination. Several groups have shown this to be a promising approach [3,6]. Including the cytokine gene together with the DNA priming vaccine may have advantages over co-expressing it with the rFPV boost, since the immunomodulatory effect of the cytokine can only be directed against the inserted HIV genes in the DNA vaccine as no other genes are expressed by plasmid vector. Fourth, we will assess administration of the DNA vaccine alone for immunogenicity and efficacy. The DNA vaccine clearly primed an immune response and the efficacy of this approach alone against a SHIV challenge is of interest. Fifth, we will evaluate further refinements of schedule (e.g., a single DNA prime) and dose. Sixth, we will assess mucosal immunity, which may be of additional benefit in protection against mucosally transmitted HIV. Seventh, we will study vaccines expressing antigens from HIV-1 subtype A/E suitable for eventual use in Asia. Last, we will progress these vaccines to clinical trials.

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References


