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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.

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 vaccine to date. HIV-specific T cell responses may facilitate control of HIV-1 infection since these responses correlate with the control of acute HIV-1 viremia [1] and depletion of CD8 T cells results in rises in viremia in simian immunodeficiency virus (SIV) infected macaques [2]. The induction of simian/human immunodeficiency virus (SHIV)-specific T cell responses in macaques also correlates with protective immunity [3].

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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 viably 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 oligonucleotides [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 immunity [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, Zn^{2+} fingers of Gag to ameliorate RNA packaging). The modified HIV-1 genome was inserted into the plasmid DNA vaccine vector pHIS-64 (Dr. Heather Davis, Coley Pharmaceuticals), containing kanamycin-selection, a CMV promoter, the bovine growth hormone polyA signal, and 14 primate-optimised CpG immunostimulatory sequences.

Vaccine rFPV, expressing mutated Gag/Pol, was constructed using the *gag*/*pol* from the 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-*gag*/*pol* 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 P.E/L promoter immediately downstream of the FPV TK gene to construct separate rFPV co-expressing these pro-T cell cytokines. Early poxvirus transcription terminators, T5NT, 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 nemestrina* 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 had 80% power (two-sided significance test of 5%) to detect 1.26-fold differences 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
Vaccine regimen and dose

Vaccine regimen	*N*	Week				
		0	3	6	10	17 (challenge)
DNA/rFPV	6	DNA[a]	DNA	rFPV[b]	rFPV	HIV-1[c]
DNA/rFPV-IFNγ	6	DNA	DNA	rFPV-IFNγ	rFPV-IFNγ	HIV-1
DNA/rFPV-IL-12	6	DNA	DNA	rFPV-IL-12	rFPV-IL-12	HIV-1
Controls	4	Placebo	Placebo	Placebo	Placebo	HIV-1
rFPV-IFNγ	4	None	rFPV-IFNγ	rFPV-IFNγ	rFPV-IFNγ	HIV-1
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: 5×10^7 plaque forming units (pfu) (as 5×10^7 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_{MN} (10 μg/mL, kindly provided by Dr. J. Lifson, National Cancer Institute (NCI), Frederick, MD), a single concentrated pool of 122 15mer HIV-1_{HXB2} 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 ^{3}H-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_{MN} 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_{LAI}, 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_{LAI} isolate K98227/W35 and maintained in RF10 media supplemented with rHuman 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^{TM}, 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 IFNγ producing cells specific for whole inactivated HIV-1 and HIV-1 Gag peptides compared to control macaques ($P < 0.05$, Fig. 1b and c). However, in the two groups receiving rFPV vaccines alone, only low levels of HIV-specific IFNγ producing T cells, that were not significantly greater than control-vaccinated macaques ($P > 0.05$), were observed (Fig. 1b).

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 not co-expressing cytokines was significantly greater than the ELISPOT responses from the DNA/rFPV-IFNγ group (Fig. 1b and c). At week 15, post-vaccination, the *P*-value for the difference between the DNA/rFPV regimen and the DNA/rFPV-IFNγ regimen was $P = 0.008$ (Fig. 1b). On day of challenge, the *P*-value between these two groups was $P = 0.04$ to whole inactivated HIV-1, and $P = 0.03$ to HIV-1 Gag peptides (Fig. 1c). Similarly, responses from the DNA/rFPV group were significantly greater than those for the DNA/rFPV-IL-12 following all vaccinations (Fig. 1b; $P = 0.01$), but were not significantly enhanced on the day of challenge (Fig. 1c; $P = 0.10$ using whole inactivated HIV-1, and $P = 0.16$ HIV-1 Gag peptides).

HIV-specific lymphoproliferative responses, which generally detect CD4+ T-helper responses, were assessed to whole inactivated HIV-1 antigen and HIV-1 P55 Gag protein. One week following two DNA vaccinations alone (week 4), no 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 ($P > 0.05$).

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; this 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 vaccination prime in only 6 of the 18 macaques receiving the DNA prime (example shown in Fig. 3b, week 6). 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

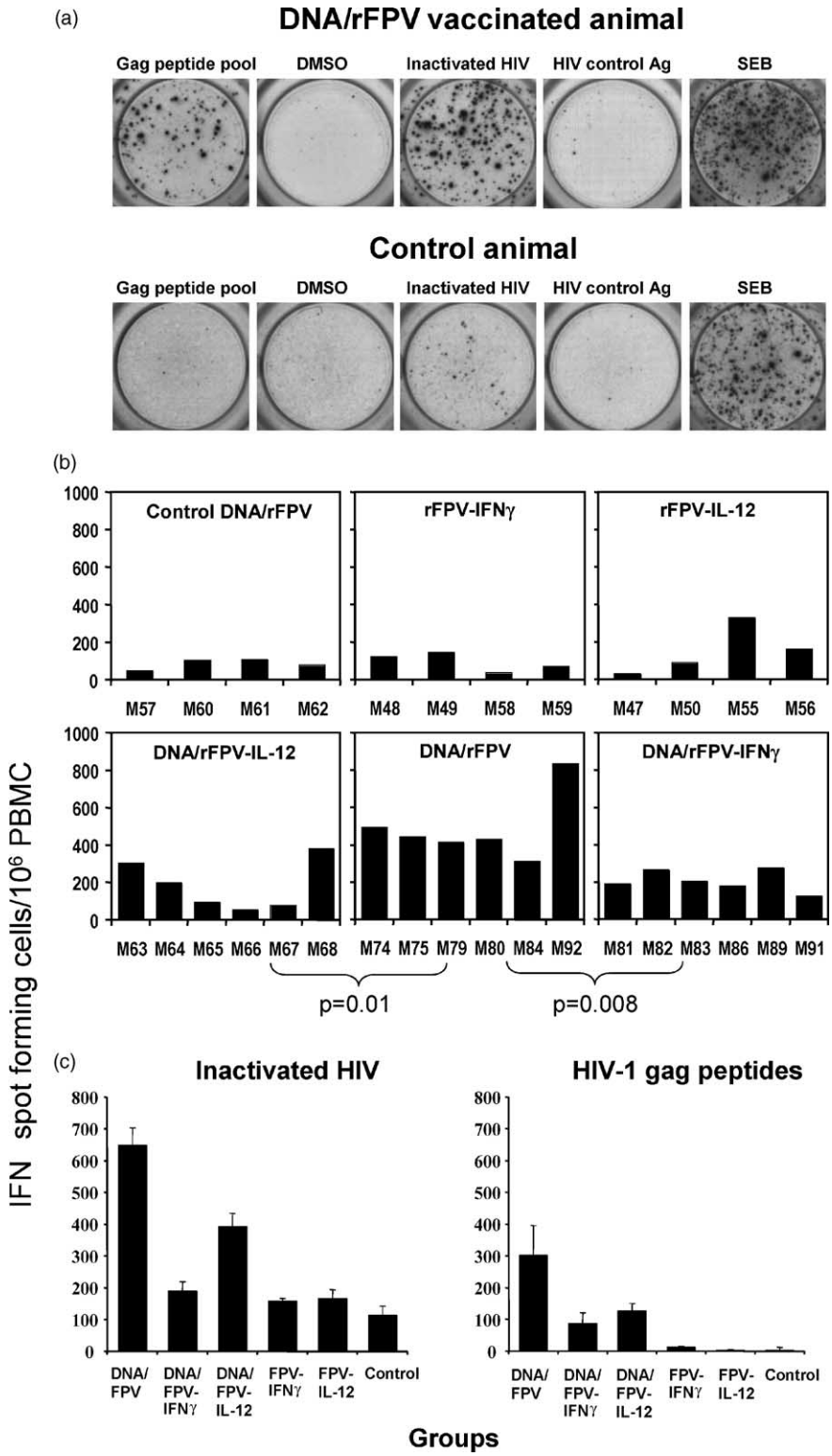

Fig. 1. (a) Example of HIV-specific IFNγ ELISPOT result to both whole inactivated HIV-1 and a single pool of HIV-1 Gag 15mer peptides in PBMC (week 17) from a macaque vaccinated with control empty DNA and rFPV vaccines (Control) and a macaque vaccinated with HIV-1 expressing DNA and rFPV vaccines (DNA/rFPV). 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.

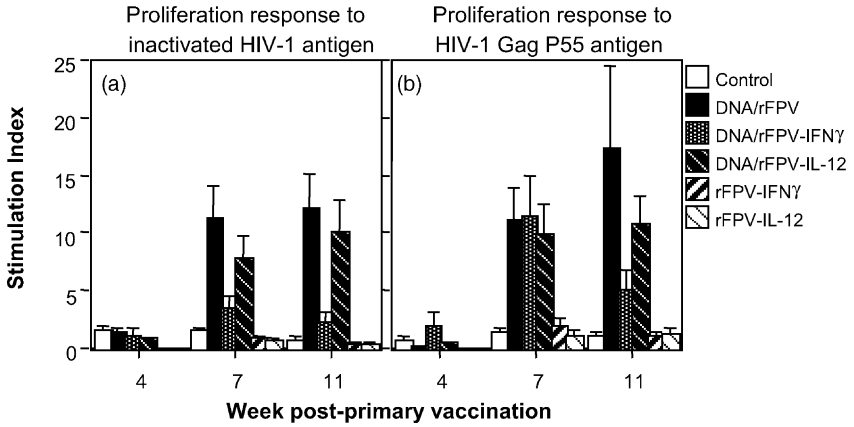

Fig. 2. Lymphoproliferative responses in PBMC of macaques to: (a) whole inactivated HIV-1; and (b) HIV-1 P55 Gag protein. Mean and standard error of the mean proliferative responses across vaccine regimens at week 4 (1 week after the second DNA vaccination in the prime/boost groups—the rFPV only groups were not analysed at this time), week 7, after the first rFPV boost, and week 11, after the second rFPV boost are shown.

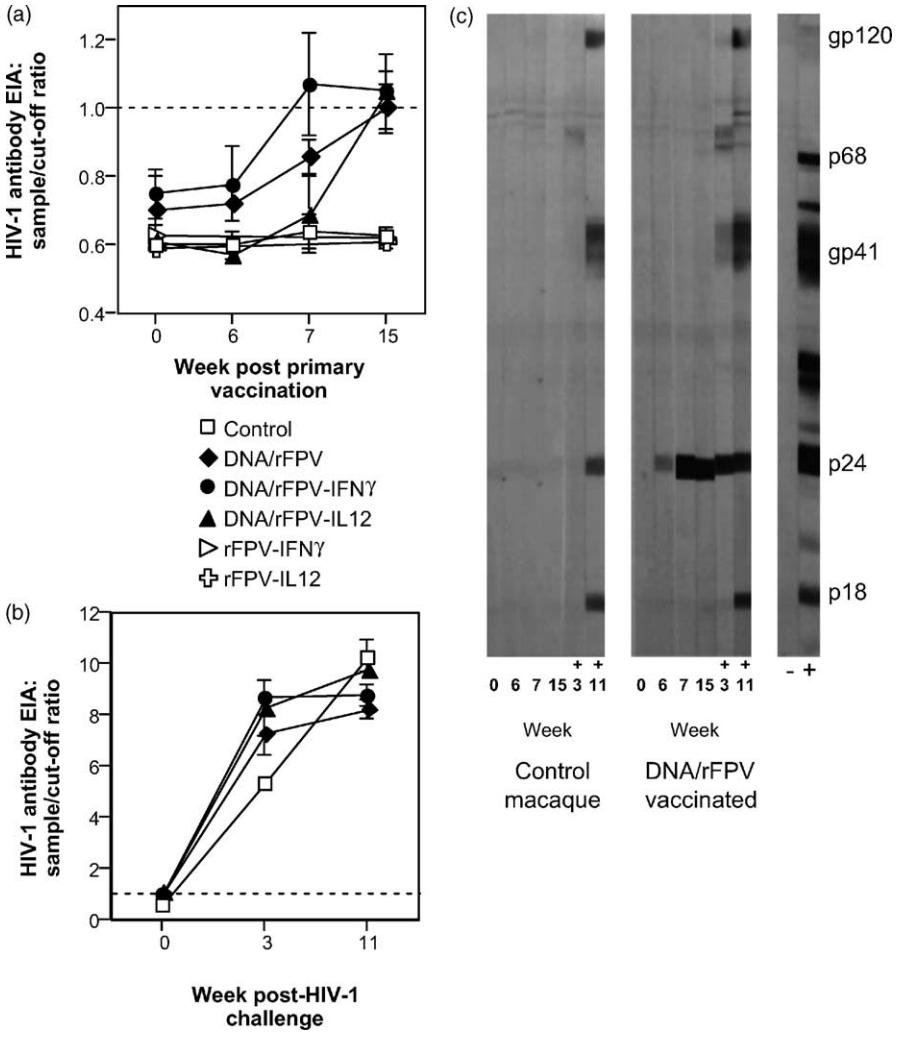

Fig. 3. (a) HIV-1 antibody responses to vaccination by EIA. Mean and standard error of the mean across vaccine regimens are shown. (b) HIV-1 antibody responses following HIV-1 challenge by EIA. (c) Example of HIV-1 Western blot in a control macaque and a DNA/rFPV vaccinated macaque prior to vaccination (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/rFPVèIFNγ ($P < 0.01$) 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-IFNγ 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.

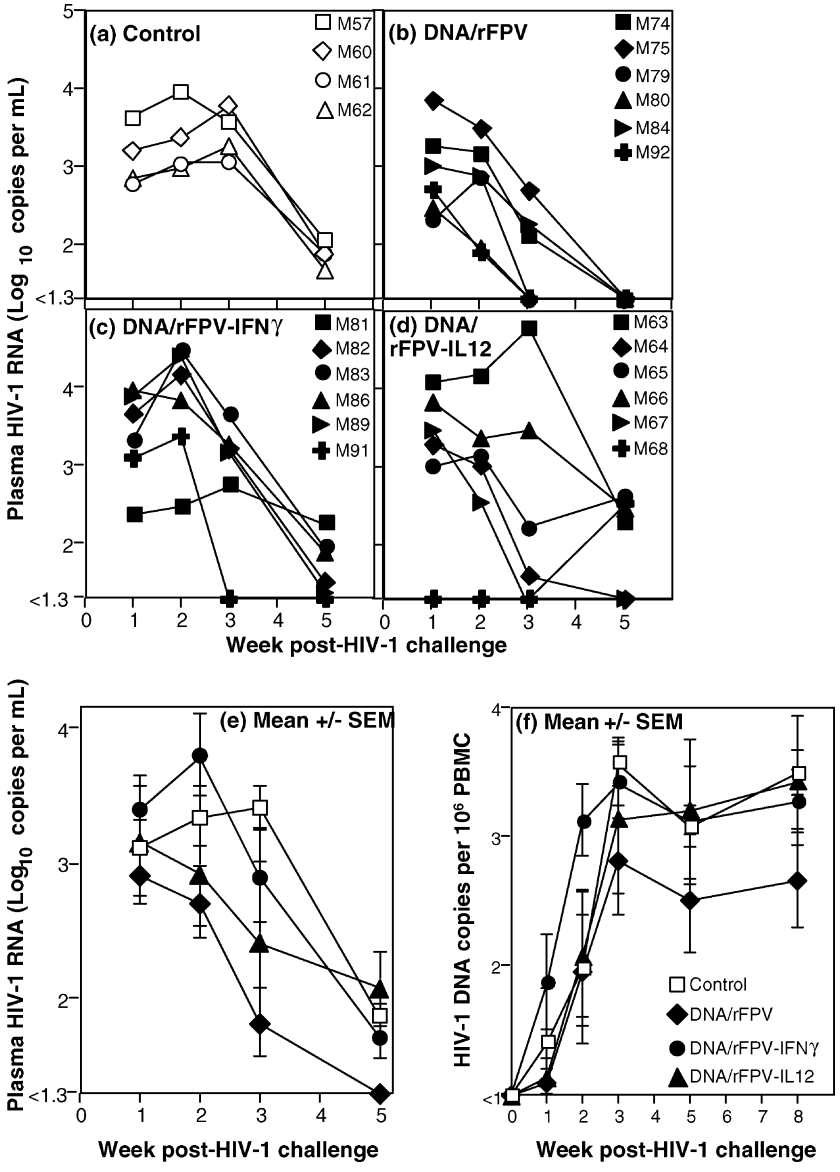

Fig. 4. Plasma HIV-1 RNA levels by RT-PCR following HIV-1 challenge. Individual time-courses are noted in (a)–(d) in four groups, and the mean and standard error of the mean for each group is shown in (e). Panel (f) shows the cellular HIV-1 DNA detected by real-time PCR (mean and standard error of the mean for each group). Symbols used in panel (e) are the same as defined in panel (f).

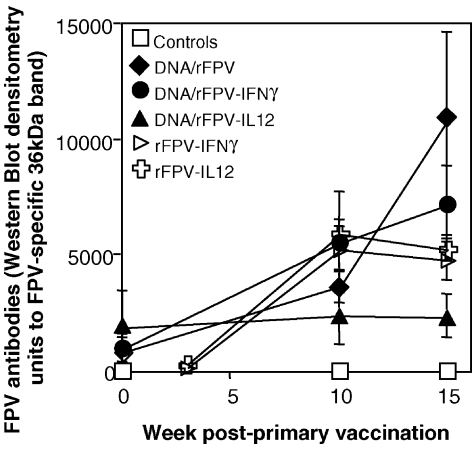

Fig. 5. FPV antibody responses following vaccination determined by densitometry of the FPV-specific 36 kDa band. Mean and standard error of the mean across vaccine regimens is presented. Control plasma was taken from macaques which did not receive DNA or FPV vaccines.

Anamnestic antibody responses to HIV-1 challenge showed more rapid rises in antibody titers in macaques receiving the three prime/boost regimens compared to controls 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 observed 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 following rFPV vaccinations (data not shown) and antibody responses 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 quantified by densitometry of the FPV-specific 36 kDa band after all vaccinations (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 observed 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 boosting 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 immunogenicity 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 significant 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, incorporating 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 detection of persisting levels of cell-associated HIV-1 DNA, even in this non-pathogenic model, is of concern given the propensity 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 induced significantly inferior HIV-specific T cell responses, suggesting a negative immunomodulatory affect. The finding 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 group were observed in comparison to the DNA/rFPV-IL-12 regimen. Co-expression of IL-12 may have enhanced the elimination of the FPV vector by FPV-specific T cells, resulting in decreased FPV and HIV antigen expression and consequently poorer immunogenicity. Although the rFPV vector is non-replicating, durable persistence and expression of the inserted antigen may be required for optimal immunity.

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 preclinical 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 [3,36]. 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 administration of the vaccines for the induction of 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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