Subtype AE HIV-1 DNA and recombinant Fowlpoxvirus vaccines encoding five shared HIV-1 genes: safety and T cell immunogenicity in macaques

Robert De Rose a,1, Socheata Chea a,1, C. Jane Dale a,1, Jeanette Reece a, Caroline S. Fernandez a, Kim M. Wilson b, Scott Thomson c, Ian A. Ramshaw d, Barbara E.H. Coupar d, David B. Boyle d, Mark T. Sullivan a,e, Stephen J. Kent a,∗

a Department of Microbiology and Immunology, University of Melbourne, Royal Parade, Vic. 3010, Australia
b National Serology Reference Laboratory, St Vincent’s Institute of Medical Research, Fitzroy, Vic. 3065, Australia
c John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia
d CSIRO Livestock Industries, Geelong, Vic. 3220, Australia
e National Centre in HIV Epidemiology and Clinical Research, University of NSW, Sydney, NSW 2010, Australia

Received 20 August 2004; received in revised form 8 October 2004; accepted 14 October 2004

Abstract

To induce broad T cell immunity to HIV-1, we evaluated the safety, immunogenicity and dose-response relationship of DNA and recombinant Fowlpoxvirus (rFPV) vaccines encoding five shared HIV subtype AE genes (Gag, Pol, Env, Tat, Rev) in pigtail macaques. The DNA (three doses of either 1 mg or 4.5 mg) and rFPV (a single boost of either 5 × 10^7 or 2 × 10^8 plaque forming units) vaccines were administered intramuscularly without adjuvants. Broadly reactive HIV-specific T cell immunity was stimulated by all doses of the vaccines administered, without significant differences between the high and low doses studied. The vaccines induced both CD4 and CD8 T cell responses to Gag, Pol, Env and Tat/Rev proteins, with CD4 T cell responses being greater in magnitude than CD8 T cell responses. The vaccine-induced T cell responses had significant cross-recognition of heterologous HIV-1 proteins from non-AE HIV-1 subtypes. In conclusion, these subtype AE HIV-1 DNA and rFPV vaccines were safe, induced broad T-cell immunity in macaques, and are suitable for progression into clinical trials.

Keywords: HIV-1; Subtype AE; DNA; Fowlpoxvirus; Macaque

1. Introduction

The precise immune correlates of protection from HIV-1 are not clear. HIV-specific T cell responses facilitate partial 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 in most studies [3]. Although the generation of broadly reactive neutralizing antibodies by vaccination would be ideal, they have not been reliably induced by any viable HIV vaccine to date.

Many HIV vaccine strategies designed to induce T cell immunity either encode a limited number of the nine HIV-1 genes (often just Env and/or Gag) and/or encode multiple individual CD8 T cell epitopes [4–6]. Both these approaches may restrict the breadth of T cell responses generated in outbred populations with diverse MHC genes. It is now clear that the smaller regulatory genes are important targets for T cell immunity [7,8]. Further, since T cell immune responses
can be avoided by sequence diversity within HIV [9–11], inducing T cell responses recognising multiple epitopes across as much of the HIV proteome as possible is likely to restrict opportunities for the selection of viral escape mutations.

The critical role of HIV-specific CD4 T cells in controlling HIV-1 infection in humans has also become clearer in recent years. HIV-specific CD4 T cell responses correlate with the control of viremia during acute infection and antiretroviral treatment interruptions [12,13], however, these cells are also common targets for virus-induced destruction and dysfunction [14]. Induction of HIV-specific CD4 T cells by vaccines is likely to assist in the generation of effective virus-specific memory CD8 T cell responses [15,16].

The majority of preclinical and clinical vaccine evaluation has been focussed on subtype B HIV-1 strains predominating in the Western world. However, by far the greatest burden of HIV-1 infection is occurring in the developing world, where non-subtype B HIV-1 strains predominate. Subtype AE HIV-1 strains are common in large epidemics in many South-east Asian countries, including Thailand. The impact of the significant sequence diversity between subtype B and non-subtype B strains on vaccine efficacy using monovalent vaccines is unknown [17]. Matching the HIV-1 subtype of the vaccine antigens with common circulating strains within communities most at risk may be helpful both for inducing the most relevant immunity and facilitating the political commitment to conduct clinical trials of such vaccines in less developed countries.

Consecutive heterologous immunisation involving priming by DNA vaccination and boosting with recombinant poxvirus vectors such as fowlpoxvirus (rFPV) encoding common HIV antigens is a useful strategy to induce T cell immunity [4,18–21]. We have previously reported the utility of subtype B vaccines expressing a narrow set of shared HIV-1 antigens (Gag and Pol) in macaques and these B subtype vaccines have entered a proof-of-principle clinical trial in Australia [22]. Although doses of 1 mg of DNA and 5 x 10^7 plaque-forming units of rFPV have been immunogenic in our previous macaque studies [22], other studies of DNA/poxvirus combinations suggest higher doses may be more immunogenic in human trials [21]. This study evaluated the safety and immunogenicity of low and high doses of HIV-1 subtype AE DNA and rFPV vaccines in macaques. The T cell immunity induced was analysed for breadth, phenotype and cross-subtype recognition.

2. Methods

2.1. Vaccines

The subtype AE HIV-1 DNA and rFPV vaccines were based on the design of similar subtype B vaccines described elsewhere [22,23] (Purcell et al., manuscript in preparation). The DNA vaccine (pHIS-HIV-AE) encoded approximately two-thirds of the AE subtype p93TH253 provirus derived from Thailand. pHIS-HIV-AE encodes sequences expressing modified Gag, modified RT, Protease, modified mRNase H, Rev, Tat, truncated Nef and Env containing a deletion in the middle one-third of the gene that included the CD4 binding region. HIV-1 genes whose function posed a theoretical risk were either deleted (Integrase, LTRs, Vif, Vpr) or mutated (RT, Zn2+ fingers of Gag to ameliorate RNA packaging). The modified HIV-1 genome was inserted into the plasmid DNA vaccine vector pHES-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.

The single rFPV vaccine (rFPV-HIV-AE) encoded identical sequences for the HIV-1 AE Gag, Pol, Env, Tat and Rev antigens as the DNA vaccine, driven off the early/late FPV promoter, the bovine growth hormone polyA signal, and 14 primate-optimised CpG immunostimulatory sequences.

The single rFPV vaccine (rFPV-HIV-AE) encoded identical sequences for the HIV-1 AE Gag, Pol, Env, Tat and Rev antigens as the DNA vaccine, driven off the early/late FPV promoter at three separate insertion sites as previously described [23]. Tat and Rev were were expressed as a fusion protein. As with the DNA vaccine, the Pol antigens in the rFPV (RT, Protease) are expressed via the native viral sequences for the HIV-1 AE Gag, Pol, Env, Tat and Rev antigens as the DNA vaccine, driven off the early/late FPV promoter at three separate insertion sites as previously described [23]. Tat and Rev were were expressed as a fusion protein. As with the DNA vaccine, the Pol antigens in the rFPV (RT, Protease) are expressed via the native viral sequences.

2.2. Macaques and vaccinations

Juvenile Macaca nemestrina were housed under physical containment level three 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 used groups of three macaques per dose as shown in Table 1.

2.3. Power and statistical considerations

The study was primarily powered to detect two-fold differences in IFN-γ ELISPOT responses between regimens of six animals receiving either a high or low dose of rFPV as per our factorial design. Previous standard deviations in ELISPOT assays between similarly immunised macaques, predicted that a six versus six-macaque comparison had 80% power (two-sided significance test of 5%) to detect less than two-fold differences in HIV-specific IFN-γ ELISPOT responses.

2.4. HIV-1 AE peptide antigens

To evaluate T cell immunity in vitro, 15 mer peptides overlapping by 11aa were constructed (>80% purified) spanning the HIV-1 subtype AE vaccine antigens modified Gag (116 peptides), Pol (modified RT, Protease, modified RNase H, 173 peptides), deleted Env (164 peptides), Tat (23 peptides) and Rev (29 peptides) (Auspep, Parkville, Australia). Peptides were solubilised in pure DMSO at high concentrations (1 mg peptide/10–40 μl DMSO) and peptides for each antigen pooled. To evaluate inter- and cross-subtype T cell responses, 15 mer Gag peptides overlapping by 11aa spanning
Table 1

<table>
<thead>
<tr>
<th>Group (n=3)</th>
<th>Dose of pHIS-HIV-AE/rFPV-HIV-AE</th>
<th>pHIS-HIV-AE (IM)</th>
<th>rFPV-HIV-AE (IM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=3)</td>
<td>Low/low</td>
<td>1.0 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0 x 10^7 pfu</td>
<td></td>
</tr>
<tr>
<td>2 (n=3)</td>
<td>Low/high</td>
<td>1.0 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 x 10^8 pfu</td>
<td></td>
</tr>
<tr>
<td>3 (n=3)</td>
<td>High/low</td>
<td>4.5 mg</td>
<td>4.5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5 mg</td>
<td>4.5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0 x 10^7 pfu</td>
<td></td>
</tr>
<tr>
<td>4 (n=3)</td>
<td>High/high</td>
<td>4.5 mg</td>
<td>4.5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5 mg</td>
<td>4.5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 x 10^8 pfu</td>
<td></td>
</tr>
</tbody>
</table>

Consensus HIV-1 subtypes A, B and C and B subtype Env peptides (HIV-1MN) were kindly supplied by the NIH AIDS reagent repository and solubilized in DMSO and pooled as above.

2.5. IFN-γ ELISPOT responses

Fresh macaque peripheral blood mononuclear cells (PBMC) were stimulated with peptide pools as above or with whole aldrithiol-2 inactivated HIV-1MN (5 μg/ml, kindly provided by Dr. J. Lifson, National Cancer Institute (NCI), Frederick, MD). PBMC were also stimulated with 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). Samples were assayed using the monkey IFN-γ ELISPOT kit (U-CyTech Bv, Utrecht, Netherlands), according to the manufacturer’s instructions and as previously described [26]. Spots were counted using an automated counter (Autoimmun Diagnostika GmbH, Strassberg, Germany).

2.6. Intracellular cytokine staining (ICS)

Intracellular IFN-γ secretion was assessed by flow cytometry as previously described [27,28]. In short, 200 μl whole blood was incubated with HIV-1 AE peptides as described above at 1 μg/ml along with co-stimulatory antibodies anti-CD28 (clone L293) and anti-CD49d (clone L25.3) (BD Biosciences, PharMingen, San Diego, CA) for 2 h at 37°C. 5% CO2, 10 μg/ml Brefeldin A (Sigma, St Louis, MO), was then added and blood incubated for a further 5 h. The cells were then stained with anti-CD4-FITC (clone M-T477), anti-CD3-PE (clone SP34) and anti-CD8-PerCP (clone SK1) (BD Biosciences) for 30 min at room temperature. Erythrocytes were lysed using FACS lysing solution (BD), and remaining cells washed with PBS and permeabilised with FACs permeabilising solution (BD). Cells were then incubated with anti-IFN-γ-APC (clone B27, BD) and formaldehyde-fixed before acquisition of flow cytometry data (BD FACScan). Antigen-specific CD4 and CD8 T cell responses were assessed as the percentage of CD3+8+ or CD3+4+ gated lymphocytes expressing IFN-γ above control-stimulated cultures.

2.7. HIV-1 antibody responses

Antibodies to HIV-1 were tested by a competitive enzyme immunoassay (EIA, Murex HIV-1.2.0, Murex Biotech, Dartford, UK) and by western blot using 7.5 μg of standardised HIV-1 subtype B viral lysate per strip [29].

3. Results

3.1. Reactogenicity of the DNA and rFPV vaccines in macaques

Four groups of three pigtailed macaques received three low or high doses of DNA priming vaccinations followed by a single rFPV booster vaccination, all at 4-weekly intervals (Table 1). The juvenile macaques remained healthy following the vaccinations and gained weight normally (Fig. 1A). No injection site reactions were observed, even at the higher doses of the vaccines. 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. A transient 1.6-fold mean elevation in total lymphocyte counts was observed after the rFPV boost, likely consistent with vaccine immunogenicity (Fig. 1B). Additional detailed pre-clinical toxicology experiments performed in mice did not reveal any organ pathology (unpublished data).

3.2. T cell immunogenicity by IFN-γ ELISpot

HIV-specific IFN-γ production from T cells was quantified longitudinally in all animals by ELISpot (Fig. 2). The vaccines were immunogenic at week 13, one week after the final vaccine, the primary endpoint. Eleven of the 12 vaccinated macaques produced a positive response to Gag (≥50 spots/10^6 PBMC). A mean (±S.D.) of 270 ± 158 HIV-1 Gag-AE-specific IFN-γ spot forming cells/10^6 PBMC was observed at week 13, compared to a mean of 4 ± 6 spot forming cells/10^6 PBMC prior to vaccination. T cell immune responses to HIV-1 Gag by ELISpot declined from the peak responses at week 13–14 to low levels by week 22.

At week 13, responses of similar amplitude were also detected to Env in all 12 animals (mean 242 ± 160 spots/10^6 PBMC) even though the Env encoded within the vaccines had a large deletion in the middle third of the protein (Fig. 2A, triangles). Interestingly, responses to Pol peaked later than Gag and Env, being greatest at week 16 (Fig. 2A, diamonds). Pol-specific responses at week 16 were detected in all 12 macaques and were of similar magnitude to peak Env and Gag responses (mean 275 ± 146 spots/10^6 PBMC).

This broad recognition of HIV-1 subtype AE antigens is also reflected in an elevated response to whole inactivated HIV virions (mean 313 ± 197 spots/10^6 PBMC) even though this reagent measures cross-subtype responses (B subtype strain, HIV-1MN). However, no significant responses were detected to Tat or Rev antigens by ELISpot, (mean 18 ± 19 and 25 ± 23 spots/10^6 PBMC, respectively, compared to 5 ± 1 and 6 ± 3 pre-vaccination). Two macaques had detectable responses (≥50 spots/10^6 PBMC) to Rev and one to Tat at week 13, but all were ≤70 spots/10^6 PBMC.

There was significant cross-subtype recognition of both Gag and Env peptide pools from HIV-1 subtypes heterologous to the vaccine antigens (Fig. 2B). Gag peptides generated from consensus HIV-1 subtypes A, B and C were all reactive in the ELISpot assay, albeit with less reactivity compared to Gag AE peptides homologous to the vaccine. Similarly, subtype B Env was also recognised. This cross-subtype reactivity suggests T cell recognition of exposure to heterologous virus strains would occur.

The comparative immunogenicity of high and low doses of the pHIS-HIV-AE DNA and rFPV-AE vaccines was evaluated at week 13, one week after the final vaccine was administered. In this 2 × 2 factorial design, six macaques received a low dose of DNA vaccine (1.0 mg) and six received a high dose (4.5 mg). Similarly, the six high dose (2 × 10^6 pfu) recipients of the rFPV-HIV-AE vaccine were compared to the six low dose (5 × 10^5 pfu) vaccinated animals (Fig. 2C). There were no statistically significant differences in T cell immunogenicity by ELISpot between doses, with a mean ±95% CI of the HIV-1 Gag AE-specific response in the six high dose pHIS-HIV-AE vaccinated animals of 177 ±69 spots/10^6 PBMC versus 363 ± 130 for the six low dose vaccines (p = 0.08). Similarly, the six high dose rFPV-HIV-AE vaccines had
Fig. 3. Phenotype and magnitude of HIV-1 AE specific T cell responses by IFNγ-ICS. A: HIV AE Gag specific CD4 and CD8 T cell responses (mean ± S.E.) over time (weeks after first vaccination) is shown. B: CD4 (closed bars) and CD8 (open bars) T cell responses (mean ± S.E.) to several HIV-1 AE proteins (Gag, Pol, Env peptide pools and a combined Tat and Rev peptide pool) at week 13, one week after the rFPV-HIV-AE boost. C: A comparison of CD4 (left side) and CD8 (right side) T cells responses (mean ± 95% CI) to HIV AE Gag at week 13, one week after rFPV-HIV-AE boost. Low dose pHIS-HIV-AE and rFPV-HIV-AE vaccinations (open bars) vs. high dose vaccinations (closed bars).

318 ± 118 versus 222 ± 132 spots/10^6 PBMC for the six low dose vaccines (p=0.31). This dose-response pattern was also observed to whole, inactivated HIV-1 particles (high dose pHIS-HIV-AE = 209 ± 118, low dose pHIS-HIV-AE = 418 ± 154 and high dose rFPV-HIV-AE = 416 ± 161 versus 211 ± 111 for the low dose).

3.3. CD4+ and CD8+ T cell immunity by IFNγ intracellular cytokine staining

To phenotype HIV-specific T cell immunity following the vaccinations, we performed intracellular cytokine staining on fresh whole blood samples (Fig. 3). All 12 vaccinated animals produced significant CD4 T cell responses to Gag at week 13 (mean percentage of responding CD4 T cells was 0.66%, 95% CI 0.40–0.93%). Seven of the 12 vaccinated animals had a detectable (>0.05%) CD8 T cell response to Gag at week 13 although these were lower than the Gag-specific CD4 T cell responses (mean of seven responses was 0.15%, 95% CI 0.07–0.23%). Mean baseline responses of CD4 or CD8 T cell responses to Gag prior to vaccination were ≤ 0.02%.

Strong Env and weaker Pol and Tat/Rev-specific T cell responses were also detected following the rFPV-HIV-AE boost (Fig. 3B). Env was the most immunogenic of the five HIV-1 proteins encoded by the vaccine, with all 12 macaques developing CD4 T cell responses to Env (mean 0.67%, 95% CI 0.34–1.00%). All 12 macaques also had Env-specific CD8+ T cell responses (mean 0.12%, 95% CI 0.07–0.16%). Responses to Pol were also prevalent at week 13 by ICS (Fig. 3B). Ten of the 12 vaccinees developed Pol-specific CD4+ T cell responses (mean of responding animals 0.09%, 95% CI 0.07–0.11%) and six of the 12 developed weak CD8 T cell responses (mean of responding animals 0.19%, 95% CI 0.05–0.33%).

Responses to Tat and Rev were recognised with better efficiency by ICS compared to ELISpot. Ten of 12 macaques generated moderate levels of CD4 T cells responding to Tat/Rev (mean of responding animals 0.26%, 95% CI 0.13–0.39%). CD8 T cell responses to Tat/Rev were also detected in the same 10 macaques (mean of responding animals 0.17%, 95% CI 0.08–0.26%).

T cell responses were assessed by ICS at week 9, one week after the third and final DNA vaccination. Weak CD4 T cell responses to Gag were detected in nine of the 10 vaccinees assayed (mean 0.16%, 95% CI 0.12–0.21%). However, CD8 T cell responses were only detected in three vaccines (mean 0.23%, 95% CI 0.03–0.42%). Although these responses were weak, significant priming of T cell immunity was likely to have been generated following the pHIS-HIV-AE vaccination.

In concordance with the ELISpot data, there were no significant differences in immunogenicity for high and low doses of each vaccine. Mean Gag-specific CD4 and CD8 T cells responses ± 95% CI in the high dose DNA vaccine recipients were 0.49 ± 0.19% (CD4) and 0.20 ± 0.23% (CD8), respectively, compared to 0.84 ± 0.48% (CD4) and 0.06 ± 0.05% (CD8) receiving the low dose DNA vaccine (Fig. 3C). Similarly, the six macaques receiving the high dose of the rFPV-HIV-AE vaccine did not generate significantly higher responses (CD4, 0.91 ± 0.42% and CD8, 0.13 ± 0.16%) compared to the low dose rFPV-HIV-AE vaccinated macaques (CD4, 0.42 ± 0.22%; CD8, 0.05 ± 0.03%).

3.4. HIV-1 antibodies

Although the primary aim of the pHIS-HIV-AE and rFPV-HIV-AE vaccines was to induce T cell mediated immunity, we also evaluated HIV-1 specific antibodies by a commercial EIA and western blot using standard subtype B proteins. At week 13, 10 of the 12 immunised animals had detectable HIV-specific antibodies by EIA [sample/cut-off ratio (S/CO) > 1] after completion of all immunisations (Fig. 4A). At week
9. one week after the third pHIS-HIV-AE vaccine, there was a rise (>0.2 S/CO) in detectable antibodies in eight of the 12 macaques and four of 12 animals were scored positive (S/CO > 1) for HIV-specific antibodies. There were no significant differences between animals administered high or low doses of pHIS-HIV-AE or rFPV-HIV-AE vaccines (data not shown). By western blot, all 12 animals developed antibody responses to Gag antigens, in particular p24, but there was no recognition of the B subtype Env in this assay (Fig. 4B).

4. Discussion

This dose-ranging pigtail macaque study evaluated novel HIV-1 subtype AE DNA prime/rFPV boost regimens intended for clinical evaluation in South-east Asia. The vaccinations were well tolerated and broad HIV-specific T cell responses were induced at all doses tested. The DNA and rFPV vaccines both encoded subtype AE Gag, Pol (minus integrase), Env (with a deletion in the middle third), Tat and Rev within single constructs. Strong T cell immunity was demonstrated by IFNγ ELISpot to Env, Gag and Pol antigens in almost all animals studied, and additional responses to the smaller Tat and Rev antigens also detected in the majority of immunised animals. By intracellular cytokine staining, most animals had both CD4 and CD8 T cells responding to the various HIV-1 proteins, with the CD4 T cell responses predominating. The very broad array of HIV-1 proteins recognised by both CD4 and CD8 T cells induced by these vaccines could, if replicated in human trials, both facilitate control of HIV-1 replication and limit opportunities for viral escape from T cell recognition.

The lack of a significant enhancement in immunogenicity with the higher doses suggests, in this macaque model, that we are reaching a dose-response plateau for this combination vaccine approach. There was a trend towards reduced T cell responses in the group receiving the lower doses of rFPV, particularly for the ELISpot and CD4+ T cell responses. Other DNA/poxvirus prime/boost studies have suggested that high vaccine doses may be required to consistently induce high levels of T cell immunity in human studies[21]. This study provides supporting safety data demonstrating it is reasonable to cautiously proceed with high dose vaccine studies in humans.

The delayed peak T cell response to Pol antigens detected by ELISpot, occurring 2–3 weeks after the peak responses to Env and Gag responses was intriguing. The Pol enzymes are expressed at much lower levels than the structural Gag and Env antigens in the vaccines, being generated from the native viral Gag/Pol frameshift events. This raises the possibility that, when multiple antigens are expressed at varying levels, the temporal induction of T cell immunity in primates may be dependent on antigen levels expressed. There was a gradual fall to memory levels of T cell immunity by 10 weeks after the last vaccination. Although it is generally assumed that peak levels of viral immunity predict levels of memory T cells in mice [30], there are suggestions that this may not always be true in macaques [31,32], and further evaluation of the functional efficacy of memory T cell responses to HIV-1 is clearly warranted.

Regulatory HIV-1 proteins are key targets of the cellular immune response, frequently recognised early during infection [7,8,33]. Significant levels of Tat- and Rev-specific immunity were detected by ICS following immunisation with these subtype AE DNA and rFPV vaccines. The lower levels of T cell immunity to Tat and Rev proteins, in comparison to Gag/Pol/Env proteins may be due either to the small nature
of these proteins, which may lack multiple T cell epitopes in this group of animals, or the lower levels of expression of these small regulatory genes from the vaccines. It was interesting that T cell recognition of Tat/Rev was more frequently detected by ICS than by ELISpot. Note, however, that the Tat/Rev peptide pools were combined together in the ICS assay whereas the pools were tested separately in the ELISpot assay and this may have increased the sensitivity of the detection of responses to these small antigens in the ICS assay.

Most (10 of 12) animals developed a positive antibody response by a standard commercial EIA after all vaccinations. Western blot utilizing B subtype HIV-1 antigens demonstrated this response was primarily recognising Gag antigens (being also detected in the two animals negative by EIA). It is likely, however, that the reliable detection of Env-specific antibodies will require subtype AE-specific protein reagents (currently being generated), since Env is much more variable than Gag across different subtypes.

The T cell recognition of non-subtype AE antigens following vaccination with HIV-1 AE encoding vaccines is encouraging and consistent with previous observations utilising B subtype vaccines [34,35]. Multiple subtypes frequently co-circulate in many regions of the world and at least partial recognition of heterologous strains should assist the development of protective immunity. Interestingly, T cell responses to consensus HIV-1 subtype A Gag peptides heterologous to the vaccine were essentially equivalent to peptides derived from consensus subtype B and C antigens (but lower than the response to the homologous vaccine antigens). The significant loss of T cell responses between strains within a subtype, but lack of significant further loss of responses across other subtypes suggests that subtype differences may play a relatively less important role in abrogation of T cell recognition.

In summary, this study utilises a novel combination of DNA and rFPV vaccines expressing five shared HIV-1 genes derived from a subtype AE Thai strain. The vaccines were safe and immunogenic at all doses studied and suitable for use in clinical trials in South-East Asia.

Acknowledgements

We thank R. Sydenham, A. Sydenham and K. Szalnowski for expert animal care and all the other members of the Australian Thai HIV Vaccine Consortium for their support and guidance. Financial support: NIH HIV Vaccine Design and Development Team award no. 1AI05395 and the Australian Commonwealth Department of Health and Aging.

References


