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Induction of HIV-1-specific T-helper responses and type 1 cytokine secretion following therapeutic vaccination of macaques with a recombinant fowlpoxvirus co-expressing interferon-gamma

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Abstract: Preventive and/or therapeutic vaccines against Human Immunodeficiency Virus (HIV-1) are urgently required. Induction of cellular immunity is favoured since these responses correlate with control of HIV-1. Recombinant fowlpoxvirus (FPV) vaccines encoding both HIV-1 gag/pol and interferon-gamma (FPV gag/pol-IFNγ) were hypothesised to enhance HIV-specific cellular immunity and were further evaluated in macaques previously infected with HIV-1. A novel assay to detect IFNγ secretion following HIV antigen stimulation of whole blood was developed to further assess the safety and immunogenicity of the FPV gag/pol-IFNγ vaccine. Immunisation with FPV gag/pol-IFNγ safely enhanced HIV-specific IFNγ secretion following *ex vivo* stimulation of whole blood, greater than that observed following FPV gag/pol vaccination not co-expressing IFNγ. Both HIV-specific IFNγ-spot-forming cells by ELISPOT and CD69 expression by $CD4^+$ lymphocytes were also enhanced following FPV gag/pol-IFNγ vaccination. Hence, the FPV-HIV vaccine co-expressing IFNγ stimulated HIV-specific T cell responses in macaques, and should be further evaluated as a therapeutic or preventive HIV vaccine.

Key words: cellular immunity – ELISOT – CD69 – interferon-gamma assay – vaccine vectors

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Introduction

A preventive HIV-1 vaccine is urgently required. In addition, a therapeutic vaccine to facilitate HIV-1 control and slow or halt progressive immunodeficiency would be of great benefit to the millions of people already infected with HIV-1 [37]. However, studies to date have shown no clinical benefit from therapeutic HIV-1 vaccines [12, 28]. This may be due to both the poor T cell immunogenicity of the vaccines evaluated to date, and evaluating vaccines in subjects not on effective antiretroviral therapy [34]. Suppressive antiretroviral drug treatment may be required to partially alleviate the HIV-1 induced immunodeficiency and to restore the host's ability to mount effective immune responses to HIV vaccines [30].

Circumstantial evidence suggests that HIV-specific CD4+ T-helper (Th) and CD8+ cytotoxic T lymphocyte (CTL) responses, rather than antibodies, are critical immune determinants of HIV-1 control. HIV-specific CTL and Th responses have been detected in HIV-exposed but uninfected persons, and are likely to have a role in protection from subsequent HIV-1 exposure [9, 32]. CTL responses temporally correlate with the decline in acute HIV-1 viremia in both humans and animal models [21, 24, 26]. Evidence that an early and vigorous selection for HIV-1 and SIV variants that escape CTL recognition occurs during infection is also consistent with a role for CTL in control of HIV-1 and SIV [3, 13]. No studies have directly addressed the requirement of Th or CTL responses to mediate protective immunity to HIV-1. In one SIV vaccine study, a correlation between SIV-specific CTL and a reduction in early viral load was made; however, this was to vaccine-induced protective immunity in only one animal [14]. Recent studies have shown that depletion of CD8+T cells with monoclonal antibodies results in a dramatic rise in SIV viremia in macaques [39].

Whether HIV-specific Th responses were likely to facilitate control of HIV-1 has been uncertain for many years. Since HIV actively infects, destroys and induces the apoptosis of activated Th cells, few HIV-1 infected individuals harbour detectable HIV-specific Th cells. Studies from HIV-1 infected subjects during early infection or following adequate antiretroviral therapy have suggested that HIV-specific Th responses correlate with the control of HIV-1 viremia [30]. This is consistent

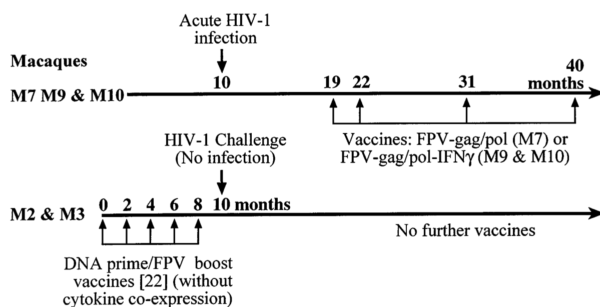

Fig. 1. Vaccine regimen and HIV-1 challenge of macaques. Macaque M2 and M3 were primed with a DNA vaccine encoding HIV-1 env/gag/pol, and boosted with FPVs encoding env and gag/pol, and all macaques were challenged i.v. with HIV-1 during the 10th month of the trial [22]. HIV-1 challenge did not lead to HIV-1 infection of macaques M2 and M3 [22]. Macaques M7, M9 and M10 became acutely infected following challenge, but this infection became latent with detection of only HIV-1 DNA, but not RNA, possible by month 19 [23]. Macaque M7 was immunised four times with the FPV gag/pol vaccine (10^8 pfu), and concurrent FPV gag/pol-IFNγ vaccines (10^8 pfu) were delivered to macaques M9 and M10.

with the general role of Th cells in the induction of CTL responses and supports animal model studies where the generation of both Th and CTL correlate with control of HIV-1 and SIV [20, 21].

Th cells that secrete the cytokines IL-2 and IFNγ (Th1 phenotype) support the generation of CTL responses more efficiently than Th cells secreting alternative cytokines (i.e. Th2, secreting IL4, IL-5 and IL-10), which support humoral immunity. The induction of Th1 responses by HIV vaccines is likely to be beneficial to the maintenance of HIV-specific CTL responses, potentially required for long-term immunogenicity and protective efficacy [19]. The vigorous Th response induced by live, attenuated SIV and HIV-1 strains also supports a role for Th responses for efficient induction of protective immunity [11, 15].

The most commonly used assays to detect and quantify antigen-specific CD8+ and CD4+ T cell responses are the ^{51}Chromium release assay and the lymphoproliferative assay, respectively. Both assays are labour intensive, require prolonged incubation periods for cell expansion, generally require the use of fresh cells for reliable results, and commonly exhibit high inter-assay variability, particularly when the responses are analysed kinetically. Simpler and more reliable assays are clearly required.

Assays developed to meet these requirements to detect and quantify antigen-specific T cells include the detection of early activation markers (e.g. CD69, [6]) and intracellular cytokines [36] by flow cytometry, detection of T cell receptor and epitope complexes by MHC Class I tetramer and flow cytometry [1, 13, 27], and detection of cytokine production by ELISPOT where cytokine-producing cells are detected on plates using biotin-labelled antibodies and the formation of visible spots [35]. MHC tetramer assays are available for detection of SIV-specific T cells in a few MHC class I molecules defined for *Macaca mulatta*, but are not yet available for HIV-1-specific T cells in *Macaca nemestrina* [13].

Another assay for T cell-dependent immunity, which has not to our knowledge been used to study HIV-1 vaccines in non-human primates to date, is an assay for HIV-specific IFNγ production from whole blood. This assay, originally developed to detect mycobacterial-specific IFNγ production in cattle [38] and subsequently humans [33], is simple, does not require the separation of peripheral blood mononuclear cells (PBMC), and has a short (20 hour) incubation period. In this report we developed and evaluated a quantitative whole blood assay for HIV-specific IFNγ production in macaques.

For the initial evaluation of the safety and immunogenicity of novel cytokine co-expressing vaccines, we utilised pigtail macaques (*M. nemestrina*) previously infected with HIV-1 [21, 22] (Fig. 1). *M. nemestrina* are susceptible to acute HIV-1 infection, but after a few months only HIV-1 DNA, and not HIV-1 RNA or culturable virus, can be detected and the animals do not progress to immunodeficiency [21]. HIV-specific T cell responses decline to low memory levels following resolution of acute HIV-1 infection of macaques [21, 23]. Although this model is a very highly attenuated lentivirus infection system, it allows the preliminary evaluation of the ability of novel vaccines to boost HIV-primed T cell responses.

Recombinant poxviruses expressing HIV-1 antigens (including recombinant vaccinia, attenuated vaccinia variants, and avian poxviruses such as canarypox and fowlpox viruses, FPV) have long been proposed as potentially useful for HIV-1 vaccines [17, 18]. Poxviruses are attractive vaccine vectors since large amounts of foreign genetic material can be inserted at a number of well-characterised sites, and the antigens are abundantly expressed intracellularly with the potential to stimulate CTL responses. In human clinical trials, however, avipox-HIV-1 vaccines have induced HIV-specific CTL responses in only a limited proportion of subjects [8]. Co-expression of cytokines by recombinant poxvirus vaccines can dramatically modulate the resulting immune responses in mice (including enhanced T cell responsiveness) [25, 29], but this approach has not been assessed in non-human primates. Insertion sites were introduced into an Australian FPV strain (FPV M3) to accommodate and express multiple foreign proteins. The FPV M3 strains have been used safely as a successful vaccine vector in chickens [16]. Interferon-gamma (IFNγ) is a critical cytokine in the induction of CTL responses and Th1 responses. Utilising a recombinant FPV expressing both IFNγ and HIV-1 gag/pol (FPV gag/pol-IFNγ), the safety and immunogenicity as a therapeutic vaccine in macaques was assessed.

Materials and methods

Vaccines

Vaccines FPV gag/pol (rFPV encoding the HIV-1 gag/pol antigens) and FPV gag/pol-IFNγ (rFPV encoding the HIV-1 gag/pol antigens and human IFNγ) have been described previously [22, 23]. Briefly, for FPV gag/pol, HIV-1_{SF2} *gag/pol* genes were inserted into the FPV M3 genome downstream of the FPV *thymidine kinase*, and expression of the HIV-1 antigens utilised the FPV P.E/L bi-directional promoter. For FPV gag/pol-IFNγ, the human IFNγ gene was cloned into the FPV plasmid vector pAF09 by PCR, and IFNγ expression was under the control of the P.E/L promoter. The *gag/pol* genes were inserted immediately downstream of IFNγ, and gag/pol expression controlled by the vaccinia virus P.7.5 promoter.

Animals and vaccinations

Five *M. nemestrina* were used in this study and have been described previously [22, 23] (Fig. 1). *M. nemestrina* were anaesthetised with Ketamine (10 mg/kg i.m.) prior to all procedures. The five macaques were either mock-immunised (monkeys M2 and M3), immunised with FPV gag/pol (monkey M7), or immunised with FPV gag/pol-IFNγ (monkeys M9 and M10), four times each (Fig. 1). A report in press describes an evaluation after the first two vaccinations, particularly CTL responses (by ^{51}Chromium release assays) and lymphoproliferative responses [23]. All animals were previously challenged intravenously with HIV-1_{LAI} 9 months prior to initiating this study (Fig. 1). Prior to the first immunisation, only HIV-1 DNA and not HIV-1 RNA or culturable HIV-1 could be detected in PBMC from the animals.

Safety assessment of vaccines

Animals were monitored twice daily following vaccination for reactions caused by the vaccines. The activity of the macaques was recorded by counting the total number of behaviour events (foraging, grooming, displacement, mounting, and individual and conspecific play activities) over 15 minutes daily as described [5]. Mean behaviour scores for 1 week prior to vaccination were compared to those following vaccinations. Body weights were also recorded regularly at the time of blood sampling.

To assess possible central nervous system toxicity, cerebrospinal fluid (1 ml) was removed from one of the macaques receiving the FPVgag/pol-IFNγ vaccination (macaque M9) by lumbar puncture 1 week following the third vaccination. The lumbar puncture was performed acceptically under Ketamine sedation using a 23G paediatric lumbar puncture needle through the L3/4 intervertebral space without complications.

Antigen stimulation of whole blood and IFNγ detection by ELISA

Prior to use in this vaccine study, an assay to detect *M. nemestrina* IFNγ secretion from whole blood was optimised using the mitogen con-

canavalin A (Sigma, St Louis, MO), titrating the concentration from 100 μg/ml to 1 μg/ml. Venous blood was collected from the animals prior to, and following, the 4th immunisation (Fig. 1). Heparin-anticoagulated whole blood (0.5 ml) was added to wells of a 48-well culture plate (Corning, Acton MA) and stimulated for 20 hours with 20 μg/ml of Aldrithiol-2 inactivated whole HIV-1 (kindly supplied by L.O. Arthur, J.D. Lifson and J.L. Rossio, AVP, SAIC Frederick, NCI-FCRDC, Frederick MD; [31]). This antigen has been shown to efficiently stimulate lentivirus-primed T cells with low or undetectable levels of stimulation in naïve macaques [2]. Untreated plasma samples from each animal, and whole blood incubated without antigen, served as negative controls in the assay. Assays were set up in duplicate for each antigen on weeks 0 and 2.

Plasma from each of the blood samples was removed following gentle centrifugation (1,000 g) of the plates, and stored at -20°C. Plasma samples and titrated recombinant human IFNγ standards (Genzyme, Cambridge MA) were batched and assayed in duplicate for antigen-specific non-human primate IFNγ using a capture ELISA (PRIMAGAM™; CSL Ltd, Parkville, Australia) utilising methods developed for mycobacterial-specific IFNγ production [10]. Briefly, samples were reacted with the IFNγ specific FA42.2B9 monoclonal antibodies (mAb) conjugated to horseradish peroxidase in ELISA wells coated with $F(ab')_2$ fragments of FA42.1F7 mAb for 2 hours at room temperature as described by Desem and Jones [10]. After washing, wells were incubated with chromophore for 30 minutes. The substrate reaction was stopped using 0.5 M H_2SO_4 and optical densities (OD) were measured using a 450 nm filter (Titertek Instruments, Huntsville AL).

Detection of IFNγ-secreting cells by ELISOPT

PMBC were isolated by density gradient centrifugation (Ficoll-Paque, Amersham Pharmacia, Uppsala Sweden) from heparin-treated blood collected from each animal and stored in liquid nitrogen. PBMC were batch-thawed and stimulated with Aldrithiol-2 inactivated HIV-1 (10 μg/ml), HIV-1_{SF2} p24 protein (10 μg/ml; NIH AIDS Research and Reference Reagent Program, MD) or with media-alone. The assay was batched for all PBMC samples collected over a 5-week period prior to, and following the 4th vaccination. Frozen PBMC, collected from each of the macaques prior to delivery of HIV-1 vaccines or HIV-1 exposure (month 0, Fig. 1; Naïve timepoint Fig. 4), were also tested. Samples were assayed using the monkey IFNγ ELISPOT kit (U-CyTech bv, Utrecht, The Netherlands), according to the manufacturer's instructions. Briefly, 150 μl antigen-stimulated PBMC were counted and incubated in ELISPOT plates pre-coated with anti-IFNγ mAb. 'Spots' were detected using labelled anti-biotin antibodies. Results were normalised for the cell counts and expressed as the number of IFNγ spot-forming cells per 10^6 antigen-stimulated PBMC.

Flow cytometric detection of HIV-1-specific $CD69^+$ lymphocytes

Fresh PBMC were stimulated with HIV-1 antigens (as above) for 16 hours. Stimulated PBMC were washed and incubated with Ortho-mune OKT-4 FITC-conjugated CD4 mAb (Ortho Diagnostic Systems, Raritan, NJ) together with mouse anti-human CD69 mAb conjugated with phycoerythrin (Becton-Dickinson, Franklin Lakes NJ). Stimulated PBMC were also incubated with the mouse γ1/γ2a simultest control antibody (Becton-Dickinson). Cells were analysed using the FACScalibur flow cytometer (Beckman Instruments, Fullerton CA), and the data analysed using Cell-Quest software (Becton Dickinson Immunocytometry Systems). Gated lymphocytes were back-gated for CD4 expression and the proportion of CD69-expressing cells determined.

Results

Safety of FPV vaccines

Systemically administered cytokines can induce significant toxicity. However, cytokines expressed from FPV act by secreting the cytokine at the local level of immune response induction, rather than systemically [29]. The safety of this novel FPV vaccine encoding both HIV-1gag/pol and IFNγ was therefore of interest in non-human primates. No significant adverse effects of the FPV vaccines were noted in the macaques studied. Utilising methodology to enumerate normal playful macaque behaviour activities, previously developed to monitor animal enrichment devices [5], the activities of the macaques were studied prior to and following the immunisations. A transient (1–2 day) reduction in activity was detected in animals immunised with either FPVgag/pol-IFNγ (macaques M9 and M10) or FPVgag/pol (macaque M7, Fig. 2a). This reduction in playful behaviour became less apparent following subsequent vaccinations. Body weights of the macaques were analysed following vaccinations and all the juvenile macaques gained weight normally (Fig. 2b).

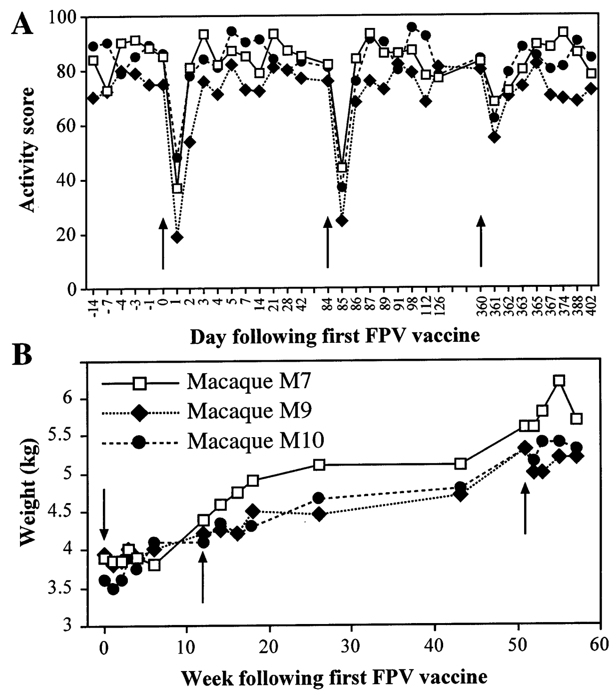

Fig. 2. Safety of FPV gag/pol and FPV gag/pol-IFNγ vaccines. **A**. The number of normal, playful macaque behavioural activities were counted daily over a 15-minute period following FPV vaccinations. **B**. The juvenile macaques gained weight normally over the course of the trial.

Since systemic IFNγ administration could have potential central nervous system toxicity [7], a lumbar puncture was performed 7 days after the third FPVgag/pol-IFNγ vaccination of one of the macaques, M9. The cerebrospinal fluid was clear and colourless and had a cell count of 1 lymphocyte/μl. The glucose and protein levels were normal as determined by automated biochemistry. Thus, no significant long-term central nervous system or behavioural abnormalities were observed following administration of the IFNγ expressing vaccine.

Detection of HIV-specific IFNγ secretion in whole blood

A simple assay was developed to detect HIV-specific macaque IFNγ secretion using whole blood. This whole blood antigen-specific IFNγ assay does not require PBMC separation and has a short incubation. Results were highly reproducible during assay development and for samples tested, with the standard error of the mean of replicate samples less than 0.05. The assay was developed prior to the 4th FPV vaccination and blood from the macaques was evaluated prior to, and following, this vaccination (Fig. 3). A rise in HIV-specific IFNγ secretion was seen by 1 week following FPV-gag/pol-IFNγ vaccinations, and this persisted for at least 4 weeks. FPVgag/pol vaccination resulted in lower HIV-specific IFNγ production, and macaques that did not receive concurrent immunisations showed no rise in HIV-specific IFNγ secretion. Unstimulated plasma samples from all animals, including FPVgag/pol-IFNγ vaccinees, showed no significant IFNγ secretion.

Enumeration of HIV-specific IFNγ producing cells

Enumeration of antigen-specific cells by detection of cytokines bound to antibody coated plates and

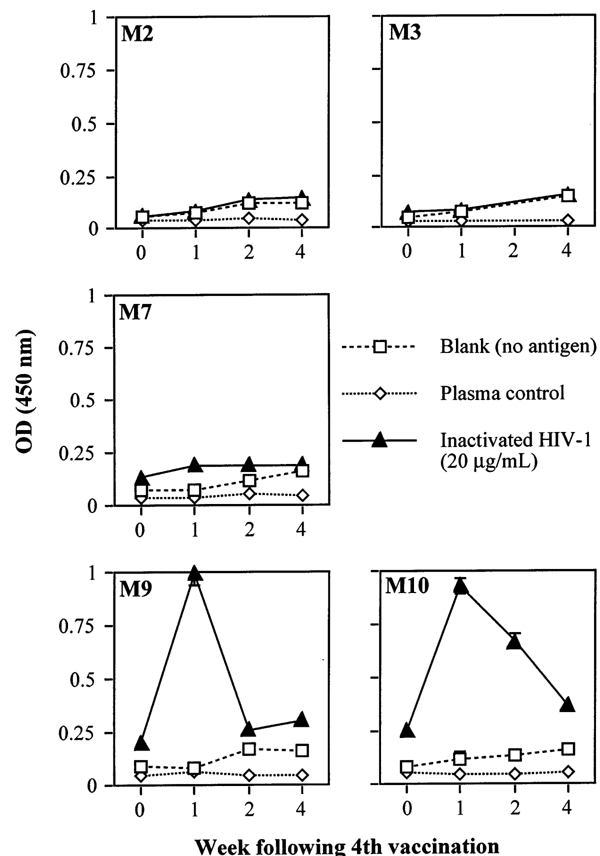

Fig. 3. Detection of IFNγ secretion following antigen stimulation of whole blood. Whole blood collected prior to (week 0), and on weeks 1, 2 and 4 following the 4th FPV gag/pol (macaque M7) and FPV gag/pol-IFNγ (macaques M9 and M10) vaccines was stimulated with inactivated HIV-1 (20 μg/ml). Control plasma samples were either not incubated, or incubated without HIV-1 antigens. Blood collected from macaques M2 and M3, not concurrently vaccinated with FPVs, was similarly treated and served as negative controls in the assay. IFNγ secretion was enhanced 1 week following immunisation with FPV gag/pol-IFNγ compared to the other animals in the study, and, although declining, IFNγ secretion could be detected 4 weeks following this immunisation. IFNγ secretion detected in unstimulated plasma samples (plasma control) did not increase following immunisation of any of the animals. From serial dilutions of recombinant human IFNγ (not shown), an OD (450 nm) of one was approximately equivalent to 300 pg/ml IFNγ in the assay.

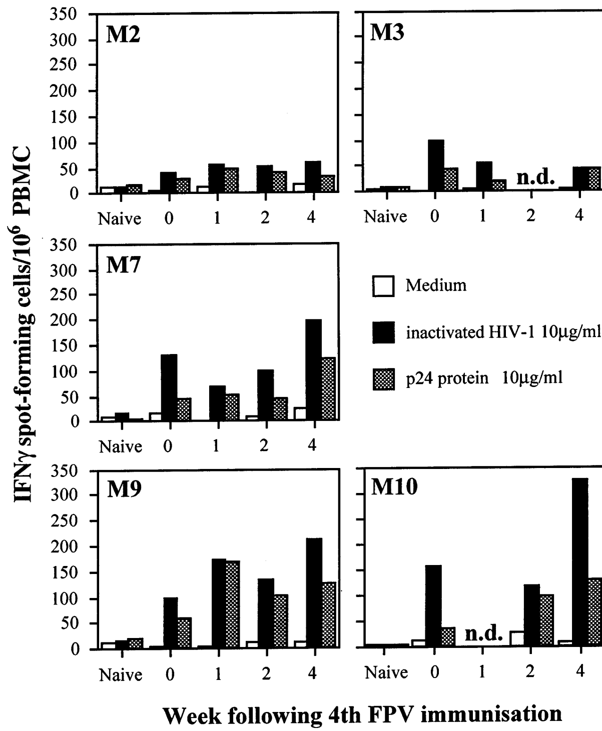

Fig. 4. Enumeration of HIV-specific IFNγ secreting cells. ELISPOT assays to detect the number of lymphocytes secreting IFNγ. PBMC collected before HIV-1 vaccines and exposure (naïve, month 0 in Fig. 1), and prior to and following the 4th FPV gag/pol (macaques M7) and FPV gag/pol-IFNγ (macaques M9 and M10) vaccination were stimulated with either inactivated HIV-1 (10 μg/ml) or recombinant p24 protein (10 μg/ml).

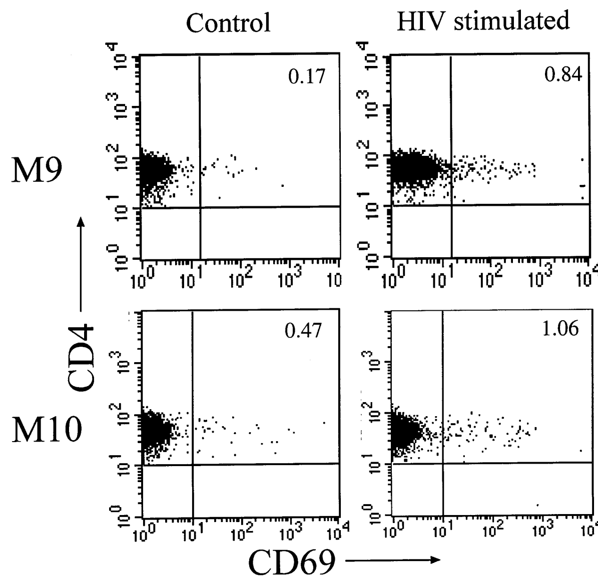

Fig. 5. HIV-specific expression of CD69 following FPV gag/pol-IFNγ immunisation. Expression of CD69 on CD4⁺ lymphocytes was evaluated when freshly isolated PBMC were stimulated with inactivated HIV-1 (10 μg/ml) 4 weeks following the 4th immunisation with FPV gag/pol-IFNγ.

detected by a second conjugated antibody is being increasingly studied in HIV/SIV vaccine experiments in macaques and humans [15, 35]. Serial frozen PBMC samples collected prior to any HIV-1 vaccines of HIV-1 exposure (naïve timepoint, Fig. 4), and prior to and following the 4th FPV vaccinations, were stimulated with two HIV-1 antigens (inactivated HIV-1 and recombinant p24 protein). Control animals (M2 and M3) had been vaccinated with DNA and FPV HIV-1 vaccines 30 months previously (see Fig. 1). IFNγ secreting cells specific for HIV-1 antigens were detected at low levels following previous vaccinations in all five animals. P24-specific IFNγ secreting cells were boosted 2.1 to 4.0-fold by week 4 following the 4th FPVgag/pol or FPVgag/pol-IFNγ vaccinations. Control monkeys, which did not receive concurrent FPV vaccines (M2 and M3), showed no significant enhancement of HIV-specific IFNγ secreting cells. Frozen PBMC samples from an additional two naïve pigtail macaques were stimulated with both HIV-1 antigen preparation and showed no significant IFNγ secreting cells (<10 spot-forming cells/10^6 PBMC, data not shown).

HIV-specific expression of CD69 following vaccination

Analysis of early activation markers on T cells, such as CD69, by flow cytometry is a reliable and simple method to detect antigen-specific T cells. We analysed CD69 expression on $CD4^+$ lymphocytes following *in vitro* stimulation of freshly isolated PBMC from macaques following the 4th FPVgag/pol-IFNγ vaccination (Fig. 5). HIV-specific $CD4^+$ lymphocytes represented 0.59–0.67% of $CD4^+$ lymphocytes 4 weeks following the 4th FPVgag/pol-IFNγ vaccination.

Discussion

This report extends a preclinical evaluation of the safety and immunogenicity of a FPV-HIV vaccine co-expressing IFNγ [23]. The FPV gag/pol-INFγ vaccine safely stimulated high levels of Th cells which secreted IFNγ in macaques. No significant systemic plasma IFNγ was detected following FPV gag/pol-IFNγ vaccination, and all safety analyses demonstrated no significant or long-term toxicity. These results are consistent with the vaccine acting to stimulate and influence the phenotype of the HIV-specific Th cells locally rather than mediating an effect by increasing systemic IFNγ levels. Given the perceived importance of the induction of HIV-1-specific Th1 responses for the maintenance of HIV-specific CTL effectors and control of HIV-1 replication [15, 30], this vaccine should be further

evaluated as a therapeutic or preventive HIV vaccine.

This initial evaluation of these novel FPV vaccines was performed in pigtail macaques previously exposed to HIV-1. This highly attenuated lentivirus infection model is not suited to analyses of the possible virologic efficacy of this vaccine since no active HIV-1 replication, and only low levels of latent HIV-1 DNA are detectable after the first few months following HIV-1 inoculation [23]. Future studies are planned to evaluate these vaccines in pathogenic SHIV macaque models. Alternatively, if ongoing studies define HIV-1 strains which induce high levels of replication and/or disease in pigtail macaques [4], this model could be re-evaluated.

A novel, simple, whole blood IFNγ production assay was developed to analyse HIV-specific macaque Th1 cells in this study. This assay clearly demonstrated the potential utility of the FPV gag/pol-IFNγ vaccine studied, with elevations of HIV-1-specific IFNγ production detected within 1 week of vaccination which persisted for at least 4 weeks following FPV gag/pol-IFNγ vaccination. Indeed, baseline levels of HIV-1-specific IFNγ production were still elevated 9 months following the third FPV gag/pol-IFNγ vaccine (Fig. 3, week 0 timepoint). Replicate samples were highly concordant, and the results consistent with previous analyses of standard lymphoproliferative assays and IFNγ production following *in vitro* stimulation of isolated PBMC for 7 days [23]. It is most likely that the antigen-specific IFNγ production detected by this assay reflects IFNγ secretion from $CD4^+$ T cells, but future depletion studies are required for this to be confirmed. One limitation of this assay is the requirement for modest volumes of fresh whole blood (0.5 ml per antigen replicate), which could be limiting in smaller macaques. Nevertheless, this assay should prove a simple and reliable addition to immunologic monitoring of macaques in lentivirus vaccine studies.

Quantification of the number of HIV-specific cells secreting IFNγ were also evaluated by ELISPOT on serial frozen cells in this report. This assay detected an increase in the number of HIV-specific T cells following the 4th FPV gag/pol or FPV gag/pol-IFNγ vaccination, but not in mock-immunised control macaques. Interestingly, HIV-specific IFNγ secreting T cells were detected both 9 months following the third FPV gag/pol of FPV gag/pol-IFNγ vaccination (macaques M7, M9 M10), and, to a lesser extent, 30 months following HIV-1 DNA and FPV prime/boost vaccine studies (macaques M2 and M3), in comparison to samples obtained prior to any vaccinations or challenges, or other HIV-naïve macaques. Taken together, these results suggest future studies of DNA vaccine priming and FPV gag/pol-IFNγ boosting could stimulate even greater levels of Th1/CTL responses. These results are consistent with previous reports suggesting ELISPOT assays are highly sensitive for the detection of HIV-specific IFNγ secretion. The pattern of enhancement of IFNγ secretion by individual HIV-1-specific T cells was different to that observed utilising the whole blood HIV-1-specific IFNγ production assay. This may reflect a number of effects, namely: (i) the longer duration of antigen stimulation in the ELISPOT assay (potentially detecting a more significant memory T cell response); (ii) that ELISPOTs do not quantify the amount of IFNγ secreted by each T cell; (iii) counting of ELISPOTs is difficult to standardise; or (iv) the ELISPOT assays were performed on frozen PBMC in this study. Used in tandem, both ELISPOT and whole blood IFNγ secretion assays should provide an accurate reflection of antigen-specific IFNγ secreting T cells.

In summary, a FPV-HIV vaccine co-expressing IFNγ stimulated high levels of HIV-specific Th1 cells in macaques. This vaccine is poised to be evaluated in further macaque studies to prevent or modulate pathogenic SHIV infections, and ultimately, in therapeutic and preventive HIV vaccine clinical trials in Australia.

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