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A comparative analysis of HIV-specific mucosal/systemic T cell immunity and avidity following rDNA/rFPV and poxvirus–poxvirus prime boost immunisations

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ABSTRACT

In this study we have firstly compared a range of recombinant DNA poxvirus prime-boost immunisation strategies and shown that combined intramuscular (i.m.) 2× DNA-HIV/intranasal (i.n.) 2× FPV-HIV prime-boost immunisation can generate high-level of HIV-specific systemic (spleen) and mucosal (genito-rectal nodes, vaginal tissues and lung tissues) T cell responses and HIV-1 p24 Gag-specific serum IgG1, IgG2a and mucosal IgG, SIgA responses in vaginal secretions in BALB/c mice. Data indicate that following rDNA priming, two rFPV booster immunisations were necessary to generate good antibody and mucosal T cell immunity. This data also revealed that mucosal uptake of recombinant fowl pox (rFPV) was far superior to plasmid DNA. To further evaluate CD8+ T cell immunity, i.m. 2× DNA-HIV/i.n. 1× FPV-HIV immunisation strategy was directly compared with single shot poxvirus/poxvirus, i.n. FPV-HIV/i.m. VV-HIV immunisation. Results indicate that the latter strategy was able to generate strong sustained HIV-specific CD8+ T cells with higher avidity, broader cytokine/chemokine profiles and better protection following influenza-$K^dGag_{197–205}$ challenge compared to rDNA poxvirus prime-boost strategy. Our findings further substantiate the importance of vector selection/combination, order and route of delivery when designing effective vaccines for HIV-1.

1. Introduction

The great majority of human pathogens are first encountered at the mucosae, leading to a renewed interest in developing vaccines that elicit mucosal immunity. Many HIV-1 "systemic vaccine trials" or vaccines delivered via the intramuscular route to the blood in humans have elicited poor outcomes [1,2], and there is now an increased awareness of the potential importance of inducing local antiviral immune responses at mucosal surfaces, particularly in the genital and rectal tissues, including the cervico-vaginal tissues in females which is the primary site of infection [3–6] and the gastrointestinal tract, a major reservoir of HIV virus replication with resultant CD4+ T cell depletion [7]. It is widely thought that vaccine-induced mucosal immunity requires that a vaccine be delivered to the mucosa, and that vaccines administered systemically do not generally induce good immune responses at mucosal sites [8].

To date a variety of plasmid DNA and/or recombinant virus heterologous prime-boost vaccine delivery strategies have been investigated as candidate vaccines for HIV-1. For safety reasons, the majority of studies have been based on virus vectors that are unable to replicate in the host. For example, avipox viruses such as fowl poxvirus (FPV) does not replicate in mammalian cells and has a highly restricted host range, although heterologous genes under the control of early promoters are expressed in mammalian cells, resulting in presentation of encoded vaccine antigen to the immune system [9–12]. To date many different heterologous prime-boost vaccine protocols have been explored, including non-replicating vaccinia virus (VV) and adenovirus (Ad) vectors. Protein antigens in combination with rDNA and/or recombinant virus vectors have also been used to augment antibody responses in prime-boost regimes [13–17]. Even though many of the rDNA prime-boost HIV-1 vaccines tested in animal models have shown promising results [18], human trials have generated poor outcomes [1,2,19,20]. It is now believed that this could be related, at least in part to sub-optimal

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Table 1
Recombinant poxviruses used in this study [28].

Recombinant	Insertion sites	
	F region	TK-ORFX or TK
FPV gag/pol (FPV-HIV)	B clade gag/pol(m)	
VV gag/pol (VV-HIV)		B clade gag/pol(m)

TK, thymidine kinase; ORFX, uncharacterised gene.

doses of DNA vaccine. Interestingly, a recent phase I clinical trial using a 4 mg primary dose of DNA vaccine with NYVAC (New York vaccinia virus, a highly attenuated strain) in a prime-boost strategy reported good immunogenicity in humans and offers some optimism for the future [21].

A critical question that has not yet been clearly addressed is the generally poor immunogenicity of DNA vaccines in humans. Another major problem for the design of effective vaccines against HIV-1 and other intractable pathogens is our poor current understanding of the immunological correlates of protection. Does "quality" or "avidity" of immune responses matter? Functional T cell avidity, the ability of antigen-specific T cells to recognise and respond to antigen, is a key component that may underpin the effectiveness of T cells in clearing infection. By definition, low avidity T cells are incapable of effector function at low concentrations of antigen, while high avidity T cells can recognise low concentrations of antigen and appear to mediate increased functional activity [22,23].

In previous studies, macaques vaccinated with multiple HIV antigens via rDNA/rFPV prime boosting either systemically or via mucosal (intranasal) delivery of the FPV booster vaccine [24] showed that the mucosally immunised animals generated local T cell responses in cervico-vaginal tissues following pathogenic vaginal SHIV (simian human immunodeficiency virus) challenge with significant reduction in acute plasma viremia, in the absence of significant circulating SHIV T cell responses prior to challenge. Furthermore, we have shown that intranasal priming with FPV vectors followed by intramuscular boosting with VV vectors generates robust long-term systemic and mucosal T cell responses against HIV-1 vaccine antigens in BALB/c mice [25], which were also of higher avidity [26]. In this current study we have directly compared a systemic/mucosal heterologous rDNA/poxvirus prime-boost immunisation regime with poxvirus/poxvirus (rFPV/rVV) prime-boost immunisation and clearly shown that the latter strategy generates T cells with greater avidity, correlating with the weight loss profiles observed following influenza-$K^dGag_{197–205}$ mucosal challenge.

2. Materials and methods

2.1. Recombinant DNA or poxvirus vaccines

The DNA plasmid pHIS (DNA-HIV), recombinant fowl pox (FPV-HIV) and recombinant vaccinia virus (VV-HIV) vaccines expressing modified B clade gag and pol genes were prepared as described elsewhere [12,27,28] (Table 1).

2.2. Immunisation of mice

Pathogen free 8–10 week old female BALB/c mice were obtained from the Animal Research Centre, Perth, Western Australia or the Animal Breeding Establishment, The John Curtin School of Medical Research (JCSMR). All animals were maintained and used in accordance with Institutional animal ethics guidelines. Mice (n = 4–5) were primed intramuscularly (i.m.) with 50 or 100 μg DNA-HIV in sterile phosphate-buffered saline (PBS), (50 μl/ per quadriceps), or intranasally (i.n.) 20 μl per mouse complexed 1:3 with Lipofectamine transfection reagent (Invitrogen, Carlsbad, CA). Two doses were given at an interval of 4 weeks. 2–4 weeks following DNA priming, mice were boosted i.n., i.r. (intrarectally) or i.m with 5×10^6 or 10^7 pfu FPV HIV as indicated in Table 2. During i.m. delivery of rFPV, 50 μl per quadriceps and during i.n. or i.r delivery 20 μl rFPV per mouse were delivered after sonication of virus as indicated below (rFPV was not complexed with lipofectamine). Further groups of mice (n = 4–5) were primed and boosted with 1×10^7 pfu FPV-HIV followed by 1×10^7 pfu VV-HIV given 2 weeks apart using either i.n./i.m. (mucosal/systemic) or i.m./i.m. (pure systemic) immunisation routes as indicated in Table 2. Mice were immunised under mild methoxyfluorane anesthesia. Prior to each immunisation, FPV-HIV or VV-HIV vaccines were diluted in PBS and sonicated to obtain homogeneous viral suspensions. To evaluate protective immunity at 6 weeks after the final vaccine booster, mice were challenged mucosally (i.n.) with a dose (50 plaque forming units (PFU)) of influenza virus PR8 expressing the $K^dGag_{197–205}$ epitope of HIV in the neuraminidase stalk. This construct was created using reverse genetic technology as described elsewhere [29,30]. Body weight was monitored for 10 days after challenge.

Table 2
Prime-boost vaccine strategies used in this study.

	Prime	Boost
1	2× i.n. DNA-HIV	2× i.m. FPV-HIV
2	2× i.n. DNA-control	2× i.m. FPV-HIV
3	2× i.m. DNA-HIV	2× i.n. FPV-HIV
4	2× i.m. DNA-control	2× i.n. FPV-HIV
5	2× i.m. DNA-HIV	2× i.n. DNA-HIV
6	2× i.m. DNA-HIV	2× i.r. FPV-HIV
7	2× i.m. DNA-HIV	2× i.m. FPV-HIV
8	2× i.m. DNA-HIV	i.m. FPV-HIV*
9	2× i.m. DNA-HIV	i.n. FPV-HIV*
10	i.n. FPV-HIV*	i.m. VV-HIV*
11	i.m. FPV-HIV*	i.m. VV-HIV*

All constructs encode HIV-1 subtype B gag/pol antigens, except DNA control. i.n., intranasal; i.m., intramuscular; i.r., intrarectal. Two DNA-HIV priming immunisations were performed in each case. In contrast as indicated some instances one (*) or two i.n. or i.m. FPV-HIV or VV-HIV immunisations were performed.

2.3. Preparation of lymphocytes

To measure T cell responses mice were sacrificed at different time intervals (4 weeks post 2nd DNA-HIV; 2–4 weeks post 1st FPV-HIV; 4,8 or 16 weeks post 2nd FPV-HIV or 2 weeks post VV-HIV, or 10 days post-challenge), spleen and genito-rectal nodes (iliac lymph nodes) were removed, and single cell suspensions were prepared in complete RPMI as described previously [25]. Splenocytes were treated with red cell lysis buffer to remove erythrocytes.

Single cell suspensions from mucosal tissues (i.e. vaginal, and lung) were prepared as follows. Tissue samples were collected in complete RPMI and were cut into small pieces and incubated at 37 °C with 2 mg/ml collagenase (Sigma), 2.4 mg/ml dispase (GIBCO) and 5 Units/ml DNAse (Calbiochem) in complete RPM for 1 h with gentle agitation and 5 ml of complete RPMI was added to each sample and was passed through 2 layers of sterile gauze to remove cell debris. Cells were then treated with red cell lysis buffer, washed twice with compete medium and particulate material was removed by passing though a cell stainer. Finally, cells were resuspended in complete medium.

2.4. Serum and lavage collection

Serum and vaginal lavages were collected from pre-immune mice, after 2 doses of DNA-HIV, after FPV-HIV the first booster, and after the 2nd FPV-HIV booster immunisations. Vaginal lavage fluids were collected by flushing the vagina with 40 μl of sterile PBS, then