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Evaluation of fowlpox–vaccinia virus prime-boost vaccine strategies for high-level mucosal and systemic immunity against HIV-1

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Abstract

We have tested the efficacy of recombinant fowl pox (rFPV) and recombinant vaccinia virus (rVV) encoding antigens of AE clade HIV-1 in a prime-boost strategy, using both systemic and mucosal delivery routes. Of the various vaccine routes tested, intranasal/intramuscular (i.n./i.m.) AE FPV/AE VV prime-boosting generated the highest mucosal and systemic T cell responses. Peak mucosal T cell responses occurred as early as 3 days post-boost vaccination. In contrast only low systemic responses were observed at this time with the peak response occurring at day 7. Current data also revealed that, due to better uptake of the rFPV, intranasal viral priming was much more effective than intranasal rDNA priming tested previously. The i.m./i.m. prime-boost delivery also generated strong systemic but poor mucosal responses to Gag peptides. Interestingly, the oral administration of AE FPV followed by i.m. AE VV delivery elicited strong systemic responses to sub-dominant Pol 1 peptides that were absent in mice that received vaccine by other routes. Moreover, priming with AE FPV co-expressing cytokine IL-12 significantly enhanced the T cell responses to target antigens, whilst co-expression of IFNγ decreased these responses. The results also indicated that the route of inoculation and the vaccine vector combination could radically influence not only the magnitude but also the antigen specificity of the immune response generated. Further, in contrast to the generally protracted HIV rDNA/rFPV multiple delivery prime-boosting, this single rFPV prime and rVV boost approach was more flexible and generated excellent mucosal and systemic immune responses to HIV vaccine antigens.

Keywords: Poxvirus; Prime-boost; Mucosal immunity; Oral/intranasal rFPV delivery; Co-stimulatory molecules; Immunodominance

1. Introduction

Novel easily deliverable vaccine strategies are urgently needed to combat the HIV-1 epidemic that is spreading at an alarming rate in the developing world. Consecutive immunisation with heterologous vectors encoding the same antigen has proven highly immunogenic in models of type 1 human immunodeficiently virus (HIV-1)/simian immunodeficiently virus (SIV) infection. Several heterologous recombinant DNA (rDNA) prime-boost vaccine strategies have elicited robust cellular and humoral responses to HIV antigens in animal models [1–6] and phase I/II prime-boost HIV-1 vaccine trials have now been conducted around the world [7–9]. Despite generating good immune responses in mice and non-human primates rDNA prime-boost strategies have so far proven to be disappointing in human clinical trials. In one such trial, using rDNA prime and recombinant modified vaccinia Ankara (rMVA) boost immunisation, only 20% of 205 recipients developed a significant level of immunity to HIV antigens (http://www.iavi.org). In another study, using rDNA/rFPV prime-boosting little or no immunity to

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vaccine antigens was detected [10]. These results possibly indicate that current rDNA vectors may be poor immunogens in humans, in contrast to the responses elicited in other species. Novel vaccines that generate broadly reactive high-avidity T cell and humoral immune responses are still in needed for HIV-1 prevention.

A compelling body of evidence suggests that virus specific CD8+ cytotoxic T lymphocytes (CTL) play a vital role in containing HIV-1 infection [11,12]. Further, vaccine-induced cytotoxic T cells have also been shown to initially control the replication of pathogenic simian immunodeficiency virus (SIV), and although there is emergence of CTL escape variants, these mutants have a replication disadvantage compared to the wild-type virus [11]. However, new data indicate that the absolute levels, breadth, durability and cytokine secretion profiles of responding CTL, may all be important for protection of humans from HIV-1 exposure [13–15].

The presence of HIV-specific immunity at major sites of virus transmission, i.e. rectal and oral tissues and particularly the cervico-vaginal tissues in females may be critical in the initial control of infection. For diseases such as HIV/AIDS, it is hence important to develop vaccine strategies that elicit immunity at the primary site of mucosal infection [16–18]. In general, systemically administered vaccine antigens, although inducing good systemic T cell responses, rarely induce optimal mucosal immune responses [16,19]. It has also been postulated that application of a vaccine at one mucosal surface can trigger immunity at local and distant mucosa [19,20]. Indeed, a number of mucosal immunisation regimes based on this approach have been tested and shown to be effective in generating HIV-1 specific mucosal cellular and humoral immunity in mice and macaques [21–27]. Mucosal (intra nasal and intrarectal) delivery of rDNA/rFPV prime-boost vaccines in mice and macaques also confirm these findings (Ranasinghe et al., unpublished data) [4].

Different vaccine delivery routes and vectors have proven to induce a diverse range of immune responses to vaccine antigens [28–30]. In recent years, a number of recombinant viral and bacterial vectors that are suitable for mucosal and systemic delivery have been tested, i.e. adenovirus [25,31], poliovirus [24] influenza viral vectors [27], *Listeria monocytogenes* [32]. More recently, a rFPV/rMVA prime-boost regime was reported to induce strong antigen-specific T cell responses to *Plasmodium berghei malaria* and *Mycobacterium tuberculosis* antigens, [30,33]. Also the co-expressions of certain cytokines and chemokines have been shown to enhance immune responses to vaccine antigens [34–39]. These data indicate that a rational combination of vectors, delivery routes and co-stimulatory molecules could play a pivotal role in modulating immune responses to vaccine antigens.

In this study we have characterised the use of rFPV and rVV expressing AE clade HIV-1 antigens, in prime-boost vaccine strategies, and have shown that these vaccines generate strong sustained mucosal and systemic T cell immunity, with mucosal responses peaking very early after boost immunisation. We have also studied cytokine co-expression to enhance immunogenicity of vaccine antigens. Moreover, we have evaluated the efficacy of oral delivery of rFPV, an important vaccine delivery approach for implementation in the developing world.

2. Materials and methods

2.1. Recombinant poxvirus vaccines

The AE FPV contained modified AE clade *gag*, *pol*, *env*, *rev* and *tat* genes. The AE IL-12-AE and AE IFNγ-AE constructs expressed Gag, Pol, Env and IL-12 or IFNγ, respectively (Table 1). Whereas, AE VV contained modified *gag* and *pol* genes only as indicted in Table 1. These recombinant viruses were constructed as described elsewhere [40–42].

2.2. Immunisation of mice

Groups of (4–5) 5–7 weeks old BALB/c (H-2^d) mice were primed with rFPV followed by a booster 2 weeks later of rVV (1×10^7 pfu) both expressing AE clade HIV-1 antigens (Table 1). Mice were sacrificed at different time intervals to evaluate immune responses to vaccine antigens. Initially, varying amounts of AE FPV (5×10^4, 5×10^5, 5×10^6, 1×10^7 pfu) were administered to the mice, to measure the dose response. However, in the subsequent experiments 1×10 pfu of AE FPV were given to each mouse in a final volume of 20–25 μl (intranasal, i.n.; intradermal, i.d.), 100 μl (intramuscular, i.m.) or 200 μl (intravenous, i.v.), except mice immunised orally received 2×10^7 pfu in a 20–40 μl volume. Prior to each immunisation the rFPV or rVV was diluted in phosphate buffered saline (PBS) and sonicated 30–40 s to obtain a homogeneous viral suspension. Note that most of these experiments were repeated over three times and the data are a representative of one experiment.

2.3. Sample collections and preparation of lymphocytes

Sera were collected from pre-immune and immunised mice. Blood was collected from tail vein or heart bleed, serum separated by centrifugation and stored at −20 °C until assayed. To measure systemic and mucosal T cell responses

Table 1
Recombinant viruses used in this study [40,41]

Recombinant	Insertion sites		
	F6,7-9	TK-ORFX or TK	REV
FPV-106 (AE IFNγ-FPV)	AE gag/pol(m)	muIFNγ	AE env(m)
FPV-107 (AE IL-12-FPV)	AE gag/pol(m)	muIL-12	AE env(m)
FPV-117 (AE FPV)	AE gag/pol(m)	AE tat-rev	AE env(m)
VV-336 (AE VV)		AE gag/pol(m)	

TK: thymidine kinase, ORFX: uncharacterised gene, and REV: reticuloendotheliosis provirus.