The novel capripoxvirus vector lumpy skin disease virus efficiently boosts modified vaccinia Ankara human immunodeficiency virus responses in rhesus macaques

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Poxvirus vectors represent promising human immunodeficiency virus (HIV) vaccine candidates and were a component of the only successful HIV vaccine efficacy trial to date. We tested the immunogenicity of a novel recombinant capripoxvirus vector, lumpy skin disease virus (LSDV), in combination with modified vaccinia Ankara (MVA), both expressing genes from HIV-1. Here, we demonstrated that the combination regimen was immunogenic in rhesus macaques, inducing high-magnitude, broad and balanced CD4+ and CD8+ T-cell responses, and transient activation of the immune response. These studies support further development of LSDV as a vaccine vector.

Human immunodeficiency virus (HIV)/AIDS remains a major public health problem globally, and long-term control of the epidemic will only be achieved with an effective prophylactic vaccine (Koff et al., 2013). Poxviruses are regarded as among the most promising HIV vaccine vectors, with the only HIV vaccine trial to show efficacy in protecting against HIV acquisition, RV 144, using a combination of a canarypoxvirus-based vaccine boosted by HIV gp120 protein (Rerks-Ngarm et al., 2009). Another replication-defective poxvirus vector in widespread development as an HIV vaccine candidate is modified vaccinia Ankara (MVA) (Gómez et al., 2012a), and vaccine regimens often include repeated administration of the same vector. Poxvirus immunity is long lived (Amara et al., 2004), and there is evidence that pre-existing immunity to vaccinia virus results in lower responses to MVA (Gudmundsdotter et al., 2009). Combinations of heterologous and homologous poxvirus vectors expressing the same HIV genes have been tested in clinical trials, and MVA in combination with fowlpox induced substantially better CD8+ T-cell immunogenicity compared with either vector alone (Keef er et al., 2011). There is an intensive search for novel and improved HIV vaccine vectors (Garber et al., 2012; Barouch, 2010), and there remains a need for the identification of additional, antigenically distinct, non-pathogenic poxvirus vectors. Lumpy skin disease virus (LSDV), a capripoxvirus, does not complete its replication cycle in non-ruminant hosts (Aspden et al., 2003), and therefore mature particles are not formed; thus, there is no risk of viral shedding. The Neethling vaccine strain of LSDV is widely used to vaccinate cattle in Africa, and there has been no report of transmission to other animals or infection of humans (Kitching et al., 1987). Furthermore, we have shown that recombinant LSDV expressing HIV genes was safe and non-pathogenic in two strains of immunocompromised mice, as well as being immunogenic in mice (Shen et al., 2011). LSDV is therefore an excellent candidate as a live replication-deficient vaccine vector.

We investigated the ability of recombinant LSDV (rLSDV) to boost immune responses to recombinant MVA (rMVA) in rhesus macaques. Both vectors expressed HIV-1 subtype C Gag, reverse transcriptase, Tat and Nef as a polypeptide, with MVA additionally expressing Env (Burgers et al., 2008; Shephard et al., 2008). Six animals that had previously been primed with two doses of 10⁶ p.f.u. rMVA were boosted with a single dose of 10⁶ f.f.u. rLSDV intramuscularly, 66 weeks after initial priming. LSDV was grown and titrated in primary fetal bovine testes cells, as described previously (Shen et al., 2011). Briefly, infected cells were lysed and virus was isolated by sucrose density-gradient centrifugation. The purified virus was resuspended in PBS. Following vaccination of macaques, rLSDV was well tolerated, and no fever or any other clinical signs were observed. One animal was euthanized prior to the experimental end point with suspected simian herpes B virus infection, and was thus excluded from further analysis. A further three animals received rLSDV only. All procedures were approved by the
Fig. 1. HIV-specific T-cell responses induced by recombinant MVA and LSDV vaccination. (a) Kinetics of IFN-γ ELISPOT responses in animals that received MVA (two inoculations of 10⁹ p.f.u. each) followed by 10⁶ f.f.u. LSDV (filled symbols) or LSDV only (open symbols). Individual animal numbers and weeks post-immunization are indicated. Responses are expressed as s.f.u. per 10⁶ PBMCs, after subtraction of background unstimulated cells. (b) Breadth of IFN-γ ELISPOT responses, indicating Gag, Nef, Pol and Tat responses. Peak responses after MVA vaccination, after boosting with a single LSDV vaccination in the same group, or after a single LSDV vaccination, are shown. Responses are expressed as s.f.u. per 10⁶ PBMCs after background subtraction. (c) Total frequency of CD4⁺ and CD8⁺ T-cells producing any cytokine (IFN-γ, IL-2 or TNF-α), as determined by intracellular cytokine staining and flow cytometry. (d) Individual cytokine responses in CD4⁺ and CD8⁺ T-cells. Symbols indicate the specificity of the response, as follows: Gag (●), Nef (▲) Pol (■), Tat (▼). (e) Polyfunctional profiles of HIV-specific T-cells. The proportion of all possible combinations of the three cytokines (IFN-γ, IL-2 or TNF-α) are shown for CD4⁺ and CD8⁺ T-cells. (f) Memory phenotype of HIV-specific cells. Cytokine-producing CD4⁺ and CD8⁺ T-cells were delineated into central (Tcm) and effector (Tem) memory cells based on CD28 and CD95 expression. Representative flow
Animal Ethics Committee of the University of Cape Town. We investigated the induction of T-cell responses using an IFN-γ ELISPOT assay, with pools of peptides spanning the vaccine-expressed genes, as described previously (Burgers et al., 2009). There was a median of 3.2 spots (range 0.3–6.5 spots per 100 000 PBMCs) in background unstimulated wells (cells and medium) for all time points tested (data not shown). After two priming doses of rMVA, two out of five macaques responded, with peak magnitudes of 334 and 403 spot-forming units (s.f.u.) per 10^6 PBMCs reached after the second rMVA dose (Fig. 1a). In contrast, after a single rLSDV boost, all five animals mounted T-cell responses to the vaccine regimen, two with response magnitudes >1000 s.f.u. per 10^6 PBMCs (Fig. 1a). The median response magnitude was 570 s.f.u. per 10^6 PBMCs. In addition, two out of three controls that received a single dose of rLSDV only mounted HIV responses. The combination of rMVA and rLSDV generated broader T-cell responses than either vector alone, targeting all the vaccine-expressed HIV genes (Fig. 1b). HIV-specific responses became undetectable in four of the five animals receiving the combination regimen by 9 weeks after rLSDV vaccination, indicating that responses following a single rLSDV vaccination were short lived. With regard to humoral immunity, no antibodies to Env were generated following vaccination with the recombinant rMVA construct (data not shown), and, as rLSDV did not contain Env, we did not test for antibodies after rLSDV boosting.

To characterize further the immune response induced by the heterologous poxvirus vector regimen, we performed intracellular cytokine staining followed by flow cytometry (Chege et al., 2013). The combination regimen elicited both CD4^+ and CD8^+ T-cell responses (Fig. 1c), which produced primarily IFN-γ and TNF-α, with lower levels of IL-2 (Fig. 1d). Polyfunctional T-cells expressing combinations of three and two cytokines simultaneously were detected in the CD8^+ subset, with CD4^+ cells being primarily single producers of IFN-γ or TNF-α (Fig. 1e). To gain some insight into the longevity of memory responses generated by the vaccine regimen, we determined the expression of CD28 and CD95 on vaccine-induced cells. The majority of HIV-specific CD4^+ responses were of a central memory (CD28^+CD95^-) phenotype, whilst HIV-specific CD8^+ T-cells were primarily effector memory cells (CD28^-CD95^+; Fig. 1f).

As our study describes the first characterization of rLSDV in non-human primates, we monitored generalized T-cell activation profiles induced by the vaccine vector. Vaccines that cause prolonged T-cell activation may increase the risk of HIV acquisition, by increasing the frequency of target cells for HIV. We monitored the level of activated T-cells in the vaccinated rhesus macaques using expression of Ki-67 and Bcl-2 (McElrath et al., 2008). Representative flow cytometry plots are shown in Fig. 2(a), with the frequency of activated Ki-67^hiBcl-2^lo T-cells tracked over time. T-cell activation peaked 1 week after LSDV immunization but returned to baseline or near-baseline by 12 weeks post-vaccination for both CD4^+ and CD8^+ T-cells (Fig. 2b). Interestingly, T-cell activation was substantially higher than HIV insert-specific responses measured by cytokine production. This could be attributed to T-cell responses directed at the vector, or indeed additional HIV-specific T-cells secreting cytokines other than those measured in our assays. The memory phenotype of total activated T-cells was consistent with HIV-specific phenotypes, with CD4^+ cells being primarily central memory cells and CD8^+ T-cells having a more balanced but primarily effector memory response by week 12 (Fig. 2c, d). Evolution of the memory response was evident particularly in the CD8^+ compartment, with an increase in activated effector memory cells and decrease in central memory cells over time (Fig. 2d). Expression kinetics of CCR5 revealed a decrease in CD4^- and CD8^+ T-cells in the majority of animals by week 12, with the exception of two animals that displayed steady increases in CCR5 expression over time (Fig. 2e, f). This may reflect a slower contraction of the immune response in these animals, evident from Fig. 1(a). Given the potential increase in HIV risk of increased CCR5 expression on CD4^+ cells, these data warrant further investigation in future studies, in particular immune monitoring beyond 12 weeks after LSDV vaccination. Nevertheless, for the most part, rLSDV induced generalized but transient activation of CD4^- and CD8^+ T-cells. Given that anti-vector immunity has the potential to limit responses to subsequent repeat immunizations, we tested whether anti-LSDV immunity developed after vaccination, or was cross-reactive with MVA-specific immunity. We failed to detect any cellular responses to the LSDV vector prior to LSDV vaccination (tested 36 weeks post MVA2) in four animals tested (data not shown). In addition, a group of animals previously found to develop strong MVA-specific responses after three MVA vaccinations failed to show any cross-reactivity of cellular responses with LSDV (data not shown). In contrast, 3 weeks after rLSDV vaccination, one of four animals tested generated LSDV anti-vector immunity (P36A, 300 s.f.u. per 10^6 PBMCs; data not shown). These results warrant further investigation in future studies, if regimens are designed to incorporate multiple rLSDV booster vaccinations.

Taken together, our results demonstrate that priming and boosting with two heterologous poxvirus vectors, namely MVA and LSDV, can elicit high-magnitude, broad T-cell
responses to the HIV antigens they express. Strikingly, the rLSDV vector was immunogenic at a dose 1000-fold lower than that of rMVA. Both CD4+ and CD8+ responses were induced, as well as a balanced central and effector memory profile, which may lead to both longevity of the memory response and the ability of cells to rapidly traffic to effector sites of exposure and virus replication.

Only four replication-deficient poxviruses are currently in clinical development as vectors for HIV vaccines, namely
the orthopoxviruses MVA and NYVAC, both derived from vaccinia virus, and the avipoxviruses ALVAC and FP9 (fowlpox virus) (de Cassan & Draper, 2013). Efforts to develop these poxvirus vectors further have focused on deletion of immunomodulatory genes, resulting in improved immunogenicity (Garber et al., 2012; Garcia-Arriaza et al., 2013; Gómez et al., 2012b; Kibler et al., 2011; Perdiguero et al., 2013). In contrast, the number of adenovirus vectors in clinical development is growing, utilizing adenoviruses that represent rare human serotypes, those of simian origin and those from chimpanzees (Barouch, 2010; de Cassan & Draper, 2013). Some of these vectors demonstrate substantially improved immunogenicity over the prototype Ad5 vector (Penaloza-MacMaster et al., 2013; Quinn et al., 2013), and the combination of adenovirus vectors and poxvirus vectors can stimulate potent immune responses in humans (Borthwick et al., 2013). These studies highlight the need for the isolation and development of new poxvirus vectors.

Our data suggest that the capripoxvirus LSDV represents an attractive vaccine vector for development as a candidate vaccine for HIV or other pathogens. Further studies investigating dose and immunization regimen to optimize immunogenicity of rLSDV are warranted. As the kinetics of responses indicated that cellular responses were relatively short-lived after a single rLSDV vaccination (as demonstrated previously for MVA responses; Burgers et al., 2009), a booster LSDV immunization may be required for expanding the population of longer-lived memory cells. Additional improvements could be the inclusion of Env in the rLSDV, or addition of an Env protein boost in a vaccination regimen, as antibodies to Env were a correlate of protection in the RV 144 trial (Haynes et al., 2012). Future clinical testing would require rLSDV to be grown under Good Manufacturing Practice conditions on chorioallantoic membranes of embryonated chicken eggs. Overall, these studies support the development of LSDV as a vaccine vector.

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References


