

REVIEW

Clinical experience with plasmid DNA- and modified vaccinia virus Ankara-vectored human immunodeficiency virus type 1 clade A vaccine focusing on T-cell induction

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Candidate human immunodeficiency virus type 1 (HIV-1) vaccines focusing on T-cell induction, constructed as pTHr.HIVA DNA and modified vaccinia virus Ankara (MVA).HIVA, were delivered in a heterologous prime–boost regimen. The vaccines were tested in several hundred healthy or HIV-1-infected volunteers in Europe and Africa. Whilst larger trials of hundreds of volunteers suggested induction of HIV-1-specific T-cell responses in <15 % of healthy vaccinees, a series of small, rapid trials in 12–24 volunteers at a time with a more in-depth analysis of vaccine-elicited T-cell responses proved to be highly informative and provided more encouraging results. These trials demonstrated that the pTHr.HIVA vaccine alone primed consistently weak and mainly CD4⁺, but also CD8⁺ T-cell responses, and the MVA.HIVA vaccine delivered a consistent boost to both CD4⁺ and CD8⁺ T cells, which was particularly strong in HIV-1-infected patients. Thus, whilst the search is on for ways to enhance T-cell priming, MVA is a useful boosting vector for human subunit genetic vaccines.

Introduction

Vaccination is the most cost-effective measure for control of infectious diseases and remains the best hope for avoiding AIDS for people living in the developing world, where both social and economic factors continue to preclude effective human immunodeficiency virus type 1 (HIV-1) prevention programmes and limit access to antiretroviral therapy (UNAIDS, 2004).

Although induction of neutralizing antibody remains the main goal of HIV-1 vaccine research, many currently tested HIV-1 vaccines focus on induction of T-cell immunity. T cells function by killing HIV-1-infected cells and producing soluble factors that can control HIV-1 spread directly and indirectly. Whilst, for prophylactic vaccination, T cells cannot prevent the first wave of transmitted virus from infecting host cells, they can limit the extent of early viral infection substantially and rapidly, and are expected to increase the dose of incoming HIV-1 necessary to establish infection. As a result, potent vaccine-induced HIV-1-specific T-cell responses could decrease tissue damage during the acute phase of infection and improve the control of the virus load, leading to a lower set point and hence delaying the development of AIDS and reducing viral transmission. Indeed, mathematical modelling suggested that a tenfold reduction in viral load would be sufficient to reduce HIV-1-associated mortality significantly in the first 20 years after the vaccine introduction (Davenport *et al.*, 2004). Vaccines that maintain a population of activated T cells at the site of entry may even achieve clearance of a lower incoming HIV-1 dose. In the therapeutic setting, vaccine-induced T-cell responses during highly active antiretroviral treatment (HAART) could control HIV-1 rebound after HAART cessation and, in the best scenario, prolong drug-free time indefinitely. Thus, induction of T-cell responses by vaccination has a definite potential to benefit both healthy and HIV-1-infected individuals. However, determining the level of protection that vaccine-induced T cells can confer against HIV-1 exposure and in already infected individuals will only be possible through development of strategies that reliably elicit vigorous T-cell responses of the desired quality and location.

Until major breakthroughs anticipated by the Global HIV/AIDS Vaccine Enterprise (2005) materialize and rational vaccine design (Douek *et al.*, 2006) brings its fruits, the search for novel approaches evoking protective responses against HIV-1 will remain at least half-empirical. This is for two main reasons. First, no single immunological correlate of protection against HIV-1 infection has been identified, with the only possible exception of passive infusion of HIV-1-neutralizing antibodies in non-human primate (NHP) models (Ferrantelli & Ruprecht, 2002). Whilst data supporting the importance of CD8⁺ T cells in HIV-1 control are accumulating, a positive correlation of a particular T-cell function with protection remains elusive and in fact might not exist, as protection is likely to be multifactorial. Without this information, evaluation of vaccine performance should include multiple parameters, such as frequency of vaccine-induced HIV-1-specific CD4⁺ and CD8⁺ T cells, their functionality (lytic activity, secretion of cytokines and ability to proliferate on antigen re-exposure), longevity and tissue/anatomical distribution. Protection against surrogate virus challenges in animal models may indicate functioning of vaccine-induced T cells *in vivo*. The overall frequency of responders to vaccination is an important measure of vaccine consistency. The second reason for the importance of empiricism

in vaccinology is our insufficient knowledge of how to manipulate the immune system to induce responses of the desired quality, specificity and vigour. Whilst different viruses, and by analogy vaccine vectors, induce different qualities of T-cell responses (Appay *et al.*, 2002; Harari *et al.*, 2004a, b) through stimulation of distinct pathways of innate immunity (Leifer *et al.*, 2003; Schulz *et al.*, 2005), there is growing evidence that the magnitude of CD8⁺ T-cell response alone is not sufficient for assessment of the CD8⁺ T-cell antiviral efficacy (Rowland-Jones *et al.*, 2001; van Baalen *et al.*, 2002; Zhang *et al.*, 2003a). Taken together, vaccine advances rely strongly on data from preclinical studies of vaccine immunogenicities in animal models and clinical trials designed to optimize vaccine dose and delivery in humans, which in turn provide feedback for preclinical vaccine improvements. A truly meaningful efficacy evaluation can only come from human studies in HIV-1-infected or -exposed individuals.

In the present article, we review the immunogenicity of candidate HIV-1 vaccines designed to test the protective role of cellular responses in the absence of any vaccine-induced virion-binding antibody, neutralizing or not. These vaccines are vectored by DNA and modified vaccinia virus Ankara (MVA), used alone and in a heterologous prime–boost regimen, and have been used in clinical trials in healthy and HIV-1-infected subjects. Parallel NHP studies with the same vaccines are also discussed.

pTHr.HIVA DNA and MVA.HIVA vaccines

Design

We have found that a successive immunization with DNA- and modified vaccinia virus Ankara-vectored vaccines expressing a common immunogen is a potent way of inducing CD8⁺ T cells in experimental animals (Hanke *et al.*, 1998b; Schneider *et al.*, 1998). We chose the DNA and MVA vectors because of their safety and acceptability for human use (Estcourt *et al.*, 2004; Im & Hanke, 2004) and applied them in a heterologous prime–boost regimen. In general, heterologous prime–boost regimens are used to avoid the build-up of anti-vector immunity resulting from repeated administration of the same complex vector, which would dampen elicitation of the desired responses against the passenger HIV-1-derived immunogens. Particularly encouraged by the immunogenicity of this approach in NHPs (Hanke *et al.*, 1999; Allen *et al.*, 2000, 2002; Amara *et al.*, 2001), we designed and constructed DNA–MVA-based vaccine candidates for clinical trials in humans (Hanke & McMichael, 2000) and developed them as part of the partnership between the UK Medical Research Council (MRC UK), the University of Nairobi, Kenya, and the International AIDS Vaccine Initiative (IAVI). The HIVA immunogen consists of consensus HIV-1 clade A Gag p24/p17 sequences and a string of CD8⁺ T-cell epitopes, including those recognized by mouse and rhesus macaque T cells, and a monoclonal antibody (mAb) epitope tag (Hanke & McMichael, 2000). The relevance of the HIVA immunogen for the viruses currently circulating in Nairobi was demonstrated by detection of positive responses in a gamma interferon (IFN- γ) enzyme-linked immunospot (ELISPOT) assay using either MVA.HIVA-infected or HIVA peptide-pulsed peripheral blood mononuclear cells (PBMCs)

in HIV-1-infected and -exposed but uninfected infants (Slyker *et al.*, 2005). The utility of the HIVA immunogen was also shown by boosting HIV-1-specific responses in individuals in Oxford, UK, infected with diverse HIV-1 clades summarized in this article (Dorrell *et al.*, 2005, 2006). The gene encoding HIVA was inserted into plasmid DNA and MVA. The DNA vector pTHr (Hanke *et al.*, 1998a; Williams *et al.*, 1998) was developed in house and uses upstream sequences derived from human cytomegalovirus enhancer/promoter/intron A, bovine growth hormone polyadenylation signal and a repressor titration system (Williams *et al.*, 1998) instead of antibiotic resistance for selection of plasmid-carrying bacteria. Parental MVA Saatvirus 575. FHE-K, v. 14.12.83, was obtained from Professor Mayr (University of München, Germany) and the HIVA gene was inserted into the thymidine kinase locus under control of the P7.5 promoter, together with a β -galactosidase gene used as a marker (Chakrabarti *et al.*, 1985). The pTHr.HIVA DNA and MVA.HIVA vaccines were produced under Good Manufacturing Practice (GMP) conditions by COBRA Therapeutics and IDT, respectively. The pTHr.HIVA and MVA.HIVA vaccines have achieved a number of 'firsts': (i) they were the first HIV-1 vaccines tested in clinic focusing on African clade A; (ii) MVA.HIVA was the first MVA-vectored prophylactic HIV-1 vaccine to enter clinical evaluation; (iii) pTHr.HIVA–MVA.HIVA was the first DNA–MVA regimen tested clinically for candidate HIV-1 vaccines; and (iv) HIVA was the first HIV-1-derived string of CD8⁺ T-cell epitopes tested in humans.

Preclinical safety and immunogenicity

Preclinical safety studies with the pTHr.HIVA DNA and MVA.HIVA vaccines were carried out in compliance with Good Laboratory Practice (GLP) at Huntingdon Life Science, UK. A combined protocol of persistence, distribution and toxicity of the pTHr.HIVA and MVA.HIVA vaccines in the BALB/c mouse was performed, which demonstrated that the vaccines were non-toxic and detectable beyond 5 weeks after administration only in the sites of injection (Hanke *et al.*, 2002). These results formed a basis for approval of phase I safety and immunogenicity clinical trials in healthy, HIV-1-uninfected volunteers. To support studies in HIV-1-infected subjects, toxicity and biodistribution of MVA.HIVA in mice with severe combined immunodeficiency (SCID) and simian immunodeficiency virus-infected rhesus macaques were determined, which demonstrated that the MVA.HIVA vaccine was non-toxic in mice and non-persistent in places other than the injection site in mice and monkeys (Hanke *et al.*, 2005).

We have extensively demonstrated T-cell immunogenicity of both the pTHr.HIVA and MVA.HIVA vaccines alone and in prime–boost combinations in mice (Hanke & McMichael, 2000; Hanke *et al.*, 2002, 2003; Nkolola *et al.*, 2004; Estcourt *et al.*, 2005a, b; Larke *et al.*, 2005; Nordström *et al.*, 2005) and rhesus macaques (Wee *et al.*, 2002; Nkolola *et al.*, 2004; Hanke *et al.*, 2005; Im *et al.*, 2006).

Safety in humans

Safety has been our prime concern. Although the long-term safety of genetic vaccines can be only established years after vaccination, the short- to medium-term safety profiles of the lower doses of MVA.HIVA administration have been mapped in about 400 healthy, HIV-1-

uninfected adult subjects (Table 1). Overall, both vaccines were safe and tolerated well. For DNA, the reaction was minimal. MVA-vaccinated volunteers experienced some local redness, with a few having skin damage, and crust and scab formation. Reactogenicity peaked at 3 days post-vaccination, resolved by 2 weeks and was milder for the second dosing (Cebere *et al.*, 2006).

Vaccinations with pTHr.HIVA and MVA.HIVA of HIV-1-infected patients on HAART (Table 1) were tolerated well and no serious adverse events were observed. CD4⁺ T-cell counts remained stable and viral loads were undetectable throughout the follow-up, which was for 1 year after the first immunization (Dorrell *et al.*, 2005, 2006).

Immunogenicity in healthy HIV-1/2-uninfected individuals

Up the learning curve. Evaluation of the T-cell immunogenicity in the first trials was primarily dependent on validated IFN- γ ELISPOT assays (Mwau *et al.*, 2002) and its modified versions, carried out on freshly isolated PBMCs. An ELISPOT assay quantifies cell-mediated immune responses by detecting cells that release cytokines upon specific antigenic/peptide stimulation (Czerkinsky *et al.*, 1984; Lalvani *et al.*, 1997). The released cytokines are first captured in the immediate vicinity of the producing cells by a mAb immobilized on the bottom of assay wells, and then visualized by combination of a second mAb coupled to an enzyme and a chromogenic substrate. The frequency of spot-forming units (s.f.u.) is estimated from the number of 'footprints' in the well and the cell input. The ELISPOT assay detecting IFN- γ release is used widely in large-scale vaccine trials for its rapidity, technical simplicity and relative sensitivity. This allows use of the *ex vivo* assay for comparison of vaccine performances among different clinical trials; however, its possible limitation is that the responding cells are usually effectors or effector memory cells, which may not be the major long-term mediators of the control of virus replication. In addition, specific depletion of a particular cell subset can be performed to identify the phenotype of IFN- γ -secreting cells. Although all collected samples were also tested in a whole-blood intracellular IFN- γ -staining (WBICS) assay, which detects IFN- γ produced in response to specific peptide stimulation in semi-permeabilized cells by utilizing a fluorochrome-conjugated mAb followed by flow cytometry (Maino & Picker, 1998), this was, in our hands, much less sensitive than the ELISPOT assay (Goonetilleke *et al.*, 2006).

Induction of HIV-1-specific T cells by administration of pTHr.HIVA and MVA.HIVA vaccines to healthy volunteers was first demonstrated in small trials IAVI 001, 003 and 005 in Oxford, UK, designed primarily to address vaccine safety, and their equivalents in Nairobi, Kenya, recruiting up to 20 volunteers each (Table 1) (Mwau *et al.*, 2004). These trials were followed by much larger, multi-arm trial designs involving up to 120 study subjects and addressing a number of dose- and schedule-related issues. Of these, the first larger, phase I/IIa ('a' indicates the fact that volunteers are at low risk of infection) trial was designated IAVI 006, in which healthy subjects received 0, 0.5 or 2 mg pTHr.HIVA DNA intramuscularly twice, followed by 4 or 16 week intervals before two intradermal doses of 5×10^7 p.f.u. MVA.HIVA. The vaccines/placebos were delivered into alternating deltoid muscles. Overall, the vaccination-

induced responses were detectable in fresh PBMCs *ex vivo* by IFN- γ ELISPOT assay in <15 % of vaccinees in the active arms and the responses were detected only transiently, mainly after the first MVA.HIVA dosing. There was no statistically significant difference for the DNA doses or timing of the rMVA boost, nor was a significant effect of DNA priming detected. Similar data, indicating a low frequency of responders, were obtained in the other phase I/IIa trials in Africa (Table 1). The reasons for these initial results probably include low vaccine doses and suboptimal timing of blood sampling. As we show below, T-cell responses peak 1 week after the first MVA.HIVA administration and these early trials first assessed the vaccine-elicited T cells 2 weeks after the vaccination, by which time the *ex vivo* responses had at least halved (Goonetilleke *et al.*, 2006). It should be noted that we used and continue to use stringent positive-response criteria, requiring an assay positive to be higher than the 99th percentile of all pre-vaccination and placebo values and at least four times higher than the assay no-peptide background. Thus, trial IAVI 006 played an important part in our learning process.

Induction of antibodies against the p24/p17 domain of HIVA was assessed in a standard ELISA assay. Of all volunteers enrolled in the IAVI 001, 003 and 005 trials, only one DNA-rMVA prime-boost recipient developed detectable Gag-specific antibodies at the titre of 1/400 (Mwau *et al.*, 2004). This weak anti-Gag and absence of anti-Env antibody induction by the HIVA vaccines may facilitate identification of true HIV-1 infections by the first-line Western blot-based test used in trials in high-risk volunteers. Antibody responses induced by the MVA vector and measured against vaccinia virus (Lister strain)-infected cell lysates were detected in all vaccinees who received prior smallpox vaccination, whereas no increase of anti-vaccinia antibody was observed in smallpox-unvaccinated subjects. Thus, intradermal recombinant MVA at the 5×10^7 p.f.u. dose did not prime anti-vector antibody responses efficiently (Mwau *et al.*, 2004).

A rapid, small clinical trial can be highly informative. Once approved, clinical protocols allow very little flexibility and may take a long time to complete. The IAVI 006 phase I/IIa trial took over 2 years. Therefore, we decided to carry out a smaller and faster trial and analyse in much greater detail the quality of the HIVA vaccine-elicited immune responses. The trial, designated IAVI 016 (Goonetilleke *et al.*, 2006), enrolled 24 volunteers divided into two groups (eight subjects receiving the active products and four receiving placebos), vaccinated with either 2 \times MVA.HIVA (MVA.HIVA) or 2 \times pThr.HIVA DNA plus 1 \times MVA.HIVA (pThr.HIVA-MVA.HIVA). In this trial, we increased the pThr.HIVA DNA and MVA.HIVA vaccine doses to 4 mg and 2.5×10^8 p.f.u., respectively, used an earlier post-vaccination time point and employed a number of *ex vivo* and cultured assays to measure the T-cell responses in a more sensitive, but also memory-focused way. The IFN- γ ELISPOT-based analyses were carried out on fresh PBMCs and included the IAVI 006-validated *ex vivo* assay, an assay supplemented with interleukin (IL)-7/IL-15 and a 10-day peptide-driven culture expansion of PBMCs prior to enumeration of IFN- γ -secreting cells (so-called cultured ELISPOT). Thus, in contrast to the *ex vivo* assay, the cultured IFN- γ ELISPOT assay measures a cell population capable of proliferation and function in response to recall antigens, which is more relevant to central immunological memory and

protection against infection. In addition, isolated PMBCs were analysed by WBICS (described above) and multi-cytokine Luminex assays. Finally, the proliferative capacity of vaccine-induced cells was assessed in [³H]thymidine-incorporation and carboxyfluorescein diacetate succinimidyl ester (CFSE)-dilution assays. The same criteria for positivity as described above were used for all assays.

The results of the IAVI 016 trial shed a new light on the vaccine immunogenicities (Goonetilleke *et al.*, 2006). First of all, at no time was a positive response detected by any of the assays in placebo recipients. By using the *ex vivo* IFN- γ ELISPOT assay, no positive responses were detected following the MVA.HIVA regimen, but pThr.HIVA–MVA.HIVA elicited HIV-1-specific T cells in four of eight individuals, with an overall group mean of 210 (range, 4–957) HIV-specific antigen-responding cells, or s.f.u., per 10⁶ PBMCs. In the cultured IFN- γ ELISPOT assay, HIV-1-specific T-cell responses were detected in five of eight and eight of eight subjects that received the MVA.HIVA and pThr.HIVA–MVA.HIVA vaccination regimens, respectively. For the pThr.HIVA–MVA.HIVA regimen, the group mean of highest measured responses in the cultured IFN- γ ELISPOT assay was 4503 (range, 388–13 694) s.f.u. per 10⁶ PBMCs, mostly measured 2 weeks after administration of MVA.HIVA. It would have been of interest to determine the cultured IFN- γ ELISPOT frequencies 1 week after the first MVA.HIVA vaccination, as that was the peak for the *ex vivo* IFN- γ ELISPOT assays; however, these assays were not performed due to lack of cells. In all positive Gag-specific responses detected in cultured IFN- γ ELISPOT assays, the summed frequencies of s.f.u. induced to smaller peptide pools were similar to those detected against one large pool of all Gag peptides combined together, therefore verifying these results internally. The pThr.HIVA–MVA.HIVA regimen elicited mainly CD4⁺ T-cell responses, although two vaccinees also had detectable CD8⁺ T cells specific for the multi-epitope region of HIVA. In all assays detecting positive responses, the responses were larger in the pThr.HIVA–MVA.HIVA recipients than in those given MVA.HIVA alone. The cultured IFN- γ ELISPOT assay was the most sensitive test. All positive responses detected in *ex vivo* IFN- γ ELISPOT or IFN- γ WBICS assays were also detected by the cultured IFN- γ ELISPOT assay, whilst cultured IFN- γ ELISPOT assay responses correlated well with the CFSE-dilution proliferation assays and to some extent with the [³H]thymidine-incorporation proliferation assay. Four assays indicated the same kinetics of the vaccine-induced T cells, which peaked at 1 week after the first MVA.HIVA vaccination and decreased to 50 % by week 2 [see Figs 1a, b, 2a and 3a of the study by Goonetilleke *et al.* (2006)]. The kinetic results are consistent with studies on the vaccine biodistribution and persistence, which showed that both pThr.HIVA and MVA.HIVA vaccine DNA became undetectable within 5 weeks after application in sites other than the site of delivery (Hanke *et al.*, 2002, 2005) and indicated that the pThr plasmid and MVA are genuinely non-persisting vaccine vectors. Furthermore, the vaccine-induced HIV-1-specific T cells readily proliferated and produced multiple cytokines following re-exposure to HIV-1-derived peptides. These measurements are more relevant to the memory T-cell compartment than the *ex vivo* IFN- γ ELISPOT assay used in our previous trials. Finally, as for the breadth of the response, vaccine-induced T cells were detected to 29 out of 90 Gag peptides with four of eight vaccinees in the pThr.HIVA–MVA.HIVA group responding to two or

more epitopes. These detected responses were relevant to natural HIV-1 infection, because most CD4⁺ T-cell epitopes recognized by PBMCs of the pTHr.HIVA–MVA.HIVA group corresponded to previously defined CD4⁺ T-cell epitopes capable of promiscuous binding to multiple HLA-DR alleles in HIV-1-infected patients.

Re-evaluation by using novel, more sensitive assays. Having developed a very sensitive and reliable assay for detection of vaccine-induced T-cell responses, we are currently reanalysing frozen samples from trial IAVI 006 to determine whether the lower doses of the pTHr.HIVA and MVA.HIVA vaccines used initially primed HIV-1-specific T-cell responses in more volunteers than were detected originally by the *ex vivo* IFN- γ ELISPOT assay. Studies are also ongoing to assess the longevity of T-cell responses induced by pTHr.HIVA–MVA.HIVA vaccination. Memory T cells will be assessed in 30 HIV-1-negative volunteers who had received the HIVA vaccines through participation in one of the five Oxford clinical trials between 1 and 5 years ago, and 10 unvaccinated subjects as negative controls. We shall employ mainly cultured IFN- γ ELISPOT and [³H]thymidine-incorporation proliferation assays and compare these results with immune responses detected in fresh PBMCs during the course of the clinical trials.

Towards an effective therapeutic vaccine

Immunogenicity in HIV-1-infected patients on HAART. International efforts to expand access to HAART for HIV-1-infected patients are a short-term solution and do not address the issues of drug toxicity, drug resistance and sustainability of treatment regimens. Drug-sparing strategies for the control of HIV-1 replication are needed urgently. Therefore, we decided to test the immunogenicity of the HIVA vaccines in a therapeutic setting and conducted three small trials in HIV-1-infected patients on HAART. The study participants had to be clinically stable, i.e. they had sustained undetectable viraemia initially below 400 and later below 50 RNA genome copies ml⁻¹ and a CD4⁺ cell count above 300 cells μ l⁻¹, and remained on their HAART regimens through out the study. The clades of the patients' viruses were diverse, coming from Africa, Asia, Europe or the Americas.

In the first therapeutic trial, a low dose (0.5 mg) of pTHr.HIVA DNA vaccine was administered intramuscularly on two occasions to 10 subjects (Ther 001; Table 1) with a median pre-existing response to the HIVA-derived peptides of 831 s.f.u. per 10⁶ PBMCs. The DNA vaccination amplified the pre-vaccination HIV-1-specific CD4⁺ and CD8⁺ T-cell responses by two- to sixfold in three of 10 recipients, which was confirmed in assays with individual peptides. However, the overall group median showed no vaccine-induced increase in response at any time point after vaccination (Dorrell *et al.*, 2005). In subsequent trials, a low dose (5 \times 10⁷ p.f.u.) of MVA.HIVA vaccine was given intradermally on weeks 0 and 4 to eight previously unvaccinated subjects (Ther 002) and eight of the DNA-alone trial volunteers 2 years after the DNA vaccine (Ther 003). Two additional subjects were recruited as controls. The median pre-existing response to peptides derived from the consensus clade A Gag domain of HIVA was 1508 s.f.u. per 10⁶ PBMCs, of which over 50 % was mediated by CD8⁺ cells. Following

MVA.HIVA vaccination, the Gag-specific T-cell responses were amplified significantly in all 16 recipients of the active product. The response group medians were increased significantly at all time points after vaccination and remained significantly elevated 6 months after the first vaccination. For example, the median responses on weeks 2 and 8 increased by 882 and 1243 s.f.u. per 10^6 PBMCs, respectively, compared with the pre-vaccination levels. Also, the mean number of recognized peptide pools increased from five pools pre-vaccination to seven pools at the peak of responses. CD8⁺ cell-depleted IFN- γ ELISPOT assay revealed that CD4⁺ cells were augmented in 13 of 15 vaccinees (one subject was excluded from the analysis due to high no-peptide background levels) (Ondondo *et al.*, 2006). By using 13 major histocompatibility complex (MHC)–peptide tetrameric complexes relevant to this study, the vaccine-driven T-cell expansions were predominantly in the CD45RA⁻ CCR7⁺ and CD45RA⁻ CCR7⁻ compartments and persisted for at least 1 year. Furthermore, the expanded T cells had marked but transient upregulation of the activation marker CD38 and perforin, and were capable of *in vitro* proliferation upon re-exposure to HIVA-derived peptides. Finally, possibly due to the low doses and low numbers of enrolled subjects, we did not observe any significant effect of the pTHr.HIVA DNA priming, although there was a trend for the responses in the DNA-primed subjects to peak after the first MVA.HIVA administration rather than after the second, as was observed in the recipients of MVA.HIVA alone. This would be consistent with our DNA–rMVA studies in healthy, HIV-1-uninfected volunteers (Dorrell *et al.*, 2006).

The immunogenicity of the MVA.HIVA vaccine in the above two trials is remarkable, despite a number of factors not being in its favour. First, according to the patients' pre-treatment CD4⁺ cell nadir of 180 cells μl^{-1} , the study cohort comprised patients with a more advanced disease prior to commencing HAART than in other reported trials (Jin *et al.*, 2002; Robbins *et al.*, 2003; Levy *et al.*, 2005, 2006; Tubiana *et al.*, 2005). Second, a relatively low dose of recombinant MVA was used compared with previously reported studies using poxvirus-vectored vaccines in humans and NHPs (Wee *et al.*, 2002; Cosma *et al.*, 2003; Santra *et al.*, 2004; Harrer *et al.*, 2005; Tubiana *et al.*, 2005). Third, the vaccine immunogen was not matched to the infecting viruses. Whilst HIVA was derived from consensus HIV-1 clade A (Hanke & McMichael, 2000), the patients enrolled in these trials were mainly infected with other HIV-1 subtypes.

Broadening of T-cell responses by addition of immunogen RENTA. The HIVA immunogen has proved extremely useful as a model immunogen for both preclinical and clinical comparative studies of a number of vaccine vectors and heterologous vaccination regimens. However, an important aspect of vaccine development is finding formulations capable of inducing T-cell responses specific for multiple HIV-1 proteins. To broaden responses induced by the existing HIVA vaccines, we designed a second immunogen designated RENTA and inserted its gene into the pTHr plasmid and MVA vectors (Nkolola *et al.*, 2004). RENTA is derived from the consensus HIV-1 clade A amino acid sequences (Korber *et al.*, 1997) of reverse transcriptase, Env, Nef and Tat. Biological activities associated with these proteins that probably contribute to HIV-1 pathogenesis were inactivated. In addition, epitopes recognized by mouse and rhesus macaque CD8⁺ T cells, as well as a mAb epitope, were added to the C terminus of the protein to

facilitate the preclinical studies. We have extensively demonstrated immunogenicity of a single delivery of either of the pTHr.RENTA DNA or MVA.RENTA vaccines and their prime–boost combinations, and shown that immune responses induced by HIVA were broadened by co-administration of RENTA in BALB/c mice and rhesus macaques (Nkolola *et al.*, 2004; Im *et al.*, 2006). We are now planning to test the two-component MVA.HIVA/MVA.RENTA vaccine in a small clinical trial in HIV-1-infected individuals on HAART to assess its immunogenicity and efficacy (Table 1). The latter will be determined by a detailed analysis of vaccine-induced T-cell responses and kinetics of the virus rebound following a supervised HAART interruption at the peak of MVA.HIVA/MVA.RENTA-induced immune responses.

Conclusions

Whilst data from larger phase I/IIa trials using the validated IFN- γ ELISPOT assay detected responses in <15 % of vaccine recipients, data from small clinical trials in both healthy and HIV-1-infected individuals on HAART using a number of more sensitive and highly reproducible, but research-grade, assays demonstrated that the pTHr.HIVA and MVA.HIVA vaccines induced HIV-1-specific T-cell responses in a majority of vaccine recipients. Whilst the pTHr.HIVA DNA alone primed consistently weak and mainly CD4⁺, but also CD8⁺ T-cell responses, MVA.HIVA delivered a consistent boost to both CD4⁺ and CD8⁺ T cells, which was particularly strong if the HIV-1-specific T cells were primed efficiently, e.g. by HIV-1 infection. In healthy subjects, the responses elicited by the pTHr.HIVA–MVA.HIVA regimen were higher than those induced by MVA.HIVA alone.

In naïve individuals, intramuscular DNA injection elicited some CD8⁺, but mainly CD4⁺ T-cell responses that were expanded by rMVA boost. This is in agreement with other published clinical studies employing the DNA–rMVA regimen (Moorthy *et al.*, 2003; Vuola *et al.*, 2005). Although CD8⁺ T cells are typically the effector cells, induction of multifunctional and rapidly expandable CD4⁺ T cells is important for both maintenance and effective stimulation of CD8⁺ T-cell responses during priming, as well as following antigenic re-exposure (Sun *et al.*, 2004; Barber *et al.*, 2006). Indeed, at least one study associated vigorous anti-HIV-1 CD4⁺ T-cell responses with control of viraemia (Rosenberg *et al.*, 1997).

Whilst MVA confirmed its position as one of the leading vaccine vectors for boosting, priming by intramuscular needle injections using 2× 4 mg DNA resulted in weak, vaccine-specific T-cell responses, which comprised mainly CD4⁺ T cells of the Th1 type. This presumably low-level expression of immunogens by DNA vaccines may favour induction of better-quality response, e.g. higher T-cell avidity (Estcourt *et al.*, 2002), but it is likely to be a limiting factor for the vigour of the DNA prime–rMVA boost-induced response. Therefore, it is essential to evaluate other priming strategies that have shown greater promise in preclinical studies in humans. Means have been sought to improve DNA immunogenicity through the use of *in vivo* electroporation (Zhang *et al.*, 2003b), biojectors (Raviprakash *et al.*, 2003), gene gun (Haynes *et al.*, 1996), aerosols generated either mechanically (Koshkina *et al.*, 2003) or electrically for inhalation, topical dermal application (Choi & Maibach, 2003), so-called DNA

tattooing (Bins *et al.*, 2005) or chemical/genetic adjuvants (Barouch *et al.*, 2000, 2002; Gursel *et al.*, 2002). An alternative way to increase the potency of priming is to employ other vaccine modalities, such as other attenuated poxvirus (Hutchings *et al.*, 2005; Vuola *et al.*, 2005; Webster *et al.*, 2005), human, non-human and chimeric adenovirus (Farina *et al.*, 2001; Gilbert *et al.*, 2002; Casimiro *et al.*, 2004; Roberts *et al.*, 2006), BCG (McShane *et al.*, 2004; Vordermeier *et al.*, 2004; Williams *et al.*, 2005), Semliki Forest virus (Hanke *et al.*, 2003; Nordström *et al.*, 2005) or protein (Gilbert *et al.*, 1999; Larke *et al.*, 2005)-based vaccines. Many of these alternative vectors have already entered or are in the pipeline for clinical-trial evaluation (<http://www.iavi.org/>).

The T-cell immunogenicity of the pTHr.HIVA–MVA.HIVA vaccines in healthy individuals (Goonetilleke *et al.*, 2006) is in a good correlation with data obtained in the NHP model. In our initial rhesus macaque study using the polyepitope immunogen HW and the same pTH and MVA vectors, we showed that the DNA prime–rMVA boost regimen was better than rMVA alone, and the tetramer frequencies of vaccine-induced T cells peaked 1 week after the rMVA dosing and decreased thereafter. The difference from the results in humans was that an efficient boost was delivered even with the second and third rMVA dosing (Hanke *et al.*, 1999). In the following study, the HIVA vaccines induced T cells releasing IFN- γ upon stimulation with multiple HIVA-derived peptides, which were, however, at least in the one monkey tested, mostly CD8⁺ (Wee *et al.*, 2002). This might be due to the difference between the relative NHP and human doses. For example, if the same vaccine dose is used in a 2 kg monkey as in a 180 cm/80 kg man, the monkey receives an approximately 20-fold higher dose per unit of body surface area (Mosteller, 1987). Furthermore, by using a four-component vaccination with DNA–MVA/HIVA–RENTA, multi-specific Mamu-A*01-restricted T cells were elicited, which readily proliferated, produced and released IFN- γ and tumour necrosis factor alpha, and lysed peptide-sensitized target cells. The same monkeys had HIV-1-specific responses detectable in both *ex vivo* and cultured IFN- γ ELISPOT assays at multiple time points up to 68 weeks post-vaccination (Nkolola *et al.*, 2004; Im *et al.*, 2006). As there is no adequate challenge model for the HIVA-RENTA vaccines other than using chimpanzee infection with HIV-1, the four-component vaccination has not been tested in NHPs for protective efficacy. Overall, a long list of similarities was detected between the NHP and human data, arguing that the NHP models can be used to better inform clinicians on vaccine potential.

To put our effort into the context of the overall HIV-1 vaccine development, to date, close to 100 investigational products have been tested in over 200 clinical trials of candidate HIV-1 vaccines. Many of the early vaccine candidates focused on induction of neutralizing antibodies; only later did the emphasis shift towards induction of T cells. Because HIV-1 live-attenuated viruses are too dangerous and inactivated HIV-1 particles do not induce CD8⁺ T cells efficiently, recombinant subunit mainly genetic vaccines, particularly in heterologous prime–boost combinations, currently attract the greatest attention. These innovative technologies include plasmid DNA, non-replicating viruses, attenuated bacteria, virus-like particles, proteins and peptides. The currently most studied viral vaccine vectors include non-replicating human adenovirus serotype 5 (HAdV-5) and its modified versions, attenuated poxviruses such as

ALVAC (canarypox), NYVAC (New York vaccinia virus attenuated by a rational deletion of 18 genes), MVA and fowlpox FWPV-9, followed closely by the alphavirus vector Venezuelan equine encephalitis virus and adeno-associated viruses (Anonymous, 2002; Davis *et al.*, 2002; Franchini *et al.*, 2004; Im & Hanke, 2004; Barouch & Nabel, 2005). All of these constructs infect human cells and deliver immunogen-derived peptides into the MHC class I presentation pathway either directly or through cross-priming, but replicate poorly or not at all. For most of these vectors, clinical immunogenicity data are only now emerging. Vigorous and sustained responses were induced by rHAdV-5; however, no human data have been published on the HIV-1 vaccine candidates, which recently entered a phase IIb proof-of-principle trial in high-risk individuals (Anonymous, 2004, 2005). For all of the attenuated recombinant poxvirus vaccines, broadly similar T-cell immunogenicities in *ex vivo* assays have been observed in small phase I/IIa trials (Ferrari *et al.*, 1997; Belshe *et al.*, 1998; Evans *et al.*, 1999; AIDS Vaccine Evaluation Group 022 Protocol Team, 2001; Gupta *et al.*, 2002; Cao *et al.*, 2003; Musey *et al.*, 2003). Larger datasets will be needed, in addition to the use of multiple validated assays, to discern any beneficial differences. Much more will be learned about these vectors in the coming years.

In conclusion, our clinical experience with the DNA- and MVA-vectored vaccines demonstrated that the perceived performance of a vaccine in humans is critically dependent on the trial design and assays employed to evaluate vaccine immunogenicity. The situation is not helped by the fact that simple correlates of protection against HIV-1 infection and/or progression to AIDS have not been identified. Whilst it is good practice to run NHP studies along with human studies, no NHP challenge experiment can replace a phase IIb proof-of-principle trial in individuals at high risk of HIV-1 infection; clinical trials are and will remain 'the proof of the pudding'. Because clinically relevant protection can be achieved by current vaccines in NHP models, which usually use higher challenge doses and more virulent challenge viruses than those that humans are exposed to, we may have vaccines that might benefit people, if only high-risk groups, in our hands today. Therefore, proof-of-principle trials employing current vaccine modalities should be included more systematically in the Global HIV/AIDS Vaccine Enterprise programme in addition to the commendable rational approach.

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Table 1. Overview of prophylactic and therapeutic trials using pTHr.HIVA, MVA.HIVA and MVA.RENTA vaccines

Trial/vaccine(s)	Site*	Subjects	Dose/route (D, mg; M, p.f.u.)†	Outcome of IFN- γ ELISPOT assay (ex vivo/cultured)‡	Publications	Comments	
IAVI 001 pTHr.HIVA	Oxford	18 healthy HIV-1 ⁻	0.1 or 0.5 mg 2 \times i.m.	78 %/ND (DD)	Mwau <i>et al.</i> (2004); Cebere <i>et al.</i> (2006)	First series of phase I clinical trials.	
IAVI 003 MVA.HIVA	Oxford	8 healthy HIV-1 ⁻	5 \times 10 ⁷ p.f.u. 2 \times i.d.	88 %/ND (MM)			
IAVI 005 IAVI 001+MVA.HIVA	Oxford	9 healthy HIV-1 ⁻	0.1 or 0.5 mg 2 \times i.m. 5 \times 10 ⁷ p.f.u. 2 \times i.d.	89 %/ND (DDMM)			
IAVI 002 pTHr.HIVA	KAVI	18 healthy HIV-1 ⁻	0, 0.1 or 0.5 mg 2 \times i.m.	15 %/ND (DD) 20 %/ND (PP)	Manuscripts in preparation		
IAVI 004 MVAHIVA	KAVI	18 healthy HIV-1 ⁻	0 or 5 \times 10 ⁷ p.f.u. 2 \times or 3 \times i.d.	25 %/ND (MM/M)			
IAVI 004+MVA.HIVA		11 healthy HIV-1 ⁻		17 %/ND (PP)			
IAVI 008 IAVI 002+MVA.HIVA	KAVI	10 healthy HIV-1 ⁻	0.5 or 1 mg 2 \times i.m. 5 \times 10 ⁷ p.f.u. 2 \times i.d.	10 %/ND (DDMM)			
IAVI 006 pTHr.HIVA+MVA.HIVA	Oxford/IC	119 healthy HIV-1 ⁻	0, 0.5 or 2 mg 2 \times i.m. 0 or 5 \times 10 ⁷ p.f.u. 2 \times i.d. 4 or 16 wk D-M interval	12 %/ND (DDMM) 6 %/ND (PPMM) 0 %/ND (PPPP)	Manuscripts in preparation		Phase I/IIa trials using improved assays and centralized reagents provided by the IAVI Core Laboratory. All of these trials were planned at more or less the same time.
IAVI 009 pTHr.HIVA+MVA.HIVA	UVRI	50 healthy HIV-1 ⁻	0 or 0.5 mg 1 \times or 2 \times i.m. 0 or 5 \times 10 ⁷ p.f.u. 2 \times i.d.	15 %/ND (DDMM) 15 %/ND (DPMM)			
IAVI 010 pTHr.HIVA+MVA.HIVA	KAVI/Kings	114 healthy HIV-1 ⁻	0.5 mg 2 \times i.m. 0, 5 \times 10 ⁶ , 5 \times 10 ⁷ or 2.5 \times 10 ⁸ p.f.u. 2 \times i.d., s.c. or i.m.	0 %/ND (PPPP) 3 %/ND (DDMM) 5 %/ND (PPPP)			
IAVI 011 MVA.HIVA	Kings/Lausanne /Soweto	81 healthy HIV-1 ⁻	0, 5 \times 10 ⁶ , 5 \times 10 ⁷ or 2.5 \times 10 ⁸ p.f.u. 2 \times i.d., s.c. or i.m.	6 %/ND (MM) 0 %/ND (PP)	Goonetilleke <i>et al.</i> (2006)		
IAVI 016 pTHr.HIVA+MVA.HIVA	Oxford	24 healthy HIV-1 ⁻	0 or 4 mg 2 \times i.m. 0 or 2.5 \times 10 ⁸ p.f.u. 1 \times or 2 \times i.d.	50 %/100 % (DDM) 0 %/63 % (MM) 0 %/0 % (PP or PPP)			
OX-LTF-01 pTHr.HIVA+MVA.HIVA	Oxford	30 healthy HIV-1 ⁻	Long-term follow-up	Ongoing	Dorrell <i>et al.</i> (2005)		
Ther 001 pTHr.HIVA	Oxford	10 HIV-1 ⁺ /HAART	0.5 mg i.m.	30 %/ND (DD)			
Ther 002 MVA.HIVA	Oxford	10 HIV-1 ⁺ /HAART	0 or 5 \times 10 ⁷ p.f.u. 2 \times i.d.	100 %/ND (MM) 0 %/ND (PP)		Dorrell <i>et al.</i> (2006); Ondondo <i>et al.</i> (2006)	
Ther 003 pTHr.HIVA+MVA.HIVA	Oxford	10 HIV-1 ⁺ /HAART	0 or 0.5 mg 2 \times i.m. 0 or 5 \times 10 ⁷ p.f.u. 2 \times i.d. 0 or 2.5 \times 10 ⁸ p.f.u. i.d.	100 %/ND (DDMM) 0 %/ND (PPPP) Ongoing			
Ther 004 Ther 002/003+MVA.HIVA	Oxford	10 HIV-1 ⁺ /HAART +HAART interruption		Ongoing	Goonetilleke <i>et al.</i> (2006)	A novel approach to evaluation of vaccine immunogenicity.	
Ther 005 MVA.RENTA	Oxford	10 HIV-1 ⁺ /HAART	0, 5 \times 10 ⁷ or 2.5 \times 10 ⁸ p.f.u. 2 \times i.d.	Year 2007			
Ther 006 MVA.HIVA/MVA.RENTA	Oxford	20 HIV-1 ⁺ /HAART +HAART interruption	To be decided	Year 2007			

*Oxford, Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK; KAVI, Kenyan AIDS Vaccine Initiative, Kenya; IC, Imperial College London, UK; UVRI, Ugandan Virology Research Institute, Uganda; Kings, Kings College London, UK; Soweto, Chris Hani Baragwanath Hospital, South Africa; Lausanne, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland.

†i.m., Intramuscular; i.d., intradermal; s.c., subcutaneous; D, pTHr.HIVA DNA; M, MVA.HIVA; P, placebo; ND, not done.

‡Percentage of recipients of the active product with detected vaccine-induced responses.