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Viral vectors as vaccine platforms: deployment in sight

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A little more than a decade after the explosion of research into recombinant live-attenuated or replication-deficient viruses as vaccine platforms, many viral vector-based vaccines have been licensed for animals. Progress has been slower for humans but 2011 will see the licensure of the first viral-vectored vaccine for humans, against Japanese Encephalitis. In addition a vaccine with a viral-vectored component showed efficacy against HIV infection in humans. Viral-based vaccines have an excellent safety profile but must deal with the potential problem of pre-existing anti-vector immunity. Recent successes reflect diverse improvements such as development of new adenovirus serotypes and better prime-boost approaches, suggesting that many viral vectors are approaching their final years as vaccine 'candidates' rather than vaccines.

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Introduction

The deployment of viruses as vaccine vectors utilizes mechanisms that evolved in these microbes for entering cells and hijacking the cellular machinery to express viral proteins [1••]. Molecular virology has sufficiently advanced that recombinant viruses have been successfully rendered highly attenuated or replication deficient, giving them an excellent safety profile in animal and clinical trials. However, the host immune system has also evolved mechanisms to inhibit viral infections: activation of innate immunity can provide adjuvant activity, but may also decrease vector efficiency [2••]. The adaptive immune responses to the vector may also block or reduce the induction of the desired responses against the vaccine antigen. This gave rise to concerns about the usefulness of such vaccines in target populations where pre-existing anti-vector immunity is high. Despite successes for veterinary use, such as the early licensure of a vaccinia virus-based vaccine against rabies, the scientific community has been awaiting clear success of such a vaccine against a human disease in clinical trials. Excitingly, a vectored-vaccine against Japanese Encephalitis for use in humans is now ready for development: Imojev (Sanofi Pasteur), a recombinant yellow fever virus encoding two structural proteins from the Japanese Encephalitis virus, was licensed in Australia at the end of 2010 for use from 12 months of age.

We describe here the encouraging progress and success observed in the past two years to circumvent pre-existing immunity, in particular for adenovirus vectors, which indicates that boosting with the same vector can overcome pre-existing immunity, greatly broadening their potential application (O'Hara *et al.*, submitted). Recent clinical trials have demonstrated that these vaccines can induce T-cell immune responses of unprecedented magnitude, and improvements in production and stabilization finally should make viral-vectored vaccines feasible for large-scale deployment.

The basics: advantages of viral-vectored vaccines

Opportunities for new vaccines exist for nearly 40 human pathogens. Most of the 30 licensed vaccines against 26 other viral and bacterial infections, based on subunits, inactivated microorganisms, live attenuated microorganisms and toxoids, protect by inducing antibodies. While these approaches have shown tremendous success in preventing disease, new technologies are required to overcome their limitations. For example, it has proved difficult to elicit antibody-mediated protection against HIV [3] while use of an attenuated HIV would generate substantial safety concerns [4].

Viral vectors offer a series of advantages over traditional vaccines: in addition to inducing outstanding antibody responses, they also elicit cytotoxic T lymphocytes (CTL) that are crucial for control of intracellular pathogens and cancer, and that are not induced by protein-based vaccines [2••]. T-cell inducing vaccines can also elicit responses against highly conserved epitopes, potentially offering protection against several strains of the

same pathogen [1••], for example to generate universal heterosubtypic influenza [5] and HIV vaccines. For the latter, this development involved the construction of a multi-clade vaccine derived from the most conserved regions of the HIV-1 consensus proteome (HIVconsv), which has proven to be highly immunogenic in macaques [6,7]. Another approach, known as mosaic antigen design, utilizes *in silico* algorithms to generate synthetic immunogens that cover genetically diverse circulating virus isolates, increasing the breadth and depth of the $CD8^+$ response compared to consensus immunogens [8,9]. For both approaches, recombinant viral vectors have played a central role in inducing high T-cell frequencies.

Many viral species have been evaluated as recombinant vectors for vaccines, including alphaviruses (such as Semliki forest virus), adeno-associated viruses, vesicular stomatitis virus, measles virus, poliovirus and hepatitis B virus. Their potentials and concerns are well described in a review by Brave *et al.* [10]. Yellow fever virus is of particular interest as it is the first to reach human licensure; however, it is currently limited to expression of flavivirus antigens, such as those of dengue virus, or small epitopes, and as a replication competent vector induces a significant viremia. Several studies describing efficiency of other vectors in animal models have been reported in the last year, including baculovirus, Newcastle disease virus, ovine atadenovirus, rabies virus, pseudorabies virus, equine herpes virus, influenza A virus and coxsackievirus. One striking publication demonstrated that the lymphocytic choriomeningitis virus can elicit CTL responses equivalent to or greater than those obtained with human adenovirus 5 (AdHu5) or vaccinia virus, and crucially without eliciting vector-specific antibodies [11•]. Such developments suggest that new viral-vectored vaccine platforms, although about 10 years behind adenoviruses (Ad) and poxviruses in clinical development, may well catch up during the coming decade. Currently however, the most widely evaluated vectors to date are AdHu5 and members of the poxvirus family (Box 1) [1••].

Tailoring the immune response to specific applications – different vectors, different routes, different outcomes

Viral-vectored vaccines induce systemic T-cell responses that have been extensively characterized, including polyfunctional cytokine-secreting $CD4^+$ and $CD8^+$ T-cells [12•]. More research is aimed at inducing immunity at the site of microbe encounter: the mucosa. Mucosal delivery has been investigated for many years, especially for adenoviruses owing to the natural tropism of many serotypes to the respiratory epithelium. However viral-vectored vaccines need not be delivered mucosally to elicit mucosal responses, and these responses can be elicited in unexpected sites: a systemic prime-boost-boost strategy (DNA-NYVAC-Adhu5) induces a anti-SIV T-cell response in the milk of lactating rhesus monkeys, as strong as that found in the blood and vaginal tract, suggesting that such a vaccine could reduce HIV transmission through breast-feeding [13]. Several studies have demonstrated that viral vector-based vaccines can impact on mucosal infection, in particular against rectal SIV or SHIV challenge in rhesus macaques immunized with a chimpanzee adenovirus prime and poxvirus boost [14], or with heterologous alphavirus prime-boost incorporating both intramuscular and mucosal delivery [15]. The differential phenotype and trafficking of vaccine-induced $CD8^+$ T cells was investigated after adenoviral-vectored vaccine delivery by different routes (intramuscular, oral, intranasal, intrarectal, aerosol). The immunization route dramatically impacted not only the quantity but also the phenotype of $CD8^+$ T-cells. In particular, intranasal immunization, despite inducing similar transgene expression as intramuscular injection, generated lower frequency and less polyfunctional T cells while respiratory administration by aerosol elicited systemic and mucosal polyfunctional $CD8^+$ T cell responses [16].

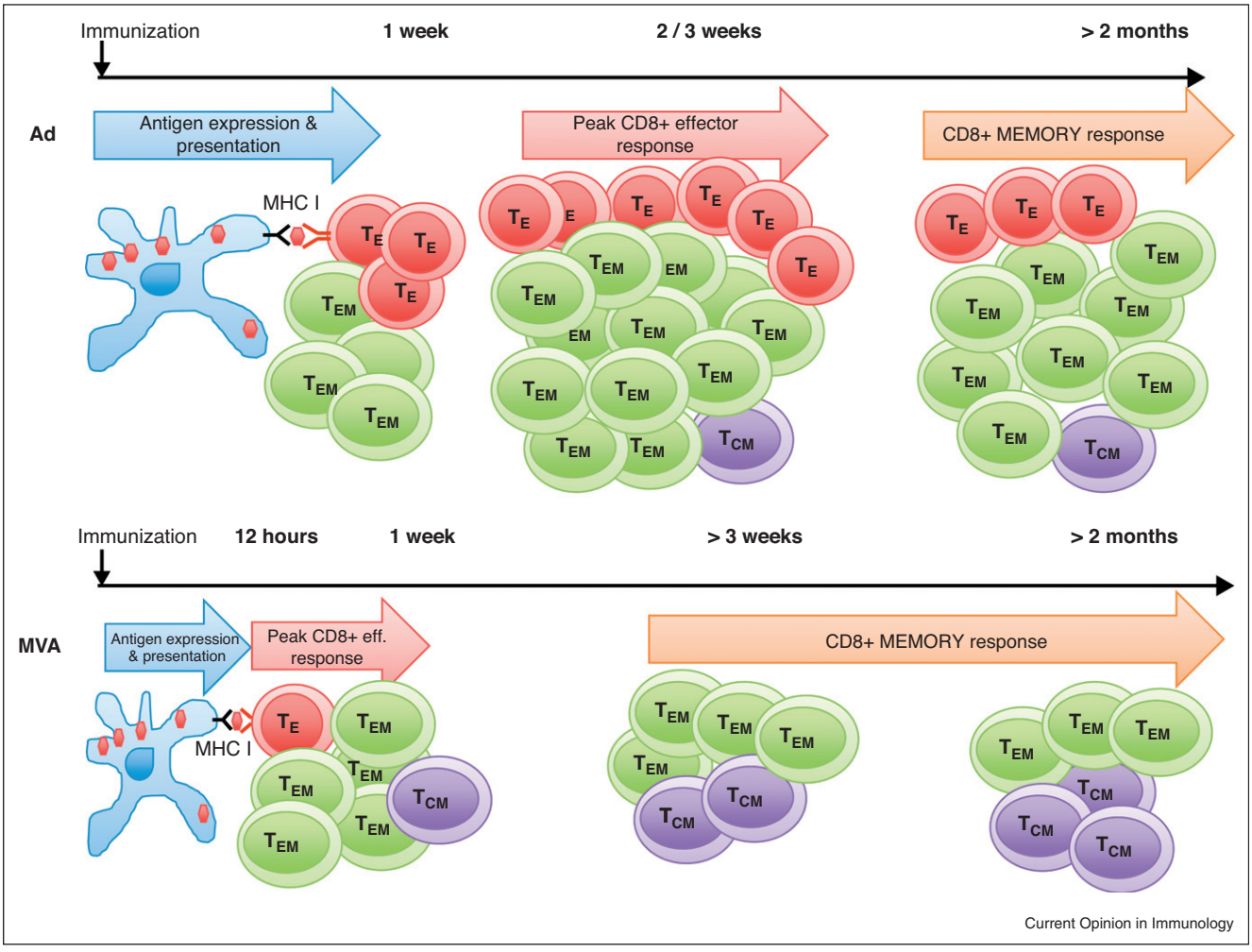
Investigation of the memory responses induced by viral-vectored vaccines is of particular importance and should be the focus of more work, as immune memory is fundamental to the durable efficacy of prophylactic vaccines. Different viral vectors can indeed influence the type of memory responses elicited against the transgene as exemplified recently in a study comparing Ad and MVA (Reyes Sandoval *et al.*, submitted). While Ad induces a strong $CD8^+$ T cell effector memory response and little $CD8^+$ T cell central memory, the opposite is induced after MVA immunization. This phenomenon, illustrated in Figure 1, is probably at least partly linked to the duration of antigen expression. In conclusion, all of these properties should be considered when selecting a vector able to induce the appropriate immune response for a particular disease. Furthermore, there is now interest in combining these vectors with known or new adjuvants, in order to increase or further modulate the immune responses elicited. This type of study will probably be increasingly important in the coming years.

Circumventing pre-existing immunity to adenoviruses: the rise of chimpanzee serotypes

Genetically modified human adenoviruses are a leading vaccine platform but their ability to induce vector-specific immune responses can result in a lack of prolonged expression of newly delivered genes upon re-administration of the same vector [14]. More importantly, despite

Box 1 *Vaccines based on poxviruses* are derived from vaccinia virus (the smallpox vaccine) or members of the avipox genus. Attenuated derivatives of vaccinia virus are used as vaccine platforms, such as NYVAC and Modified Vaccinia Ankara (MVA). The avian poxviruses infect but do not replicate in mammalian cells, and include attenuated canarypox virus (ALVAC) and fowlpox virus.

Figure 1



Difference between the Ad and MVA viral vectors in the kinetics of antigen-specific $CD8^+$ T cell expansion, contraction and generation of memory phenotype. Three major subsets of antigen-experienced $CD8^+$ T cells can be identified, based on the expression of CD62L (L-selectin) and CD127 (IL-7R α-chain): effector T cells (T_E) ($CD43^{hi}$, $CD62L^-CD127^-$), effector memory T cells (T_{EM}) ($CD62L^-CD127^+$) and central memory T cells (T_{CM}) ($CD62L^+CD127^+$). In a malaria model in mice, MVA induced lower $CD8^+$ T cell responses as compared to Ad, peaking at week one, but accelerated T_{CM} generation. In contrast, Ad vectors, which permit persistent antigen expression, resulted in delayed kinetics, with the peak of $CD8^+IFN\text{-}\gamma^+$ cell expansion at three weeks, a slow contraction phase and a prolonged T_E and T_{EM} response (Reyes Sandoval *et al.*, submitted).

the efficient induction of humoral and cellular immune responses in animal models, a major problem is that most humans have high titres of neutralizing antibodies against several Ad serotypes including AdHu5, the most widely studied adenovirus as a vaccine platform, owing to exposure since early childhood, negatively affecting their performance as vectors [14]. This has prompted the development of alternative serotypes of adenovirus not circulating in human populations such that the prevalence of neutralizing antibodies is low [17,18]. One strategy is the identification and development of rare human serotypes such as AdHu35 [19], AdHu28 [20], or modification of the AdHu5 capsid [21,22]. Adenoviruses isolated from chimpanzees (AdC) have also been well characterized and developed as vectors. Chimpanzee adenoviruses identified to date comprise a range of serotypes many of which are part of subgroup E, making them phylogenetically group phylogenectially with, but are distinct from, human species E adenoviruses [23]. The first report on the use of AdC vectors involved AdC68 expressing rabies virus glycoprotein and showed induction of high levels of antibodies protective against rabies challenge [24]. AdC vectors were subsequently utilized as T cell vaccines for HIV, inducing antigen-specific $CD4^+$ and $CD8^+$ T cell responses in mice [25] and rhesus macaques [26]. This made the AdC vectors an attractive option for other T-cell inducing vaccines, such as the pre-erythrocytic malaria vaccines, where excellent antibody and $CD8^+$ T cell responses were induced with a single dose of simian adenovirus, relative to the earlier poxviral (fowlpox virus),

MVA and the AdHu5 vectors [27]. More recently, their particular ability to efficiently prime T-cell responses has prompted their use in heterologous regimens using adenoviral vectors boosted with MVA to further augment the antigen-specific T-cell response [12•,28]. These pre-clinical studies have laid the basis for the use of the AdC vectors in human trials, especially for malaria, as discussed below.

The long-awaited success of viral-vectored vaccines in clinical trials

The past two years have seen remarkable progress in the development of viral-vectored vaccines against some of the main diseases affecting global health. After the disappointing lack of efficacy and suggested, but probably unfounded, safety concerns over the use of AdHu5 in HIV-infected subjects [29,30], the HIV field quickly rebounded. The Thai efficacy trial of a prime-boost regimen comprising a canarypox vector (ALVAC-HIV, Sanofi Pasteur) followed by a gp120 subunit in Alum (AIDSVAX B/E, Global Solutions for Infectious Diseases), showed a statistically significant trend towards preventing HIV infection in an at-risk population [31••]. As a booster vaccination, the AIDSVAX B/E vaccine achieved protective immunity, despite the previous lack of efficacy of AIDSVAX B/E alone in a Phase III trial. This highlights a key property of viral vectors as vaccine platforms in that they can be combined in a plethora of permutations to achieve the desired immunological endpoint. Despite the potential to combine viral vectors, most recently published Phase I trials of new candidate HIV vaccines employ a DNA prime, adenoviral boost regime that induces only modest T cell frequencies (median IFN-γ spot forming cells per million of 891 [32] and 237 [33]) or employ only a single vector [34]. The addition of a second viral vector such as a poxvirus, which is currently the best boosting vector, should further enhance T cell immunity, particularly the $CD8^+$ T cell frequencies that are so important to HIV immunity [35].

Table 1 illustrates how the specific properties of each of the main groups of viral vectors are employed to harness their respective advantages. For example, a Phase I trial boosting BCG-induced immunity with an MVA vector induces high frequencies of $CD4^+$ T cells with substantial durability [36]. Priming with a simian adenovirus and boosting with an MVA vector, both expressing a liver-stage malaria antigen, induced unprecedented cellular immunogenicity and a previously unattainable degree of T cell mediated protection against experimental challenge with malaria parasite in a phase IIa trial (Ewer *et al.*, submitted). In addition, the protection induced was still measurable more than 6 months later. However, vaccine-mediated protection against the erythrocytic stage of malaria remains an elusive goal despite some 35 clinical trials. Of these, only 3 employed viral vectors, of which none has yet reported results; the remainder are mostly trials of protein-in-adjuvant formulations. However, an interesting pre-clinical development is the combination of protein-in-adjuvant with viral vectors in both priming and boosting vaccinations, harnessing the ability of the former to induce extremely high antibody titres and $CD4^+$ T cell frequencies and the latter to induce protective $CD8^+$ T cell responses. In murine and macaque studies, these combinations induced significantly higher titres than prime-boost with viral vectors alone [37] and this regimen is currently entering clinical trials in the UK.

Reaching real life: improvements in design and deployability

That such a large proportion of currently licensed vaccines are based on viruses, either live-attenuated or inactivated, suggests *a priori* that there is no fundamental barrier to product-scale manufacture of viral-vectored vaccines. However, the doses utilized in recent clinical trials – over 10^{10} viral particles of adenovirus and 10^8 plaque forming units of MVA – are substantially larger than those typically employed for traditional viral vaccines (but small by gene therapy standards). Although current processes are up to the task both in practical and safety terms, and indeed in operation for MVA in its original, non-recombinant incarnation as a 'third-generation' smallpox vaccine [38], it is likely that development of products based on viral vectors will be accompanied by incremental advances in cell line and virus purification technology for manufacturing. In parallel, there is scope for enhancement of the vectors themselves, whose design for vaccine applications as tested in the clinic has changed little fundamentally since the inception of E1/E3-deleted adenovirus and recombinant vaccinia virus. In particular,

Table 1

Use of viral vectored vaccines in clinical trials registered at http://clinicaltrials.gov (accessed 20th December 2010)

Disease	All vaccine trials (*n*)	Viral vector vaccine n (%)	Type of vector			
			Adenovirus	Vaccinia virus (MVA/NYVAC)	Fowlpox virus	ALVAC
Malaria	88	15 (17)	7	11	5	0
Tuberculosis	48	23 (47.9)	3	19	1	0
HIV/AIDS	277	94 (33.9)	43	26	4	34
Cancer	932	76 (8.2)	35	14	37	9
Total	1345	208 (15.5)	88	70	47	43

the recent application of bacterial artificial chromosome 'recombineering' technology to genetic manipulation of MVA [39] and adenoviruses (M.J. Dicks *et al.*, manuscript in preparation) greatly facilitates the generation of novel vectors with the linked goals of fine-tuning innate immune stimulation for optimal immunogenicity and overcoming the immunodominance of vector antigens over transgenic antigens. Excitingly, the recent development of a membrane-based low temperature drying technology able to thermostabilize adenovirus and MVA for up to 6 months at 45 °C in carbohydrate glass presents the opportunity to deploy any future vaccine based on these vectors in a refrigeration-free, single-dose format able to drive adoption in even the most impoverished districts of underdeveloped countries [40••].

Conclusions

The field of viral vectors as vaccine platforms is evolving at high speed, and the diversity of related publications last year is an indication of how dynamic this field is and the enthusiasm of the scientific community. Although not covered in this review, research focusing on the mechanisms involved in the efficacy of viral-vectored vaccines has also been prolific recently [1••]. Therefore, after the doubts and fear induced by one HIV vaccine clinical trial [29,30], the progress made in enhancing viral-vectored vaccine-induced immunity, the development of alternative adenoviral serotypes and improved vector production and stability have culminated in several successes, most notably the Thai HIV clinical trial [31••].

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