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Vaccine 31 (2013) 4223–4230

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Utilizing poxviral vectored vaccines for antibody induction—Progress and prospects

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ARTICLE INFO

*Article history:*
Available online 5 June 2013

*Keywords:*
Viral vector vaccine
Prime–boost
Antibody
B cell
Adenovirus
Poxvirus
MVA
NYVAC
Vaccinia
Canarypox
ALVAC
DNA vaccine

ABSTRACT

Over the last decade, poxviral vectors emerged as a mainstay approach for the induction of T cell-mediated immunity by vaccination, and their suitability for human use has led to widespread clinical testing of candidate vectors against infectious intracellular pathogens and cancer. In contrast, poxviruses have been widely perceived in the vaccine field as a poor choice of vector for the induction of humoral immunity. However, a growing body of data, from both animal models and recent clinical trials, now suggests that these vectors can be successfully utilized to prime and boost B cells and effective antibody responses. Significant progress has been made in the context of heterologous prime–boost immunization regimes, whereby poxviruses are able to boost responses primed by other vectors, leading to the induction of high-titre antigen-specific antibody responses. In other cases, poxviral vectors have been shown to stimulate humoral immunity against both themselves and encoded transgenes, in particular viral surface proteins such as influenza haemagglutinin. In the veterinary field, recombinant poxviral vectors have made a significant impact with numerous vectors licensed for use against a variety of animal viruses. On-going studies continue to explore the potential of poxviral vectors to modulate qualitative aspects of the humoral response, as well as their amenability to adjuvantation seeking to improve quantitative antibody immunogenicity. Nevertheless, the underlying mechanisms of B cell induction by recombinant poxviruses remain poorly defined, and further work is necessary to help guide the rational optimization of future poxviral vaccine candidates aiming to induce antibodies.



## 1. Introduction

Vaccines remain a cornerstone for the control and prevention of infectious disease in both humans and animals. Recent times have witnessed dramatic reductions in the burden of disease as a whole range of licensed products were effectively deployed, as well as the first mass vaccination campaigns leading to eradication of two infectious organisms – variola virus – the causative agent of smallpox [1], and rinderpest – a viral disease of cattle [2]. Today, new and improved next-generation products remain under intensive development, and a third pathogen (poliomyelitis virus) stands on the brink of eradication. Although the development of each and every effective vaccine has taken extensive efforts to overcome unique series of challenges, much success has rested on the ability of vaccine formulations, consisting of killed/live-attenuated viruses or bacteria as well as some recombinant subunit protein vaccines formulated in adjuvant, to elicit robust and protective levels of immunity [3]. The latter in turn has relied on the remarkable nature of the humoral arm of the adaptive immune system to generate effective polyclonal antibody responses, with associated long-term maintenance and B cell immunological memory [4].

However, despite such laudable success, many challenges remain. It was estimated by the World Health Organization (WHO) in 2002 that 15 million deaths per annum still occurred worldwide due to infectious disease [5], and approximately 2–4 million of these are now vaccine-preventable [6], highlighting the urgency faced by those working to accelerate deployment of existing vaccines [7]. However, a significant majority remain due to human pathogens against which no licensed or highly effective vaccine is available, including the so-called "big three": *Plasmodium* malaria parasites; *Mycobacterium tuberculosis*; and the human immunodeficiency virus-1 (HIV-1). For these and many other so-called "difficult" pathogens, the development of attenuated whole organism vaccines may be unfeasible due to safety concerns, or limited by technological challenges relating to production of the organism. In other cases subunit vaccine strategies have struggled in the face of significant antigen polymorphism or a lack of knowledge about protective targets of immunity in highly complex pathogens. In many cases, protective immune mechanisms may also constitute more than a simple threshold level of neutralizing antibody, necessitating complex interplays of the cellular immune system of

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http://dx.doi.org/10.1016/j.vaccine.2013.05.091

which understanding remains limited and the ability to induce by vaccination even more so.

Few licensed vaccines are thought to protect *via* cell-mediated immunity, the most notable being the attenuated *M. bovis*, Bacille Calmette–Guérin (BCG), which has been used for over 70 years to vaccinate against human tuberculosis (TB) with variable levels of efficacy, but primarily against disseminated TB in childhood [8]. Much research has therefore focussed over the last decade on novel and alternative vaccine strategies aimed at the induction of strong T cell-mediated immunity against intracellular pathogens as well as cancer, and in this context recombinant attenuated poxviral vectors have emerged as a front-running delivery platform [9]. Such intensive research in the T cell vaccine field has highlighted both the robustness and utility of the poxviral subunit vaccine platform – large transgene insert capacity (up to 25 kbp) [10]; scope for inclusion of multiple transgenes; *in vivo* mammalian expression of foreign antigen circumventing the need for heterologous expression of difficult recombinant proteins; absence of requirement for formulation in a classical adjuvant; relative ease of generation of new recombinants including using BAC technologies [11] as well as scale-up production processes compliant with Good Manufacturing Practice (GMP); and an excellent safety track record of attenuated poxviral vectors for human use leading to a favourable outlook from regulatory agencies [9,12]. In light of such advantages, it comes as little surprise that such a platform has also been explored for the induction of humoral immunity, alongside intensive efforts focussed on T cell induction. Progress in the former endeavour will be the focus of this review.

## 2. Vaccinia virus – the first antibody-inducing poxviral vaccine

For many years, poxviruses have been widely perceived in the subunit vaccine field as a poor choice of vector for the induction of humoral immunity. This is somewhat surprising given the track record of the vaccination agent against smallpox – live replicating vaccinia [13]. Although the original Dryvax® vaccine against smallpox (a polyclonal mixture of vaccinia viruses produced in the skin of calves) is no longer manufactured, recent efforts have led to a second-generation product for human use, ACAM2000™ – a plaque-purified derivative of Dryvax®, aseptically propagated in cell culture [14,15]. Studies in mice have shown induction of strong $CD4^+$ T cell-dependent neutralizing antibody responses in response to live replicating vaccinia virus vaccination which peak at one month and are maintained out past three months [16–18]. Similar results have been observed in rhesus macaques [19], whilst in humans following vaccinia immunization neutralizing antibodies are detectable within 14 days and are raised against the intracellular mature virion (IMV) and extracellular enveloped virus (EEV) forms [20,21]. Peak responses decline over the first year, but then stabilize with a reported half-life of 92 years (similar to other replicating viral infections analyzed in the same longitudinal study) [22]. In the same manner, vaccinia-specific $IgG^+$ memory B cells appear to be maintained for more than 50 years [23]. Analysis by a proteome array of 185 vaccinia virus genes has identified 14 antigens recognized by vaccinated human serum and 21 from mice, including surface proteins from both IMVs and EEVs as well as core proteins and soluble factors [24]. Amongst these, B5R and H3L have been reported as neutralizing determinants in human sera [25,26]. Mouse models have shown that passive transfer of immune serum or monoclonal antibodies is protective against vaccinia virus challenge [27], with similar results obtained when $CD4^+$ and $CD8^+$ T cells are depleted, although antiviral T cell responses can still contribute to immunity in B cell deficient models [17]. Elegant studies in rhesus macaques have since confirmed these observations, whereby depletion of $CD20^+$ B cells in immune monkeys abrogates protective efficacy in contrast to $CD4^+$ or $CD8^+$ T cell depletion which failed to affect lethal monkeypox virus challenge outcome [28]. A conclusive demonstration that antibody is sufficient to protect against lethal infection was shown following survival of naïve monkeys administered human vaccinia immune globulin (VIG) four days prior to challenge [28], with similar documented cases in humans showing highly successful outcomes following VIG transfer to smallpox-infected patients [29].

Arguably, however, modern subunit vaccine developers are potentially discouraged by strong anti-poxviral vector immunity, and seek instead strong responses against transgenes encoded by the recombinant virus. Success in this regard was first demonstrated when a recombinant vaccinia virus vaccine was reported encoding the hepatitis B surface antigen (HBsAg) [30]. This vector induced antibody responses against the encoded transgene that were sufficient to protect chimpanzees against a hepatitis B challenge [31]. Since the report of this first antibody-inducing poxviral vectored vaccine thirty years ago, a key breakthrough has been the demonstration that replication-competence and vector immunogenicity do not necessarily go hand in hand. Intensive efforts have therefore focussed more on the utility of related but attenuated poxviral vectors, aiming to maintain immunological robustness whilst maximizing safety.

## 3. Antibody induction by attenuated poxviral vectors

A range of attenuated poxviral vaccine vectors are now available including vectors with a demonstrable safety record for human use [9]. The most widely used include the attenuated orthopoxvirus cousins of vaccinia – modified vaccinia virus Ankara (MVA) as well as New York attenuated vaccinia (NYVAC). In some fields, attenuated avipoxviruses have also been developed, including fowlpox strain 9 (FP9) [32] and canarypox (ALVAC) [33]. MVA was attenuated from the parental chorioallantois vaccinia virus Ankara (CVA) by >570 serial passages in chick embryo fibroblasts (CEFs), leading to significant deletions from the genome and loss of its ability to replicate in all other mammalian cells, with the exception of a few transformed cell lines [34–36]. NYVAC was generated by genetic deletion of 18 open reading frames from the Copenhagen strain of vaccinia, leading to replication deficiency [37].

Mice can mount significant dose-dependent anti-MVA neutralizing antibody responses, comparable to replication-competent vaccinia, but higher doses of immunizing virus are required [16,38]. These responses are mounted against both the IMV and EEV forms, and likely contribute to protective efficacy against a lethal intranasal challenge with Western Reserve (WR) strain vaccinia [16]. Given the significant safety issues and in some cases life-threatening health risks surrounding the use of live vaccinia as a smallpox vaccine, it is no surprise that the attenuated orthopoxviruses have been explored as third generation vaccine candidates, especially in light of on-going concerns surrounding bioterrorism. Currently MVA is the most advanced, with a version called IMVAMUNE® [39], developed by Bavarian Nordic, fast tracked for licensure in the USA [40], but other attenuated viruses, such as LC16m8 derived from the Lister vaccinia strain, remain under development [41]. Interestingly, fourth generation subunit vaccines, based on the neutralizing antibody determinants of the IMV and EEV forms, have shown comparable, if not better, efficacy than MVA in protection studies against lethal monkeypox virus challenge in a non-human primate model [42]. Serum from monkeys vaccinated with the subunit vaccine candidate could passively transfer immunity to mice, again confirming the critical role of antibodies in protection [43].

Recombinant attenuated orthopoxviruses have also shown comparable immunogenicity to replication-competent vaccinia in numerous animal studies [38,44–46]. Although most have focussed on T cell responses against the encoded transgenes, some have assessed antibody induction. In a comparison of MVA to WR vaccinia in mice, comparable induction of antibodies against vaccinia antigens as well as the β-galactosidase transgene were shown, although higher doses of MVA were required [38]. Other studies have shown recombinant MVA vectors to be immunogenic for antibody induction, including a vector encoding the soluble glycoprotein B (gB) of human cytomegalovirus (hCMV) under the control of the modified H5 (mH5) promoter at the deletion III locus. In this case, strong neutralizing antibody responses were induced against gB in mice following two immunizations given either intramuscularly or subcutaneously. These were maintained for six months, and could be further boosted by vaccine re-administration at this time-point [47]. Highly comparable results were also reported in mice and guinea pigs with an ALVAC vector recombinant for the same antigen [48]. Similarly, a recombinant MVA encoding the spike glycoprotein of the severe acute respiratory syndrome (SARS) coronavirus at the deletion III locus was shown to induce neutralizing antibodies after two intramuscular immunizations in mice, rabbits and rhesus macaques, with the latter protected against a pathogenic challenge [49].

MVA has also shown promise as an antibody-inducing vaccine against another viral surface protein – the haemagglutinin (HA) of influenza virus. Almost 20 years ago, MVA recombinant for H1 HA and the internal nucleoprotein (NP) antigen was shown to confer protection against lethal homologous strain influenza A infection in mice following intramuscular injection [50], similar to results ten years earlier with recombinant vaccinia virus [51]. This MVA vaccine co-expressing both antigens induced strong haemagglutination inhibition (HI) titres, as well as cytotoxic T cell responses, and (as discussed earlier) performed comparably, if not better, than WR vaccinia encoding the same antigens [50]. In the latter's case, anti-HA responses were associated with protection in separate studies in mice [52] as well as ferrets [53]. More recently, MVA vectors encoding HA from H5N1 subtypes of influenza A have been reported. In both C57BL/6 mice and cynomolgus macaques, a two-dose immunization regime was shown to induce strong and protective antibody responses with cross-reactivity to heterologous H5N1 strains [54,55]. Similar results were also shown with H5 HA encoding MVA vectors following a single immunization of BALB/c mice [56]. MVA vaccines encoding HA from the H1N1 type A pandemic influenza have also shown robust antibody responses following two immunizations of ferrets as well as protective immunity against challenge with a heterologous H1N1 strain [57]. Similar results were reported in mice, with better efficacy reported for MVA vectors encoding H1 HA, rather than N1 neuraminidase (NA) [58].

## 4. Antibody induction by prime–boost regimes – preclinical experiences

Despite the highly promising results described above for antibody induction by poxviral vectors recombinant for viral surface proteins, not all antigens have fared as well in the poxviral delivery system. The traditional approach utilized for the induction of high-titre antibody responses by subunit vaccines has been recombinant protein-in-adjuvant formulations. These technologies have performed robustly for a wide range of candidate antigens, although they rely heavily on heterologous expression systems for protein antigen production, as well as access to a suitable adjuvant. For many difficult pathogens however, such as malaria parasites, heterologous production of antigens in traditional expression systems such as *Escherichia coli* has proved extremely challenging [59]. Similarly, access to proprietary adjuvants that are both potent and demonstrate acceptable reactogenicity profiles in humans has been equally problematic for many diseases of poverty [60]. The prospects of antibody induction using vectored vaccine technologies is therefore appealing given their aforementioned advantages, coupled with their known capabilities for induction of strong cellular immunity, unlike protein-based vaccines. Indeed, it is increasingly anticipated that induction of strong cellular immunity in conjunction with antibodies may be essential to protect against some of the most challenging viral, bacterial and parasitic pathogens for which vaccines do not currently exist.

The development of viral vectored vaccines capable of inducing antibodies has been widely studied in the field of blood-stage malaria. The asexual blood-stage of the malaria parasite life-cycle sees exponential growth of parasite numbers within the host and is responsible for the associated morbidity and mortality [61]. Most vaccine developers have aimed to induce neutralizing antibodies that prevent infection of new host red blood cells by the invasive merozoite form of the parasite [62].

Early studies using a leading candidate blood-stage malaria antigen, the 42 kDa C-terminus of merozoite surface protein 1 ($MSP1_{42}$), showed little to no detectable antibody induction following immunization of BALB/c mice with recombinant MVA or FP9 vectors. In the case of MVA, antigen was inserted into the thymidine kinase (TK) locus under the control of the early/late P7.5 promoter, and in FP9 antigen was present in the terminal repeat regions of the genome under control of the same promoter. In contrast, it was shown that a single immunization in mice with a recombinant human adenovirus serotype 5 (AdHu5) vaccine could induce anti-$MSP1_{42}$ specific serum IgG responses, and whereas MVA was inefficient in priming, these adenovirus-primed responses could be significantly boosted by the recombinant MVA when administered eight weeks later [63]. This AdHu5–MVA regime was the first vectored vaccine platform to demonstrate high-titre antibody responses against $MSP1_{42}$ in mice, and these were shown to be protective against a lethal challenge with blood-stage *P. yoelii* rodent malaria parasites [63]. A heterologous DNA vaccine prime–MVA boost regime employing the same $MSP1_{42}$ antigen showed much lower levels of IgG induction, whilst a heterologous FP9–MVA poxvirus regime showed no detectable antibodies [63], consistent with poor antibody priming by poxviruses reported in another study of vectored malaria vaccines [64].

Numerous follow-up studies have shown the adenovirus prime–poxvirus boost approach to be versatile and robust with regard to antibody induction against encoded transgenes. In the malaria field, further vaccines have been developed targeting other blood-stage antigens such as apical membrane antigen 1 (AMA1) [65], as well as leading mosquito-stage transmission-blocking antigens such as Pfs25 [66]. Clinical vaccines encoding MSP1 or AMA1 from the human malaria parasite *P. falciparum* utilizing a simian adenovirus to prime (chimpanzee adenovirus 63, ChAd63) and MVA to boost have been widely tested in mice, rabbits and rhesus macaques demonstrating strong functional antibody induction [67–69]. Similar data relating to the ability of poxviral vaccines to boost antibody responses have followed on from malaria in the field of HIV-1 vaccine development. A recent study in rhesus macaques showed an AdHu26–MVA regime to afford significant protection against a highly stringent simian immunodeficiency virus $(SIV)_{MAC251}$ challenge, where antibody titres against the encoded Envelope (Env) transgene were shown to associate with vaccine efficacy [70]. Partial efficacy mediated by anti-Env antibodies has also been reported against $SIV_{SME660}$ challenge following MVA boosting of DNA vaccine primed responses. Anti-Env gp160 V3 loop antibodies were also reported in mice primed with recombinant influenza virus followed by boosting with MVA or WR vaccinia, with the highest responses seen after MVA [71].

More recently, the vectored vaccine platform has shown a key advantage with regard to antigen-screening, whereby the *P. falciparum* reticulocyte binding protein homologue 5 (PfRH5) was identified as the first blood-stage malaria antigen to be susceptible to broadly neutralizing antibody responses, following a screen of ten candidate antigens in rabbits vaccinated with recombinant AdHu5–MVA vectors [72]. In this case, two earlier studies had failed to express PfRH5 as full-length recombinant protein antigen to enable vaccine testing [73,74], in contrast to the *in vivo* mammalian expression enabled by the adenoviral and poxviral vectors.

## 5. Mechanisms of antibody induction by poxviral vectors

The ability of poxviral vectors to boost, if not prime, antibody responses in animals has thus been established against a number of difficult disease targets. Rational design of transgenes and optimization of the vectors has not been extensively reported, although it seems likely that, unlike for T cell induction where intracellular antigen can be processed and presented, antibody responses rely on targeting the encoded antigen to the secretory pathway. This can be achieved using an N-terminal signal peptide, such as that from human tissue plasminogen activator (tPA) [63], and has been reported to improve antibody immunogenicity from DNA vaccines [75], recombinant vaccinia virus [76] as well as adenoviruses [67,77] – presumably allowing for antigen expressed intracellularly to exit the infected cell and engage B cell receptors. Intriguingly though, the study with recombinant vaccinia also assessed the merits of membrane-binding *versus* secretion of the encoded *P. falciparum* MSP1 antigen and reported the highest levels of functional antibody induction when the antigen was designed to anchor in the membrane. Similar observations were made by showing that targeting a protective antigen from *Yersinia pestis* to the IMV membrane of vaccinia virus led to enhanced and protective antibody responses in mice [78]. The benefits of membrane anchoring *versus* secretion may be antigen-dependent, as well as determined by the cell type infected, but such observations may warrant further investigation as new poxviral vectors are designed for optimal antibody induction.

A prolonged (8 week rather than 2 week) prime–boost interval was also reported to be essential for high-titre antibody induction following AdHu5–MVA immunization [63], in agreement with another malaria study using AdHu5 and vaccinia virus vectors expressing the sporozoite-stage circumsporozoite protein (CSP) [79]. Such an interval presumably allows sufficient time for B cells primed by the AdHu5 vector to contract into the memory phase prior to efficient boosting. Nevertheless, an even longer 14 week interval has been reported in mice to further improve IgG induction when using ChAd63 and MVA recombinants encoding *P. falciparum* MSP1 [80], suggesting further work is necessary to establish optimal immunization schedules.

Few comparative data have been reported regarding antibody induction from poxviral vectors and choice of transgene promoter or insertion site. The TK locus has been traditionally used for insertion of a transgene cassette, along with the sites of the large genomic deletions [81], or more recently an intergenic region [82]. Promoter strength is highly variable and can constitute early and/or late activity – commonly used promoters with early/late activity include P7.5, mH5 and the short synthetic promoter (SSP) [83,84], whilst exceptionally strong intermediate/late promoters such as I1L have also been reported [85]. In one tumour vaccine study, T cell induction from vaccinia virus was shown not to associate with overall promoter activity *per se*, but instead with early promoter activity in antigen presenting cells [86]. More recently, four immediate-early endogenous promoters, utilizing their authentic genomic loci in MVA, were shown to given comparable T cell immunogenicity to the conventional promoters used at traditional insertion sites [83]. *In vivo* imaging studies in mice, utilizing vaccinia and MVA recombinant for luciferase, have also shown that the poxviral vectors lead to shorter bursts of antigen expression in comparison to a recombinant adenovirus, whilst transgene expression levels are also route of immunization dependent [87]. The merits of promoter activity and timing/duration of antigen expression remain to be investigated with regard to poxviral antibody-inducing capability, especially given many antibody responses are reportedly vaccine dose-dependent.

In other studies, antibody induction by a chimpanzee adenoviral vaccine has been shown to be sensitive to type I interferon (IFN) induction [88]. Whether such a mechanism(s) also affects antibody induction by poxviral vaccines remains to be explored. Analyses have shown MVA to stimulate innate production of IFN-β *in vitro* [89], whilst type I IFN resistance is reportedly mediated by the E3L gene product [90]. A much better understanding of how poxvirus vectors stimulate and escape host immunity should lead to new ways to explore vector modifications with potential for improved antibody and B cell immunogenicity.

## 6. Antibody induction by prime–boost regimes – clinical experiences

Few experiences have been reported in clinical trials with regard to antibody-inducing capabilities of recombinant poxviral vectored vaccine candidates, given most have been progressed to induce T cells targeting intracellular pathogens. Nevertheless, in agreement with preclinical studies, early Phase Ia clinical trials in the malaria field reported limited or undetectable serum IgG antibody responses following immunization of healthy adult volunteers in the UK with DNA–MVA [91] or FP9–MVA [92] regimes, respectively, encoding the liver-stage malaria antigen ME-TRAP (a multi-epitope string fused to the thrombospondin-related adhesion protein). A different MVA vector encoding CSP also failed to prime antibody responses in humans that could be boosted by a particulate CSP protein-in-adjuvant vaccine, as would be predicted from preclinical studies [93]. Similarly, a NYVAC vector encoding seven different antigens from *P. falciparum* showed antibody induction in rhesus macaques as well as the advantages of poxviral delivery in terms of insert capacity and genotypic stability [94], however only low level antibody induction was observed in a Phase I/IIa trial where three homologous immunizations were given to healthy adults [95]. Encouragingly, a Phase I clinical trial using an ALVAC vector recombinant for rabies glycoprotein G did show neutralizing serum antibody induction in a dose-dependent manner, although titres achieved were lower than those following immunization with the standard human diploid cell culture rabies vaccine [96]. An ALVAC prime–protein boost regime was also recently reported to induce 31% efficacy against HIV-1 in the RV144 Phase III clinical trial undertaken in Thailand [97]. No induction of broadly neutralizing antibodies was reported, however low level plasma anti-Env IgA and high levels of IgG antibody capable of binding the variable V1/V2 regions of Env were associated with reduced infection risk [98]. In a secondary analysis, low level anti-Env IgA and high levels of antibodies associated with antibody-dependent cellular cytotoxicity (ADCC) were also implicated [99]. It thus remains of clear importance to the field to establish whether qualitative differences to antibody induction occur in humans in the context of such a canarypox virus prime–protein boost regime, in comparison to approaches that elicit quantitatively improved humoral immunity.

More recently, Phase Ia clinical trials utilizing the ChAd63–MVA vectors designed to secrete *P. falciparum* MSP1 or AMA1 from

infected cells have shown significant promise. Following immunization of healthy UK adult volunteers with the ChAd63 vectors, all subjects seroconverted to the encoded transgene, and administration of the recombinant MVA vector at day 56 led to significant boosting of the serum IgG responses [100,101], as predicted from the animal models. In the case of both antigens, standardized ELISA readouts showed the peak median responses to equal 40–50 μg/mL of antigen-specific IgG [100,101]. Encouragingly, in the case of AMA1 these antibody responses were comparable to those observed with a protein vaccine formulated in either aluminium hydroxide (Alhydrogel) [102] or Montanide ISA720 [103] adjuvants as assessed by the same international reference laboratory. However, they remained lower than those seen when AMA1 protein vaccines have been administered in Alhydrogel + CpG7909 adjuvant [104] or the proprietary AS02 formulation from GSK [105], indicating there remains scope for further improvement with regard to vector-induced antibody immunogenicity. No protein-based blood-stage malaria vaccine has demonstrated significant efficacy to-date in the primary endpoint of a Phase II clinical trial [62]. In agreement with this, the antibodies induced by the vectored vaccines subsequently failed to impact on blood-stage malaria parasite growth rates in a Phase IIa efficacy study whereby vaccinated individuals were exposed to five infectious mosquito bites. However, the strong T cell responses induced in conjunction with the antibody responses appeared to have some effect on blood-stage parasite forms developing in the liver, thus reducing the liver-to-blood parasite inoculum and leading to delayed malaria diagnosis in a subset of vaccinees and sterilizing immunity in one [106]. Despite the absence of efficacy afforded against blood-stage *P. falciparum* parasites when using ChAd63–MVA vectors encoding MSP1 and AMA1, it remains encouraging that such levels of antibody can be induced in humans without the need for adjuvant or recombinant protein. Other difficult infectious pathogens are likely susceptible to antibody without need for extreme titres and as such may be more suitable targets for current vectored vaccine technologies. Pneumococcal conjugate vaccines, for example, are believed to protect against invasive disease once the anti-capsular IgG concentration exceeds a threshold of 0.35 μg/mL [107] – well within the scope of adenovirus–MVA human immunization. Two clinical reports also suggest that re-boosting of T cell responses by MVA is effective as early as six months after the preceding immunization [108,109] – somewhat encouraging for subunit vaccine developers but of potential concern regarding the longevity of protection that might be afforded by MVA as a third-generation smallpox vaccine. It remains to be seen whether the same re-boosting can be demonstrated with regard to B cell and antibody responses.

## 7. Antibody inducing poxviral vaccines for veterinary use

Given the relative success in the human field, it is unsurprising that antibody-inducing poxviral vectors have also made a major impact in the field of veterinary vaccines. Indeed, less stringent regulatory requirements and greater ease of efficacy assessment mean that at least nine recombinant poxviral vaccines (six ALVAC, two fowlpox virus (FPV) and one vaccinia virus) are licensed for veterinary use against a range of viral pathogens [110,111]: rabies virus, Newcastle disease virus, avian or equine influenza virus, West Nile virus, canine distemper virus and feline leukaemia virus. Additionally, use of recombinant FPV vaccines in poultry provides dual protection against this avian pathogen – a clear advantage in terms of vaccine cost effectiveness. All of these effectively target viral haemagglutinins or surface glycoproteins and many likely afford protection, at least in part, *via* antibody induction, mirroring the relative success of poxviral vaccines at inducing such antibody responses as described earlier for human pathogens.

## 8. Enhancing poxviral antibody-inducing vaccine immunogenicity

Adjuvantation of viral vectored vaccines has not proved straightforward, given the inherent immunogenic nature of the viral vector itself. However, some reports have demonstrated potential for improving quantitative antibody immunogenicity. Fusion of the oligomerization domain from the α-chain of complement C4b-binding protein (C4bp) to the C-terminus of an encoded protein can lead to expression of heptameric antigen. This has been shown to lead to some minor improvement in antibody induction with the malaria $MSP1_{42}$ antigen following AdHu5–MVA prime–boost immunization [63], as well as following a MVA–MVA homologous regime [112]. Co-administration of classical oil-in-water emulsion adjuvants has also been shown to improve antibody induction in pigs from a NYVAC vector encoding HA from avian influenza H5N1 [113], and similarly addition of Carbopol polymer to an ALVAC vector encoding glycoproteins from equine herpes virus-1 led to improved antibody immunogenicity and protection in ponies [114]. Furthermore, MVA and FP9 poxviral vaccines have also been reported to act themselves as adjuvants in mice for a protein vaccine against the malaria CSP antigen [115], whilst MVA, FP9, NYVAC and ALVAC were also shown to adjuvant HBsAg in a similar manner [116].

## 9. Concluding remarks

Numerous examples are now available to describe the antibody-inducing capabilities of poxviral vaccine vectors, both in animals and humans. These include the induction of strong anti-poxviral responses, as well as those against the encoded transgene. There is a clear antigen-dependence relating to the ease of antibody induction with some performing well in the context of poxviral-only regimes, whereas in other cases, the poxvirus can function as an effective antibody-boosting platform. Success in the veterinary field has led to numerous licensed products, with promising data also now emerging in numerous clinical trials with regard to poxviral vectors fulfilling a role in the context of antibody induction. However, there remains clear scope for improvement, and significant efforts are needed to better understand the mechanisms and parameters of the vector that affect B cell priming and boosting. Poxviral vectors should no longer be viewed by vaccine developers as a means of exclusive T cell induction, but instead with careful design and application can also be regarded as robust and versatile tools for the induction of antibodies.

## Acknowledgements

SJD is a UK Medical Research Council Career Development Fellow (Grant G1000527). MGC is a Fellow of the Oxford Martin School Institute for Vaccine Design. SJD and SCG are Jenner Investigators.

*Competing interests:* SJD, MGC and SCG are named inventors on patent applications covering vectored vaccines and immunization regimes.

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