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# Heterologous Prime-Boost Immunisation Regimens Against Infectious Diseases

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**Human Protection and Performance Division**
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## ABSTRACT

Development of prophylactic vaccines against infectious diseases (such as malaria, tuberculosis and HIV) has been hindered by the lack of effective immunisation strategies that induce the cellular arm of the immune system necessary for protection against these intracellular pathogens. DNA vaccines, recombinant proteins and recombinant viral vectors are all effective antigen delivery systems for inducing cellular immunity. However, when used alone, the levels of specific immune response they induce is often low. Heterologous prime – boost immunisation strategies involve using two different vaccines, each encoding the same antigen. In the past decade, numerous published reports have demonstrated that such prime – boost immunisation strategies effectively enhance cellular immunity in several different animal and disease models. Since several intracellular pathogens are considered potential biowarfare threats, the objective of this review is to assess whether prime-boost vaccination is likely to be effective in protecting against those intracellular pathogens of defence interest. This review focuses on heterologous prime – boost immunisation studies using DNA vaccines as the priming vehicle followed by either recombinant protein or recombinant viral vector boost. Included is a summary of studies up to July 2005, for a number of diseases. This paper evaluates if this approach may be applied to those intracellular pathogens considered a threat to the ADF in our vaccine development program.

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# Heterologous Prime-Boost Immunisation Regimens Against Infectious Diseases

## Executive Summary

Repeated administration of a vaccine to increase induced immunity forms the basis of traditional vaccination and is a well-recognized practice. Homologous protein based immunisation is very effective for generating humoral (antibody) immune responses, but is generally inefficient at boosting the cell-mediated immunity important for protection against infections caused by intracellular pathogens. DNA vaccines, on the other hand, are very potent at inducing cellular immune responses in mice but results to date indicate that they induce only weak responses in non-human primates and humans. In contrast recombinant viral vectors are very good at inducing cellular and humoral immunity characterised by increased CD4+ and CD8+ T cells as well as antibodies. However, none of these delivery systems have been found to induce the high levels of antigen specific T cells necessary for protection against intracellular pathogens when used alone. This has led to the investigation of heterologous prime-boost immunisation strategies that use several of these subunit vaccines in combination to induce higher levels of specific cellular immune responses.

The effective heterologous prime-boost strategies that have been examined comprise priming with a DNA vaccine followed by boosting with either recombinant viral vectors or recombinant proteins. The resulting effect is often the generation of antigen-specific memory T cells by priming followed by amplification of these cells by boosting. DNA vaccines are good priming agents since they are internalised by antigen presenting cells and can induce antigen presentation via both MHC class I and class II, thereby inducing both cytotoxic T lymphocytes and type 1-helper T lymphocytes. Successful boosting agents include recombinant proteins and recombinant viral vectors. Non-replicating viral vectors are safe and can induce high levels of protein expression, for presentation to the host's immune system. Viral vectors commonly used in vaccine trials include modified Vaccinia virus Ankara (MVA), New York vaccinia (NYVAC), recombinant Vaccinia virus (VV), attenuated adenoviruses (ADV) and attenuated poxviruses like fowlpox (FPV) and canarypox.

Heterologous prime-boost strategies have been shown to enhance cellular immunity in several different animal and disease models. The disease models include for example *M. tuberculosis*, HIV and simian immunodeficiency virus (SIV), malaria, leishmania, *Schistosoma mansoni*, hepatitis C virus, herpes simplex virus and hepatitis B virus. This review will primarily focus on the heterologous prime – boost immunisation studies using DNA vaccines as the priming vehicle followed by either recombinant protein or recombinant viral vector boost.

The vaccine development research program at DSTO is focusing on intracellular pathogens that represent a potential disease risk to ADF personnel. DSTO has constructed and tested DNA vaccines against *Burkholderia pseudomallei* and obtained significant, but low levels

of protection. The objective of this review is to assess the potential of prime-boost immunisation strategies to produce improved protection.

In summary, taking into consideration the observations contained within this report, the recommendations for the DSTO vaccine development research program are:

- Considering the promising results of the prime – boost trials outlined in this report, especially for infections caused by intracellular pathogens, DSTO should continue, as well as assess the expansion, of collaborations assessing prime-boost vaccine combinations for protection against infectious agents.

- In a number of prime - boost studies, the inclusion of growth factors or cytokines has increased the immune parameters measured. Therefore it is recommended that the DSTO vaccine program should explore the inclusion of adjuvants, growth factors and cytokines or varying the route of administration to increase the level of immune response generated in our animal models.

- Maintain a watching brief on the prime – boost vaccination trials published by the research community including those immunisation regimens that have progressed to human trials.

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*Susan Shahin, PhD, joined DSTO in 1998. Prior to DSTO, Susan worked at the University of Melbourne investigating the regulatory mechanisms of protein folding in yeast. Her work in DSTO includes the damaging effects of Sulphur mustard on mammalian cell DNA and development of rapid PCR techniques for the detection of biological warfare agents. Recently, her research has centred on the development of DNA vaccines against melioidosis and producing recombinant proteins.*

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# Contents

# 1. Introduction

Repeated administration of a vaccine to increase immunity is a well-recognized practice. Homologous protein based immunisation is very effective for generating humoral immune responses, but is generally inefficient at boosting the cell-mediated immunity important for protection against infections caused by intracellular pathogens. Since many potential biowarfare agents are intracellular pathogens, improving induced cellular immunity is important for protection. A schematic of the activation of the humoral and cellular immunity in response to pathogens is represented in Figure 1. Most adjuvant protein vaccines readily induce CD4+ T helper 2 cell responses, but there are few adjuvants that can induce strong cytotoxic CD8+ T cell responses. DNA vaccines, on the other hand, are very potent at inducing cellular immune responses in mice but results to date indicate that they induce only weak responses in non-human primates and humans [1]. In contrast recombinant viral vectors are very good at inducing both CD4+ and CD8+ T cells as well as antibodies. However, none of these delivery systems have been found to induce very high levels of antigen specific T cells when used alone. This has led to the investigation of heterologous prime-boost immunisation strategies that use several of these subunit vaccines in combination to induce higher levels of specific immune response [2].

The effective prime-boost strategies that have been examined comprise priming with a DNA vaccine followed by boosting with either recombinant viral vectors or recombinant proteins. The resulting effect is often the generation of antigen-specific memory T cells by priming followed by amplification of these cells by boosting. DNA vaccines are good priming agents since they are internalised by antigen presenting cells and can induce antigen presentation via both MHC class I and class II, thereby inducing both cytotoxic T lymphocytes and type 1-helper T lymphocytes. Successful boosting agents include recombinant proteins and recombinant viral vectors [2,3]. Non-replicating viral vectors are safe and can induce high levels of protein expression, and therefore presentation to the host's immune system. Viral vectors commonly used in vaccine trials include modified Vaccinia virus Ankara (MVA), New York vaccinia (NYVAC), recombinant Vaccinia virus (VV), attenuated adenoviruses (ADV) and attenuated poxviruses like fowlpox (FPV) and canarypox.

Heterologous prime-boost strategies have been shown to enhance cellular immunity in several different animal and disease models. The disease models include for example *M. tuberculosis* [4], HIV and simian immunodeficiency virus (SIV) [5,6] , malaria [7], leishmania [8,9], *Schistosoma mansoni* [10], hepatitis C virus [11,12], herpes simplex virus [13] and hepatitis B virus [14]. This review will primarily focus on the heterologous prime – boost immunisation studies up to July 2005 using DNA vaccines as the priming vehicle followed by either recombinant protein or recombinant viral vector boost. These studies are summarised in Table 1 and some are discussed in more detail in this review.

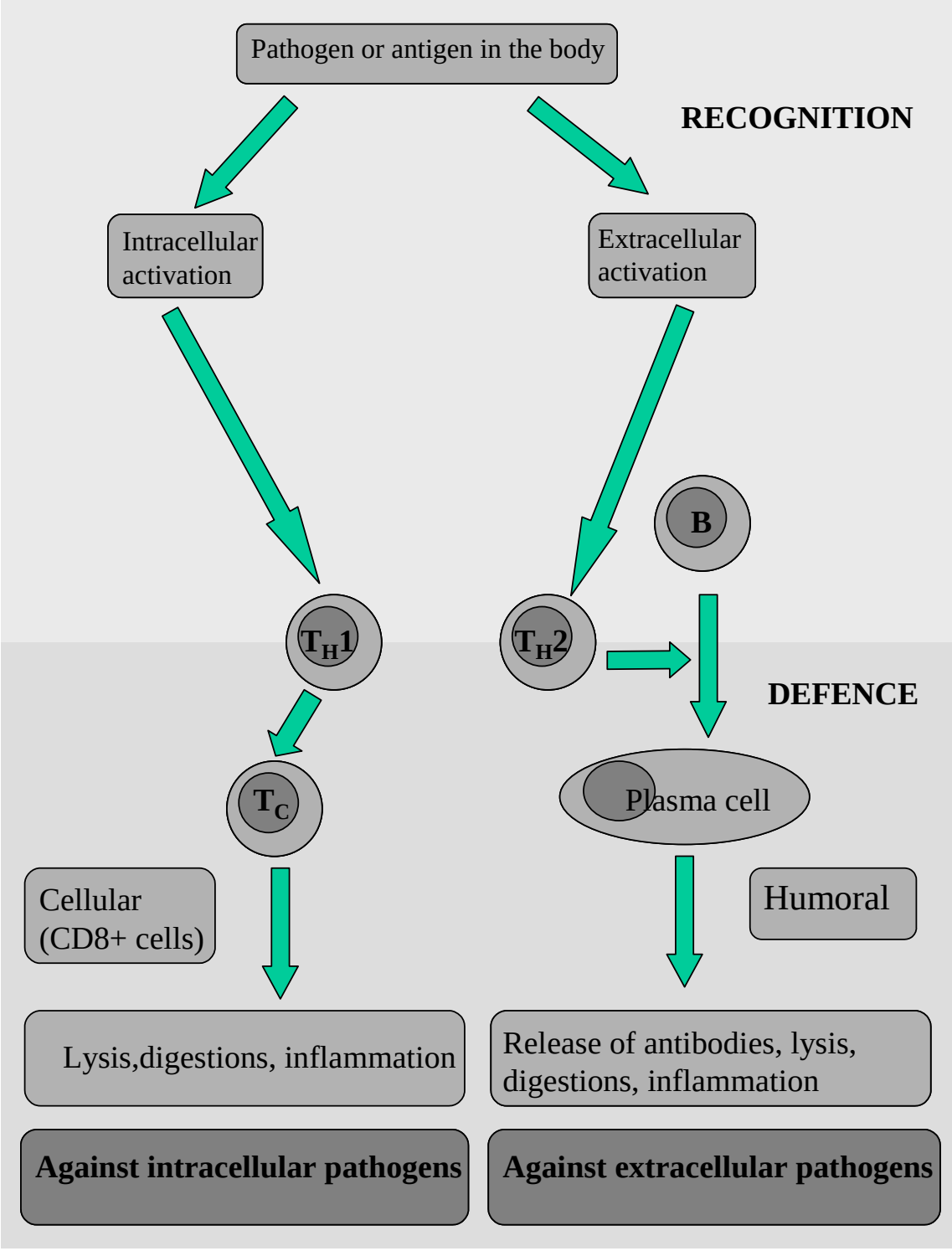


***Figure 1:*** *Intracellular pathogens result in activation of $T_H1$cells, which stimulates cellular immune responses characterised by CD8+ cytotoxic T cells ($T_c$). Extracellular pathogens activate $T_H2$ lymphocytes, which stimulate B cells to produce antibody (humoral immunity).*

# 2. Malaria

Malaria is a disease caused by *Plasmodium* species, an intracellular parasite, for which an effective vaccine is likely to require induction of both antibody and cell-mediated immune responses. Development of a malaria vaccine has been complicated by the multistage life cycle of the parasite. Most of the malaria antigens exhibit stage specificity and thus different antigens will be needed to induce immunity against the various stages of the parasite's life cycle. The first infectious stage of the malaria parasite, the sporozoite, travels to the liver of the host within minutes of infection during the blood meal of mosquito [15]. Infection of hepatocytes results in replication of the parasite followed by lysis and release of infectious merozoites into the blood stream. An effective malaria vaccine will need to induce both T-cell responses against infected hepatocytes and antibodies against blood stage parasites. It must also be effective against the pre-erythrocytic liver stage of the life cycle. Most of the vaccine candidates that have been studied to date are targeted at the initial pre-erythrocytic stage of infection with some at the blood and sexual stages. Table 1 summarises the results of several DNA prime and either protein or recombinant virus boost immunisation studies using antigens expressed at different life-cycle stages of the parasite in an attempt to increase the efficacy of the vaccines.

The assessment of heterologous prime-boost immunisations against malaria in humans has been performed using both naïve volunteers and individuals from areas with endemic malaria. DNA prime / MVA boost vaccines encoding a polypeptide fused to *P. falciparum* pre-erythrocytic TRAP antigen were administered via gene gun to healthy volunteers with no adverse effects [32]. Furthermore, individuals in this study were partially protected, as measured by delayed parasitemia, from challenge with a different strain of *P. falciparum*. Phase 1 clinical trials in Gambia in semi-immune adults have demonstrated that the heterologous DNA ME-TRAP prime/MVA ME-TRAP boost regimen is safe and induces T cell effector responses greater than those generated in naïve volunteers or by natural malaria infection [34,35]. However, the vaccine was ineffective at reducing the natural infection rate in these semi-immune Gambian adults. Another group demonstrated that priming with a DNA vaccine encoding *P. falciparum* CSP and boosting with a recombinant protein vaccine RTS,S/AS02A, induced the production of both antibodies and CD4+ and CD8+ T cell responses in healthy volunteers [36,37]. Both of these immune responses were superior to those elicited by either DNA or recombinant protein alone. These initial results provide promising evidence that heterologous prime-boost immunisation can induce the strong and broad immune response that is necessary for protection against malaria in humans.

# 3. HIV and SIV

Worldwide, it is estimated that over 60 million people are infected with HIV and over 14,000 new infections occur daily [38]. Most of these new infections are in developing countries that lack the economic resources and infrastructure to acquire and administer effective antiviral therapy. Therefore, a safe and effective vaccine is paramount for containing the spread of this highly infectious disease. However, the biology of HIV has presented vaccinologists with many challenges to overcome [39,40]. Unlike other diseases, natural infection with HIV does not result in protective immunity. HIV has proven to be difficult to neutralise with antibodies due to carbohydrate shielding of critical gp120 (virion envelope glycoprotein) epitopes, occlusion of envelope epitopes via oligomerisation, conformational masking of receptor binding sites and mutational variation of envelope variable loops [41]. Infection with HIV results in progressive destruction and impaired regeneration of CD4+ T-helper cells and the virus rapidly evolves *in vivo* to escape from cellular and humoral immune responses [41]. In addition, because of the high levels of HIV genetic diversity, it is likely that more than one vaccine preparation will be required for global use [39]. Although it is not clear what arm of the immune response correlates with protection from HIV-1 infection or disease, an effective HIV vaccine will have to stimulate a range of host defences, including mucosal and innate immunity, neutralising antibodies and cell-mediated immune responses [40].

There are numerous studies investigating different vaccine candidates and immunisation regimens for HIV/SIV (Table 1 and Table 2), some of which are discussed in detail in this review. The majority of the prime/boost approaches have been based on targeting structural HIV-1 proteins (encoded by conserved *gag, pol* and *env* genes), and others have included the regulatory/accessory HIV-1 proteins to induce immune responses able to recognize early-infected cells.

The majority of the HIV/SIV heterologous prime – boost studies, demonstrated that priming with DNA vaccine followed by boosting with either recombinant protein or recombinant viral vector expressing the same or different genes, generated significant levels of cellular immune response in animal models (Table 1). However, only a few of these studies demonstrated a humoral immune response and in those reports with increased specific antibody titres, there was rarely a corresponding increase in neutralisation activity. Heterologous prime-boost vaccination strategies have recently progressed to human trials where a DNA prime – MVA boost regimen was evaluated in phase 1 clinical trials in healthy volunteers [68]. The vaccines expressing the immunogen consisting of consensus HIV-1 Gag p24/p17 proteins fused to a string of epitopes recognised by cytotoxic T lymphocytes were safe, well-tolerated and stimulated HIV-specific T cell responses both on their own and in a prime-boost combination and one of the volunteers in the prime – boost group developed detectable Gag-specific antibodies.

# 4. Tuberculosis

Together with malaria and AIDS, tuberculosis is a major health threat, particularly in developing countries. The disease is caused by the intracellular respiratory pathogen *Mycobacterium tuberculosis.* In a recent survey, the World Health Organisation estimated that

one third of the world population is infected with the bacillus, eight million people develop the disease and two million die of tuberculosis each year [69]. This public health threat has become increasingly severe due to the emergence of multi-drug resistant strains and the AIDS epidemic, the former being responsible for a third of all AIDS related deaths [70,71]. Even though effective chemotherapy is available for the treatment of human tuberculosis, it is lengthy and expensive and difficult to implement in developing countries. Furthermore the emergence of multi-drug resistant strains of *M. tuberculosis* is severely reducing the effectiveness of the treatment. The widely used vaccine against *M. tuberculosis* bacille, Calmette-Guerin (BCG) is an attenuated strain of *Mycobacterium bovis*. BCG vaccination offers variable protection against childhood meningeal tuberculosis and systemic forms of the disease. However, the level of protection against the most common form, pulmonary tuberculosis in adults, is low and decreases with time [72,73]. Therefore, there is a need for an improved vaccine that provides long lasting protection against this fatally infectious disease.

Protective immunity against tuberculosis is dependent on the generation of a Th1-type cellular immune response, which is characterised by antigen specific T cells that produce IFN-γ. Both CD4+ and CD8+ T cells secrete IFN-γ. Protective immunity against tuberculosis is primarily mediated by CD4+ T cells [74], but CD8+ T cells are also required for effective protection against *M. tuberculosis* infection [4]. Therefore an immunisation regimen that stimulates both CD4+ and CD8+ T cells should lead to improved protection against *M. tuberculosis* infection. Heterologous prime-boost vaccination protocols in experimental tuberculosis that have been assessed include DNA prime/MVA boost; [4,75,76], DNA prime/ protein boost [77-79], DNA prime/BCG boost [80-82] and BCG prime/MVA or FP or DNA boost [83-85]. The findings of these studies are summarised in Table 1.

These reports highlight the effectiveness of heterologous prime-boost immunisation strategies at enhancing cell-mediated immunity compared to homologous immunisation. In the majority of these animal studies, the secretion of IFN-γ from antigen specific T cells, used as an immunological correlate of protection against tuberculosis, was enhanced several fold following heterologous prime-boost immunisation. Recently, the first Phase 1 clinical evaluation of the safety and immunogenicity of the BCG prime/MVA85A boost vaccination strategy in humans was completed [87]. Elevated levels of IFN-γ secreting T cells were demonstrated in both BCG naïve volunteers and volunteers that were vaccinated in early childhood. Further clinical trials using this vaccination regimen are underway [88] and the outcome of these are eagerly awaited by the research community.

# 5. Other Infectious Diseases

Prime-boost immunisation strategies have also been evaluated in numerous other infectious disease models (Table 1). While some of these studies are discussed below, inclusion of all of the studies in the text is beyond the scope of this review. A study of visceral leishmaniasis evaluated the effectiveness of immunising dogs with plasmid DNA prime – VV boost both carrying the gene LACK, and assessed the protection following challenge with *L. infantum* [8]. This study reported a 60% protection against infection in dogs immunised by DNA-LACK prime/VV-LACK boost compared to no protection in animals that were immunised with the DNA vaccine alone. A previous study using the same prime-boost strategy with the *L.*

*infantum* P36/LACK antigen dramatically reduced lesion size and parasite load in a mouse model of cutaneous leishmaniasis [9]. Since this study demonstrated a predominant Th1 response, a subsequent study looked at the effect of co-administration of the interleukins IL-12 and IL-18 with the P36/LACK antigen [89]. Following challenge with *L. major*, the group immunised with DNAp36/LACK + DNA IL-12 + DNA IL-18 and VVp36/LACK exhibited a 68% reduction in lesion size compared to 28% observed in the mice vaccinated without the cytokines. The former group also had a 36% reduction in the lymph node weight. Protection against *Shistosoma mansoni* infection in mice was evaluated following priming with DNA and boosting with VV containing genes coding for antioxidant enzymes by Shalaby et al [10]. Both the Cu/Zn cytosolic superoxide dismutase (CT-SOD) and glutathione peroxidase (GPX) afforded the same level of protection when administered as naked DNA or encoded by VV. The prime-boost strategy doubled the ability of GPX to induce protective immunity but had no effect on the immunity induced by CT-SOD.

Pancholi et al [11] investigated the potential for vaccination of mice using DNA encoding polycistronic hepatitis C virus (HCV) structural and non-structural genes either alone or combined with boost with canarypox virus expressing the same genes to generate CD8+-specific responses and provide protection against challenge. Mice immunised by HCV DNA prime/canarypox virus boost exhibited higher levels of IFN-γ secreting cells and antigen specific cytotoxic-T-lymphocyte (CTL) reactivity. When challenged with vaccinia virus expressing HCV proteins 2 months after immunisation, these animals also showed a complete reduction in vaccinia virus titres compared to HCV DNA prime/boost and sham immunised controls. However, 8 months later the protection in HCV DNA prime/boost immunised mice against HCV-vaccinia virus challenge was higher than that observed in HCV DNA prime/canarypox virus boost immunised mice. Another study evaluated the HCV E2- and core-specific T-cell responses induced by DNA and/or recombinant adenovirus vaccines and reported that a heterologous vaccination regimen elicited the highest level of T helper 1 CD4+ T-cell responses [12].

Recently, a report evaluating a hepatitis B (HB) DNA vaccine encoding HbsAg followed by a recombinant protein boost reported the induction of strong humoral and cellular immune responses [14]. This immunisation regimen elicited greater levels of IL-12 and IFN-γ in splenocytes and stronger CD8+ CTL responses compared to those responses elicited by immunisation with plasmid DNA-HBsAg or HbsAg protein alone. Effectively enhancing both arms of the immune response by heterologous prime-boost strategies have also been reported for herpes simplex virus (HSV) [13]. These investigators demonstrated greater immune responses in mice generated following an MVA-HSV-2 glycoprotein D (gD2) prime and DNA-gD2 boost than in DNA-gD2 primed mice. The other disease models summarised in Table 1 include *Chlamydia pneumoniae* [90], *Taenia solium* [91], Enterotoxigenic *Escherichia coli* [92], Human T-cell leukemia / lymphoma virus type1 (HTLV-1) [93] and *Chlamydophila abortus* [94].

# 6. Conclusion

Heterologous prime-boost immunisation strategies have demonstrated an effective way to enhance immune responses to a wide variety of pathogens. Such strategies combine the

strengths of antibody and CD4+ T cell induction by proteins, induction of cellular immunity by DNA vaccines and recombinant viral vector enhancement of CD4+ and CD8+ T cells as well as antibodies. The use of these subunit vaccines in combination with plasmid DNA results in the induction of higher levels of cell-mediated immunity important in protection against infection by intracellular pathogens. Several antigen delivery systems have been shown to be effective at boosting previously primed immune responses and the choice of boosting agent will be partially determined by the type of immune response required. The ability to stimulate both arms of the immune system is an advantage as it closely mimics natural infection. It is also more closely aligned with traditional vaccines but does not have the associated side effects. There are many examples that have shown a combination of plasmid DNA followed by a heterologous boost has generated a far superior outcome, be it immune response or protection, than either approach alone. Some of these prime-boost combinations have proceeded to human trials with some promising preliminary results and future trials are eagerly awaited.

Research in prime-boost vaccination strategies, aided by the development of sensitive quantitative assays to evaluate T cell immunity, is continually expanding. The addition of adjuvants to supplement the priming and delivery of vaccines to mucosal surfaces (intravaginal or intranasal) are examples of promising approaches that are likely to undergo expansion in the near future. Further work on the mechanism and optimisation of these prime-boost vaccination strategies can only contribute to our knowledge and accelerate development of vaccines against highly infectious and often fatal diseases.

# 7. Implications for Defence Research

The information contained in this review has several implications for the vaccine development program at DSTO. It has been well established that DNA vaccines alone are effective at increasing cellular immunity. However, the level of immunity induced is not sufficiently protective against infection caused by some intracellular pathogens. As summarised in this review, Prime – Boost vaccination combinations have been shown to increase immunity above that induced by DNA vaccines alone in experimental models. To date the level of protection has not been sufficient for full protection against a number of diseases as outlined in the studies discussed in this report. However the results from these studies have been promising and indicate that consideration of the appropriate parameters can significantly affect the level of induced cellular immunity. Therefore, the enhanced cellular immunity generated by prime – boosting can be potentially augmented by the addition of other immune reagents such as cytokines and growth factors as well as the choice of boosting agent and route of administration. In addition to the low cost associated with the production of DNA vaccines, production of the recombinant boosting agents is also cost effective. The safe and minimal side effects associated with prime – boosting regimens have been demonstrated by the human trials conducted to date [32, 68, 87]. All these factors are important when immune protection is being developed for public and/or defence use. Finally, for many diseases for which an effective vaccine has not been developed, public research is gravitating towards prime – boost combinations. Therefore a wealth of information can be gained from these studies and applied to infectious agents, which are considered a threat to the ADF.

In summary, taking into consideration the observations contained within this report, the recommendations for the DSTO vaccine development research program are:

- Considering the promising results of the prime – boost trials outlined above, especially for infections caused by intracellular pathogens, DSTO should continue, as well as assess the expansion, of collaborations assessing prime-boost vaccine combinations for protection against infectious agents.

- In a number of prime - boost studies, the inclusion of growth factors or cytokines [26, 27, 46, 49, 89] has increased the immune parameters measured. Therefore it is recommended that the DSTO vaccine program should explore the inclusion of adjuvants, growth factors and cytokines or varying the route of administration to increase the level of immune response generated in our animal models.

- Maintain a watching brief on the prime – boost vaccination trials published by the research community including those immunisation regimens that have progressed to human trials.

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# Appendix A: Selected Infectious Diseases

## A.1. Malaria

Malaria is a disease caused by Plasmodium species, an intracellular parasite, for which an effective vaccine is likely to require induction of both antibody and cell-mediated immune responses. Development of a malaria vaccine has been complicated by the multistage life cycle of the parasite. Most of the malaria antigens exhibit stage specificity and thus different antigens will be needed to induce immunity against the various stages of the parasite's life cycle. The first infectious stage of the malaria parasite, the sporozoite, travels to the liver of the host within minutes of infection during the blood meal of mosquito [15]. Infection of hepatocytes results in replication of the parasite followed by lysis and release of infectious merozoites into the blood stream. An effective malaria vaccine will need to induce both T-cell responses against infected hepatocytes and antibodies against blood stage parasites. It must also be effective against the pre-erythrocytic liver stage of the life cycle. Most of the vaccine candidates that have been studied to date are targeted at the initial pre-erythrocytic stage of infection with some at the blood and sexual stages. Table 1 summarises the results of several DNA prime and either protein or recombinant virus boost immunisation studies using antigens expressed at different life-cycle stages of the parasite in an attempt to increase the efficacy of the vaccines.

Two sporozoite surface proteins, the circumsporozoite protein (CSP) and the thromospondin-related adhesive protein (TRAP), are well-characterised antigens in terms of their biological role and for use in the development of a pre-erythrocytic stage malaria vaccine. CSP belongs to a family of 40-60kDa proteins and contains a central region consisting of tandem repeats, which is flanked by two regions of highly conserved amino acid sequences, designated region I (RI) and region II (RII) [16]. RII with flanking basic residues, is involved in recognition, binding and invasion of hepatocytes by *Plasmodium* sporozoites [17]. TRAP also known as SSP2 is a transmembrane protein and has a multi-domain organisation [18]. The ectodomain of TRAP consists of an A-domain, a region homologous to the RII of CSP and a repeat region. TRAP, like CSP, is involved in invasion of hepatocytes by sporozoites in a RII-dependent manner [19]. Bhardwaj et al [20] designed a DNA vaccine construct to encode a hybrid protein containing regions of *Plasmodium cynomolgi* (Pc) TRAP (N-terminus) and PcCSP (C-terminus) where the important biological and immunological parts of CSP and TRAP are located. DNA prime-recombinant protein boost immunisation generated antigen-specific humoral and cellular immune response in mice and rhesus monkeys. Following challenge with *P. yoelii* sporozoites, the vaccinated mice were protected significantly compared to those animals vaccinated with control DNA. Anderson et al [21] investigated the use of attenuated fowlpox virus (FPV), as recombinant vaccine vectors for eliciting CD8+ T cells against liver-stage *Plasmodium berghei* (Pb) infection in mice. The gene encoding the *P. berghei* circumsporozoite protein (PbCSP) was inserted into a commercially available FPV vaccine strain and the attenuated strain FP9. This combination was more immunogenic than DNA/MVA prime boost immunisation [21]. However, Schneider et al [22] reported complete protection of mice against *P. berghei* sporozoite challenge following immunisation with DNA and MVA vectors expressing PbCSP and PbTRAP. In the Aotus monkey model of *P. falciparum* (Pf) infection, a DNA prime-protein boost induced higher levels of antibodies to the *P. falciparum* erythrocyte-binding protein (EBA-175) region and higher levels of protection against challenge than

homologous immunisation with either vaccine [23], thus highlighting the potential advantages of heterologous boosting.

Rogers et al [24] demonstrated that a multi-antigen, multi-stage DNA priming and poxvirus boosting vaccine containing both pre-erythrocytic and erythrocytic stage vaccine targets can protect rhesus monkeys against a lethal challenge with *P. knowlesi* sporozoites. Addition of cytokine encoding plasmids (GM-CSF, IL2, TNF-α) in the DNA priming dose, did not significantly enhance the immune response [25]. Another study using chimpanzees vaccinated with DNA vaccines encoding PfTRAP and liver stage specific antigen-1 (PfLSA-1) together with GM-CSF followed by MVA-PfTRAP boost demonstrated increased specific antibody responses and IFN-γ secreting antigen-specific T cells to TRAP [26]. Similarly, in a mouse *P. yoelii* (Py) malaria model, priming with PyCSP DNA vaccine mixed with pGM-CSF and then boosting with either vaccinia virus - or poxvirus - PyCSP induced excellent anti-PyCSP IFN-γ and cytotoxic T lymphocyte (CTL) responses [27-29]. These studies also demonstrated enhanced protection in both mice immunised as neonates and as adults.

A malaria vaccine construct expressing six *P. falciparum* pre-erythrocytic antigens linked together to produce a polyprotein termed L3SEPTL was designed to generate protective T cell responses[30]. Immunisation with plasmid DNA encoding L3SEPTL failed to prime the MVA-L3SEPTL boost. However, priming with DNA vaccines encoding the single antigens contained in L3SEPTL followed by boosting with MVAs encoding the same single antigens or the full-length polyprotein, induced strong anti-TRAP and anti-LSA1-specific CD8+ T cell responses. A prime boost vaccination regime with FPV prime and MVA boost, both encoding L3SEPTL, induced IFN-γ secreting cells specific for each of the six antigens. However, the number of IFN-γ secreting cells induced by both vaccine regimens (DNA prime/MVA boost and FPV prime/MVA boost) was similar [30].

Transmission blocking vaccines target the sexual stages of the malaria parasite and therefore prevent further development within the mosquito halting the transmission of the parasite. Coban et al [7] found that immunisation with DNA encoding Pfs25 (a *P. falciparum* surface protein) elevated mainly IgG1 in rhesus macaques. Following a protein boost, the IgG1 responses were increased further and IgG2 and IgG4 anti-Pfs25 antibodies were also detected. The ability of the sera to block oocyst formation in mosquito midguts and thus block malaria was assessed by membrane feeding assays. Only the sera obtained after the protein boost effectively blocked parasite infectivity. Kongkasuriyachai et al [31] immunised mice with the *Plasmodium vivax* (Pv) zygote/ookinetic surface proteins (Pvs25 and Pvs28) and evaluated DNA prime/DNA boost, DNA prime/protein boost and protein prime/protein boost vaccination strategies. The heterologous prime-boost combination compared to homologous delivery was slightly more effective at eliciting strong and functionally active antibody responses with high transmission-blocking activity.

The assessment of heterologous prime-boost immunisations against malaria in humans has been performed using both naïve volunteers and individuals from areas with endemic malaria. DNA prime / MVA boost vaccines encoding a polypeptide fused to *P. falciparum* pre-erythrocytic TRAP antigen were administered via gene gun to healthy volunteers with no adverse effects [32]. The polypeptide referred to as ME-TRAP encodes a string of T and B cell epitopes (14 CD8+ T cell epitopes, one CD4+ T cell epitope, and two B cell epitopes) from six pre-erythrocytic *P. falciparum* antigens and two non-malarial CD4+ T cell epitopes, fused in

frame to the entire TRAP antigen. This heterologous prime-boost immunisation elicited five to ten fold higher levels of IFN-γ secreting, antigen-specific T cells in humans than those responses induced by the DNA vaccine or MVA alone [33]. Furthermore, individuals in this study were partially protected, as measured by delayed parasitemia, from challenge with a different strain of *P. falciparum*. Phase 1 clinical trials in Gambia in semi-immune adults have demonstrated that the heterologous DNA ME-TRAP prime/MVA ME-TRAP boost regimen is safe and induces T cell effector responses greater than those generated in naïve volunteers or by natural malaria infection [34,35]. However, the vaccine was ineffective at reducing the natural infection rate in these semi-immune Gambian adults. Another group demonstrated that priming with a DNA vaccine encoding *P. falciparum* CSP and boosting with a recombinant protein vaccine RTS,S/AS02A, induced the production of both antibodies and CD4+ and CD8+ T cell responses in healthy volunteers [36,37]. Both of these immune responses were superior to those elicited by either DNA or recombinant protein alone. These initial results provide promising evidence that heterologous prime-boost immunisation can induce the strong and broad immune response that is necessary for protection against malaria in humans.

## A.2. HIV and SIV

Worldwide, it is estimated that over 60 million people are infected with HIV and over 14,000 new infections occur daily [38]. Most of these new infections are in developing countries that lack the economic resources and infrastructure to acquire and administer effective antiviral therapy. Therefore, a safe and effective vaccine is paramount for containing the spread of this highly infectious disease. However, the biology of HIV has presented vaccinologists with many challenges to overcome [39,40]. Unlike other diseases, natural infection with HIV does not result in protective immunity. HIV has proven to be difficult to neutralise with antibodies due to carbohydrate shielding of critical gp120 (virion envelope glycoprotein) epitopes, occlusion of envelope epitopes via oligomerisation, conformational masking of receptor binding sites and mutational variation of envelope variable loops [41]. Infection with HIV results in progressive destruction and impaired regeneration of CD4+ T-helper cells and the virus rapidly evolves *in vivo* to escape from cellular and humoral immune responses [41]. In addition, because of the high levels of HIV genetic diversity, it is likely that more than one vaccine preparation will be required for global use [39]. Although it is not clear what arm of the immune response correlates with protection from HIV-1 infection or disease, an effective HIV vaccine will have to stimulate a range of host defences, including mucosal and innate immunity, neutralising antibodies and cell-mediated immune responses [40].

There are numerous studies investigating different vaccine candidates and immunisation regimens for HIV/SIV (Table 1 and Table 2), some of which are discussed in detail in this review. The majority of the prime/boost approaches have been based on targeting structural HIV-1 proteins (encoded by conserved *gag, pol* and *env* genes), and others have included the regulatory/accessory HIV-1 proteins to induce immune responses able to recognize early-infected cells. The regulatory proteins, Tat and Rev, are essential for virus replication whereas the accessory proteins (Nef, Vif, Vpr, Vpx and Vpu) while not required for virus growth *in vitro* are essential for viral replication and pathogenesis *in vivo* [42]. Immunising baboons with DNA expressing *env* genes generated low levels of anti-gp140 antibodies and low lymphoproliferative responses whereas following a booster with Env protein, both were increased significantly [43]. However, no viral neutralising activity against virus challenge

was detected. Priming with DNA plasmid and boosting with MVA expressing several HIV-1 and SIV proteins induced strong cellular immune responses in macaques [5]. Gherardi et al [44] using a similar DNA prime-MVA boost immunisation strategy encoding the HIV *env* gene, demonstrated an enhanced mucosal immune response, following intranasal administration in mice when cholera toxin was co-administered in both the prime and boost vaccinations. Several recent studies have demonstrated that priming with plasmid DNA vaccines and boosting with recombinant viral vectors such as replication-defective adenoviruses [45-48], vaccinia viruses [6,49-52], and poxviruses [6,53] generate potent virus specific cytotoxic T-lymphocyte responses and offer significant control of pathogenic SHIV challenge in rhesus monkeys [45,51,52]. The use of SHIV-mimicking virus like particles (VLP) as a boost following either a plasmid DNA or FPV expressing *gag/pol* or *env* genes prime, in rabbits, was investigated by Radaelli et al [54] and Zanotto et al [55]. These studies demonstrated that the FPV vector was less efficient than plasmid DNA at inducing an anti-Gag humoral response and that priming with both plasmid DNA and FPV followed by VLP boosting generated greater levels of both IFN-γ and IL-4. Effective VLP boosting of a DNA vaccine prime was also demonstrated against HIV-1 *gag* in mice [56]. In this study, the VLP boost resulted in a 4-fold increase in the number of Gag peptide-specific CD8+ IFN-γ memory cells compared to a 1.5 fold increase when the DNA prime was boosted with the same DNA vaccine.

Other boosting vectors that have been assessed in animal models include herpes simplex virus type-1 (HSV-1) [57] and replication-defective sendai virus (SeV) [58]. Immunisation with DNA/HIV-1 gp120 followed by HSV-gp120 boost tripled the gp120-specific cellular immune responses as determined by tetramer staining and greatly enhanced the long term memory compared to DNA-gp120 alone [57]. In a rhesus macaques model, following priming with DNA-Gag and boosting with SeV-Gag, protection against intravenous challenge with SHIV was demonstrated 4 months after boosting [58]. All of the vaccinated monkeys were protected from acute AIDS progression and some were protected from CD4+-T-cell depletion.

Since the use of recombinant viral vectors in humans will be limited somewhat by pre-existing anti-vector immunity, co-administration of different cytokine adjuvants have been tried to increase the efficacy of DNA based vaccines. Using mice with pre-existing anti-adenovirus immunity, Barouch et al [46] clearly demonstrated that the cytokines, GM-CSF and MIP-1α, when combined with the DNA-HIV-1 Env prime and either ADV-HIV-1 Env or VV-HIV-1 Env boost greatly elevated the induced immune responses as indicated by a 5 fold increase in the number of specific CD8+ T lymphocytes. Gomez et al [49] constructed a DNA vector expressing a polyprotein termed TAB13 which contained the V3 region of gp120 from eight different HIV-1 isolates. The use of the DNA-TAB13 in the priming and either VV or MVA expressing the same antigen in the booster resulted in a 60-fold enhancement of CD8+ T cell responses against V3 epitopes compared to homologous vaccination in mice. Further enhancement of specific IFN-γ secreting cells was seen following the co-administration of plasmid vector expressing the cytokine IFN-γ with the DNA-TAB plasmid prime/MVA-TAB boost.

DNA prime – recombinant virus or protein boost vaccination regimes are the most explored in animal models of HIV/SIV. However, other investigators have also assessed the efficacy of different prime – boost combinations. For comparative purposes some recent studies are included in this review (Table 2). Vazquez- Blomquist et al [59] reported that priming mice

with MVA and boosting with FPV, both expressing HIV-1 multi-epitope polypeptides resulted in a 2-fold increase in the number of IFN-γ secreting CD8+ T-cells compared to homologous combinations. This combination was also more effective than a DNA prime – poxvirus boost, resulting in a 3-fold enhancement of the number of IFN-γ secreting T cells. Priming with replicating adenovirus recombinants encoding SIV *env/rev, gag* and/or *nef* genes followed by boosting with SIV gp120 resulted in a 52 –fold reduction in viral load following intra-rectal infection of rhesus macaques with SIV [60]. However, even though viral clearance was correlated with increased T cell responses, this immunisation regimen failed to produce neutralising antibodies. To investigate whether this protection is mediated by antibody-dependent cellular cytotoxicity, Gomez-Roman et al [61] collected sera from animals immunised with adenovirus recombinants encoding SIV genes/gp120 prime/boosted at the time of challenge. The investigators demonstrated that the serum, mediated killing of SIV infected H9 cells *in vitro*. Other immunisation regimens include the use of cytosine phosphate guanosine oligodeoxynucleotide (CpG) as an adjuvant with protein as a prime followed by adenovirus boost [62]; a rabies virus (RV) vaccine strain as the prime and a recombinant vesicular stomatitis virus (VSV) expressing HIV envelope protein as the boost [63]; a Semliki Forest virus (SFV) prime with a FPV boost bearing the HIV-1 TAB9 multiepitope polypeptide minigene [64]; and priming with SFV expressing the HIVA protein followed by MVA-HIVA boost [65]. Gherardi et al [66] investigated immunisation regimens that generate anti-HIV cytotoxic T cells at mucosal tissues or in lymph nodes draining the genital and rectal tracts in mice, which may be of use in limiting the spread of HIV after initial infection. In these studies the recombinant influenza virus expressing the CD8+ T-cell epitope from the V3 loop of HIV-1 was used to target the mucosal tissue and prime the host. The boost consisted of MVA or VV expressing the entire HIV-1 Env protein. The influenza – MVA combination when administered intranasally induced a 4-fold higher CD8+ T cell response in the spleen and a low but significant response in the genitorectal lymph nodes compared to MVA immunisation alone. A similar prime – boost strategy using influenza – VV expressing Gag protein demonstrated enhanced SIV Gag-specific CD8+ T cell responses in both spleen cells and lymphocytes derived from cervical and mediastinal lymph nodes [67].

In summary, the majority of the HIV/SIV heterologous prime – boost studies, demonstrated that priming with DNA vaccine followed by boosting with either recombinant protein or recombinant viral vector expressing the same or different genes, generated significant levels of cellular immune response in animal models (Table 1). However, only a few of these studies demonstrated a humoral immune response and in those reports with increased specific antibody titres, there was rarely a corresponding increase in neutralisation activity. Heterologous prime-boost vaccination strategies have recently progressed to human trials where a DNA prime – MVA boost regimen was evaluated in phase 1 clinical trials in healthy volunteers [68]. The vaccines expressing the immunogen consisting of consensus HIV-1 Gag p24/p17 proteins fused to a string of epitopes recognised by cytotoxic T lymphocytes were safe, well-tolerated and stimulated HIV-specific T cell responses both on their own and in a prime-boost combination and one of the volunteers in the prime – boost group developed detectable Gag-specific antibodies.

## A.3. Tuberculosis

Together with malaria and AIDS, tuberculosis is a major health threat, particularly in developing countries. The disease is caused by the intracellular respiratory pathogen *Mycobacterium tuberculosis.* In a recent survey, the World Health Organisation estimated that one third of the world population is infected with the bacillus, eight million people develop the disease and two million die of tuberculosis each year [69]. This public health threat has become increasingly severe due to the emergence of multi-drug resistant strains and the AIDS epidemic, the former being responsible for a third of all AIDS related deaths [70,71]. Even though effective chemotherapy is available for the treatment of human tuberculosis, it is lengthy and expensive and difficult to implement in developing countries. Furthermore the emergence of multi-drug resistant strains of *M. tuberculosis* is severely reducing the effectiveness of the treatment. The widely used vaccine against *M. tuberculosis* bacille, Calmette-Guerin (BCG) is an attenuated strain of *Mycobacterium bovis*. BCG vaccination offers variable protection against childhood meningeal tuberculosis and systemic forms of the disease. However, the level of protection against the most common form, pulmonary tuberculosis in adults, is low and decreases with time [72,73]. Therefore, there is a need for an improved vaccine that provides long lasting protection against this fatally infectious disease.

Protective immunity against tuberculosis is dependent on the generation of a Th1-type cellular immune response, which is characterised by antigen specific T cells that produce IFN-γ. Both CD4+ and CD8+ T cells secrete IFN-γ. Protective immunity against tuberculosis is primarily mediated by CD4+ T cells [74], but CD8+ T cells are also required for effective protection against *M. tuberculosis* infection [4]. Therefore an immunisation regimen that stimulates both CD4+ and CD8+ T cells should lead to improved protection against *M. tuberculosis* infection. Heterologous prime-boost vaccination protocols in experimental tuberculosis that have been assessed include DNA prime/MVA boost; [4,75,76], DNA prime/ protein boost [77-79], DNA prime/BCG boost [80-82] and BCG prime/MVA or FP or DNA boost [83-85]. The findings of these studies are summarised in Table 1.

McShane et al [4] selected 2 secreted antigens, early secreted antigenic target 6 (ESAT6) and mycobacterial protein tuberculosis 63 (MPT63). MPT63 is present in both *M. tuberculosis* and some strains of *M. bovis* whereas ESAT6 is specific to *M. tuberculosis.* Using a DNA construct encoding the polyprotein and recombinant MVA encoding both antigens, they demonstrated that heterologous prime-boost regimens with the two vaccines generated up to four-fold enhancement of CD4+ T-cell responses when compared to homologous boosting. However, the order in which the vaccines were administered made no difference to the magnitude of the immune response but importantly, priming with DNA seemed to be necessary for protecting the mice following challenge as assessed by the bacterial load in the lungs. The effectiveness of this DNA prime – MVA boost was also demonstrated in cattle using the secreted mycobacterial protein Ag85 [76]. In this study the majority of the antigen specific IFN-γ secreting T cells generated were CD4+. Taracha et al [76] also demonstrated that a population of memory cells were generated by the initial prime boost immunisation as T cell responses were boosted with a second administration of MVA months later. Both the mycobacterial proteins Ag85 [77] and antigen Hsp65 [78] when used to boost the DNA vaccine prime, induced a stronger immune response than vaccination with DNA or protein alone in mice and cattle respectively. Tanghe et al [77] demonstrated a 2 to 4 fold increase in Ag85 specific IL-2 and IFN-γ responses in the spleens of DNA primed/protein boosted mice, compared to those

immunised with DNA or protein alone. Calves immunised with the antigen Hsp65 in a DNA prime / recombinant protein boost regimen exhibited enhanced specific lymphocyte proliferation and IFN-γ secretion when compared to those calves immunised with DNA or protein alone [78]. In contrast, similar DNA prime – protein boost immunisation strategies with the mycobacterial antigens MBP70 and MBP83 failed to protect cattle from an intratracheal virulent *M. Bovis* challenge even though the elicited T-cell responses were higher than those seen in the DNA alone vaccinated group of animals [86]. However, in this trial only the BCG vaccinated group displayed consistently strong T-cell responses and reduced lung lesions. Another study by Wang et al [79] showed that boosting mice with ESAT6 protein after priming with DNA encoding ESAT6 increased the parameters associated with cellular immunity compared to DNA-DNA or protein-protein immunisation.

Priming with a DNA vaccine encoding antigen 85B and boosting with BCG was more effective than BCG immunisation in protecting mice against *M. tuberculosis* aerosol challenge [80]. Depletion of CD8+ T-cells impaired this protection suggesting that CD8+ T cells may partially mediate this improved efficacy. In this study, priming with DNA and boosting with either recombinant vaccinia virus or protein did not improve upon the partial protection conferred by DNA alone. Immunisation with three DNA vaccines encoding the *M. tuberculosis* Apa (alanine-proline rich antigen) and the immunodominant Hsp65 and Hsp70 mycobacterial antigens combined with a BCG boost was assessed for protection against tuberculosis in mice [82]. This demonstrated specific CD4+ and CD8+ T cell responses in mice vaccinated with the DNA vaccines and BCG boost compared to the BCG vaccinated animals. In addition, following challenge with a virulent strain of *M. tuberculosis*, the group of mice that received all three DNA vaccines/BCG boost had significantly less bacteria in the lungs than either the DNA vaccines or the BCG immunised mice. Skinner et al [81] reported enhanced protection against *M. Bovis* infection in cattle using the same priming cocktail of Apa/ Hsp65/ Hsp70 DNA vaccines and BCG boost. This demonstrated that even though similar levels of antigen specific IFN-γ producing cells were induced by DNA vaccines/BCG and BCG immunisation, the DNA vaccines/BCG vaccinated animals exhibited less lung and lymph node lesions and lower lymph node bacterial counts.

A number of studies have evaluated the efficacy of BCG priming followed by either DNA or recombinant viral boost in animal models. Goonetilleke et al [83] demonstrated that MVA expressing *M. tuberculosis* Ag85A strongly boosts BCG induced specific CD4+ and CD8+ T cell responses in mice. Following aerosol challenge, the protection seen in the lungs was associated with Ag85A-specific, IFN-γ secreting T cells. Using the same antigen, Vordermeier et al [84] assessed the immune response generated following priming with BCG and boosting with either MVA or FPV in calves. There was a stronger and more specific T cell response in those calves boosted with MVA85A than in the BCG/BCG vaccinated calves. Such enhanced protection against tuberculosis was also demonstrated following priming with BCG and boosting with DNA vaccine Rv3407 [85].

These reports highlight the effectiveness of heterologous prime-boost immunisation strategies at enhancing cell-mediated immunity compared to homologous immunisation. In the majority of these animal studies, the secretion of IFN-γ from antigen specific T cells, used as an immunological correlate of protection against tuberculosis, was enhanced several fold following heterologous prime-boost immunisation. Recently, the first Phase 1 clinical evaluation of the safety and immunogenicity of the BCG prime/MVA85A boost vaccination

strategy in humans was completed [87]. Elevated levels of IFN-γ secreting T cells were demonstrated in both BCG naïve volunteers and volunteers that were vaccinated in early childhood. Further clinical trials using this vaccination regimen are underway [88] and the outcome of these are eagerly awaited by the research community.

# Appendix B: Tables

*Table 1. Heterologous prime (DNA) - boost immunisation regimens in animal trials*

| Pathogen | Boost | Antigen | Measurement | Animal Model /Outcome | Ref. |
|---|---|---|---|---|---|
| **Malaria** | | | | | |
| *Plasmodium falciparum* | MVA | PfTRAP PfLSA-1/GM-CSF | IgG, IFN-γ CD8+ T cells, CTL, challenge | Chimp / ↑T cell & Ab responses to pfTRAP | [26] |
| *Plasmodium falciparum* | Protein | EBA-175 region II | IgG, Growth/invasion inhibition, challenge | *Aotus* monkeys / ↓parasite load | [23] |
| *Plasmodium falciparum* | MVA or FPV | L3SEPTL polyprotein | CD8+ T cells, IFN-γ secreting cells, tetramer staining | Mice / ↑CD8+ T cells with single antigen prime | [30] |
| *Plasmodium falciparum* | Protein | pfs25 | IgG, transmission blocking assays | Rhesus Macaques /↑IgG1, ↓90% oocyte number in mosquito | [7] |
| *Plasmodium berghei* | MVA | PbCSP, PbTRAP | IFN-γ CD8+ T cells, challenge | Mice / ↑IFN-γ CD8+ cells, ↑protection | [22] |
| *Plasmodium berghei* | FPV | PbCS | IFN-γ CD8+ cells, challenge | Mice / poor protection | [21] |
| *Plasmodium knowlesi* | Canarypox, VV | PkCSP, PkSSP2, PkAMA1, PkMSP1p42 | Specific Ab, IFN-γ CD4+ T cells, challenge | Rhesus Macaques / ↑Ab, ↑ IFN-γ response, ↓parasite load | [24] [25] |
| *Plasmodium vivax* | Protein | Pvs25 and Pvs28 | Specific Ab, transmission blocking assays | Mice / ↓87-96% oocyte number in mosquito | [31] |
| *Plasmodium cynomolgi* | Protein | PcTRAP, PcCS | Specific Ab, Lymphocyte proliferation, IFN-γ secreting cells, challenge | Mice & monkeys/↑IgG2a, ↑lymphocyte proliferation ↓parasite load | [20] |
| *Plasmodium yoelii* | VV, Poxvirus | PyCSP/GM-CSF | Specific Ab, challenge, CD8+ T cells, IFN-γ secreting cells, CTL | Mice, neonatal mice / ↑IFN-γ & CTL induction, ↑protection (70-90%) | [27] [28] [29] |
| *Plasmodium falciparum* | MVA | Influenza nucleoprotein, *P. falciparum* T cell epitopes | IFN-γ secreting cells, CTL, *P. berghi* & influenza A virus challenge | Mice / ↑CD8+ T cells, ↑CTL responses, ↑protection (90-100%) | [95] |
| **HIV / SIV** | | | | | |
| HIV | MVA | HIV-1 IIIB *env, gag, RT, rev, tat, nef* & SIV *gag/pol* | CD8+ T cells, IFN-γ secretion, serum Ab, plasma viral RNA levels following SHIV challenge | Cynomolgus macaques / high Abs, CTL & proliferation, ↓viral RNA | [5] |
| HIV | MVA | HIV-1 Env IIIB Ag | CD8+ T cells, cytokine & chemokine production, Ab against gp160 | Mice / ↑in all parameters when cholera toxin used in prime and boost | [44] |
| HIV | ADV | HIV *gag- pol- nef* ± *env* | Neutralisation assays, IFN-γ secreting cells, CD4+ cells, viral RNA levels after SHIV challenge | Rhesus monkeys / 2.5 fold ↑ IFN-γ secreting cells, +env ↑protection against CD4+ depletion | [45] |
| HIV | ADV, VV | HIV-1 Env IIIB gp120 | P18-specific CD8+ cells, cytotoxicty assays, IFN-γ production, cytokine secretion, serum Ab, | Mice / ↑CD8+ cells & CTL induction, ↑IFN-γ & IL-2, ↑gp120 Ab (10 fold) | [46] |
| HIV | MVA, VV | TAB 13 | IFN-γ secretion cells, IFN-γ & IL-10 levels, specific Abs | Mice / ↑IFN-γ secreting cells, ↑IFN-γ & IL-10 | [49] |
| HIV | Protein | HIV Env proteins, Gag | Serum Ab, lymphocyte proliferation, virus neutralisation | Baboons /↑Ab (75fold) &↑lymphoproliferation but weak neutralisation | [43] |

| | | | | | |
|---|---|---|---|---|---|
| HIV | VLP | HIV-1 subtype C *gag* | IFN-γ CD8+ T cells, cytotoxicity, IFN-γ secretion | Mice / ↑IFN-γ CD8+ T cells (4 fold), ↑cytotoxicity | [56] |
| HIV | ADV | HIV-1 env (gp150, gp140, gp120) | Specific Ab, neutralisation assays, IFN-γ secretion | Monkeys / ↑Ab (10 fold), ↑specific T cells, neutralisation detected after boost | [47] |
| HIV | HSV | HIV-1 gp120 | CD8+ T cells by tetramer staining, cytotoxicity assay, specific Ab | Mice / 3 fold ↑CD8+ T cells (acute and long term) | [57] |
| HIV | ADV | *gag* of HIV-1 clade B | Gag specific CD8+ T cells, | Mice / no change compared to adenovirus alone | [48] |
| SIV | VV | SIV *gag/pol* | Lymphocyte proliferation, cytokine production, IFN-γ secreting cells, CTL assays, gag Ab, challenge with wild type VV expressing SIV *gag/pol* | Mice / ↑IFN-γ CD4+ T cells & proliferation, ↑CTL induction, 300 fold ↓viral load | [50] |
| SIV | VV, MVA, FPV | SHIV89.6P *env* SIVmac239 *gag* | IFN-γ secretion, Ab against gp120, CD8+ & CD4+ T cells, viral RNA after challenge with SHIV | Rhesus monkeys / ↑CTL responses (10 fold), no diff. in viral load or CD4+ T cell depletion from DNA boost | [6] |
| SIV | FPV | SIV gag/pol & HIV-1, *tat, rev, vpu & env* FPV *gag/pol boost* | IFN-γ secreting cells, lymphocyte proliferation, neutralisation Ab, SHIV challenge | *Macaca nemestrina* monkeys/ ↑IFN-γ secreting cells (10 fold) & proliferation (3 fold), ↓viral load, | [53] |
| SIV | VV | SHIV genes & Env gp160 prime Vv SHIV Gag/Pol or Env gp160 boost | Specific Ab, SHIV challenge, viral load, IFN-γ secretion | *Macaca nemestrina* / ↑ anti-Env Ab, slower decline of CD4+ T cells, ↑neutralising Ab and survival | [51,52] |
| SIV | VLP | SIV *gag, pol* FP-HIV-1 *env* | Ab against gag or env proteins, virus neutralisation, lymphocyte proliferation, IFN-γ & IL-4 expression | Rabbits / ↑Ab titre, no difference in lymphocyte proliferation or neutralisation | [54,55] |
| SIV | Sendai virus | SIV Gag | SHIV challenge, virus specific IFN-γ induction, CD4+ depletion, | Rhesus macaques / ↑CD4+ & CD8+ T cells, protection from CD4+ T cell depletion | [58] |
| HIV | Protein | env | Specific Ab, Lymphocyte proliferation, challenge | Monkeys / high Ab titres, protection | [96] |
| HIV | Protein | HIV-1-gp160 prime, gp41 boost | IgG & IgA Ab, virus neutralisation, T cell proliferation | Mice / high Ab titres, long lasting neutralisation Ab, | [97] |
| HIV | Protein, VV | env | Ab levels – IgG, IgA, IgM, viral neutralisation | Mice / long lasting specific Ab responses, IFN-γ secreting $CD4^+$ cells, viral neutralisation | [98] |
| SIV | VV | gag, pol, env, nef, rev, vpr, vpx, tat, vif | Lymphocyte proliferation, Ab neutralisation, viral load, challenge | Monkeys / specific Ab, $CD4^+$ and $CD8^+$ cells, ↓viral load, no protection | [99] |
| HIV | VV | CTL epitopes | CTL response, IFN-γ secretion | Monkeys / ↑ $CD8^+$ specific cells, | [100] |
| SIV | Poxvirus | gag, env, pol | Lymphocyte proliferation, CTL, Specific Ab | Monkeys / ↑$CD8^+$ specific cells, ↑T cell proliferation against gag & env proteins | [101] |
| SIV | Protein | gp160 | Specific Ab, virus neutralisation, CTL, Lymphocyte proliferation, viral load, challenge | Monkeys (newborn) / Ag specific & neutralising Ab, ↓viral load, limited containment of infection | [102] |
| SIV / HIV | VV | SIV-gag, pol, vif, vpx, vpr, HIV- env, tat, rev | Viral load, specific Ab, challenge, Lymphocyte proliferation | Monkeys / virus specific $CD4^+$ & $CD8^+$ T cells, low Ab levels, low viral load, controlled infection | [103] |
| SHIV | Sendai virus | Prime ($env^-$, $nef^-$) Boost gag | Viral load, SHIV challenge | Monkeys / ↓ viral load, ↓CD4+ T cell depletion | [104] |
| HIV / SIV | VV | CTL epitopes | Lymphocyte proliferation, viral load, challenge | Mice, monkeys / high $CD8^+$ proliferation, ↓viral load, limited protection | [105] [106] [107] |

| | | | | | |
|---|---|---|---|---|---|
| HIV | VV | Tat-rev-nef- gp41 | Lymphocyte proliferation, IFN-γ secreting cells | Mice, monkeys / specific T cell responses | [108] |
| HIV | Protein | HIV-1 gp120 | Specific Ab, neutralising Ab activity, CTL activity | Mice, guinea pigs / ↑Ab (25 fold), ↑neutralising activity (2-100 fold) | [109] |
| SIV | Listeria | SIV Gag & Env | IFN-γ CD8+ & CD4+ T cells, challenge, anti-SIV Ab | Monkeys / ↑CD8+ & CD4+ T cells, ↓viral load, | [110] |
| **Tuberculosis** | | | | | |
| *Mycobacterium tuberculosis* | MVA | ESAT6 MPT63 | IFN-γ CD4+ cells, CD8+ & CD4+ T cell depletion, challenge | Mice / ↑IFN-γ CD4+ cells, same protection as BCG | [4] |
| *Mycobacterium tuberculosis* | MVA | Ag85A | IFN-γ secreting T cells, cell depletion, challenge | Mice / ↑ CD4+ and CD8+ T cells, ↑protection | [75] |
| *Mycobacterium tuberculosis* | Protein | Ag85A<br>Ag85B | IgG, cytokine production, challenge | Mice / ↑IFN-γ CD4+ cells, ↑IgG2a, ↓CFU in lungs | [77] |
| *Mycobacterium tuberculosis* | Protein | ESAT6 | IgG, lymphocyte proliferation, IFN-γ & IL-4 production, Cytotoxicity | Mice / ↑IFN-γ, ↑CTL induction, ↑IgG2a:IgG1 | [79] |
| *Mycobacterium tuberculosis* | BCG | Ag85B | Bacterial load, CD8+ T cell depletion | Mice / ↑protection | [80] |
| *Mycobacterium tuberculosis* | BCG | Hsp70, Hsp65, Apa | CD4+ and CD8+ T cells, cytokine production, challenge | Mice / ↓bacterial load | [82] |
| *Mycobacterium tuberculosis* * | MVA | Ag85A | IFN-γ secreting T cells, bacterial load after aerosol challenge | Mice / ↑ CD4+ and CD8+ T cells, ↑protection | [83] |
| *Mycobacterium tuberculosis* * | DNA | Rv3407 | IFN-γ secreting T cells, serum Ab, bacterial load after aerosol challenge | Mice / ↑protection | [85] |
| *Mycobacterium Bovis* | Protein | Hsp65 | IgG, Lymphocyte proliferation, IFN-γ production | Cattle /↑ IFN-γ & lymphocyte proliferation | [78] |
| *Mycobacterium Bovis* | MVA | Ag85A | IFN-γ CD4+ and CD8+ T cells, Lymphocyte proliferation, | Cattle / ↑ IFN-γ secreting T cells & proliferation | [76] |
| *Mycobacterium Bovis* | BCG | Hsp70, Hsp65, Apa | IFN-γ & IL2, IFN-γ producing T cells, challenge | Cattle / ↑ IFN-γ & protection | [81] |
| *Mycobacterium Bovis* | Protein | MPB70, MPB83 | Challenge, serum Ab, IFN-γ & IL-2 production, cytokine gene expression, IL-2 receptor | Cattle / no protection | [86] |
| *Mycobacterium Bovis* * | MVA and/or FPV | Ag85A | IFN-γ secreting T cells, intracellular staining of CD8+ T cells | Cattle / ↑ IFN-γ secreting T cells | [84] |
| **Other Diseases** | | | | | |
| Hepatitis C Virus | ADV | E2, virus core | E2 & core specific Th1 CD4+ T-cells, IFN-γ producing cells, CTL activity. | Mice / ↑CD4+ T cells | [12] |
| Hepatitis C Virus | Canarypox | capsid/E1/E2/NS2/NS3 proteins | Specific Ab, IFN-γ producing cells, cytotoxicity | Mice / ↑ CD8+ IFN-γ producing cells (3-8 fold) | [111] |
| Hepatitis C Virus | Canarypox | capsid/E1/E2/NS2/NS3 , NS3/NS4/NS5 | CTL assay, IFN-γ secreting cells, cytokine gene expression, challenge | Mice / complete reduction in virus titres | [11] |
| Hepatitis C Virus | ADV | HCV-core | CTL induction, viral clearance | Mice / reduced vaccinia titres | [112] |
| *Equine herpesvirus1* | baculovirus | EHV-1 glycoprotein D | EHV-1 specific Ab, virus clearance from lungs. | Mice/ Both parameters higher in prime boost | [113] |
| *Herpes simplex virus 2* | MVA | Glycoprotein D | Specific Ab, cytokine levels, challenge, neutralisation assays | Mice / ↑ Ab and cytokine levels | [13] |
| *Cowdria ruminantium* | Protein | MAP1 | MAP1 Ab, lymphocyte proliferation, cytokine levels, challenge. | Mice / survival rate up from 13-27% to 67% | [114] |
| *Hepatitis B Virus* | Protein | HB surface antigen | Cytokine production, CD4+ and CD8+ cells, cytolytic activity, tumour protection | Mice / stronger Th1-type cellular immune responses, 80 % protection | [14] |
| *Chlamydia* | SFV | MOMP or Omp2 | Specific IgG1 & IgG2a, | Mice / ↓ bacterial load, ↑ IFN-γ | [90] |

| | | | | | |
|---|---|---|---|---|---|
| *pneumoniae* | | | lymphocyte proliferation, IFN-γ secreting T cells, cytotoxic activity, lung clearance, challenge | secreting T cells | |
| *Taenia solium* | Protein | cC1 / GST-cC1 | Challenge and cyst counts, lymphocyte proliferation, IgG1 & IgG2 | Pig / longer lasting increased protection (PP – 53%, ↑IgG1; DP –79%, ↑IgG2) | [91] |
| Enterotoxigenic *Escherichia coli* | Protein | FAEG | Lymphocyte proliferation, specific Ab | Pig / weak immune response | [92] |
| Human T-cell leukemia/ lymphoma virus type1 (HTLV-1) | NYVAC | DNA HTLV-1 *env* prime NYVAC-HTLV-1 *env+gag* boost | Specific Ab, challenge, lymphocyte proliferation | Monkey / 100 % protection 8 months after challenge | [93] |
| *Bacillus anthracis* | Protein | Protective antigen (PA), Lethal factor (LF) | Ab against PA, aerosol challenge | Rabbit / protection similar to protein alone | [115] |
| Classical swine fever virus | ADV | gp55 / E2 | Specific Ab, challenge, | Pigs / 100% protection | [116] |
| *Chlamydophila abortus* | Protein | dnaK (heat shock protein) | Specific Ab, challenge, bacterial titre in spleen | Mice / Increased Ab but no protection | [94] |
| *Leishmania infantum* | VV | LACK | Intravenous challenge, cytokine mRNA levels, lymphocyte proliferation, specific Ab, parasite load | Dogs / 60% protection, ↑IL-4, ↑IFN-γ & ↑IL-12 mRNA. | [8] |
| *Leishmania infantum* | VV | P36/LACK | Parasite load/footpad lesion size, cytokine production, specific Ab, CD8+ T cells | Mice / ↓70% lesion size, ↓parasite load (1000 fold), ↑IFN-γ cells | [9] [89] |
| *Schistosoma mansoni* | VV | CT-SOD / SP-SOD / GPX | Challenge & worm burden | Mice / 85 % protection | [10] |
| *Schistosoma mansoni* | Protein | Sm23 | Challenge & worm burden, specific Ab | Mice / ↑IgG1:IgG2a ratio, no protection | [117] |

** Those trials using BCG as the prime followed by the indicated boost.*
*Abbreviations: MVA; modified virus Ankara, ADV; adenovirus, NYVAC; New York vaccinia, VV; vaccinia virus, FPV; fowlpox virus, HSV; Herpes simplex virus, VLP; virus like particles, SFV; Semliki Forest virus, IFN-γ ; interferon gamma, IL; interleukin, CTL; cytotoxic T lymphocyte, Ab; antibody, CFU; colony forming units. ↑↓; denotes increase and decrease respectively*

*Table 2. Other prime - boost immunisation combinations for HIV/SIV*

| Pathogen | Immunisation Strategy | | Antigen | Measurement | Animal Model /Outcome | Ref. |
|---|---|---|---|---|---|---|
| | **Prime** | **Boost** | | | | |
| HIV | MVA | FPW | Central region of V3 loop of gp120 of HIV-1&IIIB (TAB13) | IFN-γ secreting cells, | Mice / ↑IFN-γ secreting CD8+ T cells (2 fold), | [59] |
| HIV | SFV | MVA | HIVA immunogen from HIV-1 clade A | IFN-γ secreting cells, CTL assay | Mice / ↑IFN-γ secreting cells, longer lasting CTL induction | [65] |
| HIV | CpG + protein | ADV | HIV-1 Gag | Intracellular cytokine staining, gag-specific Abs | Mice / ↑IFN-γ secreting CD8+ T cells (7 fold), | [62] |
| HIV | Rabies virus | VSV | HIV-1 Env | Challenge, specific Ab, IFN-γ secreting cells, neutralisation activity | Mice / ↑Abs, ↑IFN-γ secreting cells (4 fold), ↑neutralisation activity | [63] |
| HIV | SFV | FPV | HIV-1 TAB9 multiepitope | Challenge, IFN-γ secreting cells, viral titre in ovaries | Mice / ↑IFN-γ secreting T cells (15 fold), ↓viral titre (60% protection) | [64] |
| HIV | Influenza virus | VV, MVA | V3 loop of gp120 of HIV-1 IIIB prime HIV-1 IIIB gp160 boost | Ab against V3 loop of gp160, CD8+ IFN-γ secreting cells, T-cell proliferation, | Mice / ↑IFN-γ secreting CD8+ T cells (60 fold), ↑IgG2a:IgG1 | [66] |
| SIV | ADV | Protein | SIV*env/rev/gag/nef* SIV gp120 | Challenge, IFN-γ secreting cells, T-cell proliferation, CD8+ antiviral activity, serum antibodies | Rhesus macaques / ↓viral load (21-52 fold), ↑survival time | [60] |
| SIV | Influenza virus | VV | SIV Gag | CD8+ IFN-γ secreting cells, challenge, viral titre | Mice / ↑IFN-γ secreting cells (5 fold) | [67] |
| SIV | ADV | Protein | SIV gp120 | Ab-dependent cellular cytotoxicity (ADCC) | Rhesus macaques / ↑ADCC against SIV infected H9 cells | [61] |

*Abbreviations: MVA; modified virus Ankara, ADV; adenovirus, VV; vaccinia virus, FPV; fowlpox virus, SFV; Semliki Forest virus, VSV; Vesicular stomatitis virus, IFN-γ ; interferon gamma, Ab; antibody, CpG; cytosine phosphate guanosine oligodeoxynucleotide. ↑↓; denotes increase and decrease respectively.*

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Susan Shahin and David Proll

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DNA vaccine, heterologous prime-boost, infectious diseases, malaria, tuberculosis, HIV/SIV

19. ABSTRACT

Development of prophylactic vaccines against infectious diseases (such as malaria, tuberculosis and HIV) has been hindered by the lack of effective immunisation strategies that induce the cellular arm of the immune system necessary for protection against these intracellular pathogens. DNA vaccines, recombinant proteins and recombinant viral vectors are all effective antigen delivery systems for inducing cellular immunity. However, when used alone, the levels of specific immune response they induce is often low. Heterologous prime – boost immunisation strategies involve using two different vaccines, each encoding the same antigen. In the past decade, numerous published reports have demonstrated that such prime – boost immunisation strategies effectively enhance cellular immunity in several different animal and disease models. Since several intracellular pathogens are considered potential biowarfare threats, the objective of this review is to assess whether prime-boost vaccination is likely to be effective in protecting against those intracellular pathogens of defence interest. This review focuses on heterologous prime – boost immunisation studies using DNA vaccines as the priming vehicle followed by either recombinant protein or recombinant viral vector boost. Included is a summary of studies up to July 2005, for a number of diseases. This paper evaluates if this approach may be applied to those intracellular pathogens considered a threat to the ADF in our vaccine development program.