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Review

Prime-boost strategies for malaria vaccine development

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Summary

Malaria is an intracellular pathogen, for which an effective vaccine is likely to require induction of cell-mediated immunity. Immunisation approaches that stimulate strong and persistent levels of effector T-cells are being sought by many researchers. DNA vaccines, recombinant protein and viral vectors were amongst the vaccine delivery systems that appeared promising for the generation of cellular immunity, and in some initial studies in small animals this goal was achieved. However, clinical trials of these candidate vaccines when used alone or in repeated homologous boosting regimes have been disappointing, with short-lived low levels of induced specific T-cell responses. Recent years have seen the development of immunisation strategies using a combination of different antigen delivery systems encoding the same epitopes or antigen, delivered at an interval of a few weeks apart. This sequential immunisation approach with different vectors is known as heterologous prime-boosting and is capable of inducing greatly enhanced and persistent levels of CD8+ T-cells and Th1-type CD4+ T-cells compared to homologous boosting. This review will summarise the key pre-clinical studies of prime-boost strategy and outline recent progress in clinical trials of this approach. Possible mechanisms of action and potential improvements to existing delivery systems will be discussed. The prime-boost approach represents an encouraging step towards establishing an effective preventative vaccine to one of the world's greatest killers.

Key words: vaccine, malaria, prime-boost strategy, T-cell.

Introduction

Malaria remains one of the world's biggest killers, despite decades of research. Each year up to 2.7 million people are estimated to die as a result of *Plasmodium falciparum* infection, and two billion of the world's population live in malaria-exposed regions (Marshall, 2000). The socio-economic impact of malaria is immense and areas with holoendemic malaria are almost all poor with continued low economic growth (Gallup and Sachs, 2001). Therefore a safe and effective vaccine is urgently required to enhance existing malaria control measures. As malaria is an intracellular parasite, it is likely that a protective vaccine would activate the cellular arm of the immune response and be effective against the pre-erythrocytic liver stage of the life cycle.

An important potential role for CD8+ T-cells in protection against *P. falciparum* malaria was suggested by studies in mice (Schneider et al., 1998) and humans (Hill et al., 1991). Established vaccines in current clinical use act predominantly by induction of antibodies, and stimulating strong cellular immunity has proved harder to achieve. In particular, many studies have shown that non-particulate antigens adjuvanted with alum do not induce significant levels of CD8+ cytotoxic T-lymphocytes (CTLs). A number of alternative antigen delivery systems have the potential to activate cell-mediated immunity, including DNA vaccines, recombinant viral and bacterial vectors, protein-in-adjuvant formulations and recombinant virus-like particles. For DNA and recombinant virus subunit vaccines, the DNA sequence for the antigen(s) of choice is inserted into an *Escherichia coli*-derived purified plasmid or the genome of a double-stranded DNA virus such as vaccinia. Host CD4+ and CD8+ responses can then be induced following intracellular synthesis, processing and HLA (Human Leukocyte Antigen) presentation of class I and II T-cell epitopes.

In the mid-1990s there was much international optimism about the potential for DNA vaccines to be effective preventative and therapeutic vaccines for a range of intracellular diseases including malaria, tuberculosis, HIV and cancer. Many murine studies demonstrated their ability to stimulate both humoral and cellular immunity, including protection against *Plasmodium yoelii* by PyCSP, a DNA vaccine encoding the *P. yoelii* circumsporozoite antigen (Hoffman et al., 1994). Human studies confirmed the safety of

the approach and the ability to elicit antigen-specific CD8+ CTLs (Wang et al., 1998). However, a much more limited magnitude of T-cell response, which was insufficient to be protective against malaria challenge, was observed in these clinical trials. Strategies to improve the immunogenicity of DNA vaccines have been reported by many groups, including co-administration of cytokine- and chemokine-encoding plasmids (Doolan and Hoffman, 2001; Gurunathan et al., 1998; Sedegah et al., 2000) and ubiquitination approaches such as N-end rule targeting (Tobery and Siliciano, 1999). However, these modifications have yet to result in enhanced cellular immunity of sufficient magnitude to confer protection of humans against challenge.

In parallel with the development of DNA vaccines has been the emergence of recombinant viral vectors, such as poxviruses and adenoviruses, as vaccine delivery systems. Ideally such vaccines should be unable to replicate in human cells, to minimise side effects and allow use in immunocompromised individuals. Poxviruses are good candidates as they show high species specificity; for example, avipox viruses are unable to replicate in mammalian cells (Paoletti, 1996). Protective T-cell responses in small animals induced by recombinant vaccinia viruses were first reported in the 1980s (Panicali and Paoletti, 1982; Smith et al., 1983). The highly attenuated recombinant vaccinia viruses MVA (modified vaccinia virus Ankara) (Sutter and Moss, 1992) and NYVAC (New York vaccinia) (Tartaglia et al., 1992) have been shown to have excellent immunogenicity. MVA was developed by over 500 serial passages in chicken embryo fibroblasts and was used as a smallpox vaccine in 120 000 people in the 1970s, including immunocompromised individuals (Mayr et al., 1978), and appears to have an excellent safety profile. Due to an acquired replication defect at a late stage of virion assembly MVA does not replicate in human cells, but is able to express recombinant genes, making it an excellent candidate viral vector, like NYVAC, which was derived from the Copenhagen strain of vaccinia virus and is molecularly attenuated. However, these recombinant viruses when used singly or with repeated administration (homologous boosting) do not produce the levels of CD8+ T-cells required for high-level protection against malaria in murine models (Lanar et al., 1996; Pye et al., 1991; Schneider et al., 1998; Sedegah et al., 1990).

Induction of CD8+ T-cells requires introduction of antigen into the MHC (Major Histocompatibility Complex) class I presenting pathway. Soluble proteins and peptides do not induce CD8+ T-cells when administered alone, probably because the antigen does not enter class I processing pathways and does not provide a sufficient ‘danger signal’ to trigger innate immune responses. Therefore protein-based vaccines frequently employ adjuvants for delivery, to act as immune stimulants. Other approaches to facilitate intracellular delivery of protein material include the use of bacterial toxins (Donnelly et al., 1993), liposomes (Lipford et al., 1994), lipopeptides (Deres et al., 1989) and virus-like particles (VLPs) such as the yeast-derived Ty-VLP (Gilbert et al., 1997).

Heterologous prime-boost strategy

Much research has been conducted into ways of improving the efficacy of DNA and recombinant viruses, and it was logical to try combining different approaches. Li et al. (1993) reported protection of mice against *P. yoelii* challenge when a priming immunisation with a recombinant influenza virus expressing an epitope from the circumsporozoite protein of *P.yoelii* was followed by a boosting immunisation of a recombinant vaccinia virus expressing the same epitope (Li et al., 1993). This sequence of immunisation was crucial because homologous boosting or the opposite order of immunisation failed to induce protection. This early example of protection by heterologous prime-boost immunisation appeared to be mediated by predominantly CD8+ T-cells, as the anti-malaria immunity was abolished by treatment of the immunised mice with anti-CD8 monoclonal antibody. In some other studies in the field of HIV vaccine research, combining different antigen vectors (DNA vaccine boosted by a protein-in-adjuvant formulation) resulted in enhanced antibody function, but by an additive rather than synergistic effect, which did not result in greatly enhanced effector T-cell induction (Gorse et al., 1994; Letvin et al., 1997), signifying that not all heterologous prime-boost strategies are effective at generating a synergistic enhancement of T-cell responses.

Other heterologous combinations have emerged that confirm the ability of certain prime-boost approaches to enhance cellular immunity with a variety of antigen delivery systems. Although many vector agents are able to prime an immune response, not all are effective at boosting. Priming the response requires induction of specific T-cells, including a population that persists as antigen-specific memory cells beyond elimination of the antigen, which then undergoes rapid expansion upon re-exposure to the same antigen in a boosting immunisation. The nature of an antigen delivery system determines its ability to boost the cell-mediated immune response. In general DNA plasmids, protein-in-adjuvant formulations, virus-like particles and lipopeptides are excellent priming agents but relatively ineffective as boosting agents. Recombinant viruses including MVA, NYVAC, attenuated fowlpox strain 9 (FP9) and non-replicating adenovirus strains appear capable of either priming or boosting when used in heterologous regimens. Immunisation with recombinant viruses results in expression of the vaccine antigens inside infected cells, and hence their efficient delivery to MHC class I and II antigen-processing pathways *via* endogenous pathways. Protein-in-adjuvant and other particulate vaccines that result in exogenous antigen delivery may not access the class I antigen processing pathway as efficiently (Belshe et al., 2001). However, this does not explain why delivery systems that prime well fail to boost as well as poxviruses and adenoviruses (Gilbert et al., 2002). Part of the explanation may be simply an immunodominance effect. The overall immunogenicity of a recombinant poxvirus (Harrington et al., 2002) or adenovirus is substantially greater that that of a plasmid DNA or a lipopeptide vaccine. However, when used

alone or in homologous prime-boost regimes, much of the immunogenicity of these recombinant viruses is targeted at vector components. By priming with a different vector, synergistic prime-boost immunisation may generate memory T-cells to the insert with the priming immunisation that are then amplified by the booster immunisation in preference to vector-specific T-cell responses that were not primed. Many groups have now reported enhanced CD4+ and CD8+ T-cell induction by prime-boost strategies in a range of disease models, including tuberculosis (McShane et al., 2001), HIV (Hanke et al., 1998), human papillomavirus (van der Burg et al., 2001) and Ebola (Sullivan et al., 2000).

Assays of cellular immunity

Evaluation of T-cell immunity has improved in recent years, due to development of sensitive quantitative assays. The chromium release assay was previously the established assay of effector function, but had the disadvantages of low sensitivity, requirement of radio-labelling of target cells, and the need for *in vitro* culture over several days. Sensitive assays now available include the *ex vivo* and cultured ELISPOT (enzyme-linked immunospot) assays of cytokine secretion (Herr et al., 1996), intracellular cytokine staining by FACS analysis (Murali-Krishna et al., 1998), tetramer staining studies (Altman et al., 1996) and commercially available multiple simultaneous cytokine detection systems (De Jager et al., 2003). Due to its relative ease of use, low cost, good reproducibility and ability to detect responses across a 1000-fold range, the *ex vivo* γ-interferon ELISPOT is now widely used to quantify CD4+ and CD8+ T-cell responses in pre-clinical and clinical studies.

Antigen selection

The recent publication of the entire *P. falciparum* genome sequence identified about 5300 probable genes (Gardner et al., 2002), highlighting the difficulties in selecting the best antigen(s) for inclusion in a vaccine. The parasite life cycle involves multiple stages, expressing largely different antigens at different times, and with so many antigens it is difficult to know which to focus on. Many major antigens show genetic variation and a few major blood stage antigens display antigenic variation, or clonal switching, further aiding evasion by the parasite of the host immune response. Identification of immune correlates of protection against malaria would greatly aid antigen selection. Studies of T-cell effector function in mice (Doolan and Hoffman, 2000), malaria-exposed humans (Dodoo et al., 2002) and vaccinated malaria-naïve populations (Lalvani et al., 1999) have highlighted the complexity and diversity of T-cell immunity. Many candidate vaccines in pre-clinical and clinical studies have used whole or part of the well-characterised circumsporozoite protein (CS), which is expressed on the extracellular sporozoite and the intracellular hepatic stages of the parasite (Nardin and Nussenzweig, 1993; Nardin et al., 1982). Thrombospondin-related adhesion protein (TRAP), a pre-erythrocytic antigen necessary for gliding motility and infectivity of liver cells (Sultan, 1999) is a protective antigen in both *P. berghei* and *P. yoelii* and has been used in several candidate vaccines to date. T-cell responses to this antigen generated by natural exposure to malaria have been characterised in studies in The Gambia and Kenya (Flanagan et al., 1999).

Murine studies

Researchers at the University of Oxford conducted a series of immunisation studies comparing the immunogenicity in mice of several potential vaccine delivery systems (recombinant particles, peptides, plasmid DNA, numerous adjuvants and recombinant vectors such as recombinant BCG, *Salmonella*, adenovirus, MVA and attenuated fowlpox), encoding malaria epitopes and antigens (Gilbert et al., 1999; Plebanski et al., 1998; Schneider et al., 1998). Only modest levels of T-cell immunogenicity, as measured by *ex vivo* γ-IFN ELISPOT, were induced by most of these approaches, which did not protect against sporozoite challenge. Repeated administration of the same antigen delivery system (homologous boosting) did not enhance CD8+ T-cell induction or protection (Schneider et al., 1998). However, a priming immunisation with either Ty-virus like particles encoding a major CD8 T-cell epitope (Gilbert et al., 1997) or plasmid DNA encoding the entire *P. berghei* circumsporozoite antigen followed by a boosting immunisation with recombinant MVA carrying the same antigen, induced a five- to tenfold higher level of CD8+ T-cells compared to either the DNA or the recombinant MVA vector alone in homologous regimens (Fig. 1). This greater T-cell response was associated with an increase of observed efficacy against *P. berghei* sporozoite challenge from 0–20% to 80–100% (Table 1). Administering both DNA and MVA together failed to produce this enhancement, with a minimum interval between the immunisations of 9 days required. The US Naval Medical Research group has reported similar enhanced T-cell induction and protection against *P. yoelii* malaria with DNA prime followed by NYVAC as the boosting immunisation (Sedegah et al., 1998).

Table 1. *Heterologous prime-boost immunisation and protection against* Plasmodium berghei *sporozoite challenge*

Immunization 1	Immunization 2	Number infected	Protection (%)
DNA-CS+TRAP	DNA-CS+TRAP	5/5	0
MVA-CS+TRAP	MVA-CS+TRAP	4/5	20
DNA-CS	MVA-CS	0/10	100
DNA-TRAP	MVA-TRAP	2/16	88
DNA- CS+TRAP	MVA-CS+TRAP	0/10	100
MVA-CS+TRAP	DNA-CS+TRAP	5/5	0
DNA-epitope	MVA-epitope	0/10	100
None	None	9/10	10

TRAP, thrombospondin-related adhesion protein; MVA, modified vaccinia virus Ankara; CS, circumsporozoite protein.

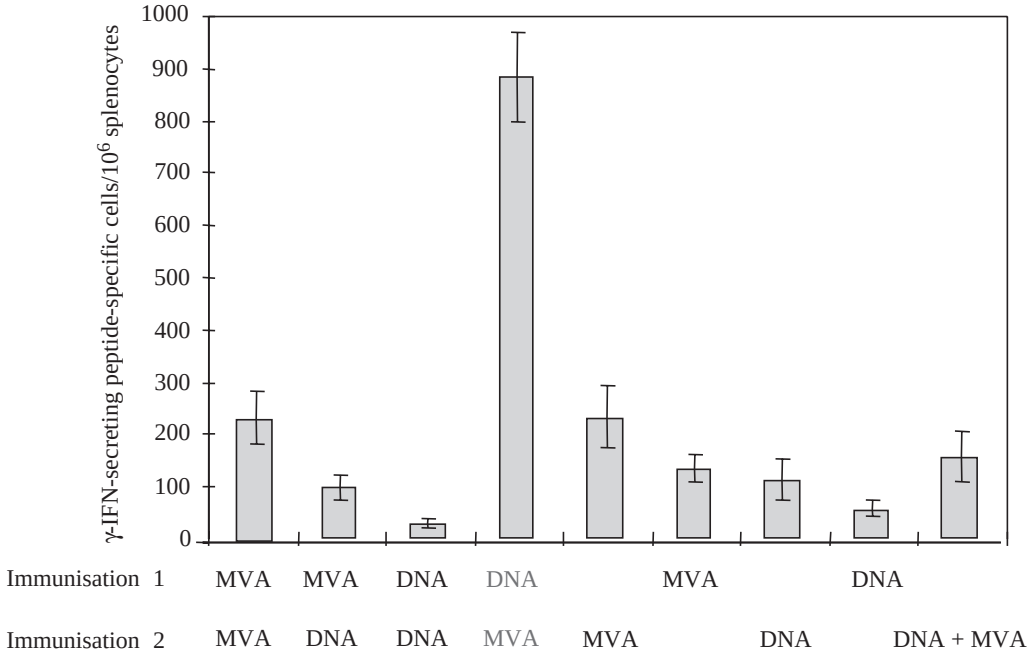

Fig. 1. Immunogenicity following different prime-boost immunization regimens. The CD8 T-cell response to a nonamer Kd-restricted epitope in the CS protein of *P. berghei* was measured by gamma-interferon ELISPOT assay of Balb/c mouse splenocytes after various immunisation regimens, shown on the *x*-axis. The interval between immunisations was 14 days. Each bar represents the mean number of spot forming cells per 10^6 splenocytes from three mice assayed individually. Reproduced with permission from Schneider et al. (1998).

These studies underlined the importance of immunisation order, as priming with the MVA vector and boosting with the DNA vaccine resulted in no improvement in immunogenicity or protection over MVA alone. The route of inoculation for the MVA vector was shown to be a factor, with intravenous and intradermal significantly better than intramuscular, subcutaneous or intraperitoneal delivery. Additionally the boosting ability of the two non-replicating vaccinia recombinant viruses MVA and NYVAC were compared the replication-competent WR (Western Reserve) strain, when delivered after the DNA vaccine. Interestingly, high levels of protection were only seen when boosting with the non-replicating poxviruses. Further murine studies in Oxford demonstrated enhanced immunogenicity and protection using Ty-virus-like particles followed by MVA boosting (Gilbert et al., 1997; Plebanski et al., 1998), and recently even better protection with recombinant adenovirus priming followed by MVA boosting (Gilbert et al., 2002). Importantly, the latter study demonstrated that a non-replicating adenovirus vector could boost efficiently as well as acting as a strong priming vaccine vector. The induced T-cell responses have been shown to persist to some extent. In the DNA-MVA prime-boost regimen in mice, protective efficacy against *P. berghei* fell from 100% at 14 days post MVA to 60% at day 150 (J. Schneider, personal communication). Durability of protection was characterised in more detail for prime-boost regimens in the *P. yoelii* model, with a drop in efficacy from 70–100% at 20 weeks to 30–40% at 28 weeks (Sedegah et al., 2002).

Additional vectors have also been evaluated. Studies at the New York School of Medicine revealed that non-replicating adenoviruses expressing CS can prime to induce complete protection against *P. yoelii*, when followed by a boosting recombinant vaccinia virus expressing the same protein (Bruna-Romero et al., 2001). As noted above, studies with *P. berghei* CS antigen demonstrated that such non-replicating adenoviruses can either prime or boost CD8+ T-cell responses (Gilbert et al., 2002). Recombinant FP9, an attenuated fowlpox, has also been developed in Oxford, and in murine studies this was also more immunogenic than DNA as a priming agent, with higher levels of induced T-cells and better protection against *P. berghei* challenge (R. J. Anderson, C. M. Hannen, S. G. Gilbert, S. M. Laidlaw, E. G. Sheu, S. Korten, R. Sinden, M. A. Skinner and A. V. S. Hill, submitted). Triple vector immunisation with sequential delivery of three heterologous vectors encoding the same antigen is an appealing next step but, perhaps surprisingly, there is no evidence from studies using triple combinations of DNA, MVA, FP9 and adenovirus for any improvement over the best regimens employing two vaccines (Gilbert et al., 2002).

Non-human primate studies

The difference in immunogenicity and efficacy between small animals and humans of many candidate vaccines for malaria and other diseases has underlined the potential importance of non-human primate studies in providing a better guide to what should be expected in clinical trials. DNA-prime and MVA-boost regimens were evaluated in chimpanzees as early as 1995–1996, using constructs expressing the *P. falciparum* TRAP antigen (Schneider et al., 2001). T-cell responses to TRAP were not detected after DNA priming, but MVA boosting induced high levels of T-cell and a significant antibody response, with one of two animals producing strong peptide-specific CTLs. However these responses did not confer protection against a heterologous *P. falciparum* intravenous challenge. Good immunogenicity and impressive protection of some animals against *P. knowelsi* challenge were observed in a study in rhesus macaques using multi-stage constructs

encoding two pre-erythrocytic antigens and two blood-stage antigens, with better protection obtained with a DNA/NYVAC regimen than a DNA/canarypox prime-boost regime (Rogers et al., 2001).

Experience with non-human primate models in other intracellular diseases is of potential relevance to malaria. The efficacy of synergistic heterologous prime-boost strategies against SIV (simian immunodeficiency virus) and SHIV (simian-human immunodeficiency virus) has been assessed in rhesus monkeys, as a model for HIV. Several studies have reported impressive boosting of CD4+ and CD8+ T-cell responses primed with DNA and boosted with recombinant poxviruses (Allen et al., 2000; Kent et al., 1998), and in one case survival from challenge 7 months after challenge with the highly virulent SHIV 89.6P virus (Amara et al., 2001). The observed protection appeared to be mediated by cellular immunity, as no neutralising antibodies were detected (Robinson et al., 1999). Promising data from studies of prime-boost immunisation in non-human primates have been generated for other diseases including hepatitis B (Pancholi et al., 2001) and Ebola (Sullivan et al., 2000).

Human clinical trials

Since 1999, a series of heterologous prime-boost vaccine trials have been underway in Oxford to assess the safety, immunogenicity and efficacy against sporozoite challenge of several combinations of vectors in healthy malaria-naïve volunteers. The initial vaccine candidates used were DNA and MVA constructs encoding a Multiple-Epitope ('ME') string and the entire PfT9/96 strain TRAP antigen. The polyepitope string includes 14 CD8+ T-cell epitopes from six pre-erythrocytic *P. falciparum* antigens, and six further B-cell or T-cell epitopes. The DNA vaccine was delivered at doses of up to 2 mg by intramuscular injection or 4 μg by intraepidermal 'gene gun'. The MVA vaccine was administered by intradermal injection at doses up to 15×10^7 p.f.u. (plaque-forming units). An excellent safety profile for the vaccines alone or sequentially has been seen in over 150 subjects in total (Moorthy et al., 2003).

Cellular immune responses were assessed chiefly by the summed *ex vivo* γ-IFN ELISPOT to overlapping peptide pools covering the entire vaccine insert (McConkey et al., 2003). Responses after repeated vaccination with DNA alone were small, but were markedly increased following boosting with MVA, with summed pools of over 1000 S.F.C. (spot forming cells) 10^{-6} PBMCs (peripheral blood mononuclear cells) in some cases using high doses (Fig. 2). These changes from baseline were highly significant (P=0.0006, adjusted for multiple comparisons). CD8+ and CD4+ responses were detected, mostly to TRAP antigen rather than the polyepitope string, and were detectable to all peptide pools tested. There was substantial cross-reactivity of the T-cell response to peptide pools from the heterologous Pf3D7 strain. Depletion studies suggested the responses were mainly CD4+ rather than CD8+, and antibody responses to the antigen insert were low or not detected. Stronger responses were seen when the interval between DNA and MVA was three weeks rather than eight weeks (P=0.026). Although the peak level of induced response dropped significantly from one to 4 weeks after the MVA vaccination, the elicited immune responses were persistent and were still detectable 5–11 months after the peak.

Vaccine efficacy was assessed using an adapted *P. falciparum* sporozoite challenge model (Chulay et al., 1986). The challenge was heterologous because the strain of parasite used for challenge (Pf3D7) differed from the strain of the vaccine TRAP antigen (PfT9/96) by approximately 6% of the TRAP amino acid sequence (Robson et al., 1990). Subjects immunised with DNA prime–MVA boost regimens showed a highly significant delay in time to blood-stage parasitaemia (thick film positive) compared to subjects receiving homologous regimens and unvaccinated control subjects (P=0.013). Although this does not represent sterile immunity, based on a presumed eightfold multiplication rate of blood-stage parasites in one 48 h cycle in human blood (Simpson et al., 2002), a 2 day delay to parasitaemia can be estimated to correspond to an approximately 87% reduction in parasites emerging from the liver. The best estimate of the mean reduction induced by DNA–MVA vaccination in this study was 78%. Such evidence of vaccine efficacy against the parasite is encouraging, given that this challenge model may deliver ten times more sporozoites than a natural mosquito bite in the field. Nevertheless this level of protection does not reach the levels of sterile immunity achieved by vaccination of malaria-naïve subjects with RTS,S/AS02A (Kester et al., 2001), where antibody-induced protection may be of most importance.

On the basis of the Oxford studies, Phase I trials have been undertaken in semi-immune adults in The Gambia (V. S. Moorthy, T. Imoukhuede, M. Pinder, W. H. H. Reece, K. Watkins, S. Atabani, C. Hannan, K. Bojang, K. P. W. J. McAdam, J. Schneider, S. C. Gilbert et al., submitted). The DNA and MVA vaccines have shown good safety profiles in this population. As expected from previous immunological studies in endemic areas, baseline levels of T-cell responses to the TRAP insert in these subjects were low; at approximately 25 S.F.C. 10^6 PBMCs. Prime-boost vaccination enhanced the immunogenicity to levels at least as high as achieved in the Oxford trials. A double-blind phase IIb study of 372 semi-immune Gambian adults receiving either DNA–DNA–MVA or rabies vaccine has been conducted successfully (V. S. Moorthy, T. Imoukhuede, M. Pinder, W. H. H. Reece, K. Watkins, S. Atabani, C. Hannan, K. Bojang, K. P. W. J. McAdam, J. Schneider, S. C. Gilbert et al., manuscript in preparation) very recently.

In 2001 clinical trials commenced in Oxford evaluating recombinant fowlpox (FP9) encoding the same 'ME-TRAP' insert as the DNA and MVA vaccines. Several combinations were assessed and challenged (D. Webster, S. Dunachie, J. Vuola, S. McConkey, I. Poulton, L. Andrews, R. Ebensen, T. Berthoud, S. Keating, P. Bejon, et al., manuscript submitted

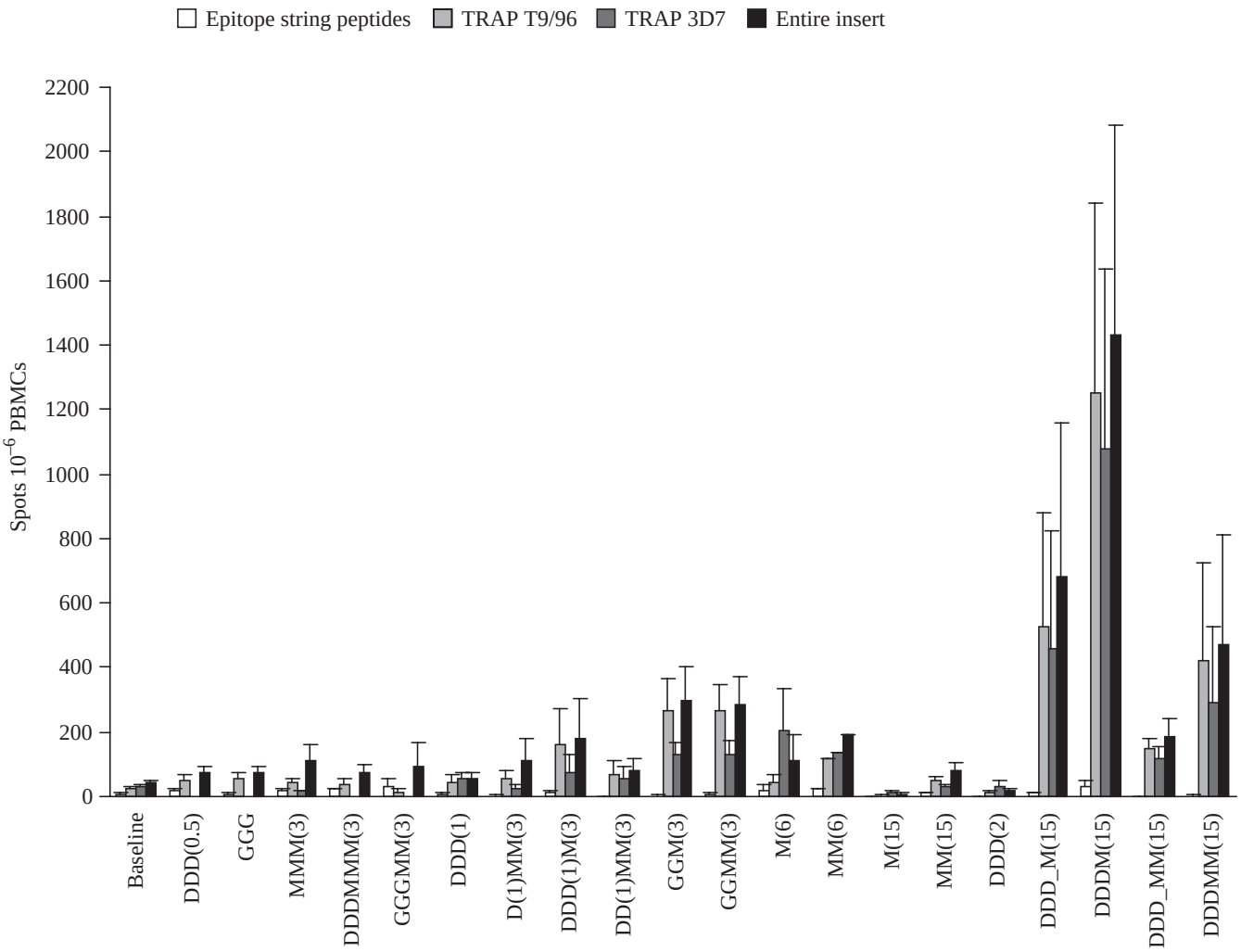

Fig. 2. T-cell responses as measured by γ-interferon ELISPOT to pools of peptides from the ME-TRAP malaria insert seven days after various DNA and/or MVA vaccination regimes. Summed net responses to pools of peptides from all of the vaccine insert, from the T9/96 strain of TRAP encoded in the vaccine and from TRAP from another *P. falciparum* strain, 3D7, are shown. The arithmetic mean of the responses for the subjects in that group are presented with an error bar to indicate the standard error of the mean. D, DNA-ME TRAP given by intramuscular injection into the deltoid muscle; G, DNA-ME TRAP given epidermally by needleless delivery device; M, MVA-ME TRAP given by intradermal injection. The numerals included in parentheses in the regimen names correspond to the dosage of vaccine, in mg for DNA and p.f.u.×10^7 for MVA. For example, the last column DDDMM(15) shows summed ELISPOT responses for subjects vaccinated with three priming doses of 2 mg DNA-ME TRAP i.m. followed by two boosting doses of MVA-ME TRAP at 1.5×10^8 p.f.u.

for publication). Further studies have commenced using MVA encoding the circumsporozoite protein (CS) and RTS,S/AS02A in prime-boost combinations, as well as trials to evaluate ICC-1132, a recombinant protein vaccine comprising of several CS epitopes fused to the Hepatitis B core antigen, produced by Apovia Inc. Ongoing work includes a collaboration with the US Navy employing DNA and MVA vaccines encoding CS, and prime-boost studies using FP9-CS.

Discussion

These preliminary clinical trials provide an encouraging start to efforts towards developing a malaria vaccine that induces protective T-cell responses. All the mechanisms contributing to the marked T-cell immunogenicity of heterologous prime-boosting have yet to be elucidated, but there are several likely contributors. Firstly the delivery of the epitope of choice in two different forms may facilitate focussing of the response on the desired foreign epitope(s), minimising distraction by the antigenicity of the delivering vector. Boosting with a different vector having only the relevant epitope in common with the priming immunisation may allow preferential expansion of pre-existing memory T-cells to the malaria epitope(s) of interest. Secondly, using a different vector to boost the initial response avoids the effects of anti-vector humoral and cellular immunity dampening down the response to further stimulation. The use of different antigen delivery systems may minimise host immune-evasion methods acquired by the vector. In general, it

may be easier to prime an immune response than amplify it because of a negative feedback effect of pre-existing cytolytic T-cells on antigen presenting T-cells that are attempting to boost a response. Hence, it may be that only powerfully immunogenic viral vectors, which may actually retain some evolved mechanisms for avoiding such control, can succeed in boosting. Recently, interesting evidence that there may an important role for T-cell crowding and competition around antigen-presenting cells has been provided (E. G. Sheu, R. J. Anderson, C. M. Hannan, J. T. Hu, S. C. Gilbert, J. Schneider and A. V. S. Hill, manuscript submitted for publication). Finally, presenting the epitope of choice in the context of different agents could amplify the host immune response by increased activation of non-specific and specific costimulatory responses by the vector, giving a favourable milieu for antigen presentation. These theories may help to explain why some simple agents like DNA make good primers, inducing a CD8+ T-cell response focussed on a few immunodominant epitopes, whilst boosting requires the 'danger signals' of a viral vector to amplify the response *via* rapid expansion of the primed antigen-specific memory T-cells.

Although prime-boost immunisations can induce strong CD8+ responses, in some cases depletion studies have revealed predominantly a CD4+ response. The precise pattern and levels of cellular immunity required for protection against malaria remain unknown. A major obstacle in malaria vaccine research is the identification of correlates of response to vaccination and protection against malaria. As a result of multiple prior episodes of parasitaemia, semi-immune people living in endemic areas show a repertoire of humoral and cell-mediated immune responses to malaria that varies between individuals. The opportunity to conduct malaria challenge studies in malaria-naïve subjects allows greater scrutiny of the relationships between vaccination, induced immunity and protection.

Heterologous prime-boost immunisation can be seen as the sequel to the widespread use of DNA vaccines in the 1990s. Intensive research continues to further improve both the priming and boosting components of this approach, for example by vector modification, and significant advances are likely. The sequencing of the *P. falciparum* genome heralds a new age in the identification of vaccine antigens, and combining pre-erythrocytic vaccines with blood-stage and transmission-blocking vaccines could amplify the progress to powerful efficacy. Some of the regimens currently under study may appear too complex for widespread use across Africa, requiring a range of vaccines delivered by a variety of routes at different times. However, given the enormity of the malaria problem internationally, and decades of thwarted efforts to find a vaccine, the current strategy is to obtain good efficacy first and then develop ways to simplify the regimen. Novel delivery devices allowing reliable intradermal delivery, and technology allowing controlled product release over a time period of weeks are under development, which could facilitate deployment of the regimens described in this review. Although most deployed vaccines prevent a significant majority from getting any disease, a vaccine protecting 50% of recipients for 6 months in children less than 5 years of age in Africa would be worthwhile given the high mortality from malaria. This is a realistic goal, but will require much greater international financial support than that currently available. Clearly the final product must be affordable to those who need it most, namely children in developing countries. Increased international collaboration in recent years has accelerated progress, and further advances in immunology and vaccine design are expected to contribute enormously over the next few years. There is therefore good reason to look forward to the next few years of malaria vaccine development with great anticipation, although the timeline is unpredictable.

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