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Prime-boost: the way forward for recombinant vaccines against apicomplexan parasites. A *Theileria* perspective

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Abstract A good $CD8^+$ response is required for immunity to many intracellular pathogens. Traditional antigen delivery using isolated sub-units or killed pathogens and adjuvant stimulates strong antibody responses but weak T-cell reactivity. Attenuated vaccines are usually more effective. The prime-boost delivery system based on immunisation with a naked DNA antigen–gene construct followed by boosting with an attenuated vaccinia virus expressing the same antigen is proving to be a powerful way to stimulate antigen-specific CD8 responses. Success using single epitopes is possible in malaria and we believe this approach holds much promise for other apicomplexan parasites such as *Theileria* spp.

Introduction

To date there is no useable subunit or recombinant vaccine against any protozoan parasite. However, the prospects for malaria, after two decades of molecular biological based research, are looking brighter (Good and Doolan 1999; Taylor-Robinson 2000). A central problem in vaccine development in general has been difficulty in generating high levels of antigen-specific T cells, particularly $CD8^+$ T lymphocytes, by immunization with purified antigens. Administration of soluble protein alone generally does not induce $CD8^+$ T cells, presumably because the antigen is not introduced into the MHC class I presentation pathway (Schneider et al. 1999). Furthermore, protein subunit vaccines in combination with a variety of adjuvants, such as alum or mineral oils, generally stimulate very high titre antibody responses, which will neutralise extracellular pathogens but have little effect on intracellular pathogens (Bowersock and Martin 1999; Makela 2000). Many pathogens, notably apicomplexans, are intracellular for large periods in their mammalian host and the key effectors against these are often $CD8^+$ T cells (McKeever et al. 1994; Abrahamsen 1998; Khan et al. 1999; Preston et al. 1999; Doolan and Hoffman 2000; Taylor-Robinson 2000).

It is now becoming possible to generate potent T-cell responses using novel routes of antigen delivery. One of the main strategies bearing fruit is the prime-boost technique and, in particular, one permutation of this approach which utilises naked DNA and vaccinia virus as vehicles for antigen delivery (Hanke et al. 1998; Schneider et al. 1998).

The prime-boost regimen

The prime-boost regimen depends on priming the immune system with a particular antigen via a naked DNA construct and subsequent boosting with an alternative antigen-delivery system, such as recombinant pox virus vectors.

A naked DNA vaccine consists of an expression plasmid containing a protective antigen gene linked to a mammalian promoter (Donnelly et al. 1997; Hasan et al. 1999). It induces persistent cell-mediated and humoral immune responses to the antigen, depending on the route of administration and the nature of the antigen. The route of inoculation can affect the efficacy of the response, although success has been achieved with all the main routes (Haensler et al. (1999). Furthermore, it has recently been demonstrated that intradermal biolistic delivery of the DNA can allow a ten-fold reduction in the optimal dose (Dégano et al. 1999).

This type of vaccine has been shown to provoke protective immune responses to a variety of pathogens, but there are instances where it simply fails to work (Schneider et al. 1998). In some cases, this failure can be

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overcome by boosting with vaccinia virus recombinants encoding the same antigen (Hanke et al. 1998; Schneider et al. 1998). Vaccinia virus stimulates protective cellular immune responses in its own right (Paoletti 1996; Sutter and Moss 1992). An important point to note is that live recombinant vaccinia vectors (and other pox vectors) that can replicate in vivo are unlikely to be sufficiently safe for widespread use as prophylactic vaccines in domestic livestock. Therefore, highly attenuated and replication-deficient vaccinia virus vectors, such as the modified vaccinia strain Ankara (MVA), have been developed as delivery vectors for heterologous antigens. MVA has been attenuated by multiple serial passages, which has resulted in the loss of 31 kb of DNA, including host range genes and genes encoding cytokine receptors, and has been safely used in the Smallpox Eradication Programme (Sutter and Moss 1992).

A combination of a priming dose of antigen in the form of a naked DNA construct followed by a booster in the form of a vaccinia recombinant has been shown to be superbly effective against *Plasmodium berghei* in mice (Schneider et al. 1998; Sedegah et al. 1998). In the former study, the vaccine was based on the well-characterised CSP and TRAP antigens. Indeed, protection was achieved in mice (C57BL/6) strains which were previously unable to respond to other routes and which usually succumb to the malaria. In addition, protection was also possible using a single T-cell epitope (Schneider et al. 1998). Enzyme-linked immunospot (ELISPOT) analysis revealed a very high frequency of $CD8^+$ in the circulation in these immune animals. It is thought that stimulation of the $CD8^+$ T-cell response is a result of the viral vector and nucleic acid vaccines being presented to MHC class I via both the endogenous and the exogenous processing pathways.

One crucial factor in achieving success is the order in which the immunisation is performed. Thus, success against *P berghei* was only achieved when the naked DNA was used as the priming dose and vaccinia was used as the boost. DNA:DNA, vaccinia:vaccinia or vaccinia:DNA were not effective. The same phenomenon was observed when immunising with recombinant Ty particles and MVA containing a single *P. berghei* epitope (Plebanski et al. 1998). Ty followed by MVA elicited 95% protection against malaria in mice, whereas the reciprocal sequence (MVA-Ty) or homologous boosting was not protective. The explanation for this directionality is not clear, although various hypotheses have been put forward. It is suggested that the DNA prime, being relatively antigenically simple, focuses the response on the relevant epitope. In addition, the relatively low, but persistent, expression of the immunogenic proteins in vivo serves to provide prolonged immune stimulation and the possible induction of high-affinity T cells. The antigenically complex, but highly immunogenic, vaccinia virus then induces a strong antigen-specific secondary response, amplifying the primary response substantially. Presenting vaccinia first results in an unfocused polyclonal primary response, which confounds specific boosting (Schneider et al. 1999).

The prime boost regimen results in a greatly enhanced $CD8^+$ responses, with up to ten times as many $CD8^+$ cells being detected when compared with homologous prime-boost schedules (Schneider et al. 1999). In addition, MVA has been shown to elicit better boosting protection than other viruses (Irvine et al. 1997; Schneider et al. 1999). One possible explanation for the superior boosting effect of vaccinia virus may be the ability to infect professional antigen-presenting cells (APCs) including dendritic cells. It has been shown that, in response to MVA infection, fibroblasts secrete type 1 interferon (Blanchard et al. 1998). Type 1 interferons have been suggested in other studies to provide a protective signal that promotes long-term survival of $CD8^+$ T cells (Tough et al. 1996).

The *Theileria* perspective

Much of the protective immunity against the bovine *Theileria*s is cell-mediated and directed to the schizont-infected cell. Evidence for this in *T. parva* has been accrued and the most incisive data is the passive transfer analysis performed by McKeever (McKeever et al. 1994; Boulter and Hall 2000). For *T. annulata* such positive transfer data are not available, but cell-mediated responses to the schizont-infected cell are generally thought to be responsible for protective immunity. It is possible to vaccinate against *T. annulata* with an attenuated schizont-infected cell line and since this anti-schizont immunity cannot be transferred by serum, then by deduction the response is cell-mediated (reviewed in Boulter and Hall 2000). In addition, for *T. annulata,* a wealth of evidence suggests a crucial role for the macrophage in the longer term (Preston et al. 1999). However, since this macrophage activity could be underpinned by interferon-gamma secreting T cells, then the $CD8^+$ cell may be crucial in both parasites. Thus, the naked DNA–vaccinia prime-boost system would appear to be ideally suited to vaccine delivery with respect to bovine *Theileria* spp.

The main problem in the search for a *Theileria* subunit vaccine is then shifted to the nature of the candidate antigens. For *T. annulata,* we have previously undertaken a number of vaccination trials in cattle, utilising the major sporozoite-surface antigen (SPAG-1) and the merozoite antigens TAMS 1–1 and TAMS 1–2 (Boulter and Hall 2000). As both these antigens are extracellular, we have previously concentrated on vaccine-delivery systems which induce potent humoral responses. The results of these trials, whilst not astounding, are promising, with partial protection to sporozoite challenge being achieved. Similar results have been acquired upon immunisation with p67, the SPAG-1 homologue of *T. parva*.

Until now, however, we have lacked suitable schizont antigens for inclusion in our vaccination campaigns.

A new class of *T. annulata* schizont-derived, host-located molecules, with appropriate signal sequences, are currently being defined (Swan et al. 1999). Six of these are being included in naked DNA and MVA vectors with the aim of vaccinating cattle in the near future. Similarly, trials have just begun utilising the prime-boost system for various *T. parva* antigens, and the results are expected soon (Gilbert et al. personal communication).

The genome-sequencing projects for *T. parva* and *T. annulata* currently being undertaken will allow the complete portfolio of potentially protective antigens to be documented and eventually tested. With the emergent technologies allowing us to focus the immune system in constructive ways, and with our ability to induce high levels of antigen-driven $CD8^+$ T cells, we expect that marked enhancement of the potency of many vaccines will result.

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