

## Editorials

### Designing a malaria vaccine

Considerable progress has been made in the 20 years since the first malaria vaccine trials were conducted in establishing the feasibility of and capacity for vaccine development. Nevertheless, there is still not a single vaccine that is likely to be introduced into control programmes in the foreseeable future. The reasons are many and complex. Forty per cent of the world's population lives at risk from malaria but it is not a uniform risk. The heartland of malaria is Africa south of the Sahara where 90% of the 500 million clinical cases and most of the 2-3 million deaths each year occur. This does not mean that the burden of disease elsewhere in the world is not severe; the seemingly inexorable spread of drug resistance is nowhere more advanced than in parts of southeast Asia. The main aim of vaccination is to eliminate or lessen the occurrence of clinical disease. Because of the complexity of the life cycle of *Plasmodium* spp, there are several different ways in which this can be attempted, and most of them are under extensive study.<sup>1</sup>

#### Sporozoite invasion and intrahepatic development

Infection occurs when an infected mosquito injects sporozoites that circulate briefly in the blood but must then penetrate liver parenchyma cells. Considerable attention has been focused on the sporozoites as vaccine targets because when attenuated by radiation they provide strong protection against subsequent sporozoite induced challenge. The immunogenic circumsporozoite protein (CSP) on the sporozoite surface contains a long series of tandem repeats of amino acids. In *Plasmodium falciparum* this consists of about 40 copies of the sequence asparagine-alanine-asparagine-proline (NANP). Natural antibody responses are mainly directed towards this region, and a series of peptide and recombinant forms of it have been tested in clinical trials with a variety of different adjuvants, but without great success, despite the induction of high titres of anti-NANP antibodies.<sup>2</sup>

This prompted a more detailed examination of the effector mechanisms induced by the attenuated sporozoites and to the developing intrahepatic forms. Experimentally, cytotoxic T cell production and a range of other cellular responses, some facilitated by interferon (IFN) $\gamma$  production, were shown in addition to antibody dependent parasite killing. Subsequent vaccine design incorporated T helper and cytotoxic T cell epitopes from regions of the CSP flanking the tandem repeats, T cell epitopes from other antigens, and a wide range of expression systems. Some of these vaccine candidates have shown enhanced protective efficacy in model infections but cannot yet be considered for clinical application.<sup>3,4</sup> However, a CSP recombinant vaccine produced in yeast and consisting of the tandem repeat region and C-terminal T cell epitopes of the malarial antigen fused to the hepatitis B surface antigen is highly immunogenic. Experimentally, the particulate product induced strong antibody, cytotoxic T cell, and interleukin-2 and IFN $\gamma$  responses. Seven volunteers were given three doses of the vaccine together with a potent adjuvant consisting of oil in water emulsion, monophos-

phoryl lipid A and Quillaia saponin; six of the volunteers were completely protected against subsequent challenge with the homologous parasite.<sup>5</sup> The numbers are small but the results are encouraging and a phase I/II field trial is to be performed in African adults. The success or failure of the field trials, in the face of heterologous strain challenge, may depend on whether protection is mediated by the response to the conserved tandem repeats or the polymorphic T cell epitopes.

The one phase of the malaria life cycle at which a cytotoxic T cell response could be effective is during intrahepatic development and, while its feasibility as a vaccine strategy has yet to be established, it is being investigated with in vitro presentation of epitopes from selected liver stage antigens.<sup>6</sup>

#### Blood stage vaccines

The products of development in the liver, the merozoites, invade erythrocytes. Here, further asexual cycles of multiplication and merozoite release occur every two or three days and these are responsible for the pathogenic effects that cause clinical disease and, in *P falciparum*, death. In principle, there are various ways in which vaccination could be used to modulate this phase of the life cycle. The surface of the infected erythrocyte is greatly modified, and expressed parasite antigens can be targeted to achieve antibody-mediated removal of the affected red blood cells. *P falciparum* blood stage trophozoites and schizonts cytoadhere to endothelial cells as a natural part of their development; blocking this ability to bind would enhance parasite clearance; however, the *P falciparum* erythrocyte membrane protein 1 (Pf EMP 1) molecules that mediate binding constitute a large family of proteins characterised by their great variability.<sup>7</sup> It is a feature of these blood stages that antigens are polymorphic, and the parasite has the capacity for extensive antigenic variation.

Most attention at this stage of infection has focused on molecules that are involved in initial invasion of erythrocytes by merozoites. Two of these, apical merozoite antigen 1 (AMA 1) and merozoite surface protein 2 (MSP 2), plus a third antigen (Pfl55/RESA), are currently in a clinical vaccine trial. A further antigen, MSP 1, is considered by some to be the best vaccine candidate for invasion inhibition. It is cleaved into fragments during merozoite formation; the C-terminal portion remains on the surface of the penetrating merozoite, and antibody responses to it have been shown to correlate with protective immunity.<sup>8</sup>

An MSP 1 N-terminal sequence is one of the four components of the polymerised synthetic peptide SPf 66, the one *P falciparum* candidate vaccine to have been tested extensively in clinical trials. The other components are NANP repeats from the circumsporozoite protein, and sequence from two blood stage antigens, Pf35 and Pf55, about which little is known. The vaccine gave 30-35% protection against clinical malaria in trials in South America, where transmission is low, and in 1-5 year old children in Tanzania where transmission is intense.<sup>9</sup> By contrast, no protection was demonstrable when infants

under 1 year old were vaccinated in The Gambia<sup>10</sup> or in 1–15 year olds in Thailand.<sup>11</sup> A further trial in infants is underway in Tanzania as this is the age group in Africa most at risk. While the concept of combining epitopes from different antigens and stages is a good one, this particular peptide is unlikely to be used outside South America, and even here will probably be superseded.

### Multiple antigen-epitope-gene approach

The multiple antigen-epitope-gene approach to malaria vaccine development is widely perceived as the only way to achieve good antiparasitic and disease immunity. An important element of such an approach is likely to be an immune response to the sexual cycle that begins with the male and female gametocytes produced in the blood and is completed within the feeding mosquito. The acquired sexual stage immunity prevents mosquitoes becoming infected and, in model infections, it is possible to induce a very strong transmission blocking immunity with sexual stage antigens expressed by gametes or by post-fertilisation ookinetes. One small safety and immunogenicity phase I clinical trial has been done so far with a recombinant form of Pfs 25, the dominant post-fertilisation surface antigen of *P. falciparum*. Such a vaccine could be used alone in some low endemic situations or in combination with other vaccines or other control measures, when it would serve particularly to reduce the transmission of vaccine or drug resistant mutants.<sup>12</sup>

Seven *P. falciparum* antigens, three pre-erythrocytic, three asexual blood stage, and one sexual stage have been expressed simultaneously in recombinant form in an attenuated vaccinia virus called NYVAC 7; the first clinical trial with it has been done. DNA vaccines giving expression of CSP and other pre-erythrocytic stage antigens, which have given promising results in animal studies, will also soon be tested clinically.<sup>13</sup>

What can be expected in the next five to 10 years? A fairly safe prediction is that no vaccines will go into control programmes, although the need for these increases

progressively. However, alongside the continuing basic research there are now organised attempts to develop what we have in concert with the pharmaceutical industry and to prepare field trial centres. The absolute requirement is for long and sustained investment.

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## Is proficiency testing in cervical cytology proficient?

The proficiency test in cervical cytology may not be proficient, whether aimed at assessing individuals or laboratories as a whole, according to arguments from Dr C J R Stewart in a recent issue.<sup>1</sup> Stewart's article follows a recent editorial on the same subject in *Cytopathology*<sup>2</sup> and comes at a time when the draft guidelines for the NHS cervical screening programme (NHSCSP) are being revised for proficiency testing. Stewart's views, and those of Slater,<sup>2</sup> reflect widespread scepticism about the way proficiency testing is carried out in the UK. Indeed, the British Society for Clinical Cytology and the Royal College of Pathologists had rejected the original draft guidelines, although supporting the need for some form of external quality assessment.

In an article referred to by Stewart, Valente says candidly that one of the goals of proficiency testing is to "weed out the incompetent", although he points out that there is no evidence that proficiency testing improves laboratory performance.<sup>3</sup> He also says that "common sense would

indicate that the recognition of accepted diagnostic criteria is a valid measure of competence" and that "we must not lose sight of the education role of proficiency testing".<sup>3</sup> Stewart suggests that internal quality control, accreditation, and comparison of performance and outcome might equally or even better be able to identify poor performance. Is an external assessment needed as well?

Stewart must be justified in saying that there is no evidence that mortality from cervical cancer is affected by proficiency testing. Laboratory performance would have to be uniformly substandard for a long time to be reflected in mortality, which is difficult to compare in small populations. More to the point, he says that proficiency does not reflect laboratory false negative rates. Sensitivity of primary screening is proportional to the number of abnormalities known to be present<sup>4</sup> and would be expected to be high in a set of 10 slides almost half of which were known to be abnormal. Sensitivity of primary screening can be monitored by re-screening negative slides, but ultimately



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