Selecting Candidate Antigens for Malaria Vaccines*

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The prime targets for development of vaccines against human malaria are the extracellular forms of the parasite, namely the sporozoite, the merozoite and the gamete, which come into contact with the immune system. In addition the intraerythrocytic schizont is also a potential target, in so far as parasite determined antigens appear on the surface of the infected erythrocyte.

The necessity to develop pure antigen vaccines or to synthesise antigenic peptides has long been seen as essential to overcome the difficulties intrinsic to the nature of malarial parasites, their intimate association with human host tissues and the difficulties of growing large numbers of parasites in culture.[1] By virtue of the success with culture,[2] *Plasmodium falciparum* has been the species of initial attention, although *P. vivax* merits similar interest when it becomes technologically approachable.

The immediate objective is to identify, purify and produce the antigens most appropriate as vaccine candidates. An important constraint is the limited scope of animal models and the limited supply of those monkeys which provide the best available animal model. With these considerations in mind, it is essential to devise strategies for the characterisation of parasite antigens that will give the most complete information preliminary to animal trials.

The cloning and expression in *E. coli* of the sporozoite coat antigen of *P. knowlesi*[3] has given confidence that fairly standard gene cloning routes should be open for the production of at least some malaria protein antigens. The sporozoite is unusual in that this antigen forms most of the coat of this parasite stage and thus the corresponding antigen from *P. falciparum* will be a major vaccine candidate when it has been successfully produced in quantity. Some uncertainty concerning the use of a sporozoite vaccine alone must be entertained by virtue of the ability of even a small number of sporozoites which escape immune surveillance to establish an infection in the host.

From the viewpoint of human populations, gamete antigens are also attractive vaccine candidates, since gametocytes are exposed for sufficient time to the immune system and their inactivation would eliminate transmission by the vector. The ability to induce the formation of large numbers of gametocytes in culture[4] has led to important studies on their surface antigens and the search for inhibitory antibodies which recognise them, so to facilitate the necessary molecular analysis.

In terms of the clinical disease the asexual blood stages are of prime interest, although for a number of reasons they present some difficulties in terms of exploitation of their antigens as vaccine candidates. During much of the erythrocytic cycle the parasite is protected from immune attack by the erythrocyte membrane. However, trophozoites and schizonts synthesise proteins that are associated with the erythrocyte membrane[5,6] and these antigens may have important functions such as attachment to capillary surfaces that render them important potential candidates for use in immunoprophylaxis. Particularly attractive, however, are antigens exposed on the surface of the merozoite or at some stage during the invasion process, since immunological inhibition of this event will lead to substantial lessening of the clinical disease.

At this stage of our knowledge it would seem likely that candidate vaccines will need to be cocktails of antigens or antigenic peptides from

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all or most of these stages. The asexual blood stages present particularly difficult problems which are discussed further below.

Recognition of important blood stage antigens

Two approaches have been used to identify malaria antigens capable of eliciting a protective response. One is to compare the antigens recognised by antibodies in sera from individuals exposed to malaria, and correlate particular antigens so recognised with the degree of protective immunity exhibited by the sera. This approach has led to the identification of antigens with molecular weights of 96 kDa[7] and 200, 137 and 115 kDa[8] in *P. falciparum*. However, the number of antigens recognised by immune and nonimmune sera as judged by immunoprecipitation is considerable[9] and a great variability has been noted by many investigators.

A second approach is to use hybridoma technology to produce murine monoclonal antibodies – or human monoclonal antibodies – which inhibit the growth of malaria *in vitro*, then to identify the corresponding antigen. This approach has led to the production of inhibitory antibodies directed against the sporozoites of several species;[10] the blood froms of *P. yoellii*,[11] *P. knowlesi*[12] and *P. falciparum*[13,14] and from gametes of *P. gallinaceum*.[15] A number of other *P. falciparum* antigens, recognised by monoclonal antibodies have been described[16,17] but the ability of these monoclonal antibodies to inhibit parasite growth has not been reported.

Holder and Freeman[18] used a single antigen of *P. yoellii* identified by monoclonal antibodies as a vaccine in mice to elicit immunity and have attempted to draw parallels between this antigen and one from *P. falciparum*[16] although the latter antigen is not known to elicit protective antibodies.

Theoretically blood stage antigens to be considered as potential candidates for vaccine development could have one or more of the following characteristics: location on the schizont or merozoite surface, recognition by antibodies that block invasion in culture, recognition by antibodies that interfere with intraerythrocytic development, location on the surface of infected erythrocytes, association clinically with protection, or other special properties.

Inhibitory monoclonal antibodies

Possibly the most compelling information that can be derived from experiments using cultured parasites is that associated with antibodies which inhibit a specific function, such as invasion, growth or attachment to capillary surfaces. We have developed semi-automated assays to assess the initial invasion step separately from intraerythrocytic growth,[19,20] using purified schizont inputs.[21] These assays allow the screening of large numbers of monoclonal antibodies for their functional effects.

Thus, monoclonal antibodies have been produced against a *P. falciparum* schizont antigen doublet, Mr 220,000, that inhibit invasion by merozoites.[14,21] At a concentration of 1 mg/ml invasion is inhibited by nearly 80%, which is remarkable for an antibody which presumably recognises a single epitope. In collaboration with R. Anders, we have demonstrated that this antigen is indeed an S antigen of the type originally described by Wilson *et al.*[22] More recently we have examined a much larger number of monoclonal antibodies directed against *P. falciparum* and have found several more to exhibit extensive inhibition of invasion. Preliminary characterisation indicates that these antibodies recognise antigens other than the S antigen. These antibodies, and those reported by Perrin *et al*,[13] appear to be the main inhibitory ones so far available but their properties give rise to confidence that following detailed structural characterisation of the corresponding antigens the latter must be ranked as vaccine candidates at this stage.

Conceivably either murine or human monoclonal antibodies could be assessed for passive protection in the host as a prelimianry indication of the possible usefulness of the corresponding antigens as candidate vaccines.

Structural assessment of candidate antigens

There are two major approaches to the next stage, namely the production of parasite antigens in sufficient quantities to use for vaccination trials. One is to identify expressed antigens in cDNA libraries. The availability of a good monoclonal antibody made this possible for the *P. knowlesi* sporozoite surface antigen.[3] Here also, a repetitive sequence facilitated recognition of antigenic determinants in a composite protein product. More random approaches[23] have been used for the cloning of schizont/merozoite antigen cDNA followed by screening with immune sera. This system is promising but requires improvement to reduce breakdown of protein products. Specific probes are needed and in this context our large library of anti-parasite monoclonal antibodies is being used to screen cDNA libraries for this purpose.

The second approach is to utilise more directly the information available from the interaction between inhibitory monoclonal antibodies and their corresponding antigens. If inhibitory monoclonal antibodies recognise antigenic determinants that can induce protection *in vivo* – a notion that can be tested by comparison with immune sera – then these determinants are themselves candidates for vaccine development. Protein antigens recognised by monoclonal antibodies can be purified by affinity chromatography using these antibodies bound to a solid support. Fragments of these protein antigens can

be produced by standard enzymatic or chemical procedures and amino acid sequences determined in the relevant region. These in turn can be used to build synthetic oligonucleotide probes for searching through genomic DNA libraries or for cloning specific messenger RNA species.

Ultimately chemically synthesised peptide determinants can be explored as candidate vaccines, such as have been developed for foot-and-mouth disease virus.[24] In this case cloning technology is used as an intermediate step. Generalised rules have been formulated which allow a reasonable prediction to be made from the DNA sequence of the synthetic peptides most likely to give rise to a suitable antigenic response.[25]

Antigenic variation

An important shadow hangs over the enthusiastic efforts to select candidate antigens for malaria vaccines. This is the matter of antigenic variation. Brown and Brown[26] demonstrated conclusively for the first time intra-strain antigenic variation in *P. knowlesi*. More detailed analyses of this phenomenon have been reported recently by Howard *et al.*[27]

Monoclonal antibodies provide useful tools to assess the diversity of malaria parasite antigens. Studies by McBride *et al*[28] and Schofield *et al*[14] have shown that great diversity does indeed exist for some antigens among established parasite lines from different regions of the world. In the latter study, variant antigens were shown to be recognised by inhibitory antibodies, suggesting that some antigens putatively associated with protective immunity can exhibit variation. This is perhaps not so surprising, since evolutionary pressures would be expected to generate variation mechanisms for evasion of the host immune response in just those antigens of importance to the parasite in the invasion process. Hommel and co-workers[29] have also reported variation of surface antigens in infected erythrocytes.

Thus, a most important component of the process of selecting candidate antigens or antigenic peptides for vaccine development is extensive testing for variance or otherwise. While it is difficult in *P. falciparum* to assay intra-strain variation, it is feasible to screen for geographic variation of epitopes in primary isolates using monoclonal antibodies. Recent studies by Schofield in Papua New Guinea (unpublished data) have demonstrated both variant and apparently invariant epitopes by this means. Such studies need to be extended to the global scale for each inhibitory monoclonal antibody to allow the selection of invariant antigens or epitopes.

Conclusion

The approaches available at present for the selection of candidate antigens for malaria vaccines are potentially fruitful. Indeed, several such antigens are currently being investigated in depth. Using inhibitory monoclonal antibodies it is possible to identify antigens that are putative inducers of protective immunity. This applies equally to sporozoites, schizonts/merozoites and gametes of *P. falciparum*. At this point both DNA cloning technology and synthetic peptide technology are applicable to the production of antigens for vaccine trials. A major constraint is the extent of antigenic variation in *P. falciparum*, necessitating a search for invariant antigens recognised by inhibitory antibodies.

Summary

The quest for vaccines against malaria parasites presents a number of challenges. Sporozoites, merozoites and gametes are all legitimate targets. Because of the complexity of the parasite, peptide antigens rather than whole parasites are seen as the appropriate vaccine candidates. Both recombinant DNA cloning techniques and peptide synthesis can be used to produce suitable peptides. Several monoclonal antibodies produced against shizonts of *Plasmodium falciparum* that recognise specific peptide antigens have been shown to inhibit invasion of merozoites in culture. These antigens are candidates for vaccine development. A major problem has been identified in the form of antigenic diversity/variation. Selection of candidate vaccine antigens/epitopes require the recognition of those which exhibit minimal variation in structure.

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