Progress towards a Malaria Vaccine

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The failure of conventional prophylactic measures to control malaria in tropical and sub-tropical areas of the developing world has focused attention upon the great benefits that would accrue from the availability of effective malarial vaccines. However, some features of malarial immunity, as it develops in its natural hosts including man, have long cast doubts upon the plausibility of such a goal. Thus, in areas of sustained malaria transmission, clinical immunity develops slowly over perhaps a decade of repeated exposure to infection. In addition, the immunity achieved is incomplete in the sense that low levels of parasitaemia are seen intermit-tently in subjects who are clinically immune. Such observations have suggested that malaria parasites may be antigenically diverse so that clinical immunity prevails only after exposure to a wide spectrum of antigenic variants. A high degree of variation within a given plasmodial species could seriously jeopardise attempts to develop malaria vaccines.

However, certain features of acquired malarial immunity do encourage the hope of vaccine development. For example, immunoglobulin from immune African adults effectively clears parasitaemia from infected infants, including those resident in geographically remote areas. In addition, specific immunoglobulins can be shown to prevent, in vitro, the entry of sporozoites into liver cells and merozoites into red cells. As anticipated from such observations, both sporozoites and merozoites, appropriately inactivated, have provided the basis for stage specific vaccination against various species of experimental malaria, including Plasmodium falciparum, the cause of malignant tertian malaria in man. But the large-scale production and standardisation of such vaccines is not practical. These difficulties have been circumvented by two major recent technological developments which have facilitated the production of vaccines comprising individual antigenic constituents of malaria parasites. These are hybridoma technology for production of monoclonal antibodies, and gene cloning which permits the detailed characterisation and in vitro production of specific plasmodial polypeptide antigens with potential for inducing immunity to natural infection. These advances have revolutionised prospects for practical vaccine development.

The Malaria Cycle

More than 100 plasmodial species have been described, which cause malaria in a wide range of vertebrates and exhibit narrowly defined host specificity, only four species being naturally infective for man. Infection is initiated by the bite of a female anopheles mosquito which inoculates saliva containing sporozoites. These motile organisms, 11-12 μm long, are rapidly cleared from the circulation and initiate the tissue stage of primary exo-erythrocytic (ee) development, which in mammalian malarias is confined to liver parenchymal cells. Exo-erythrocytic forms become multinucleate and within about 10 days they rupture to liberate 100-30,000 ee merozoites. In some species, including P. vivax in man, ee forms may remain latent for several months before maturation. Blood infection is initiated by merozoite invasion of red cells. This involves a sequence of random adherence to the erythrocyte, attachment of the apical prominence of the merozoite to a specific red cell receptor and induction of endocytosis by the red cell. Intracellular growth proceeds through stages of ring, trophozoite and schizont and terminates with rupture of infected cells at 24-72 hour intervals, liberating 10-30 merozoites which initiate a further cycle of development. The clinical signs and symptoms of malaria are associated exclusively with cyclical blood stage development.

After a period of purely asexual multiplication of erythrocytic parasites, a proportion of newly invaded merozoites differentiate into male and female gametocytes. These mature without undergoing cell division over a period of about 10 days. When ingested into the mosquito stomach, the male (microgamocyte) undergoes exflagellation, liberating eight microgametes, and fertilisation occurs. The zygote differentiates in the wall of the mosquito gut and sporozoites finally accumulate in the insect salivary glands to complete the sexual cycle of development. Current attempts at prophylactic vaccine development are concentrated on two separate stages of the parasite, which induce stage-specific immunity. These comprise the sporozoite and the blood-stage merozoite.

Sporozoite Vaccines

Natural sporozoite infection does not lead to acquired immunity in man or any experimental animal. However, the inoculation of sporozoites attenuated by irradiation does induce effective immunity in experimental rodent and human malarials; this protection is strictly stage specific and without effect upon the blood-stage of infection. Sporozoites for vaccine preparation can be isolated
from salivary glands of infected mosquitoes by laborious dissection but a practical vaccine based on such material cannot be envisaged. The induction of stage-specific clinical immunity by attenuated sporozoites is nevertheless of considerable interest and has stimulated attempts to identify protective sporozoite antigens.

Monoclonal antibodies secreted by hybridoma cell lines ‘recognise’ an immunodominant surface antigen, the circumsporozoite (CS) antigen, which occurs in distinct but analogous forms in several species of plasmodia, including the human parasites P. falciparum and P. malariae. The protective nature of the antigen is indicated by the fact that specific monoclonal antibodies directed against it are able to neutralise sporozoite infectivity both in vitro and in vivo.

The molecular structure of CS antigens has been established in two species by cloning the genes which control their synthesis. In the case of P. falciparum, the entire gene was isolated from a genomic DNA library and shown to encode a protein, of 412 amino acids, which includes an unusual central region consisting of 41 tandem repeats of a tetrapeptide that constitutes the immunodominant portion of the molecule. The simian parasite, P. knowlesi, has a CS antigen of analogous structure (Fig. 1) with a central segment of repeat sequences of a dodecapeptide. This peptide has been synthesised and, after attachment to a carrier protein, used to immunise. Although the vaccinated animals produced antibody reactive with the CS protein, there are no reports of induced protection against sporozoite challenge, probably because an essential component of immunity against exo-erythrocytic stages of malaria may be cell-mediated and directed against parasites developing in hepatic parenchymal cells (Fig. 2). Thus most mice rendered B-cell deficient by repeated injection of anti-IgM serum can be successfully vaccinated with irradiated sporozoites; the failure of these immunised animals to produce measurable levels of specific antibody indicates that cell-mediated effector mechanisms have a role in anti-sporozoite immunity. Effective vaccination may therefore require both the CS antigen to induce antibody which limits the number of sporozoites entering the liver, and other as yet unidentified antigen(s) which induce cell-mediated immunity against the developing hepatic forms of the parasite.

**Blood-Stage Antigens**

As the clinical manifestations of malaria are associated exclusively with the blood-stage of parasite development, this stage is a prime target for any malaria vaccine. Immunity to blood-stage infection does develop naturally after long and repeated exposure to infection and has been actively induced in experimental malarias by using isolated erythrocytic merozoites as vaccines. The development of more practical and refined vaccines is compli-
cated by the considerable antigenic complexity of the erythrocytic parasite and the apparent absence of any immunodominant surface structure as seen in sporozoites. In addition, there is considerable antigenic diversity of blood-stage plasmodia within a given species.

The phenomenon of antigenic diversity can be illustrated by the properties of a component found on the surface of schizont infected red cells in several species of malaria. This antigen, recognised by monoclonal antibodies, is synthesised late in the cycle of asexual blood-stage development as a high molecular weight protein (about 200 kD) and is localised on the surface of schizont infected erythrocytes. At the time of rupture of the parasitised red cell the protein is cleaved to a series of smaller peptides present on the surface of newly released merozoites. The protective role of this antigen is shown by the fact that monoclonal antibodies directed against it inhibit parasite multiplication in vitro and in vivo; in addition, vaccination with the antigen confers significant immunity in rodent malaria. Analysis of the antigen in different isolates of *P. falciparum* reveals that it contains a region of invariant structure while other portions of the molecule vary in different parasite strains. It is the variable part of the molecule which seems to be expressed on the red cell surface and it is this heterogeneity which seems likely to limit the value of the antigen as a potential vaccine component.

Fortunately, other protective antigens of blood-stage parasites appear to have an invariant structure in all isolates of a given species. One such antigen (150 kD molecular weight) appears to be lodged in the membrane of ring-infected red cells during merozoite invasion. Antibodies reactive with this antigen inhibit parasite multiplication in vitro. Part of the gene controlling the synthesis of this antigen has been analysed and found to code for a series of repeat peptides enclosing a non-repeating amino acid sequence. This antigen remains invariant among geographically remote isolates of *P. falciparum* and therefore has considerable interest as a putative vaccine. However, its stability must be established by direct testing since another antigen found to be common to several strains of the simian parasite *P. knowlesi* underwent variation when subject to the immune pressure which followed its use as a vaccine.

Apart from the complication of antigenic plasticity among malaria parasites, the development of blood-stage vaccines may, as suggested above for sporozoites, require the induction not only of anti-merozoite antibody, but also of specific cell-mediated immunity active against intra-erythrocytic parasites (Fig. 3). Specific T-cells reactive with plasmodial antigens are known to stimulate macrophages to release such parasiticidal agents as tumour necrosis factor and oxygen-derived free radicals which can kill parasites developing within red cells. Such agents might be most effective when parasitised cells come in close contact with activated macrophages as would occur in the splenic circulation; this may explain the prime importance of the spleen in malarial immunity. The identification of blood-stage plasmodial antigens reactive with T-cells and able to induce cell-mediated effector mechanisms against intracellular blood-stage parasites, may ultimately be mandatory for successful vaccine development.

**Conclusion**

Acquired immunity to malaria is stage and species specific; it depends in part upon antibodies that block sporozoite attachment to hepatocytes or inhibit merozoite invasion of red cells. Additional mechanisms, mediated by T-cells and macrophages, killing intracellular parasites through oxidative and other mechanisms, are involved in the maintenance of immunity. Some forms of experimental vaccination, including the use of irradiated sporozoites and non-viable extracellular blood-stage merozoites, induce effective immunity, but are impractical for mass human vaccination. The isolation of individual plasmodial antigens is currently being expedited by the use of monoclonal antibodies and gene cloning techniques. Sporozoites of all species have a uniform (but unique) circumsporozoite protein which has the properties of a protective antigen. No comparable immunodominant protective antigen has been identified in blood-stage parasites, which are antigenically extremely complex. However, a variety of antigens with protective properties are being delineated; many of them are associated with mature schizonts and some appear in a partially degraded form on the surface of merozoites. This work has led to the cloning of several genes controlling the synthesis of protective polypeptide antigens, and is concentrated on those common to all strains of a given species.

Some immunodominant antigenic peptides of these antigens have been synthesised and show promise as components of an ultimately practical and effective vaccine against the exo-erythrocytic and erythrocytic stages of human malaria. The remaining areas of uncertainty in this endeavour concern the antigenic stability of individ-

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**Fig. 3.** Immunity to the blood-stage of malaria requires the presentation of antigens by accessory cells (A) to Th cells. These induce B-cells to make antibody which neutralises free merozoites and also activate macrophages (Mφ) to produce oxidative and other products, such as tumour necrosis factor, which kill intra-erythrocytic parasites.
ual protective antigens when subject to immune pressure following vaccination and the requirement in any vaccine for T-cell reactive antigenic determinants able to induce cell-mediated immunity active against intracellular parasites.

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Dr Shephard Thomas Taylor

Besides his two important books, described in the next paragraph, the College has all the surviving papers of Dr Shephard Thomas Taylor, 1840–1936. His father was a Norfolk farmer, of Dillham Hall, near Hippisburgh, an early expert on farming machinery and a skilled carver of ivory.

Dr Taylor kept a diary all his life, in a legible copper-plate hand. He was apprenticed to Dr F. Bateman of Norwich and worked in the Norfolk and Norwich Hospital, and subsequently went to King’s College Hospital in London. In 1927 and 1930 he published the volumes describing his medical education, as The Diary of a Norfolk and Norwich Hospital Student, 1858–1860, and The Diary of a Medical Student, 1860–1864. They are the best descriptions of their subject, profusely illustrated with photographs, and are most amusing. They were completed by his postgraduate period in Berlin in Reminiscences of Berlin during the Franco-Prussian War 1870–71; interesting, but written in ‘journalese’, which he thought proper for books. This is not in the Library, but is in the London Library.

He was later on physician to the Norfolk and Norwich, and the Jenny Lind, Hospitals from about 1880 to 1910, and Medical Officer of Health, one of his primary interests: on p.1 of the book on his apprenticeship, he describes how the first thing he did when allotted his room was to measure it to see if it came up to standard volume and floor-area (it did not).

As a youth he was interested in a great many things, became a highly competent botanist, collected epifaths in churchyards, got into mischief and never wasted a moment. While at King’s, which was then in Portugal Street off the Strand, he had lodgings near King’s Cross and occupied himself in 1863 by sketching the iron coal hole lids in the London pavements. He collected 150 different designs seen between his lodgings and the hospital, taking in Portland Place to Gray’s Inn Road. This unique collection of Victoriana was eventually brought to the attention of the editor of The Ironmonger in 1929. That journal carried a short account of the work and then published all the drawings in a pamphlet aptly titled Opercula, (London coal-hole Plates, sketched by Aesculapius Junior), a copy of which is in the library.

He left a fair copy of all the lectures he gave at the Norfolk Students Society, bound in a volume, written in beautiful handwriting, in the most unfortunate ‘literary’ English, and other MS volumes of lists of plants, medical cases, etc. When he had published the student diaries in 1930 he destroyed all the originals, except the current volume, probably because they contained notes of the pretty girls he saw (one survives from the age of 18, and another at 85!). The last volume, 1895–1926, is so interesting that it looks as though we lost a heritage as valuable as Parson Woodforde’s diary.

He was a first-class linguist, and read widely in French, German, Italian, Latin and Greek, with most interesting comments: e.g. Erasmus’ Colloquies ‘most delightful reading, abounding in inimitable wit’; ‘but a little coarse here and there, which adds to their attraction, although perhaps I ought not to say so’. Of Carlyle’s Sartor Resartus, almost the most revered book of his day, he observed: ‘one of the most tedious and exasperating books I have ever read’ (23rd December, 1924); and of Tristram Shandy, which I for one would include in the top ten essential books: ‘clever and witty, but tedious reading, being much ado about nothing’ (16th September 1924). He played the eider and taught Edith (a servant) to play it: he also taught Katie, the cook, German.

He retired to The Mount, Edgefield, near Holt, added a large area of woods and cleared the brambles and weeds with his own hands at 90. It was characteristic of him that he recorded the exact number of dock plants he dug up and burned each day. The last volume of the dairy gives a clear picture of an impressive old man. When the editor of The Ironmonger went to see him in 1929 he wrote of hearing ‘light and rapid footsteps in the hall’ and being greeted by ‘a small elderly gentleman with an almost imperceptible stoop and a merry twinkle in his eye’, adding that he ‘had met a man who was infinitely more remarkable than his hobby’.

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