Malaria

Blood-stage antigens cloned

from K. N. Brown

THE development of DNA cloning and bacterial expression techniques has opened up a new approach to studying the antigenicity of malaria parasite and, perhaps, to developing a malaria vaccine. Five groups[1-5] have recently achieved expression of cloned genes encoding sporozoite and blood-stage proteins of the human malaria parasite (*Plasmodium falciparum*). Intriguingly, four of the antigens contain short repetitive sequences. The significance of these sequences is not known, but they may have a role in generating antigenic diversity and preventing host immunity.

Two papers[1,2] were discussed recently in these columns[6]. Of the others, Coppel *et al.*[3] have cloned an antigen associated with the membrane of infected erythrocytes. Immunoprecipitation and immunoblotting identified the antigen as a 155,000-molecular-weight (155K) acidic protein although some sera also reacted with a 210K polypeptide. The smaller molecule is found predominantly in the immature 'ring'-stage parasites and the 210K antigen in mature parasites. This may indicate a precursor–product relationship, particularly since the mRNA appears to be abundant only in fully mature parasites. Presumably the 155K portion is carried into erythrocytes by invading merozoites. Sequence data show a subunit structure of five repeats encoding Glu-Glu-Asn-Val-Glu-His-Asp-Ala. Immediately C-terminal are repeats of Glu-Glu-Asn-Val codons with Glu-Glu-Val interspersed irregularly. There is so far no evidence that antibody reacting with the membrane antigen of ring-infected cells is protective.

Another gene encoding a repetitive sequence, in this case one of nine amino acids, Glu-Glu-Val-Val-Glu-Glu-Val-Val-Pro, and associated with the membrane of erythrocytes infected with mature parasites, has been isolated by Koenen *et al.*[4] (see page 382 of this issue). Early work showed that erythrocytes infected with mature trophozoites and schizonts had exposed antigens. Two of the antigens on schizont-infected cells have been characterized: an antigentically diverse polypeptide possibly associated with the 'knobs' of *P. falciparum*-infected cells[7] and the phenotypically varying SICA antigen of *P. knowlesi*[8]. Whether the sequence identified by Koenen *et al.* relates to either of these polypeptides, and indeed how these two types of antigen are related, remains to be determined.

Of the cloned antigens of asexual erthrocytic parasites, the only ones known from *in vivo* evidence to have some significance for protective immunity belong to a family of polypeptides occurring in all species of mammalian parasite so far examined and varying in size from 190K to 250K. The effectiveness of protection induced varies greatly with the host–parasite combination used[5,9]. In this issue of *Nature* (page 379), Hall *et al.* report that vaccination of Saimiri monkeys with one member of this family, the *P. falciparum* p190 antigen, provides slight protection[5]. This antigen shows extensive diversity between isolates, although there are common epitopes present on the molecule, and many different serotypes can be isolated from one patient[10]. The antigen, or a processed part of it, is present on the merozoite. Hall *et al.* have cloned what is probably its C-terminal end. The sequence is non-repetitive, but it would be surprising if repetitive sequences did not occur elsewhere in the molecule since merozoites and sporozoites are similar in ultrastructure and in function, both being designed to penetrate cells, and the sporozoite surface antigen has well characterized repeat sequences.

Extensive phenotypic variation during chronic infection has been demonstrated for several surface-exposed parasite antigens, including possibly the 190–250K family. It may well be that variable sequences are inserted into all the exposed antigens of malaria parasites, which would provide a solution to two problems: restriction of numbers to ensure host survival and continued parasite survival for vector transmission. Phenotypic antigenic variability is seen in protozoa other than the malaria parasite. The ciliate *Paramecium* is a classic example of a free-living protozoan which adapts its surface macromolecules to environmental change; the changes are readily detectable serologically and indeed can be triggered by antibody. It seems likely that this immunologically important variation results from a general capacity for genetic rearrangement found throughout the protozoa which is exemplified strikingly in the variant surface glycoprotein genes of *Trypanosoma brucei* and has recently been inferred in other parasites[11-13]. In the case of malaria parasite, some of the diversity detected serologically, whether genotypic or phenotypic, may not necessarily be a response to immune pressure, but rather may reflect the requirement of an obligate parasite to enter and survive in genetically diverse hosts.

If genetic diversity and rearrangement are part and parcel of malaria parasites, what hope is there for a malaria vaccine? Given the epidemiology of malaria it is hardly surprising that with the exception of a few highly-selected laboratory systems, immunization with crude or purified antigen is relatively ineffective. Protection has not so far been achieved with the sporozoite surface antigen — is this a result of antigenic diversity[6], immune evasion by antigen capping or inappropriate vaccination technique? Perhaps immunization with vaccinia virus-containing sporozoite sequences[14] will answer the last question. The requirement for draconian adjuvants and the antigenic diversity of the merozoite surface antigen do not encourage much hope for protective cross-reactivity through common epitopes, although there is preliminary evidence that isolates can be ranked for cross-protective activity. Good immunity to virulent blood-stage infection can, however, be obtained using avirulent K^- *P. falciparum* lines[15] and γ-irradiated non-replicating parasitized cells without adjuvant. These results may indicate that parasite antigen seen in the context of modified erythrocyte is the required immunogen. Could such an immunogen be produced in quantity? Does the report of the insertion of new genetic material into haematopoietic stem cells[16] at last begin to approach the level of technical sophistication which immunization against malaria parasite requires, leading ultimately to *in vitro*-grown erthrocytes derived from precursors containing parasite-antigen-encoding inserts in their DNA?

In order to exploit the developments in the molecular biology, a reliable assay of the protective immunogenicity of and responses elicited by the various forms of malaria parasite immunogens *in vivo* will be essential. This is a difficult problem, however, as protective immunity is rarely complete; for example, around 50 per cent of immune adults in holendemic areas may have parasites detectable in their blood at any one time, and recent or current low-level parasitaemia may be a prerequisite for preventing clinically significant infection. Many attacks of malaria over several years are required to generate clinical immunity, and immunity is in part strain specific. The diversity of malaria parasites and the complexity of the epidemiology also make the interpretation in terms of protective immunity of the new epitope and sequence data on surface antigens extremely speculative at this stage. □

1. Dame, J.B. *et al. Science* **225**, 593 (1984).
2. Enea, V. *et al. Science* **225**, 628 (1984).
3. Coppel, R.L. *et al. Nature* **310**, 789 (1984).
4. Koenen, M. *et al. Nature* **311**, 382 (1984).
5. Hall, R. *et al. Nature* **311**, 379 (1984).
6. Maddox, J. *Nature* **310**, 541 (1984).
7. Leech, J.H. *et al. J. exp. Med.* **159**, 1567 (1984).
8. Howard, R.J. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4129 (1983).
9. Freeman, R.R. & Holder, A.A. *Clin. exp. Immun.* **54**, 609 (1983).
10. Thaithong, S. *et al. Trans. R. Soc. trop. Med. Hyg.* **78**, 242 (1984).
11. Cowman, A.F. *et al. Cell* **37**, 653 (1984).
12. Vaidya, A.B. *et al. J. Immun.* **132**, 3126 (1984).
13. De Lange, T. *et al. Nucleic Acids Res.* **12**, 3777 (1984).
14. Smith, G.L. *et al. Science* **224**, 397 (1984).
15. Langreth, S.G. & Peterson, E. *Proc. 2nd int. Conf. Malaria Babesiosis*, 117 (Marcel Merieux, 1983).
16. Williams, D.A. *et al. Nature* **310**, 476 (1984).

K.N. Brown is at the National Institute for Medical Research, Mill Hill, London NW7 1AA.