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Serotyping *Plasmodium falciparum* from acute human infections using monoclonal antibodies

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Summary

Monoclonal antibodies specific for the schizont and merozoite stages of *Plasmodium falciparum* have been used to demonstrate antigenic differences between parasites obtained from individual patients with acute malaria. Parasites from East Africa are shown to share some strain-specific antigenic determinants with culture-adapted isolates from Africa as well as from Asia and Papua New Guinea.

Introduction

In considering methods for immunological control of *Plasmodium falciparum* malaria it is essential to know more about antigenic diversity within and between natural populations of the parasite from major endemic areas. A simple antigen typing assay is needed which can be used to screen large numbers of samples freshly obtained from clinical cases, and which allows comparisons of results from different laboratories. No widely applicable typing assay exists, primarily because standardized antibody typing reagents have only become available with the advent of monoclonal antibodies.

We have developed a typing assay based on the use of monoclonal antibodies in indirect immunofluorescence tests and have used it to demonstrate a considerable serological diversity among isolates of *P. falciparum* adapted to growth in culture (MCBRIDE *et al.*, 1982). This work suggested that the parasite existed as a number of strains characterized by their distinct combinations of monoclonal-defined antigenic specificities, and that at least some such strains might have a worldwide distribution. However, since laboratory strains can undergo antigenic change during longterm cultivation (LANGRETH *et al.*, 1979) it needs to be established that comparable antigenic diversity also exists among natural populations of parasites and thus is not a result of adaptation to culture.

Here we report results of serotyping *P. falciparum* parasites obtained from clinical cases using these antibodies, and discuss some applications of this new method of strain characterization.

Materials and Methods

Patients

Case J.P. A Scotsman, aged 60 years, took no malaria prophylaxis on his trip to Aden, Comoros Islands and return via Dar-es-Salaam. He first complained of fever, headache and body pains 12 days after his return and was admitted to Edinburgh City Hospital with symptoms of cerebral malaria 21 days after leaving Africa. A sample of venous blood was collected when approximately 20% of red cells were parasitized with small pigment-less ring-forms; these matured to schizonts within 46 hours *in vitro*. This patient's blood also contained small numbers of circulating mature schizonts from which an additional antigen preparation for serotyping was made.

Case I.R. A Scotsman, aged 37 years, took malaria prophylaxis (300 mg chloroquine weekly) regularly on his visit to Malawi. *P. falciparum* malaria was diagnosed clinically and microscopically eight days after his return to the U.K. This patient's venous blood contained approximately 1% of erythrocytes infected with pigmented young trophozoites which matured into schizonts after 26 hours *in vitro*.

Maintenance of parasites and indirect immunofluorescence assay (IFA)

Synchronous young forms of the parasite were obtained as peripheral blood before administration of antimalarial chemotherapy. They were allowed to mature to schizonts in short-term cultures as follows: heparinized (10U/ml) blood was centrifuged, plasma and buffy-coat removed, and the red cells washed twice in medium RPMI 1640. Cultures were prepared with 0·3 ml of packed erythrocytes in 3 ml of culture medium RPMI 1640 supplemented with 15% (V/V) human AB serum using the TRAGER & JENSEN (1976) Petri dish method. Parasite maturity was assessed at intervals by examination of Giemsa-stained blood films. When most parasites became mature schizonts (segmenters), a sample of the culture was harvested while the remainder was incubated for a further 48 hours to obtain a second harvest of schizonts which had developed entirely *in vitro*. The schizont-infected red cells were washed twice in saline to remove human serum, antigen slides were made and serotyping by IFA was performed as described by MCBRIDE *et al.* (1982). Approximately 10^4 schizonts were examined for each parasite and antibody combination. The reactions were scored as negative when no fluorescing organisms were observed and as positive when most of the parasites were stained; it is not excluded that the latter reactions may have contained a small minority of non-reactive organisms but in practice these would not be detected. Within the limits of the test, all isolates examined in the present work appeared to be homogeneous in their reactions with all antibodies and none was identified as a mixture of antigenically different organisms. *P. falciparum* isolates and clones maintained in long-term culture were used as antigen standards in the serotyping test. The standards included Thai parasites K1, T9/clone 96 and T9/clone 102, PB1/clone 5, (THAITHONG & BEALE, 1981; ROSARIO, 1981; Thaithong *et al.*, unpublished work), Ugandan Palo Alto and isolate MAD 20 from Papua New Guinea (kindly provided by Dr. R. J. M. Wilson of NIMR, Mill Hill, London and Dr. Graham Knowles of PNGIMR, Madang, Papua New Guinea, respectively).

Monoclonal antibodies against K1, PB1 and T9, three antigenically different uncloned isolates of *P. falciparum* from Thailand, were raised in mice as described (MCBRIDE *et al.*, 1982). 17 strain-specific antibodies known to react

Table I—Serotypes of *P. falciparum* schizonts obtained from patients J.P. and I.R.

P. falciparum		Reactivity by indirect immunofluorescence[a]															
		anti-K1 antibodies						anti-PB1 antibodies					anti-T9 antibodies				
Code	Origin	*5·1*	*6·1*	*7·3*	*7·6*	*7·1*	*9·2*	*9·7*	*10·3*	*10·4*	*9·4*	*9·5*	*9·11*	*12·1*	*12·2*	*12·3*	*12·5*	*12·7*
K1[b]	Thailand	+++	+++	+++	+++	+++	−	−	−	−	+	−	−	−	±	−	−	+
PB 1/clone 5[b]	Thailand	−	−	−	−	±	+++	+++	+++	++	++	+++	+++	−	−	+++	+++	+++
MAD 20[b]	Papua New Guinea	+++	−	−	−	−	+++	+++	+++	++	++	+++	+++	+++	−	−	−	+
Patient J. P. (STS)[c]	Comoros or Tanzania	−	−	−	−	−	+++	+++	+++	−	++	+++	+++	−	−	−	−	+
Patient I. R. (STS)[c]	Malawi	−	−	−	−	−	+++	+++	+++	+	+	−	−	−	+	+++	+++	+++
Palo Alto[b]	Uganda	−	−	−	−	−	+++	+++	+++	++	+	−	−	−	+	+++	+++	+++
T9/clone 102[b]	Thailand	−	−	−	−	−	+++	+++	+++	++	−	−		+++	+	+++	+++	+++
T9/clone 96[b]	Thailand	+++	−	−	−	−	+++	+++	+++	++	−	−	−	+++	+++	+++	+++	+++

[a]acetone-fixed schizonts: +++ and ++ strong positive reactions comparable to those of homologous isolates; + dull reactions; − negative reactions.
[b]isolates adapted to long-term growth *in vitro*.
[c](STS) short-term schizonts, i.e., peripheral blood parasites after 24-96 hours *in vitro*.

with restricted groups of parasite isolates, i.e., which recognized antigenic determinants expressed by some but not all parasites, were used in this study. With the exception of antibody 5·1, all the monoclonals react specifically with the surface of schizonts and merozoites (McBRIDE *et al.*, 1982; HALL *et al.*, 1983; McBride, unpublished results).

Results and Discussion

Table I shows antigenic phenotypes of parasites obtained from the two patients with acute *P. falciparum* malaria, and these are compared to phenotypes of several established laboratory isolates and clones of the parasite. The two fresh isolates clearly differ from each other in their reactivities with eight antibodies, although they also share several specificities. It is of interest that the differences as well as the similarities between these two East African parasites are detected by reagents made against parasites from Thailand. The phenotypes of the two wild strains have been compared to those of over 40 laboratory strains of varied geographical origins (McBride & Walliker, unpublished results) and examples of isolates found to be the most similar to the strains are given in the table. I.R. was almost identical to Ugandan Palo Alto, and closely similar to Thai T9/Clone 102 which differed in only one specificity. J.P. had a combination of antigenic specificities not found among the cultured strains; it was more similar to MAD 20, from Papua New Guinea, than to any other isolate inclusive of three African ones. These results lend support to our earlier view that some of the strain-restricted *P. falciparum* antigens are shared by parasites from different endemic areas (McBRIDE *et al.*, 1982). This has also been indicated by studies on geographical distribution of soluble S-antigens detected by double-diffusion techniques (WILSON & VOLLER, 1970). However, our conclusion is based on the reactions of a limited number of monoclonal reagents and it cannot be excluded that antigens unique to certain geographical regions exist as was proposed by SCHOFIELD *et al.* (1982).

Schizonts of *P. falciparum* are not usually found in the peripheral circulation and thus are not available for direct typing. Therefore in this study we prepared schizonts from younger blood stages which were cultivated for 24 to 96-hour periods *in vitro*. Patient J.P. whose peripheral blood contained a few schizonts provided us with the opportunity to compare the antigenic composition of naturally developed schizonts with schizonts resulting from *in vitro* maturation of peripheral blood ring-forms, and with schizonts derived from the next asexual cycle which took place entirely in culture. We obtained identical serotyping results with all three preparations indicating that schizonts from the short-term cultures still expressed antigenic phenotypes representative of those of natural schizonts *in vivo*.

Protective immunity to *P. falciparum* is in part strain-specific (McGREGOR *et al.*, 1963; JEFFERY, 1966; SADUN *et al.*, 1966) suggesting that diversity in one or several of the parasite's putative protective antigens occurs. The apparently extensive serological diversity recognized by our monoclonal antibodies is due to participation of several antigenic entities (HALL *et al.*, 1983), but we do not know if any of them stimulate protective immune response *in vivo*. Some of the antibodies (7·3, 7·6, 9·2) recognize prominent schizont polypeptide(s) with approximate

molecular weight (MW) of 190-200,000 daltons (HALL *et al.*, 1983; McBride, Newbold & Hall, unpublished data). These may be the same or related to a 195,000 dalton antigen recently described by HOLDER & FREEMAN (1982): their apparent molecular weights are very similar, they are stage-specific and associated with schizont membrane(s) and the surface of merozoites, and they seem to be specificaly processed to smaller fragments. Holder and Freeman discussed the evidence which, by analogy with rodent Plasmodia, indicates that this polypeptide may be a protective antigen of *P. falciparum*. It is of interest that strain-specific monoclonal antibodies which inhibit the *in vitro* growth of some but not all *P. falciparum* isolates are said to be directed also against protein(s) of around 200,000 MW (SCHOFIELD *et al.*, 1982).

In view of these results it seems certain that a system for serotyping *P. falciparum* based on the diversity of defined antigens will facilitate studies on the antigenic composition of different populations of this malaria parasite. We are using a preliminary classification scheme based on limited data obtained with laboratory isolates, and aim to establish a more definite system when a large number of natural parasite strains from several endemic areas have been screened using the approach outlined in this report. We hope that this may contribute to the eventual development and application of malaria vaccines.

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References

Hall, R., McBride, J. S., Morgan, G., Tait, A., Zolg, J. W., Walliker, D. & Scaife, J. (1983). Antigens of the erythrocytic stages of the human malaria parasite *Plasmodium falciparum* detected by monoclonal antibodies. *Molecular and Biochemical Parasitology*, **7**, 247-265.

Holder, A. A. & Freeman, R. R. (1982). Biosynthesis and processing of a *Plasmodium falciparum* schizont antigen recognised by immune serum and a monoclonal antibody. *Journal of Experimental Medicine*, **156**, 1528-1538.

Jeffery, G. M. (1966). Epidemiological significance of repeated infections with homologous and heterologous strains and species of *Plasmodium*. *Bulletin of the World Health Organization*, **35**, 873-882.

Langreth, S. G., Reese, R. T., Motyl, M. R. & Trager, W. (1979). *Plasmodium falciparum*: Loss of knobs on the infected erythrocyte surface after long-term cultivation. *Experimental Parasitology*, **48**, 213-219.

McBride, J. S., Walliker, D. & Morgan, G. (1982). Antigenic diversity in the human malaria parasite *Plasmodium falciparum*. *Science*, **217**, 254-257.

McGregor, A., Carrington, S. P. & Cohen, S. (1963). Treatment of East African *P. falciparum* malaria with West African human γ-globulin. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **57**, 170-175.

Rosario, V. (1981). Cloning of naturally occurring mixed infections of malaria parasites. *Science*, **212**, 1037-1038.

Sadun, E. H., Hickman, R. L., Wellde, B. T., Moon, A. P. & Udeozo, I. O. K. (1966). Active and passive immunization of chimpanzees infected with West African and Southeast Asian strains of *Plasmodium falciparum*. *Millitary Medicine*, **131**, 1250-1262.

Schofield, L., Saull, A., Myler, P. & Kidson, C. (1982). Antigenic differences among isolates of *Plasmodium falciparum* demonstrated by monoclonal antibodies. *Infection and Immunity*, **38**, 893-897.

Trager, W. & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science*, **193**, 673-675.

Thaithong, S. & Beale, G. H. (1981). Resistance of ten Thai isolates of *Plasmodium falciparum* to chloroquine and pyrimethamine by *in vitro* tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**, 271-273.

Wilson, R. J. M. & Voller, A. (1970). Malarial S-antigens from man and monkey infected with *Plasmodium falciparum*. *Parasitology*, **61**, 461-464.

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