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# ANTIGENS OF *PLASMODIUM VIVAX* BLOOD STAGE PARASITES IDENTIFIED BY MONOCLONAL ANTIBODIES

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Monoclonal antibodies have been obtained that react with antigens of *P. vivax* blood stage parasites and infected erythrocyte membranes. Some define external surface antigens of the merozoite while other appear to be confined to the merozoite rhoptry organelles. Other monoclonal antibodies appear to recognize parasite induced components of the infected erythrocyte membrane.

One monoclonal antibody, 3F8.A2 probably recognizes the merozoite surface glycoprotein of *P. vivax* which is analogous to antigens found in rodent (Holder & Freeman, 1981), simian (Epstein et al., 1981), and human malaria species, (Holder & Freeman, 1984). At least three other antigens appear to be on the surface of *P. vivax* merozoites, two of which are of 170 Kd and 140 Kd in molecular weight as judged by SDS-Page analysis of immunoprecipitates.

Two monoclonals recognize two different antigens both of which appear to be associated with the rhoptry organelles of *P. vivax*. These two antigens are of 160/140 Kd and 53 Kd.

Five monoclonal antibodies appear as speckles over the infected erythrocyte and recognize two antigens of 95 Kd and 70 Kd. These monoclonal antibodies probably recognize parasite antigens in the caveola-vesicular complexes which are associated with the membrane of *P. vivax* infected erythrocytes, (Aikawa, Miller & Rabbage, 1975).

Analysis by immunofluorescence assay also indicates that all or some of these monoclonal antibodies cross-react with *P. simium, P. cynomolgi* and *P. knowlesi*, which may reflect their evolutionary relationship with *P. vivax*. In fact, since all twelve of the monoclonal antibodies cross-react with *P. simium*, it suggests that this species is antigenically closely related with *P. vivax* which may have only in the recent past become adapted to Brazilian monkeys.

*Plasmodium vivax* is a human malaria parasite species of major importance and of wide distribution. In many areas of the world the prevalence of *P. vivax* exceeds that of *P. falciparum* and the other two human malaria species, *P. malariae* and *P. ovale*. Although, not generally causing a lethal infection, as may be the case in some rapidly evolving *P. falciparum* infections, *P. vivax* infections cause severe morbidity, and malarial fever attacks are frequent due to relapses and reinfection.

There have been very few studies on *P. vivax* blood stage antigens and the available information does not permit us to define those which may be involved in the protective host response. This situation is primarily due to the inability to culture this human malaria species *in vitro* and is further agrravated by the impossibility to obtain suitable amounts of parasite material from the most frequently used simian host, i.e., Aotus monkeys. We have bypassed the latter difficulty by adapting several strains of *P. vivax* to splenectomized *Saimiri* monkeys which develop relatively high peak parasitemias of 2 to 10%. The parasite material we have collected from these monkeys was used to immunize mice for the production o monoclonal antibodies in order to characterize *P. vivax* blood stage antigens and to identify potential immunogens.

We have obtained numerous hybridomas which secrete monoclonal antibodies reactive with the blood stage parasites of *P. vivax*. Several of these monoclonal antibodies have been characterized by their stage specificity, the pattern they produce by the immunofluorescence assay (IFA), and relative eletrophoretic mobility of the protein antigens(s) cognized by these monoclonal antibodies. In addition, studies of the cross-reactivities between different isolates of *P. vivax*, and other malaria species have revealed some findings of interest. Table I gives the general characteristics of the monoclonal antibodies and the antigens they recognize.

Five monoclonal antibodies react with mature schizonts and merozoites. These monoclonals, 3F8.A2, 1G5.D9, 4E4.D9, 1E9.D5 and 1C2.F10 when assayed on mature schizonts, all produce an immunofluorescence pattern that rims or circumscribes the outline of the developing or developed merozoites. This suggests a surface membrane or surface coat location for the antigens they recognize. 3F8.A2 gives the brightest fluorescent signal at a standard concentration of 10 $\mu$g of antibody per ml and also circumscribes the surface of late trophozoites and early devel-

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TABLE I

Characteristics of *P. vivax* blood stage antigens detected by a series of monoclonal antibodies

| Monoclonal Antibody | Isotype | Parasite Stage Reactivity | IFA Pattern | Probable Location of Parasite Antigen | Mr of Antigen (Kd) |
|---|---|---|---|---|---|
| 3F8.A2 | $IgG_1$ | MZ/SZ# | surface outline | merozoite membrane | 210* |
| 4E4.D9 | $IgG_1$ | MZ/SZ | surface outline | merozoite membrane | 170 |
| 1G5.D2 | $IgG_2$ | MZ/SZ | surface outline | merozoite membrane | 140 |
| 1C2.F10 | $IgG_1$ | MZ/SZ | surface outline | merozoite membrane | ND** |
| 1E9.D5 | $IgG_1$ | MZ/SZ | surface outline | merozoite membrane | ND |
| 4B10.D5 | $IgG_1$ | MZ(Int.) | punctate | rhoptry | 160 |
| 4E1.D9 | $IgG_1$ | MZ(Int.) | punctate | rhoptry | 53 |
| 2H8.D3 | $IgG_1$ | infected erythrocyte | speckled | CVC+ | 95 |
| 2H12.B4 | $IgG_1$ | infected erythrocyte | speckled | CVC+ | 95 |
| 1H4.B6 | $IgG_1$ | infected erythrocyte | speckled | CVC+ | 95 |
| 4C12.G4 | $IgG_1$ | infected erythrocyte | speckled | CVC+ | 95 |
| 1A3.G4 | $IgG_1$ | infected erythrocyte | speckled | CVC+ | 70 |

* The 3F8.A2 antigen metabolically incorporates $^3$H-glucosamine.
\+ CVC = caveola-vesicle complexes (Schuffner's stippling).
\# MZ = merozoite, SZ = schizont, Int. = Internal.
** ND = Not determined.

oping schizonts. It precipitates from detergent extracts of biosynthetically labeled schizonts a protein band of 205 to 210 Kd. The antigen also incorporates $^3$H-glucosamine biosynthetically which suggests the protein is glycosylated. Mab 1G5.D9 gives a weaker fluorescent signal at 10 $\mu$g Ab per ml and immunoprecipitates a biosynthetically ($^{35}$S-methionine) labeled protein band of approximately 140 Kd. This protein is labile, even with the addition of numerous protease inhibitors into the detergent extract and with careful handling, and breaks down into a few bands of a step-ladder pattern on SDS-Page. 4E4.D9 also gives a fairly strong fluorescent signal circumscribing schizonts and merozoites. The 4E4.09 Mab precipitates a $^{35}$S-methionine labeled protein of about 170 Kd. This antigen, like the 140 Kd protein defined by Mab 1G5.D2, also, under conditions of limited proteolytic digestion, breaks down into a pattern of numerous bands resembling a 'step-ladder'. If this 'step-ladder' pattern is produced by proteases that recognize specific cleavage sites, it may indicate the presence of repetitive amino acid sequences. The Mabs 1E9.D5 and 1C2.F10 also give a fluorescent signal which surrounds the merozoite. We have not yet been able to identify the molecular moieties recognized by these monoclonal antibodies, neither by immunoprecipitation, followed by SDS-Page analysis, nor by Western immunoblotting.

Two monoclonal antibodies react with *P. vivax* merozoites by producing a characteristic discrete punctate dot immuno-fluorescence pattern that sometimes is a double dot of fluorescence in a single merozoite. This pattern of staining has been interpreted as an indication that the antigens are localized in the rhoptry organelles of merozoites (Oka et al., 1984). One Mab, 4B10.D5, immunoprecipitates a $^3$H-leucine labeled doublet of bands of approximately 160 and 140 Kd. 4E1.F2 immunoprecipitates a band of approximately 53 Kd which appears to be weakly labeled with $^3$H-histidine or proline.

Another set of monoclonal antibodies produce a completely different pattern of staining by IFA. These Mabs, 1A3.G4, 2H8.D3, 2H12.B4, 1H4.B6, and 4C12.G4, produce a staining pattern of small bright speckles or spots of unequal size and intensity over the entire parasitized erythrocyte. This pattern is reminiscent of Schuffner's stippling that is typically present in Giemsa stained thin films of *P. vivax* and vivax-like malaria species. 1A3.G4 immunoprecipitates a 70 Kd $^{35}$S-methionine labeled band, whereas the other four monoclonals recognize a 95 Kd band which can be most readily extracted in 0.5 – 1.0% SDS and only minimally extracted with the anionic detergent Triton X100. Surface fluorescence occurs with two of these Mabs, 2H8.D3 and 2H12.B4, when combined with unfixed intact suspensions of trophozoite – infected erythrocytes. This suggests the epitope(s) on the 95 Kd protein which are recognized by these two Mabs maybe exposed on the external surface of the erythrocyte, presumably within the caveolar depression.

All the Mab antibodies discussed in this report react not only with the Belem strain used for immunization but also with other isolates of *P. vivax* (North Korean and Thai). A comparison of the cross-reactivity of these monoclonal antibodies with other human or simian malaria species is given in Table II. None of the monoclonal antibodies react with *P. brasilianum* or *P. falciparum* blood stage parasites. Some of the Mabs react with the simian malarias *P. cynomolgi* and *P. knowlesi*. However, all the Mabs react with a simian New World malaria species so far found only in some areas of Brazil, *P. simium*. This would suggest a very close evolutionary relationship, if not identity, between *P. simium* and *P. vivax*.

TABLE II

Reactivity of *P. vivax* blood stage monoclonal antibodies with other human and simian malaria species by immunofluorescence assay

| | *P. simium* | *P. cynomolgi* | *P. knowlesi* | *P. brasilianum* | *P. falciparum* |
|---|---|---|---|---|---|
| 3F8.A2 | +++* | ++ | – | – | – |
| 4E4.D9 | +++ | – | – | – | – |
| 1G5.D2 | ++ | + | + | | |
| 1C2.F10 | ++ | +++ | ++ | – | – |
| 1E9.D5 | +++ | +++ | ++ | – | – |
| 4B10.D5 | +++ | – | – | – | – |
| 4E1.F2 | ++ | – | – | – | – |
| 1A3.G4 | +++ | – | – | – | – |
| 2H8.D3 | +++ | – | – | – | – |
| 2H12.B4 | +++ | – | – | – | – |
| 1H4.B6 | +++ | +++ | ++ | – | – |
| 4C12.G4 | +++ | +++ | +++ | – | – |
| | 100.0%+ | 50% | 42% | 0 | 0 |

* Increasing number of plus signs indicates the intensity of the fluorescent signal from weak (1+) to strong (3+).
\+ Per cent of monoclonal antibodies which react with a particular species of *Plasmodium*.

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