BABESIA ARGENTINA, PLASMODIUM VIVAX AND P. FALCIPARUM: ANTIGENIC CROSS-REACTIONS

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An indirect fluorescent antibody test was used to analyze the antigenic relationships between *Babesia argentina*, a parasite of cattle, and two human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. Elevated antibody titers to *P. falciparum* were found in cattle infected with *B. argentina*. Some persons infected with *P. falciparum* or *P. vivax* were found to produce antibodies to *B. argentina*. Implications for the occurrence of these cross-reactions are considered.
Babesia argentina, Plasmodium vivax and P. falciparum: Antigenic Cross-Reactions

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Ludford, C. G., Sulzer, A. J., Wilson, M., and Hall, W. T. K. 1972 Babesia argentina, Plasmodium vivax, and Plasmodium falciparum: Antigenic cross-reactions. Experimental Parasitology 32: 317-326. An indirect fluorescent antibody test was used to analyze the antigenic relationships between Babesia argentina, a parasite of cattle, and two human malaria parasites, Plasmodium falciparum and Plasmodium vivax. Elevated antibody titer to P. falciparum was found in cattle infected with B. argentina. Some persons infected with P. falciparum or P. vivax were found to produce antibodies to B. argentina. Explanations for the occurrence of these cross-reactions are considered.

Index descriptors: Indirect fluorescent antibody, Babesia argentina; Plasmodium vivax; Plasmodium falciparum; Serology; Human hosts; Cattle; Antigen antibody reactions; Immunology; Antigens.

The reports of human cases of babesiosis, recently reviewed by Garnham et al. (1969), have attracted interest to the Babesia, a genus once thought to have a host range restricted to wild and domestic animals. Such infections might be confused with malaria, and correct diagnosis is important for both clinical and epidemiological reasons.

It is of historic interest that, Wilson and Chowning (1904) believed that a babesia that they termed Piroplasma haemina was the causative organism of Rocky Mountain spotted fever. Although this belief was erroneous, their description and illustrations are convincing that they saw babesia in some of their patients, all of whom had been bitten by ticks. This would then be the first record of babesia infecting man.

Four cases of babesiosis in human beings have been reported since 1957. The first three were in splenectomized persons. Skrabo and Demovic (1957) reported a case believed to be due to Babesia bicornis; Scheflen et al. (1968) a case in whom an unidentified species of Babesia was seen; and Fitzpatrick et al. (1968, 1969) a case where the causative organism was Babesia microti.
gos. Babesia microti was isolated from the fourth case, a non-splenectomized person, described by Westen et al. (1970). Serum from the latter case was found by one laboratory to give a positive indirect fluorescent antibody (IFA) reaction with P. falciparum antigen.

Babesia differ from plasmodia in vectors, method of multiplication, and in metabolic activity. In view of these differences, Cox and Miller (1968), seeking an explanation of cross-protection between Babesia odocoilei, Plasmodium chabaudi, and Plasmodium berghei, suggested that immune mechanisms were in part non-specific and independent of the species-specific or genus-specific antigens. However, Cox and Turner (1970) have recently shown by IFA tests that some degree of relationship does occur between parasite antigens of Plasmodium vivax, P. chabaudi, P. berghei, and B. odocoilei and B. microti.

Two of the human cases of babesiosis (Skoklelo and Deamore 1957; Fitzpatrick et al. 1968, 1969) were caused by cattle Babesia species. The availability of B. odocoilei, a third cattle parasite, prompted an investigation of the relationship of this Babesia and human Plasmodium. This paper describes IFA reactions of antisera to Babesia odocoilei, and to the human species Plasmodium falciparum and Plasmodium vivax with both homologous and heterologous parasite antigens.

Materials and Methods

Antigens

Simian-adapted strains of P. falciparum, P. vivax and P. berghei were used to prepare washed-cell, thick-smear antigens (Sulzer et al. 1959).

Blood with 2% of the erythrocytes parasitized by the "C" strain of B. odocoilei was produced in a splenectomized calf in Australia by the technique of Callow and Mellors (1966). Erythrocytes were washed three times in fetal calf serum by centrifuging at 750 g and resuspended in fetal calf serum. Penicillin and streptomycin were added to a final concentration of 100 units ml to inhibit contamination. The washed cells were sent, cooled on wet ice, by air to the United States. Thick-smear antigens were then prepared as described for the malaria parasites (Sulzer et al. 1969).

Babesia microti. Gray strain, isolated from a human case of babesiosis by Westen et al. (1970), can be passaged through monkeys and hamsters. Antigen slides of this strain were prepared from the blood of an infected rhesus monkey by the same technique used for malarial parasites.

Antisera

Serum samples collected from 23 non-immune patients suffering a primary attack of induced P. falciparum and P. vivax malaria were obtained from Dr. David F. Clyde, Director, Institute of International Medicine, University of Maryland, Baltimore, Md. Preinfection samples were taken from each individual; additional samples were collected approximately every 5 days throughout the course of infection. Samples were tested from three cases of induced P. falciparum malaria and 20 cases of induced P. vivax malaria. Serum submitted to the Center for Disease Control (CDC) for malaria IFA tests from 21 cases of slide-proven malaria were also examined. Four were from P. falciparum cases, one from a P. ovale case and 17 from P. vivax cases.

Antisera against B. odocoilei were produced in cattle in Australia and sent on ice by air to the United States. On Day 1, 10 Australian Hulawar Shorthorn steers 12-18 months of age were exposed to infection from 2 g, i.e., about 40,000, larval Haemaphysalis microplus ticks carrying B. odocoilei strain C. Five blood samples were collected from the jugular veins of each of the 10 cattle before tick infestation and at Days 13, 26, 35, and 61. Serum samples were stored at -20° C before shipment to the United
States. Upon receipt they were again frozen until tested.

**Indirect Fluorescent Antibody Tests**

Indirect fluorescent antibody (IFA) tests were performed by the technique described by Wilson et al. (1970), using a 1-fold dilution series. All *B. argentina* serum samples from infected cattle initial serum dilution, 1:8, were tested at the same time against a particular antigen. Serum samples from individual malarial cases were tested with all antigens simultaneously but all cases were not tested on one day because of the large number of specimens.

All slides were randomized, coded, and read on a Leitz Ortholux fluorescent microscope equipped with Schott BG-12 and RG-2 exciter filters and a Leitz K170 secondary filter. Fluorescence of schizonts in *Plasmodium* spp. antigens was sought, as mentioned by Targett et al. (1970). *B. argentina* was readily recognized as a bright fluorescent body surrounded by the fluorescent ring of the parasitized erythrocyte, similar to its description by Ludford (1960) after direct fluorescent staining. In the present work, however, using the indirect technique, morphological details were not as apparent as with the direct technique. Intensity of fluorescence was graded from 0 to 4+, as described by Wilson et al. (1970).

Preinfection serum samples from the induced cases served as negative controls. As a control, saline was incubated with antigen slides in place of serum dilutions. Human and bovine sera known to react with *Plasmodium* spp. and *B. argentina* antigens, respectively, were included as positive controls.

**Results**

*B. argentina Antibody*  
As shown in Table 1, *B. argentina* was found in blood smears of eight of 10 cattle, 12–18 days after infestation with ticks. Antibodies to *B. argentina* were detected in the sera of these eight cattle on the third bleeding and remained with little alteration in titer until the end of the experiment. The reactions of the sera of the 10 cattle against *P. luteum* antigen are also shown in Table 1. Sera from nine of the 10 cattle reacted at low levels before infestation with ticks carrying *B. argentina*. However, by Days 26 or 35, a 16-fold rise in titer occurred in five cattle: Nos. 1, 2, 4, 7 and 9, their titers falling again by Day 61. A 4-fold rise in titer was found in cattle Nos. 3,
5, 8, and 10; the latter animal, however, did not have anti-
B. argentia. Animal 6 had no antibody against B. argentia and
did not show a rise in titer to P. falciparum antigen.

None of the serum samples from the 10 cattle reacted with P. vivax antigen. Representative samples from two cattle (Nos. 4 and 5) reacted with B. morsitans antigen. The preinfection sample from animal 4 was negative at the 1:4 dilutions, but that from animal 5 reacted at 1:4. Samples from both collected on Day 35 reacted at 1:4 and 1:16. Preinfection samples and samples taken on Day 35 from all eight remaining animals were negative at 1:4.

P. falciparum Antisera (Induced)

Serum samples from one induced P. falciparum malaria infection, case B2, gave positive results in the IFA test with B. argentia antigen. The results of the IFA tests are shown in Fig. 1. Tests on Days 1, 8, and 15 were negative, but on Day 22, positive reactions were found to all Plasmodium antigens and B. morsitans. The titer to B. argentia was 4-fold greater than that against P. falciparum. Thereafter, up to Day 55, titers against B. argentia were always greater or equal to those against P. falciparum. On Days 71 and 85, reactions against the heterologous malarial parasites P. brasiliense and P. vivax had noticeably declined, although those against B. argentia remained high.

Serum samples from two P. falciparum cases, B11 and B20, did not react with B. argentia. The serological results from these two cases against the three malaria antigens are given in Table II. As in case B2, the titer against P. falciparum rose to 1:256.

P. vivax Antisera (Induced)

Of 20 induced P. vivax infections, serum samples from three cases, Nos. B4, B9, and B12, reacted in the IFA test with B. argentia antigen. Results of these tests are shown in Figs. 2, 3, and 4, respectively. In all three cases, antibody to P. vivax and to B. argentia appeared together and remained throughout the course of infection. In case B4, titers to both B. argentia and P. vivax reached 1:4096, although on Days 36, 57, and 71, titers to B. argentia were higher than those to P. vivax. Similarly, in case B12, the maximum titer to both parasites was 1:256, but on Days 23 and 38, the titer to B. argentia exceeded that to P. vivax. In case B9, the titer to B. argentia rose to 1:4096, 16-fold higher than the titer of 1:256 to P. vivax. Case B4 showed a residual titer of 1:4 to P. vivax after 328 days, but antibody to B. argentia was not

Fig. 1. Case B2 P. falciparum, Thailand strain, mosquito induced.
then detectable. All three cases also had antibody to *P. brasiliense* and *P. falciparum*.

Serum samples from 16 cases of induced *P. vivax* malaria did not react with *B. argentina*. Results of IFA tests with malaria antigens on these are summarized in Table 11. Three strains of *P. vivax* were used for inoculation. In all cases except B15, antibody titers to *P. vivax* were greater than 1:16, and all cases formed antibody to *P. brasiliense* and *P. falciparum*.

Serum samples from one case, B7, gave
unusual results when tested for antibody against *B. argentina*, although reactions with *P. vivax* were normal. As shown in Table III, there was little or no reaction with *B. argentina* at 1:4 dilution and only partial reactions by some serum samples at 1:16, 1:64, and 1:256 dilutions. The titers to *P. vivax* during infection were 1:256 and 1:1024.

*Naturally Acquired Malaria*

Table IV shows the results of IFA tests for malaria and *B. argentina* antibodies in serum samples from 22 naturally acquired cases of malaria. Sixteen samples had antibody titers of 1:64 or greater to the infecting species of parasite. The six remaining samples with low antibody titers were all from *P. vivax* infections; two of these showed higher titers to a heterologous parasite, *P. falciparum*. Samples from three *P. vivax* infections reacted with *B. argentina* antigen.

**Discussion**

The results of blood smears and IFA tests showed that eight out of 10 cattle became infected with *B. argentina*. Although preinfection serum samples of most of the cattle reacted in the IFA test with *P. falciparum* antigen at low dilutions, the 16-fold rise in titer in five animals appeared to be a response from infection with *B. argentina*. However, the 4-fold rise in three infected animals is of doubtful significance, as the same rise was found in animal 10 in the
absence of antibody to \textit{B. argentina}. The reactions between sera of two cattle and \textit{B. microti} antigen suggest an antigenic cross-reactivity between this parasite and \textit{B. argentina}. Ristie et al. (1971) showed by the use of IFA tests that this strain of \textit{B. microti} was antigenically related to \textit{Babesia capitis}.

The reactions of human sera with \textit{B. argentina} antigen differed from the reactions of the cattle sera and \textit{P. falciparum} antigen. Serum from only one of seven \textit{P. falciparum} cases reacted with the \textit{Babesia} antigen. Although no reaction was detected between \textit{B. argentina} antisera and \textit{P. vivax} antigen, serum samples from six of 37 cases of \textit{P. vivax} malaria reacted with \textit{B. argentina} antigen. Malarial antibody patterns of sera positive to \textit{B. argentina} appeared in no way different from those which did not react with \textit{B. argentina}. Although the numbers are small, the incidence of reactions to \textit{B. argentina} appears to be similar in infections from the two malaria species. If results indicate an antigenic cross-reaction, it is likely to be between components of the \textit{babesia} and antibody to a malarial parasite antigen present in both \textit{P. falciparum} and \textit{P. vivax}.

The cross-reactivity was best studied in the induced cases, in which antibody responses to \textit{B. argentina} and the malarial parasites were followed throughout the course of infection. Production of antibody to \textit{B. argentina} differed in a number of ways from that of antibody against heterologous malarial parasites. In malaria infections, Glenson et al. (1971) showed that 4-fold differences in titer of sera with homologous and heterologous antigens indicated the infecting species in 77% of the sera submitted for routine diagnosis. In the present work, tests with malaria antigens in cases B9 and B12 indicated, by the above criterion, the infecting species on most occasions. The results with sera from case B2 indicated the infecting species on four of eight occasions. However, results obtained with sera from case B4 indicated the correct species in only one of seven samplings. Reactions to \textit{B. argentina} in these four cases followed a different pattern. On all except the final sample from case B4, the serum titer to \textit{B. argentina} equaled or exceeded that to the infecting parasite. The strongest reaction to \textit{B. argentina} did not occur in case B4, where a strong heterologous response against \textit{P. brasilianum} equaled or exceeded that against the homologous species, \textit{P. vivax}. It occurred, however, in case B9, where the antibody pattern against the malaria species correctly identified the infecting species as \textit{P. vivax}. Strong reactions against \textit{B. argentina} were seen with serum samples taken from two of three natural cases of malaria reacting with this parasite, as shown in Table IV.

The irregular occurrence of antibody reacting with \textit{B. argentina} in the malaria antisera was an outstanding feature. Although numbers were small, the frequency of response to \textit{B. argentina} appeared to be about the same, one reactor in seven cases, in both induced and natural malaria. It occurred with both blood-induced and mosquito-in-

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Reactions of serum dilutions to \textit{B. argentina}</th>
<th>Reciprocal Titer to \textit{P. vivax}</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
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<td>1</td>
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<td>15</td>
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</tr>
<tr>
<td>86</td>
<td>1, 2, 3</td>
<td>1, 2, 3</td>
</tr>
</tbody>
</table>

*Reactions graded from -- (negative) to 4 (strong positive).*
TABLE IV

Results of Tests for Plasmodium and Babesia argentina Antibody on Serum Samples from 22 Naturally Occurring Cases of Malaria

<table>
<thead>
<tr>
<th>Serum number</th>
<th>Infecting species</th>
<th>HFA test results (reciprocal of titer)</th>
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</thead>
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<tr>
<td></td>
<td>P. vivax</td>
<td>P. falciparum</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>256</td>
<td>64</td>
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<tr>
<td>4</td>
<td>256</td>
<td>64</td>
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<td>5</td>
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<td>6</td>
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<tr>
<td>9</td>
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<td>11</td>
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<td>256</td>
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<tr>
<td>22</td>
<td>16</td>
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</tr>
</tbody>
</table>

*Not tested.

duced infections and with more than one strain of P. vivax. It differed noticeably from the common response of malaria patients to homogenous plasmodia as well as to the infecting species (Collins et al. 1963). The reasons for the irregular occurrence of this reaction are not clear from our work. It is a common immunological phenomenon that not all subjects react alike and that differences may be found in cross-reactions of serum samples from different individuals (Landsteiner 1962). When antisera against some antigens are being prepared, many animals may be needed to find individuals producing a good response. Such responses, however, are usually graded, but the reaction between malaria antiserum and B. argentina antigen was “all or none.” The possible exception was in case B7 (Table III) where the reactions resembled a prozone. This may represent the production of antibody of poor reactivity or avidity to B. argentina.

Increased globulin production occurs during malaria infection: not all of this globulin is specifically directed against the parasite (Curtain et al. 1964). Kitchen et al. (1939) demonstrated positive Wasserman reactions in many malaria patients, and Oliver-Gonzales and Torregrosa (1944) found isoeugglutinin titers increased above the normal range in persons who had suffered repeated attacks of malaria. Tick transmission of B. argentina to cattle was used in this study rather than blood passage, to prevent the production of isoeutshibodies that might possibly interfere in IFA tests.

The production of certain antibodies may be related to the presence of Fossman anti-
gen in invading perivascular. Cox et al. (1966) described the presence of agglutinins for trypanosomized erythrocytes in P. berghei infections of rats. Kreher (1969) has described a similar agglutinin in P. gallinaceum infections of chickens. The reactions described in this paper between B. argentina antigens and sera from some malaria cases may be non-specific or related to the presence of Forssman antigens in these parasites. The occurrence, however, of elevated titers to P. falciparum antigens in some cattle infected with B. argentina must also be considered as indicating a possible antigenic relationship between the two parasites. The present observations suggest that further investigation is required in this area.

The question of the taxonomy of Babesia and their position among the protozoa has been repeatedly examined. In 1964 the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists removed the Babesia from the spirochaetes and established them as class Plasmodia under the superclass Sarcodina (Honigberg et al. 1964). At this time the Babesia were thought to differ markedly from the plasmodia. More recently the subject has been reviewed by Levine (1971) who placed the Babesia in class Plasmodia, which, with class Sporozoa, formed the Subphylum Apicomplexa. Electron microscope studies (Simpson et al. 1967, Ferris 1970) have shown that Babesia possess an apical complex resembling that of coccidia, plasmodia, and toxoplasmas. The occurrence of common or related antigens in Babesia and plasmodia is further evidence that these parasites are not as widely separated phylogenetically as was previously believed.

Whether the cross-reactivity between B. argentina and P. falciparum and P. vivax is specific or not, it should be kept in mind when interpreting IFA test results from persons thought to be infected with malaria, especially when there has been contact with cattle and ticks.

References


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