ANTIGENIC RELATIONSHIPS BETWEEN THE MALARIA PARASITES
AND PIROPLASMS OF MICE AS DETERMINED BY THE
FLUORESCENT ANTIBODY TECHNIQUE

by

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1. Introduction

The fluorescent antibody technique has been used on a number of occasions to
determine antigenic similarities or differences between malaria parasites and between piro-
plasms. In the case of the parasites of rodents the results obtained have been fairly clear
cut. Voller (1965), summarizing his work up to that time, stated that fluorescein-labelled
antisera against Plasmodium berghei berghei reacted well with P. b. berghei as an antigen, less
intensely with P. vinckei, very little with the parasites of primates and not at all with
avian malaria parasites. These results indicated that the malaria parasites of rodents,
P. b. berghei and P. vinckei, were antigenically similar. El-Nahal (1967) extended these
findings and showed that antisera against P. b. berghei and P. b. yoelii reacted similarly
with these two parasites as antigens but less strongly with P. chabaudi and P. vinckei.
Similarly, antisera against either P. chabaudi or P. vinckei reacted strongly with
P. chabaudi and P. vinckei antigens but less strongly with P. b. berghei and P. b. yoelii.
El-Nahal's results indicated that the four parasite species of rodents examined could be
classified into two antigenically distinct groups: P. chabaudi and P. vinckei on the one
hand and P. b. berghei and P. b. yoelii on the other. This grouping corresponded with the
protective cross-immunity between P. chabaudi and P. vinckei and between P. b. berghei and
P. b. chabaudi, but not between the two pairs, which had been demonstrated by Cox & Voller
(1966). The piroplasms of rodents have not been so intensively studied, but Ludford (1969)
found that antisera against Babesia rodhaini reacted strongly with this parasite but either
weakly or not at all with B. argentina, B. bigemina or B. canis. From these results two
things are obvious. Firstly the fluorescent antibody technique appears to be specific
even enough to enable one to distinguish between a variety of parasites in mice. Secondly it
seems possible that similarities detected by this technique might also reflect patterns of
protective cross-immunity. The fluorescent antibody technique was therefore used to
examine the antigenic similarities and differences between four malaria parasites,
Plasmodium vinckei, P. chabaudi, P. b. berghei and P. b. yoelii and two piroplasms,
Babesia rodhaini and B. microti, in the hope that the results obtained might throw some light
on the high degree of heterologous protective immunity which has been shown to exist between
these parasites (Cox, 1970).

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2. Materials and methods

The following strains of parasites were used: *Plasmodium vinckeii* (Katanga 52), *P. chabaudi* (54X), *P. berghei berghei* (173K), *P. berghei yoelii* (RCA 17X) *Babesia rodhaini* (Antwerp) and *B. microti* (King's 67). All six parasites were maintained in mice and used both as antigens and for the production of antisera. Further details of these parasites and the methods used for obtaining immune animals are given by Cox (1970).

The fluorescent antibody technique employed was an indirect one which utilized thin films of infected blood as the antigen, sera from immune mice as the antibody and antisera to mouse immunoglobulins prepared in rabbits and labelled with fluorescein isothiocyanate. Thin films of blood from mice infected with the parasite being studied were fixed in 0.3N HCl for five minutes and washed in tap water and phosphate-buffered saline (PBS) at pH 7.5. Films were taken during the ascending logarithmic phase of the infection and in the cases of both *P. b. berghei* and *P. b. yoelii* it was important to make the films during the first five days of the infection. Immune sera were obtained by bleeding mice which had overcome their infections. The sera were taken from mice infected with *Plasmodium vinckeii* 45 days after infection, *P. chabaudi* 48 days after infection, *P. b. berghei* 34 days after infection, *P. b. yoelii* 36 days after infection, *Babesia rodhaini* 34 days after infection and *B. microti* 21 days after infection. The sera were left in contact with the antigens for 30 minutes at 20°C. After washing, the fluorescein-labelled immunoglobulins were applied for 30 minutes at 20°C. The labelled immunoglobulin used was specific rabbit antimouse Ig. Fuller details of this serum and its preparation are given by Cox, Crandall & Turner (1969). The fluorescein-labelled parasites were then washed, counterstained with 0.1% Evans Blue, mounted in 90% glycerine in PBS and examined under ultra-violet light. In order to obtain quantitative results the sera from immune mice were titrated using serial double dilutions until no fluorescence was visible.

The six sera from immune mice were reacted with each of the six parasites, and the antibody titres determined using labelled anti-Ig. This produced a total of 36 titres and the patterns of reaction are shown in Table 1.

In order to see if the results obtained were reproducible, sera from a group of mice immune to *P. vinckeii* were taken 30 days after infection and pooled. This pooled serum was reacted with the six parasite antigens and the results obtained were compared with those obtained with the *P. vinckeii* antisera described above. This experiment was repeated with specific labelled anti-IgM and IgG.

3. Results

The results obtained are shown in Table 1 and Figs 1 and 2.

The results from experiments using labelled anti-Ig show that a considerable degree of cross-reaction exists between the six parasites used as antigens and the heterologous antisera. Nevertheless the highest titres were obtained with the homologous antisera and these titres were significantly higher than any of the others. For each antisera the second highest titre was obtained with the related heterologous antigen, that is *Plasmodium chabaudi* in the case of anti-*P. vinckeii* serum and vice versa and similarly *P. b. berghei* and *P. b. yoelii* and *Babesia rodhaini* and *B. microti*. These titres were no higher than those obtained with less closely related parasites, *P. b. berghei* in the case of *P. vinckeii* antisera, *P. vinckeii* in the case of *P. b. berghei* antisera and all parasites, except *P. b. yoelii*, in the case of *Babesia microti* antisera. The lowest titres were obtained with malaria antisera and piroplasms as antigens and vice versa. The two parasites in each pair behaved like one another with respect to each of the six antisera. In general the titres were highest in the homologous situation, lower with the related heterologous parasite, still lower with the less related parasite belonging to the same genus and lowest with parasites belonging to another genus.
The results obtained using a second sample of *P. vinckei* antiserum are shown in Fig. 2. The second antiserum gave a lower homologous titre than the first but the pattern of reaction was almost exactly the same.

4. Discussion

The six parasites used in this investigation fall into three distinct groups: *Plasmodium vinckei* and *P. chabaudi*, *P. b. berghei* and *P. b. yoelii* and *Babesia rodhaini* and *B. microti*. In each pair the first named species is virulent and always causes a fatal infection in mice while the second is less virulent and causes an infection from which the majority of animals recover. Phylogenetically, *Plasmodium vinckei* and *P. chabaudi* are closely related and may even be two subspecies of *P. vinckei*; *P. b. berghei* and *P. b. yoelii* are closely related and belong to the same subgenus as *P. vinckei* and *P. chabaudi*; *Babesia rodhaini* and *B. microti* are morphologically similar to one another but only remotely related to the malaria parasites. The experiments described in this paper have revealed a high degree of serological cross-reaction between these parasites and this indicates antigenic similarities. The highest titres were obtained with the homologous parasites and antisera and this result was expected. In all cases the titres were in the region of 1/1280 to 1/2560 which are relatively high. With heterologous situations the results were less clear cut, but in general the degree of cross-reaction reflected the accepted phylogenetic affinities, the titres being lowest in reactions between the two genera. The relationships between *B. rodhaini* and *B. microti* have not been examined before and the results obtained in this study indicate that they have considerable affinities but nevertheless are serologically distinct. The serological relationships between *Plasmodium b. berghei* and *P. vinckei* were studied by Voller (1965), using a direct fluorescent antibody technique, who concluded that they could be grouped together on the basis of the results obtained. A more detailed study of the serological relationships between *P. vinckei*, *P. chabaudi*, *P. b. berghei* and *P. b. yoelii* was made by El-Nahal (1967). El Nahal concluded that there were affinities between all four parasites but that the cross-reactions were greatest between *P. vinckei* and *P. chabaudi* which formed one group and *P. b. berghei* and *P. b. yoelii* which formed another, thus confirming the classically accepted situation. Within each group El-Nahal found similar antibody titres regardless of the antigen or antiserum used. In the present study the two groups recognized by El-Nahal were apparent but less obvious than he suggested, while there were considerable differences between *P. vinckei* and *P. chabaudi* and between *P. b. berghei* and *P. b. yoelii*. These differences may be in part due to the fact that El-Nahal used rats and we used mice, but it is more likely that our technique, which produced much higher titres, was more sensitive and thus picked out minor antigenic differences.

The main point of this investigation, however, was not to study the affinities between these parasites but to see if serological studies could throw any light on the considerable degree of protective cross-immunity which has been demonstrated between malaria parasites and between malaria parasites and piroplasms by Cox (1970). The results of the experiments described in this paper have shown that the six parasites do have a number of antigens in common and these could form a basis for the protective immunity. These results are in contrast with those recorded for other species of malaria parasites; for example *P. cynomolgi bastianellii* and *P. c. ceylonensis* in monkeys do not protect against one another although the fluorescent antibody technique has revealed affinities between them (Voller, Garnham & Targett, 1966).
TABLE 1. FLUORESCENT ANTIBODY TITRES OBTAINED WITH SIX PARASITES AS ANTIGENS AND THE HOMOLOGOUS AND HETEROLOGOUS ANTISERA
(Each figure represents the reciprocal of an individual titre)

<table>
<thead>
<tr>
<th>Antiserum to</th>
<th>Plasmodium vinckei</th>
<th>P. chabaudi</th>
<th>P. b. berghei</th>
<th>P. b. yoelii</th>
<th>Babesia rodhaini</th>
<th>B. microti</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vinckei</td>
<td>2 560</td>
<td>640</td>
<td>640</td>
<td>320</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>P. chabaudi</td>
<td>640</td>
<td>2 560</td>
<td>320</td>
<td>160</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>P. b. berghei</td>
<td>160</td>
<td>80</td>
<td>1 280</td>
<td>160</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>P. b. yoelii</td>
<td>40</td>
<td>80</td>
<td>160</td>
<td>2 560</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>B. rodhaini</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>20</td>
<td>2 560</td>
<td>80</td>
</tr>
<tr>
<td>B. microti</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>1 280</td>
</tr>
</tbody>
</table>

SUMMARY

An indirect fluorescent antibody technique, using specific anti-Ig was employed to determine the antigenic relationships between Plasmodium vinckei, P. chabaudi, P. berghei berghei, P. b. yoelii, Babesia rodhaini and B. microti in mice. The results showed that the reactions were greatest between homologous antisera and antigens, less between heterologous parasites of the same genus and least between the piroplasms and malaria parasites. Although all the parasites possessed antigens in common the antibody titres could not be correlated with the heterologous immunity which has been demonstrated between these parasites.

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RESUME

On a utilisé une technique indirecte aux anticorps fluorescents avec des anti-Ig spécifiques pour déterminer les relations antigéniques entre Plasmodium vinckei, P. chabaudi, P. berghei berghei, P. b. yoelii, B. abesia rodhaini et B. microti chez les souris. Les résultats montrent que les réactions ont été maximales entre antisérums et antigènes homologues, moindres entre parasites hétérologues du même genre et minimales entre piroplasmes et parasites du paludisme. Bien que tous les parasites aient possédé des antigènes en commun, il n'a pas été possible d'établir de corrélation entre les titres d'anticorps et l'immunité hétérologue qui a été mise en évidence entre ces parasites.
REFERENCES


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FIG. 1. FLUORESCENT ANTIBODY TITRES OBTAINED WITH SIX PARASITES AS ANTIGENS AND THE HOMOLOGOUS AND HETEROLOGOUS ANTISERA. EACH HISTOGRAM REPRESENTS THE RECIPROCAL OF THE ANTIBODY TITRES OBTAINED USING SPECIFIC ANTI-Ig.

V = Plasmodium vinckei; C = P. chabaudi; B = P. b. berghei; Y = P. b. yoelii; R = Babesia rodhaini; M = B. microti

Anti-vinckei

Anti-chabaudi

Anti-berghei berghei

Anti-berghei yoelii

Anti-rodhaini

Anti-microti
FIG. 2. FLUORESCENT ANTIBODY TITRES OBTAINED USING SIX PARASITES AS ANTIGENS AND TWO SEPARATE BATCHES OF PLASMODIUM VINCELE ANTISERA. EACH HISTOGRAM REPRESENTS THE RECIPROCAL OF THE ANTIBODY TITRES OBTAINED USING SPECIFIC ANTI-IMMUNOGLOBULINS. THE WHITE HISTOGRAMS REPRESENT THE TITRES OBTAINED WITH THE ANTISERUM DESCRIBED IN THIS PAPER AND SHOWN IN FIG. 1. THE BLACK HISTOGRAMS REPRESENT ANOTHER ANTISERUM WITH A LOWER HOMOLOGOUS TITRE.

V = P. vinckei, C = P. chabaudi, B = P. b. berghei, Y = P. b. yoelii, R = Babesia rodhaini, M = B. microti