Antigenic Relationships between the Malaria Parasites and Piroplasms of Mice as Determined by the Fluorescent-antibody Technique*

by F. E. G. Cox[1] & Sheila A. Turner[2]

The fluorescent antibody technique has been used on a number of occasions to determine antigenic similarities or differences between malaria parasites and between piroplasms. 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 malaria parasites 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 to 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 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).

Materials and methods

The following strains of parasites were used: *Plasmodium vinckei* (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 5 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 5 days of the infection. Immune sera were obtained by bleeding mice which had overcome their infections. The sera were taken from mice infected with *P. vinckei* 45 days after infection, *P. chabaudi* 48 days after infection, *P. b. berghei* 34 days after infection, *P. b. yoelii* 36 days after infection, *B. 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.

* This investigation received financial support from the World Health Organization (F.E.G.C.) and from the Wellcome Trust (S.A.T.).

[1] Reader in Zoology, Department of Zoology, University of London King's College, London, England.

[2] Sometime Research Associate of King's College, London, England.

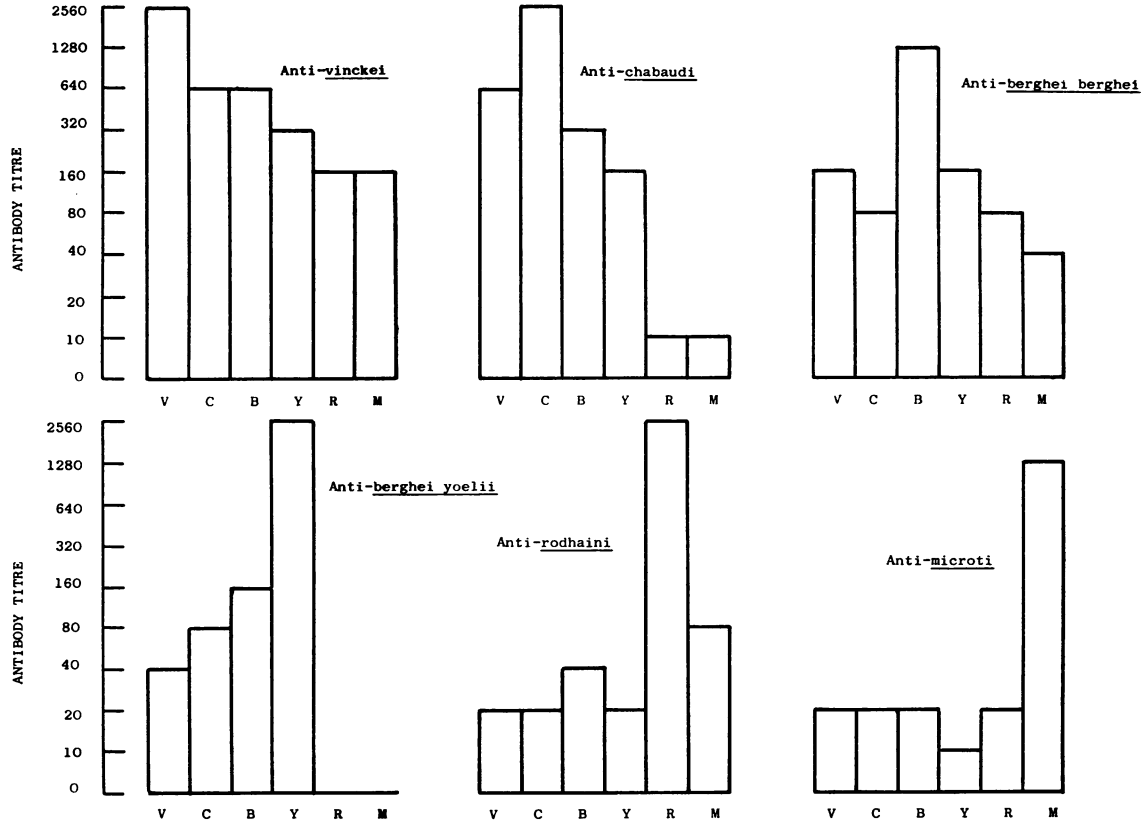
FLUORESCENT-ANTIBODY TITRES OBTAINED WITH 6 PARASITES AS ANTIGENS AND THE HOMOLOGOUS AND HETEROLOGOUS ANTISERA [a]

Antiserum to:	Antigen					
	Plasmodium vinckei	*P. chabaudi*	*P. b. berghei*	*P. b. yoelii*	*Babesia rodhaini*	*B. microti*
P. vinckei	2 560	640	640	320	160	160
P. chabaudi	640	2 560	320	160	10	10
P. b. berghei	160	80	1 280	160	80	40
P. b. yoelii	40	80	160	2 560	neg.	neg.
B. rodhaini	20	20	40	20	2 560	80
B. microti	20	20	20	10	20	1 280

[a] Each figure represents the reciprocal of an individual titre.

FIG. 1

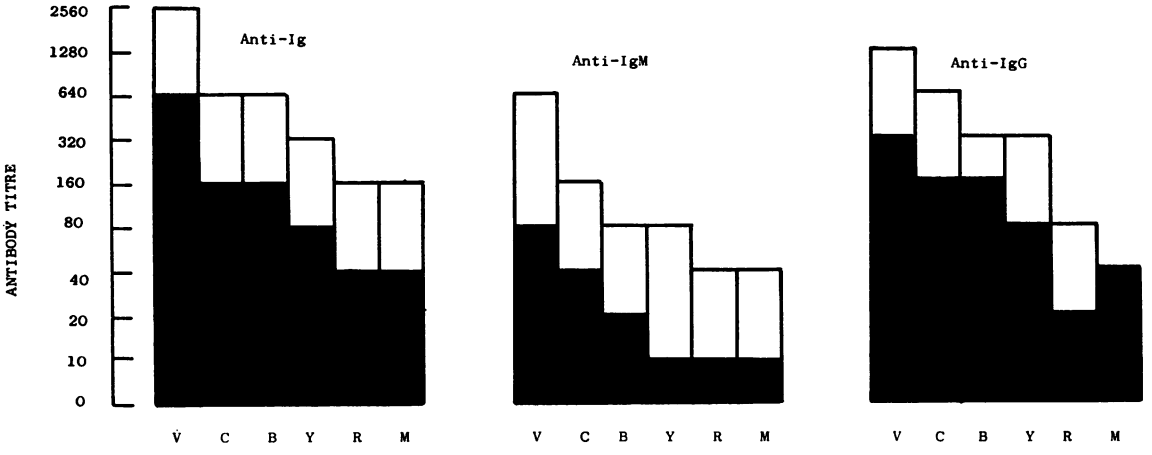
FLUORESCENT-ANTIBODY TITRES OBTAINED WITH 6 PARASITES AS ANTIGENS AND THE HOMOLOGOUS AND HETEROLOGOUS ANTISERA [a]



[a] Each histogram represents the reciprocal of the antibody titres obtained using specific anti-immunoglobulin. V = *Plasmodium vinckei*; C = *P. chabaudi*; B = *P. b. berghei*; Y = *P. b. yoelii*; R = *Babesia rodhaini*; M = *B. microti*.

FIG. 2

FLUORESCENT-ANTIBODY TITRES OBTAINED USING 6 PARASITES AS ANTIGENS AND 2 SEPARATE BATCHES OF *P. VINCKEI* ANTISERA [a]



[a] 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. Letter symbols as in Fig. 1.

After washing, the fluorescein-labelled immunoglobulins were applied for 30 minutes at 20°C. The labelled immunoglobulin used was specific rabbit antimouse immunoglobulin. 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% glycerol in PBS and examined under ultraviolet light. In order to obtain quantitative results, the sera from immune mice were titrated using serial double dilutions until no fluorescence was visible.

The 6 sera from immune mice were reacted with each of the 6 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 the accompanying table.

In order to see whether the results obtained were reproducible, sera from a group of mice immune to *P. vinckei* were taken 30 days after infection and pooled. This pooled serum was reacted with the 6 parasite antigens and the results obtained were compared with those obtained with the *P. vinckei* antiserum described above. This experiment was repeated with specific labelled anti-IgM and anti-IgG.

Results

The results obtained are shown in the table and in Fig. 1 and 2.

The results from experiments using labelled anti-Ig show that a considerable degree of cross-reaction exists between the 6 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 antiserum the second highest titre was obtained with the related heterologous antigen, that is *P. chabaudi* in the case of anti-*P. vinckei* serum and *vice versa*, and similarly *P. b. berghei* and *P. b. yoelii* and *B. 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. vinckei* antiserum, *P. vinckei* in the case of *P. b. berghei* antiserum, and all parasites except *P. b. yoelii* in the case of *B. microti* antiserum. 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 6 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.

Discussion

The 6 parasites used in this investigation fall into three distinct groups: *P. vinckei* and *P. chabaudi*, *P. b. berghei* and *P. b. yoelii*, and *B. 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, *P. 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*; *B. 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 1/1280–1/2560, 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 *P. 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 whether 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 6 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 each other although the fluorescent-antibody technique has revealed affinities between them (Voller, Garnham & Targett, 1966).

ACKNOWLEDGEMENTS

We wish to thank Dr C. A. Crandall for her help in the preparation of the labelled immunoglobulins used.

REFERENCES

Cox, F. E. G. (1970) *Bull. Wld Hlth Org.*, **43**, 325-336
Cox, F. E. G., Crandall, C. A. & Turner, S. A. (1969) *Bull. Wld Hlth Org.*, **41**, 251-260
Cox, F. E. G. & Voller, A. (1966) *Ann. trop. Med. Parasit.*, **60**, 297-303
El-Nahal, H. M. S. (1967) *Bull. Wld Hlth Org.*, **36**, 423-429
Ludford, C. G. (1969) *Exp. Parasit.*, **24**, 237-335
Voller, A. (1965) *Ann. Soc. belge Méd. trop.*, **45**, 385-396
Voller, A., Garnham, P. C. C. & Targett, G. A. T. (1966) *J. trop. Med. Hyg.*, **69**, 121-123