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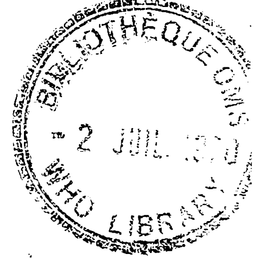

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THE SPECIFICITY OF IMMUNOGLOBULIN G AND IMMUNOGLOBULIN M IN THE FLUORESCENT ANTIBODY TEST FOR MALARIA PARASITES IN MICE[1]

by

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1. INTRODUCTION

The fluorescent antibody technique has been widely used for the detection of malaria parasites or immune sera but the potential use of the technique for the actual identification of particular species has not been exploited because of the considerable degree of cross-reaction which occurs. Serological cross-reactions, as detected by the fluorescent antibody technique, have been reported between species of malaria parasites in humans by Diggs & Sadun (1965), between simian and human species by Tobie et al. (1962, 1963), Coudert et al. (1965) Meuwissen (1968) and Collins, Skinner & Coifman (1967), between simian species by Collins et al. (1965), Collins, Skinner & Guinn (1966) and Voller, Garnham & Targett (1966), between rodent species by El-Nahal (1967) and Cox & Turner (1970a) and between avian and human species by Kielman & Weiss (1968). These results were based on observations made with the parasites themselves as antigens, whole antisera and fluorescein-labelled antiglobulins. It has been shown repeatedly that both immunoglobulin M (IgM) and immunoglobulin G (IgG) are involved in the immunological response to malaria parasites. It therefore follows that these immuno-globulins might be more specific in detecting the homologous reaction than whole immunoglobulin (Ig) and thus increase the sensitivity of the fluorescent antibody technique. IgM and IgG can be utilized in one of two ways. First, the parasites can be exposed to fractionated antisera and labelled with anti-Ig or, secondly, the parasites can be exposed to whole antisera and labelled with specific anti-IgM or IgG. In either case the degree of reaction with IgM or IgG can be estimated. In the experiments reported in this paper the second alternative was used.

Cox & Turner (1970a) have shown that there are antigens in common between four malaria parasites and two piroplasms in mice as detected by the fluorescent antibody technique using fluorescein-labelled anti-Ig. The degree of reaction was greatest between antisera and homologous parasites, less between the same antisera and heterologous but related parasites and least between the antisera and the most distantly related parasites. These results provide the basis for a useful model to test the possibility that fluorescein-label ed anti-Igm or anti-IgG might be more specific than anti-Ig in detecting the homologous reaction.

2. 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). The parasites were maintained in mice and used both as antigens and for the production of antisera. Further details of the methods used for obtaining immune mice are given by Cox (1970).

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The antisera were obtained from mice which had recovered from infection with one of the six parasites. The sera were taken from mice immune to Plasmodium vinckei 45 days after infection, P. chabaudi 48 days after infection, P. berghei berghei 34 days after infection, P. berghei yoelii 36 days after infection, Babesia rodhaini 34 days after infection and B. microti 21 days after infection.

The fluorescent antibody technique used was an indirect one based on that of Voller (1964) and has already been described by Cox, Crandall & Turner (1969) and Cox & Turner (1970a). Briefly, thin films of infected blood were fixed in 0.3N HCl, washed, exposed to the appropriate antisera, washed and finally exposed to fluorescein-labelled anti-Ig, anti-IgG or anti-IgM before being examined under ultra-violet light. Antibody titres were determined by the serial dilution of the antisera.

The fluorescein-labelled immunoglobulins used were those described by Cox, Crandall & Turner (1969). Three were used: these detected Ig, IgG and IgM and were specific.

The six sera from immune mice were reacted with each of the six parasites and the antibody titres determined using labelled anti-Ig, anti-IgM and anti-IgG. This produced a total of 108 titres. The reproducibility of the results was tested by comparing them with results previously obtained with anti-Ig (Cox & Turner, 1970a) and with other samples of anti-P. vinckei and anti-P. chabaudi sera and by using a random series of replicates.

3. RESULTS

The results obtained are given in Table 1. These results show that the antibody titres obtained using fluorescein labelled anti-IgG were in most cases the same as those obtained using anti-Ig or were only one dilution below. The titres obtained using anti-IgM were considerably lower. The IgG titres differed most markedly from the Ig titres in those cases where P. berghei berghei was involved either as the antigen or in the production of the antiserum. In the 25 combinations in which P. berghei berghei was not involved 19 of the titres obtained using anti-IgG were the same as, or one dilution less than, the titres obtained with anti-Ig. In the 11 reactions in which P. berghei berghei was involved only two were this close and one of these was the homologous reaction. The only other situations in which the IgG titres were significantly lower than the Ig titres were three in which Babesia microti was the antigen, two in which B. rodhaini was the antigen and one in which Plasmodium vinckei was the antigen and the antiserum was anti-B. microti.

Results obtained using the same six parasites as antigens and second samples of anti-P. vinckei and anti-P. chabaudi sera are shown in Table 2. The antibody titres were comparable with those obtained using the first samples of antisera.

4. DISCUSSION

The results obtained from the experiments outlined in this paper show that the fluorescent antibody technique as it is normally performed for the detection of malaria parasites can be modified by using fluorescein-labelled anti-IgG or anti-IgM instead of anti-Ig. The antibody titres obtained were similar in the case of anti-Ig, or lower in the case of anti-IgM, to those obtained using anti-Ig. In all cases the highest titres were obtained with homologous antigens and antisera. These results indicate that the specificity and sensitivity of the fluorescent antibody technique cannot be improved by using labelled anti-IgG or anti-IgM instead of anti-Ig. In view of the difficulties inherent in the production of anti-IgG and anti-IgM sera there seems to be no point in advocating a change in procedure.

Although the use of fluorescein-labelled anti-IgG and anti-IgM do not improve the specificity of the fluorescent antibody technique, it is possible that the use of these antisera might provide information as to how recently a malaria infection had been acquired, as suggested by Collins & Skinner (1968) who drew attention to the fact that in human malaria infections both

the IgM and IgG levels rose early in the infection but the IgM level then fell. The relative levels of IgG and IgM should then indicate whether or not the infection was a recent one. In fact, the information available on the occurrence of IgM and IgG in human malaria infections is limited (Abele et al., 1965 and Tobie et al., 1966) and does not really lend itself to such an analysis. On the other hand, in infections in mice, recent experiments with P. chabaudi and P. vinckei (Cox et al., 1969), P. berghei yoelii (Cox & Turner, 1970b) and the piroplasm Babesia microti (Cox & Turner 1970c), did not show any significant decline in IgM levels during the course of infection and therefore the relative levels of IgM and IgG could not be used to indicate, with these parasites, how recently the infection had been acquired.

In view of the reported differences between immunoglobulin responses in mice and in humans it is obvious that the experiments reported in this paper will have to be repeated with malaria parasites in man, but it seems unlikely that the results obtained will be any different.

SUMMARY

The fluorescent antibody technique has been used to determine antigenic similarities and differences between four species of malaria parasites, Plasmodium vinckei, P. chabaudi, P. berghei berghei and P. berghei voelii and two piroplasms, Babesia rodhaini and B. microti. All six parasites cross-react with heterologous sera and presumably possess antigens in common. The titres obtained using fluorescein-labelled anti-IgG were similar to those obtained using anti-Ig but those obtained using anti-IgM were considerably lower. The use of fluorescein-labelled anti-IgM or anti-IgG did not improve the specificity of the technique.

RESUME

La technique d'immunofluorescence a été utilisée pour déterminer les similitudes et les différences antigéniques entre quatre espèces de parasites du paludisme : Plasmodium vinckei, P. chabaudi, P. berghei berghei et P. b. yoelii et deux piroplasmes : Babesia rodhaini et B. microti. Ces six parasites donnent tous des réactions croisées avec les sérums hétérologues et possèdent vraisemblablement des antigènes communs. Les titres obtenus avec des anti-IgG marquées à la fluorescéine étaient analogues à ceux que l'on déterminait à l'aide d'anti-Ig non marquées mais les anti-IgM donnaient des titres nettement inférieurs. L'emploi d'anti-IgM ou d'anti-IgG marquées à la fluorescéine n'a pas amélioré la spécificité de la technique.

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TABLE 1. FLUORESCENT ANTIBODY TITRES OBTAINED WITH THE SIX PARASITES AS ANTIGENS AND THE HOMOLOGOUS AND HETEROLOGOUS ANTISERA. EACH FIGURE REPRESENTS THE RECIPROCAL OF AN INDIVIDUAL TITRE

Antiserum to:	Antigen																	
	Plasmodium vinckei			P. chabaudi			P. b. berghei			P. b. yoelii			Babesia rodhaini			B. microti		
	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG
Plasmodim vinckei	2 560	640	1 280	640	160	640	640	80	320	320	80	320	160	40	80	160	40	40
P. chabaudi	640	80	320	2 560	320	1 280	320	10	80	160	neg	80	10	neg	neg	10	neg	10
P. b. berghei	160	40	10	80	20	10	1 280	160	640	160	40	40	80	10	neg	40	neg	neg
P. b. yoelii	40	40	20	80	20	40	160	40	40	2 560	80	1 280	neg	10	neg	neg	neg	neg
Babesia rodhaini	20	10	10	20	20	10	40	10	10	20	neg	neg	2 560	640	1 280	80	20	10
B. microti	20	10	neg	20	10	neg	20	neg	neg	10	neg	neg	30	neg	20	1 280	320	320

TABLE 2. FLUORESCENT ANTIBODY LEVELS OBTAINED WITH TWO SEPARATE SAMPLES OF ANTI-P. VINCKEI SERUM AND TWO SEPARATE SAMPLES OF ANTI-P. CHABAUDI. EACH FIGURE REPRESENTS THE RECIPROCAL OF AN INDIVIDUAL TITRE

Antiserum to:	Antigen																	
	Plasmodium vinckei			P. chabaudi			P. b. berghei			P. b. yoelii			Babesia rodhaini			B. microti		
	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG	Ig	IgM	IgG
P. vinckei (1)	2 560	640	1 280	640	160	640	640	80	320	320	80	320	160	40	80	160	40	40
(2)	640	80	320	160	40	160	160	20	160	80	neg	80	40	10	20	40	10	40
P. chabaudi (1)	640	80	310	2 560	320	1 280	320	10	80	160	neg	80	10	neg	neg	10	neg	10
(2)	160	80	80	2 560	320	640	80	20	80	160	20	80	20	10	neg	10	10	neg

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