Miscellanea : "Plasmodium gallinaceum" as antigen in immunofluorescence antibody studies

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Plasmodium gallinaceum as Antigen in Immunofluorescence Antibody Studies

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Recently TODOROVIC et al. (1) and FERRIS et al. (2) have shown that, using the slide gel precipitation test, a soluble antigen obtained from the sera of chickens infected with *Plasmodium gallinaceum* reacted with a variety of heterologous malarial antisera.

In this study we describe cross reactions between an erythrocyte-bound antigen of *P. gallinaceum* and sera recovered from patients with acute *P. falciparum* and *P. vivax* infections, by use of the indirect immunofluorescent antibody technique.

Method

The method employed was essentially the one described by KUVIN et al. (3). Blood smears from chickens with *P. gallinaceum* infection were made from the 7th to 10th day post infection at which time 80% to 90% of erythrocytes were found to be infected. The films were air dried then stored at — 20°C until use.

Sera from five patients with acute Malaria (three with *P. vivax,* two with *P. falciparum*) were reacted with the slide antigens in dilutions ranging from 1/10 to 1/160. Tests were carried out before and after treatment, which consisted of Chloroquine × 5 days and Primaquine × 14 days. Titers were derived from the final dilution still giving a definitive fluorescence as compared to the non-fluorescence of a preparation using the serum of a healthy individual as antiserum.

In a second group of tests, the erythrocytes from a heavily parasitized chicken were separated from the serum, washed three times in physiological saline and resuspended in serum from a nonparasitized healthy animal. The serum from the parasitized chicken in turn was then mixed with the erythrocytes of the healthy chicken. Blood films were subsequently made as above and allowed to react with *P. falciparum* and *P. vivax* antisera.

Results

In four cases of acute Malaria initial reciprocal titers ranging from 80 to 160 were obtained (Table 1). In one case with *P. falciparum* infection, the initial

TABLE 1

Patient	Antisera to Plasmodium	titer prior to treatment	titer after 5 d. chloroquine	titer after 5 d. Chl. + 14 d. Primaquine
A *	*P. falciparum*	80	—	—
B	*P. falciparum*	10	160	± 10
C	*P. vivax*	160	—	20
D	*P. vivax*	80	—	10
E	*P. vivax*	80	—	20

* Patient succumbed prior to treatment.

reciprocal titer was only 10. The diagnostic blood smear taken at the same time as the serum, showed in this case only few trophozoites (0–2 per field when enlarged 300 ×). This serum was retested 5 days later, the patient meanwhile having received a total of 2100 mg Chloroquine (Nivaquine). This time the reciprocal titer had risen to 160.

By the 20th day of treatment (radical and consolidation cures) the titers had fallen rather markedly, as also demonstrated by COUDERT et al. (4).

When sera B (after 5 days of Chloroquine treatment) and C (prior to treatment) were allowed to react with the antigen preparation containing serum from a chicken heavily parasitized with *P. gallinaceum* and red blood cells from a healthy animal, no fluorescence could be detected. By contrast, identical results to the first experiment were obtained when the antigen consisted of parasitized red blood cells suspended in serum of a nonparasitized animal (Table 2).

TABLE 2

Patient serum	Antiserum to Plasmodium	Titer	
		Antigen from infected RBC and noninfected serum	Antigen from non-infected RBC and infected serum
B	*P. falciparum*	160	0
C	*P. vivax*	80	0

Additional sera from 10 healthy human individuals as well as antisera to Amoeba, Bilharzia, Filaria, Leishmania, Trypanosoma, Ancylostoma and Ascaris gave no fluorescence.

Discussion

As these preliminary results indicate, an antigen from *P. gallinaceum* appears to react serologically with heterologous antisera to *P. falciparum* and *P. vivax*. Although as has been shown by VOLLER (5), the reverse does not seem to hold true. Thus he could detect no serologic activity when he reacted *P. gallinaceum* antiserum with smears of a variety of malaria parasites as antigen. In accordance with TOBIE (6) the titers obtained were lower than those obtained by VOLLER & BRAY (7) with a homologous system, but compare well with the results obtained by COUDERT et al. (4), who tested *P. vivax* antiserum against films of *P. cynomolgi bastianelli* as antigen.

In contrast to TODOROVIC (1), who, on the basis of his experiments, has shown the erythrocyte antigen to be species specific and the serum antigen to be genus specific, our findings seem to indicate an erythrocyte-bound genus specific antigen, the nature of which is unclear as yet.

Investigations are under way to:

a) determine the degree of reaction with more and other human plasmodia as well as eventual cross reactions with different protozoa;

b) establish the specificity of the reaction and elaborate, if possible, a procedure for the serologic diagnosis of malaria by means of the indirect immunofluorescence antibody technique using *P. gallinaceum* as source of antigen;

c) try to determine the nature of the antigen in question.

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