

10. Title:

MALARIA IMMUNITY

Principal Investigators: Robert Desowitz, Ph.D., D.Sc.
Katchrinnee Pinswasdi, M.D.

Assistant Investigator: Barnyen Permpnich

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Objective

A program has been established to study various aspects of the immune response in malaria. Investigations now in progress are: serology and the related characterization of the immune response; antigenic architecture and the effect of antimalarials on protein electrophoretic fractions; factors associated with innate immunity and non-specific immunologic responses. Trials on experimentally induced immunity have been initiated but are at too early a stage to warrant reporting at this time. It is to be noted that these studies were begun in October 1965.

The term malaria encompasses a mosaic of many biological entities. There is a large array of Plasmodium species, each species having its particular host predilections and, in respect of pathological effect, different host-parasite relations. It is our overall aim to make a comparative study of rodent, simian and human malaras. We should like to determine their antigenic relationships as well as the immune response they evoke in their various hosts. Concurrent studies on the physiology of the hosts and of the parasites are also being undertaken in order to give a picture of the inter-related factors of the host-parasite system. It is hoped that these investigations will give us a better understanding of the immunologic and pathologic processes in human malaria. In addition, by examining a variety of animal malaras using, as far as possible, the same techniques as those for human malaria studies, information should be forthcoming that would allow for the selection of the most suitable model systems.

Description and Summary

1. Serology:

Attempts have been made to produce more efficient methods of antigen preparation and tanned cell sensitization for the indirect hemagglutination test. Instead of using water hemolyzed, glass tissue-grinder extracts we have hemolyzed the rbc with dilute saponin solutions. After washing the deposit, it is sonicated for 1/2 hr followed by centrifugation at 15,000 rpm/10'. The supernatant is used as antigen. This sonified antigen seems to give sharper endpoints and somewhat higher sensitivity. For example with block titration the following titres, are obtained with P. berghei antigen.

Antigen dilution

	$\frac{1}{100}$		$\frac{1}{150}$		$\frac{1}{200}$		$\frac{1}{250}$	
	WG	SS	WG	SS	WG	SS	WG	SS
Normal human serum	200	400	200	200	200	100	200	200
Serum malaria patient	800	800	800	6400	200	1600	200	800
Immune rat	52,200	51,200	51,200	51,200	51,200	51,200	51,200	51,200
* WG = Water hemolyzed, ground antigen								
SS = Saponin — Sonicated antigen Titres (reciprocals) with <u>P. berghei</u> antigens								

We have also tried tanning and sensitizing by a new bulk preparation method:

Tanning: 1 vol packed formalized cells: 2 vols PBS (pH 7.2): 3 vol 0.005% tannic acid. Incubate mixture at 37°C/5'. This is followed by washing 2X in PBS and resuspending to 2.5% concentration. Sensitization is by mixing the suspension 1:1 with an antigen dilution and incubating at 37°C/30'. This is followed by the conventional handling of the cells after sensitization. The results with this new method are still equivocal but in some tests the end points have been good and titres higher than by the conventional method. Further work is in progress on these modifications and also the adaptation of the IHAT to a microtitre technique.

Undoubtedly existing serological techniques are too complicated and unreliable for routine laboratory use. Preliminary trials have been carried out by other techniques. We have tried to adapt the latex agglutination by a slide screening method without success. Further work on this test is in progress using tubes and different antigen concentrations. It would be an ideal test if it can be made to work. Agglutination in the presence of antibody following absorption or adsorption onto various types of Sephadex particles has also been tried with slight agglomeration noted. This too is in its early stages and further trials are being carried out.

Passive cutaneous anaphylaxis (PCA) would be a useful tool in assessing the immune response since it is supposed to be mediated by a 7S gamma-globulin antibody. PCA trials have been carried out in guinea pigs using immune rat serum and P. berghei antigen. One faint reaction was obtained after a 4 hr. latent period. Further trials are in progress using various antigen concentrations and latent times. The tests will be extended to human immune sera. We have also obtained one positive skin test reaction in an immune rat with 0.02 ml saponin-sonicated crude antigen. There was no pronounced wheal but an immediate (15') reaction occurred producing a 2.5 cm erythema. This did not occur in the control rat. Further studies on this reaction are also in progress. The aim is to correlate all these serologic techniques with the immune response and the state of functional immunity.

2. Antigenic constitution and the effect of chloroquine on protein electrophoretic fractions.

Disc electrophoresis has the ability of resolving protein mixtures into sharply defined bands. The method was applied to the P. berghei antigen used in IHAT. A typical pattern is shown in fig. 1. There appears to be one broad relatively immobile band that is composed of at least two peaks. There is a second broad mobile band and finally a third, sharply defined fraction which is the most mobile of all the fractions. The electrophoretic architecture resembles that of cell-free extracts of African trypanosomes (Desowitz, 1959; Williamson and Desowitz, 1961). In those extracts the broad immobile fraction is probably a nucleoprotein. Work is now in progress to determine the chemical nature of the plasmodial electrophoretic fractions and determine which fraction or fractions might participate in the IHAT.

It has been shown that basic trypanocidal drug combine with and precipitate protein of trypanosomal extracts (Desowitz, 1960). By electrophoretic analysis it was found that the nucleoprotein fraction seemed to be the specific site of combination (Desowitz, 1960). Furthermore the drug-protein combination exerted greater prophylactic activity than either the drug or antigen alone. In view of this it was thought of interest to determine whether a similar effect was produced by chloroquine on cell-free extracts of P. berghei.

Cell-free extracts of P. berghei were prepared from washed suspensions of heavily infected mouse erythrocytes. The rbc were hemolyzed twice with 1:10,000 saponin and the deposit of parasites washed four times in phosphate buffered saline. The washed parasites were thoroughly ground in a Griffiths tube and this was followed by sonication, in the cold, for 30'. The material was centrifuged, under refrigeration, for 15' at 20,000 rpm x G. The supernatant was divided into several aliquots, one aliquot set aside as control, and varying amounts of chloroquine sulphate, in powder form, added to the others. The aliquots were then electrophoresed in polyacrilamide gels (Canalco apparatus) stained and patterns constructed with the Canalco scanning and recording apparatus. A progressive decrease, with chloroquine concentration, in the electrophoretic fractions, particularly the fast moving band and the broader band preceding it was observed. Fig. 1 illustrates the control extract pattern and that of the extract to which 1 mg of chloroquine was added to 1 ml of extract, the maximum concentration used. Work is now in progress to determine whether the decrease in these fractions is due to actual binding and possibly denaturation with chloroquine or is a non-specific effect influencing the electrophoretic procedure.

3. Non-specific immunologic factors.
 - a. ABO blood group titers in malaria.

It is believed that the intravascular agglutination of erythrocytes contributes to the clinical manifestations in malaria, particularly cerebral malaria due to P. falciparum. The cause of this agglutination is unknown but two hypothesis are; (1) an autoimmune reaction and (2) a plasmodial surface antigen present on the membrane of the infected cell. The relationship of blood group to susceptibility has not been adequately resolved either. For example Green (1929) found no relationship to blood type and malaria infection but Myatt et al (1954) that the average prepatent period to blood induced P. vivax in group O subjects was 11.3 days while in other blood group subjects it was 3.7 days.

CONTROL EXTRACT PATTERN



1 MG CHLOROQUINE / 1 ML. EXTRACT PATTERN.



FIG. 1. THE EFFECT OF CHLOROQUINE ON THE ELECTROPHORETIC FRACTIONS OF CELL-FREE EXTRACTS OF P. BERGHEI

Our initial approach to the problem has been to actually measure the ABO titer by a simple hemagglutination test in Kahn tubes. Blood group type was first determined with Eldon blood group cards. The analysis of many hundreds, if not thousands, of sera from both normal controls and malaria patients is required before any reliable pattern can be determined but the results of our initial tests are shown in the accompanying table.

The preliminary data indicates a much wider range of titers, for the malaria group than for the normals. There is also a notably greater percentage of sera that are of high titer amongst the malaria group as compared to the controls. However it must again be emphasized that many more samples are required to confirm this pattern and to determine if there is any relationship of titer to blood group or stage and species of infection.

Hemagglutination tests have also employed trypsinized erythrocytes in order to determine whether an incomplete blood group antibody might be present. No differences in titer were noted between untreated and trypsinized erythrocytes and sera negative with untreated cells remained negative when tested against trypsinized rbc.

	No. Sera tested	ABO Titre (reciprocal)												
		Neg	10	40,50	80	160	200	320	640	800	1280	1600	6400	12,800
Normal sera	19	12 (63%)	0	2 (5%)	3 (16%)	0	0	2 (5%)	0	0	0	0	0	0
Malaria pts.	39		1 (2.5%)	6 (15%)	1 (2.5%)	3 (8%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	2 (5%)	1 (2.5%)	2 (5%)	2 (5%)	4 (10%)

4. Attempts to infect heterologous hosts.

These investigations were begun by Dr. Weinman and have been continued since his departure. Blood from malaria patients at the Phrabuddabat and Cholburi hospitals was inoculated into chick and duck embryos; infant mice, splenectomized and/or cortisone treated mice, and infant and splenectomized hamsters and rats. Routes were intra-vascular except in infant animals in which it was intra-peritoneal or intra-cerebral. Whole heparanized blood was used as well as washed erythrocytes. Some animals received normal human serum before and after inoculation since it has been shown that normal-host serum will support growth of trypanosomes in a heterologous rodent host (Desowitz and Watson, 1952). P. falciparum infected blood, usually about 1% infection, was used in all cases with the exception of one trial with P. vivax.

As expected, in almost all animals the plasmodia were rapidly removed from the circulation; the few successes are possibly important.

In these instances, persistence of P. falciparum was observed and with persistence possibly, development. Relatively, infant mice seemed to be the best host, and in one such animal schizonts with 9-12 segments were observed. In addition there were a number of more immature schizonts with 2 or 3 nuclei. This development was seen 5 days after injection of blood in which only ring forms were seen.

Further experiments are planned along these lines and a technique is now being worked out that will permit the transfusion of large amounts of infected blood into the recipient host.

Inoculation of P. berghei into a tree shrew (Tupaia glis) failed to produce infection.

Intravenous and intraperitoneal inoculation of 5 ml. blood from an untreated American infected with P. vivax failed to produce infection in two splenectomized rhesus monkeys of Thai origin.

5. Miscellaneous:

Several hundred slides from birds taken during the MAPS project have been examined. One bird had a Plasmodium infection but this was very scanty and the parasite is unidentifiable. Many birds were found to be infected with Haemoproteus sp. Microfilariae were seen in one blood film.

Bats captured in Chiangmai were found to be infected with a Hepaticystis-like parasite. Further study of the organism is required before definite identification can be made.

The Parasitology Department has continued to examine blood films sent by other departments. Blood films and stool specimens from Dr. Rucknagel's project are also being examined.

References

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