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Mechanisms of immunity to malaria*

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The erythrocytic phase of malarial infection provides a potent stimulus for the production of specific malarial antibody. Serologic tests do not provide any indication of immune status, so that much specific antibody has no protective function. The role of serum antibody in acquired malarial immunity has, however, been established by passive transfer tests, and in the case of P. knowlesi *by specific inhibition of the cyclic growth of parasites* in vitro. *The inhibitory antibody appears to combine with merozoites and prevents their attachment to red cells, thus interrupting the cyclic proliferation of the parasite. The inhibitory antibody response is predominantly variant-specific, but cross-reacting antibody occurs in sufficient amount to suppress proliferation of most other variants of the species. The occurrence of cross-immunity between variants is encouraging from the point of view of vaccination. If it were possible to isolate cross-reacting antigens, these could provide the basis for a malarial vaccine effective against erythrocytic forms of the parasite.*

The clinical manifestations of malaria are, in many instances, effectively controlled by the specific immune response of the host. Clinical and experimental observations and, in particular, *in vitro* studies on cultured parasites have elucidated the nature of these immune protective mechanisms. However, acquired malarial immunity is often slow to develop and in many species is associated with persistent low-grade parasitaemia, a phenomenon referred to as "premunition". It is therefore apparent that plasmodia effectively sensitize the host's immune system and yet may survive for long periods. The mechanisms that allow parasites to evade the potentially lethal consequences of immunization remain a central problem of malarial immunity.

In this paper, our present understanding of the nature of immune effector responses is outlined; the nature of malarial immunity and the mechanisms for its evasion by parasites are discussed; and finally, on the basis of these findings, the outlook for malarial vaccination is briefly assessed.

IMMUNE EFFECTOR MECHANISMS

Adaptive immune responses are initiated by the interaction of an antigen with specific receptors on the surfaces of lymphoid cells. Two major classes of lymphocytes are recognized and these react differently after induction by the antigen. T-lymphocytes undergo transformation and mitosis to produce a population of cells specifically reactive with the inducing antigen; B-lymphocytes differentiate into plasma cells which secrete humoral antibody, but this reaction usually requires cooperation with T-cells. The effector mechanisms generated by these cellular responses (summarized in Table 1) therefore fall into two major categories. The first is mediated by specific humoral antibody and the second by specifically sensitized T-lymphocytes (cell-mediated immunity). While it is true that antibody action may, in certain circumstances, be independent of cells (reactions 1 and 2, Table 1) and that certain specific cell-mediated reactions are independent of antibody (reaction 6, Table 1), there remain several immune effector mechanisms that require the interaction of specific antibody with cells such as macrophages (reactions 3 and 4, Table 1) or mast cells (reaction 5, Table 1). In addition, specific interaction of antigen with T lymphocytes may liberate cytotoxins and macrophage activating factors and so damage cells or organisms that do not carry the specific antigen (reactions 7 and 8, Table 1).

THE IMMUNE RESPONSE TO MALARIA

Specific malarial antibody

Chronic malarial infection in man provides a most potent stimulus for Ig synthesis (1, 2, 3). The reduced IgG production observed in West Africans main-

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Table 1. Immune effector mechanisms

SPECIFIC MECHANISMS				
Reaction	Effector Ig	Effector cell	Effector complex [b]	Biological effect
1	all classes	—	Ant–Ab	neutralization activation
2	IgG3 IgG1 IgG2 IgM	—	Ant + Ab + C	complement-dependent cytolysis
3	IgG1 IgG3 IgM SMAF [a]	macrophage (phagocytic)	Ant + Ab(+C) combined with macrophage	immune adherence endocytosis
4	Ig	K-cell (nonphagocytic)	Ant + Ab combined with K cell (nonphagocytic)	Ab-mediated cytolysis
5	IgE	mast-cell	Ant + Ab combined with mast cell	immediate hypersensitivity
6	—	T-cell	Ant + T-cell	cell-mediated cytotoxicity
NONSPECIFIC MECHANISMS				
Reaction	Cooperating cell	Effector cell	Effector complex	Biological effect
7		T-cell	Ant + T-cell→ cytotoxin	nonspecific lymphocyte-mediated cytotoxicity
8	T-cell	macrophage	Ant + T-cell→ Mediators→ activated macrophage	nonspecific macrophage-mediated cytotoxicity

[a] SMAF = specific macrophage activating factor, a cytophilic product of T cells with antigen specificity.
[b] Ant = antigen; Ab = antibody; C = complement.

tained on malarial prophylactic therapy (3) suggests that in subjects exposed to chronic infection about a third of the circulating immunoglobulin might consist of malarial antibody. Specific antibody can be demonstrated in immune sera by several serologic tests including precipitation, agglutination, opsonization, antibody fluorescence, and complement fixation. The fact that the serologic cross-reactions between malarial species revealed by these tests cannot be correlated with cross-immunity indicates that much of the specific antibody formed during infection does not have a protective function.

Protective malarial antibody

Acquired immunity in malaria is directed mainly against the asexual parasite cycle in the blood. Circulating gametocytes of *P. falciparum* are apparently unaffected by immune serum (3), although in some monkey malarias immunity is associated with a loss of gametocyte viability in the mosquito host. Immunity to the erythrocytic stage of infection does not modify the exoerythrocytic development of malaria parasites in man, chimpanzee, or monkey (4), but suppresses the subsequent phase of erythrocytic development.

The role of serum antibody in acquired malarial immunity has been established by passive transfer tests in monkey and human infections, and, to a lesser degree, in rodent malaria (5). These studies suggested that protective antibody acts against either mature schizonts or extracellular merozoites, and provided some information about the classes of Ig associated with immune protection (3). However, passive transfer tests do not provide a suitable basis for detailed investigations on the mechanism of malarial immunity.

Serum from rhesus monkeys immune to *P. knowlesi* has been shown to inhibit the cyclic proliferation of the parasite maintained *in vitro* (6). Parasite growth was assessed by incorporation of ^{3}H-leucine into parasite protein using cultures giving average multiplication rates of at least sixfold in 24 hours. Immune serum had no effect upon the growth of intracellular parasites but inhibited the

cycle of development that followed schizogony. This effect was species-specific, as was shown by the failure of serum from a monkey immune to *P. cynomolgi bastianellii* to inhibit *P. knowlesi*. The immune serum appears to act directly on the parasites (Table 1, reaction 1), and its effect is not complement-dependent (Table 1, reaction 2) being unaffected either by heating at 56°C for 3 h or by the addition of fresh normal monkey serum as a source of complement; moreover, the $F(ab')_2$ fragment of immune IgG is actively antiparasitic. The degree of parasite inhibition is dose-dependent. In the sera studied, protective antibody was associated with both IgG and IgM, but not with IgA or IgE (7). The failure to demonstrate activity in monkey IgE is of interest because levels of IgE are high in human and monkey parasitic infections, and this, together with earlier observations on the common occurrence of immediate type sensitivity, had suggested a possible protective role for IgE in parasitic diseases (Table 1, reaction 5).

There has long been a need in malarial research for a dependable *in vitro* technique for the detection of protective malarial antibody. The schizont-infected red cell agglutination test (SICA) (9) and the assay of inhibitory antibody described above have been proposed for this purpose (6). Comparison of these antibody activities with the clinical immune status of rhesus monkeys (18) reveals that SICA antibody titres of appropriate specificity may be high in susceptible animals or undetectable in immune animals; on the other hand, inhibitory antibody levels correlate with immune status in all situations that have been tested (Table 2).

These observations indicate that the inhibitory antibodies assayed during *in vitro* culture initiate specific protective immunity *in vivo*. This antibody is probably directed against merozoites and its action *in vitro* is independent of complement or cells and is analogous to viral neutralizing antibody (Table 1, reaction 1). Inhibitory antibodies are predominantly IgG and, since this class is cytophilic for macrophages and K-cells, it seems certain that cell-mediated killing of parasites must also occur in the living animal (Table 1, reactions 3 and 4). The phagocytic activity of macrophages has long been recognized in malaria, and the role of specific antibody in promoting macrophage ingestion of parasites has been demonstrated *in vitro* and *in vivo*. Synergism between cells and antibody is suggested by the finding that immune splenic cells confer greater protection than serum when passively transferred to rats challenged with *P. berghei* (14). Similarly, the antimalarial action of passively transferred immune serum in rats is greatly diminished by previous splenectomy of the normal recipients (22).

Role of cell-mediated immunity in malaria

The proven role of serum antibody, outlined above, does not exclude the possibility that cell-mediated immunity, dependent upon actively sensitized lymphocytes of thymic origin (Table 1, reaction 6), may play a part in specific acquired resistance to malaria. Attempts to demonstrate this have rested upon the following findings:

(*a*) Thymectomized rats are more susceptible than control animals to subsequent infection with *P. berghei* (10).

Table 2. Clinical immunity to *P. knowlesi* variants in relation to SICA and inhibitory antibody titres in rhesus monkeys [from Butcher & Cohen (18)]

Immunization [a]	Challenge variant	Antibody to challenge variant		Clinical immunity
		SICA	Inhibitory	
repeated infection with W1 strain	W1	++	++	+
repeated infection with W1 strain	W2	0	+	+
repeated infection with G strain	W1	0	++	+
one infection with W1	W1	++	+ or 0	+ or 0

[a] *P. knowlesi* strains: W, supplied by Walter Reed Army Institute; G, supplied by London School of Hygiene and Tropical Medicine.

(*b*) Anti-rat thymocyte serum (ATS) raised in rabbits reduces the resistance of rats to subsequent infection with *P. berghei* (11).

The interpretation of these findings solely in terms of cell-mediated responses is complicated by the fact that the majority of antigens are now known to require cooperation between thymus (T) and marrow (B) cells for induction of specific serum antibody. It follows that thymectomy or ATS may render animals susceptible to malarial infection by reducing the serum antibody response.

(*c*) Lymphoid cells transferred from immune rats to inbred nonimmune animals confer resistance to *P. berghei* (12). This finding cannot, however, be taken as evidence for cell-mediated immunity alone since such cells are capable of producing specific antimalarial antibody (13).

Since the published studies (14) and our experiments show that immune lymphocytes are not antiparasitic when tested *in vitro*, it must be concluded that cell-mediated immunity has no defined role in specific malarial resistance.

SURVIVAL OF PARASITES IN THE IMMUNIZED HOST

The mechanisms that lead to a state of "premunition" in malaria, i.e., clinical immunity associated with continued low-grade infection, have long been a subject of speculation. Interest in this phenomenon is increased by recent data revealing the potent immunogenicity of the parasite, the successful passive transfer of immunity (3), and the effective *in vitro* inhibition of cyclic plasmodial growth by specific antibody (6). Under natural conditions, the apparent survival of the organism may in fact be related to reinfection with distinct strains of the species or represent a relapse derived from persistent preerythrocytic stages of the plasmodium. In addition, however, laboratory studies have shown that recrudescences do arise from preexisting systemic infection. Such recrudescence could be accounted for by soluble immune complexes or free antigen blocking the action of antibody in a manner analogous to the blocking associated with neoplasms. Alternatively, the immunosuppressive effect of malarial infection or the action of protozoal factors that specifically inhibit host defence mechanisms (15) might lead to recrudescent infection. One significant mechanism for parasite survival is connected with the ability of the organism to undergo antigenic variation.

The occurrence of antigenic variants during the course of the asexual cycle (5) has been described in *P. berghei* infections of mice, in the monkey malaria parasites *P. knowlesi* and *P. cynomolgi bastianellii*, in the avian malaria parasites *P. lophurae* and *P. gallinaceum*, and in the human malaria parasite *P. falciparum*. In *P. knowlesi*, a wide spectrum of antigenic variants is recognizable on the basis of the schizont agglutination (SICA) test (9). Schizont agglutinins are also present in *P. gallinaceum* and *P. falciparum* infections, but cannot be demonstrated in many other species in which the occurrence of serologic variation is deduced from infectivity tests. Antigenic variation could account for many features of premunition mentioned above, since each relapse during a chronic *P. knowlesi* infection is associated with a distinct variant (9). Recent experiments indicate that SICA antibodies, which as mentioned above are not protective, induce antigenic variation of *P. knowlesi* (Table 1, inductive reactions) in a manner analogous to that observed in free-living ciliates (16). These antibodies may therefore promote the survival of plasmodia in the immunized host. However, other mechanisms must also be operative since SICA antibodies cannot be detected in many malarial species that show antigenic variation.

The importance of antigenic variation as a cause of relapse must be assessed in the light of the following facts.

(*a*) Spontaneous relapses in *P. knowlesi* infections are usually well controlled by the immunized host and parasitaemia rarely rises above 1%; nevertheless, the relapse variant produces a rapidly fatal infection if inoculated into an unimmunized recipient.

(*b*) After repeated challenge with a single variant of *P. knowlesi*, rhesus monkeys are equally resistant to the same and several other variants (17, 18). For example, monkeys repeatedly challenged with a single variant (W1) of *P. knowlesi* were immune to W1 and several serologically distinct variants (e.g. W2, W3). Sera from these animals showed inhibitory antibody directed predominantly against the specific variants to which the animal had been exposed, but also contained lower levels of cross-reacting antibody directed against variants that had never been patent. Animals were therefore sensitized for a rapid protective antibody response when infected with new variants. The presence of this cross-reacting antibody explains why *P. knowlesi* parasites, which arise by antigenic variation during chronic infections, produce mild parasitaemia in the host and yet are fully virulent in normal monkeys.

PROSPECTS FOR VACCINATION AGAINST ERYTHROCYTIC FORMS OF MALARIA

An ability to evade the lethal consequences of the host's immune response constitutes an essential requirement for obligate parasitism (15). As shown above, parasites sensitize the host's immune system and their survival depends upon evasion of effector immune mechanisms. Our knowledge of how this is achieved in malaria is incomplete, but in the case of *P. knowlesi* the importance of antigenic variation is established. Other possible mechanisms, including the presence of serum blocking factors, have not been investigated. The circumstances that favour parasite survival in natural infections may be equally operative in determining the outcome of artificial vaccination and it is not surprising that attempts to induce immunity have achieved only limited success. The occurrence of cross-immunization between variants of *P. knowlesi* is however encouraging from the point of view of vaccine production. A vaccine containing cross-sensitizing antigens may be expected to induce a degree of clinical immunity similar to that observed after chronic infection. Our own search for such antigens involves (*a*) the isolation of soluble plasmodial fractions that effectively absorb out inhibitory antibody of several variant specificities from immune sera (20) and (*b*) the production of extracellular merozoites in high yields from malaria cultures using appropriate antisera or lectins to precipitate red cells and intracellular parasites (21). The immunizing properties of these protective antigen fractions and pure merozoite preparations are at present being tested in this laboratory.

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RÉSUMÉ

MÉCANISMES DE L'IMMUNITÉ DANS LE PALUDISME

La phase érythrocytaire de l'infection paludéenne est un puissant stimulant de la production d'anticorps antipaludiques spécifiques. Les épreuves sérologiques ne fournissent aucune indication sur le degré d'immunité, ce qui montre qu'une grande partie des anticorps ne jouent aucun rôle protecteur. La contribution des anticorps sériques à l'immunité acquise a cependant été mise en évidence par des épreuves de transfert passif et, dans le cas de *Plasmodium knowlesi*, par l'inhibition spécifique de l'évolution cyclique du parasite *in vitro*. En se combinant avec les mérozoïtes, les anticorps inhibiteurs les empêchent d'adhérer aux érythrocytes et interrompent la multiplication cyclique du parasite. La réponse en anticorps inhibiteurs est essentiellement spécifique de variant, mais la production d'anticorps à réactivité croisée est suffisante pour inhiber la prolifération de la plupart des autres variants d'une espèce.

L'existence d'une telle immunité croisée est un facteur encourageant en ce qui regarde la vaccination. Si l'on parvient à isoler des antigènes à antigénicité croisée, on disposera des éléments de base pour la mise au point d'un vaccin actif contre les formes érythrocytaires du parasite.

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DISCUSSION

MOLINEAUX: With respect to the persistence of low parasite densities among immune persons in endemic areas, I would argue that the circulating population of parasites must be equal to the ratio between the number added per unit time and their death rate. It would therefore be zero only if the rate of addition became zero or if the death rate became infinite; if immunity effects neither, that by itself could explain persistence of a small parasite population.

COHEN: I doubt whether sucn a rigorous but simple mathematical model could be applicable to such a complex problem.

BRUCE-CHWATT: What is the explanation of the fact that the injection of heterologous antilymphocyte serum into Balb/C mice infected with *P. berghei yoelii* (which normally controls the infection) increases parasitaemia and lengthens the course of the infection without changing the immunofluorescent antibody pattern of immune response?

COHEN: Antilymphocyte serum acts against T-lymphocytes. Antibody response requires the cooperation of T-cells—hence if T-cell populations are destroyed no further antibody response of that particular type occurs. But, even after the injection of antilymphocyte serum, the fluorescent antibody response would persist for some time because the serum has no effect on plasma cells already induced to produce antibody.

NUSSENZWEIG: How can one explain the higher inhibitory antibody titres against the heterologous *P. knowlesi* strain in the laboratory-bred natural host and the absence of heterologous reactions in rhesus? Does the rhesus monkey respond to a more restricted population of antigenic determinants?

COHEN: The levels of antibody in the rhesus during the first week of infection probably do not reach significant levels. Those in the natural host are significant and of broad specificity.

NUSSENZWEIG: Are merozoites inactivated more rapidly by immune serum than the time taken for them to invade a new erythrocyte and is the activation complete or can invasion occur in immune serum?

COHEN: Merozoites attach themselves to erythrocytes in 10–15 minutes. Agglutination is faster and prevents attachment.

LUZZATTO: How is it determined whether merozoites penetrate erythrocytes and is there early destruction of the parasites after penetration?

COHEN: In *in vitro* culture, penetration of new cells does not occur in immune serum. Some penetration did occur, with bush-baby erythrocytes, but the parasites did not develop.

BRAY: The erythrocyte is not the only area of susceptibility. First there is the tissue barrier—i.e., the hepatic cells in mammalian malaria. Then there is penetration of erythrocytes but no development unless the spleen is removed, as is the case with *P. falciparum* in the chimpanzee. Is there any evidence of acquired resistance to invasion by an erythrocyte already invaded?

COHEN: No, the erythrocyte—unlike the fertilized ovum—can be invaded again.

BRAY: Is there any correlation between McGregor's and Wilson's analysis of placental antigen and an antigen giving rise to Cohen's antimerozoite globulin?

McGREGOR: There is a group of antibodies that pass across the placenta and react with heat-labile antigens. These would be our choice for further investigation in this respect.

VAN DER KAAY: Is there any evidence of the prolongation of the asexual cycle in immune serum?

COHEN: We have not found any.

MEUWISSEN: Why are so many schizonts found in the placenta of pregnant women who are immune? Are there conditions in which a merozoite from one erythrocyte can pass to another erythrocyte through immune serum, for instance in the clogged capillary of the brain in falciparum malaria?

COHEN: The question of the immune response in pregnancy is very difficult. Some responses may be suppressed. Whether a travelling merozoite would effectively combine with an erythrocyte or with antibody is a question of relative concentrations and affinities of reactants and cannot be answered in simple terms.

BRAY: Does Professor Cohen mean that cross-immunity between variants resides in the merozoites and their variant common antigens, whereas Neil Brown ascribes the cross-immunity to T-cell memory and to T–B-cell interaction?

COHEN: It depends on what antibody one thinks is protective. We think antimerozoite antibody is effective. Neil Brown thinks schizont agglutinating antibody is effective and has produced the T-cell theory to explain observed clinical cross-immunity.

GRAMICCIA: If parasitization of red blood cells is due to the presence of available receptors, why does splenectomy cause susceptibility in some normally nonsusceptible species of host and why does a single sojourn in the splenectomized host then give rise to a strain of parasites that will infect the intact host? Does this mean that there can be changes in the merozoite receptors after experiencing a new host?

COHEN: One can only say that splenectomy must eliminate the early antibody responses, both natural and acquired. There may be a range of affinities on the surface of merozoites and selection for high affinity receptors may occur during infection.