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Malaria: immunity, vaccination and immunodiagnosis

by L. Perrin, A. Perez and C. Chizzolini

Geneva Blood Center, WHO Unit, Department of Medicine, Geneva University Hospital, CH-1211 Geneva 4 (Switzerland)

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The range of investigations in the field of immunology of malaria is immense and covers various areas from immunopathology to both natural and specific immunity, immunodiagnosis and vaccination trials in experimental animal models. Over the last few years, a number of excellent reviews have been published on several of these aspects[5, 15, 16, 18, 44, 54, 70, 74]. The present review will therefore be restricted to major concepts, will mention more specialized reviews and concentrate on recent developments, particularly those concerning *P. falciparum*, the most lethal species for man.

I. Immunity

The development of resistance to malaria in man depends on the frequency and duration of exposure to the parasite[49, 55]. In non-immune human volunteers, *P. falciparum*, *P. vivax* and *P. ovale* infections have a duration of more than 2 years and *P. malariae* may persist for over 30 years. In endemic areas, babies from immune mothers are resistant to malaria during their first 3 months of life. Later, they suffer from severe and recurrent attacks and most deaths due to malaria occur in young children[49]. From adolescence to adulthood, there is a progressive decrease in the severity and frequency of malaria attacks and clinically significant malaria becomes infrequent in adults, except pregnant women. Resistance in humans therefore builds up slowly following the course of successive infections, but sterile immunity is probably never achieved and low grade parasitemia is still observed from time to time in adults living in endemic areas[49, 55]. These observations suggest that humans and, more generally, vertebrate hosts can raise a protective immune response but that plasmodia,

like other parasites, have developed ways of partially escaping the protective effector mechanisms of their hosts.

1. Natural immunity

There is clear evidence that the high frequency in malaria endemic areas of several single gene disorders affecting the RBC and its components such as sickle cell anemia, the thalassemias, and glucose 6 phosphate deficiency, is due to the protection which is afforded to heterozygote carriers against malaria[44,65]. Impairment and retardation of parasite growth and/or higher sensitivity to oxydant stress has been observed in the RBC carrying the defects mentioned above[23,65]. Other factors involved in natural resistance to malaria are related to the efficiency of merozoite attachment and invasion of RBC[54]. Malaria merozoites can invade only the RBC of certain host species and have a preference for a given age-related subpopulation of red blood cells[54]. The attachment of merozoites to RBC involves the presence of an erythrocyte receptor; for example, individuals lacking the duffy blood group antigen are resistant to *P. vivax* infection[53,54] and it has been postulated that an antigen closely related or linked to the duffy blood group acts as a receptor for *P. vivax* merozoites[53,54]. For *P. falciparum,* it has been suggested that the initial binding of merozoites to the RBC reflect a lectin-ligand type interaction in which the parasite binds to a cluster of oligosaccharides present on glycophorin A and B[38]. In this context, it has been found that in African endemic areas, there is a high incidence of individuals who lack the duffy blood group or who have a glycophorin B deficiency[38,54].

Malaria has probably selected not only for genes which influence innate resistance but also for genes which control the amplitude of the specific immune response. This is suggested by the increased frequency of HLA A2 and B17 found in malarious areas of Sardinia while no excess of any HLA genes was detected in areas of Sardinia unexposed to malaria[64].

2. Acquired immunity

During most of the malaria parasite life cycle, its development is intracellular and the parasite is therefore protected from direct contact with effector immune mechanisms of the host such as antibodies and effector cells. However, parasite components are exposed to host immune mediators each time it invades a new host cell and during fertilization. The invasive forms of the parasite (sporozoites, merozoites) and gametes are highly differentiated. Sporozoites and merozoites are coated with stage-specific components which are specifically designed for the recognition of the specific cell type to be invaded. The 3 extracellular developmental forms of the parasite are the obvious targets for immune attack by the host. These elements explain the well demonstrated stage specificity of acquired immunity. For example, mice immunized with *P. berghei* sporozoites are resistant to sporozoite challenge but not to challenge with asexual blood stages[61]. Similarly, immunity to gametocytes may develop without effect on asexual blood stages[8,50]. Immunity to malaria has therefore to be approached separately for the 3 extracellular stages. In addition to its stage specificity, acquired immunity is predominantly species-specific and, for some malaria species including *P. falciparum,* to some extent isolate-specific[3,15,37].

The immune response to plasmodia is complex and the respective roles of its various components in the development of an acquired immunity to malaria are still not elucidated, but the available data point towards complex mechanisms involving both humoral and cellular immune reactions.

a) Humoral response

There is considerable evidence to show that specific antimalarial antibodies play a major role in resistance to malaria in man and other vertebrates[11,15]. Antibodies present in the sera of individuals repeatedly infected with malaria react with parasites of various developmental stages[88]. Using sera from experimental animals vaccinated with parasites of different developmental stages, it has also been demonstrated that antimalarial antibodies recognize both antigens common to the various developmental stages and stage-specific antigens; the latter appear to be involved inprotective immunity.

A protective role for antisporozoite antibodies is suggested by a variety of experimental data, mainly obtained using murine malaria and showing: 1) a reduction of sporozoite infectivity after incubation with immune serum[60]; 2) an increase in the rate of sporozoite clearance after injection of immune sera in normal mice[62]; 3) a protection of mice from a lethal sporozoite infection following passive injection of monoclonal antibodies reacting with the surface coat of sporozoite[102]. This particular monoclonal antibody also inhibits the in vitro attachment and penetration of sporozoites into their target cells[32].

There is ample evidence that antimalarial antibodies directed against asexual blood stages have a protective effect against malaria infection[5,14,15,22,52]. Passive transfer of immunoglobulins from the sera of adults with a high degree of immunity to malaria caused a dramatic fall in the number of asexual forms of *P. falciparum* and *P. malariae* in infected children, but the number of gametocytes remained unaffected[11]. Similar transfer experiments have been successfully conducted in experimental animal models using malaria species infecting monkeys and mice[22,76]. Evidence from in vitro studies indicate that human antimalarial antibodies may agglutinate merozoites and schizonts[26], prevent invasion of RBC by merozoites[73], enhance phagocytosis of schizonts and merozoites[10,40], and may destroy *P. falciparum*-infected RBC in presence of lymphocytes[7]. Each of these mechanisms is likely to influence the outcome of malaria infection but their importance in vivo is not known. Direct demonstration of the role of anti-merozoite antibodies in vivo has come from experimental studies using monoclonal antibodies: monoclonal antibodies directed against *P. yoelii* merozoites but not those directed against *P. yoelii* trophozoites were protective in vivo following passive transfer in normal mice[22].

Gametes mature and fertilize in the midgut of mosquitoes after ingestion of a blood meal. Antigamete antibodies present in the ingested blood can block the fertilization of gametes and therefore block transmission of the disease (transmission blocking immunity)[8, 30, 50]. This effect has been demonstrated in vivo by immunization of various vertebrates with gametes before induction of malaria infection and mosquito feeding[8, 30] and in vitro by feeding mosquitos with blood containing gametocytes and antigamete antibodies[9, 79].

b) Cellular response

While the role of humoral immunity in malaria is relatively easily demonstrated, the role of cell-mediated responses has been difficult to evaluate in man and has been investigated mainly in murine malaria. The involvement of T cells includes a variety of functions such as cooperation with B cells for antibody synthesis, specific T cell cytotoxicity and production of mediators which recruit and activate other cells including macrophages and NK cells. The role of T cells in malaria has been investigated by using animals selectively depleted in T or B cells[4], cell transfer experiments[25, 87] and correlation between T cell dependent response and immunity[91]. In rodent models, these kinds of experiments indicate that antibody is certainly protective against sporozoite or blood stage-induced infection but that resistance can also be induced in its absence, for example, mice treated with anti-μ chain serum which suppresses humoral immunity can be successfully immunized with sporozoites or merozoites[11]. In rats immune to *P. berghei,* both T and B cells can confer protection[25]. Good indirect evidence for the role of T cells is provided by experiments using nude mice or T cell-deprived mice which cannot develop immunity after immunization with sporozoites and cannot control a normally non-lethal blood-induced *P. yoelii* infection[88, 95]. The involvement of T cells has also been suggested by delayed hypersensitivity tests which have shown some correlation with protection. In human, simian and murine malaria in vitro lymphocyte transformation has also been demonstrated upon exposure to homologous parasite antigens[91, 101]. There is also increasing evidence that soluble mediators such as interferon[36] and macrophage necrotizing factor[89] induced by T cell activation or activated macrophages contribute to the elimination of malaria parasites and are probably involved in the intracellular parasite damage observed at the time of recovery of malaria infection.

c) Other factors

The course of malaria infection is influenced by a number of non-specific factors inducing the release of lymphokines such as BCG and *C. parvum,* polyclonal antibody formation[80], circulating immune complexes[67], alteration in the number and traffic of lymphocytes, enhanced phagocytic activity of macrophages and monocytes and splenomegaly. Some of these factors, e.g. macrophage activation[43], enhanced phagocytic activity of macrophages[27], have a protective effect but others, e.g. polyclonal antibody formation and immune complexes, may contribute to the prolonged survival of the parasite for a long period of time. Other factors which may be responsible for the long survival of the parasite include its intracellular development, the existence of variant antigens at the surface of asexual blood stages *(P. knowlesi)*[3], or of a diversity of these antigens within the same species *(P. falciparum)*[1, 35, 47, 83], the release of soluble parasitic antigens in high concentration[97, 100], a parasite-induced immunosuppression of the host's immune response[28], and the presence of a parasite-associated mitogen[29].

d) Immunological response to defined malarial antigens

Up to 10 years ago, studies on the immune response of infected hosts towards malaria infection had a phenomenologic character and were aimed at the dissection of the respective roles of natural immunity and specific cellular or humoral immunity. Recent advances in biochemistry, molecular biology, cell culture and the development of monoclonal antibody technology have provided new tools for immunologists and parasitologists for the identification of target antigens of immune effector mechanisms. Several recent articles have been devoted to detailed reviews of this approach combining identification, characterization and study of the biological role of defined malarial antigens[16, 18, 69, 71]. A few investigations will be mentioned here to illustrate this kind of approach.

Monoclonal antibodies raised against the major surface component of *P. berghei* sporozoite (circumsporozoite protein of 44 kdaltons) have been shown to protect mice, following passive transfer, from sporozoite challenge infection[101]. In vitro studies have also indicated that monoclonal antibodies directed against circumsporozoite antigens inhibit penetration of sporozoite in their vertebrate target cells[32]. Antigens of the same class, responsible for the circumsporozoite reaction have been identified for *P. knowlesi, P. falciparum* and *P. vivax* sporozoites[13, 59]. Part of the gene coding for *P. knowlesi* circumsporozoite antigen has been cloned and its structure elucidated[24, 63]. The circumsporozoite antigen has an unusual structure comprising, in its central part, 12 tandemly repeated aminoacid peptide units, which may be the only part of the circumsporozoite antigen exposed to immune effector mechanisms[24]. More recently, the gene coding for the major epitope of the polypeptide expressed at the surface of *P. falciparum* parasites has been cloned[21]. The DNA sequence of the complementary DNA insert encodes a four amino acid sequence: proline-asparaginase-alanine-aspuragine, tandemly repeated 23 times[21]. Whether or not such a tandem repeat structure will affect the efficiency of immune effector mechanisms of the infected host remains to be elucidated.

Passive injection of monoclonal antibodies directed against *P. yoelii* merozoites into normal mice protected them from lethal blood-induced challenge[11]. The antigen (235 kd) recognized by this particular monoclonal antibody was purified by affinity chromatography. Mice immunized with this antigen were protected from lethal blood-induced challenge[33].

P. falciparum schizont- and/or merozoite-specific antigens have been identified using metabolic labeling and

surface labeling techniques[19, 31, 34, 41, 42, 51, 58, 66, 70]. The main component of the membrane of schizonts of 190–200 kd has been identified using monoclonal antibodies. This polypeptide is processed into lower molecular weight products of 83, 42 and 19 kd which are expressed at the surface of merozoites[34]. Antigenic diversity at the level of the 190–200 kd polypeptide has been shown using monoclonal antibodies[47]. Monoclonal antibodies directed against *P.falciparum* schizont- and/or merozoite-specific antigens expressed at the surface of asexual blood stages (apparent Mr of 200, 140, 82 and 41 kdalton) inhibit the growth of *P.falciparum* cultures in vitro[68, 70, 81].

Other studies have shown, using sera from human individuals with various degrees of immunity towards *P.falciparum* infection, that there is a correlation between resistance to malaria and specific recognition of schizont- and/or merozoite-specific polypeptides of 200, 140, 105, 96, 82 and 41 kdalton[6, 69, 70]. The specific recognition of these polypeptides seems also to be linked with the capacity of these sera to inhibit the growth of *P.falciparum* asexual blood stages in vitro[6] and with an enhancement of in vitro antibody-mediated phagocytic activity of monocytes and granulocytes, towards *P.falciparum* schizonts[10].

P.falciparum merozoites specifically attach to and penetrate red blood cells. The red blood cell receptor for merozoites consists probably of a cluster of oligosaccharides present on glycophorin A and B[38]. *P.falciparum* polypeptides of apparent molecular weight 140 kd (probably identical to the 140 kdalton polypeptide recognized by inhibitory monoclonal antibodies), 70 and 35 kdalton bind to isolated glycophorin A bound to insoluble support[65]. Antibodies directed against the portion of these polypeptides (possibly a tetramer polypeptide) responsible for the binding to glycophorins, should be able to block invasion of red blood cells by merozoites. Other studies involved the characterization of S antigens responsible for antigenic diversity among various isolates of *P.falciparum* using sera from individuals living in endemic areas[1, 97]. The recent cloning of part of the gene coding for the S antigens has shown that part of this polypeptide is composed of tandem repeats of 11 to 4 amino acids which vary among various isolates of *P.falciparum*[17, 48] and that adjacent portions of the polypeptides have preserved amino acid sequences. S antigens are present in high concentrations in sera of infected individuals[99, 100] and antibodies are probably directed mainly against the variant part of the S antigens since isolate diversity may be detected using sera from repeatedly infected individuals using radial immunodiffusion[98]. It seems therefore that the amino acid repeats are strongly immunogenic; whether or not they play a role in favoring parasite survival by scrambling the immune response of the infected host remains to be determined.

Two monoclonal antibodies reacting with surface antigens of both male and female gametes of *P.gallinaceum* have been shown to synergistically mediate transmission blocking immunity[79]. More recently, the target antigens of transmission blocking immunity on gametes of *P.falciparum* have been identified[79].

Most of the studies concerning the immunological response to defined plasmodial antigens are related to the humoral immune response. It is possible, however, that some truly protective antigens may elicit primarily a T cell protective response with little or no antibody protection; this area of research clearly needs further investigation.

II. Immunization and vaccination

Immunization studies have been conducted over the last 50 years in a variety of host-plasmodia combinations including experimental monkeys infected with *P.falciparum*. Under optimal experimental conditions, it has been shown that protective or partially protective immunity against malaria may indeed be induced by immunization using sporozoites or merozoites (see references 16, 86, 95 and 12, 56, 57, 60, 61, 77, 78, 85, 90). The acquired immunity is stage- and species-specific and, for some plasmodia species, isolate-specific[37]. In addition, some forms of immunization induce a more complete immunity than that which follows repeated natural infections. The extent and duration of the acquired protective immunity is dependent on the parasite-host system, on the immunogens and on the adjuvants used. Immunization with gametes is usually without effect on the development of asexual blood stage parasitemia but blocks transmission of the disease by inhibiting fertilization in the gut of the female mosquito[8, 9, 30, 50].

The production of whole sporozoites, merozoites, schizonts or gametes in sufficient amounts for large-scale immunisation programmes is not possible. In addition, these preparations contain foreign material such as salivary glands and red blood cell membranes which may induce adverse autoimmune reactions in the immunized host[90]. In addition, crude antigenic preparations may contain immunosuppressive factors and/or elicit a 'diffuse' immune response[96]. It appears necessary, therefore, to use as a vaccine pure and well characterized plasmodial antigens. Candidate antigens have been described in the previous chapter. The present limitations concern the purification of adequate quantities of potentially 'protective' antigens for immunization studies. The recent successful cloning of the genes coding respectively for surface antigens of *P.knowlesi* and *P.falciparum* sporozoites[21, 24] and of the genes expressing *P.falciparum* schizont and merozoite specific polypeptides[17, 48] may solve this problem in the near future and render possible the mass production of malaria antigens either using the DNA recombinant technology or biochemical synthesis of polypeptides after elucidation of the DNA sequence of the malaria cloned genes.

A limited number of investigations have been conducted using defined malarial polypeptides. For example, mice immunized with a surface protein of *P.yoelii* merozoites have been protected from a lethal blood-induced challenge[33]. Similarly, Rhesus monkeys immunized with a 74 kdalton polypeptide expressed at the surface of *P.knowlesi* schizonts were also protected from a lethal blood-induced challenge[82]. In these 2 instances, sterile immunity was not induced by immunization an the vaccinated animals developed a relatively high transient parasitemia. Similar results were achieved

in Saimiri monkeys immunized with low amounts of preparations containing several soluble *P.falciparum* antigens purified from schizonts and merozoites grown in in vitro cultures[20,72]. More recent experiments indicate that immunization with either the 200, 140 or the 41 kdalton *P.falciparum* schizont and merozoite-specific polypeptides also induces at least a partial protective immunity in Saimiri monkeys (Perrin et al., J. exp. Med. in press; and unpublished data). Similar experiments are underway in several laboratories and it may be expected that in the near future such experiments will be conducted using material produced by DNA recombinant technology or by biochemical synthesis and that these investigations will be extended to sporozoites and gametes. Despite encouraging perspectives, several problems remain to be solved before an effective anti-malaria vaccine can be developed for human use, such as the development of adjuvants acceptable for human use, the production on a large scale of adequate malarial antigens, the definition of the role of antigenic diversity among various isolates of *P.falciparum,* and the extent of cross-protection with other plasmodia species infecting man.

Another question relates to the strategic choice between sporozoite, merozoite or gamete vaccines. Many aspects of the answer will depend on the efficacy of each particular vaccine, but one may consider that an ideal sporozoite vaccine will produce sterile refractoriness against plasmodial infection (for how long?), a gamete vaccine will decrease transmission (without protective effect on the vaccinated host), a merozoite vaccine will protect against clinical manifestations and reduce the gametocyte number (efficacy? sterile immunity?). Obviously, the antigens contained in these various vaccines may be mixed together or alternatively may have different indications: a sporozoite vaccine may be ideal for an individual willing to spend a few months in a malaria endemic area while a merozoite/gametocyte vaccine may be the best choice for children living in endemic areas.

III. Immunodiagnosis

Immunodiagnostic tests have 2 main fields of application: 1) epidemiological study of communities in areas endemic for malaria; 2) various applications in research laboratories. The test systems required for these 2 applications are different in many ways but some requirements apply for both applications, e.g. antigen production and purification, standardization of reagents and reproducibility. The immunodiagnostic test aims at the detection of antibodies or at the detection of antigens; these 2 aspects will be considered separately.

a) Antibody detection

The detection of antimalarial antibodies for immunodiagnostic purposes has certain limitations since the presence of antibodies in serum does not necessarily indicate an active disease. They are therefore of little help in the diagnosis of active disease in individual cases but may be used for follow-up of patients and have applications, for example, in blood transfusion centers of non-endemic areas to determine whether individuals coming back from endemic areas may be accepted as regular blood donors. At the present time, the main interest of these tests is that they may serve as tools for the epidemiological surveillance of malaria incidence. They have been used to determine the geographical distribution and prevalence of malaria and for the evaluation of the transmission of the disease[88]. This latter aspect is particularly important for the evaluation of the impact of malaria eradication programs. The advantages of the presently available test for antimalaria antibody detection is the relatively low cost and the sensitivity and simplicity, but the problems of standardization are still not completely solved. The most widely used assays for antimalarial antibody detection are the indirect immunofluorescence technique (IFA)[88,93], indirect hemagglutination (IHA)[39], and a variety of solid phase immunoassays[94].

The indirect immunofluorescence antibody technique (IFA) was introduced for malaria in the early 1960s and has been widely used for epidemiological studies which have clearly established the age-related variation in antibody titres in endemic areas and have helped in the evaluation of remaining malaria foci following eradication programmes[88]. More recently, IFA has been used for the selection of monoclonal antibodies reacting with malaria antigens present on defined anatomical sites[22,68,101]. The main advantages of IFA are its rapidity, relative simplicity and the possibility to identify the antibody binding site on defined anatomical structures of the parasites.

For the indirect hemagglutination test[39], erythrocytes coated with malarial antigens are incubated with a dilution of sera to be tested. IHA has been used successfully in epidemiological studies but its use in tropical areas is hampered by false positive results due to frequent non-specific erythrocyte agglutination[39].

Solid-phase immunoassays are based on the property that most of the antigens become firmly attached to plastic surfaces and preserves their reactivity after attachment. Following antigen absorption, dilutions of the sera to be tested are incubated in the wells of plastic microplates and the amount of antibody is determined in a second step by measuring the binding of an enzyme labeled anti-immunoglobulin antibody (enzyme-linked immunoabsorbent assay = ELISA)[94] or the binding of radiolabeled anti-immunoglobulin antibody (radioimmunoassay = RIA)[2]. These tests are used increasingly since they combine sensitivity, low cost and possible automation.

Stage-specific or functional assays such as schizont-infected cell agglutination assay (SICA)[3], in vitro growth inhibition of asexual blood stages[73], inhibition of binding of sporozoites to their target cells[32], and inhibition of gamete fertilization[9] are applied for research purposes but are not easily applicable to immunodiagnosis.

One of the critical points for the development of immunodiagnostic tests and particularly for antibody detection is the production and standardization of antigens. The main source of antigens used are: blood from infected individuals or experimental animals, sera from infected patients, and parasites obtained from in vitro cultures. Other sources of antigens such as sporozoites and gametocytes are not available in sufficiently large

amounts. These sources represent a wide range of antigens and are normally used as total extracts. Therefore, measurement of antibody levels using whole parasitic extract as a source of antigen represents only a mean assessment and does not differentiate between the various antibody specificities involved.

Recent studies have shown that individuals with a high degree of resistance to malaria preferentially react with some defined antigenic determinants[3,69] and vaccination trials against malaria may be developed in the future. In these 2 instances, the availability of purified defined antigens for antibody measurement would represent an essential tool. Progress in the characterization and identification of single malarial antigens has been achieved in recent years and several malarial antigens have been expressed in *E. coli* plasmids using DNA recombinant technology[17,24,63]. One may therefore expect that, in the near future, antibody measurement against a single antigen would be easily achievable and allow for a more precise and quantitative evaluation of the degree of resistance of populations living in endemic areas and of vaccinated individuals.

b) Detection of antigens

The detection of antigens in biological fluids is widely used in microbiology for the detection of recent or ongoing infection[92]; such tests are usually extremely specific and sensitive. A variety of techniques have been similarly developed for the detection of malaria antigens, mainly for the detection of *P. falciparum* antigens in the serum and/or in the blood of infected patients[2,45,46,98–100].

The antigens associated with asexual blood stages of malaria include soluble antigens present in the serum[98–100] and intraerythrocytic antigens. Immunoprecipitation in gel[100] and counterimmunoelectrophoresis[67,84] have been used for the detection of soluble malaria antigens during or after *P. falciparum* infection. These antigens have been characterized according to their resistance to heating at different temperatures. There is a sequential appearance of different antigens even in the serum of a single individual. The analysis of heat stable (S) antigens in Gambian children has shown a heterogeneicity of this group of antigens, suggesting an antigenic diversity within the *P. falciparum* species[99,100]. Indeed, this technique allows for the diagnosis of recent infection but is limited by its low sensitivity.

The simplest way to detect intraerythrocytic antigens in the study of individual cases is obviously the examination of blood films. However, in endemic areas, infected individuals frequently have very low parasitemia and in large scale epidemiological studies the method is time-consuming and requires highly skilled personnel. To bypass this problem, solid-phase inhibiton RIA and ELISA assays have been developed[2,45,46]. In the presently available assays, extracts of patients' RBC are incubated with enzyme or radiolabeled antimalarial antibodies and, after centrifugation, the supernatants are incubated in plastic tubes coated with malaria antigens. The fixation of the antibody to the coated tube is specifically inhibited by the prior reaction of the antibody with lysed infected cells. The measurement of the amount of radioactivity bound to the tube or the measurement of the absorbence of the enzyme activity coated to the antimalarial antibodies allows for the determination of a specific binding inhibition. These tests are very sensitive since parasites were detected at a level of 8 parasites/10^6 normal RBC[46]. There are however some limitations related to the standardization of the tests: the relatively complicated manipulations including washing of red blood cells to eliminate the antimalarial antibodies contained in the patient's blood, since they compete with the labeled antibody, and the specificity, since correlation between microscopic parasite detection and significant binding inhibition has still to be improved.

There are suggestions that some of these problems may be solved by the use of anti-*P. falciparum* monoclonal antibodies which have been developed by several laboratories. Pools of monoclonal antibodies directed against selected determinants would help in the standardization of the test and may be less sensitive to competition with natural antibodies present in the blood of infected patients. Another possible modification would be the selection of a sandwich assay using polyclonal antibodies or a pool of monoclonal antibodies for the coating of the plastic well on which lysate of parasitized blood would be applied. The final step would be the application of a pool of enzyme labeled or radiolabeled monoclonal antibodies of known specificities. Obviously, this technology can be developed 1) for *P. vivax, P. malariae* and *P. ovale* antigens which may cross-react with *P. falciparum* antigens; 2) for the detection of soluble malarial antigens present in the serum and 3) for the determination of isolate-specific antigens since monoclonal antibodies with isolate specificity have been recently described[47]; 4) for the detection of single antigens by using polyclonal sera or monoclonal antibodies directed against several epitopes expressed on the same antigen. An interesting recent development concerns a test for the detection of single epitopes of the anti-sporozoite protein of *P. berghei* (P.b.44). The principle of this solid-phase assay (inhibition of idiotype anti-idiotype interaction) is that the antigen (P.b.44) inhibits the interaction between 2 monoclonal antibodies. The first antibody (monoclonal) is directed against the antigen to be tested and the second antibody (also monoclonal) is directed against the idiotype of the first antibody. Introduction of the antigen (P.b.44) into the system inhibits the binding of the second antibody. Obviously, similar assays may be applied to the detection of other malaria antigens[75].

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