(54) Title: METHOD AND COMPOSITION FOR KILLING MALARIA PARASITES

(57) Abstract

A composition and method for killing malaria parasites by selective delivery of parasiticidal agents, including antibodies and antibody-toxin conjugates are provided.
**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AT</th>
<th>Austria</th>
<th>FI</th>
<th>Finland</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU</td>
<td>Australia</td>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>IE</td>
<td>Ireland</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KP</td>
<td>Democratic People’s Republic</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>CS</td>
<td>Czechoslovakia</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>ES</td>
<td>Spain</td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>MR</td>
<td>Mauritania</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>PT</td>
<td>Portugal</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>RU</td>
<td>Russian Federation</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
<td>SK</td>
<td>Slovak Republic</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
<td>SU</td>
<td>Soviet Union</td>
</tr>
<tr>
<td>TD</td>
<td>Chad</td>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>UA</td>
<td>Ukraine</td>
<td>US</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
METHOD AND COMPOSITION FOR KILLING MALARIA PARASITES

INTRODUCTION

The invention described herein was made in the course of research supported in part by the National Institutes of Health under Grant No. AI27247. The Government has certain rights in this invention.

BACKGROUND

Malaria is a serious parasitic disease spread by the bite of Anopheles mosquitoes. The World Health Organization (WHO) recognizes malaria as the world's major primary health problem, causing more morbidity and mortality than any other disease. Although the WHO has undertaken a massive program of malaria control, little progress has been made in the past 20 years. Mosquitoes have developed resistance to insecticides and the parasites have developed resistance to drugs.

The organisms responsible for malaria are parasitic protozoans of the genus Plasmodium. The species that cause the disease in humans are P. falciparum, P. vivax, P. ovale, and P. malariae, each producing a different form of malaria. Prevalent throughout the tropics, the global incidence of malaria is now approaching 500 million cases annually, with P. falciparum responsible for nearly half of all cases. Although malaria has been virtually eliminated in the United States, Canada, Australia and Europe, it is spreading in India, Pakistan and parts of Africa because both parasites and the mosquitoes which carry the disease have developed mutations resistant to drugs and insecticides once effective against them. The spread of drug-resistant P. falciparum and the
resistant to drugs and insecticides once effective against them. The spread of drug-resistant *P. falciparum* and the lack of efficacious vaccines based on current strategies emphasizes the need to develop new agents and vehicles for controlling this disease.

Malaria parasites (plasmodia) spend part of their life cycle in the *Anopheles* mosquitoes and part in humans. Mosquitoes become infected with malaria parasites by ingesting blood containing the sexual forms of the protozoa. After developing in the mosquito, plasmodia are transmitted by bites of the mosquito whose saliva carries the spore of the parasite. After an infectious spore (merozoite) enters a victim's blood, it migrates to the liver. After developing further, the parasite enters the blood stream and infects red blood cells (erythrocytes). Infected erythrocytes then burst within 48-72 hours, releasing the parasites in a further stage of development which can attack other cells or develop into forms that can infect mosquitoes. *P. falciparum*, which causes the most severe type of malaria, infects all ages of red blood cells (other species attack only young or old cells) and therefore, affects a greater proportion of the blood cells. Falciparum malaria can be fatal within a few hours of the first symptoms because so many blood cells have been destroyed that they block blood vessels in vital organs, especially the kidneys. Destruction of blood cells may lead to hemolytic anemia. Kidney and liver failure are common complications of falciparum malaria as well.

There is no vaccine yet against malaria but therapeutic medications are available. Since the seventeenth century, quinine has been used against malaria but is not a drug of choice because of its unpleasant side effects. The most currently used drug is chloroquine, however, it must be taken regularly for continuous protection. Chloroquine can reduce fever in about 24 hours and destroy the parasites in the blood in about 48 hours. However chloroquine-resistant falciparum malaria is now
widespread in many tropical areas. In such cases, combinations of drugs, such as chloroquine and proquanil or pyrimethamine and sulfadoxine or dapsone may be given. Some of these drugs act upon the parasites before they reach the red blood cells. Others interfere with the reproduction of the parasite in the mosquito that bites a person taking these medicines. In extreme cases, exchange blood transfusions have been investigated as a life-saving effort.

Current strategies for developing vaccines against pre-erythrocytic stages of the malaria-causing parasite have so far proven ineffective due to antigenic diversity on the parasite. In addition, even if only one sporozoite survives, the potentially lethal erythrocytic stage of the disease will proceed unabated. Experimental blood-stage vaccines designed to block infection of erythrocytes by circulating merozoites have been shown to have modest protective effects *in vitro*, but would be impractical for immunizing humans since an enormous quantity of antibody with high titer is required. There remains a long felt need for an effective anti-malarial vaccine.

**SUMMARY OF THE INVENTION**

A composition and method for killing blood-stage malaria parasites by selective delivery of parasiticidal agents, including antibodies and antibody-toxin conjugates, are provided. This novel approach to treating malaria is made possible by the inventors' discovery of a parasitophorous duct which directly exposes the intraerythrocytic parasite to the serum during the 48 hour blood-stage life cycle. By exploiting the newly discovered parasitophorous duct, selected chemotherapeutic agents or antibody raised by vaccination would be required in relatively small amounts. In addition, the parasite would be vulnerable to treatment for a relatively long period of time (days). Finally, even if the parasitemia (% of erythrocytes infected with malaria) is not totally
eliminated but greatly reduced, it may prove beneficial for acquiring immunity.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the typical time course for accumulation of rhodamine-labeled dextran (10K) in the parasite of trophozoite-stage infections.

Figure 2 is a graph showing the accumulation of rhodamine-dextran in the parasite of trophozoite-stage infections as a function of dextran concentration.

DETAILED DESCRIPTION OF THE INVENTION

We have found that intraerythrocytic, trophozoite-stage (the initial stages of asexual reproduction), Plasmodium falciparum parasites endocytose macromolecules through a newly discovered parasitophorous duct. Passage through this duct to the vacuolar space was found to be temperature and energy independent, suggesting that it is not a transient structure. The parasite internalized macromolecules from the aqueous compartment surrounding it by fluid-phase endocytosis. It is believed that this system provides the parasite with uninterrupted access to essential nutrients, lipids, protein-bound fatty acids from the extra-erythrocytic fluid phase. It is not yet clear whether the parasitophorous duct represents an invagination of the erythrocyte membrane which persists from the time of merozoite invasion, or a membrane-bound pathway formed at a later time in the parasite's life cycle.

Our observation that an IgG antibody was endocytosed by the parasite may explain some hitherto enigmatic immunological observations. IgG antibodies and crisis-form factor from human immune serum have been found to retard the in vitro growth of P. falciparum. The parasitophorous duct may provide the pathway for these molecules to reach the intracellular parasite. Until now, surface antigens on trophozoites and schizonts (a parasite developed from a trophozoite which undergoes multiple fission to form merozoites) have not heretofore been
considered as important for development of blood-stage malaria vaccines or targets for toxic compounds, since they were considered to be inaccessible to the serum. We believe that the parasitophorous duct which we have discovered will provide the means through which potentially lethal antibodies or antibody-toxin recombinants (immunotoxins) can be delivered specifically to the intraerythrocytic parasite.

We believe that the parasitophorous duct can be used to (1) deliver monoclonal or polyclonal antibodies to inhibit parasite growth and/or agglutinate segmented schizonts, preventing reinvasion by binding to specific antigens on the parasite plasma membrane; (2) selectively deliver immunotoxins to kill trophozoite or schizont stage parasites; (3) inhibit parasite growth by blocking the entrance to the duct.

Worldwide prophylactic vaccination against infectious diseases has led to complete or nearly complete eradication of many of these diseases in developed countries. Smallpox and polio are two impressive examples. However, the development of an effective vaccine against malaria has proven elusive because most attempts have focused on preventing infectious sporozoites from invading liver cells or blocking merozoite invasion into red blood cells. The former has been hampered by antigenic diversity on the surface of the sporozoite and the latter, by the requirement for enormous sustained amounts of a high antibody titer. In accordance with our invention, a blood-stage malaria vaccine will be developed wherein vaccination will be achieved either by immunization with isolated, prematurely lysed parasites (which would no longer be infectious), killed parasites or purified parasite surface antigens from ring, trophozoite or schizont-stage infections. We have demonstrated for the first time that monoclonal antibodies against parasite surface antigens passively diffuse from the serum into the parasitophorous duct where they bind parasite surface antigens. We believe
that following antibody binding to the parasite, complement will be activated and the parasite killed. We believe that the immune system does not kill intraerythrocytic parasites in vivo, since the duct is too narrow to permit entry of B lymphocytes necessary to initiate an immune response. However, vaccination with parasite surface antigens will stimulate the immune system to produce antibodies that will enter the duct, bind the parasite, activate complement and kill the parasite.

10 Identification Of The Parasitophorous Duct

Cell suspensions of trophozoite-stage P. falciparum-infected cells were incubated with rhodamine-dextrans (relative molecular mass, 10,000) at 37°C and washed. Confocal fluorescence imaging microscopy (CFIM) revealed heavy labeling of the parasite after 5 minutes of incubation. When infected cells were incubated with rhodamine-dextran at 4°C and washed, no cell-associated fluorescence was detected. Similar labeling patterns were obtained with a 70,000 (70K) rhodamine-dextran. This result could be due either to the blocking of fluid phase endocytosis at reduced temperature, or to the fact that at 4°C the uncharged rhodamine-dextran enters the erythrocyte but cannot bind to the membrane and is removed by washing. To distinguish between these possibilities, we compared the labeling at 37°C and 4°C with rhodamine- and fluorescein-dextrans (10 or 70K) that have several positively charged binding sites (lysine residues) for binding to negatively charged membranes. We found that the parasite is labeled more heavily at 37°C than at 4°C. We obtained similar results with the large (42K) globular protein rhodamine-protein A. CFIM images of infected cells incubated in tissue culture with fluorescein-protein A for 2 hours at 37°C and 4°C showed that the parasites are labeled at both temperatures.

To distinguish external and internal membrane labeling, we incubated cells labeled with fluorescein-protein A or fluorescein-dextran-lysine with an anti-
fluorescein IgG at 4°C. When this antibody binds fluorescein, more than 95% of the fluorescein is quenched. Parasites labeled at 37°C with fluorescein-protein A were fluorescent, while those labeled at 4°C were not. Similar results were obtained for the fluorescein-dextran-lysine labeled cells. These results demonstrate that at 4°C, only the external membrane (erythrocyte, parasitophorous vacuolar and parasite plasma membranes) were labeled, while at 37°C internal parasite structures were also labeled.

To identify membranes within infected red blood cells, living trophozoite-stage infections were labeled with either C₆ NBD-PC (1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3-diazole)-amino-caproyl phosphatidylcholine); C₆-NBD-phosphatidylethanolamine (PE); or C₆-NBD-ceramide and examined by CFIM. Irrespective of the phospholipid headgroup, tubular extensions of membrane appeared to bridge the erythrocyte and parasite vacuolar membranes. These structures, termed by us "parasitophorous ducts" appear to interconnect the parasitophorous vacuole and the external medium to provide a path by which macromolecules in the serum can passively diffuse to the parasite.

Visualization of the parasitophorous duct was not restricted to NBD-labeled phospholipids. When infected erythrocytes are incubated at 4°C or 37°C with 30-nm, highly charged fluorescent latex beads, tubular extensions from the parasitophorous vacuole, were seen to traverse the erythrocyte cytoplasm.

**Passage Of Macromolecules Through The Parasitophorous Duct And Subsequent Endocytosis By The Parasite**

Several lines of evidence suggest that once serum macromolecules diffuse through the parasitophorous duct, they can be endocytosed by the parasite at 37°C. Parasites in infected erythrocytes incubated with rhodamine-dextran at 37°C are fluorescent, while those incubated at 4°C are not. Parasites in infected red blood cells incubated with rhodamine-dextran-lysine, rhodamine-protein A, or fluorescein-protein A are more heavily labeled at 37°C than
at 4°C. Furthermore, trophozoite-stage parasites incubated in vitro with a rhodamine-goat anti-mouse IgG 2a antibody at 37°C are heavily labeled, but are devoid of any antibody labeling at 4°C.

The kinetics of uptake of rhodamine-dextran (10K) into the parasite at 37°C were shown to be characteristic of endocytosis. Accumulation of rhodamine-dextran was inhibited by depletion of ATP. The accumulation of rhodamine-dextran in the parasite of trophozoite-stage cells was also measured as a function of rhodamine-dextran concentration, and a linear relationship, typical of fluid-phase endocytosis, was observed.

Our results show passive diffusion of macromolecules in serum through a newly identified parasitophorous duct, with subsequent endocytosis of these macromolecules by the parasite. This system provides the parasite with an energy-efficient pathway to obtain nutrients and protein-bound fatty acids and lipids from the serum. Our observation that an IgG antibody has access to the parasite may explain some hitherto enigmatic immunological observations. IgG antibodies and crisis-form factor from human sera are known to retard the in vitro growth of P. falciparum. The parasitophorous duct may provide the pathway for these molecules to each the intracellular parasite. Until now, surface antigens on trophozoites and schizonts have not been considered as important for development of blood-stage malaria vaccines. IgG antibodies elicited by vaccination, specific for antigens on the parasite plasma membrane, may be parasiticidal when coupled to toxins or other agents that compromise the biochemical viability of the malaria parasite.

The present invention is further illustrated by the following examples, which are not intended to be limiting in any way.

**EXAMPLES**

**Example 1:** Confocal Fluorescence and Transmitted Light Examinations of Malaria-Infected Red Blood Cells
A. Malaria-infected red blood cells were incubated in tissue culture with (a) rhodamine-dextran (10K) for 2 hours at 37°C; (b) rhodamine-dextran (10K) for 2 hours at 4°C; (c) rhodamine-dextran-lysine (10K) for 2 hours at 37°C; and (d) rhodamine-dextran-lysine (10K) for 2 hours at 4°C. In all cases, cells were washed 5 times at 4°C before microscopy. The C-5 clone of the FCR-3/PMG African strain of *P. falciparum* (Green et al. (1985), *Am. J. Trop. Med. Hyg.* 34:24-30) was cultured in vitro as described in (Trager et al. (1976), *Science* 193:673-675). Ring-stage Gambian human isolates 249, 251, K86 and K41 were cultured by this method for only one cycle. Labeling patterns in the continuously cultured parasites and the human isolates were similar. Uninfected erythrocytes did not take up dextran, as mature erythrocytes do not endocytose. Cell viability was always greater than 98% using a live/dead viability kit (Molecular Probes, Inc., Eugene, Oregon). Fluorescence images were obtained with a Bio-Rad MRC 500 confocal imaging system using an Olympus IMT-2 inverted microscope.

B. Malaria-infected red blood cells were also incubated in tissue culture with fluorescein-protein A (10 mg/ml; Sigma) for 2 hours at (a) 37°C or (b) 4°C. Cells were washed 5 times at 4°C before microscopy. Following CFIM, 1 U of anti-fluorescein IgG antibody was added to cells from (a) and (b). To ensure that labeling of the parasite at 4°C was not caused by an alteration of the structure, or permeability properties of the erythrocyte by the fluorescent dye bound to the protein A, erythrocytes were incubated at 2 hours at 4°C with unlabeled protein A-biotin. Cells were washed 4 times and incubated with rhodamine-avidin at 4°C. The labeling patterns were identical to those obtained when fluorescein-protein A was the primary label.

C. Living, trophozoite-stage infected red blood cells were incubated with NBD-labeled phospholipids for 30 minutes at 37°C or highly charged, carboxylate-modified,
red fluorescent 30-nm latex FluoSpheres (Molecular Probes, Inc., Eugene, Oregon) for 1 hour at 37°C. The spatial and vertical (z-axis) resolution of the confocal microscope under the conditions used in these experiments was approximately 0.5 microns. In NBD-labeled cells, the parasite was found to be heavily labeled compared with the plasma membrane of the erythrocyte due to the density and proximity of labeled intraparasitic membranes and organelles. Tubular membranes, which we refer to as parasitophorous ducts, were seen interconnecting the parasite vacuolar and erythrocyte membranes. Cells were also incubated at 1 hour at 37°C with carboxylate-modified fluorescent latex beads, diluted 200 times with PBS which contained 1% bovine serum albumin and 3% of 40K dextran. Ducts projecting from the parasitophorous vacuolar membrane (PVM), which traverse the erythrocyte cytoplasm, were clearly visible.

Example 2: Accumulation of Rhodamine-Labeled Dextran in the Parasite of Trophozoite Stage Infections

A. Cells were incubated in tissue culture with rhodamine-dextran (1 mg/ml) at 37°C. For each time point, cells were taken from culture, washed 4 times at 4°C to remove external rhodamine-dextran, and the fluorescence intensity in the parasite compartment quantified by CFIM.

The results are shown in Figure 1. Each fluorescence value is the average from 70-100 infected cells. Fluorescence values were obtained at fixed photomultiplier tube gains and expressed in arbitrary units. To deplete ATP (\(\overline{U}\)), infected cells were incubated in glucose-free tissue culture medium, supplemented with 50 mM deoxy-glucose and 5 mM NaN₃, for 30 minutes at 37°C.

B. Cells were incubated with increasing concentrations of rhodamine-dextran for 1 hour at 37°C, washed 4 times at 4°C and the fluorescence in each compartment quantified by CFIM. The results are shown in Figure 2. Each value represents the average fluorescence from 70-100 infected cells.

Example 3: Labeling of Parasites with Monoclonal Antibodies
A. An IgG monoclonal antibody, IA2, specific for the parasite surface antigen found during all stages of infection was purified from Ascites fluid. An aliquot of the antibody was labeled with rhodamine (Rh). The Rh-IA2 was characterized and did not contain any unassociated rhodamine or contaminating proteins. When the Rh-IA2 was incubated at 4°C with trophozoite-stage infected red blood cells, labeling of the parasitic plasma membrane was apparent. When the temperature was raised to 37°C, it appeared that internal structures in the parasite also became labeled. This is important because the immunotoxin must be endocytosed by the parasite to be efficacious. Controls done with unlabeled IA2 indicate that the addition of Rh does not alter the binding properties of the antibody.

B. Trophozoite-stage infected red blood cells will be incubated with Rh-IA2 at 4°C (to prevent parasite uptake by fluid-phase endocytosis) with serial dilutions of the antibody to determine the minimum amount of antibody necessary for visualization by CFIM. Having determined this, we will examine the ability of Rh-IA2 to label parasites in vitro at different developmental stages. Infected cells will be incubated at 4°C with Rh-IA2 and examined by CFIM for reactivity. The presence of the antigen will be confirmed by Western blotting. To identify the membranes that Rh-IA2 is associated with, cells will be fixed, sectioned and reacted with a gold-conjugated goat anti-mouse IgG and examined by electron microscopy. When the incubation is done at 4°C, only the parasite plasma membrane should be labeled. If the incubation is done at 4°C, the cells are washed and the temperature raised to 37°C, IA2 should also be associated with vesicles/organelles in the parasite cytosol if it has been endocytosed.

Example 4: Determination of Antibody Toxicity

Little is known about the biochemical function of the proteins in the parasite plasma membrane. If the
antigen that binds IA2 performs a vital function, antibody binding will interfere with its function and be toxic to the parasite. Cultures will be incubated for two cycles with IA2 in the culture medium. As a control, identical cultures without IA2 will also be maintained. New antibody will be added to the culture when the medium is changed. Throughout the life cycle, an aliquot of infected cells will be removed and viability assayed using a viability assay (Molecular Probes, Inc., Eugene, Oregon). Parasite stage will be determined by Giemsa-stained blood smears. Infected red blood cells will also be examined by CFIM. At each new invasion, the parasitemia of the culture containing the antibody will be measured and compared to controls. Cell killing will be expressed as % dead infected cells versus time (hours). If cell killing is achieved, serial dilutions of antibody will be incubated with infected red blood cells to determine the LD$_{90}$ (amount of antibody necessary to kill 50% of the infected cells). Our preliminary results indicate that a significant amount of parasite killing occurs during the trophozoite stage of the life cycle. As designed, this experiment will distinguish between direct killing of the parasite (by examination of cell viability as a function of stage) and blocking of re-invasion by agglutination (which should be obvious in segmented schizonts) of released merozoites.

Example 5: Evaluation of Antibody-Toxin Conjugates (Immunotoxins)

Immunotoxins are cytotoxic agents prepared by linking cell-reactive antibodies to potent toxins or their A chains. Ricin, a disulfide-bonded heterodimeric glycoprotein consisting of a B chain (which binds galactose-containing molecules on cell surfaces) and an A chain (which kills cells by enzymatically inactivating the 60 S ribosomal unit) is the most widely used toxin. In order for ricin-antibody conjugates to be lethal, the complex must be endocytosed, the antibody-ricin link broken, and free ricin released to the cytosol. The procedures for conjugating ricin A chain to monoclonal are
well known in the art. Using standard methods, a ricin A-
IA2 immunotoxin will be prepared. The toxicity of ricin A
plus B chain, ricin A chain, and ricin A-IA2 will be
established in our cell system as described in previous
examples. We expect that ricin will kill the parasite
since we have shown that the parasite actively endocytoses.
Cultures will be incubated for two cycles with
ricin A plus B, ricin A, and ricin in combination with a
selected antibody in the culture medium. As a control,
identical cultures without the toxins will also be
maintained. New aliquots of toxins and ricin-antibody will
be added to the culture when the medium is changed. Ricin
A chain agglutinates erythrocytes at concentrations above
20 micrograms/ml. This is well above the concentrations
(10^{-7}-10^{-13} M) where ricin A-toxins have been shown to be
toxic. Throughout the life cycle, an aliquot of infected
cells will be removed and viability will be assayed using a
viability assay (Molecular Probes, Inc., Eugene, Oregon).
Parasite stage will be determined by Giemsa-stained blood
smears. At each new invasion, the parasitemia of the
culture containing the antibody will be measured and
compared to controls. Cell killing will be expressed as %
dead infected cells versus time (hours). If cell killing
is achieved, serial dilutions of ricin-antibody will be
incubated with infected red blood cells to determine the
LD_{50}. 
What is claimed:

1. An anti-malaria composition comprising a selected parasiticidal agent of a selected size for passage through the parasitophorous duct of the malaria parasite.

2. The composition of claim 1 wherein said selected parasiticidal agent comprises an immunotoxin.

3. The composition of claim 2 wherein said immunotoxin comprises an antibody-toxin conjugate.

4. The composition of claim 2 wherein said immunotoxin comprises ricin A-IA2.

5. The composition of claim 1 wherein said selected parasiticidal agent comprises antibodies against a selected parasite surface antigen.

6. The vaccine of claim 5 wherein said antibodies are IgG.

7. An anti-malaria vaccine comprising an antigen from a blood-stage malaria parasite which is capable of eliciting antibodies of a selected size for passage through the parasitophorous duct of a malaria parasite for binding intracellular surface antigens.

8. The vaccine of claim 7 wherein said antigen is selected from the group consisting of a purified surface antigen and an intracellular parasite.

9. An anti-malaria vaccine comprising a selected parasiticidal agent of a selected size to block passage of materials through the parasitophorous duct of the malaria parasite.

10. A method for killing a selected malaria parasite comprising delivering a selected parasiticidal agent of a selected size through the parasitophorous duct of the malaria parasite.

11. The method of claim 10 wherein said selected parasiticidal agent comprises an antibody.

12. The method of claim 10 wherein said selected parasiticidal agent comprises an immunotoxin.
13. The method of claim 12 wherein said immunotoxin comprises an antibody-toxin conjugate.


15. The method of claim 10 wherein said selected parasiticidal agent comprises antibodies elicited against a selected parasite surface antigen.

16. The method of claim 15 wherein said antibodies are IgG.

17. A method for inhibiting the growth of a selected malaria parasite comprising blocking the entrance to the parasitophorous duct of said parasite with a selected parasiticidal agent of a selected size.
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION No.**
PCT/US92/07380

**A. CLASSIFICATION OF SUBJECT MATTER**

| IPC(S) | A61K 39/00, 35/14, C07K 15/00, 17/00 |
| US CL | 424/85.8; 530/389.5, 388.4 |

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S.: 424/85.8; 530/389.5, 388.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**DIALOG**
search terms: malaria, antibody, parasitophorous duct

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Science, Vol 252, issued 21 June 1991, Waldmann &quot;Monoclonal Antibodies in Diagnosis and Therapy&quot;, pages 1657-1662, see entire document.</td>
<td>1-17</td>
</tr>
<tr>
<td>Y</td>
<td>US, A, 4,867,973 (Geors et al) 19 September 1989, see entire document.</td>
<td>2-5, 8, 9, 12-14 and 17</td>
</tr>
</tbody>
</table>

* Further documents are listed in the continuation of Box C.  

**See patent family annex.**

**Date of the actual completion of the international search**
05 October 1992

**Date of mailing of the international search report**
12 NOV 1992

**Name and mailing address of the ISA/Commissioner of Patents and Trademarks**
Box PCT  
Washington, D.C. 20231

**Facsimile No.** NOT APPLICABLE

**Authorized officer**
DONALD E. ADAMS, PH.D.

**Telephone No.** (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)