tion and on the ratio between the CA-DM group and the carrier (Fig. 1). Finally, since the antibody responses induced by hapten-coupled Ba or Ficoll were independent of antigen-specific T cells, it is likely that our immunotoxin acts directly on B cells.

We next assessed the relevance of the acid-sensitive spacer CA to the immunosuppressive potency of the antigen-DM conjugate. Normal splenocytes were incubated with medium alone, TNP_3-OVA-CA, TNP_3-OVA-$DM_{5.4}$, TNP_3-OVA-(CA-DM)$_{5.4}$, or or a haptenated conjugate in which DM was replaced by butacaine (BC) (a compound of similar molecular weight as DM but without cytotoxic activity). After incubation, the cells were washed and challenged in vitro with TNP-Ba. Immunosuppression resulted from DM-antigen treatment only if the antibiotic was linked to the carrier protein by the acid-sensitive spacer group (Fig. 2).

To determine the potency of our antigen–(CA-DM) conjugate as a specific immunosuppressant in vivo, we injected CBA/CaJ mice intravenously with saline or with various conjugates. After 24 hours, the mice were simultaneously challenged with both TNP-Ba and FLU-Ba and assayed for AFC 5 days later. The conjugate containing CA-DM was markedly and specifically immunosuppressive without visibly affecting the health of the animals during the experiment (Fig. 3).

We next extended our studies to the use of monoclonal antibody as the target-specific carrier of CA-DM. Spleen cells were incubated with anti-Thy 1.2 that had been coupled to CA-DM, for 2 hours at 4°C. The cells were then washed and cultured in the presence of Con A or the B-cell mitogen lipopolysaccharide (LPS). After 3 days, cellular uptake of [^{3}H]thymidine was determined as a measure of DNA synthesis (*18*). At concentrations that completely suppressed the T-cell response to Con A, anti-Thy 1.2–(CA-DM) had no marked effect on the B-cell response to LPS (Fig. 4). Incubation of spleen cells with mixtures of the target-specific carrier and free DM resulted in nonspecific suppression.

Our experiments with antigen as the target-specific ligand indicate that immunosuppression may be obtained not only in vitro, but also in vivo with little or no adverse side effects to the animal. The construction of target-specific immunosuppressive compounds that contain small molecular weight cytocidal drugs may have the potential for human clinical use in bone marrow transplantation and the treatment of autoimmune disease. In our experiments, antigen-specific immunosuppression was obtained in mice that had not previously been exposed to antigen (Fig. 3). In cases where autoantibody is the principal cause of an autoimmune disorder, neutralization by such antibody of an autoantigen-containing immunotoxin may not occur if nonspecific immunosuppression is induced prior to immunotoxin therapy. Autoimmune diseases such as multiple sclerosis, which is thought to be mediated by T lymphocytes, may be particularly suited for therapy with an immunotoxin whose targeting moiety is a monoclonal antibody directed at the appropriate T-cell subset.

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Antigens Induced on Erythrocytes by *P. falciparum*: Expression of Diverse and Conserved Determinants

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Red blood cells that are infected with the malaria parasite *Plasmodium falciparum* express new antigens on their surface. In a study of these antigens in the erythrocytes of naturally infected children in the Gambia, an antibody-mediated agglutination assay revealed an extreme degree of antigenic diversity. Serum samples from each of ten children in the convalescent stage of malaria infection reacted with infected cells from the same child but generally not with infected cells from the other children. The Gambian children's erythrocytes also expressed shared determinants: sera from Gambian adults often reacted with the surface of infected cells from all of the children and were shown by adsorption and elution experiments to contain antibodies that recognized several isolates. Conserved determinants exposed on infected erythrocytes may be important for development of antimalarial immunity either naturally or through vaccination.

ASEXUAL BLOOD STAGES OF THE MALARIA parasite induce numerous changes in the morphology, functional properties, and antigenicity of the surface membrane on the parasitized erythrocyte (*1*). In the simian parasite *Plasmodium knowlesi*, a malarial protein was shown to be inserted into the erythrocyte membrane and responsible for the phenomenon of antigenic variation at the surface of these infected cells (*2*). Antigens specific for the surface of infected erythrocytes could be potential targets for vaccination against malaria if they expressed determinants that were conserved among isolates from different geographical regions. An early immunological and electron microscopy study (*3*) provided evidence for such an antigenically conserved determinant in *Plasmodium falciparum*–infected erythrocytes from the mon-

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key *Aotus*. Antibodies from monkeys immunized with any one of three isolates cross-reacted with the antigen (or antigens) on erythrocytes infected with the other isolates. More recent studies have indicated the presence of antigenically diverse determinants on parasitized erythrocytes (*4*). Antibodies in the sera of animals infected with isolates from different geographical regions recognized diverse rather than common determinants. The determinants were identified by indirect immunofluorescence assay of squirrel monkey erythrocytes infected with *P. falciparum* (*4*). Furthermore, antibodies that blocked or reversed the cytoadherence of *P. falciparum*–infected erythrocytes to amelanotic melanoma cells (a model for in vivo cytoadherence to capillary endothelium) were shown to be isolate-specific (*5*). When sera from immune *Aotus* or from adults in malaria-endemic areas were absorbed with infected erythrocytes, the blocking activity against the same isolate (or occasionally another isolate), but not against all *P. falciparum* isolates, was removed.

We now report an analysis of antigen phenotypes on the surface of *P. falciparum*–infected erythrocytes from naturally infected Gambian children. We found that the children developed isolate-specific antibody responses against the surface antigens of their own infected erythrocytes. Sera from uninfected Gambian adults contained antibody that cross-reacted with the surface antigen (or antigens) of many isolates.

Infected erythrocytes were obtained from children with acute *P. falciparum* malaria at the Outpatients Department, Medical Research Council Laboratories, Fajara. After parental consent had been obtained, a sample of venous blood was collected and chloroquine treatment was administered. The erythrocytes were washed in RPMI 1640 medium and cryopreserved in liquid nitrogen (*6*); serum was stored at −20°C. The children were seen again between 21 and 28 days after treatment, at which time samples of convalescent serum were obtained. The erythrocyte and serum samples were all collected in the rainy season between mid-October and mid-November 1984, when malaria transmission was high.

Serum antibodies to infected erythrocytes were detected by an antibody-mediated agglutination assay that is specific for parasite-infected cells (*7*). Parasitized blood was thawed and cultured for 22 to 28 hours until the parasites were late trophozoites or early schizonts. Some samples were not examined because the parasitemia was too low (less than 0.5 percent infected cells). Agglutination assays were performed with mature parasites that were still within the original erythrocytes taken from the patient and had not completed the first growth cycle in vitro. Culture was performed in plastic petri dishes with RPMI 1640 medium containing 10 percent fetal calf serum (FCS), 20 m*M* Hepes, p*H* 7.2, gentamicin (25 μg/ml), and 24 m*M* sodium bicarbonate (*8*). Because only restricted amounts of blood were available, the results are shown as a semiquantitative index of the degree of agglutination at a fixed serum dilution. Such results agree well with more extensive analyses of individual sera at multiple dilutions (*9*). Samples of homologous blood obtained at the acute and convalescent stages of infection from seven of the ten children were also titered (*10*). Only erythrocytes infected with mature asexual parasites were agglutinated, in clumps containing between 10 and roughly 100 cells each (*9*). Agglutination was prevented by treatment of intact infected erythrocytes with trypsin, but unaffected by neuraminidase treatment (*9*), suggesting that agglutination requires antibody recognition of a cell-surface protein. We could not compare directly the antigen reactivity of the different isolates because the parasitemia varied (from 2 to 8 percent) and the degree of agglutination is affected by parasitemia in this range (*9*).

The samples of erythrocytes obtained at the acute and chronic stages of infection from the ten children (numbered 176 through 235) were tested in a checkerboard manner with the acute and convalescent sera from each child (Fig. 1). Thus, homologous reactions are shown along the diagonal in Fig. 1 and heterologous reactions off the diagonal. Each of the serum samples obtained during the convalescent stage of infection reacted with the surface of infected erythrocytes from the same child. In eight of the ten children the homologous acute sera were negative. In the other two children the acute sera caused specific agglutination of infected erythrocytes from the same child, but in each case the titer increased at least six times with the convalescent serum. In three instances (patients 179, 188, and 189) heterologous isolates were recognized by a pair of acute and convalescent sera. In these examples (except serum from child 189 and parasites from child 179) these were relatively weak agglutination reactions and they formed no discernible pattern. We emphasize that the acute sera in these three pairs did not react at all with the surface of the child's own infected erythrocytes (patients 188 and 189) or reacted only weakly with the child's own infected cells (patient 179). Although the acute and convalescent sera from child 189 were positive against six of the nine isolates tested, this was not the oldest child studied.

We conclude from Fig. 1 that in natural infections of *P. falciparum* in children, the parasite expresses extremely diverse antigenic determinants on the infected erythrocytes. Furthermore, the children develop isolate-specific antibodies to their own infected erythrocytes during convalescence. The results also suggest that some children experience one or more earlier infections that elicit specific antibodies that cross-react with surface antigen phenotypes, as shown here.

When the infected erythrocytes from the same children were tested against a panel of sera from Gambian adults the results were very different (Fig. 2). Except for the serum

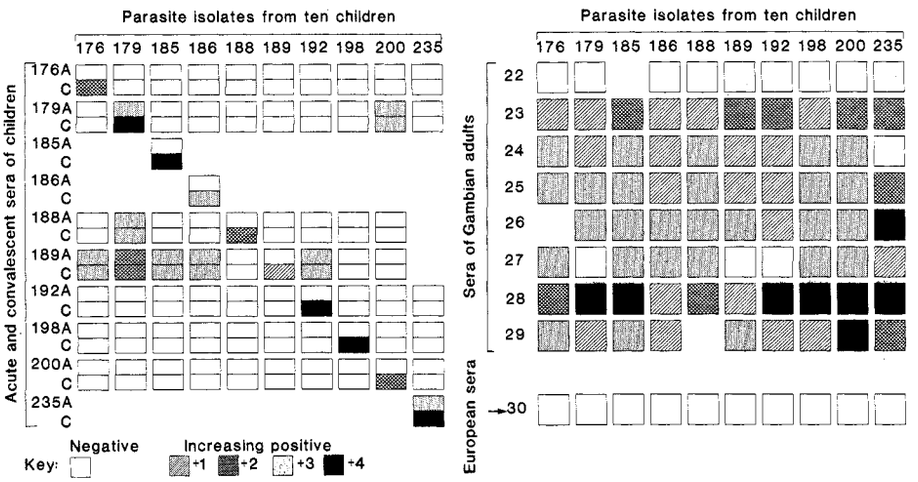

Fig. 1 (left). Agglutination with infected erythrocytes from Gambian children and acute (A) and convalescent (C) sera from the same children. The agglutination assay (*7*) was scored on a semiquantitative scale. In seven cases the homologous acute and convalescent sera were titered out (*10*). Fig. 2 (right). Agglutination with infected erythrocytes from Gambian children and sera from noninfected Gambian adults (numbers 23 to 29), a Gambian adult from an urban area after an acute *P. falciparum* infection (number 22), and from adult Europeans (results for ten sera are shown as number 30). The agglutination assay (*7*) was scored as for Fig. 1.

Table 1. Agglutination of infected erythrocytes with adsorbed sera and eluted antibody: evidence for a shared antigen on infected erythrocytes from children naturally infected with *P. falciparum*. Agglutination is scored semiquantitatively (0, no agglutination; +, agglutinates of approximately 20 infected erythrocytes; ++, agglutinates of approximately 50 to 100 infected erythrocytes).

Source of serum	*P. falciparum* isolate*							
	232	244	274	281	340	192	198	GAM 83/1
European adult	–	–	–	–	–	–	–	–
Gambian adult (GA4)	++	++	++	++	++	++	++	–
GA4 adsorbed on isolate 340 and eluted	+	+	+	+	+	ND†	ND†	–
GA4 absorbed with isolate 340	0	0	ND†	ND†	0	0	0	ND†
GA4 adsorbed on isolate GAM 83/1 and eluted	0	0	0	0	0	ND†	ND†	ND†

*Isolates 232 to 340 and 192 and 198 were from Gambian children with acute infections and were cultured <30 hours in vitro. Isolates 192 and 198 were also studied in Figs. 1 and 2. Isolate GAM 83/1 was originally from a Gambian child but had been adapted to continuous culture in vitro. †Not determined.

from adult 22, the sera were from individuals who were not infected with malaria. We presume that these adults have a greater immunity to *P. falciparum* than the children. Adult 22 was living in an urban area and had recently experienced an acute malaria infection. This individual's convalescent serum failed to recognize any of the isolates from children. In contrast, sera from four of the other seven adults recognized all of the ten isolates and sera from the other three recognized some but not all of the isolates. Serum samples from ten European adults failed to agglutinate the infected erythrocytes from the ten children.

The differences in reactivity between the sera from the children and the adults could be due to the adults having experienced previous infections with many different malaria "strains." Alternatively, the adults may have developed immune responses against antigenically conserved regions of the surface antigen on infected erythrocytes that were not recognized by the children's sera or against a different, less immunogenic antigen on the erythrocyte surface. We used isolates of *P. falciparum* from other children at the same clinic to study this question. Antibody was adsorbed from the serum of a Gambian adult (GA4) onto the surface of purified infected erythrocytes from an acutely infected child (isolate 340), and then unbound antibody was removed by washing. The antibodies attached to the cell surface were eluted at *p*H 3 with an isotonic glycine buffer (*11*). Adsorption and elution were also performed with control infected erythrocytes from a culture-adapted isolate (GAM 83/1) that had lost the capacity to be agglutinated by the same and other sera from Gambian adults (*12*). The eluted antibodies from these two types of cells were compared for their capacity to agglutinate erythrocytes from five children with acute malaria infections and from a culture of GAM 83/1 (Table 1). Antibodies eluted from the erythrocytes from patient 340 agglutinated the isolates from the five children to the same degree. In contrast, the control eluate agglutinated none of these isolates. In another experiment, 250 μl of serum GA4 at a one in ten dilution in phosphate-buffered saline was extensively adsorbed against erythrocytes from one of the children (patient 340) by incubation with an equal volume of packed cells for 30 minutes at 37°C. This was repeated six times. Whereas the unadsorbed serum GA4 agglutinated erythrocytes from five acutely infected children (patients 232, 244, 340, 192, and 198), the adsorbed serum did not agglutinate any of these isolates (Table 1).

The antigenic diversity expressed by infected erythrocytes probably reflects a parasite strategy for evasion of the host's immune responses. Studies in vitro have shown that antibody recognition of new antigens on infected erythrocytes can mediate parasite destruction through opsonization (*13*) or antibody-dependent cytotoxicity (*14*); studies in vitro (*15*) and in vivo (*16*) have shown that such antibody can block or reverse the binding of infected cells to endothelial cells, thereby leading, it is suggested, to destruction of infected cells in the spleen. According to this concept of immune evasion, the susceptibility of the acutely infected children to *P. falciparum* was due, at least in part, to the fact that they had not previously experienced infection with the same "strain" of malaria. The antigenic diversity expressed by the infected erythrocytes from the ten different children (Fig. 1) could be generated by multiple stable independent phenotypes of *P. falciparum*, or by a process of antigenic variation within a single isolate similar to that observed with cloned *P. knowlesi* infections in monkeys (*17*). The common antigen shared by many isolates must have another role for the malaria parasite. We have suggested elsewhere (*1*) that the intracellular malaria parasite alters the host erythrocyte membrane in order to facilitate the uptake and removal of metabolites (*18*) as well as adherence to endothelial cells (*19*). While some of these membrane changes might vary according to the parasite isolate, other functional changes may be conserved and less immunogenic, requiring many years and a mature immune system for the induction of specific antibodies.

It is not known whether the surface membrane antigens identified in this study are derived directly from the malaria parasite or from altered host components. In previous studies we identified malarial membrane proteins that were responsible for antigenic variation in *P. knowlesi* clones (*2*), and found antigenic diversity in laboratory isolates of *P. falciparum* (*20*). We have also identified high molecular weight surface proteins that are present on the surface of *P. falciparum*–infected erythrocytes from Gambian children (*21*), including those in the current study (*22*), but are not present in uninfected cells. Taken together, the data indicate that the antigenically diverse epitopes are derived from malarial proteins. The presence of a shared antigen on the surface of infected erythrocytes from naturally infected malaria patients raises the possibility that such an antigen may be an important target for immunity.

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Rat Resistance to Schistosomiasis: Platelet-Mediated Cytotoxicity Induced by C-Reactive Protein

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In rats infected with the parasite *Schistosoma mansoni*, the concentration of C-reactive protein in the serum increases after the lung stage of infection and is at its highest at the time of terminal worm rejection. The peak of platelet-mediated cytotoxicity induced by infected serum that has been heated (and is free of immunoglobulin E) as well as the time course for the development of platelet cytotoxic activity in infected rats was found to be correlated with the concentration of C-reactive protein. Rat and human platelets treated with homologous serum obtained during an acute phase of inflammation or with purified C-reactive protein were able to kill the immature forms of the worm in vitro. Platelets treated with C-reactive protein were furthermore capable of conferring significant protection against schistosomiasis in transfer experiments. Collectively these data indicate that a system that includes C-reactive protein and platelets participates in the natural resistance of the rat to schistosomal infection.

SCHISTOSOMIASIS AFFECTS AN ESTIMATED 200 to 300 million people. Vaccination appears to be the most favorable approach to controlling the disease, but before a vaccine can be developed there must be a greater understanding of the nature of the protective immune responses in humans and experimental animals (*1*). The mouse and rat have been widely used as experimental models for schistosomiasis; both animals show some degree of resistance to reinfection. The mouse, described as a permissive host, allows schistosomes to complete their life cycle to full maturity during a primary infection. In this case, resistance to reinfection is directly proportional to the degree of granulomatous inflammation that occurs in response to eggs trapped in the liver (*2*). Inflammation is associated, for example, with a sharp and long-lasting increase in serum amyloid P-component (SAP), the major acute-phase reactant in the mouse (*3*). In contrast, the rat, termed a nonpermissive host, demonstrates a natural resistance to schistosomiasis which is manifested as a 95 percent terminal rejection of immature worms at 4 weeks of a primary infection (*4*). No clear and definite explanation has been provided to account for this phenomenon. To determine whether inflammation plays a role in natural resistance of the rat to schistosomiasis, and possibly in resistance to reinfection, we studied the development and the potential role of serum C-reactive protein (CRP), the homolog of murine SAP, during the course of infection.

Rat CRP closely resembles human CRP in its amino acid composition, in having five subunits per molecule, and in its electron microscopic appearance as a pentameric annular disk (*5*). CRP is a major acute-phase reactant in most species and has the property of calcium-dependent binding to the phosphoryl choline and to phosphoryl choline–containing substances. Among the characteristics that differentiate rat CRP from that of other species is that rat CRP is unable to activate homologous complement. CRP is synthesized by hepatocytes and is probably under the influence of humoral mediators such as interleukin-1 (*6*), which is actively synthesized by monocytes and macrophages when they are stimulated by microorganisms, by microbial products, and by activated T lymphocytes or their lymphokine products (*5*). CRP has been shown to interact with cells, noticeably with platelets (*7*), and to affect their cellular functions. The functions of CRP are poorly known (*5*). Several biological activities have been measured in vitro with purified CRP (*5*) that are consistent with a role in host defense, but the difficulty lies in extending them to good models of host resistance in vivo.

As shown in Fig. 1A, Fischer rats developed a marked and prolonged elevation of the serum concentration of CRP as measured by immunoelectroassay (*9*), with a peak at day 28 after infection with *Schistosoma mansoni*. The high levels persisted almost until day 41.

The role of CRP in protection against schistosomiasis was investigated as follows. A rat serum was obtained 24 hours after the subcutaneous injection of turpentine (0.5 ml per 100 g of body weight), which is known to increase the serum concentration of acute phase proteins. This serum was unable to kill the immature worms (schistosomula) in vitro either alone or in association with macrophages or eosinophils. However, platelets from normal rats incubated with 10 percent serum from turpentine-injected rats, but not with 10 or 20 percent serum from normal rats, were larvicidal in vitro (Table 1). Furthermore, the

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