T15H(50–73) is an efficient inhibitor of the binding of labeled T15 to insolubilized PC antigen (*13*). However, two monoclonal anti-idiotypic antibodies to T15 (*6, 14*) do not bind to the peptide T15(50–73) (*13*).

The self-binding of the T15-M603 family demonstrates a novel property of the Ig family. A functional site that exhibits self-complementarity and can induce self-binding was identified in the V_H chain region. The self-binding site is proximal to the antigen-binding site, since the specific hapten PC effectively competes with T15 binding. The self-binding site should be viewed in the wider context of the biological properties of molecules in the Ig supergene family (*15*). Members of this family are integral membrane proteins that play different roles in the immune system. Major histocompatibility complex (MHC) proteins and T cell receptors are different from Igs because they have a second active site in addition to the antigen-binding site. The second site is designed to interact with proteins (MHC molecules) encoded by so-called immune response genes. Since the architecture of MHC molecules and T cell receptors is similar to the structure of Igs, it should not be surprising to detect a self-binding property on the T15 antibody that is not obviously related to its antigen specificity. The analogy to the immune response site on T cell receptors extends further into the character of the self-binding site of T15, which appears to be of low affinity and dependent on quaternary interactions such as intermolecular valencies. One might speculate that the T15 self-binding site is related to the intermolecular recognition site in non-antibody molecules in the Ig supergene family. The proximity of the antigen-binding site and the self-binding site in T15 seems to be another feature shared with the dual recognition specificity of T cell receptors.

The biological function of a self-binding antibody within the idiotypic network remains to be elucidated. As the self-binding site is functionally related to the antigen-binding site, a simple feedback mechanism in the immune response can be envisioned. Such a model could be extended to the role of B cells as antigen-presenting cells whereby circulating antibody could block antigen presentation to T cells. The biological relevance of self-binding antibodies would be strengthened if it could be demonstrated with other antibodies.

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Aldolase Activity of a *Plasmodium falciparum* Protein with Protective Properties

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Immunization with a 41-kilodalton blood stage antigen (p41) of *Plasmodium falciparum* induces immunity to malaria in monkeys. However, antigenic polymorphism and repetitive amino acids commonly found in protective antigens complicate vaccine development. The gene encoding p41 has now been cloned and analyzed. Sequencing and hybridization studies revealed that the gene structure is highly conserved in 14 parasite isolates from three continents. This finding and the lack of repetitive amino acids in the translated DNA sequence may indicate that p41 has an essential function. In this study the protein was found to be 60 percent homologous to the key glycolytic enzyme aldolase from vertebrates, and the affinity-purified p41 protein from parasites showed aldolase activity.

IMMUNIZATION OF MONKEYS WITH IRradiated merozoites and schizonts of the human malaria parasite *Plasmodium falciparum* induces a marked degree of protection against challenge with viable parasites (*1, 2*). Purified proteins of these blood stages of the parasite induce a similar degree of protection (*3, 4*). Among these antigens is a 41-kD polypeptide termed p41 that is expressed preferentially at the end of maturation in the asexual blood stage of the parasite. It is detected in association with membrane preparations of parasitized red blood cells and is found in increased concentrations in polar secretory organelles or rhoptries of the parasite (*5, 6*). Antibodies to the 41-kD rhoptry component react with a large number of *P. falciparum* isolates by immunofluorescence, suggesting that at least parts of the sequence are common in these isolates (*5, 7*). A malaria vaccine based on p41 might thus be active against a large number of parasite variants; we therefore analyzed the gene encoding p41.

We constructed λGT11 phage expression libraries with randomly sheared genomic

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Table 1. Reactivity of five mAbs to parasite p41 on microplates coated with affinity-purified 41-kD protein expressed in *E. coli* (*18*). The mAbs 31 cl3, 28 cl1, 58 cl1, 46 cl3, and 81 cl6 immunoprecipitate p41 protein from parasite lysates (*3*). In addition, 31 cl3 and 28 cl1 block parasite invasion in vitro (*19*); 88 cl1 and 79 cl3 are control antibodies recognizing a 195-kD merozoite surface antigen (*32*). Abbreviations: NMS, normal mouse serum diluted 1:50; and $OD^{492\ nm}$, optical density at 492 nm. Average values of ten experiments are shown (SE was less than 2.4% of reported value).

mAb	$OD^{492\ nm}$
31 cl3	1.325
28 cl1	0.723
58 cl1	0.772
46 cl3	0.801
81 cl6	1.549
88 cl1	0.201
79 cl3	0.210
NMS	0.168

DNA of the Thai (K1) isolate of *P. falciparum* (*8–10*). With the use of rabbit antiserum to affinity-purified p41 (*11*), we detected clone 41-2. Rescreening of the same library with the 41-2 insert DNA (*12*) resulted in clone g41-D. An open reading frame in the DNA of clone g41-D encodes a 41-kD protein (*13*). The sequence lacks repetitive amino acid motifs such as those commonly found in *P. falciparum* surface antigens (*14, 15*). Typically, the coding region is embedded in AT-rich flanking sequences (*14*).

To prove that the open reading frame of clone g41-D encodes p41, we determined a partial amino acid sequence of two peptides obtained by V8 protease cleavage of affinity-purified p41 protein from parasites (*16*). Both peptide sequences matched the deduced amino acid sequence for p41. *Escherichia coli* cells transformed with a pUC18 (*17*) plasmid containing the cDNA sequence of clone M25-13 expressed a 41-kD recombinant protein. This protein was purified by affinity chromatography with mouse immunoglobulin G (IgG) to p41 (*3*) from an *E. coli* lysate (*18*). Five monoclonal antibodies (mAbs) to parasite p41, including two that block parasite invasion in vitro (*19*), recognized the 41-kD recombinant antigen in enzyme-linked immunosorbent assays (ELISA) (Table 1).

To analyze allelic polymorphism of the p41 gene we constructed a cDNA library of the M25 isolate in λGT10 (*3, 20–22*) and a genomic library from RO-33 (*23*) in λGT11. A probe from the central part of the K1 gene detects positive clones in both libraries (*12*). Surprisingly, the RO-33 sequence determined was identical to K1, and only two amino acid changes and a silent mutation in the coding region distinguished the M25 cDNA from the genomic K1 sequence.

We then probed Dra I–digested genomic DNA of 14 parasite isolates (*24*) with the insert of clone g41-D. Even under stringent hybridization conditions, four bands detected by the probe in all isolates had the same size and intensity as in the K1 control DNA. As expected, the rabbit antiserum to p41 detected a single band of 41 kD in protein immunoblots with lysates of the same parasites. The lack of repetitive amino acids and the absence of allelic polymorphism distinguish the p41 gene from other genes encoding protective blood stage antigens (*15*).

To locate functional domains in p41 we compared the amino acid sequence with all entries of the National Biomedical Research Foundation (NBRF)–protein database (*25*). When aligned, the sequence had significant identity (>60%) with fructose-1,6-diphosphate aldolase from chicken, rabbit, rat, and human. A similar degree of homology was obtained when the aldolase sequence of the human parasite *Trypanosoma bruceii* (*26*) was compared with the vertebrate sequences. Consequently, we assayed the p41 protein for aldolase activity (Fig. 1A). Active forms of mammalian aldolase isoenzymes are composed of four allelic subunits with a molecular mass of about 40 kD (*27*). We show in Fig. 1B that p41 subunit formation is essential for aldolase activity. When a lysate of parasite-infected erythrocytes was passed through an affinity column with mouse IgG to p41 (*3*), 95% of the total aldolase activity was absorbed and recovered in the eluate. The aldolase activity present in the flow-through of the column corresponded to lysate levels of uninfected blood.

Aldolase is a key enzyme of the glycolytic pathway and catalyzes the cleavage of fructose-1,6-diphosphate into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. The replication and maturation process of *P. falciparum* is highly energy dependent, and the glucose consumption of infected erythrocytes is 10 to 25 times that of normal cells (*28*). This level of consumption requires an increase in glycolytic enzymes. Indeed, aldolase synthesis in the monkey parasite *P. knowlesi* is cell cycle dependent and reaches a maximum in the trophozoite and schizont stages when multiplication of the parasite occurs (*29*). The maximal rate of p41 synthesis is found in the same developmental stages of *P. falciparum* (*6*).

Antibodies to p41 are commonly found in

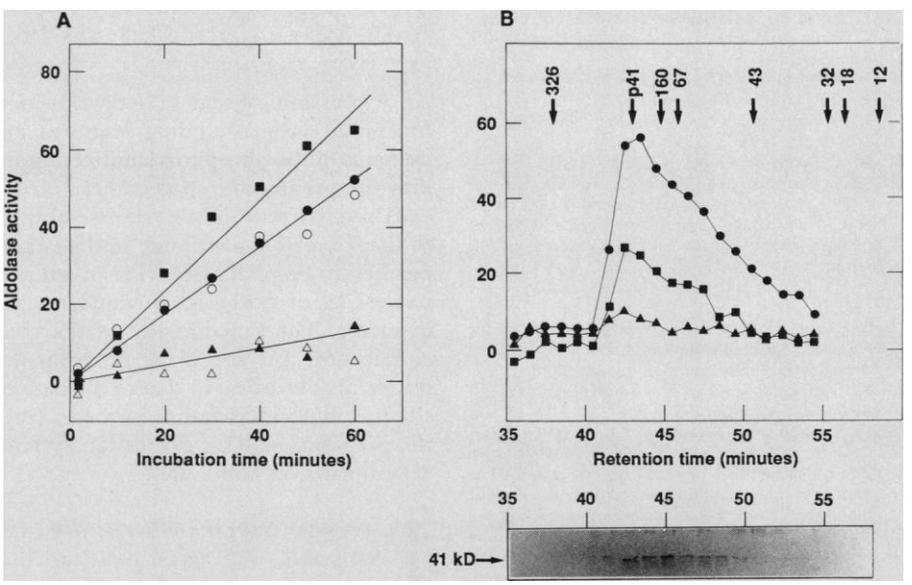

Fig. 1. (**A**) Inhibition of p41 aldolase activity by antibodies. A *P. falciparum* lysate and purified p41 protein were assayed for fructose-1,6-diphosphate cleavage in the presence and absence of rabbit antiserum to p41 (*33*). The aldolase activity [measured as described in (*33*)] is strongly inhibited by antiserum to p41. Parasite lysate without (○) and with (△) rabbit antiserum to p41, p41 without (●) and with (▲) rabbit antiserum to p41, and (■) p41 with preimmune serum (*34*). The specific activity of p41 (7 unit/mg) is comparable to a commercial preparation of rabbit aldolase (28 unit/mg; Boehringer, FRG). (**B**) Subunit structure of enzymatically active p41 protein. The molecular mass of purified, enzymatically active p41 protein was determined under nondenaturing conditions by gel permeation chromatography (*35, 36*). Molecular markers are indicated in kilodaltons. After extensive washing of the column the experiment was repeated and the column connected to a fraction collector. Half of each fraction was assayed for enzyme activity [(▲), 10 min; (■), 70 min; and (●), 4 hours] and the remainder was concentrated, denatured, and analyzed by immunoblotting after electrophoresis on a 10% SDS-polyacrylamide gel (*37*). A single band of 41 kD is detected by rabbit antiserum to p41 (*11*), and its intensity is proportional to the aldolase activity. The relevant part of the blot appears below the diagram.

sera from humans in endemic areas (*30*) and may play a role in protective immunity (*19*). This suggests that p41 is exposed to the host's immune system at a certain point of the infectious cycle, probably when the content of the rhoptry organelles is secreted onto the erythrocyte membrane (*31*). At this step, antibodies could inactivate the rhoptry proteins that are believed to participate in the invasion process (*7, 19*).

Certain features of the p41 sequence suggest two independent approaches to the control of malaria. First, the need for functional aldolase may reflect the strong conservation of the sequence among different isolates; it is unlikely that parasite mutants evolve that escape immune attack by protective antibodies against p41. Second, the common function of the enzyme yet the relatively high degree of sequence diversity between human and parasite aldolase suggests the possibility of finding compounds that specifically inhibit the parasite enzyme.

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33. K1 parasites were cultivated and extensively washed as described (*23*). The parasites were lysed by eight cycles of freeze-thawing. Lysis was monitored microscopically. Membranes and debris were removed by centrifugation at 12,000 rpm for 10 min at 4°C. Aliquots of this lysate or purified p41 protein (*3*) containing 50 units (1 unit is 1 μmol of dihydroxyacetone phosphate formed per minute and per milligram of protein at 37°C) of aldolase were assayed with a commercial aldolase kit (Sigma, procedure 752). For inhibition, 25 μl of rabbit antiserum to p41 (*11*) or preimmune serum were preincubated with the sample for 5 min at 25°C.
34. The increment in the preimmune serum control is due to aldolase and triose-phosphate isomerase activity present in rabbit serum.
35. Sizing of active p41 was performed on a 60-cm LKB TSK-G 3000 SW HPLC column connected to a 7.5-cm precolumn in 0.1*M* sodium phosphate, *p*H 7.5, at a flow rate of 0.4 ml/min. The column was calibrated with leucine aminopeptidase (326 kD), rabbit muscle aldolase (160 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), human immune interferon (32 kD), myoglobin (18 kD), and cytochrome c (12 kD). Proteins were detected after *o*-phthalaldehyde derivatization (*36*).
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Assembly of a Functional Immunoglobulin F_v Fragment in *Escherichia coli*

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An expression system was developed that allows the production of a completely functional antigen-binding fragment of an antibody in *Escherichia coli*. The variable domains of the phosphorylcholine-binding antibody McPC603 were secreted together into the periplasmic space, where protein folding as well as heterodimer association occurred correctly. Thus, the assembly pathway for the F_v fragment in *E. coli* is similar to that of a whole antibody in the eukaryotic cell. The F_v fragment of McPC603 was purified to homogeneity with an antigen-affinity column in a single step. The correct processing of both signal sequences was confirmed by amino-terminal protein sequencing. The functionality of the recombinant F_v fragment was demonstrated by equilibrium dialysis. These experiments showed that the affinity constant of the F_v fragment is identical to that of the native antibody McPC603, that there is one binding site for phosphorylcholine in the F_v fragment, and that there is no inactive protein in the preparation. This expression system should facilitate future protein engineering experiments on antibodies.

IMMUNOGLOBULINS (IGS) ARE A FAMILY of stable and similar molecules that can bind to a large number of different antigens. They constitute promising targets for investigating protein-ligand interactions since the overall folding of the domains seems to be independent of the structure of the binding site. Several three-dimensional structures of antibodies or their Fab fragments have been determined, and their common features have been compared (*1*). The essence of their architecture is a framework of fairly constant residues (arranged in a sandwich of β-sheets) linked by three hypervariable loops [complementarity-determining regions (CDR)] per chain that determine the specificity for antigen recognition. An early insight into enzyme catalysis by Pauling (*2*) was used to search for catalytic antibodies (*3*). An easy access to genetically engineered, functional antibody proteins would permit new approaches for studying antibody structure and function and the essentials of enzymatic catalysis (*4*).

Despite numerous investigations, the

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