Polymorphic Antigens in *Plasmodium falciparum*

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A PROMINENT FEATURE of host responses to infection with malaria parasites is an antibody response that includes specificities to a large number of different protein antigens of the asexual blood stages. During the last 5 years, a large number of these antigens have been cloned so that they may be investigated for their potential as components of a malaria vaccine. As a result of the sequencing studies on these cloned genes or gene fragments, a large amount of information has been obtained concerning the primary structural characteristics of *Plasmodium falciparum* proteins.

For several protein antigens that exist in antigenically distinct forms in different isolates of *P. falciparum*, a number of different genes have been sequenced. A comparison of these sequences has revealed a remarkable degree of diversity among allelic genes for several different asexual blood stage antigens. As the asexual stages of plasmodia are haploid, the sequences of these allelic genes define the protein expressed in the different parasite isolates. One of these antigens is a heat-stable soluble antigen known as the S-antigen that can be detected in the serum of infected individuals. Two other highly polymorphic antigens are integral membrane proteins associated with the surface of the merozoite, the life cycle stage that is released from rupturing schizont-infected erythrocytes and subsequently invades another erythrocyte. The structural basis of diversity in these and other *P. falciparum* antigens is described in this review.

S-Antigens

The studies of Wilson et al established that different isolates of *P. falciparum* are characterized by antigenically distinct heat stable antigens that are released into the serum of infected individuals or experimental animals. Despite the extensive diversity of S-antigens, these early studies indicated that individual S-antigens were stable markers for different parasite strains.

One of the first asexual blood stage antigens to be cloned had the characteristics of an S-antigen. Although there are numerous soluble, heat-stable, parasite antigens, the term “S-antigen” should now be restricted to the protein encoded by the gene corresponding to this original cDNA clone. Sequencing of the insert in this clone (from isolate FCQ27/PNG[FC27]) showed that the corresponding fragment of the protein was composed of a tandem array of 23 copies of an 11 amino acid sequence. Subsequent studies showed that this S-antigen has a central block of approximately 100 repeats flanked by short nonrepetitive sequences. Overall, more than 90% of this large polypeptide chain is composed of a repetitive array of the 11 residue sequence (Fig 1). The FC27 S-antigen has a typical N-terminal signal sequence, but no C-terminal hydrophobic membrane anchor sequence consistent with the observation that this antigen is secreted into the parasitophorous vacuole surrounding the intracellular parasite and released into the plasma or culture medium at the time the schizont infected cell ruptures.

The genes for five different S-antigens have now been sequenced and each contains a single exon with a large central block of repeats. A comparison of the repeats in the different S-antigens showed remarkable differences with variations in both the length and sequence of the repeat unit (Fig 1). For example, in contrast to the approximately 100 repeats of the 11 amino acid sequence in FC27, the S-antigen of the Ghanaian isolate NF7 is characterized by an 8 amino acid sequence that repeats approximately 40 times. Furthermore, whereas the repeat sequence in FC27 is conserved, there are two different 8 amino acid repeats in the NF7 S-antigen because of a substitution of leucine for arginine at one of the eight positions in the repeat. At the C-terminal end of the major block of repeats the NF7 S-antigen contains two copies of a 15 amino acid sequence. Although the amino acid sequences differ, the repeats of the various S-antigens are related, particularly at the level of the nucleotide sequence. This is most clearly seen when the major repeats are compared with a second repeat of 15 amino acids that is found at the C-terminal end of the major repeat in NF7, K1, and V1. For example, the sequence of the 11 amino acid repeat found in the V1 S-antigen was totally contained...
Major Repeat Sequences:  
- FC27: PAKAQGQLED x 100
- NF7: ASDAEAE x 43
- K1: GSDQEVKQKEQ x 22
- V1: GPPQEPKQ x 19
- Wellcome: GPNSDGDK x 65

Minor Repeat Sequence: NF7, K1 & V1: SEAGTEGPKGTGGPG

**Fig 1.** Structural diversity in the S-antigen of *P. falciparum*. Schematic diagrams are given for three types of S-antigen gene sequenced to date.° S-antigens of K1 and V1 are closely related to those of NF7.

The repetitive sequences comprise the major portion of the S-antigen polypeptide chain but they are flanked on the N-terminal and C-terminal sides by approximately 65 and 40 residues, respectively, of nonrepetitive sequence. The length of these flanking sequences is conserved in the different S-antigens, but the size of the repetitive region can vary enormously because both the size of the repeat unit and the number of repeats differ in various S-antigens. These differences are the cause of the remarkable size polymorphism that has been observed in the S-antigen system with relative molecular masses ranging from M, 50,000 to M, 250,000,°,15

Although the nonrepetitive flanking sequences in S-antigens are more conserved than the repeats, there is still considerable sequence diversity in these regions of the S-antigen polypeptides. The five known sequences fall into three groups with respect to these flanking sequences. The sequences in V1, K1, and NF7 are nearly identical but they differ significantly from FC27 and Wellcome.°,9

In the alignment of the FC27, NF7, and Wellcome sequences by Nicholls et al,° there are bp differences or deletion/insertions in one or more of the isolates at 19% of the positions. At these sites with differences, Wellcome and FC27 are the same at 65% of the positions, whereas the similarity between Wellcome and NF7, and NF7 and FC27, is 55% and 35%, respectively.

S-antigens appear to be concentrated in the parasitophorous vacuole surrounding merozoites in schizont-infected cells and are released as soluble antigens at the time of schizont rupture. No function has yet been attributed to S-antigens. It is difficult to imagine a function for a soluble protein with such extensive variation throughout the polypeptide chain. All other known protozoan proteins that show allelic sequence differences of this magnitude are associated with parasite surfaces, examples being the variable surface glycoprotein (VSG) of African trypanosomes° and the merozoite surface antigens of *P. falciparum*.°,22° S-antigens lack the primary structural features of a membrane protein in that although there is an N-terminal signal sequence there is no other hydrophobic sequence to provide a trans-membrane domain or a signal for attachment of a glycosyl phosphatidylinositol anchor as is found in the VSG° and merozoite surface antigens.°,22°

The early studies of Winchell et al and Wilson, using polyclonal antisera containing multiple specificities, indicated that there were a very large number of different S-antigens and that at least some of the individual S-antigen serotypes of *P. falciparum* were widely dispersed.°,22° The worldwide distribution of individual S-antigen serotypes has been confirmed with the more defined reagents that are now available for characterizing S-antigens. For example, a monoclonal antibody (MoAb) specific for the FC27 S-antigen has been used to detect parasites of this serotype in Papua New Guinea, Southeast Asia, South America, and Africa.° However, in a study of the parasites infecting...
individuals in neighboring villages in the Madang region of Papua New Guinea, Forsyth et al found that the prevalence of the FC27 serotype varied dramatically in both space and time.25,26

From the prevalence of the FC27 serotype of *P. falciparum* in the Madang area of Papua New Guinea (and elsewhere for that matter) it seems likely that all adults living in areas of continuous transmission would have been infected at some time with parasites of this serotype. Nevertheless, antibodies to this S-antigen are only found in a minority of individuals.26 There are several possible explanations for this, possibly the most obvious being that not all individuals are readily infected with all S-antigen serotypes of *P. falciparum*. This seems unlikely in view of the worldwide distribution of different serotypes. A second explanation could be that antibody responses to S-antigens may be relatively short-lived. This must be at least partially true because antibodies to S-antigens are most prevalent in adults and are not often found in children, although they may have been infected numerous times over several years.23 The observed pattern of antibody responses could also arise if a single infection with parasites of a given S-antigen serotype did not necessarily result in a detectable antibody response. Factors that could influence the antibody response include the level of the parasitemia and a variety of host factors such as age, nutritional status, and genotype. Several different genetic traits may influence the antibody response to S-antigens and other malarial antigens. Variant genes that result in phenotypic changes in the erythrocyte such as α thalassemia27 and ovalocytosis28 provide some resistance to malaria and may therefore limit antibody responses because of the relatively lower parasitemias. Genes located at the major histocompatibility locus may be a more important determinant of antibody responsiveness. The antibody responses to S-antigens are essentially against a single repetitive epitope (or a small number of epitopes encoded by an eight-to-12 amino acid sequence repeat). T-cell epitopes have not yet been identified in S-antigens, so it is not known whether the T cells that provide help for antibody responses to S-antigens see epitopes within the repeat or nonrepeat regions. Nevertheless, it seems likely that individuals of different major histocompatibility complex (MHC) haplotypes will vary in their ability to mount immune responses to these unusual antigens.

Another factor that may contribute to the pattern of S-antigen antibody responses is interactions between different infections. Different S-antigens characterizing different infections vary in their antigenic relatedness. For example, cross-reactions among the K1, NF7, and V1 S-antigens have been seen with some antisera, but the other characterized S-antigens are antigenically distinct.4,5 However, the FC27 S-antigen cross-reacts with the ring-infected erythrocytes surface antigen [RESA], an asexual blood-stage antigen that does not exhibit diversity among different isolates of *P. falciparum*.29 If stable S-antigen antibodies in adults reflect the cumulative effect of more than one infection, the sequence and timing of infections with more-or-less cross-reactive S-antigen serotypes may determine whether or not an individual has detectable antibodies of a particular specificity.

**Merozoite Surface Antigen (MSA) 1**

Attempts to characterize the surface of *P. falciparum* merozoites by surface radiolabeling indicated that there were more antigens on the merozoite surface than on the surface of sporozoites.30,31 It is now clear that several of these antigens are derived by proteolytic processing of a large precursor polypeptide at about the time of schizont rupture.32,33,34 This molecule has been given many names but in this review it will be referred to as MSA 1. Immunofluorescence and immunoelectronmicroscopic studies have provided additional evidence that this molecule is located on the merozoite surface.35-37 The primary structure of this molecule, deduced from the nucleotide sequence of the gene, confirmed that it is a membrane protein. The N-terminal sequence has the characteristic features of a cleavable signal peptide and there is a hydrophobic sequence at the C-terminal end of the polypeptide.38,39 Several lines of evidence indicate that this C-terminal hydrophobic sequence is not a transmembrane domain in the mature protein but is probably cleaved posttranslationally at the time of attachment of a glycolipid moiety (GPI anchor) that serves as the membrane anchor for the mature protein. The evidence that MSA 1 is modified in this way is: (1) the observation that the antigen can be radiolabeled with mannose, glucosamine, and myristate; (2) the pattern of glycosylation of MSA 1 fragments which indicates that all the sugar is associated with the C-terminal M, 42,000 to 45,000 fragment; and (3) the release of the incorporated fatty acid when the molecule is subjected to mild alkali hydrolysis or the action of phospholipase C.23 Although there is no single sequence that provides a signal for the attachment of a GPI anchor to membrane proteins there are some C-terminal sequence similarities between MSA 1 and other protozoan proteins that have a GPI anchor.40,41 Studies on various isolates of *P. falciparum* with MoAbs to MSA 1 indicated that this antigen contains both variable and conserved epitopes.35,46,47 Seven antigenically distinct forms of MSA 1 were defined by the pattern of reactivity with a panel of MoAbs. None of these antibodies recognized epitopes that were unique to any individual isolate and the large number of antigenically different forms recognized reflected the fact that the antibodies recognized epitopes in three non-overlapping segregating sites.39 The sequences of the MSA 1 genes from a number of different *P. falciparum* isolates have provided an understanding of the structural basis of the antigenic complexities exhibited by the MSA 1 of *P. falciparum*.17,18,30,39,48-53 From a comparison of the available sequences, the polypeptide could be divided into 17 blocks that were either variable, conserved, or semi-conserved (Fig 2).17,53 Interestingly, the variable blocks (and the limited variation in the more conserved regions of the molecule) have only two forms, which suggests that the existing alleles of MSA 1 have been generated largely as a result of intragenic recombination between two parental alleles. It appears that the recombinational events that lead to the diverse MSA 1 alleles were confined to the N-terminal constant regions (blocks 3 and 5) as there has been no reassortment of the two types of variable regions on the C-terminal side of block 5, which would have occurred if...
recombination occurred within conserved block 12. This dimorphic model does not explain all the variation seen in MSA 1, as two isolates have been described recently that have additional variation within variable block 2. This region contains a set of degenerate tripeptide sequence repeats, and although the repeats in MSA 1 form a relatively small part of the polypeptide chain, this region is the most variable part of the molecule.

There is much evidence from studies with the *P. falciparum* antigen and the homologous antigen in a number of other *Plasmodium* species that MSA 1 is capable of inducing protective immune responses. Although there is extensive diversity in MSA 1, the limits to this diversity imposed by the dimorphic model and the apparent selection against recombinants that would create further diversity in the C-terminal region of the molecule allow some optimism that it will be possible to generate a vaccine based on MSA 1 effective against diverse strains of *P. falciparum*.

*MSA 2.* Another merozoite surface antigen has been identified in the detergent phase when infected erythrocytes were fractionated by temperature-dependent phase separation using Triton X-114. This antigen, which has been designated MSA 2, is a much smaller polypeptide than MSA 1, having a relative molecular mass determined by sodium dodecyl sulphate-polyacrylamide electrophoresis of 45,000, but a molecular mass calculated from the amino acid sequence of close to 28,000 daltons. However, like MSA 1, MSA 2 can be labeled with glucosamine and myristate, evidence that it is also posttranslationally modified by the addition of a C-terminal GPI moiety that acts as the membrane anchor of the mature polypeptide. The primary structure of the molecule is consistent with such a posttrans-
lational modification: there is a hydrophobic sequence close to the C-terminus, but, as in other proteins that have a GPI anchor (e.g., the trypanosome VSG), there are no charged residues C-terminal to this sequence. Furthermore, there is some sequence similarity on the N-terminal side of the hydrophobic sequence with some other parasite antigens modified in this way.

Several groups have described a small molecular mass merozoite surface antigen that probably corresponds to MSA 2.\(^6\) MoAbs to this antigen have in some instances been shown to inhibit merozoite invasion.\(^6\)\(^8\)\(^9\) In order to identify the epitope recognized by an inhibitory MoAb against the MSA 2 of isolate FC27, the antibody was reacted with a complete overlapping set of peptides and bound antibody detected by ELISA. This “Geyser Scan” identified a 4 amino acid sequence STNS as being central to the epitope recognized by the inhibitory monoclonal antibody.\(^7\) This sequence occurred twice in MSA 2 as it was within a 32 amino acid sequence that occurs in two tandem copies in the MSA 2 of FC27. This same epitope was identified in an otherwise unrelated antigen a fragment of which was expressed by a \(\text{gpl} 11\)-Amp3 clone that reacted with the anti-MSA 2 MoAb.\(^9\) This antibody and other MoAbs presumably directed against MSA 2 do not react equally with all isolates of \(P\) falciparum. Thus MSA 2, like MSA 1, is antigenically diverse.\(^6\)\(^7\)\(^9\)

There are now sequences available for the MSA 2 gene of three isolates, and a comparison of these sequences suggests that antigenic differences among the MSA 2s of different isolates largely reflect different repeat structures (Fig 2). As mentioned above, the MSA 2 of FC27 contains two copies of a 32 amino acid sequence.\(^9\) In contrast, the MSA 2 of isolate Indochina I and the cloned line 3D7 contain a 4 amino acid repeat (GGSA), with 12 copies in Indochina I and 5 copies in 3D7.\(^3\)\(^0\)\(^3\)\(^1\) As well as the different repeats, there are sequence differences in the regions flanking the repeats. These differences are particularly marked on the C-terminal side of the repeats and there are extensive deletions in the Indochina I sequence relative to the 3D7 sequence which compensate for the longer repeat sequence so that the overall size of the three forms of MSA 2 is preserved. At either end of the MSA 2 polypeptide chain there are very conserved sequences. The N-terminal 43 residues are identical in all three isolates and within the C-terminal 74 residues there is only one position where there is a difference.

DNA probes for the two types of repeats in the three MSA 2 genes that have been sequenced have been used to probe DNA amplified by the polymerase chain reaction from other isolates, and it is clear that although the majority of isolates hybridized with either the 32 or 4 amino acid repeat probes there are forms of MSA 2 containing other types of repeat sequences.\(^3\)\(^0\)\(^3\)\(^1\)

Some individuals from endemic areas have high titre antibodies reacting with a synthetic peptide (GGSA), corresponding to three copies of the repeat found in the Indochina I and 3D7 MSA 2.\(^7\)\(^3\) It remains to be established whether naturally occurring antibody responses recognize epitopes in the conserved or variable sequences flanking the repeats. Nevertheless the diversity in MSA 2 appears more analogous to that in S-antigens than that in MSA 1, as both MSA 2 and S-antigens contain a central block of immunogenic sequence repeats that can vary in sequence, length of the repeat unit, and number of repeats.

Polymorphic Antigens Associated With the Membrane of \(P\) falciparum-Infected Erythrocytes

\(Pf\) EMP 1. During the intracellular maturation of the asexual blood stages of \(P\) falciparum, several protein antigens are synthesized and then transported out of the parasite to become associated in various ways with the erythrocyte membrane. One of these antigens, the \(Pf\) EMP 1, is exposed on the external surface of the infected erythrocyte and may mediate the cytoadherence of these cells to the vascular endothelium.\(^7\)\(^4\)\(^7\)\(^8\) Different isolates of \(P falciparum\) express antigenically different forms of \(Pf\) EMP 1 as shown by immunoprecipitation experiments with \(Pf\) EMP 1 labeled by the surface radiodiagram of infected erythrocytes.\(^7\)\(^3\)\(^7\)\(^1\) Pf EMP 1 is probably the antigenically diverse molecule recognized on the surface of infected erythrocytes by indirect immunofluorescence or agglutination.\(^8\)\(^3\)\(^4\) These studies suggest that the diversity in \(Pf\) EMP 1 is very extensive.

\(Pf\) EMP 1 has not yet been cloned and therefore the structural basis of diversity in this molecule is unknown. In addition to antigenic diversity, \(Pf\) EMP 1 is polymorphic in size varying from \(\sim M, 240,000\) to \(\sim M, 350,000\).\(^7\)\(^8\) When cloned populations of parasites were selected for increased or decreased ability to cytoadhere, a change was observed in the \(M\), of the dominant antigen on the surface of infected erythrocytes with the ability to cytoadhere correlating with the expression of a higher \(M\), molecule presumed to be \(Pf\) EMP 1.\(^7\)\(^8\) These rapid changes in size, which were reversible, are consistent with \(Pf\) EMP 1 also containing an extensive repeat structure.

\(Pf\) EMP 2. A second polymorphic antigen that associates with the erythrocyte membrane has been called the mature parasite-infected erythrocyte surface antigen (MESA).\(^3\) or \(Pf\) EMP 2.\(^3\)\(^6\) MESA is also a very large polypeptide (\(\sim M, 240,000\) to \(\sim M, 300,000\)) but differs from \(Pf\) EMP 1 in not being exposed on the erythrocyte surface.\(^7\)\(^3\)\(^7\) MESA is a phosphoprotein\(^7\)\(^3\)\(^8\) and has been localized on the cytoplasmic face of the erythrocyte membrane by immunoelectronmicroscopy.\(^8\)

A partial sequence of MESA derived from a cDNA clone of isolate FC27 contains an extensive set of hexapeptide repeats having the sequence GESKET.\(^8\) Antibodies to MESA are abundant in sera from infected humans or experimental animals\(^8\)\(^3\)\(^8\) and the hexapeptide repeat encodes a dominant epitope. A rabbit antisera to a dodecameric synthetic peptide corresponding to two repeat units reacted with all isolates of \(P falciparum\), so at least some of these repeats are invariably present in MESA. Variable reactivity of affinity purified antibodies with different isolates may be due to a variable number of repeats or reactivity with other epitopes that are not conserved in all isolates.\(^8\) The antibody
studies have not established which isolates of *P. falciparum* are expressing antigenically distinct forms of MESA; however, the dramatic restriction fragment length polymorphisms shown by the MESA gene and the variation in size of the antigen among different isolates suggest that there are many different MESA alleles.

**Pf11-1.** This is a third large molecular mass polymorphic antigen associated with the erythrocyte membrane. This antigen, which was identified with antibodies affinity purified on the cloned antigen, is polymorphic in size ranging from M, 260,000 to M, 350,000.\(^{99,100}\) The gene sequence predicts a polypeptide that contains three blocks of tandem sequence repeats composed of 3, 6, and 9 amino acids. The six amino acid repeats are either different or are absent from some isolates because a probe for this region of Pf11-1 failed to hybridize with DNA from 2 of 7 *P. falciparum* isolates. The repeats in Pf11-1 are immunogenic but the extent of the antigenic polymorphisms in Pf11-1 remain to be defined.

**Histidine-Rich proteins (HRPs)**

Two of the three *P. falciparum* HRPs that have been identified interact at some stage with components of the host cell membrane. Pf HRP 1 (or KAHRP, the knob-associated histidine rich protein) is localized in the membrane knobs,\(^{91-94}\) whereas Pf HRP 2 is transported to the erythrocyte membrane and then released from the infected cell.\(^{95}\) Each of the HRPs exhibit size polymorphisms among different isolates of *P. falciparum*. In the cases of Pf HRP 1 and Pf HRP 2 this reflects differences in the number of sequence repeats as observed for many other antigens.\(^{93,96-99}\) However, to date there has not been any report of antigenic differences between HRPs of different isolates.

**Polymorphisms in Other Antigens**

Not all the antigenic diversity among different isolates of *P. falciparum* reflects extensive sequence differences of the type described above. Studies on a small protein with the primary structural features of an integral membrane protein have shown antigenic differences due to single amino acid substitutions. This antigen, which has been called CRA,\(^{100}\) or exp 1,\(^{101}\) was first detected with an MoAb (MoAb 5.1) that also reacted with the CS protein of *P. falciparum*.\(^{102,103}\) The deduced amino acid sequence of CRA does not contain any true repeats, but there is a region of internal homology that includes the sequences NANP (the dominant sequence repeat in the CS protein) and the related sequence NADP (which contains one of the two substitutions found in the minor repeat of the CS protein). Some *P. falciparum* isolates fail to react with MoAb 5.1 and the CRA gene has been sequenced for four such isolates.\(^{101}\) In each case the aspartic acid residue in the tetrameric sequence NADP was replaced by glycine as a result of an A → G transition mutation. The sequences are known for the CRA genes of five isolates of *P. falciparum* and there are amino acid differences at only two other positions; one of these mutations also leads to antigenically distinct forms of CRA (A. Saul, personal communication, January 1989).

**Antigenic Diversity and Immunity**

Antigenic diversity is well-established as a mechanism that enables pathogens to survive in previously exposed hosts who have mounted protective immune responses. The best studied example among pathogenic protozoa is the African trypanosome. In a process known as antigenic variation, a single trypanosome changes its antigenic identity as a result of synthesizing a different variant surface glycoprotein (VSG). Complex genomic rearrangements regulate the expression of alternate VSG genes and the process enables a trypanosome to survive in the face of an antibody response that eliminates parasites of the original variant antigen type.\(^{105}\) Much evidence indicates that *P. falciparum* is also capable of undergoing antigenic variation. Although the *P. falciparum* antigen involved in this process remains to be identified with certainty, considerable evidence, particularly from other species of *Plasmodium*, indicates that the variant antigen is associated with the external surface of red cells containing mature parasites.\(^{106-109}\) (The possibility that PfEMP1, one of the polymorphic antigens discussed above, is the variant antigen of *P. falciparum* cannot yet be excluded.) The antigen polymorphisms discussed in this review should not be confused with antigenic variation. The diverse forms of the S-antigens or merozoite surface antigens are encoded by allelic genes that are relatively stable and, at least in some cases, appear widely dispersed.\(^{106,107,108}\)

If antigenic variation does occur in *P. falciparum*, what is the significance of the polymorphisms generating considerable antigenic diversity in other proteins expressed by the asexual blood stages of *P. falciparum*? In particular, are these polymorphic antigens involved in the induction of immunity in individuals naturally infected with *P. falciparum*? Much experimental evidence indicates that merozoite surface antigens can induce anti-parasitic immune responses, and because they are natural immunogens it seems probable that they do contribute to the development of immunity against malaria. Evidence from longitudinal field studies\(^{109}\) has associated antibodies to antigens on the red cell surface with resistance to infection, but no such evidence exists for the other antigens discussed here and such studies are urgently required.

It has been argued that the high frequency of mutations leading to amino acid changes (nonsynonymous mutations) in malaria antigens is “more likely to be a consequence of a relative absence of selective constraints—rather than the presence of biological selection.”\(^{110}\) Although there is obviously great permissiveness with respect to the primary structure of some of these antigens, the observation that the sequenced genes for each of these polymorphic antigens can be grouped into “allelic families” indicates that some selective constraints apply. As others have argued for the variation that occurs in nonrepetitive regions of the *P. falciparum* circumsporozoite protein,\(^{111}\) the different allelic forms of these antigens could not become fixed in the parasite population and be widely dispersed unless they provide a biologic advantage for the parasite. The advantage to the parasite of varying the sequence of antigens, such as merozoite surface antigens, which probably induce and/or are probable targets...
of immune responses, is obvious. The case for immune responses providing the selection pressure for variation in MSA 1 and MSA 2 would be even more compelling if the variable rather than conserved regions of the molecule were shown to be preferred locations of T- or B-cell epitopes. As discussed above, the epitope recognized by one inhibitory MoAb to MSA 2 is located in the sequence repeat, but it is not yet established whether the repeats in this molecule are as immunodominant as those in some other antigens.

The biologic advantage of diversity in other antigens that are not obvious inducers or targets of protective immune responses (eg, S-antigens) may also derive from effects on the host immune response. In addition to the diverse repetitive sequences in the allelic gene products discussed above there are many other antigens of P falciparum that contain immunodominant repeat sequences. The diversity and immunodominance of these repeats led to the suggestion that the immune system of an individual confronted with frequent infections with different parasites would be overloaded, and therefore respond poorly to critical protective epitopes. The observation of sequence relationships among different repetitive sequences in P falciparum antigens and a network of cross-reactions between these repeats suggested a mechanism whereby this diversity of repeat epitopes could modify host immune responses to the parasite’s advantage.

The normal maturation of high-affinity antibody requires the selective proliferation of B cells which have sustained somatic mutations that increase the affinity for the triggering epitope. In malaria, the network of cross-reacting repeat epitopes provides an array of structural analogues for many epitopes that trigger antibody responses. We hypothesized that reaction with one of these structural analogues may cause continued proliferation and eventual antibody production by clones other than those that sustain somatic mutations leading to higher affinity binding sites. Because many more progeny of proliferating B cells would undergo clonal expansion, a large amount of low-affinity antibody would be produced, which would explain the hypergammaglobulinemia associated with chronic malaria. Competition between clones would limit the expansion of clones sustaining multiple somatic mutations (Fig 3). We have not yet been able to directly test this hypothesis, but in recent years additional examples of cross-reactions have been documented, providing additional support for our belief that cross-reactions in some way modify immune responses to these antigens.

The host immune response may not be the only source of selection pressure that has been responsible for the diversity in antigens of P falciparum. Some of the diversity in these malarial proteins may have evolved to enable P falciparum to parasitize efficiently host erythrocytes of different phenotypes. Malaria has been a major selective pressure in the evolution of the human erythrocyte and is responsible for the prevalence of genetically determined pathologic conditions such as sickle cell anemia, thalassemia syndromes, and G6PD deficiency. Given the extent of the diversity in human erythrocytes, and the specificity of the interaction between parasite and erythrocyte, it would be surprising if parasites of different genotypes did not vary in their ability to invade and grow in different erythrocytes. Indeed, studies using mutant or enzyme-treated erythrocytes have shown that there are at least two pathways for invasion of erythrocytes by P falciparum. Which parasite molecules are involved in these different invasion pathways is not clear, but it seems likely that at least one of the polymorphic merozoite surface antigens is involved in the invasion process.

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