The circumsporozoite protein of *Plasmodium*: a mechanism of immune evasion by the malaria parasite?

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Sporozoites of malaria are covered with a repetitive surface antigen, the circumsporozoite (CS) protein. This antigen also appears to be a major target of the host immune response. The natural immunogenicity of the CS protein has led to attempts to develop the molecule as a vaccine candidate. It seems paradoxical, however, that a successful parasite should present to the host an immunogenic surface molecule which would induce protective immunity. In this paper we suggest that the CS protein is not the target of protective immunity under natural conditions, and that naturally immunogenic repetitive antigens in malaria and other parasites have evolved as a mechanism of immune evasion, via the induction of thymus-independent B-cell responses.

Introduction

In the decade since the first identification of the circumsporozoite (CS) protein of malaria sporozoites (51), there have been a large number of studies devoted to various aspects of the biology, genetics, and immunology of this protein. Included in this body of work have been several attempts to vaccinate against the parasite using CS analogues. Indeed, the hope that the CS will serve as an anti-malarial vaccine is the primary reason for the amount of attention given to this molecule. Despite this considerable research effort, however, an essential question remains unanswered, namely “what is the functional role of the CS protein in the natural history of the parasite?” An answer to this question is surely a basic prerequisite for the rational, as opposed to empirical, approach to vaccine design.

In this paper, we will review briefly some of the salient features of the biology and immunology of the CS protein, and describe how this molecule came to be chosen as a vaccine candidate. We will then present some novel data that arose from our attempts to investigate the regulation of the immune response to the CS protein. These data support the conclusion that one primary role of the molecule is to act as a mechanism of immune evasion for the parasite.

The proposition that the CS protein may function as a mechanism of immune evasion is strongly at odds with the suggestion that this antigen is the target of naturally acquired immunity to malaria. However, there is little evidence to support the latter view, and it is helpful to review this issue before turning to the question of the function of the protein in the natural history of the parasite.

Do sporozoites induce protective immunity?

The solid protective immunity induced under certain experimental conditions by irradiated sporozoites is not a feature of all host/malaria parasite systems. It is best described in two rather unusual host/parasite models. In more natural host/parasite combinations, the protection is more difficult to obtain. For example, exposure of chickens to ultra-violet irradiated *Plasmodium gallinaceum* sporozoites successfully immunized against subsequent viable challenge (34). These observations were later extended to X-irradiated sporozoites of *P. berghei* in mice (35). However, this level of protection was considerably harder to achieve in comparable immunization studies using human, simian or other rodent malaria sporozoites. Ward and Hayes (1972) immunized three rhesus monkeys (*Macaca mulatta*) against *P. cynomolgi* by three rounds of bites of X-irradiated infected mosquitoes over 42 days, which failed to provide any degree of protection against infective bite (47). Similarly, two rhesus monkeys immunized with *P. cynomolgi* were not protected upon challenge (9). In another study, 12 rhesus monkeys immunized intravenously with *P. cynomolgi* were challenged when high levels of circumsporozoite precipitin antibodies were detected in their sera. Of these animals, 2 resisted challenge and 10 were susceptible (5). After extensive immunization of human volunteers by multiple exposure to irradiated sporozoites of *P. falciparum*, more than half remained susceptible to the bite of infected mosquitoes.

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while only two of five could be protected against *P. vivax* (7).

Of great importance is the recent finding that some rodent strains cannot be successfully immunized with irradiated sporozoites of *P. yoelii* (50). Both MHC and other loci play a role in determining the acquisition of immunity. This is in marked contrast to the *P. berghei/mouse* model, where functional immunity may be obtained in all mouse strains tested (25). This difference apparently is not due to varying degrees of immunogenicity exhibited by the sporozoites of each species. Furthermore, it is unlikely that such similar sporozoites will prime very different immune effector mechanisms. Rather, it is most likely that this reflects instead the very different degrees of natural infectivity of the sporozoites for the host. It is clear that *P. berghei* has a very low degree of infectivity for laboratory mice as compared with *P. yoelii*, which, for example, gives rise to pre-erythrocytic liver schizonts easily detectable by microscopy. The difference in infectivity may be as great as two logs. Thus there are good grounds for proposing that acquired immunity operates best in circumstances where a high degree of innate immunity produces conditions inimicable to the parasite; in contrast, when natural infectivity of sporozoites for their host is high, acquired immunity is subject to threshold effects and the pattern of protection will be variable, being under polygenic control.

The proposition that solid immunity operates best when innate immunity is already high is obvious and unremarkable. However, it does seem that these conditions apply in the host/parasite model upon which much anti-sporozoite vaccine research is based, namely the *P. berghei/laboratory mouse* model. Those host/parasite combinations in which acquired immunity is more difficult to obtain appear to resemble more closely the balance as it is found under natural conditions, i.e., they are the true co-adapted host/parasite pair, or at least provide more accurate models of pre-erythrocytic malaria. This raises the question of how far the irradiated *P. berghei* sporozoite type of protective immunity may be extended to humans. The limited evidence so far indicates that exposure of humans to sporozoites provides considerably lower protection than that obtained in the *P. berghei/mouse* model.

The question of whether sporozoites induce any degree of protective immunity under conditions of natural transmission is difficult to answer conclusively. Several studies suggest that naturally acquired antibodies are non-neutralizing (26, 31). Even adults chronically exposed to natural transmission appear to be susceptible to new infections, although they are capable of controlling the infection at the blood-stage level. It is generally considered that naturally acquired immunity does not operate against sporozoites, although this must remain somewhat moot until put to experimental test.

**Does the CS protein induce protective immunity?**

Notwithstanding the limited suitability of the *P. berghei/laboratory mouse* system as a model for acquired immunity in the human malarias (and other co-adapted host/parasite combinations), it has nonetheless been worthwhile to dissect the functional immunity obtained in this model as a first step in vaccine research. Although providing considerable proof of the natural immunogenicity of the CS protein, there has been little direct evidence from this or other models demonstrating that the CS protein is responsible for inducing protective immunity against sporozoites. For example, multiple immunizations with attenuated sporozoites lead to high levels of antibody, directed almost exclusively to the repetitive domain of the CS surface antigen. Nonetheless, the high titre of anti-repeat antibody obtained in this way is ineffectual in neutralizing parasites. Sporozoites escape the antibody and penetrate the liver, where protective immunity to the parasite can result from cellular responses (41, 49). Indeed, parasite-induced antibody is invariably ineffective in neutralizing parasites when passively transferred into naïve recipients (36, 41).

When considering the biological function of the CS protein, and the involvement of this antigen in natural or experimentally acquired immunity, it is essential to avoid data derived from experiments that are fundamentally irrelevant to the natural history of the parasite. For example, pre-incubation of infectious sporozoites for 30 minutes or longer periods with anti-CS antibodies will inhibit their ability to penetrate hepatocytic target cells *in vitro*, and neutralize their infectivity for susceptible hosts. These antibodies, however, are routinely unable to provide protection when passively transferred into the same hosts. Thus, although some antibody preparations may be sufficient to neutralize sporozoite infectivity under certain experimental conditions, this evidence cannot be extrapolated to imply that the CS protein is a target of protective humoral immunity under conditions of natural exposure to the parasite. Similarly, whereas monoclonal antibodies directed towards the repetitive domain of the *P. berghei* CS protein can confer protection in passive transfer (39), it should be clear that this represents a situation not truly found during the natural immune response to sporozoites. Of course, these conditions may be those we wish to achieve for a successful vaccine, and the failure of polyclonal antibodies derived against the parasite to
neutralize infectivity in vivo does not necessarily invalidate the hope for an effective humoral vaccine. However, the data clearly indicate that under natural conditions of exposure to malaria, and even after immunization with irradiated sporozoites, the CS protein does not induce neutralizing antibodies.

The immunity that does result from immunization with irradiated P. berghei sporozoites is clearly directed towards the developing pre-erythrocytic schizonts, and may be mediated by cytotoxic T cells (41, 49), lymphokines (41), or other undescribed mechanisms (42). It is not known if the CS protein is either the target or trigger of this effector phase. Apparently, large numbers of CS-specific CD8+ T cells are sufficient to provide protection against P. berghei upon adoptive transfer into the somewhat resistant white mouse host (40). However, this T-cell clone does not protect against the more infectious P. yoelii sporozoites although there can be cross-reactivity with the corresponding P. yoelii sequence (40). As outlined above, protection can be obtained in a similar fashion by transfer of monoclonal antibodies directed towards the repetitive domain of the CS protein. Clearly, these observations cannot be taken as demonstrating that cytotoxic T cells or antibodies directed against the CS protein provide protection against malaria under natural conditions or following experimental exposure to irradiated sporozoites.

The function of the repetitive domain: an hypothesis

Included within the general issue of the role of the CS protein is the more specific question of the function of the immunodominant repetitive domains found within the molecule. The role of conserved, tandemly repetitive regions within protein antigens poses an unsolved question that applies to a multitude of the higher eukaryotic parasites such as Plasmodium, Toxoplasma, Leishmania, Trypanosoma, Schistosoma, etc., which share in common this unusual feature. As observed in a recent review, of 30 malarial antigens sequenced to date, 29 contained tandem repeats (48).

These repetitive domains are strong B-cell epitopes, being often the only antigenic sites on these molecules recognized by the humoral immune system of the host. The repetitive (NANP)n sequence of the falciparum CS protein is able to absorb the entire serological reactivity of human immune sera against the sporozoite stage of the parasite, demonstrating that this region alone is the single immunodominant epitope (53). Indeed, the natural immunogenicity of these antigens appears to be the major reason for their selection as vaccine candidates. Notwithstanding the repeated failure of polyclonal antisera to transfer immunity from donor to recipient (36, 41), these same antibodies or their monoclonal derivatives have been used to identify antigens expressed in recombinant DNA libraries. These naturally immunogenic molecules were subsequently developed as vaccine candidates, under the assumption that antibodies would confer protective immunity. Thus there are currently attempts to immunize against malaria by utilizing synthetic peptide or recombinant protein analogues of the repetitive domains of the CS protein and the ring-infected erythrocyte surface antigen (3, 8, 24).

The suggestion that repetitive antigens may induce neutralizing antibodies raises a central paradox: why would the parasite readily display on its surface highly immunogenic antigens which might induce parasiticidal antibody? This question is closely related to one more fundamental: what is the functional role, or selective (Darwinian) rationale of repetitive antigens in Plasmodium and other parasites? One hypothesis postulates that each unit of a repetitive domain acts as a ligand for a host protein, the multimeric nature of the repeats serving to increase greatly the affinity of the interaction (37). However, (a) structurally distinct repeats occur among proteins that are phylogenetic homologues, despite the fact that they must perform similar functions; (b) the hypothesis does not explain the immunogenicity of the repeats; and (c) the central requirement of this hypothesis remains unfulfilled, because despite extensive investigation no evidence exists that repeat regions are ligands for host cells such as hepatocytes or erythrocytes.

Clearly, questions concerning (a) the functional role of these repetitive domains, (b) the reasons underlying their immunodominant nature, and (c) whether their interaction with the host immune system leads to protective immunity, assume central importance, given the predominance of such elements and their candidacy as malaria vaccines. We suggest that recent experimental findings, outlined below, might provide some answers to these questions, and provide a plausible selective (Darwinian) rationale for the presence of repetitive domains in protein antigens of malaria and other eukaryotic parasites. These findings, which suggest that these vaccine candidates may actually function as a novel mechanism of immune evasion, arose from the elucidation of the pathway of antibody production to the repeats, as follows:

The anamnestic antibody response to synthetic peptides and most proteins is under 1r gene control. Following the studies of Chestnut & Grey, which demonstrate the ability of B cells to serve as antigen-presenting cells, the requirements for hapten-carrier linkage and MHC-restricted physical interaction among lymphocytes during antibody production is thought to result from the ability of antigen-specific B cells to take up antigen by surface Ig, and present
the relevant immunogenic peptides to antigen-specific helper T cells in the context of self MHC (4). This framework is assumed to apply in the case of antibody responses to the repetitive domains of malarial antigens. The major impetus for accepting this view was the finding that a synthetic peptide corresponding to the immunodominant (NANP)_n repeat sequence of the P. falciparum CS protein was a poor inducer of antibody formation in human volunteers for vaccination. It was also shown that all mice except those bearing the H2b haplotype were unable to respond to the (NANP)_n peptide (12, 15). This nonresponsiveness resulted from the failure of the synthetic peptide B-cell epitope to associate with MHC class II molecules and induce a helper T-cell response. In contrast, conjugation of (NANP)_n to a carrier such as tetanus toxoid allowed a response to the repeat to develop during immunization (24). Moreover, mice of all MHC haplotypes develop antibody to (NANP)_n upon exposure to parasites. It was inferred therefore that in responses to intact parasites, one or more helper T-cell sites on the CS protein provide the capacity for antibody production via cognate T/B-cell cooperation.

In accordance with these premises, considerable effort has been devoted to mapping CS-specific T-cell sites, by the use of synthetic peptides which induce a proliferative response from peptide-primed murine lymphocytes, or act as carriers in Ir gene-controlled responses to synthetic constructs containing CS repetitive B-cells epitopes (15, 16, 18–20, 22, 44, 45). This is deemed to be essential to the development of anti-malarial vaccines, as it is hoped that these putative T-cell sites will enable T-cell activation and Ir gene-controlled boosting of antibody in response to parasites. However, this inferential logic rests on untested assumptions. This is because in all studies to date, the mapping of T-cell sites has been solely by homologous immunization and challenge with synthetic peptide or recombinant constructs. These studies therefore demonstrate that certain regions are non-self and can associate with Class II MHC, but do not address the question of their involvement in responses to intact parasites. Thus, the studies to date do not undertake the necessary tests required to show that parasite-immunized mice acquire CS-specific helper activity, or that any of these putative helper sites actually mediate Ir gene-controlled antibody formation in response to intact parasites. Indeed, Ir gene control of the antibody response to the native CS protein of sporozoites is itself a premise that remains to be validated by experimental test. This is critically important because cognate interaction among T and B cells is not the only mechanism by which antibody can be generated to protein antigens. In contrast to synthetic peptides or monomeric protein antigens, there exist cases in which antibody formation to complex or repetitive protein antigens, and some lipoproteins, is not under Ir gene control (I, 33).

Accordingly, we sought to test the assumption that cognate T-cell help is required for the production of antibodies to the repetitive domains of the P. falciparum and P. berghei CS proteins, by utilizing a variety of classical protocols originally designed to elucidate the phenomenon of Ir gene-controlled MHC-restricted cognate interactions among antigen-specific T and B cells. First among those we chose was the protocol of Katz et al., which allows Ig production among MHC matched, but not mismatched, T and B lymphocytes in irradiated, semiallogeneic F1 recipients (27). Using intact irradiated sporozoites as the immunizing antigen, the experiments demonstrate that the anamnestic IgG response to the repetitive region of native CS proteins does not require cognate interaction with sensitized T cells, and is not MHC restricted (43).

In simple adoptive transfer experiments using primed T and B cells from syngeneic donors, we were able to show that the secondary response to the native CS protein of sporozoites was thymus-independent, i.e., proceeded without requirement for antigen-specific helper T cells (Table 1). In addition, functional helper T cells from donors immunized with homologous sporozoites, conserved CS T-cell epitope peptides, or prototype CS vaccines, were unable to influence the magnitude of the thymus-independent response to the native antigen of parasites (Tables 1 and 2).

However, as euthymic animals produce IgG in response to sporozoites, it is clear that some T-cell information is required for the class switch. We have obtained evidence that this T-cell contribution is non-cognate (MHC-unrestricted help), by the use of irradiated, allogeneic bone-marrow chimeras. These animals, constitutively unable to mount cognate cooperation among their MHC mismatched T and B cell populations, were nonetheless able to respond to priming and boost with sporozoites with the production of anti-CS IgG, as were syngeneic controls (unpublished data).

One may question the use of the term T-independent when an MHC-unrestricted contribution by T cells may be evident. However, an in vivo T-1 response may be defined as one in which antigen drives antibody formation directly, without requirement for the MHC-restricted cognate interaction of antigen-specific T and B lymphocytes in secondary responses to antigen. That a factor-driven contribution may be sufficient to influence Ig isotype does not alter the T-independent status of an antigen.

Finally, we determined that exposure of mice to sporozoites, which leads to high levels of anti-CS Ig,
Table 1: Antigen-specific T cells are not required for secondary response to the CS protein of sporozoites

<table>
<thead>
<tr>
<th>T cells</th>
<th>B cells</th>
<th>Challenge</th>
<th>CS</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (naive)</td>
<td>B (naive)</td>
<td>SPZ</td>
<td>64</td>
<td>ND</td>
</tr>
<tr>
<td>T (naive)</td>
<td>B (SPZ + TT)</td>
<td>TT</td>
<td>ND</td>
<td>128</td>
</tr>
<tr>
<td>T (SPZ + TT)</td>
<td>B (SPZ + TT)</td>
<td>SPZ</td>
<td>4096</td>
<td>32</td>
</tr>
<tr>
<td>T (SPZ + TT)</td>
<td>B (SPZ + TT)</td>
<td>TT</td>
<td>ND</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8192</td>
<td>32</td>
<td>65536</td>
</tr>
</tbody>
</table>

a A/J mice were primed with 10 µg TT in FCA i.p., or 10^6 irradiated P. berghei sporozoites i.v. After four weeks, some were sacrificed and splenic lymphocytes from tetanus toxoid (TT) and sporozoite-primed donors were mixed. Adherent cells were removed by plating at 37°C, and B-cells removed by serial panning on goat anti-mouse Ig (H+L specific) coated plates; 1.5 x 10^7 T-cells were inoculated i.v. into naive A/J recipients. After 24 hours, these animals were irradiated (600 rad) and inoculated i.v. with 1.75 x 10^7 purified B-cells, prepared by mixing equal numbers of sporozoite and TT-primed spleen cells followed by two cycles of anti-Thy-1 + C treatment and centrifugation on Lympholyte M (Accurate Chem. Co.). T-cell lysis (>98%) was monitored by surface fluorescence assay. Twenty-four hours after transfer of B-cells, separate groups of mice (n = 4) were boosted with 10 µg TT in IFA i.p., or 10^6 irradiated P. berghei sporozoites i.v. After 8 days, sera were drawn and pooled, titrated serially 2-fold in 1% BSA/PBS, and the IgG (gamma-chain specific) titre to TT or repeat region of P. berghei CS determined by RIA.

Table 2: Lack of boosting by recCS-specific T cells

<table>
<thead>
<tr>
<th>T cells</th>
<th>B cells</th>
<th>SPZ</th>
<th>recCS</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (naive)</td>
<td>B (naive)</td>
<td>64</td>
<td>64</td>
<td>256</td>
</tr>
<tr>
<td>T (naive)</td>
<td>B (SPZ + TT)</td>
<td>2048</td>
<td>128</td>
<td>2048</td>
</tr>
<tr>
<td>T (SPZ + TT)</td>
<td>B (SPZ + TT)</td>
<td>4096</td>
<td>256</td>
<td>262144</td>
</tr>
<tr>
<td>T (SPZ + TT)</td>
<td>B (recCS + TT)</td>
<td>4096</td>
<td>256</td>
<td>262144</td>
</tr>
<tr>
<td>T (naive)</td>
<td>B (recCS + TT)</td>
<td>4096</td>
<td>256</td>
<td>2048</td>
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<tr>
<td>T (recCS + TT)</td>
<td>B (recCS + TT)</td>
<td>2048</td>
<td>16384</td>
<td>262144</td>
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<tr>
<td>T (recCS + TT)</td>
<td>B (SPZ + TT)</td>
<td>4096</td>
<td>65536</td>
<td>262144</td>
</tr>
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</table>

a A/J mice were primed with 10 µg recCS or TT in FCA i.p., or 10^6 P. falciparum sporozoites i.v. After four weeks, some were sacrificed and 2 x 10^7 T-cells from each donor group, purified by panning on goat anti-mouse IgG (H+L specific) coated plates, were inoculated i.v. into unprimed syngeneic recipients. After 24 hours, recipients were irradiated, and inoculated with 2 x 10^7 sporozoite, TT, or recCS-primed B-cells, prepared by treatment with anti-Thy 1 + complement and centrifugation over Lympholyte M (TT-primed lymphocytes mixed with recCS or sporozoite-primed lymphocytes prior to lysis). Twenty-four hours later, animals received 10^6 P. falciparum sporozoites i.v., or 10 µg recCS or TT i.p. After 7 days, anti-TT and anti-(NANP), titres were determined by RIA.

b Titres to (NANP)_{10} determined for recipients of sporozoites or recCS only; titres to TT determined for TT recipients only.

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does not appear to result in the sensitization of anti-CS T-cells, i.e., such mice show little carrier-specific helper activity in response to the T-dependent recombinant CS analogue (43).

Thymus-independent antigens are typically polymers with simple, repetitive, antigenic structures. Although often thought of as being comprised only of carbohydrates, this class does include proteins with a repetitive B-cell epitope, such as collagen and polymeric flagellin. Repetitiveness is understood to provide an activation signal to antigen-specific B cells by cross-linking of hapten-specific surface Ig. Both the degree and duration of cross-linking influence the B-cell response. By careful manipulation of the various side-chains of acrylamide, Dintzis et al. demonstrated that cross-linking of 12-16 immunoglobulin receptors by regularly spaced haptenic groups was sufficient to induce T-independent activation of hapten-specific B cells (13). Interestingly, the repetitive domain of the P. falciparum CS protein contains 15 highly reiterated haptenic groups (NANP)₃, and may therefore behave as proteinaceous bacterial flagellae (14) by inducing thymus-independent antibody responses.

In addition to the “cross-linking” models of thymus-independent B-cell activation (13, 14, 33), other models (10) propose that some thymus-independent antigens are constitutive mitogens. In accordance with this view, certain lipoproteins (30) are able to induce thymus-independent B-cell responses; in these cases, the lipid moiety activates B cells upon internalization following recognition of the protein.
antigen by surface Ig. Recent evidence suggests that many repetitive malarial antigens are acylated (23, 46). Sequence analysis reveals that the CS protein shares the amino acid motif, which is thought to represent the acylation site, exhibited by these acylated malarial antigens and the Trypanosoma brucei VSA (46).

We conclude from these findings that there exist two pathways for the production of IgG to the CS protein of malaria sporozoites: a T-dependent response to synthetic peptide and recombinant protein analogues of the CS proteins (i.e., current vaccines); and a thymus-independent response induced by the parasite itself. This appears to develop in the absence of functional helper T cell sensitization to the CS protein; a non-cognate contribution by T cells, however, may be responsible for the formation of IgG. These features allow us to propose a plausible selective (Darwinian) rationale for immunodominant repetitive antigens in eukaryotic parasites (i.e., as a mechanism of immune evasion), and suggest that immunodominant repetitive domains within malarial proteins are involved in the induction of thymus-independent responses, either by cross-linking of surface Ig, or by acting as potent B-cell epitopes to concentrate a PBA moiety on B cells of the appropriate specificity. This is proposed to constitute a mechanism of immune evasion, and confer a selective advantage to the parasite, as follows:

(1) In T-dependent responses, somatic mutation of V_H genes and competition among B-cell clones to present antigen to T cells leads, through clonal selection of B cells, to an affinity maturation of Ig. This process is also responsible for T and B memory cell formation and regulation of Ig isotype. In contrast, thymus-independent responses result in few memory cells, and antibodies that are homogeneous and of low affinity. Qualitative differences of this type may explain why anti-CS antibodies generated through the T-dependent route by a synthetic vaccine may protect against sporozoite challenge (17), whereas antibodies raised against sporozoites are invariably ineffectual (36, 41, 49).

(2) The (NANP)_3 sequence is capable of absorbing almost the entire serological reactivity of human immune sera against falciparum sporozoites: thus the repetitive surface domain is the major immunodominant epitope (27). As B cells of different specificities compete for antigen they are preferentially clonally expanded (clonal selection). Immunodominant repetitive domains, whilst the target of thymus-independent antibody responses, may suppress the development of antibody to adjacent epitopes by a process of B-cell clonal dominance (epitopic suppression); a strategy of immunodominance may succeed if the immunodominant epitope itself does not induce neutralizing antibody, which is apparently the case under conditions of natural exposure to malaria.

(3) Antigen-specific B cells have been shown to be required for antigen presentation and helper T-cell priming in vivo (28). B cells reactive with CS protein repeats, induced into a thymus-independent differentiation pathway, may fail to present antigen to T cells, thereby suppressing T/B-cell cooperation. This would account for the failure to detect CS specific helper T-cell sensitization in association with T-independent antibody responses, and the failure to boost these responses with functional helper cells.

### Immune evasion via allelic polymorphism?

No discussion of immune evasion by the CS protein can be complete without reference to the observation that a large number of amino acid substitutions exists in certain regions of the molecule outside the repetitive domain (11). It has also been shown that T cells were specific for one of two variants at position 339 (20). It was therefore suggested that the observed amino acid substitutions represent polymorphisms maintained by natural selection, resulting from variants escaping destruction by immune T cells.

A recent study has shown that the amino acid substitutions are a widespread feature of natural populations of the parasite (29). However, it is not clear that this polymorphism is maintained by a selective pressure exerted by MHC-restricted immune T cells (2, 42). This hypothesis requires accepting a number of stringent conditions, namely:

- these regions must be the target of a protective herd immunity involving simultaneous recognition and destruction of parasites;
- the selective advantage offered to variants must be approximately equivalent to that accruing to drug resistance mutations, to account for the strong preferential fixation of non-synonymous substitutions;
- the amino acid substitutions must lead to a loss of recognition by the polyclonal repertoire of T-cell receptors available in the immune hosts.

It should be stressed that the observed variants cannot be maintained by MHC polymorphism in the host population. This is because MHC polymorphism does not provide a mechanism for frequency-dependent selection. To the contrary, the distribution of host MHC haplotypes remains constant over the time scales under consideration here, and thus would provide a force promoting conformity of CS alleles (maximum fitness accruing to those able to associate the least with any MHC haplotypes).
Despite the uncertainty surrounding the mechanism whereby allelic polymorphism is maintained in the parasite population, it is clear if these regions are the only areas accessible to cytotoxic T cells, a vaccine based upon CD8+ T-cell recognition of the CS protein is unlikely to be effective.

Conclusions
The proposition that the repetitive domain of the CS protein has evolved to be a good B-cell epitope and to be the target of a thymus-independent antibody response is, in our opinion, the most plausible selective rationale for this unusual structure yet forwarded. In addition to providing an explanation for a large number of disparate observations, it makes the following predictions:

1. Closely related taxa may contain divergent repeat sequences within a conserved CS framework, as there are a few constraints on the repetitive domain other than it be repetitive and able to function as a good B-cell epitope.
2. The immunogenicity and immunodominance of the repeats are a predictable consequence of this strategy of immune evasion.
3. The antibodies produced by this mechanism under natural conditions are likely to be non-neutralizing.
4. Low levels of helper T-cell activation will result from T-1 responses.

The question of whether antibodies against the CS protein (and other antigens of malaria) can protect against infection should only be addressed with clear reference to the source of antibodies and the experimental conditions. Antibodies produced under natural circumstance, or by immunization with the parasite itself, are unlikely to provide protection, but antibodies generated through other pathways may yet prove effective. Thus the use of repetitive domains in anti-malarial vaccines is not necessarily excluded as a result of these findings, as several effective vaccines currently use T-1 antigens (e.g., pneumococcal vaccines). However, the vaccination strategy to be pursued should be rationalized accordingly.

References


