Modulation of host cell receptors: a mechanism for the survival of malaria parasites

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SUMMARY

Intra-erythrocytic stages of malaria parasites can alter the surface of their host cells and release toxins which induce the production of cytokines, which in turn can up- or down-regulate the expression of adhesion receptors on the surface of microvascular endothelial cells. New adhesion receptors on endothelial cells provide the parasite with increased chances of survival despite an increasing level of host immunity. In order to take advantage of these new opportunities for survival, the parasite itself needs to make best use of its considerable ability to vary its surface antigens and adherent molecules. The paper describes the various players in this survival game and articulates a working hypothesis to explain how it may all fit together.

Key words: *Plasmodium falciparum*, *P. berghei*, *P. chabaudi*, cytoadherence, sequestration, cytokines, cerebral malaria, tumour necrosis factor, intercellular adhesion molecule-1, microvascular endothelial cells, malaria toxins, polar lipids, antigenic variation, haemozoin, chondroitin-sulphate A.

INTRODUCTION

In the course of their development, malaria parasites interact in a variety of ways with receptor molecules on the surface of host cells. Apart from the fact that intracellular stages of the parasite induce changes in the surface membrane of their host cells, a variety of molecules are released by the parasite some of which have a systemic effect on the host and either lead to an ineffective immune response and improve the chances of parasite survival, or contribute to the pathophysiology of malaria. This paper concentrates on the erythrocytic cycle of malaria parasites and examines, in particular, the interaction between molecules at the surface of infected erythrocytes and molecules at the surface of endothelial cells. Although, at first sight, this appears to be a relatively simple receptor–ligand interaction, it is becoming increasingly obvious from recent published literature that this represents a very dynamic form of interaction, where each side possesses a very considerable capacity to change not only its own set of surface receptors but also to induce receptor switching on the other. While previous reviews on this topic have concentrated mostly on whether or not cytoadherence could be a determining factor for virulence and disease severity (Goldring & Hommel, 1992; Berendt, Turner & Newbold, 1994), it is suggested here that the modulation of receptor expression may represent a mechanism evolved by the parasite to adapt to a continuously changing cellular environment and, thus, improve its chances of survival.

The players in this survival game are: the sticky molecules expressed on the surface of infected erythrocytes, the molecules on the surface of endothelial cells, the molecules (or 'toxins') released by the parasite, the cytokines released in response to toxins which, in turn, can induce an up- and a down-regulation of receptors on endothelial cells. By modulating the expression of endothelial adhesion molecules and, at the same time, switching the expression of its own molecules on the surface of infected erythrocytes to adapt to this changing environment, the parasite improves its means of survival despite increasing immune pressure. Since the mechanism by which the parasites modulate host receptor expression (via the release of toxin-inducing cytokines) is relatively random and difficult to control, depending as it does on the host's genetic make-up and its immune status, the parasite needs to make use of its own considerable biodiversity and its capability for rapid switching from one set of sticky molecules to another to adapt best to the changing environment. From such a viewpoint, what had been described earlier as 'antigenic variation' (Hommel, David & Oligino, 1983; Su *et al*. 1995; Smith *et al*. 1995), may in fact represent an elegant process of host–parasite adaptation rather than a mere 'escape mechanism'.

RECEPTORS ON THE SURFACE OF INFECTED ERYTHROCYTES

After infection by the malarial parasite, the red cell surface undergoes structural changes, which may substantially alter its function, appearance and antigenicity (Hommel & Semoff, 1988). The alterations identified so far on *Plasmodium falciparum*-infected cells include: a visible change of shape and

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reduced deformability; the presence of electron-dense protrusions or 'knobs' (Trager, Rudzinska & Bradbury, 1966); the expression of new sugar moieties, particularly galactose (David, Hommel & Oligino, 1981); the cytoadherence to endothelial cells or rosetting with normal erythrocytes (Wahlgren, Carlson & Udomsangpetch, 1987); the presence of new metabolic channels (Ginsburg *et al*. 1985); the evidence of new parasite-specific antigens associated with the red cell membrane (Hommel *et al*. 1983) and the reorganization of normal erythrocyte components (e.g. dimerization of erythrocyte Band 3 to form 'Pfalhesin') (Winograd & Sherman, 1989). The precise molecular organisation of the surface of infected erythrocytes is not yet known, but is generally believed to consist of a combination of red cell membrane alterations and the insertion of parasite molecules into the altered membrane (including PfEMP-1, sequestrin, rosettins, HRP-1 and PfEMP-3) (Leech *et al*. 1984; Rock *et al*. 1988; Ockenhouse *et al*. 1991; Helmby *et al*. 1993; Pasloke *et al*. 1993). As a result of this complex reorganization of the infected red cell membrane, new receptors are expressed on the surface, whose antigenic and cytoadherence properties are highly variable not only from one parasite isolate to another, but also within a given isolate due to a rapid phenotypic switching.

It is usually accepted that the severe forms of malaria in humans are due to ability of mature forms of *P. falciparum* to adhere to microvascular endothelium, a process referred to as 'sequestration' and which does not occur in the mild forms of malaria due to *P. vivax* or *P. malariae*. A crucial issue has been whether sequestration causes severe malaria pathology directly (e.g. by reducing blood flow) or indirectly (by producing a concentrated toxin release which influences the local cytokine environment), or whether sequestration is altogether a side-effect secondary to the up-regulation of endothelial cells receptors. The major difference between severe falciparum malaria and the severe murine malaria model is that, although both produce coma, sequestration of infected red cells occurs only in falciparum malaria, a feature which has raised questions about the relevance of the murine model for the study of the pathophysiology of human cerebral malaria (Porta *et al*. 1993). The observation that mixed infections *P. chabaudi*/*P. berghei* may be able to re-create the conditions for cerebral malaria with brain sequestration not only of leukocytes but also of infected erythrocytes (Dennison & Hommel, 1993) offers interesting prospects.

A number of adhesion receptors has been identified on the surface of endothelial cells and incriminated as cytoadherence receptors for *P. falciparum*-infected cells, including thrombospondin, CD36, ICAM-1, VCAM-1, chondroitin sulphate A, thrombomodulin, E-selectin, P-selectin and a N-linked glycosaminoglycan) (see reviews by Hommel, 1993; Rogerson & Brown, 1997). Pfalhesin has strong binding affinities for CD36 on endothelial cells (Crandall, Land & Sherman, 1994), sequestrin appears to have a specific affinity for CD36 (Ockenhouse *et al*. 1991), while rosettins probably recognize thrombospondin (Wahlgren *et al*. 1987). Of the parasite molecules expressed at the surface of infected red cells, PfEMP-1 is the best known: a polymorphic, high molecular weight protein (250–300 kDa), encoded by the *var* genes (Su *et al*. 1995; Smith *et al*. 1995); each parasite clone has approximately 50 genes of this family and is able to switch rapidly from one to another (Roberts *et al*. 1992). Variants of PfEMP-1 are capable of adherence to all the above endothelial cell receptors, but also capable of producing rosettes of normal erythrocytes via a binding to complement receptor-1 (Newbold *et al*. 1997; Rowe *et al*. 1997). Although PfEMP-3 and HRP-1 are not expressed on the surface, these molecules contribute to the structure of knobs and a change in the balance between these two molecules may affect the expression of other surface ligands (Le Scanf *et al*. 1997). Whether a single infected red cell can adhere to only one or to a variety of different endothelial receptors has not been finally established since most experiments have been performed on populations of parasites rather than single cells. Thus, the finding of a differential sensitivity to proteolytic enzymes has been interpreted as suggesting either that more than one red cell receptor may be involved (Chaiyaroj *et al*. 1994) or that a given PfEMP-1 molecule may possess different binding domains (Gardner *et al*. 1996).

ANTIGENIC VARIATION AND INHIBITION OF CYTOADHERENCE

Antigenic variation was first described in the *P. knowlesi*/rhesus monkey model (Brown & Brown, 1965), but has since been shown to exist in *P. chabaudi*, *P. falciparum* and *P. fragile* (McLean, Pearson & Phillips, 1982; Hommel *et al*. 1983; Handunetti, Mendis & David, 1987); and it is reasonable to assume that the phenomenon is widespread among malaria parasites. Many of the studies on antigenic variation have concentrated on the molecules expressed on the surface of infected erythrocytes (e.g. SICA in *P. knowlesi* and PfEMP-1 in *P. falciparum*). Switching from one variant antigenic type to another is a random event, which apparently occurs without any external stimulus or pressure. The rate of switching from one antigenic phenotype to another is thought to be very fast. In a study of *P. chabaudi* in mice, 40% of the parasites present at the peak of infection were already different from the original inoculum (Brannan, Turner & Phillips, 1994), while in an *in vitro* study of *P. falciparum* it was estimated that 2% of parasites

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switched to a new antigen in every erythrocytic cycle (Roberts *et al*. 1992), which is comparable to the *in vivo* results with *P. chabaudi*. The rapid switching of the expression of PfEMP-1 may be a consequence of the sub-telomeric position of *var* genes on almost all the chromosomes of the parasites, a particularly unstable chromosomal area, where genes are frequently reorganized in these parasites (Thompson *et al*. 1997). Switching from one antigenic phenotype to another is associated with a change in the cytoadherence properties of the infected cells and there is an undeniable relationship between antigenic phenotype and adherent phenotype, even if only based on circumstantial evidence. The consequence of antigenic variation is that, even in a cloned population of parasites *in vitro*, the parasites present at any particular time always constitute a mixture of different variant populations with different cytoadherence properties. Over time in culture, the relative proportion of the different variant populations present continuously change. If the situation is complex in a cloned population *in vitro*, it is even more complex in a natural infection, when parasites injected by a single mosquito consist usually of more than one clone and where an individual may receive more than one infective bite each night (Thaithong *et al*. 1984; Paul *et al*. 1995).

The molecules expressed on the surface of infected erythrocytes (whether pfEMP-1, rosettins, sequestrin or Pfalhesin) are all immunogenic and capable of inducing a potent, long-lasting immune response in infected individuals (Hommel, 1985; Marsh & Howard, 1986; Treutiger *et al*. 1992; Crandall *et al*. 1995). After primo-infection, only antibodies to the homologous isolate of parasites can be detected and the repertoire of antibodies to different variants increases with each further infection. What is important here is that variant-specific antibodies recognize the sticky molecules on infected cells, and can inhibit or reverse cytoadherence to the corresponding endothelial cell receptors. This had first been demonstrated as inhibition/reversal of cytoadherence to amelanotic melanoma cells (David *et al*. 1983) and later to more purified ligands (e.g. purified thrombospondin or CD36 attached to plastic, or ICAM-1-transfected CHO cells) (Roberts *et al*. 1985; Berendt *et al*. 1989; Oquendo *et al*. 1989). It may be of interest to note that *in vitro* cytoadherence to monocytes/macrophages can neither be inhibited nor reversed using antimalarial hyperimmune serum (Goldring & Hommel, 1992), since this may explain the absence of effect of passive transfer of hyperimmune serum in humans on sequestration (Taylor *et al*. 1992) which contrasts to the reversal of cytoadherence that had been observed *in vivo* in a primate model (David *et al*. 1983). The immune response to surface antigens on infected erythrocytes represents, therefore, a potent selection pressure for cytoadherence molecules and, after many years of exposure to malaria, an individual would eventually be protected from further re-infection when the repertoire of cytoadherent molecules of the endemic parasite population has been exhausted. While infection still occurs in adults living in endemic areas (i.e. no 'sterile immunity'), the level of parasitaemia always remains low and clinical manifestations are unusual. It looks as if, by continuously changing the adherence receptors expressed on infected erythrocytes, the parasite can extend its survival despite immunity; by the same token, this means that, over a period of time, the parasite needs to recognize a series of different endothelial cell molecules, but it also means that it will eventually run out of molecules to bind to. For parasites like *P. falciparum*, there is no possible survival in the absence of sequestration, with the exception of infection in splenectomized individuals, where sequestration does not take place (Israeli, Shapiro & Ephros, 1987), or infection during pregnancy, where new adherence receptors become available in the placenta (Fried & Duffy, 1996; Maubert, Guilbert & Deloron, 1997).

PARASITE 'TOXINS'

Various molecules may be released by malarial parasites during the intra-erythrocytic stage of their life cycle which can influence the function of host cells, alter the development of immunity or be, directly or indirectly, responsible for pathological events. Here we use the term 'malarial toxins' to describe this entire group of bio-active molecules, although some authors prefer to restrict the use of the term to molecules capable of inducing fever (Kwiatkowski, 1995). The release of 'toxins' is most likely to occur when a mature meront (or segmenter) opens up to allow new merozoites to escape, but it is conceivable that true excretion of parasite-made molecules (or 'exo-antigens') through the erythrocyte-membrane may occur via the parasitophorous vacuole, the tubo-vesicular membrane network and a transient 'duct' (Haldar, 1994), while the parasite develops within the red cell.

Mitogens

Malarial antigens can induce polyclonal proliferation of B cells (Greenwood & Vick, 1975; Rosenberg, 1978) and of $\gamma\delta$ T cells (Ho *et al*. 1994). While the molecular nature of the former has not yet been identified, it appears that the malarial 'superantigens' responsible for the proliferation of Vγ9/Vδ2 $\gamma\delta$ T cells are phosphorylated molecules, similar to isopentenol pyrophosphate from *Mycobacterium tuberculosis* (Behr *et al*. 1996). The polyclonal activation of B cells is generally considered to represent one cause of immunosuppression during malaria; the stimulation of $\gamma\delta$ T cells, which is

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particularly intense during malaria in naive individuals, is believed to contribute to create a Th1-type environment, which may be one of the prerequisites for the development of severe forms of malaria (Grau & Behr, 1994).

Apoptosis

The ultimate modulating effect the malarial parasite can exert on the cells of its host is the induction of apoptosis, but there is no evidence that the parasite uses this process as a mechanism of defence. Extracts of *P. falciparum* schizonts can induce apoptosis in human lymphocytes (Touré-Balde *et al*. 1996) and unusually high levels of spontaneous apoptosis are observed in short-term cultures of lymphocytes from individuals with clinical malaria, akin to what has been described in asymptomatic HIV-infected individuals. The nature of apoptosis-inducing malarial toxins has not yet been identified.

Toxic proteins

Early work on exo-antigens of *P. falciparum* had concentrated on proteins found in culture supernatants capable of inducing the secretion of inflammatory cytokines, including the Ag7 complex, PfMSP-1, RAP-1 and RESA (Jakobsen *et al*. 1993; Picot *et al*. 1993), very little can be concluded from such work based on crude preparations and probably contaminated with the far more potent polar lipids of malaria parasites.

Polar lipids

Polar lipids extracted from malarial parasites and malaria culture supernatant are responsible for a variety of effects on host cells, including the production of inflammatory cytokines by monocytes and macrophages (Bate, Taverne & Playfair, 1988; Jakobsen *et al*. 1995) and the induction of lipogenesis by adipocytes (Taylor *et al*. 1992). The biological activity is heat stable and pronase resistant and has, for the most, been assigned to phospholipids particularly phosphatidylinositol (Bate *et al*. 1992). The glycosylphosphatidylinositol (GPI) anchor of merozoite surface antigens MSP-1 and MSP-2 has been claimed to account for a large portion of the biological activity ascribed to malarial phospholipids (Schofield & Hackett, 1993; Schofield *et al*. 1996), but this view is controversial and others believe that different molecules may be responsible for the various biological effects observed (Taverne *et al*. 1995). The phospholipids that induce lipogenesis by adipocytes *in vitro* also stimulate the release of insulin *in vivo* (Elased & Playfair, 1994) and cause a rapid drop in blood glucose when injected into mice, but these effects seem distinguishable from the TNF-inducing effects of malarial polar lipids.

Haemozoin

Malarial pigment is found in the parasite food vacuole and represents the residue of haemoglobin digestion (crystalline β-haematin) combined with a variety of compounds of parasite and host origin (including aggregated proteins, lipids and phospholipids) (see review by Arese & Schwarzer, 1997). On schizont rupture, the residual body containing haemozoin is released and is rapidly taken up by phagocytic cells where it remains undigested for long periods of time. The belief that haemozoin was responsible for fever in malaria dates back to a paper by Brown in 1912, but the concept was later challenged and forgotten, only to be revived in recent years. It was observed that malarial pigment was not only responsible for the release of cytokines by monocytes (Pichyangkul, Saengkrai & Webster, 1994), but also seriously impaired the functionality of phagocytic cells (reduced phagocytic activity, failure to produce oxidative burst, failure to kill invading microorganisms) (Arese, Turrini & Ginsburg, 1991; Schwarzer *et al*. 1992).

Endotoxin and Mycoplasma *contamination*

Early work on malaria toxins was confused by the belief that the biological effects observed were the consequence of a contamination of samples by bacterial lipopolysaccharide endotoxin; some studies succeeded in detecting endotoxin or endotoxin-like molecules by using the *Limulus* amoebocyte lysate (Tubbs, 1980; Jakobsen, Baeck & Jepsen, 1988), while others failed to do so (Greenwood, Evans-Jones & Stratton, 1975). The recent finding of a frequent contamination by *Mycoplasma* of *Plasmodium* lines maintained *in vitro* (Turrini *et al*. 1997) has raised different questions about the possible artefactual nature of some or all work describing so-called 'malaria toxins' and published over the past ten years. Since the *Mycoplasma* themselves secrete highly potent 'toxic' molecules (including glycolipids, lipoglycans and proteins) with a wide range of effects, from the induction of inflammatory cytokine secretion by macrophages to mitogenic effects on B cells and immunoglobulin production, it will take considerable efforts to disentangle what, in the published literature on 'malarial toxins' can truly be ascribed to *Plasmodium*.

CYTOKINES

In the course of malaria, cytokines are produced as a result of the action of malarial toxins on host cells, particularly macrophages/monocytes and $\gamma\delta$ T cells, but also as a result of the recognition of malarial antigens by T cells. Although these different modes of cytokine production are described separately here,

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the two are obviously linked and one may either synergise or antagonise the other; macrophages will, for example, respond very differently to malaria toxins if they have previously been stimulated by T cell cytokines or not.

Effect of malaria toxins on cytokine production

A feature common to most malaria toxins is the ability to induce the production of inflammatory cytokines (TNFα, IL-1 and IL-6) by macrophages and, to a lesser extent, monocytes (as reviewed by Jakobsen *et al*. 1995). The identification of TNF-inducing malaria toxins was first performed using murine models (Bate, Taverne & Playfair, 1988) and the *in vivo* effects have been particularly well analysed in the murine malaria models using CBA/A mice infected with *P. berghei* Anka (Grau *et al*. 1988). By extension of the murine model of severe malaria many conclusions have been drawn on the pathophysiology of severe falciparum malaria in man and, although there are substantial differences between the two, there appears at least to be a clinical correlation between increased levels of circulating TNFα and disease severity (Grau *et al*. 1989). The most interesting feature about inflammatory cytokines induced by malarial toxins is that this pathway may actually be interrupted by antibodies against the toxins; this was first demonstrated with the serum of mice vaccinated against *P. yoelii* which was shown to block the induction of TNFα release (Bate, Taverne & Playfair, 1988). Immunization experiments with crude *P. yoelii* toxin have interesting features, in as much as they have shown that such immunity is T-independent, short-lived and predominantly IgM, but also that it is not *P. yoelii*-specific but cross-reacts with the toxins of other malaria parasites (e.g. *P. vivax*). Such experiments have formed the basis for the concept of an anti-toxic or 'anti-disease' vaccine against malaria (Playfair *et al*. 1990). Together with the notion of the existence of an anti-toxin immunity, the variability of different parasites isolates in their ability to induce TNFα (Allan, Rowe & Kwiatkowski, 1993) may represent an important feature for our understanding of the diversity of parasite–host interactions.

T cell cytokines

As immunity to malaria develops, a variety of cytokines is produced (Kumaratilake & Ferrante, 1994) and studies in murine models have shown that the nature of anti-malarial immunity changes over time, switching from an initial Th1 response to an essential Th2 response. Evidence that a Th1 to Th2 switch occurs in man is best demonstrated by studies performed in Gabon, where young children with acute malaria have high levels of IFN-γ, while the levels of IFN-γ decrease drastically in school-age children in whom IL-4 then becomes detectable (Mshana *et al*. 1994). This is important since Th1 cytokines will act in synergy with inflammatory cytokines while Th2 cytokines will essentially have an 'anti-inflammatory' effect. Studies with the *P. berghei* ANKA model had shown that if TNFα had a central role in the pathological events, this only occurs in the presence of IFNγ (Grau *et al*. 1988; Grau & Behr, 1994) and that no cerebral malaria could be induced in IFNγ-R-deficient mice (Rudin *et al*. 1997). The Th1 environment that prevails early in the disease may, to some extent, be the result of a considerable stimulation of $\gamma\delta$ T cell activity and the related increase of IFNγ and TNF (Grau & Behr, 1994; Ho *et al*. 1994). Conversely, levels of IL-4 and TGF-β are significantly reduced in cases of severe malaria (Wenisch *et al*. 1995), but in situations where these cytokines are experimentally increased this correlates with a reduced pathology.

UP- AND DOWN-REGULATION OF RECEPTORS BY CYTOKINES

The expression of host cell receptors on microvascular endothelial cells is, to a large extent, modulated by the micro-environment generated by the prevailing local balance of different cytokines. Inflammatory cytokines, IFNγ, GM-CSF and IL-3 have all been shown to be capable of significantly increasing the *in vitro* cytoadherence of *P. falciparum*, particularly when acting in synergy with one another (Ringwald, Le Bras & Savel, 1992). This increased cytoadherence reflects a change in the receptors expressed at the surface of the cells used in such *in vitro* cytoadherence assays. It has been shown that, depending on the cytokines present, there could either be an up-regulation of ICAM-1, V CAM-1 and E-selectin (in the presence of TNFα, IFNγ or IL-1, as well as a direct effect of certain malaria toxins, e.g. GPI) (Berendt *et al*. 1989; Schofield *et al*. 1996) or an up-regulation of CD36 and thrombospondin (in the presence of IL-4 and GM-CSF) (Yesner *et al*. 1996). The relative cytokine balance prevailing in the microvascular bed is more difficult to assess, but *in vivo* up- and down-regulations of host cell receptors clearly affect the level of sequestration and the degree of malaria severity. In IFNγ-R-deficient mice, for example, the absence of cerebral malaria has been associated with an absence of ICAM-1 up-regulation (Rudin *et al*. 1997). In humans who died of cerebral malaria, the study of *P. falciparum* cytoadherence and relative expression of adherence receptors has shown an increased expression of ICAM-1 and E-selectin in brain capillaries where cytoadherence occurred (Turner *et al*. 1994). This type of study is fraught with difficulties since the up-regulation of receptor molecules may be a consequence rather than a cause of parasite cytoadherence. Thus, while there is no

suggestion that TNF receptors on endothelial cells play any role in cytoadherence *per se*, there is a clear correlation between the expression of TNFR2 (but not TNFR1) in microvascular endothelial cells and cerebral malaria (Lucas *et al*. 1997) with the suggestion that the expression of TNFR2 is directly implicated in the up-regulation of ICAM-1. The topic of adhesion molecules on endothelial cells and the up- and down-regulation of these molecules in response to local stimulation by cytokines is a fast-evolving one, where many questions still need to be answered. We know that large vessel endothelial cells behave very differently from microvascular endothelial cells in response to cytokines, but how different are the brain microvascular endothelial cells from those of other tissues which are easier to study? Are we concentrating on certain adhesion molecules because we have the reagents, while we should be looking for less promiscuous receptors which may explain unique cytoadherence characteristics? We must not lose sight of the fact that in every *in vitro* model of cytoadherence comparing large numbers of wild *P. falciparum* isolates, there is always a percentage of isolates that do not bind to any of the cytoadherence receptors presented in the assay (while they had clearly been sequestered *in vivo*) (Goldring *et al*. 1992), which suggests that our inventory of host adhesion receptor is still not yet complete.

HOST FACTORS

The genetic make-up of the host will influence the constitutional expression of adhesion receptors on endothelial cells, the ability to respond to malarial toxins, the relative ability to produce certain cytokines and the intrinsic ability to respond to certain malarial antigens and develop immunity. Host differences have been clearly established in murine models (e.g. CBA/A, but not BALB/C, mice develop severe malaria with *P. berghei* ANKA (Grau *et al*. 1988) and some studies suggest that comparable overall differences in susceptibility to severe malaria or to the development of immunity to malaria may exist in humans (Hill, 1992; McGuire *et al*. 1994; Riley, 1996).

MODULATION OF HOST RECEPTORS AND PARASITE SURVIVAL

The concept that the dynamic relationship that exists between the parasite and its host could actually improve the chances of survival of the parasite may be articulated in the following ways. (1) The parasites continuously switch the expression of their erythrocyte-associated antigens (e.g. by switching *var* gene expression) and such a switching is random, independent of any external pressure. In consequence, at each new erythrocytic cycle, a small percentage of the parasite population present will express a new phenotype of variant adhesive molecules. In order to survive *in vivo*, all the variant adhesive molecules expressed will need to bind to a corresponding adhesion receptor on endothelial cells; any parasite that expresses a variant adhesive molecule that corresponds to an endothelial receptor not expressed at that particular moment will be eliminated. (2) Host microvascular endothelial cells express constitutively a given repertoire of adhesive receptors which provides the parasite with a 'basic' vascular bed for sequestration; this would, for instance, be used by parasites early in infection, before the development of inflammatory responses or immunity. Basic receptors may include CD36 and thrombospondin. (3) As the malaria infection progresses, the excretion of malarial toxins will bring the inflammatory cytokines into play, which will induce a series of up- and down-regulations of receptors and, hence, produce an opportunity for different variant populations to cytoadhere (and thus survive). Since the total parasite load is finite, the availability of new receptors will cause a redistribution of the infection to new sites of sequestration. Within these new sites of 'upregulated' sequestration, the presence of cytoadherence itself will further stimulate upregulation. ICAM-1 is probably the model for such receptors. (4) Once immunity to malarial antigens has been initiated, the essentially Th1 environment that is produced will enhance the production of inflammatory cytokines and Th1 cytokines will act in synergy with inflammatory cytokines for an enhanced upregulation of adhesion receptors. This will offer further opportunities for a selection of new variant parasite populations to cytoadhere; at this point, parasites that only adhere to molecules which are not constitutionally expressed on 'resting' endothelial cells (e.g. E-selectin, VCAM-1, P-selectin) will now be able to survive. Both in terms of intensity and specific location this is probably the time during infection when cerebral forms of the disease are most likely to occur. (5) With repeated malarial infections, immunity against erythrocyte-associated surface antigens will start to develop and variant-specific antibodies will inhibit the cytoadherence of more and more variants; this form of immunity is solid and long-lasting and those variants which can no longer cytoadhere will be eliminated. It is conceivable, in the natural evolution of immunity, that antibodies to variants adherent to 'upregulated' receptors may develop earlier than against variants adherent to constitutional receptors, or that the repertoire of variants of adherent to constitutional receptors is substantially larger than the up-regulated type, or that there may be different degrees of binding affinity (to lowest affinity remaining operative the longest). The result of this, and regardless of the actual mechanism involved, would

be an early reduction of severe complications, while mild infections may continue to occur. (6) After long-time exposure to serial malaria infections, such as would occur in an individual living in a malaria-endemic area, there will be an effective immunity to most of the variant populations to which the host has been exposed and parasites will no longer be able to take a hold and develop above a certain threshold, presumably related to the lag-time required for boosting immune memory. Of importance may be the observation that similar *var* gene transcripts may be shared, different parasites having a particular adherence phenotype in common (Smith *et al*. 1995); this may help to explain why immunity eventually transcends strain-specificity. (7) In an adult living in a hyperendemic area, this state of immunity will effectively persist for life, except for malaria occurring during pregnancy. From a cytoadherence point of view, pregnancy is a situation where new adhesion receptors become available, not on endothelial cells but on syncytiotrophoblasts, and these new receptors offer a new opportunity for cytoadherence of malarial parasites, despite an otherwise functional immunity. Hence in pregnancy, it would be rational to expect to find only variants capable of adherence to unusual receptors, unique to the placenta (therefore new to the host), since immunity to previously experienced variants would prevent them from developing. This hypothesis has the advantage over the older immunosuppression hypothesis (Rasheed *et al*. 1993; Matteeli *et al*. 1997) to provide an explanation for the differences in malaria severity between primigravida and multigravida, since the host becomes less and less susceptible to the 'placenta adhesive' variants with each of the subsequent pregnancies. The finding of a high frequency of parasites cytoadherent to chondroitin-sulphate A and thrombomodulin produces the first evidence to support this hypothesis (Fried & Duffy, 1996; Maubert, Guilbert & Deloron, 1997; Rogerson & Brown, 1997).

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