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Selected Problems of Malaria Blood Stage Immunity

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Summary

Both antibody dependent and cell mediated mechanisms contribute to immunity in malaria. The parasites vary in sensitivity to antibody mediated inhibition due to underlying antigenic variation. When *Plasmodium falciparum* isolates are tested with antibodies from the donor originally harbouring the parasites or with those from other donors, growth inhibition is usually lowest in the autologous combinations. Parasites with decreased sensitivity are also generated *in vitro* by culturing them for prolonged periods in the presence of certain anti-plasmodial antibodies. When the antibodies are removed, a successive return of sensitivity develops. The decrease in sensitivity to inhibition may either be due to down-regulation of synthesis of the antigen or a selection of parasites with low antigen expression from the heterogeneous original populations.

Both T lymphocytes carrying α/β and γ/δ antigen-receptors play a role in malaria immunity. However, although γ/δ T cells may expand 40-fold or more in the peripheral immune system in acutely infected humans and also inhibit parasite growth *in vitro* and *in vivo*, their relative importance for protection or pathogenicity is presently unclear. Of the two major T cell subsets ($CD4^+$, $CD8^+$) carrying α/β T cell receptors, the role of $CD8^+$ T cells in blood stage infections appears to be limited. Instead, $CD4^+$ T cells are of major importance. These cells comprise at least two functionally different subsets (Th1, Th2), distinguished on the basis of lymphokine secretion. In some rodent malaria models, Th1 cells producing primarily IL2 and IFNγ give rise to protection in early infection while Th2 cells producing IL4 are essential for parasite clearance in late infection. In other mouse strains, the same parasites induce a strong Th2 response in early infection, resulting in a lethal course. $CD4^+$ T cells of either Th1 or Th2 type also have regulatory functions in human *P. falciparum* malaria.

Most humans living in areas of high endemicity have significantly elevated blood levels of IgE, reflecting a skewing of the underlying T helper cell ratio in favour of Th2, responsible for the switch in immunoglobulin isotypes. Less than 5% of the IgE in malaria represents antibodies to *P. falciparum*. IgE elevation is highest in patients with severe and particularly cerebral malaria and is frequently associated with an elevation of tumour necrosis factor alpha (TNF). The release of this cytokine from monocytes/macrophages may reflect cross-linking of their low affinity receptors for IgE (CD23) by IgE containing immune complexes from malarial sera. Local overproduction of TNF is considered a major pathogenic mechanism, responsible for fever and tissue lesions in severe malaria. Although TNF overproduction in malaria is generally assumed to be due to direct stimulation of effector cells by certain parasite derived toxins, the present results suggest that IgE elevation constitutes yet another mechanism contributing to the pathogenicity of *P. falciparum* in human malaria.

Introduction

Malaria infection gives rise to immune responses with the ultimate goal to free the host from the infecting parasites. However, immune responses may also elicit reactions that are harmful to the host and the balance between protective and pathogenic responses will greatly affect the final outcome of an infection. In this brief survey we will discuss

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selected aspects of the immune mechanisms regulating protection or pathogenicity in malaria blood stage infection, that is the phase responsible for mortality and morbidity of the disease.

Blood stage immunity and host genetics

Immunity to malaria infection is stage- and species specific. Acquisition of efficient blood stage immunity is relatively slow, age dependent and immunity is not lasting, i. e. acquired protection may be lost upon cessation of exposure (1). The reasons for this are multiple but the genetic diversity and variability of potentially protective parasite antigens is thought to be of major importance (2, 3). The development of immunity is also dependent on the genetics of the human host. Thus, the importance of particular HLA alleles for resistance to severe malaria has been reported several years ago (4). However, other genetic factors are also involved (5, 6). Of particular interest are the recent findings by Modiano *et al* (7) who studied *P. falciparum* malaria in populations belonging to three ethnic groups but living under similar conditions of hyperendemic malaria exposure in Burkina Faso. Of these groups, individuals belonging to one of them (Fulani) were apparently less susceptible to *P. falciparum* malaria as reflected by both lower parasitemias and fewer clinical episodes. Furthermore, this partial resistance was associated with an increased capacity to form antibodies against the parasite (8). As these enhanced humoral immune responses were polyspecific and directed against many parasite antigens, it is unlikely that the heightened reactivity reflected a genetic regulation by the MHC system. Further elucidation of the molecular-genetic basis of this improved responsiveness (which may not be unique for plasmodial infections) will be of great interest.

Antibodies and antigen expression

Although antibodies have long been known to protect against human *P. falciparum* malaria, the relative importance of different antibodies requires further explorations (for review and references see (9)). Moreover, by exhibiting antigenic diversity and variation, the parasites have developed mechanisms to escape the immune response (10). This may be illustrated by recent studies by Wåhlin *et al.* (11) who investigated merozoite invasion/growth inhibition *in vitro* of antibodies from children living in a *P. falciparum* malaria hyperendemic area of Burkina Faso. When comparing inhibitory antibody activities on autologous or heterologous parasites, a significantly lower inhibition was seen in autologous combinations. Principally similar results were recently obtained by J. Iqbal *et al.* (12) who cultured a *P. falciparum* laboratory strain (F32) *in vitro* under prolonged periods in the presence of suboptimal concentrations of a human monoclonal antibody against the blood stage antigen Pf332 (13, 14). In freshly isolated parasites, this antibody inhibited growth but prolonged culturing resulted in relative resistance to inhibition. No change in growth inhibition was seen when parasites cultured with this monoclonal antibody were tested for growth inhibiton by antibodies specific for other antigens. Continued culturing of the parasites in the absence of antibody indicated that resistance to inhibition was reversible (12). Both examples illustrate how the parasites may defend themselves against the host's immune response. Whether this defense is based on direct down-regulation of antigen by antibody pressure or reflects selection of parasites with low antigen expression out of a heterogeneous population is presently unclear.

There are many pathways for antibody mediated protection in malaria, with opsonization of infected erythrocytes and ensuing phagocytosis or cytotoxicity by various effector cells probably being among those of greatest importance (15). There is presently no evidence that invasion/ growth inhibition by antibodies without effector cell involvement plays any major role in protection. However, the capacity of the parasites to change their antigenic set-up under pressure as described above is obviously of general validity regardless of which antibody mediated mechanisms may be involved in protection.

T-cell regulation and cell mediated immunity in malaria

γ/δ T cells. Normally, T cells equipped with γ/δ T cell receptor (TCR) comprise 5-10% of the circulating T cell pool. However, in acute infection this percentage may rise

up to 40%, reflecting not only a relative but also an absolute increase in numbers (16-18). This elevation which also has been reported for *P. vivax* infection (19) may persist for many weeks. Similar γ/δ T cell elevations have also been seen in other infections but the function of these cells is still a matter of speculation. Frequently, activation of γ/δ T cells leads to the release of Th1 cytokines (20) and an associated overproduction of IFNγ and TNF may become pathogenic. However, transfer of cloned γ/δ T cells from mice immunized with *P. yoelii* sporozoites to α/β TCR deficient mice has been shown to inhibit parasite development in the liver (21). There is no clear evidence suggesting that γ/δ T cells protect against blood stage infection. Nevertheless, γ/δ T cells from malaria naive humans activated by PHA or by culture with *P. falciparum* lysates also inhibit parasite growth in RBC *in vitro* in a cell contact requiring reaction (22). The crucial question of what these killer T cells see on the infected erythroctes is unresolved. Considering what is known from other systems, it could be heat shock proteins exposed on the stressed (i. e. infected) erythroctes or, rather, some phosphate containing glycolipids (23, 24). Findings to this effect have recently also been made in malaria (25, 26).

α/β T cells. The γ/δ T cell activities described above require no foregoing immunization and are not MHC-restricted. Thus, γ/δ T cells are considered to constitute a first line of defense which is rapidly mobilized. However, most known T cell activities in plasmodial infections involve T cells equipped with α/β receptors. These cells which normally are the majority of the peripheral T cells appear to have a larger repertoire than the γ/δ T cells and are MHC restricted in their reactivities with antigens. They have both cytotoxic and regulatory functions.

In malaria, T cell cytotoxicity by MHC class I restricted $CD8^+$ T cells (27) and perhaps also by MHC class II restricted $CD4^+$ T cells (28) appears to play a role in the defense against pre-erythrocytic infection in the liver. However, in the ensuing blood infection, cytotoxic T cells seem to have no role, in line with the poor expression of MHC antigens on the erythrocytes, the main targets of the parasites. Thus, in the immune response during the erythrocytic phases of plasmodial infections, regulatory $CD4^+$ T cells are the main players (29).

T cell polarization

In the mouse it has been known for several years that long lasting stimulation of $CD4^+$ T cells results in functional polarization into at least two (but probably more) cell types, the so-called T helper 1 (Th1) and T helper 2 (Th2) cells. Later, it has become clear that similar distinctions are true for human $CD4^+$ cells as well (30, 31) and can also be made for $CD8^+$ T cells (32). Th1 and Th2 cells are distinct with regard to the cytokines they produce and, therefore, with regard to function (33). Upon activation, all T cells produce a large variety of cytokines. $CD4^+$ Th1 cells that are involved in macrophage activation, cellular cytotoxicity and delayed type hypersensitivity, produce the key cytokines interferon gamma (IFNγ) and interleukin 2 (IL2). For Th2 cells which control humoral immunity and have major roles in allergy, asthma and certain infections, the key lymphokines are IL4, IL5 and IL13 (30, 34).

In "classical" studies performed by J. Langhorne and colleagues about 10 years ago (35) it was shown in a non-lethal mouse malaria system (*P. chabaudi chabaudi AS*) that the first immune response in naive mice against the parasite was a Th1 response, dominated by IFNγ and IL2 producing lymphocytes in the spleen. After 10-14 days when the infection had peaked, this response changed into a Th2 response dominated by IL4 and certain antibodies, finally resulting in complete clearance of the infection.

Host- and parasite factors affecting T cell responses

Several factors have been shown to affect the course of T cell responses and thereby the outcome of malaria infections. Studies similar to those referred to above but performed in mice differing in resistance of susceptibility to the same parasite (*P. chabaudi*) demonstrated the importance of host genetics (36). Thus, while in resistant mice the sequence of responses was as above (Th1 followed by Th2), in susceptible mice the

first response was Th2; such mice died of severe anemia and fulminant parasitemia. These results reflect the fact that protection against liver infection requires cell mediated reactions involving $CD4^+$ T cells of Th1 type and cytotoxic $CD8^+$ T cells.

Not only host- but also parasite factors will affect the development of the immune response. This may be exemplified by infection of mice with *P. vinckei* which is lethal and causes an illness involving overproduction of NO and TNF (37), i. e. a harmful Th1 response. When rescued by drug cure, they develop a solid immunity against reinfection, independently of antibodies or $CD8^+$ cells (38). Rather, it is due to a protective Th1 response, characterized by splenocytes that upon antigen stimulation produce IFNγ but no IL4 (39). Why *P. vinckei* that closely resembles *P. chabaudi* only elicits Th1 responses while *P. chabaudi* infected mice appear to be rescued by the shift of a Th1 into a Th2 response is an important but unresolved issue.

IgE in human *P. falciparum* infection

More than 85% of individuals living in malaria endemic areas have elevated blood levels of IgE, roughly corresponding to the endemicity of the region (40, 41). Of this IgE less than 5% represents antibodies against many different plasmodial antigens. An IgE elevation appearing after repeated infections has also been found in the murine *P. chabaudi* model (42).

The formation of IgE and other immunoglobulin isotypes is controlled by T cells. Thus, the Ig isotype patterns found in serum may reflect various T helper cell reactivities (43). The switching of IgM (and IgG) to IgE is induced by the cytokines IL4 and IL13, released from activated Th2 cells (44). In line with this, *in vitro* activation of T cells from peripheral blood of malaria sensitized donors with antigen or mitogen (PHA) results in an increased ratio of IL4- over IFNγ-releasing cells, reflecting the presence of increased numbers of Th2 cells (45). The Th1 to Th2 switch is controlled by many factors including antigen dose and structure, length of antigen exposure, mode of immunization and host genetics (46). The latter is illustrated by studies of African twins, in whom IgE levels were very similar in homozygous twin pairs but varied widely in heterozygous pairs (M. Troye-Blomberg *et al.*, manuscript in preparation). These results confirm previous more general results on antibody and T cell responses of malaria exposed homozygous or heterozygous twins (5, 6). The genes regulating these responses were located both within and outside the MHC region.

Role of IgE in malaria infection

In infectious disease IgE usually acts on parasites or parasitized cells through the intervention of effector cells. Interaction of IgE containing immune complexes with Fcε receptors on different types of effector cells is known to induce a variety of responses which can be protective and/or harmful for the host (47). There are two major types of Fcε receptors mediating IgE dependent cellular reactions. The so-called high affinity IgE receptor, Fcε RI, occurs primarily (but not exclusively) on mast cells and basophils and is the major madiator of immediate hypersensitivity reactions (48). The other receptor, Fcε RII or CD23, occurs primarily on monocytes/macrophages but also on B cells and other hematopoietic cells (49). This receptor, also called low affinity receptor, is induced to elevated expression by IgE and/or IL4 (50, 51) and may mediate IgE dependent phagocytosis, cellular cytotoxicity and adhesion (52-54). IgE dependent reactions encountered, for example, in helminthic infections but also in malaria, are believed to be primarily madiated by CD23.

While IgE's possible protective effects in malaria have not as yet been investigated, there is evidence for its involvement in pathogenesis. This is based on the initial findings in cerebral as well as in severe non-cerebral malaria of significantly higher IgE concentrations than in the blood of uncomplicated cases. This IgE elevation included both total and anti-parasitic IgE while no similar elevations were found for IgG (41, 55). With regard to specificity, the anti-plasmodial IgE antibodies in severe malaria cases appear to be qualitatively the same as in uncomplicated cases, indicating that the elevations seen in severe malaria were not due to IgE responses to some "disease specific" antigens.

IgE and TNF in malaria pathogenesis

Two not mutually exclusive reaction patterns are usually implicated in the pathogenesis of cerebral malaria: one involves obstruction of blood flow in the postcapillary microvasculature in the brain (56) due to sequestration of parasitized erythrocytes and resulting in ischaemia, acidosis, anoxia, ring hemorrhages and tissue necrosis. The other involves tissue damaging effects of cytokines, reactive nitrogen or oxygen, induced by the interaction of parasites with various leukocytes or vessel endothelium (57). One of the important cytokines released under such conditions is tumor necrosis factor α (TNF). Although TNF may protect against parasites (58-60), elevated concentrations are correlated with disease severity (61-63) and in cerebral malaria even with mortality (64). It is also a major factor inducing malaria fever (65).

TNF is released from monocytes, activated by, for example, bacterial lipopolysaccharides. In malaria, parasite pigment, various antigens and toxins released from the parasite have also been implicated in TNF induction (66, 67). Recently, major attention has been given to the GPI (=glycosylphosphatidyl-inositol) molecules that are anchoring many polypeptides, including malaria exoantigens, in the cell membrane (68-70). However, monocytes can be activated to produce cytokines and various effector functions via different pathways. Thus, as already indicated, the surface expression on monocytes/macrophages of Fcε RII, CD23, is upregulated by IL4 and/or IgE (50, 51) and cross-linking of this receptor by IgE containing immune complexes results in the production of nitric oxide and TNF (53) which both are effector molecules in the defense against the parasite. In line with this, IgE and TNF elevation frequently parallel each other in serum of severe malaria patients (55). At the same time, sequestration of *P. falciparum* infected erythrocytes to small vessels of the brain in cerebral malaria will also result in the deposition of IgE containing immune complexes (Y. Maeno *et al.*, manuscript in preparation), assumedly leading to local overproduction of TNF and ensuing tissue lesions. It should be emphasized here that it is not the high levels of TNF alone that causes the lesions typical for cerebral malaria. Other human malaria parasites such as *P. vivax* do not cause cerebral malaria but may induce much more TNF than *P. falciparum* (71). Rather, it would seem to be the combination of sequestration to capillaries and post-capillary venules of this parasite with local monocyte activation that is the basis for these serious complications of *P. falciparum* malaria.

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