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Review

Premunition in *Plasmodium falciparum* malaria

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Malaria parasites have evolved to maintain a well-balanced relationship with their human hosts. This implies that they can partially escape from protective effector mechanisms of their hosts, but also that hosts can develop partial immunity to the parasite. This immunity requires repeated infections, takes years to develop and is usually of short duration. However, protective immunity to clinical malaria rather than infection may be of long duration. This natural acquired immunity is called premunition since a low parasitemia mostly persists in the presence of circulating antibodies to the various stages and in the absence of clinical disease. In children who do not have circulating antibodies to the parasite, premunition is probably caused by antitoxic immunity. These poor and slowly developing immune responses to malaria are partly due to immune evasion strategies of the parasite caused by antigenic polymorphism, shedding of parts of parasite proteins, cross-reactive epitopes of antigens of different developmental stages, prolonged exposure to endemic malaria and widespread restricted immunogenicity to defined antigens. Premunition relies on the cooperation between the parasite and human antibodies, leading to the induction of antibody dependent cellular inhibition (ADCI) of the intra-erythrocytic growth of the parasite. The immunity, however, is not a sterilizing type in that the infection persists longer than the symptoms and individuals can exhibit relapses or recrudescences or become reinfected.

Key words: Intra-erythrocytic stage, parasitemia, malaria, chronic stage, human antibodies, immunity.

INTRODUCTION

The most unique characteristic of a parasite when it is in its normal host is its ability to make itself tolerated, which clearly indicates that it has sophisticated means to ensure the neutrality of its host. This is true in the case of *Plasmodium falciparum*, since after numerous malaria attacks, equilibrium is reached with a chronic stage of infection, characterized by a relatively low parasitemia and low or no disease. This natural defense mechanism known as premunition relies on the cooperation between the parasite and circulating human antibodies to the various stages, leading to an antibody dependent cellular inhibition (ADCI) of the intra-erythrocytic growth of the parasite (Pérignon and Druilhe, 1994). In children who do not have circulating antibodies to the parasite yet, premunition could probably be caused by 'antitoxic immunity' (Day and Marsh, 1991).

Malaria parasites have evolved to maintain a well-balanced relationship with their human hosts. This implies that they can partially escape from protective effector mechanisms of their hosts, but also that hosts can develop partial immunity to the parasite. This immunity requires repeated infections, takes years to develop and is usually of short duration, although protective immunity to clinical malaria rather than infection may be of long duration (Deloron and Chougnet, 1992).

These poor and slowly developing immune responses to malaria are partly due to immune evasion strategies of the parasite caused by antigenic polymorphism, shedding of parts of parasite proteins, cross-reactive epitopes of antigens of different developmental stages, prolonged exposure to endemic malaria and widespread restricted immunogenicity to defined antigens (Mendis et al., 1991;

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Mercereau-Puijalon et al., 1991; Conway, 1997). Premunition has been described in malaria hyperendemic areas of Africa and Papua New Guinea, while its occurrence in Asia is said to be doubtful (Soe et al., 2001).

In view of the high prevalence of malaria, its threat to life and search for the development of effective vaccine, it is of utmost interest therefore to gather an understanding of the underlying mechanisms of premunition and of their target antigens, in order that awareness may be created as to the existence of this mode of natural protective immunity, its elimination by indiscriminate and uncontrolled use of antimalaria drugs and its possible strategic role in vaccine development against the blood stage of the malaria parasite.

PREMUNITION IN *Plasmodium falciparum*

In areas with heavy transmission, areas regarded as holoendemic or hyperendemic, malaria infection occurs throughout the year, with children having high parasitemia and spleen rates (> 50%), while adolescents and adults generally having low parasitemia rates. The incidence of malaria disease is therefore high in younger children who are developing immunity and the pregnant especially the primigravida. The older population generally has immunity and premunition to malaria (that is, they have asymptomatic parasitemia). Conversely, in areas of seasonal and/or low malaria transmission, the population generally does not develop solid immunity and premunition to malaria. The infection and disease can therefore occur in both children and adults and following periods of reduced malaria transmission, there could be the danger of malaria epidemics developing (Druilhe and Khusmith, 1987).

Immune response against malaria parasite could be directed at either the pre-erythrocytic or erythrocytic stages of the parasite's life cycle. However, the erythrocytic stage of the life cycle is probably the most important in terms of clearing the parasite and lessening the disease. Due to the lack of HLA molecules on the surface of the parasite or the erythrocyte, it is usually assumed that antibody will play a key role in blood-stage immunity. Possible effector mechanisms for antibody include: blocking erythrocyte invasion by merozoites, antibody-dependent cellular killing mediated by cytophilic antibodies, or increased clearance of infected erythrocytes due to binding of antibodies to parasite antigens exposed on the erythrocyte surface. All these immune activities lead to a lower parasitemia (Bahouroun-Tayoun et al., 1990).

Almost always, a person will exhibit severe symptoms on their initial exposure to malaria, while subsequent ones will be less severe, providing the appropriate environment for immunity against the disease which is slow and requires multiple exposures, to develop (Baird et al., 1991).

Malarial paroxysms become less severe and irregular in periodicity as the host develops immunity. This immunity, however, is not a sterilizing type in that the infection persists longer than the symptoms and individuals can exhibit relapses or recrudescences or become reinfected. If untreated, *P. falciparum* is capable of producing a chronic, severe and lethal infection (Smith et al., 1999; Soe et al., 2001).

Characteristics

By the World Health Organisation (WHO) definition, there are 2 types of relapses (that is, renewed parasite and clinical activity) which are the most striking features of chronic malaria. These are recurrence and recrudescence. The later form of relapse is due to the persistence of indetectable erythrocytic forms in the blood of the patient after a primary attack of malaria leading to the induction of humoral immune responses (IgG and IgM) in response to parasitic antigens. These responses are effective against the asexual blood forms (mature schizonts and free merozoites), keeping them at a low subclinical level; they are however, not effective against persisting intrahepatic forms of parasites. A balance is achieved between infection and resistance- the stage of immune infection or premunition. This balance can be disturbed by intercurrent illness such as diarrhoea and malnutrition or long absence from the endemic area (and therefore lack of continuing antigenic stimuli) or splenectomy. As a result, after this period of low parasite density, the erythrocytic parasites, undergo repeated erythrocytic multiplication and become numerous enough to cause a renewed clinical paroxysmal attack. This form of relapse occurs with the four species of human plasmodia (Da Silva, 2002; Males et al., 2008).

1. Premunition is not a sterilizing type of immunity: chronic infection persists, although the maximum parasite load reached is low. Even if it adds a little in terms of reduction of parasite load as compared to innate resistance, this additional immunity is substantial in terms of morbidity as it keeps the parasite load low, below the threshold of pathogenicity. Superinfection can occur, but it remains at a low grade.
2. It is seen in holo and hyperendemic areas, mainly in Africa (and in some regions in Papua New Guinea).
3. It is independent of transmission levels provided it occurs at least once a year.
4. It is rapidly lost: exactly one year without re-challenge is enough to lose this protective state.
5. It is strain independent.
6. It is clearly immunoglobulin G (IgG) dependent.
7. The delay of acquisition is remarkably long, compared to the rate of transmission. Epidemiological studies in the above areas have helped to define three clinical periods: a short period of 0 - 5 years where mortality can occur; a long period of 0 to 15-20 years where morbidity is frequent

(though decreasing in frequency with age); thereafter a longer period of premunition where the disease in any form is absent. However such epidemiological studies do not allow distinguishing the respective importance of the immunological competence of the subjects related to age and of the long term exposure of the parasite.
8. It is the strongest type of immunity developed by humans against the asexual blood stage (ABS) infection (Pérignon and Druilhe, 1994).

Mechanism

The poor and slowly developing immune responses to malaria are partly due to immune evasion strategies of the parasite. Among these strategies, antigenic polymorphism could be considered the most important requiring that after infection an individual will acquire immunity only to that specific isolate, but not to heterologous parasites (Mendis et al., 1991; Mercereau-Puijalon et al., 1991; Conway, 1997). Among the many polymorphic antigens of *P. falciparum* the polymorphism in the erythrocyte membrane proteins of the EMP-1 family is very extensive and serves immune evasion tremendously. These EMPs are found in the knob structures which are involved in attachment of infected erythrocytes to vascular endothelium allowing the parasite to avoid splenic death (Baruch et al., 1996). The EMP-1 proteins are encoded by a comprehensive set of genes, known as *var* (for variation) genes, of which already 150 different genes scattered over the entire *P. falciparum* genome are known (Borst et al., 1995; Baruch et al., 1995; Su et al., 1995; Smith et al., 1995).

In each infected red blood cell only one or at most a few *var* genes are expressed and the rate of switching between the genes may be as high as 2.4% per generation (Smith et al., 1995). This enables the parasite to evade immune attack. Similarly, many other antigens may have a high antigenic diversity which may be the product of multiple copies of genes (Miller et al., 1996). In addition, shedding of parts of parasite proteins may have immune evasion purposes as well. Consequently, the 310 kDa large sexual stage proteins Pfs230 could originnate from a 360 kDa precursor (Williamson et al., 1995, 1996) while the 50 kDa protein that is shed contains very immunogenic amino acid repeats (Riley et al., 1995). The immune response, as a result is diverted to this irrelevant epitope by a deceptive effect, thereby suppressing the formation of high-affinity anti-Pfs230 antibodies. It is thought that the many cross-reactive epitopes of antigens of different developmental stages are also a heritage of the parasite's ability to divert the immune system (Moelans et al., 1992). Very often these cross-reactive epitopes confer immunodominant amino acid repeats, with the implication that the formation of antibodies to important adjacent areas is suppressed (Moelans et al., 1992).

There is also evidence that prolonged exposure to endemic malaria lowers the immune responsiveness of populations to malarial antigens (Riley et al., 1989; Goonewardene et al., 1990; Mshaba et al., 1990). This down-regulation, accompanied with an increased frequency of CD8+ cells, elevated levels of free CD8 and a decreased frequency of CD4+ T cells, may also be the result of a (cytokine-mediated) defective activation of helper T cells, activation of suppressor T cells and of lymphocyte reallocation, but the underlying mechanisms are not understood (Hviid et al., 1992). Finally, a widespread restricted immunogenicity to defined antigens is being reported (Quakyi et al., 1989). To this non-malaria disease, immunity and vaccine development responsiveness may be partially due to genetic factors. The implication is that malaria vaccine strategies limited to one single antigen subunit approach will be inadequate.

There is a clear cut evidence of the role of protective antibodies in the induction of premunition *in vivo*. The efficacy of these antibodies is however dependent on their cooperation with effector cells like monocytes, macrophages and the polymorphonuclear (PNM) phagocytes. These phagocytes readily opsonize merozoites *in vivo* (Lunel and Druilhe, 1989). However in a study to determine the cooperation of various cell types with protective antibodies and their effect on parasite survival within the erythrocyte, only monocytes were found to interact with ADCI to inhibit the growth of *P. falciparum* within the erythrocyte.

In a study to determine the *in vitro* mechanism of premunition, Bahouroun-Tayoun et al. (1990) used biological materials derived from IgG passive transfer experiment, to further support the relevance of ADCI in protective immunity against malaria. They observed that IgG enhanced the growth of *P. falciparum* and that monocytes on their part did not inhibit the parasite. However, when these immune cells were allowed to act together, a very strong inhibition rate was reportedly observed prompting the workers to conclude that their study was the first direct demonstration of *in vitro* inhibitory effect of antibodies with proven *in vivo* protective efficacy. In the activities of ADCI to bring about premonition,

a) Monocytes are triggered by cytophilic antibodies directed against a merozoite surface antigen.
b) This is followed by the release of tumor necrosis factor (TNF), interferons (IFN) and other soluble inflammatory mediators.
c) These soluble mediators then block the division of intra-erythrocytic parasites at the trophozoite stage (Pérignon and Druilhe, 1994).

Several other workers (Sabchaeron et al., 1991; Soe et al., 2001; Vardo et al., 2007) have also investigated the activities of ADCI in detail and have reported that the delay in immunoglobulin inoculation and the beginning of the decrease of parasitemia is not the same in all patients.

They further posited that the rupture of mature schizonts was required to trigger the mechanism mediated by antibodies *in vivo* without being able to distinguish between schizonts and merozoites. The report concluded that there was a correlation *in vivo* between the parasite reduction rate induced by IgG and the initial level of parasitemia, further supporting earlier findings that parasites are involved in the triggering of ADCI.

ADCI as a defense mechanism triggered by merozoites and acting on intra-erythrocytic parasites also provides an understanding of the chronicity of malaria. According to this finding the intra-erythrocytic parasite matures, possibly for several cycles, until the number of released merozoites reaches the threshold sufficient to trigger some monocytes. The parasite being both the trigger and the target of ADCI, parasitemia will never go to zero but will rather fluctuate at very low levels (Druilhe and Khusmith, 1987).

ADCI has also been found to be associated with one important feature of premunition, that is, absence of strain specificity. Their activities are extended to even mutants devoid of specific targets to protective antibodies, provided monocytes are triggered by “wild type” parasites (Bahouroun-Tayoun and Druilhe, 1992).

AGE DEPENDENT ACQUIRED PROTECTION

Susceptibility and death rates in *P. falciparum* malaria are high during childhood. Parasitemia and recurrent episodes of clinical malaria are also common among this age group. Gradually the children begin to have intermittent absence of parasitemias, followed by lower density parasitemias, splenomegaly and finally premunition. Adolescents and adults on the other hand sometimes have parasitemia and occasionally clinical symptoms of malaria. Conversely, pregnant women especially primigravida (first pregnancy) are highly susceptible to malaria infections and serious disease since their natural defense mechanisms are reduced during pregnancy. Besides, immunological influences, malaria infection could also be controlled by antimalarial treatments. Inadequate antimalarial treatments may prolong the presence of malaria parasites in the host and this may serve as a prerequisite for the acquisition of protective immunity against re-infection (White, 1998; Ai Long et al., 2002). However, use of anti-malaria drugs has been reported to delay the process of developing malaria immunity, with people who use one form of malaria prophylaxis or another being liable to develop severe clinical disease on re-exposure (Baird et al., 1991).

Acquired antibody-mediated immunity has also been reported to be transferred from mother to feotus across the placenta. This passively transferred immunity is lost within 6 to 9 months, as is the immunity in adults if they leave a malarious area and are no longer exposed to Plasmodia (Hoffman, 1992).

CONCLUSION

In humans repeated infection by *P falciparum* induce a progressive modulation of the immune response, eventually leading to an anti-parasite immunity characteristic of premunition. This progressive modulation of the immune response, clearly exemplified by the acquisition of *in vivo* protective antibodies able to promote *in vitro*, an ADCI effect, is important to consider, as it implied that antimalarial immunity should be assessed not only on quantitative terms (the higher the titer the best), but also from a qualitative point of view (the right antibody co-operating with the right cell). Besides immunological influences, malaria infection could also be controlled by antimalarial treatments; but inadequate antimalarial treatments may prolong the presence of malaria parasites in the host and this may serve as a prerequisite for the acquisition of protective immunity against re-infection. However, use of anti-malaria drugs has been reported to delay the process of developing malaria immunity, with people who use one form of malaria prophylaxis or another being liable to developing severe clinical disease on re-exposure.

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