

TRANSMISSION IMMUNITY IN MALARIA: REFLECTIONS ON THE UNDERLYING IMMUNE MECHANISMS DURING NATURAL INFECTIONS AND FOLLOWING ARTIFICIAL IMMUNIZATION

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Malaria transmission-blocking immunity has been studied in natural malarial infections in man, during infections in animals and following artificial immunization of animals with sexual stage malaria parasites. Effective immunity, which prevents infectivity of a malarial infection to mosquitoes, has been observed under all of these circumstances. Two general types of effector mechanism have been identified. One is an antibody mediated mechanism which acts against the extracellular sexual stages of the parasite within the midgut of a blood feeding mosquito. The other is a cytokine mediated mechanism which inactivates the gametocytes of the parasites while still in the circulation of the vertebrate host. Both effects have been observed during natural infections and following artificial immunization. The basis of induction of transmission-blocking immunity, including the nature of the memory for such immunity, however, may be very different in different host/parasite systems and during natural infection or following artificial immunization. Following artificial immunization a strong immune memory for transmission blocking immunity has been observed in animal systems. By contrast, following natural infections in man immune memory for transmission blocking immunity has been found to be weak and short lived if it occurs at all. It is suggested that the immunogens which induce natural transmission blocking immunity may be CD4⁺ independent.

Key words: malaria – transmission – gametocyte – sexual stage – parasite – antibody – cytokine – immune memory

This essay is not intended to be a comprehensive review of our current knowledge of malaria transmission blocking immunity. Its purpose is to examine some of the observations which have been made on the induction of malaria transmission immunity during natural infections and following artificial immunization in order to suggest guidelines for future research orientations.

STUDIES ON NATURAL HOST/PARASITE COMBINATIONS: *PLASMODIUM CYNOMOLGI* IN THE TOQUE MONKEY AND *P. VIVAX* AND *P. FALCIPARUM* IN MAN

Plasmodium cynomolgi and *P. vivax* are two very closely related species of malaria parasite one of which has as its natural host the Toque monkey (*Macaca sinica*) of Sri Lanka and the other of which is a wide spread pathogen of man. Both species of parasite have been studied in their natural hosts in Sri Lanka, *P. cynomolgi* in Toque monkeys providing the material for controlled studies on the course of

malarial infections in a single infected individual, and *P. vivax* for studies on naturally acquired infections in man in conditions of endemic malaria transmission.

The studies on *P. cynomolgi* in Toque monkeys have demonstrated the course and the nature of infectivity to mosquitoes during a natural malarial infection (Naotunne et al., 1990). This system allowed in particular the study of the early period of an infection which in humans is asymptomatic and, therefore, not accessible to study. In *P. cynomolgi* infections in the Toque monkey the early period is characterized by low gametocytaemia but high infectivity of the parasites to mosquitoes. This high infectivity appears to be mediated to a large extent by the presence of anti sexual stage antibodies at low concentrations. As the parasitaemias peak and decline, usually within the first week of patency, infectivity to mosquitoes declines. This may be partly due to the decline in gametocyte densities, but it is also

accompanied by the rise in titre of anti sexual stage antibodies which convert from transmission enhancing to transmission blocking in their effects. In studies with monoclonal antibodies against *P. vivax* sexual stage parasites, the same antibodies which enhanced infectivity at low concentrations mediated transmission blocking activity at high concentrations (Peiris et al., 1988). Membrane feeding studies have demonstrated that intraerythrocytic gametocytes are unaffected within the circulation of the host. Both the infectivity enhancing and the infectivity suppressing effects of serum are mediated only when the sexual stage parasites become extra cellular in the midgut of a blood fed mosquito.

In Toque monkeys which have been splenectomised prior to infection with *P. cynomolgi* much higher parasitaemias are achieved than occur with infections in spleen intact monkeys. Infections of *P. cynomolgi* in such monkeys enter a phase known as "crisis" as peak parasitaemias are reached. Crisis is associated with the appearance on the day of peak parasitaemia of dead parasites within the infected red blood cells. There is an abrupt decline in parasitaemia and a total loss of infectivity of the parasites to mosquitoes extending over a period of up to a week. Thereafter the low densities of remaining parasites recover infectivity to mosquitoes. It has been shown that the loss of infectivity of gametocytes of *P. cynomolgi* during the period of crisis is due to the effects of the cytokines TNF and gamma IFN acting in conjunction with some as yet unidentified factors to kill the gametocytes within the red blood cells while they are still in the circulation of the host (Naotunne et al., 1991). This mechanism of suppression of infectivity of the parasites to mosquitoes is, therefore, quite distinct from the antibody mediated transmission blocking or enhancing effects described above which leave the circulation gametocytes unaffected and act only after the parasites have become extracellular in a mosquito blood meal.

Similar parasite killing factors capable of suppressing the infectivity of gametocytes to mosquitoes are transiently present during the period of paroxysm in *P. vivax* infections in non-immune individuals (Mendis et al., 1990). It has been observed in our laboratory that *P. vivax* patients will not infect mosquitoes at or shortly after the period of paroxysm while high

levels of infectivity may be found on the following day in the same individuals. These observations no doubt reflect the effects of paroxysm-induced inactivation of the gametocytes.

Serum/antibody mediated malaria transmission blocking and enhancing effects acting against the extracellular sexual stages of the parasites in the midgut of a blood fed mosquito have been observed during infections of *P. vivax* in man (Mendis et al., 1987). Such transmission blocking effects are found in primary as well as during subsequent infections. Should a second malarial episode occur within a period of about four months after a previous episode the anti sexual stage antibody levels are elevated and the infectivity of such a second *P. vivax* infection to mosquitoes is considerably reduced compared to that of the first infection (Ranawaka et al., 1988). It is questionable, however, whether there is a true immunological boosting of the transmission blocking antibodies based on activation of immune memory cells from the first infection. Thus reinfection after more than four to six months, roughly the period within which titres of anti sexual stage antibodies return to preinfection levels, leads to levels of infectivity to mosquitoes similar to those in a primary infection. Moreover, individuals who have lived for many years in a malaria endemic area generate levels of transmission blocking antibodies no more effective than those experiencing a primary infection (Gamage-Mendis et al., unpublished).

Observations have also been made on the elaboration of transmission blocking antibodies during *P. falciparum* infections in man (Graves et al., 1988). Such antibodies can be demonstrated following primary *P. falciparum* infection and individuals having life long exposure to intense malaria transmission as in lowland Papua New Guinea. There is, however, little evidence to suggest that anti *P. falciparum* transmission blocking immunity becomes more effective with age and duration of exposure to malarial infection. An early study on infectivity to mosquitoes of individuals living under conditions of intense malaria transmission in West Africa found that the decline in infectiousness to mosquitoes which was observed with age, closely followed the decline in densities in gametocytes as age acquired immunity to the blood stage parasites led to an overall reduction in the densities in blood stage

parasites (Muirhead-Thomson, 1957; Carter & Gwadz, 1980). Thus, as has been found for *P. vivax* infections in an endemic area, there is no evidence that transmission blocking immunity against *P. falciparum* becomes more effective with duration of exposure to infection.

These observations have led us to propose that malaria transmission immunity may be induced during natural infections by mechanisms which do not involve an effective immune memory. Immune memory cells, usually believed to involve a major contribution from helper CD4⁺ T cells, may have little involvement in the natural induction of malaria transmission blocking antibodies. This could be because the target antigens are nonprotein in nature and are, therefore, unable to be recognized by CD4⁺ helper T cells which function via the specific interaction of peptide fragments from a protein antigen which has been processed and presented by an antigen presenting cell. Alternatively the target antigens could be protein in nature but prevented from being processed and/or recognised by helper T cells by a mechanism such as that proposed by Schofield (1991) to account for the T independent induction of antibodies to the repeats of the malaria circumsporozoite protein.

STUDIES ON ARTIFICIAL IMMUNIZATION IN ANIMAL MODELS WITH SEXUAL STAGES OF MALARIA PARASITES TO INDUCE TRANSMISSION BLOCKING IMMUNITY

The first such studies were done with the avian malaria parasites *P. fallax* in turkeys (Huff et al., 1958) and *P. gallinaceam* in chickens (Gwadz, 1976; Carter & Chen, 1976). Formalin killed or X irradiated preparations containing sexual stage parasites from infected blood were inoculated intravenously without adjuvant. An effective transmission blocking immunity was produced which, on subsequent challenge, considerably reduced or eliminated the infectivity of the immunized birds to mosquitoes. The effect of the immunity was highly specific to the sexual stages and appeared to be entirely mediated by serum (antibody) factors acting against the extracellular sexual stages after their emergence into the midgut of a blood fed mosquito.

A similar method of immunization, i.e. intravenous inoculation without adjuvant of formalin killed sexual stage parasites, was effective in inducing transmission blocking immu-

nity against the rodent malaria parasite *P. yoelii nigeriensis* in mice (Mendis & Targett, 1979). Intravenous immunization without adjuvant was, however, completely ineffective in inducing transmission blocking immunity in Rhesus monkeys immunized with preparations containing sexual stages of *P. knowlesi* (Gwadz & Green, 1978). Effective immunity in this combination was achieved by intramuscular immunization using Freund's Complete Adjuvant. In the same series of experiments it was noted that repeated blood infections of *P. knowlesi* in Rhesus monkeys failed to give rise to transmission blocking immunity in these animals.

These experiments indicate that there can be fundamental differences in different host parasite systems in the way in which transmission blocking immunity may be induced. Other studies suggest that there are equally significant differences in the ways in which such immunity may be expressed. Most studies on transmission blocking immunity have convincingly demonstrated the role of antibody mediated mechanisms of transmission blocking immunity which act as mentioned above by interacting with the sexual stage parasites only after their emergence from the host red blood cells in the midgut of a blood fed mosquito. This is undoubtedly a major mechanism in all the studies on artificial immunization mentioned above. One study, however, on *P. y. nigeriensis* in mice, has provided evidence for a cell mediated immune mechanism which acts against the intracellular gametocytes of the parasite in the host's circulation (Harte et al., 1985). The effect could be passively transferred by CD4⁺ T cells. It is suggested by the authors that these immune T cells, induced as described above by intravenous immunization with sexual stage parasites without adjuvant, may be activated in an antigen specific way by sexual stage antigens in a challenge blood infection. The result of this activation could be the release of cytokines capable of inducing macrophages to release parasite killing factors as described above during malaria infection crisis in *P. cynomolgi* infections.

In contrast to the evidence from natural malaria infections, there is evidence in several animal models for an effective, probably cellular, immune memory for transmission blocking immunity following artificial immunization. Thus, in spite of the evidence referred to above that infections such as *P. knowlesi* in the Rhe-

sus monkey and indeed *P. gallinaceum* in chickens fail to induce detectible transmission blocking immunity during a blood infection, such infections can strongly boost transmission blocking immunity in previously immunized animals. This has been shown in challenge experiments up to six months post immunization in the case of *P. gallinaceum* in chickens (Carter et al., 1979) and up to six years post immunization with *P. knowlesi* in Rhesus monkeys (Gwadz & Koontz, 1984). In these experiments pre-challenge sera were without detectible levels of transmission blocking antibodies. Rapidly after challenge, however, anti sexual stage transmission blocking antibodies rose to high levels usually resulting in complete suppression of the challenge infection to mosquitoes.

The results reported for *P. y. nigeriensis* in mice are of particular interest (Harte et al., 1985). Here mice challenged with a blood infection 6 to 12 months after immunization, as described above, were completely non-infectious to mosquitoes even though titres of anti sexual stage antibodies had disappeared within 4 months post immunization. Although levels of anti sexual stage antibodies were eventually boosted following challenge it was convincingly shown that the suppression of infectivity of the challenge infections to mosquitoes was due rather to the presence of a CD4⁺ T cells population induced by the original immunization enabling the intra erythrocytic killing of gametocytes in the blood circulation.

CONCLUSIONS

It now appears that there are two general types of mechanism whereby anti sexual stage malaria transmission blocking immunity can be mediated. One involves anti sexual stage antibodies acting against the extracellular sexual stages of the parasites in the midgut of a blood fed mosquito; the other involves non-antibody immune cell products, including cytokines and probably other factors, which kill the intra erythrocytic gametocytes in the blood circulation. Both appear to be capable of being induced by immunization with sexual stage antigens. How and which type of immunity is induced and with what effect appears to vary according to the host/parasite system involved and the nature of the immune challenge.

There has been some discussion concerning the significance and importance of immune memory and boosting of transmission blocking

immunity by a natural infection following immunization with a synthetic vaccine. The considerations raised here present a confusing picture of the role of immune memory in transmission blocking immunity. All systems involving immunization with killed parasite material appeared to result in effective immune memory. This was shown by rapid reactivation of antibody producing cells following a challenge infection or by the direct demonstration of persisting cellular immunity. By contrast transmission blocking immunity induced by natural infection showed no evidence of persisting immune memory. It is possible that natural infections which fail to induce a lasting immune memory, do so primarily by inducing an antibody response to non-protein antigens or to protein antigens which somehow elude the involvement of MHC and CD4⁺ T cell recognition. Alternatively some other feature of induction of immunity by a live malarial infection, as opposed to the dead parasites used in artificial immunization, may undermine the induction of immune memory. However, once cellular memory has been induced by appropriate immunization, the live malarial infection can provide a potent immunological stimulus to its activation.

The range of mechanisms and effects of anti sexual stage immunity is now known to be greater than was perhaps originally envisaged. Both antibody and cell mediated mechanisms of transmission blocking immunity occur. Moreover, there appear to be critical differences in the mechanisms by which such immunity is induced during natural infection and following immunization with non living material. Hitherto, largely for reasons of the available technology, but also influenced by prevailing views concerning immunity to malaria, all attention has been focused upon proteins as target antigens of transmission blocking and other forms of anti-malarial immunity. In view of the evidence for a poor immunological memory for transmission immunity induced by natural malarial infections in man it may be appropriate to direct a part of our efforts towards the study of CD4⁺ T cell independent transmission blocking immunity in general and CD4⁺ T cell independent and non-protein target antigens in particular.

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