Parasite Immunology, 1995: **17**: 55–59

Point of view

Development of immunity to malaria may not be an entirely active process

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SUMMARY

It has never been explained why it takes so long for humans to develop immunity to malaria, although factors such as antigenic variation, antigenic polymorphism, and poor immunological responses to critical antigens are thought to be important. Models of malaria, particularly in rodents, have not been helpful. The course of malaria infection differs considerably between humans and rodents. Mice rapidly develop immunity whereas for most humans it takes several years of exposure for this to occur. Mice typically exhibit high parasitaemias whereas humans typically do not. A significant difference in the immune response of humans and mice to malaria parasites might, in part, explain these differences. Most humans have a pre-existing population of activated malaria parasite-specific T cells (cross-reactive T cells) which we have referred to as 'natural' T cells, but such cells have not been observed in mice. These cells, many of which secrete interferon-γ, might control parasitaemia early in the infection, but a by-product of their further activation by malaria parasites might be disease symptoms. Development of immunity has been thought of as an active process—acquisition of specific antibody and effector T cell responses. However, it might in part reflect induction of tolerance *of this pre-existing population of disease-inducing T cells as a result of chronic parasitaemia. The initial presence of these Th1-like cells may also impede the development of a Th2-like response necessary for the production of protective antibodies. Persistent cross-reactive stimulation may significantly impede this process.*

Keywords P. falciparum, *immunological tolerance, cytokines, malarial immunity*

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Received: 4 October 1994
Accepted for publication: 16 November 1994

INTRODUCTION

In previous articles we postulated that pre-existing cross-reactive T cells may skew the development of malaria specific T cells following exposure to malaria parasites, through a type of 'original antigenic sin' (Good & Currier 1992, Good *et al.* 1993). It was postulated that aberrant homing of T cells might result and that disease might be a consequence. The possible effects that these cross-reactive T cells may have on the development of immunity are further explored in this article, where it is hypothesized that tolerance of this population may be required in order to develop immunity.

Man and mouse

Immunity in mice to various malaria parasites can develop in a period of weeks following infection of animals followed by drug cure (Kumar *et al.* 1989), but rapid acquisition of immunity to malaria is not observed in humans. Children in endemic areas can take several years to develop effective clinical immunity (Greenwood *et al.* 1987). Adults may develop immunity more quickly (Baird *et al.* 1991), but generally a number of years is required. A second obvious difference between the course of infection in both species is that parasitaemias are typically much greater in mice than in humans. One explanation for the difference between mice and humans is that rodent malarias are not natural parasites for mice, rather for tree rats, and that basic biological factors have not evolved that lend to a harmony of co-existence of mouse and parasite. This may undoubtedly be significant, but the effect of this on acquisition of immunity is difficult to prove or disprove. However, it has also been suggested that falciparum malaria has evolved from bird malarias and has only 'recently' been a parasite of humans.

The consensus view of anti-parasite immunity of humans to malaria

Certainly the processes involved in natural immunity to malaria are not well understood—a factor contributing in no small way to the difficulties that we face in attempting to develop a malaria vaccine. However, a consensus view (which may be correct or incorrect) is starting to emerge. According to this view, but based largely on experimental studies in animals, both antibodies (Bouharoun-Tayoun *et al.* 1990, Newbold *et al.* 1992) and effector T cells (Brake *et al.* 1988, Quaky *et al.* 1994) play important roles. Antibodies recognize opsonic determinants on the surface of the infected red cell (leading to phagocytosis of cells by macrophages or neutrophils), or recognize merozoites and inhibit red cell invasion. Antibodies of the former specificity have been shown to correlate with the acquisition of clinical immunity in humans. A major observation was that antibodies from immune adults could passively transfer protection to non-immune recipients (Cohen *et al.* 1961, Bouharoun-Tayoun *et al,* 1990) and that these antibodies were of the cytophillic classes (Bouharoun-Tayoun & Druihle 1992).

$CD4^+$ T cells (Brake *et al.* 1988, Kumar *et al.* 1989) have been shown to be effectors of cellular immunity in animals. Cloned lines can adoptively transfer protection, and abrogation of immunity following depletion of $CD4^+$ T cells from mice immunized by repeated infection and cure suggests that T cells alone may be able to afford significant protection. Effector T cells are probably effective within the immune spleen (Kumar *et al.* 1989). Human T cells, in the presence of monocytes and restricted by HLA molecules, are able to inhibit growth of *Plasmodium falciparum in vitro* (Brown *et al.* 1986, Quakyi *et al.* 1994, Fell *et al.* 1994). Results from a number of different model systems suggest that while T cells can control parasitaemia, antibody may be required to completely eliminate parasites (Roberts & Weidanz 1979, Kumar *et al.* 1989, von der Veid & Langhorne 1993, Taylor-Robinson & Phillips 1994), but the process is under T cell control. Elegant studies in a murine model have shown that inflammatory-type T cells (Th1) are involved initially in control of parasitemia, but that helper T cells (Th2) then develop resulting in the antibody that ultimately clears the infection (Langhorne *et al.* 1989, Taylor-Robinson *et al.* 1993). It has been suggested that the switch from Th1 to Th2 may be due to the switch in antigen-presenting cells (from macrophages to B cells (von der Weid & Langhorne 1993, Taylor-Robinson & Phillips, 1994). Some studies in humans are consistent with, but do not prove, this idea. In particular, malaria-specific interferon-γ responses and proliferative T cell responses to various malaria antigens are commonly observed to be higher in non-immune or semi-immunes than in immunes, while specific antibodies are more common in the immune population (Chizzolin *et al.* 1990, Zevering *et al.* 1991, Doolan *et al.* 1994). These studies have not been necessarily performed using the most appropriate target antigens (since these are not known) but the data with various antigens or crude parasite preparations are consistent with this view.

T cells and disease

The above discussion refers to anti-parasite immunity (the ability of immune mechanisms to inhibit parasite growth). However, when discussing immunity in malaria, it is impossible not to consider disease pathogenesis. Disease manifestations in malaria are protean, but one or more of anaemia, cerebral malaria, or pathological manifestations in the lungs, liver, kidney and bone marrow occur frequently in individuals prior to the acquisition of immunity or during pregnancy in previously immune women (particularly a first pregnancy). The pattern of pathology can differ between different strains of the parasite and between different geographical settings (Miller *et al.* 1994). While both parasite and host factors can affect the expression of disease, in this article I wish to focus on host immunological factors. Studies in animals have suggested that T cells, in particular Th1-type cells, can be critical to disease pathogenesis (de Kossodo & Grau 1993). While expression of pathology can differ between animal models and humans, the association between pathology in humans and cytokine expression (particularly TNFα) is convincing (Kwiatkowski *et al.* 1990). TNFα is typically thought to arise as a result of monocyte activation by malaria toxins, and antibodies to these toxins can prevent TNFα secretion by monocytes *in vitro* (Taverne *et al.* 1990). We have argued, that in humans, T cell stimulation by parasites might be equally effective in triggering TNFα secretion and consequent disease (Currier *et al.* 1992, 1994 (submitted)). Why, however, would parasites stimulate T cells in non-immune donors (with none or very little prior exposure to malaria)? Data from others and ourselves have shown that activated ($CD4^+$ T cells from non-immune donors can respond vigorously to malaria parasites *in vitro* (Jones *et al.* 1990, Currier *et al.* 1992), and we have raised $CD4^+$ T-cell clones to malaria parasites from non-exposed donors that respond to environmental organisms (Currier *et al.* 1992, 1994 (submitted)), and clones to environmental organisms that respond to malaria parasites (A.Fell and M.F.Good, unpublished). Thus, these cells are likely to have arisen as a result of cross-reactive

stimulation. T cells from non-exposed donors can respond to parasites at a density of 1 parasitized red cell per μl of blood or less—similar to the density when non-exposed donors can initially suffer disease symptoms in malaria. These T cells typically secrete interferon-γ and either secrete themselves or direct the secretion of TNFα. We have postulated that these cross-reactive T cells which we find in over 90% of non-exposed adults and 50% of children may contribute significantly to disease pathogenesis following activation *in vivo* by malaria parasites.

Thus, there is reason to believe that T cells can play a significant role in parasite elimination, but a consequence of this elimination is the activation of T cells and liberation of cytokines that result in disease.

The hypothesis

Acquisition of immunity in humans to malaria has largely been thought of as being an active pheonomenon. Lymphocytes engage malaria antigens and specific humoral or cellular factors arise which can control the parasite's growth. Similarly, disease is thought to lessen with increasing exposure by an active process—the development of antibodies to malaria toxins. However, we would argue that anti-parasite immunity (the ability of the immune system to control parasite load) is a composite of: (i) what we have referred to as 'natural' T cells (T cells that have arisen as a result of cross-reactive stimulation, but which may circulate in inappropriate locations (Currier *et al.* 1992, Good & Currier 1992); (ii) T cells that have arisen as a result of parasite stimulation; and (iii) antibodies (probably the cytophillic classes) specific for agglutinating antigens and which have also arisen as a result of parasite stimulation (Bouharoun-Tayoun & Druihle 1992). The relative contribution of cellular immunity ('natural' plus parasite-induced) compared to antibody-mediated immunity is not known. However, the ability of cellular immunity to protect rodents, and of human specific and cross-reactive T cells to kill parasites *in vitro* (in association with monocytes) argues that human cellular immunity can be effective in killing parasites. The fact that humans take many years to develop clinical immunity does not necessarily argue against the role of cellular immunity (which may be pre-existent in many people) in favour of specific humoral immunity, but might argue that other factors are critical. One of these factors might involve *elimination* or *tolerance* rather than induction of malaria-specific T cells which have the capacity to promote disease.

Chronic antigenaemia can result in peripheral T cell tolerance, which may involve anergy or actual deletion of the specific T cells. This has been convincingly shown in mice following infection with lymphocytic chroriomeningitis virus (LCMV) (Moskophidis *et al.* 1993). We have recently observed that infection of mice with *P. berghei* can tolerize a Th1 *P. berghei*-specific T-cell line that had been adoptively transferred (C.Hirunpetcharat & M.F. Good, in preparation). This line has a similar phenotype to many clones of cross-reactive T cells generated from non-exposed donors (TCRα/β, CD4$^+$) secretes interferon-γ), and a similar phenotype to those T cells associated with disease pathology. The berghei-specific T cell line can also reduce parasitaemia. Thus, a rodent parasite infection can negatively affect murine T cells which have a similar phenotype to human malaria-reactive T cells which are found in most non-exposed individuals. A plausible explanation for the acquisition of immunity is that infection in humans with malaria parasites is required to tolerize or physically eliminate parasite-specific T cells (which also happen to be parasiticidal). This would be consistent with the observation by Chizzolini *et al.* (1990) that T cells from immune individuals produced much less interferon-γ and proliferated less following parasite stimulation than cells from non-immune individuals. In non-immunes, T cells may initially be activated, resulting in the control of parasitemia, but with associated disease symptoms. T cells may then be exhausted, particularly if as a result of the absence of suitable antibody the parasite cannot be cleared completely. Malaria infection of humans is often characterized by a period of parasitaemia associated with disease symptoms followed by a period of parasitaemia without disease symptoms (Powell *et al.* 1972). The initial presence of Th1-like (cross-reactive) cells may also directly inhibit the development of Th2-like helper cells required for production of protective antibodies, via secretion of interferon-γ (Gajewski & Fitch 1988), IL-12 (Manetti *et al.* 1993) or other cytokines. It may take a number of infections to eliminate these cells which may also regenerate between infections as a result of further cross-reactive stimulation. It may seem paradoxical that malaria parasites could result in tolerance of malaria-specific T cells while these cross-reactive cells persist between infections—presumably as a result of cross-reactive stimulation. Chronicity of antigen stimulation seems to be crucial to maintenance of tolerance (Ramsdell & Fowlkes 1992) and this may not be a feature of the exposure to the various organisms responsible for malaria cross-reactive stimulation.

Why does this scenario not occur in mice, which rapidly develop immunity? The host-parasite relationship may be inappropriate, as discussed; however,

relevant to the outlined hypothesis it is important to note that we have not been able to find 'natural' malaria parasite-cross-reactive T cells in mice, possibly because mice are too young when studied. A consequence of this might be the high initial parasitaemia (since the pre-existing Th1 cells are not abundant when the parasite initially infects). A parasite-induced Th1 response will be stimulated, but the absence of a strong pre-existing repertoire of Th1-like T cells may favour the more rapid development of Th2-like cells.

ACKNOWLEDGEMENTS

I wish to thank Dr Allan Saul for critically reviewing this manuscript. My laboratory receives support for malaria research from NHMRC (Australia), UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease, and the Cooperative Research Centre for Vaccine Technology.

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