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Mediators and Mechanisms Associated with Paroxysm in *Plasmodium vivax* Malaria

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The Clinical Manifestations and Biological Basis of Paroxysm and its Relationship to Other Malaria Pathology

The paroxysm of human malaria is most clearly expressed in infections with that species of parasite whose effects were recognized in early times as "benign tertian" fevers and which, since the end of the last century, has been known as *Plasmodium vivax*. Infections with *P. vivax* were termed benign, or as would be said today, uncomplicated, in distinction from the severe morbidity and occasional mortality associated with infections due to the other highly prevalent species of human malaria, *Plasmodium falciparum*. The severe pathologies of *P. falciparum* infection involve organ dysfunction (e.g. cerebral malaria, renal failure, severe anaemia) and often irreversible tissue damage. The pathology of *P. vivax* infections, on the other hand, is almost invariably transient and with no detectable tissue damaging consequences. The symptoms of *P. vivax* infection are malaise, anorexia and tendency to prostration with periodic episodes of acute fever which may be accompanied by headache, nausea and vomiting and moderate to severe muscle, joint and back pain.

Neither the physiological basis of the severe and complicated pathologies found among *P. falciparum* infections, nor that of the benign pathology of paroxysm in *P. vivax* infections are understood. There are, however, biological differences between the parasites which certainly account, at least in part, for their different pathological manifestations. Thus in *P. falciparum* infection there is invariable sequestration of the maturing asexual blood-stage parasites by ligand-mediated attachment to the endothelial lining of the post-capillary microvasculature. This results in virtually continuous aggravation of the endothelial tissues in every organ affected and presumably contributes, in the minority of cases, to induction of the mediators of severe pathology.

By contrast, *P. vivax* blood-stage parasites do not sequester to any detectable degree at any stage in their development. Being intracellular parasites of red blood cells (RBC) their presence in the body is registered and responded to, particularly following the period of schizont rupture, an event which occurs synchronously at almost exactly 48-h intervals (or, as not infrequently occurs, at 24-h intervals when two distinct broods of the parasites are present in the blood). The first 2–3 rounds of schizont rupture, following release of the parasites in the blood from the sporozoite-induced (mosquito-inoculated) liver stage of the infection, are largely asymptomatic (the prodromal period). Thereafter, the full-blown clinical symptoms of a paroxysm are suddenly manifest following the next schizont rupture event, usually when parasite densities are still less than one parasitized RBC per 100 000 uninfected RBCs.

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The first symptoms of a *P. vivax* paroxysm, which are felt within 1–2 h after the beginning of schizont rupture, are a sudden feeling of chill; within a few minutes the victim takes to bed and seeks to cover him or herself with a sheet or blanket, adopting a hunched or foetal position. Within 5–10 min the sufferer is shaking with considerable violence and, although experiencing a sensation of great cold, is in fact undergoing a steep rise in body temperature. What has happened is that the body's thermostat has been reset to several degrees above normal accounting for both the sensation of cold and for the rising temperature as the body adjusts to reach the reset level. About 1 h after the first feeling of chill, the victim looses the cold sensation and one of internal warmth returns soon proceeding to one of high fever, the bed clothes being cast aside in an attempt to lose heat. Headache, muscle, joint and back pains may now become prominent and even severe; nausea and vomiting may occur. Within 3–4 h after onset, the temperature, which peaks typically at 39–40°C, begins to fall; this is usually accompanied by profuse sweating which can leave the bedclothes literally drenched with perspiration. As other symptoms slowly remit, the exhausted sufferer may fall into peaceful sleep perhaps 4–6 h after the onset. Within 8–10 h the temperature has returned to normal.

This account would be typical of a paroxysm in a non-immune sufferer. The exact picture can vary, especially in the associated symptoms. However, the chill, rigor (shaking) and soaring temperature are almost invariably experienced. The mediators and mechanisms of the paroxysm of *P. vivax* malaria are certainly different from those of the severe pathologies associated with *P. falciparum* malaria. Nevertheless, experimental investigations and speculation have identified certain substances of host or parasite origin as being generally implicated in malarial disease. Prominent among these are the cytokine tumour necrosis factor alpha (TNFα) (Clark et al 1992) and the products of the parasites released during schizont rupture (Kwiatkowski 1995).

TNFα has long been the subject of investigations which have sought to define it in a causative role in the severe and complicated pathologies of *P. falciparum* malaria, notably in cerebral malaria. While several studies have shown an association between circulating TNFα levels and severity of disease in *P. falciparum* malaria (Grau et al 1989; Kern et al 1989; Kwiatkowski et al 1990), there are no experiments which demonstrate a direct causal relationship between the activity of this cytokine and severe pathology in malaria. An experimental clinical intervention in severe malaria cases with an anti-TNFα monoclonal antibody did not protect against fatal outcome

(Kwiatkowski et al 1993) but did, on the other hand, achieve significant reduction in fever. Consistent with a role for TNFα as a key pyrogen in malarial infection is the finding that circulating levels of the cytokine in cases of *P. vivax* malaria rose sharply at the onset of a paroxysm and declined in close parallel with the fall in temperature after the paroxysm (Karunaweera et al 1992b) (Fig. 1). Moreover, the circulating levels of TNFα during paroxysms in *P. vivax* infections often exceeded those seen in the severest cases of *P. falciparum* malaria. Thus the circumstantial evidence is consistent with the role of TNFα as an endogenous pyrogen of malarial fevers but mitigates against TNFα as a sole and sufficient mediator of severe pathology of *P. falciparum* malaria.

The other class of mediators of malaria pathology which has received much attention is represented by the parasite products released following schizont rupture. The concept of a malaria toxin must have arisen in the minds of the early malariologists and it acquired a specific biological context when it was discovered that the periodic fevers of malaria are co-ordinated with the synchronous rupture of the blood stage schizonts of the parasites. This relationship was investigated experimentally by a working party of the United States Public Health Service at Vera Cruz in Mexico in 1904 (Rosenau et al 1905). These workers demonstrated that the full blown symptoms of a *P. vivax* paroxysm could be passively transferred to a healthy individual by intravenous injection of serum taken at the height of a paroxysm from a *P. vivax* sufferer; serum taken at times other than during the paroxysm had no effect on a recipient. In their report of these experiments the investigators were cautious not to claim discovery of the malaria toxin itself. This was wise, as we now realize that other mediators, such as TNFα itself, could have been, and probably were, directly responsible for the effect. Nevertheless, the experiments proved that potent pyrogens were transiently present shortly after the rupture of the blood-stage schizonts and, while admiring the caution of these American workers, it would be hard not to conclude from their experiments that the products of the schizont rupture were either the pyrogens themselves, the inducers of the pyrogens or both the inducers and, in some synergistic way, the co-mediators with those they had induced, of the febrile and other events of the paroxysm.

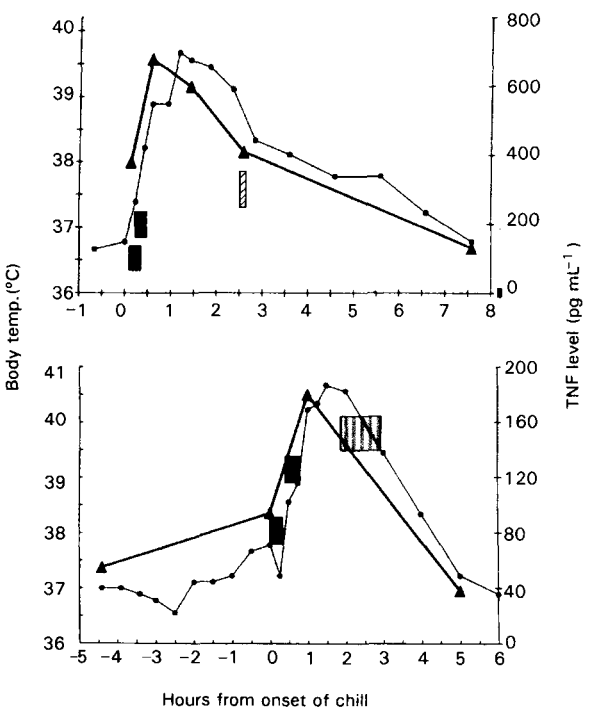

FIG. 1. Body temperature (measured orally) (●), plasma TNF level (▲) and the period and duration of the chill (hatched box), rigor (black box) and sweating (grey box) during the course of a single paroxysm in two of the nine *P. vivax* patients studied. The parallel between changes in body temperature and plasma TNF levels was as close as is shown here in eight of the nine patients studied. Reproduced by permission of the Proceedings of the National Academy of Sciences, USA (Karunaweera et al 1992b).

Recent investigations with the products of schizonts of malaria parasites prepared in-vitro, have shown that these products are, indeed, potent stimuli of TNFα production by human peripheral blood monocytes in culture (Bate et al 1992; Allan et al 1993). There is now significant literature which has explored the nature of the parasite products which are involved in such TNFα induction (Playfair et al 1990; Mendis & Carter 1995; Kwiatkowski 1995). There is general agreement that these products are glycolipid in nature, do not involve protein in their activity and, by antigenic criteria and biological activity, cannot be readily distinguished between different species of malaria parasite, including the two human species *P. falciparum* and *P. vivax*.

While no-one, today, can claim comprehensive insight into the mechanisms of malaria pathogenesis, the following general scenarios for the involvement of soluble mediators are commonly at the centre of contemporary investigation and discussion.

Severe pathology

TNFα, induced by parasite products, perhaps locally at the sites of deep vascular sequestration, acts secondarily on local tissues, e.g. venous endothelium, to up-regulate expression of ligands involved in binding of parasitized RBCs and parasite sequestration, and to induce secondary mediators such as nitric oxide or oxygen free radicals, or both, which inflict direct organ-malfunction and tissue damage.

Paroxysm (non-severe pathology)

Parasite products released into the blood circulation at the time of schizont rupture induce circulating monocytes to produce TNFα and possibly other pyrogenic cytokines, IL-1 and IL-6, which act on the thermoregulatory centre in the hypothalamus to reset the body thermostat and induce the fever of paroxysm.

As already stated there is apparent overlap between the general hypotheses for malarial paroxysm and for severe pathology in malaria in that both invoke the induction of TNFα by the action of parasite products on (it is generally assumed) blood monocytes. If this is, indeed, a true representation of the two very different types of pathological condition, it will require detailed investigation of the other mechanisms involved to achieve an understanding of why the clinical manifestations are so different. In seeking to understand the basis of malaria pathogenesis we have devoted the investigations presented here to exploring the mechanisms of the paroxysm of *P. vivax* malaria.

The Biological Significance of the Paroxysm of *P. vivax* Malaria

In the early stages of a malarial, or indeed any, blood infection in a previously unexposed host, it has been generally assumed that there can have been no time for an effective pathogen antigen-specific protective immune response to mature. The host is, therefore, presumed to be dependent for the prevention of uncontrolled parasitic infestation, upon its ability to mount innate, parasite density-controlling responses. As has been pointed out (Kwiatkowski 1995), such a response, in a human host, to the early stages of malarial infection can be reasonably argued to be represented by the paroxysm of malaria, of which the role of high body temperature in killing malaria parasites has been emphasized. In the case of malaria it appears that the mature forms of the parasite are those most sensitive to high temperature as has been demonstrated in-vitro (Kwiatkowski 1989). As a consequence, the fever of paroxysm presumably has the effect not only of culling the parasites but of doing so by eliminating all but the youngest parasites newly invaded into red blood cells. Since it is the most mature forms of the parasites, the rupturing schizonts, which initiate the paroxysm, the net effect is to tightly synchronize the blood infection by leaving only the youngest parasites alive shortly after schizont rupture. While the temperature sensitivity of mature asexual blood-stage malaria parasites, and indeed of mature malaria gametocytes, can be readily demonstrated in-vitro, the parasite-killing effects of paroxysm in-vivo have been taken on faith. Some observations made in our laboratory in Sri Lanka on the infectivity of naturally acquired primary infections of *P. vivax* to mosquitoes, however, provide direct evidence that such an effect may occur during a malarial paroxysm. Thus, mosquitoes became infected when fed on the arms of *P. vivax*-infected volunteers when they were without symptoms but those fed on the same patients during a paroxysm almost invariably failed to acquire infection (unpublished data).

This observation suggested that blood-stage parasites were indeed being killed or inactivated during malarial paroxysm and raised the question as to whether the infectious stages, the gametocytes, were being inactivated only by the fever itself or whether other factors were involved. To test this question we collected plasmas from *P. vivax* patients during the peak of their paroxysms. We then collected *P. vivax*-infected blood from volunteers who were without symptoms, and whose gametocytes were in an infectious state. The infectious gametocytes were cultured in-vitro for 3 h with medium containing paroxysmal plasma and subsequently resuspended in normal serum and fed to mosquitoes through a membrane. Almost invariably it was found that the infectivity of the gametocytes pre-incubated in the paroxysm plasmas was reduced by 70 to 80% compared to that of equivalent samples of the same gametocyte-infected blood pre-incubated in normal plasmas from healthy control donors (Karunaweera et al 1992a). Plasmas drawn a few hours before the onset of a paroxysm and plasmas drawn following its resolution had only a slight suppressive effect on the infectivity of the gametocytes to mosquitoes (Karunaweera et al 1992a; Wijesekera et al 1996).

These results showed that, independent of any effects due to temperature, there are active substances present in plasma during a paroxysm that mediate the suppression of gametocyte infectivity to mosquitoes. Moreover, the parasite-inactivating mediators are rapidly induced around the time of onset of the paroxysm, and, with similar rapidity, cease to be effective after the paroxysm has passed.

It was evident that the experimental system which we had devised to test the effects of plasma on the infectivity of gametocyte-infected blood to mosquitoes, was, in fact, a precise assay for the presence of some, as yet undefined, mediators whose activity coincided exactly with the period of a paroxysm of *P. vivax* malaria. The investigations summarised in this article are from studies, using this assay, on mediators and mechanisms within the circulating blood cells and plasma during the clinical event of paroxysm.

Investigations on the Paroxysm of *P. vivax* Malaria

Gametocyte inactivation by P. vivax *paroxysm plasma depends upon the presence of blood monocytes and appears to be mediated, at least in part, by nitric oxide*

In order to test whether the activities of soluble mediators, whose presence we deduced in the paroxysm plasmas, were dependent upon the presence of nucleated blood cells, we conducted the following experiments. Before incubating *P. vivax* gametocyte-infected blood cells with paroxysm plasma, we depleted the cells either of T cells (with antibodies to the pan T-cell surface antigen CD2, conjugated to magnetic beads—Dynabeads from Dynal) or of monocytes (with Dynabead-conjugated antibodies to the CD14 antigen – which has, in fact, been identified as the endotoxin receptor of these cells). Depletion of T cells was without effect on the activity of the paroxysm plasma; depletion of the monocytes, on the other hand, removed the effect almost completely. Thus the active factors in the paroxysm plasmas that mediated suppression of gametocyte infectivity, were totally dependent for their effect upon the presence of peripheral blood monocytes (unpublished data).

Data from other studies have indicated that the inactivation of malaria gametocytes by activated white blood cells is mediated by nitric oxide (NO) (Naotunne et al 1993).

The activity of P. vivax *paroxysm plasma is totally dependent upon the presence of the cytokine TNFα and upon the presence of products which can be neutralized by antibodies against* P. vivax *antigens*

As discussed above, there are two types of mediator which might immediately be suspected to have a role in a monocyte-mediated activity co-incident with the paroxysm of malaria. They are the products of the parasites themselves acting as endotoxin, and the cytokine TNFα, the prime candidate for the endogenous pyrogen in the paroxysm of malaria. These possibilities were tested by pre-incubating paroxysm plasma with antibodies raised in rabbits against extracts of *P. vivax* schizonts and with a neutralizing monoclonal antibody against human TNFα. Both treatments completely removed the activity of the paroxysm plasmas, indicating that both TNFα and parasite products were essential to induce monocytes to inactivate *P. vivax* gametocytes (Karunaweera et al 1992a; Wijesekera et al 1996).

These results are entirely compatible with the favoured view of malaria paroxysm which envisages that parasite products released at schizogony act as an endotoxin to induce circulating blood monocytes to produce TNFα and that this cytokine

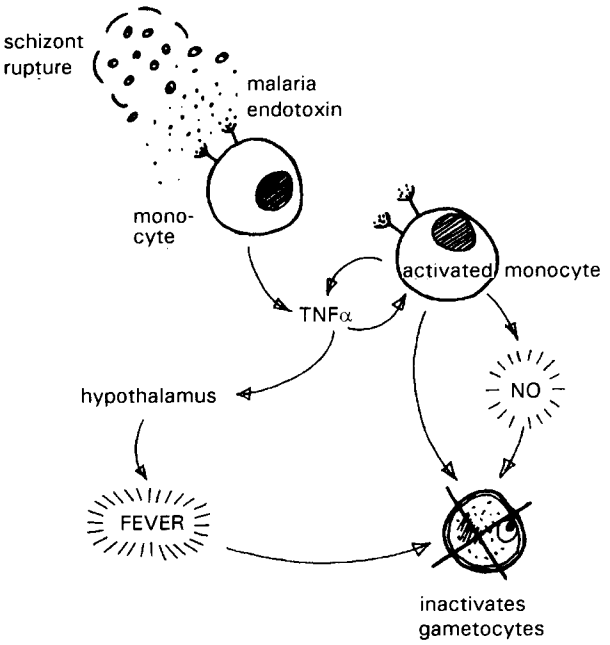

FIG. 2. Scheme representing cellular events and mediators during a *Plasmodium vivax* paroxysm.

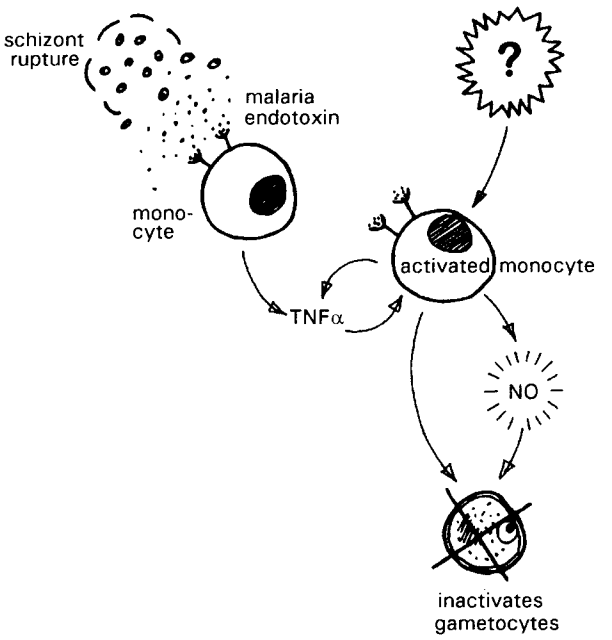

FIG. 3. Scheme representing cellular events and mediators during a *Plasmodium vivax* paroxysm showing that Fig. 2 does not fully account for the parasite-killing activity of the blood monocytes during a paroxysm.

is the endogenous pyrogen which mediates the induction of the clinical events of the paroxysm (Fig. 2). The next set of experiments, however, showed that this is not a sufficient description of the mediators and mechanisms which must be involved, at least as regards the gametocyte inactivating effects of paroxysm plasma.

Parasite products and TNFα are not sufficient to induce human blood monocytes to carry out gametocyte inactivation

If TNFα and parasite products were all that were necessary to induce blood monocytes to mediate the inactivation of *P. vivax* gametocytes, then it should be possible to induce such inactivation by addition of these to normal human plasma. Such addition, however, was totally without effect on the infectivity of *P. vivax* gametocyte-infected blood (Wijesekera et al 1996). On the other hand, their addition to plasma drawn shortly after a paroxysm (which of itself has little or no effect on the infectivity of gametocyte-infected blood) caused the post paroxysm plasma to bring about potent inactivation of the gametocytes (Wijesekera et al 1996). Addition of the parasite extracts and TNFα to plasmas drawn just before a paroxysm had a moderate but much lower effect (unpublished data).

These results proved that there are additional factors, absent in normal human plasma, which begin to appear in plasma from just before a paroxysm and which are highly active in plasma immediately following a paroxysm. The parasite products and TNFα are absolutely dependent upon these factors to be able to induce blood monocytes to mediate gametocyte inactivation (Fig. 3).

Activation of monocytes to suppress infectivity of gametocytes to mosquitoes during a paroxysm is absolutely dependent upon the presence of IL-2 and GM-CSF

To search for the unidentified mediator(s) upon which the monocytes were dependent to suppress gametocyte infectivity during a *P. vivax* paroxysm, we tested the effects of neutralizing antibodies against most of the remaining human cytokines for which such reagents were available. Of these, including antibodies to IL-1α and β, IL-6 and IFNγ, only antibodies against IL-2 and GM-CSF (granulocyte–macrophage-colony stimulating factor), mediated reversal of the effects of paroxysm plasma (unpublished data). IL-2 is the product, virtually exclusively, of activated T cells and of no other cell type (except NK cells) and GM-CSF is a maturation-stimulatory cytokine which acts on macrophages/monocytes, neutrophils and eosinophils and which is the product of T cells, B cells and monocytes.

To test the involvement of IL-2, we reconstituted normal human plasma with parasite extract and TNFα (a combination which we had previously shown does not induce monocytes to inactivate gametocytes) and added, in addition, IL-2. This combination now induced monocyte-dependent inactivation of *P. vivax* gametocytes as effectively as did paroxysm plasma itself (unpublished data). Confirmation that this almost uniquely T-cell-derived mediator, IL-2, is essential to recreate the effects of paroxysm plasma, leads us to the hypothesis that the parasite-killing aspect of monocyte activation during a malaria paroxysm is under the direct control of activated T cells (Fig. 4).

The cellular origin of the GM-CSF in the paroxysm plasma is uncertain at this stage; it could be either a monocyte or a T-cell product, or both. Its likely function in the parasite killing events would be to activate monocytes and granulocytes around the location of the parasitized cells.

The parasite products in paroxysm plasma which induce white blood cells to inactivate gametocytes are antigenically distinct and parasite species-specific

Immune sera raised in rabbits against freeze-thawed extracts of the blood-stage schizonts of either *P. vivax* or *P. falciparum*

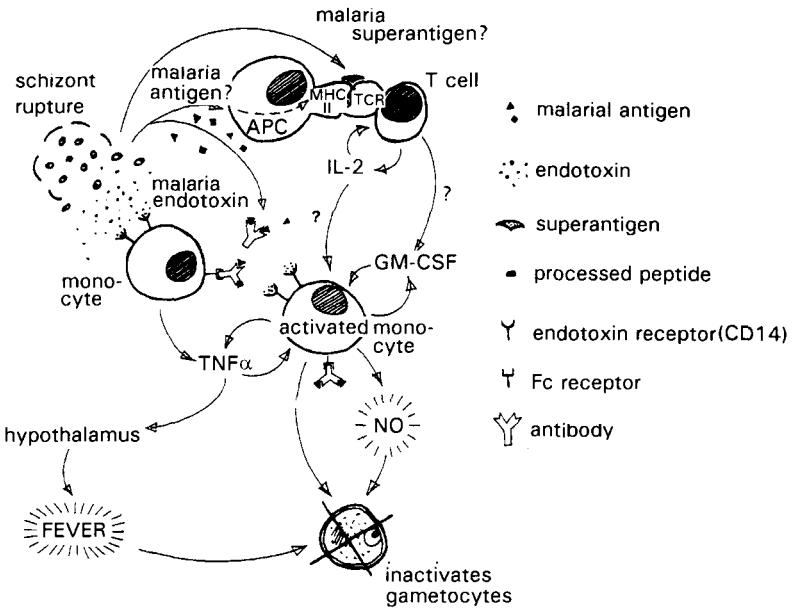

FIG. 4. Scheme representing the putative role of antigen-activated T cells and of parasite antigens in the activation of monocytes during a paroxysm of *Plasmodium vivax* malaria.

have been tested for their ability to reverse the ability of *P. vivax* paroxysm plasma to inactivate gametocytes of this parasite. Paroxysm plasma to which antisera against *P. vivax* extracts were added no longer suppressed infectivity of gametocyte-infected blood to mosquitoes. Antisera against extracts of blood schizonts of *P. falciparum*, on the other hand, were completely without effect (Wijesekera et al 1996). These findings indicate that the parasite products in paroxysm plasma which are active in stimulating the anti-parasitic activity of the white blood cells are species-specific antigens of their respective parasites.

This result stands in striking contrast to the evidence that the parasite products which induce TNF from monocytes in-vitro are antigenically indistinguishable between the two species of parasite (Bate et al 1992).

Discussion

Our investigations have enabled us to define and follow the effects of soluble mediators elaborated during the paroxysm of *P. vivax* malaria. We have used an assay which monitors the ability of peripheral blood monocytes to suppress the infectivity of gametocytes of the parasites to mosquitoes; this is, in effect, an assay of monocyte-mediated parasite killing during paroxysm.

We have shown that the active mediators of monocyte-dependent gametocyte inactivation present in paroxysm plasma include products of the parasites themselves, presumably released during schizont rupture preceding the paroxysm. Another essential mediator in activation of the monocytes is the cytokine TNFα. This cytokine is known to reach high levels during a paroxysm and is probably also the key endogenous pyrogen responsible for the induction of the clinical events of a paroxysm. It is also clear from our results, that the anti-parasitic activity of monocytes during a paroxysm, is absolutely dependent upon the presence of the activated T-cell product IL-2. Moreover, this factor becomes active no sooner than the onset of a clinical paroxysm, a fact which can be reasonably accounted for only if the T cells are activated to secrete IL-2 by parasite products released at the time of the schizont rupture.

If this conclusion is correct we can no longer regard all the events which take place during a paroxysm as simply the reflex, or innate, response of blood monocytes to the abrupt release of a parasite-derived endotoxin. At least certain aspects of these events, namely those involved in cell-mediated killing of the parasites, are now seen to fall under the control of antigen-activated T cells. There are two general possibilities for the nature of the putative antigen–T-cell relationship involved.

One possible way of explaining the activation of the T cells is to invoke the existence of malaria superantigen. Superantigens have been described from certain micro-organisms, notably *Staphlococcus aureus* B enterotoxin; their distinctive immunologic feature is that they cross-link between a major histocompatibility complex (MHC) II molecule and a T cell receptor, not by binding in the groove of the MHC molecule as do the processed peptides of conventional protein antigens, but by binding across the outer faces of the two molecules. Superantigens are able to interact with about 10–20% of a T-cell population. If this were the basis of T-cell activation during paroxysm there would be no necessity for T-cell priming by prior exposure to antigen. A paroxysm would presumably be initiated once the blood parasites passed some critical lower threshold for recognition. As far as we are aware, however, there is no evidence that malaria parasites possess material having superantigen type properties (Jones et al 1990).

The alternative is that the T cells are recognizing and responding to parasite products processed by antigen-presenting cells through conventional pathways and presented in association with an MHC class II molecule for recognition by an antigen-specific, MHC-restricted T-cell receptor. Such a scenario would require the priming and expansion of antigen-

specific T-cell clones by previous exposure to the malarial antigens. The lag that would be involved in generating such expanded antigen-specific clones could account for the prodromal period during which two to three rounds of parasite replication occur in the blood without inducing clinical symptoms in the naive individual. It is also possible that these antigens are acting on T-cell clones that have been previously primed and expanded by cross-reacting antigens of non-malarial origin (Currier et al 1995).

We have also found that the monocyte-mediated inactivation of gametocytes induced by *P. vivax* paroxysm plasma, can be reversed by immune sera raised against schizonts of *P. vivax* but not by immune sera raised against schizonts of *P. falciparum*. This finding, however, does not seem to relate to the antigens which activate T cells to produce IL-2 during a paroxysm. We can state this because IL-2 is already active in paroxysm plasma, but the parasite-killing effect of the plasma is still totally dependent upon the presence of the species-specific antigens. It is possible that these antigens are in fact endotoxins, although this rather complicates the evidence from other findings that the endotoxin of malaria is not species-specific (Bate et al 1992). Another possibility could be that the species-specific antigens activate monocytes in the form of complexes with cytophilic antibodies (Fig. 4). Antimalarial antibodies can, in fact, be detected in the serum of malaria-infected persons at the time their plasma shows parasite killing effects during a paroxysm (unpublished observations).

It is a notable consequence of these hypotheses that aspects of what has usually been represented to be an innate, non-cognitive, response to early parasitization—namely the paroxysm of malaria—may, be controlled by antigen-specific cognate immune processes.

If this situation is true, it is also probable that the parasite products, or antigens, to which the paroxysm-controlling T cells respond, and also the hypothetical monocyte-activating, immune-complexed, species-specific antigens in paroxysm plasma, are of different chemical composition to the endotoxin-like product responsible for TNFα induction by peripheral blood cells in-vitro (Dick et al 1996). As already mentioned, results from such in-vitro investigations upon endotoxin-like products of blood-stage malaria schizonts suggest that these are glycolipid in nature, do not involve protein and are antigenically similar between different species of malaria parasites. A product which activates T cells via conventional MHC antigen presentation is, virtually by definition, a protein. If the antigens which prime and activate the T cells which control *P. vivax* paroxysms are protein, then it is almost certain that the relevant molecules are also antigenically distinct between different species of malaria parasite. Likewise, the active parasite products whose presence we have detected in the paroxysm plasma are antigenically distinct and species-specific; these too may well be proteins. Our experiments provide no direct evidence that the monocyte-mediated, T cell–IL-2-controlled events of parasite-killing (gametocyte inactivation) that take place during a *P. vivax* paroxysm are causally related to the induction of the clinical symptoms of the paroxysm. It would, indeed, be quite consistent with our present data to postulate that a purely endotoxin-dependent induction of TNFα was responsible for the clinical symptoms of paroxysm. The parasite-killing events, whose mediators and components we have investigated, could thus represent an almost entirely separate, but concurrent, sequence of events having only the common cause of schizont rupture and the release of active parasite products to connect it with the clinical experience of the paroxysm. There could, on the other hand, be close causal linkage between the initiators and mediators of the parasite-killing and clinical events of paroxysm. Experience with the use of IL-2 therapy in cancer patients has shown that this cytokine, central to the events that we have described in association with paroxysm, induces a malaria-like paroxysm with chill and high fever about 2 hours following its administration. Investigation of the phenomenon showed that the IL-2 was not itself a pyrogen but that it rapidly induced pyrogenic activity which could be entirely accounted for by the presence of TNFα (Mier et al 1988). These findings bear obvious relevance to the possible interpretations of our own.

The hypotheses which we have presented from our data, that events associated with the paroxysm of *P. vivax* malaria are under the control of (presumably antigen-specific) T cells, and that species-specific antigens are involved in the immediate activation of paroxysm-associated, parasite-killing events, may be relevant to the regulation of other types of pathogenesis in malaria and, indeed, to anti-blood-stage immunity to malaria in general. It is striking that the immuno-physiological responses of the host during a paroxysm of *P. vivax* malaria are essentially anti-parasitic and, however acutely distressing to the infected host, result in little or no permanent tissue damage. It is interesting to contrast this situation with the destructive pathologies that can arise during *P. falciparum* infections and to speculate upon the mediators and their control, or lack of it, which may be involved.

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