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A malaria parasite toxin associated with *Plasmodium vivax* paroxysms

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SUMMARY

We have previously demonstrated a correlation between clinical paroxysms in *Plasmodium vivax* malarial infections and the appearance in patients' plasma of factors that kill blood stage parasites (gametocytes). This activity was, as previously shown, dependent on the presence in paroxysm plasma of tumour necrosis factor-alpha (TNF-α), which acts in conjunction with other 'complementary' factors. Here we have identified a parasite component which is essential for this activity and functions as a 'complementary' factor together with TNF, and a third component of unknown origin. The *P. vivax* parasite component present in paroxysm plasma can be substituted for by a blood-stage schizont extract of either *P. vivax* or *P. falciparum*. This was demonstrated by restoring the parasite-killing activity to post-paroxysm plasma (from which it was absent) with the addition of the extracts together with TNF. The active materials in these extracts, however, are different from the natural components in *P. vivax* paroxysm plasma, i.e. while the schizont extracts are immunologically cross-reactive between species, the activity of the natural *P. vivax* toxin(s) in patients' plasma is neutralized only by the homologous antisera. *Plasmodium falciparum* infections have neither distinct paroxysms nor parasite-killing activity in plasma. The pronounced paroxysms of *P. vivax* infections may thus be due in part to a species-specific toxin(s).

Keywords *Plasmodium vivax* malaria paroxysms malaria toxin pathogenesis clinical immunity

INTRODUCTION

Paroxysms in human *Plasmodium vivax* malaria infections are prominent, acute episodes of fever accompanied by chills, rigours and sweating which usually occur at regular intervals of 48 h, coinciding with the rupture of schizont-infected erythrocytes. These episodes are characterized by the transient appearance in patients' plasma of active mediators. These are known to include the cytokine tumour necrosis factor-alpha (TNF-α), which has been implicated in the causation of malarial paroxysms and fever by the following evidence: (i) the sharp rise and fall of fever during a *P. vivax* paroxysm is strongly correlated with sharp changes in TNF levels in patients' plasma [1]; (ii) a clincal trial with an anti-TNF-α MoAb conducted in African children with *P. falciparum* malaria resulted in a decrease in fever [2].

That other ('complementary') factors are involved during a paroxysm in *P. vivax* infections has been shown by demonstrating a transient paroxysm-associated parasite-killing activity in plasma for which TNF is necessary but not sufficient [3]. This 'complementary' factor-dependent activity results in the inactivation of intraerythrocytic sexual stage parasites, rendering them non-infective to mosquitoes [3]. This activity is a more precise marker of the pathogenesis underlying a paroxysm than plasma TNF alone, for the following reasons: (i) unlike TNF, which can remain elevated for several hours after a paroxysm, the parasite-killing activity is uniformly absent within 4 h of the peak of a paroxysm [3]; (ii) the intensity of the parasite-killing activity parallels that of a paroxysm [3]. In further support of the relevance of the parasite-killing phenomenon to the pathogenesis of malaria is the fact that reactive nitrogen intermediates have been shown to be mediators of parasite killing [4] and also implicated elsewhere in malaria pathogenesis [5].

In this report we have studied the 'complementary' factors involved in parasite-killing activity in *P. vivax* paroxysm plasma. We present evidence that these include components of parasite origin, and that they may underlie species-specific aspects of clinical disease and immunity in human malaria.

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PATIENTS AND METHODS

Human subjects

The study invovled material from two groups of patients: (i) malaria non-immune adults who were admitted to the General Hospital Colombo (GHC) [6] during a *P. vivax* infection. All such patients were residents of Colombo and its suburbs where malaria transmission does not occur; they had acquired their infection following travel to a malaria-endemic

region of Sri Lanka. These individuals had few or no previous recorded malaria infections and they generally experienced intense clinical disease; (ii) malaria semi-immune adults who were residents of Kataragama, a malaria-endemic region in southern Sri Lanka [7] who reported to an out-patient clinic with *P. vivax* infections. All had experienced several previous *P. vivax* infections and experienced generally mild clinical disease.

A diagnosis of malaria was made by microscopic examination of thick and thin blood smears stained with Giemsa's stain. Blood was collected only from subjects from whom informed consent was obtained. In every patient a complete history was obtained, and a clinical evaluation was performed by the use of a questionnaire which had been previously validated by us and used before ([8], Karunaweera *et al*., Clinical disease and anti-disease immunity to *Plasmodium vivax* malaria, 1996, submitted for publication).

Paroxysm plasma

Plasma was collected during or after the period of a *P. vivax*-induced paroxysm from non-immune patients admitted to the GHC, as follows. After diagnosis, informed volunteers completed a single paroxysm without drug treatment. Blood was collected for plasma into 0·1% EDTA containing a protease inhibitor (Aprotinin, containing 0·6 trypsin inhibitor units/ml; Sigma Chemical Co., St Louis, MO) either at or within 1 h of the peak of a paroxysm (paroxysm plasma) and/or 4–5 h after a paroxysm (post-paroxysm plasma). Plasma samples were aliquoted and stored at -20°C until further use.

Clinically distinct paroxysms were much rarer in *P. falciparum* patients, and, for ethical reasons, they could not be kept for long under observation while witholding treatment, due to the potentially life-threatening nature of the infection. However, we obtained plasma samples from two *P. falciparum* patients who presented to us during high fever.

Preparation of schizont extracts of P. vivax *and* P. falciparum

Schizont-infected blood was collected from patients attending the GHC having a synchronous infection of *P. vivax*. The schizont-infected erythrocytes were isolated from uninfected erythrocytes using a Percoll gradient as described previously [9]. *Plasmodium falciparum* schizonts were obtained from laboratory-maintained *in vitro* cultures as described elsewhere [4]. Pellets of schizont-infected erythrocytes were suspended in a small volume of PBS and freeze-thawed three times. The entire procedure was carried out under sterile conditions, and the extract was stored at -20°C.

Human sera

Immune convalescent sera were obtained from adults resident in Kataragama, 4–6 weeks after drug cure of a confirmed *P. vivax* infection, at a time when they were free of a blood infection as confirmed by microscopy. Similarly, non-immune sera were obtained from residents of Colombo who were convalescing from a confirmed *P. vivax* infection. Normal human serum was obtained from healthy non-malarial subjects resident in Colombo.

Immune rabbit sera

Rabbits were immunized, each with 12 doses of freeze-thawed *P. vivax* or *P. falciparum* schizont extracts, each dose comprising 1×10^7 parasites, and administered intravenously at 4 day intervals. For control sera, rabbits were immunized with uninfected human erythrocytes using the same immunization protocol. Animals were bled for serum 6 days after the last immunization.

Testing the effect of patient plasma on the infectivity of P. vivax *gametocytes to mosquitoes*

Gametocyte-infected blood was obtained from acute *P. vivax* malaria patients by venepuncture when they were not experiencing a paroxysm. Blood was diluted in 10 v of suspended activation (SA) solution (10 mM Tris, 170 mM NaCl, 10 mM glucose pH 7·2) and washed free of serum. The infected blood was resuspended at a haematocrit of 10% in culture medium (RPMI 1640, 25 mM $NaHCO_3$) containing 20% test or control plasma and pre-incubated at 37°C in an atmosphere of 5% CO_2 and 95% air in 24-well Linbro plates (Flow Labs, Irvine, UK). After 3 h of incubation, gametocyte-infected blood was centrifuged and the pellet was resuspended to a haematocrit of 50% in normal human serum (NHS) and fed to *Anopheles tessellatus* mosquitoes in a water-jacketed membrane feeding apparatus circulating water at 40°C. A week later fed mosquitoes were dissected for mid-gut oocyst stages. The average (arithmetic mean) number of oocysts per midgut of fed mosquitoes was taken as the measure of infectivity of the gametocytes to mosquitoes.

Evaluating the effect of immune human and rabbit sera on reversing the gametocytocidal activity of paroxysm plasma

Paroxysm plasma (0·4 ml) was pre-incubated for 30 min with an equal volume of either immune human or immune rabbit serum prior to adding to gemetocyte-infected blood. Normal human serum, a rabbit serum raised against uninfected human erythrocytes and normal rabbit serum were used, as appropriate, as controls. Gametocyte-infected blood was then treated as described above and fed to mosquitoes after a 3-h incubation period.

Conferring gametocytocidal activity on P. vivax *post-paroxysm plasma with recombinant human TNF and a freeze-thawed schizont extract*

Recombinant human (rh) TNF (0·013 mg/ml; Genzyme, Cambridge, MA) and a freeze-thawed schizont extract of 5×10^6 schizonts/ml of either *P. vivax* or *P. falciparum* were added to post-paroxysm plasma before incubating with gametocyte-infected blood. As controls the rhTNF and the parasite extract were added to normal (non-malarial) plasma.

RESULTS

We have studied plasma taken during acute infections of *P. vivax* and *P. falciparum* in non-immune patients. All of the 57 non-immune *P. vivax* patients studied here gave a clear verbal history consistent with paroxysms; when followed through, 49 of them developed clinically defined paroxysms under our observation. In contrast to the *P. vivax* patients, only about half of the 25 *P. falciparum* patients reported having experienced a paroxysm during their current illness. Of three such patients whom we were able to follow, none developed a paroxysm during the period of our observations. This is consistent with the general view that clinical paroxysms are much less well defined episodes in *P. falciparum* infections than they are in *P. vivax*.

Gametocytes incubated with plasma taken during a *P. vivax* paroxysm consistently lost their infectivity to mosquitoes compared with controls incubated with normal plasma (Table 1). As previously shown, this activity was dependent on

Table 1. Parasite inactivating effects of *Plasmodium vivax* and *P. falciparum* paroxysm plasma*

Paroxysm plasma* (serial no.)		Infectivity of gametocytes to mosquitoes after incubation in paroxysm plasma (expressed as % of that in normal plasma) Experiment number 1	2	3	Mean
P. vivax					
SKW70	(1)	32·5	21·89	—	27·2
SKW73	(2)	0·17	0·24	58·39	19·6
SKW75	(3)	32·41	57·51	—	45·0
SKW76	(4)	57·63	–	—	57·6
SKW84	(5)	15·47	14·62	26·67	18·9
SKW90	(6)	1·38	—	—	1·4
SKW93	(7)	0·45	65·42	—	32·9
SKW99	(8)	13·38	—	—	13·4
SKW100	(9)	51·17	2·61	—	26·9
SKW102	(10)	21·2	—	—	21·2
SKW103	(11)	32·33	44·22	—	38·3
SKW104	(12)	54·54	—	—	54·5
SKW105	(13)	49·9	—	—	49·9
SKW106	(14)	15·92	—	—	15·9
Average (±s.e.m.)					30·2 ± 4·3
P. falciparum					
PF1	(15)	67·9	159·33	95·32	107·5
PF2	(16)	94·04	121·5	—	107·8
Average (±s.e.m.)					107·6 ± 0·1

*The paroxysm plasmas have the individual designations given in column 1; the 'serial numbers' in parentheses are provided for ease of cross reference only in the context of the present study. The experimental data are extracted from Tables 2–6, together with some not reported elsewhere in this study.

Table 2. Effect of immune human serum on the parasite-inactivating activity of *Plasmodium vivax* paroxysm plasma

Reagent(s)*	Infectivity of gametocytes to mosquitoes after incubation in the reagents indicated Experiment number 1	2	3	4	5
Infectivity in normal plasma expressed as mean oocysts/gut (number of mosquitoes infected/number dissected)					
NP	43·57 (17/19)	32·21 (17/19)	64·72 (11/18)	83·4 (13/16)	24·19 (16/22)
Infectivity expressed as % of that in normal plasma (number of mosquitoes infected/number dissected)†					
Pv PP	$32{\cdot}50^{1}$ (19/21)	$21{\cdot}89^{1}$ (13/19)	$0{\cdot}17^{2}$ (2/17)	$0{\cdot}24^{2}$ (2/20)	$57{\cdot}63^{4}$ (14/17)
Pv PP + IHS	$77{\cdot}12^{a}$ (13/20)	$70{\cdot}48^{a}$ (11/12)	$93{\cdot}80^{a}$ (17/19)	$32{\cdot}45^{b}$ (14/16)	155.68^{a} (14/19)
Pv PP + NIHS	$24{\cdot}44^{c}$ (7/17)	$2{\cdot}42^{c}$ (5/18)	$1{\cdot}16^{d}$ (4/12)	$3{\cdot}32^{c}$ (5/13)	$75{\cdot}07^{c}$ (15/19)
Pv PP + NHS	$47{\cdot}56^{e}$ (12/18)	$43{\cdot}06^{e}$ (17/20)	ND	ND	ND
NP + IHS	ND	ND	$82{\cdot}39^{f}$ (15/19)	ND	ND

*Reagents in which gametocytes were incubated. NP, Normal human plasma; Pv PP, non-immune human *P. vivax* paroxysm plasma; IHS, immune malaria convalescent human serum; NIHS, non-immune malaria convalescent human serum; NHS, normal (non-malarial) human serum.

† Numerical superscript assigned to a value (in row 2) refers to the serial number of the paroxysm plasma (PvPP) used in that experiment as indicated in Table 1. Character subscript assigned to a value (in rows 3–6) refers to an individual from whom a serum, either IHS, NIHS or NHS which was used in that experiment, was taken, to indicate the number of different sera of a kind that were used.

ND, Not determined.

the presence of TNF-α acting in conjunction with other unidentified plasma components present in paroxysm plasma [3] (data not shown for the present study). In contrast, plasma taken from two of the *P. falciparum* patients during periods of heightened fever had no significant effect on the infectivity of gametocytes (Table 1). This is consistent with the view previously developed [3] that the gametocyte killing activity is associated with clearly defined paroxysms which, as indicated above, are found in non-immune *P. vivax* patients but rarely in *P. falciparum* patients.

The suppression of gametocyte infectivity mediated by paroxysm plasma from the *P. vivax* patients was consistently reversed by pre-incubating the plasma with *P. vivax* convalescent human sera from partially immune individuals from the endemic area (Kataragama) (Table 2). Neither convalescent serum from non-immune (Colombo) patients nor serum from uninfected controls had any effect on the gametocyte-inactivating activity of the paroxysm plasmas. These findings suggested the possibility, among others, that *P. vivax* convalescent sera from the malaria-endemic patients had antibodies which neutralized parasite-derived components essential for the paroxysm plasma-mediated killing.

We investigated this hypothesis with a rabbit anti-serum raised against a freeze-thawed extract of *P. vivax* schizonts; this anti-serum, as did the convalescent immune human sera, reversed the parasite-killing activity of *P. vivax* paroxysm plasma (Table 3), indicating that parasite components present in the paroxysm plasma are indeed essential for its parasite-killing activity. Rabbit serum raised against a freeze-thawed extract of uninfected human whole blood cells did not reverse the effect, confirming that activity involved in the parasite-killing was specific to the parasites.

To confirm this, we made use of another observation respecting *P. vivax* infection plasma. This is that plasma taken 4 h after a paroxysm (post-paroxysm plasma) has lost the gametocyte-killing activity (Table 4) [3]. The addition to post-paroxysm plasmas of a combination of a freeze-thawed extract of *P. vivax* schizonts and rhTNF consistently restored potent gametocyte-inactivating activity. On their own, either the parasite extract or rhTNF conferred gametocyte inactivating activity on some, but not on all, post-paroxysm plasmas (Table 4). This may have been due to the persistence of either TNF or the parasite products, respectively, in these patients' post-paroxysm plasmas.

Thus, component(s) of parasite origin and TNF-α (as we have previously shown [3]) appear to act in synergy during a paroxysm to produce parasite killing. However, even these two components together are insufficient in themselves to mediate this effect because, when added to normal human plasma, a combination of rhTNF and a freeze-thawed extract of *P. vivax* failed to induced

Table 3. Effect of immune rabbit serum on the parasite-inactivating activity of *Plasmodium vivax* paroxysm plasma

	Infectivity of gametocytes to mosquitoes after incubation in the reagents indicated				
	Experiment number				
Reagent (s)*	1	2	3	4	5
Infectivity in normal plasma expressed as mean oocysts/gut (number of mosquitoes infected/number dissected)					
NP	64·72 (11/18)	4·71 (9/17)	24·19 (16/22)	91·35 (19/20)	104·7 (17/21)
Infectivity expressed as % of that in normal plasma (number of mosquitoes infected/number dissected)†					
Pv PP	$0·17^{2}$ (2/17)	$58·39^{2}$ (9/16)	57.63^{4} (14/17)	$1·38^{6}$ (3/19)	$15·47^{5}$ (7/20)
Pv PP + Pv IRS	86·43 (15/16)	87·26 (14/18)	145·43 (9/11)	79·68 (11/14)	69·67 (10/17)
Pv PP + rbc RS	0·29 (2/21)	43·74 (10/17)	ND	1·09 (5/18)	32·59 (7/16)
Pv PP + NRS	28·49 (13/18)	7·01 (2/18)	33·65 (10/21)	0·51 (3/17)	31·3 (9/16)
NP + Pv IRS	71·59 (15/18)	171·97 (18/20)	99·21 (13/15)	31·82 (8/14)	83·47 (13/17)
NP + rbc RS	71·63 (12/13)	84·00 (10/14)	ND	69·30 (14/15)	43·04 (10/15)

* Reagents in which gametocytes were incubated. NP, Normal (non-malarial) human plasma; Pv PP, non-immune human *P. vivax* paroxysm plasma; Pv IRS, anti-*P. vivax* rabbit serum; rbcRS, anti-uninfected human erythrocyte rabbit serum; NRS, normal rabbit serum.

† Superscript assigned to a value (in row 2) refers to the serial number of the paroxysm plasma (PvPP) used in that experiment as indicated in Table 1.

ND, Not determined.

parasite killing (Table 4). Therefore, a third essential component must be required which is present in the post-paroxysm plasma, but is absent from normal plasma.

Having demonstrated the role of *P. vivax* parasite products in the parasite-killing activity of *P. vivax* infection plasma we next investigated the species-specificity of this effect with a freeze-thawed extract of schizonts of *P. falciparum*. Like those of *P. vivax*, such extracts were indeed capable of conferring gametocyte-inactivating effects on *P. vivax* post-paroxysm plasma (Table 5). Moreover, immune rabbit sera raised against freeze-thawed schizont extracts of either *P. vivax* or *P. falciparum* were equally capable of reversing the inactivating activity conferred on post-paroxysm plasma by either species, in a species cross-reactive manner (Table 5). This indicates that the type of parasite component(s) which are essential for gametocyte inactivation are represented in schizont extracts of both *P. falciparum* and *P. vivax*, and that the active components found in these extracts are immunologically cross-reactive between the two species.

Our findings thus far implied that the parasite component involved in gametocyte-killing activity of *P. vivax* infection plasma could be due to species cross-reactive component(s) present in schizonts of both *P. vivax* and of *P. falciparum*. The experiments represented in Table 5, however, were conducted entirely with artificial parasite extracts. We wished to determine whether the properties of these extracts were the same as those of the naturally occurring parasite products found in *P. vivax* paroxysm plasma.

To do this, the rabbit anti-sera raised against either *P. falciparum* or *P. vivax* were tested for their ability to reverse parasite-inactivating effects of *P. vivax* paroxysm plasma itself. Anti-sera against the *P. vivax* schizont extract completely abolished the gametocyte-inactivating effects in *P. vivax* paroxysm plasma, whereas the anti-*P. falciparum* serum had no effect (Table 6). Thus, contrary to our findings with schizont-extracts of *P. vivax* and *P. falciparum*, the parasite components which are naturally present in paroxysm plasma were not immunologically cross-reactive between the species. These, therefore, must be different from the active components present in the artificial parasite extracts.

DISCUSSION

We have previously demonstrated that patients' plasma during *P. vivax* paroxysms has transient parasite-inactivating effects; this is mediated by TNF acting in conjunction with other 'complementary' factors [3]. Here we demonstrate that one of these 'complementary' factors is a parasite component. We infer this from two independent observations: (i) a rabbit anti-serum raised against *P. vivax* schizonts reversed the parasite-inactivating properties of paroxysm plasma; and (ii) the addition of a *P. vivax* parasite extract and rhTNF restored this effect to post-paroxysm plasma in which this activity was otherwise lost.

We have thus shown that a parasite component present in paroxysm plasma is essential, together with TNF, for parasite killing. The induction of TNF itself is known to be dependent on a parasite component, and one might infer that the dependence of the killing activity on the parasite component is for its ability to induce TNF. This, however, cannot be the case, for the following reasons: (i) because TNF is already present in high concentrations in paroxysm plasma [1,3], it cannot be that the dependence on the parasite component for the killing activity is for the induction of TNF; (ii) only when the parasite extract and rhTNF were added together to post-paroxysm plasma was the full parasite-killing activity restored. This is not to say that the parasite component implicated here does not induce TNF, but that its role in parasite killing, as identified here, is to act in synergy with TNF to produce the final mediators of parasite killing (Fig. 1). The production of these mediators, which include reactive nitrogen intermediates, as shown in our previous work [4], is dependent on the presence of nucleated blood cells.

We have argued that TNF and the parasite factor(s) are independent, co-essential agents of the parasite-killing activity in paroxysm plasma. Our results, however, demonstrate that there is a second category of 'complementary' factor(s) without which killing cannot be mediated (Fig. 1). We do not know the nature of this factor(s) which is present during and post-paroxysm, but it is absent from non-malarious plasma. It appears to be induced or released during the paroxysm and to persist beyond the time for which TNF and the parasite component(s) are active in plasma.

The parasite-killing activity was also mediated by extracts of *P. falciparum* schizonts. Immune sera against extracts of schizonts of either *P. falciparum* or *P. vivax* neutralized the activity in both species. This indicated that a species-conserved component(s)

Table 4. Restoration of gametocyte-inactivating activity to *Plasmodium vivax* post-paroxysm plasma with freeze-thawed *P. vivax* shizont extract and recombinant human tumour necrosis factor (rhTNF)

	Infectivity of gametocytes to mosquitoes after incubating in the reagents indicated							
	Experiment number							
Reagent(s)*	1	2	3	4	5	6	7	8
Infectivity in normal plasma expressed as mean oocysts/gut (*number of mosquitoes infected/number dissected*)								
NP	7·62 (7/13)	32·15 (12/20)	10·12 (14/17)	22·00 (13/14)	126·6 (12/15)	42·14 (4/14)	67·78 (8/9)	26·06 (13/18)
Infectivity expressed as % of that in normal plasma (*number of mosquitoes infected/number dissected*)†								
Pv PP	32·41[3] (9/19)	14·62[5] (7/20)	57·51[3] (10/17)	0·45[7] (1/21)	26·67[5] (9/17)	65·42[7] (3/7)	13·38[8] (3/14)	2·61[9] (4/18)
Pv POP	157·48 (9/17)	58·07 (11/20)	97·04 (9/17)	33·23 (6/16)	54·22 (11/14)	53·84 (10/16)	89·47 (11/14)	13·12 (4/20)
Pv POP + *P. vivax*	5·51 (3/19)	22·71 (12/23)	11·26 (3/15)	11·23 (6/19)	ND	0·00 (0/11)	54·18 (10/18)	0·00 (0/10)
Pv POP + rhTNF	95·93 (6/13)	33·87 (11/19)	28·16 (3/15)	2·55 (1/18)	ND	0·00 (0/15)	9·09 (9/17)	27·13 (6/14)
Pv POP + *P. vivax* + rhTNF	0·00 (0/20)	3·42 (5/20)	3·95 (4/20)	8·82 (5/17)	1·35 (7/21)	1·59 (2/12)	16·70 (9/18)	0·61 (1/19)
NP + *P. vivax* + rhTNF	139·76 (10/17)	60·72 (16/23)	116·70 (14/21)	51·64 (9/14)	71·15 (12/15)	40·93 (6/12)	124·49 (12/19)	42·71 (6/15)

* Reagents in which gametocytes were incubated. NP, Normal (non-malarial) human plasma; Pv PP, *P. vivax* paroxysm plasma; Pv POP, *P. vivax* post-paroxysm plasma; *P. vivax*, a freeze thawed extract of *P. vivax* schizont-infected erythrocytes.

† Superscript assigned to a value (in row 2) indicates the serial number of the paroxysm plasma used in the experiment, as indicated in Table 1. PoPs have been obtained from the same patient as the PvPP used in a given experiment.

ND, Not determined.

present in blood stage schizonts of the parasites was responsible for the parasite-killing activity in the extracts. In *P. vivax* paroxysm plasma itself, on the other hand, the activity was neutralized only by immune sera against schizonts of its own species. The natural parasite component in paroxysm plasma must, therefore, be distinct from that in the parasite extracts. This is because, in contrast to the materials in the extracts, the natural component is immunologically species-specific.

Other studies devoted to the identification of malarial toxin(s) have used TNF induction as a marker of toxin activity ([10], for review see [11]); the source of material for these studies has been extracts of blood stage parasites prepared *in vitro*. The results presented here show that the TNF-inducing ability may be only one of several roles played by parasite components in events leading to parasite inactivation and pathogenesis. Moreover, in our parasite killing assay we have shown that the properties of parasite extracts do not correspond to those of the natural toxin(s), i.e. the active parasite products in the schizont extracts were immunologically species cross-reactive, whereas those in paroxysm plasma were species-specific.

Glycosylphosphatidylinositol (GPI), isolated from schizonts of *P. falciparum*, has been implicated as a malarial toxin on the basis of its ability to induce TNF [12]. For the reasons given above there is no obvious connection between the parasite component whose activity is shown here (a synergist with TNF) and GPI (a TNF inducer). Inducers of TNF, be they GPI or related molecules ([10], for review see [11]), have been shown to be immunologically species cross-reactive, as indeed is the TNF synergizing activity of our extracts. However, the relevance of these species cross-reactive components to the natural toxin(s) of malarial infections is unclear.

Whereas clearly defined paroxysms and parasite-killing activity in plasma were found consistently in *P. vivax* infections, neither were detectable in this study in *P. falciparum* infections. This may reflect a basic difference between the pathogenesis of *P. vivax* and *P. falciparum*. The findings of this study raise the possibility that *P. vivax* infections may carry toxin(s) which uniquely produce the clinical features of this infection. It is also likely that differences in biological features between the two species of parasite, such as sequestration of *P. falciparum* erythrocytic schizonts in post-capillary venules, underlie some of these clinical differences.

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Table 5. Effect of anti-parasite rabbit serum on the parasite-inactivating activity conferred to *Plasmoidium vivax* post-paroxysm plasma

	Infectivity of gametocytes to mosquitoes after incubation in the reagents indicated				
	Experimental number				
Reagents*	1	2	3	4	5
Infectivity in normal plasma expressed as mean oocysts/gut (*number of mosquitoes infected/number dissected*)					
NP	50·82 (10/17)	6·00 (2/8)	73·15 (8/13)	62·84 (11/19)	35·39 (13/18)
Infectivity expressed as % of that in normal plasma (*number of mosquitoes infected/number dissected*)†					
Pv POP	17·71[v] (6/13)	33·33[v] (9/16)	95·69[x] (9/13)	168·56[y] (10/14)	166·71[z] (8/11)
Pv POP + *P. vivax* + rhTNF	1·97 (4/15)	1·00 (1/6)	5·47 (3/17)	26·46 (6/19)	50·00 (7/17)
Pv POP + *P. vivax* + rhTNF + anti-Pv IRS	14·40 (6/19)	37·66 (3/8)	24·14 (8/14)	140·83 (8/13)	144·36 (7/11)
Pv POP + *P. vivax* + rhTNF + anti-Pf IRS	ND	22·17 (5/15)	ND	153·56 (11/16)	129·28 (7/12)
Pv POP + *P. falciparum* + rhTNF	3·25 (6/20)	0·00 (0/12)	5·04 (6/16)	36·16 (9/18)	55·30 (6/14)
Pv POP + *P. falciparum* + rhTNF + anti-PvIRS	ND	27·00 (4/13)	17·61 (7/16)	67·15 (9/15)	232·69 (10/18)
Pv POP + *P. falciparum* + rhTNF + anti-PfIRS	ND	6·67 (1/15)	ND	168·68 (10/16)	115·68 (7/12)

* Reagents in which gametocytes were incubated. NP, Normal (non-malarial) human plasma; Pv POP, *P. vivax* non-immune post-paroxysm plasma; *P. vivax*, freeze-thawed extract of *P. vivax* schizont-infected erythrocytes; anti-Pv IRS, anti-*P. vivax* rabbit serum; anti-PfIRS, anti-*P. falciparum* rabbit serum; *P. falciparum*, freeze-thawed extract of *P. falciparum* schizont-infected erythrocytes.

† Superscript assigned to value refers to an individual from whom PvPOP used in that experiment was obtained.

ND, Not determined.

Table 6. Species-specific effect of anti-parasite rabbit serum on parasite-inactivating effect of *Plasmodium vivax* paroxysm plasma

	Infectivity of gametocytes to mosquitoes after incubation in the reagent indicated						
	Experiment number						
Reagent(s)*	1	2	3	4	5	6	7
Infectivity in normal plasma expressed as mean oocysts/gut (*number of mosquitoes infected/number dissected*)							
NP	82·25 (7/8)	6·0 (2/8)	73·15 (8/13)	24·0 (11/14)	41·89 (4/7)	62·84 (11/19)	35·39 (13/18)
Infectivity expressed as % of that in normal plasma (*number of mosquitoes infected/number dissected*)†							
Pv PP	21·20[10] (8/18)	32·33[11] (6/16)	44·22[11] (8/20)	51·17[9] (8/17)	15·92[14] (8/18)	54·54[12] (6/10)	49·90[13] (5/18)
Pv PP + anti-Pv IRs	ND	93·33 (5/10)	61·91 (10/14)	99·99 (4/10)	54·71 (8/13)	81·95 (10/16)	147.23 (17/21)
Pv PP + anti-Pf IRS	23·40 (8/16)	18·83 (3/15)	24·11 (6/11)	27·08 (2/10)	9·55 (6/20)	81·44 (5/11)	13·37 (2/15)

* Reagents in which gametocytes were incubated. NP, Normal (non-malarial) plasma; Pv PP, *P. vivax* non-immune human paroxysm plasma; anti-PvIRS, anti-*P. vivax* rabbit serum; anti-Pf IRS, anti-*P. falciparum* rabbit serum; PfPP, *P. falciparum* non-immune paroxysm plasma; ND, not determined.

† Superscript assigned to a value refers to the serial number of the paroxysm plasma (PvPP) used in that experiment as indicated in Table 1.

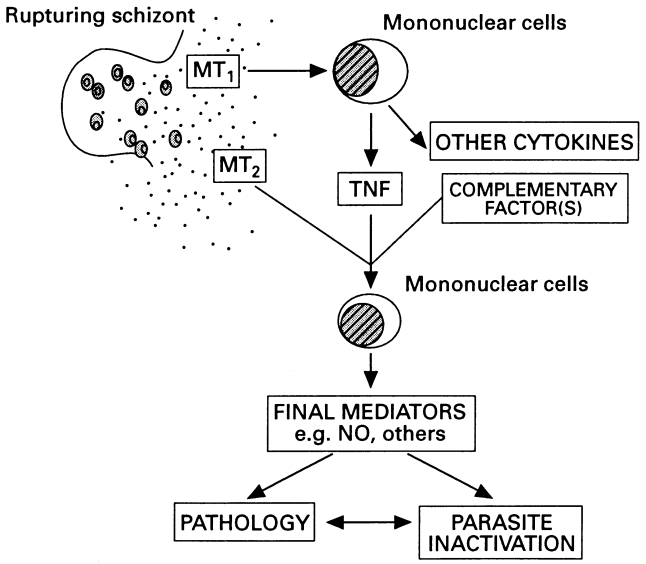

Fig. 1. A hypothetical scheme of events underlying a malarial paroxysm. Schematic view of events which could underlie a *Plasmodium vivax* paroxysm. MT, Malarial toxin. It is not known whether MT_1 which is implicated in the induction of tumour necrosis factor (TNF), and MT_2, which has been identified in this study as an essential co-factor which acts with TNF for parasite inactivation, are the same, or different, parasite products

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