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Host–parasite interaction and morbidity in malaria endemic areas

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SUMMARY

Severe morbidity due to *Plasmodium falciparum* is a major health problem in African children. The patterns of morbidity in endemic areas are modified by the immune response, and vary markedly with transmission intensity. Severe disease falls into three overlapping syndromes: coma, respiratory distress, and severe anaemia. Recently, it has become clear that metabolic acidosis plays a major role in the pathogenesis of severe disease and is particularly important in the overlap between the different clinical syndromes. We propose that the different manifestations of severe malarial morbidity arise from the interaction of a limited number of pathogenic processes: red cell destruction, toxin-mediated activation of cytokine cascades, and infected cell sequestration in tissue microvascular beds. The pattern of severe morbidity varies with age within any one endemic area, with severe anaemia predominating in the youngest children and coma having its highest incidence in older children. Between endemic areas there is a marked variation in mean age of children with severe malaria, and therefore in the importance of different clinical syndromes. The shift in mean age is due to a combination of increased challenge and more rapid development of immunity at higher levels of transmission. Recent comparative studies indicate that at higher levels of transmission the net effect of these shifts may be a paradoxical reduction in total severe malarial morbidity.

1. INTRODUCTION

In naive individuals infection with malaria parasites inevitably leads to clinical illness. By contrast, in malaria-endemic areas the majority of individuals are parasitized at any given time, but only a minority of episodes is associated with disease, and an even smaller proportion with severe life-threatening disease (Marsh 1992). Although many factors combine to act on the risk of developing disease (Greenwood *et al*. 1991), acquired immunity has a dominant effect and its most obvious manifestation is the virtual absence of life-threatening disease in older children and adults. Immunity to malaria not only modifies the age-specific prevalence of disease but also has major effects on the clinical spectrum of resulting pathology. In this paper we first consider the most important manifestations of morbidity in African children, concentrating on recent changes in our understanding of the clinical spectrum in endemic areas in relation to current concepts of how host–parasite interactions lead to disease. In the second part of this paper we consider the impact of variations in endemicity on the pattern of malarial morbidity in more detail.

2. SEVERE MALARIA IN ENDEMIC AREAS

Compared with other major causes of mortality there has, until recently, been a surprising paucity of data on the clinical features of severe malaria in African children. Most studies have concentrated on cerebral malaria as the most dramatic form of severe disease (Molyneux *et al*. 1989; Brewster *et al*. 1990). Cerebral malaria is defined in clinical terms as the presence of coma due to malaria (Warrell *et al*. 1990), but the term has also been considered synonymous with a pathognomonic histopathological appearance: the packing of the cerebral microvasculature with infected red cells (Macpherson *et al*. 1985). Consequently, many attempts to explain the pathogenesis of severe malarial disease have been dominated by two linked paradigms, i.e. severe malaria equals cerebral malaria equals cerebral microvascular sequestration. Recent interest in a second area of host–parasite interaction, the induction of cytokine responses, does not fit so easily into this framework in so far as it concerns systemic responses. It is, however, possible to align these two areas, for instance in the effect of cytokines on sequestration and the consequences of sequestration for local cytokine release (see below).

In the following sections, we summarize briefly evidence relating to the two main sets of hypotheses (cytoadherence and cytokine activation) which have been advanced as explanations of how parasitization may lead to morbidity. We then summarize recent changes in our understanding of the clinical spectrum of severe morbidity in African children and consider

how these can best be reconciled with hypotheses on pathogenesis.

(*a*) *Cytoadherence and severe malaria*

During its blood-stage cycle, *P. falciparum* undergoes successive cycles of asexual division, each cycle from uninucleate ring to multi-nucleate schizont taking *ca.* 48 h. However, only the earliest stages are seen on peripheral blood smears from patients with malaria, red cells containing parasites more than *ca.* 14 h into the cycle withdraw or 'sequester' in deep microvascular beds. This process is mediated by binding between receptors on the luminal surface of vascular endothelial cells and ligands on the infected red cell surface. Sequestration is seen at its most dramatic in the classical post-mortem picture of cerebral microvessels packed with parasitized red cells (Macpherson *et al*. 1985; Oo *et al*. 1987), but infected cell cytoadherence occurs in many tissues and is a normal part of the biology of the infection. Several putative endothelial receptors have been identified, including thrombospondin (Roberts *et al*. 1985), CD36 (Barnwell *et al*. 1989), ICAM 1 (Berendt *et al*. 1989), VCAM and E-selectin (Ockenhouse *et al*. 1992). On the infected red cell side of the interaction there is evidence that both modified host band three (Sherman *et al*. 1992) and a conserved parasite molecule, sequestrin (Ockenhouse *et al*. 1991), may play a role in binding to CD36. However, increasingly strong evidence suggests that most cytoadherence interactions are mediated by a family of hypervariable parasite proteins, Pfemp1, inserted into the host cell membrane (Magowan *et al*. 1988; Baruch *et al*. 1995; Gardener *et al*. 1996).

There is considerable variation in parasite phenotype with regard to receptor usage; most parasites seem able to adhere to thrombospondin or CD36 (although often to widely differing degrees), whilst the ability to adhere to others is often less common (Newbold *et al*. 1997). Heterogeneity in the binding phenotype of parasites raises the possibility that differences in either degree or site of cytoadherence *in vivo* may give rise to different pathological consequences. The fact that expression of some receptors is up-regulated by cytokines released during malaria infection (Udeinya & Akogyeram 1993), and that there is both variation in the ability of parasites to stimulate cytokine responses (Allan *et al*. 1993) and in the control of the human cytokine response, adds several layers of complexity. Thus, it has been suggested that cerebral malaria reflects the chance occurrence of the wrong parasites in the wrong host (Berendt *et al*. 1989). In practice, it has been difficult to relate particular cytoadherence phenotypes with specific clinical syndromes (Marsh *et al*. 1988; Ho *et al*. 1991; Ringwald *et al*. 1993). This is perhaps not surprising given the complexity of the situation, and may have been exacerbated by false assumptions of homogeneity of clinical syndromes themselves (see below). However, several pieces of evidence do hang together, hence the observation that despite marked variation parasites from patients with cerebral malaria showed greater ability to bind to ICAM 1 (Newbold *et al*. 1997), fits well with the finding of a strong correlation between ICAM 1 expression and site of attachment in capillaries of patients dying of cerebral malaria (Turner *et al*. 1994). One cytoadherence phenotype that does correlate with severity of disease is the phenomenon of rosetting or binding to uninfected red cells (Carlson *et al*. 1990; Rowe *et al*. 1995). However, it is not clear whether uninfected red cell binding is pathogenic *per se* or whether it serves as a marker for an, as yet, undescribed cytoadherence property. A dramatic model of how specific cytoadherence properties could lead to organ-specific pathology is provided by the recent identification of chondroitin sulphate as a receptor on syncytiotrophoblasts (Fried & Duffy 1996). Thus, it seems that placental malaria, an important cause of morbidity in otherwise immune women in endemic areas, may be due to a subgroup of parasites with a relatively rare cytoadherence phenotype being able to utilize a niche where none of the other known cytoadherence receptors are expressed.

There continues to be debate over the sequence of events following the obstruction of capillaries by infected red cells. At the simplest level such obstruction might be expected to lead to undersupply of oxygen and other essential molecules to surrounding tissues. Alternatively, the cytoadherence of infected cells could act as a stimulus to both endothelial cells and other surrounding cells for the local release of cytokines, or for the production of nitric oxide (Clark & Rockett 1996). Clearly, these mechanisms are not mutually exclusive; and although the exact role of infected cell sequestration in any given clinical situation may prove difficult to delineate, it seems reasonable to continue to assume that the ability of the parasite to concentrate in particular microvascular beds must play a central role in the pathogenesis of malarial disease.

(*b*) *Cytokines and severe malaria*

Malaria infection leads to a potent acute phase response, which is best regarded as an early, non-specific attempt to protect the host against invading parasites. In addition to the activation and mobilization of the cellular elements required for both non-specific and specific immunological responses, some cytokines are directly inhibitory to malaria parasites (Clark *et al*. 1990). Moreover, fever, a direct result of the acute phase response, is itself inhibitory to mature parasites (Kwiatkowski 1995). However, there is a balance between protective and pathogenic effects for many components of the immune response; and over the last ten years, a large body of experimental and clinical evidence has built up which implicates excessive cytokine production and its downstream consequences as being important factors in the pathogenesis of severe malaria.

Much interest has centred around the role of TNF-alpha. In experimental models, this cytokine has a clear causal role in the pathogenesis of both murine cerebral malaria (Grau *et al*. 1988, 1989), and other features of severe disease (Clark *et al*. 1992). There are important differences between the pathology of cerebral malaria in humans and the syndrome in mice, and untangling cause from effect, when confronted with a complex web of linked responses which typically have short half lives, is difficult. However, there is a

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clear relationship between serum levels of TNF-alpha and outcome in human malaria (Grau *et al*. 1989; Kern *et al*. 1989; Kwiatkowski *et al*. 1990), and the hypothesis that this is a causal relationship rather than an epiphenomenon is strengthened by the finding that human genetic polymorphisms in the promoter region of the gene for TNF-alpha (which, therefore, presumably affect the level of the response) are associated with increased susceptibility to severe morbidity (McGuire *et al*. 1996).

Several parasite components, including phosphatidylinositol moieties (Schofield *et al*. 1993; Bate & Kwiatkowski 1994) and haemozoin (Sherry *et al*. 1995), have been implicated as malaria 'toxins', and there is considerable variation between parasite isolates in their ability to induce TNF production by macrophages (Allan *et al*. 1993, 1995). This, together with genetic polymorphisms in the human population that are likely to affect cytokine responses (McGuire *et al*. 1996) provides an obvious basis for the idea of a spectrum running from protection to pathology arising from host–parasite interactions.

Several mechanisms have been proposed by which excess cytokine levels could lead to the clinical features of severe malaria (Clark *et al*. 1989). These include both direct and indirect, and systemic and local effects. The idea that the most important effects may be local is strengthened by the fact that infection with another malaria species, *Plasmodium vivax*, leads to very high levels of TNF but that this does not usually result in the sort of downstream pathology typical of *P. falciparum*. A scenario that emphasizes local effects and links the activation of cytokine responses with infected cell sequestration is that cytokines induce up-regulation of some endothelial receptors for infected cell cytoadherence (Pober *et al*. 1988), and that parasite sequestration may then lead both to a further increase in endothelial activation (Udeinya & Akogyeram 1993; Schofield *et al*. 1996), and local excess cytokine production (Clark & Rockett 1996). Recently, much interest has been focused on the potential role of nitric oxide as a final common mediator for effects of cytokine activation in malaria (Clark & Rockett 1996). Indirect measurement of levels of nitric oxide production in severe malaria has produced apparently conflicting results, with some studies apparently showing an association of high levels with poor outcome (Nüssler *et al*. 1994; Al Yaman *et al*. 1996) and others reporting the opposite effect (Anstey *et al*. 1996). However, Clark *et al*. (1996) have argued that these results are consistent with the idea of a spectrum running from protection to pathology. The final proof of the utility of current concepts of the role of cytokines in the pathogenesis of severe malaria will probably come, as for any other hypothesis about pathogenesis, with the demonstration that outcome may be altered by interventions based on these concepts.

(*c*) *The clinical spectrum of severe malaria*

We attempted to define the clinical spectrum of severe malaria in African children by performing a detailed descriptive study in 1844 consecutive admissions to a district hospital with a primary diagnosis of malaria, without prior assumptions as to diagnostic categories (Marsh *et al*. 1995). The results are summarized in figure 1. Despite its undoubted pathophysiological complexity, severe malaria fell into three reasonably distinct, though overlapping, syndromes. Malaria with disturbed consciousness is essentially synonymous with cerebral malaria, as described by others (Molyneux *et al*. 1989; Brewster *et al*. 1990). However, it is notable that the mortality of this syndrome was considerably lower than usually reported, other than when it overlapped with a second syndrome, malaria with respiratory distress. The latter emerged as a distinct entity which can be defined reproducibly by a range of observers (English *et al*. 1995), and which is associated with a high mortality. As expected, children with severe malarial anaemia formed a numerically important group, but again with a much lower mortality than has been reported previously (Lackritz *et al*. 1992). In the case of both malaria with disturbed consciousness and severe malarial anaemia, the associated mortality was very much higher when associated with respiratory distress. For coma, this seems to be a synergistic interaction, whereas mortality in anaemic children with respiratory distress was the same as in those children with respiratory distress alone. There were marked differences in the mean age at which different clinical syndromes presented, with severe anaemia predominating in young children and malaria with neurological impairment in older children (figure 2).

The central role of the syndrome of respiratory distress was all the more surprising as it had not featured in standard descriptions of malarial morbidity in African children. Respiratory distress is a descriptive term, and could theoretically reflect a number of pathogenic mechanisms, including direct sequestration of malarial parasites in the lung, fluid overload, or congestive cardiac failure. The last mechanism might be considered the most likely and certainly this has been assumed to be the case in children with concurrent severe anaemia. But, subsequent studies showed clearly that in the large majority of cases of severe malaria, respiratory distress is a manifestation of severe underlying metabolic acidosis (English *et al*. 1996*a*,*d*).

(*d*) *Acidosis in severe malaria*

Severe acidosis has emerged as a feature of severe malaria in a number of different sites in Africa (Taylor *et al*. 1993; Krishna *et al*. 1994; Marsh *et al*. 1995). In subsequent studies at Kilifi we have confirmed that it is a major determinant of mortality (English *et al*. 1996*d*). Several mechanisms may play a role in the pathogenesis of the metabolic acidosis of malaria, including impaired renal clearance of fixed acids (English *et al*. 1996*c*) and in some cases ingestion of exogenous acids in the form of salicylates (English *et al*. 1996*b*). However, accumulation of lactate is probably the major single factor (Krishna *et al*. 1994; English *et al*. 1996*d*), and several features of severe malaria may contribute to this. These are likely to include reduced hepatic blood flow leading to impaired

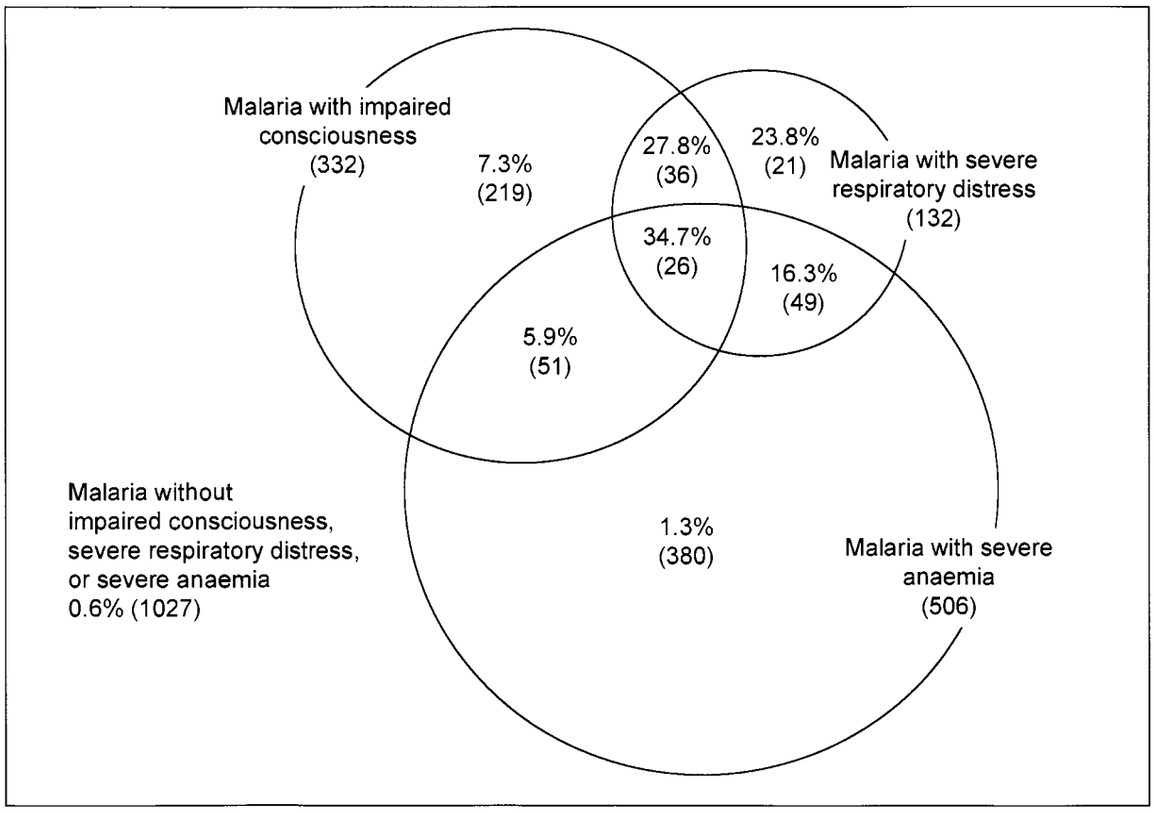

Figure 1. The clinical spectrum of severe malaria in African children. Figures in parentheses are numbers of children, and case fatality for groups are given as percentages. (Adapted from Marsh *et al.* (1995).)

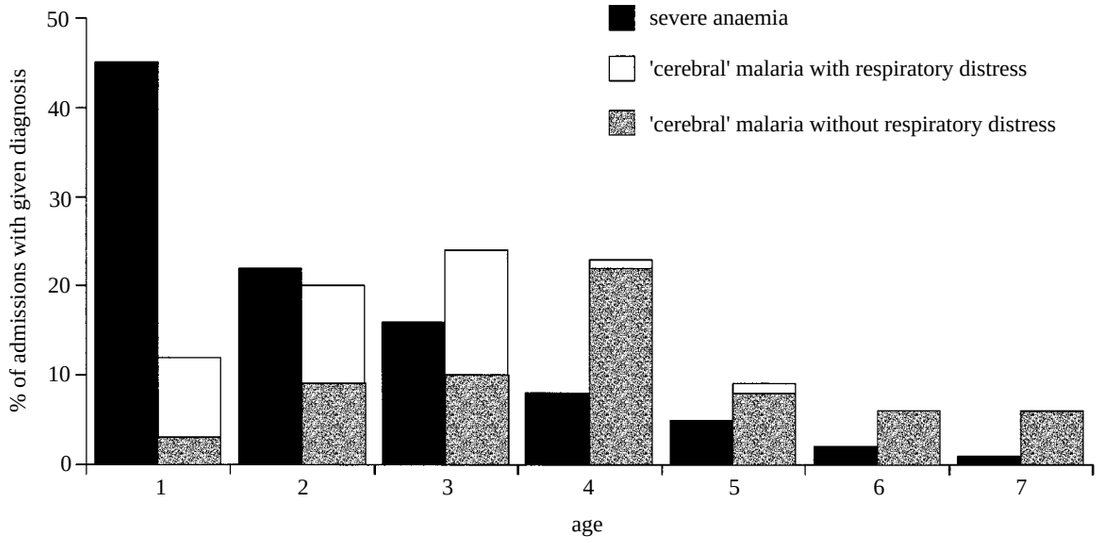

Figure 2. The age distribution of clinical syndromes in children presenting to hospital in Kilifi, Kenya.

clearance (Molyneux *et al.* 1989*b*), production of lactate by metabolically active parasites (Jensen *et al.* 1983), and direct effects of cytokines (Clark & Rockett 1996). But in most pathological settings excess lactate production is associated with anaerobic tissue metabolism, and three features of severe malaria are particularly likely to lead to decreased oxygen delivery to tissues: red cell destruction, hypovolaemia, and microvascular obstruction. Thus, we hypothesized that decreased delivery of oxygen to tissues is a major factor in the pathogenesis of severe malaria (Marsh *et al.* 1996). In recent studies in children with severe malaria who were receiving blood transfusions, we found a strong positive correlation between increases in oxygen uptake measured by

calorimetry and blood lactate concentration at the beginning of transfusion, indicating that in at least some children with severe malaria, an oxygen debt is likely to be a major cause of the observed lactic acidosis.

(*e*) *Cerebral malaria*

As indicated above, cerebral malaria has usually been considered to be a homogeneous clinical syndrome with a site-specific and pathognomonic histopathological correlate. The identification of a subgroup with severe acidosis and a higher mortality does not of itself necessarily argue against this view, but the subsequent demonstration that in many cases vigorous correction of the acid–base status leads to rapid resolution of coma (often within a few hours) (English *et al*. 1996*a*) argues that, in these cases, coma may be the brain's response to a metabolic insult rather than stemming from a brain-specific primary lesion. The idea of a distinct pathogenic entity is strengthened by the observation that the mean age of this group of children is much lower than for those without acidosis (figure 2). Subsequent studies at Kilfi indicate that even in those children with coma in whom acidosis is not a dominant feature, several different pathogenic mechanisms may operate (Crawley *et al*. 1996). Two groups are particularly important. (i) In some children, coma seems to be an abnormally prolonged post-ictal state following epileptic seizures; recovery takes place within a few hours, and these children have a good prognosis. (ii) In other children, coma is actually due to (rather than sharing a common cause with) electrical seizure activity, which persists in the absence of gross clinical signs; appropriate treatment with anti-epileptic drugs leads to rapid restoration of consciousness (Crawley *et al*. 1996). The importance of these two groups, as with those children who have a metabolic encephalopathy secondary to acidosis, is that the rapid recovery following appropriate management is hard to reconcile with the idea that they have extensive cerebral microvascular obstruction of the sort seen in classical post-mortem descriptions.

Thus, the clinical syndrome of cerebral malaria should probably be viewed as a collection of syndromes with differing aetiologies. Although there is inevitably overlap, for instance, not all acidotic comatose children recover consciousness rapidly, these clinical–pathophysiological entities are seen sufficiently often in a 'pure' form to make the distinction worthwhile. Although there are obvious management implications of such distinctions, the real importance for understanding how host–parasite interactions lead to morbidity is that it may be inappropriate to try to force disparate pathological entities into the straight-jacket of a single explanatory hypothesis. For instance, the idea that cerebral malaria is due to the chance occurrence of parasites with a particular cytoadherence phenotype in individuals with an increased propensity to express the corresponding ligand on cerebral endothelium (Berendt *et al*. 1989), will not be appropriately tested using parasites from children whose cerebral symptoms are unlikely to be the consequence of a primary brain event.

(*f*) *Severe malarial anaemia*

All clinical attacks of malaria are associated with red cell destruction, and thus a degree of anaemia. This is arbitrarily considered to be severe if the haemoglobin concentration is less than $5\,g\,l^{-1}$ (Warrell *et al*. 1990). As can be seen from figure 1, severe anaemia forms a large part of the morbidity due to *P. falciparum* infections in Africa. In addition to the loss of red cells due to parasite invasion there is also considerable destruction of uninfected cells (Looareesuwan *et al*. 1987). A range of potential mechanisms, including immune sensitization and damage by oxygen radicals (Phillips & Pasvol 1992), have been advanced to explain this. At the same time, it has been reported that malaria infection leads to dyserythropoeisis and hypothesized mechanisms include suppression by chronic release of cytokines such as TNF (Clark & Chaudhri 1988). These findings have led to the idea that malarial anaemia may be a chronic process as well as an acute event (Abdalla *et al*. 1980); and in a sense this is undoubtedly true in that it has often been demonstrated that the mean haemoglobin of the childhood population rises following successful control of malaria (Molineaux 1985). However, the question of whether life-threatening presentations with severe malarial anaemia are essentially acute episodes related primarily to uncontrolled exponential parasite growth, or whether they are children with chronically progressive anaemia who finally cross some sort of threshold, remains contentious.

Interest in the pathogenesis of severe malarial anaemia has centred on the causes of the drop in haemoglobin rather than on the downstream events that actually lead to morbidity or mortality. It is generally assumed that these latter events are due to congestive cardiac failure, but if this were true, it should be most evident in those children in figure 1 who form the overlap group between anaemia and respiratory distress, where practically all the deaths associated with severe malarial anaemia are concentrated. However, this is not the case: in these children respiratory distress is due, as in the less anaemic children, to severe metabolic acidosis (English *et al*. 1996*a*), once again emphasizing its importance as an underlying feature of severe malarial morbidity.

(*g*) *Summary of severe morbidity*

Recent clinical studies at a number of centres have led to important shifts in our perception of the clinical picture of severe malarial morbidity in African children. We have proposed that these changes are best viewed in the light of current hypotheses concerning host–parasite interactions, to produce a synthesis in which a few key processes lead to severe malarial disease, and in which the clinical manifestations differ depending on the balance between these processes (Marsh *et al*. 1996). Thus, we hypothesize that exponential parasite growth, leading to red cell destruction, microvascular obstruction, and toxin release, combine to lead to two main outcomes: activation of cytokine cascades (with a variety of downstream effects) and

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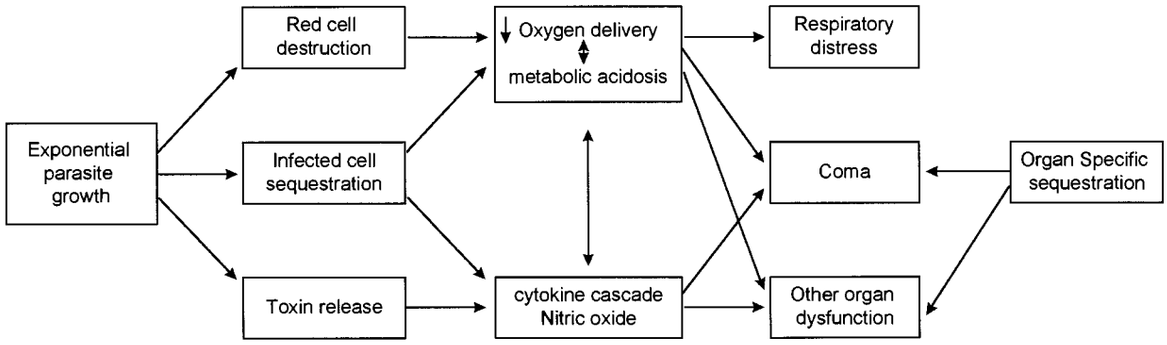

Figure 3. A conceptual model for the development of severe malaria in African children. (Adapted from Marsh *et al.* (1996).)

tissue hypoxia leading to metabolic acidosis. These two processes lead to most of the clinically severe manifestations of severe malaria, with the effect of organ-specific sequestration of parasitized red cells bringing into focus these events (figure 3).

3. THE EPIDEMIOLOGY OF SEVERE MALARIAL MORBIDITY

Figure 4 illustrates two manifestations of malarial infection in a malarial endemic area: parasitization and severe disease. In each case there is clear evidence of age-specific acquisition of immunity, though it is striking that they are temporally discordant. So the prevalence of parasitization *per se* is still rising at the point where deaths due to malaria have essentially ceased, emphasizing the need to be very specific in defining the terms by which one describes anti-malarial immunity. A comparison of parasite prevalence curves under varying levels of transmission pressure indicates that, while the basic shape of the curve remains the same, it may be shifted to the left or the right at higher or lower levels of transmission, respectively (Molineaux & Gramiccia 1980). It is, therefore, of obvious interest to ask whether the same is true with respect to patterns of malarial morbidity.

In striking contrast to the mass of available data on patterns of parasitization in endemic areas, there is a dearth of corresponding data on disease, reflecting the almost complete absence of interest in malarial morbidity in approaches to the epidemiology of malaria for much of the last 50 years. Fortunately this deficit is now being addressed in an exciting atmosphere of collaboration between theoretical, field, and basic science researchers (Gupta *et al.* 1994; Molineaux 1996). If the age pattern of severe malarial morbidity is shifted with transmission intensity, the observation of differences in the mean age of different clinical syndromes in a single area (figure 2) would suggest that the relative importance of these syndromes might also vary markedly between areas. Once again, there is little comparative data, though anecdotally it has been a commonplace belief amongst malariologists that there are marked differences in the pattern of morbidity in different areas.

To approach the question of whether the burden of malarial morbidity varies with transmission intensity, we abstracted all published data on malaria-specific mortality in African communities where estimates were provided for the annual entomological inoculation rate (Snow & Marsh 1995). It is important to note that the techniques used to describe malaria-specific mortality, using interviews with bereaved relatives, have been shown to lack both sensitivity and specificity (Snow *et al.* 1992; Todd *et al.* 1994), and that entomological measures of transmission intensity have inherent problems of sensitivity, particularly in areas of low vector abundance (Dye & Hasibeder 1986; Mbogo *et al.* 1995). These qualifiers and the paucity of data illustrate how difficult it is to tackle this question. Nonetheless, the data suggested that the relationship between transmission and mortality is not linear. Initially there is a steep rise in mortality with relatively small increases of transmission, thereafter, depending on the weight given to the disparate data, it could be argued that mortality subsequently rises less steeply, plateaus, or even falls under higher levels of transmission. This latter possibility could have particularly important consequences for control methods that partially reduce transmission.

To examine further the relationship between the intensity of transmission and ensuing morbidity from severe malaria, we have recently completed (Snow *et al.* 1997) a series of epidemiological studies across a wide range of endemicities in Africa (from hypoendemic malaria in The Gambia to holoendemic malaria in Kenya). Clinical surveillance from five demographically-defined populations over 3–5 years was maintained to determine the age-specific rates (up to the tenth birthday) of malaria morbidity warranting intensive clinical management. Data for other major causes of paediatric morbidity indicated comparable community utilization of the referral centres and epidemiological features of these disease systems. In contrast, significant declines in the mean ages of malaria morbidity were observed with increasing transmission intensity, ranging from 49 months of age in the community with the lowest intensity of transmission to 17 months of age in the community with the highest intensity of transmission. For all categories of hospitalized malaria morbidity the cumulative incidence was lowest among the population with an extremely low force of transmission, thereafter, the cumulative incidence rose to its highest levels among the populations

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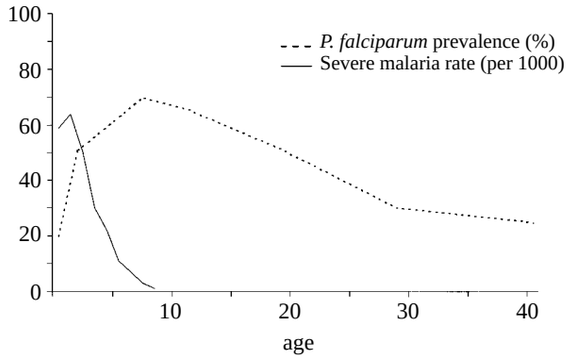

Figure 4. Malarial parasitization and severe malarial disease in an area mesoendemic for malaria (Kilifi District, Kenya).

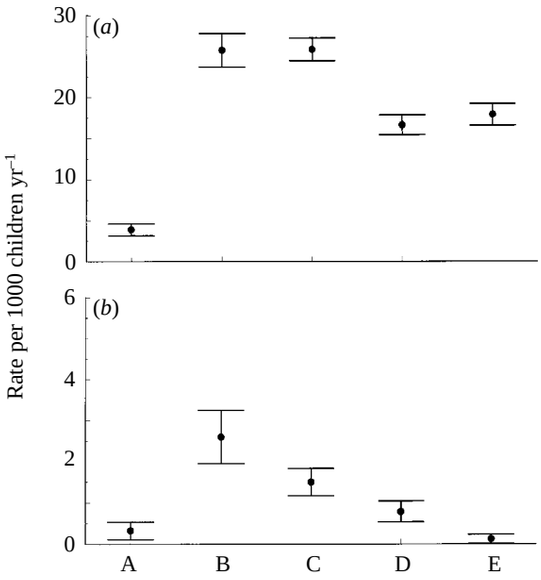

Figure 5. Period prevalence rates (95% confidence intervals) of hospitalization with (*a*) severe malaria and (*b*) cerebral malaria for children aged below the age of ten, in areas with differing forces of infection. (Force of infection (h): A: 0.00097; B: 0.034; C: 0.050; D: 0.093; E: 0.176.)

exposed to low-to-moderate intensity of transmission, and showed evidence of a decline among the populations exposed to the highest intensities of transmission (figure 5). This paradoxical decline in incidence throughout childhood was most striking for cases of severe malaria with cerebral involvement (figure 5). The undoubted difficulties in defining malarial morbidity and taking account of the many factors that may modify the outcome of infection make all such ecological comparisons difficult. Nonetheless, it now seems clear that the age pattern, clinical spectrum, and total burden of severe malarial morbidity vary considerably with transmission intensity.

It is not clear why age *per se* affects the clinical manifestations of severe disease. It has been suggested that the considerably smaller red cell mass of young children means that a larger proportion is destroyed by any given degree of exponential growth of a parasite population (Molineaux 1996). Whilst this seems plausible we would argue that the reason cerebral malaria is much less frequent at younger ages is that it takes longer to develop, i.e. severe anaemia supervenes before the events leading to cerebral manifestations can be expressed. However, there is little evidence to support this. On the contrary, the mean time to the development of cerebral symptoms is less than that to presentation with severe anaemia (Koram *et al*. 1995). As well as a difference in the prevalence of severe anaemia and cerebral malaria, there is a decline in the mean age of children with the latter syndrome with increasing transmission. We hypothesize that this is due to differences in the contribution of the different components of this heterogeneous syndrome, with the metabolic encephalopathy associated with acidosis (which has younger peak prevalence) being more important at higher levels of transmission. But this still leaves unexplained why any given syndrome of cerebral malaria should occur at a later age, and in particular why the 'core' syndrome of coma without respiratory distress or acidosis should occur with a peak incidence in the fourth or fifth year of life. Certainly at this stage the victim will have already experienced many episodes of infection and mild disease. This might be thought to indicate a possible immune pathology, though it has been reported that children with cerebral malaria do not differ in their prior experience of malaria compared with other children (Erunkulu *et al*. 1992), and there is no direct evidence of immune mechanisms that could explain the clinical manifestations. An alternative explanation might be that distinct clinical syndromes are caused by distinct strains of the parasite (Gupta *et al*. 1994). If the strains able to cause cerebral malaria were relatively rare, then one would expect the mean age of encountering them to be relatively later; however, despite convincing theoretical arguments of how a strain structure could be maintained (Gupta *et al*. 1996), there is to date no direct evidence to support this. Alternatively, the age differential in susceptibility to different syndromes may have a physiological basis, for instance in an age-related difference in the expression of specific receptors on vascular endothelium. Again, evidence is lacking.

The apparent reduction in total load of severe morbidity with increasing transmission raises important questions and highlights our poor understanding of how antimalarial immunity works. The natural history of malarial disease in endemic areas, continuing parasitization punctuated by episodes of disease which decrease in frequency with age (Marsh 1992), strongly supports the view that naturally acquired immunity is in part 'strain-specific', and that responses to a repertoire of strains has to be built up. Data from induced malaria in humans, where isolate specific homologous immunity was quickly acquired with little evidence of immunity to heterologous isolates (Boyd 1949), supports this conclusion. However, in this case one might expect that under higher transmission pressure the total burden of disease would be the same, but that it would simply be compressed into a shorter timeframe. One possible explanation for a reduced total load of morbidity is that active immunization to

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malaria can occur under the passive protection of maternal antibodies (Sehgal *et al*. 1989). The higher the transmission pressure, the greater the proportion of the entire parasite 'strain' repertoire that would be encountered in the period of passive protection. An alternative, though not mutually exclusive, explanation for a reduction in severe morbidity under increasing transmission is that an element of protective immunity is directed to conserved or cross-reactive targets, and that the experience of more intense and continuous challenge leads to more rapid development of these responses. We believe that it is likely that both of these suggested mechanisms play a role and that the potential importance of the relationship between transmission, immunity, and total morbidity highlights the need for a better understanding of the mechanisms by which humans in endemic areas acquire protective immunity to malaria.

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