RELATIONSHIPS OF MALARIA MORBIDITY WITH EXPOSURE TO PLASMODIUM FALCIPARUM IN YOUNG CHILDREN IN A HIGHLY ENDEMIC AREA

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Abstract. To study incidence of clinical Plasmodium falciparum malaria in relation to exposure to parasites, attendance of children less than eighteen months old at a village dispensary in a highly endemic area of Tanzania was recorded. Entomologic inoculation rates (EIRs), estimated as a function of time period and place of residence, exceeded one sporozoite positive bite per adult per night in some village neighborhoods during the wet season. Incidence of clinical P. falciparum malaria, defined either as fever with parasitemia or as fever with hyperparasitemia, increased with the EIR over the whole range of exposures. Each 10-fold increase in incidence of fever plus parasitemia (95% confidence interval = 1.4–2.0). Therefore reduction of human-vector contacts will probably reduce morbidity incidence even at very high exposures. Incidence showed little relationship to estimated cumulative numbers of inoculations since birth, but decreased steeply with estimated cumulative time infected with trophozoites. This suggests that clinical immunity depends mainly on the extent of exposure to blood-stage antigens, not on the diversity of inocula seen, and thus temporary reductions in human-vector contacts are unlikely to result in subsequent increases in morbidity.

Reducing human mosquito contact by using insecticide-impregnated mosquito bed nets is becoming an important component of malaria control strategies in sub-Saharan Africa. Such interventions reduce exposure to infectious mosquito bites and their effectiveness therefore depends on the existence of a relationship between exposure to mosquito bites and morbidity and mortality. Impregnated bed nets have been shown to reduce both malaria morbidity1–3 and mortality4–6 in sites covering a range of different malaria endemicities. However, at the very highest exposures the relationships of morbidity and mortality to the frequency of bites from sporozoite-infected mosquitoes (the entomologic inoculation rate [EIR]) remain uncertain.8

Children in a highly endemic area acquire clinical immunity to malaria during the first few years of life. This immunity has been hypothesized to be the result of continual exposure to poorly immunogenic, conserved epitopes on asexual stages of the parasite. Evidence for this was the results of artificial challenges during malaria therapy, which conferred partial clinical protection both to homologous and heterologous inocula.9 Passive transfer studies have also demonstrated also that sera from west Africans can confer protection against east African P. falciparum parasites.10 In contrast to this view, the slow pace at which anti-parasitic mechanisms develop has been used to suggest that plasmodial populations comprise many antigenically distinct genotypes, that clinical immunity is largely specific to genotypes, and that the child becomes immune as he or she is exposed sequentially to different genotypes.11–14 This would imply that reduction in exposure by the use of impregnated bed nets could interfere with the acquisition of clinical immunity. Even when a short-term (e.g., 12-year follow-up) study indicated that the nets were protective, longer-term effects might be less favorable.15

Despite the availability of molecular techniques for parasite typing and for the assay of immunologic responses, there is no consensus on which specific responses should be measured as a proxy for clinical immunity, nor is it feasible to use molecular techniques to track all the infections in any substantial cohort of individuals. Moreover, randomized controlled trials of bed nets with very long follow-up are unethi-

MATERIALS AND METHODS

Study site. The study took place in the village of Idete, which has been described previously,15,17 close to the town of Ifakara in southern Tanzania. A total of 4,758 inhabitants and 868 houses (divided into 14 different neighborhoods) were enumerated in the village during a census conducted between October 1992 and February 1993; 45% of the residents were less than 15 years of age and 5% were less than one year of age.

All subsequent births of children to village residents were registered by project field workers and the infants were issued identification cards including photographs and unique identification numbers. Children included in the present analysis are all those less than 18 months of age in the village at any time during the one-year study period beginning in September 1993. These children were too young to be included in the phase III SPf66 malaria vaccine trial,18 which was carried out in the village at the same time.

Procedures for obtaining informed consent were as described previously.16,17 Ethical clearance was provided by the Medical Research Coordinating Committee of the National
Institute for Medical Research (NIMR) and research clearance was obtained from the Tanzanian Commission of Science and Technology per ref. NSR/RCA 90.

**Morbidity surveillance.** In conjunction with the SPf66 trial, the village dispensary was strengthened and refurbished. The dispensary was the predominant source of antimalarial drugs, which were supplied free of charge.

Morbidity was determined among children that reported sick to the dispensary. Children were identified and screened by project personnel. Axillary temperatures were determined with an electronic thermometer (Münchner Büro Organisation, Munich, Germany). If they were \( \geq 37.5^\circ C \), or if fever was reported during the previous 24 hr, thick and thin blood films were prepared.\(^{15}\)

For the purposes of the present analyses, clinical malaria was defined as either reported fever, or axillary temperatures \( \geq 37.5^\circ C \) together with *P. falciparum* parasitemia. Reported fevers were included because many infants with clinical malaria do not present with fever at the time of examination.\(^{18, 19}\) The inclusion of even cases with low-level parasitemia was justified by analyses (Vounatsou P, unpublished data) using the relative risk of fever in relation to parasite density using an established approach,\(^{20}\) which indicated that this case definition was appropriate in these very young children. The same case definition was used irrespective of age, season, or area of the village. Parasite density cut-off values of 10,000 parasites/µl, 20,000 parasites/µl, and 40,000 parasites/µl were used to define hyperparasitemic clinical malaria cases.

**Estimation of exposures.** Mosquito were caught in Centers for Disease Control and Prevention (Atlanta, GA) miniature light traps suspended inside bedrooms of 139 houses on 1,056 trapping occasions. Exposures for different areas of the village were estimated as described previously.\(^{17}\) As in that study, estimates of exposure to *P. falciparum* were determined every two weeks for 11 of the 14 village neighborhoods over the period from March 1992–September 1994. Three different measures of exposure were used.

1) **Entomologic inoculation rate during the preceding four weeks.** The EIR was estimated as described previously.\(^{17}\) The estimates are intended to be applicable to adults; thus, the true exposure of small children is likely to be considerably lower than these nominal values.\(^{17}\) The estimation procedure gave separate values of the EIR for each neighborhood and each two-week period. The value assigned for the present analyses to each day falling in two-week period \( i \) was the mean of the EIR for the two-week periods \( i-1 \) and \( i-2 \).

2) **Cumulative EIR since birth.** All children in the study were born after the start of the entomologic surveillance; thus, the cumulative number of inoculations since birth could be estimated by summing the relevant neighborhood and the two-week period–specific EIR estimates over the child’s life. This was used as an indirect measure of the number of parasite genotypes to which a child has been exposed.

The cumulative number of inoculations is much greater than the number of distinct infection events because not all bites from sporozoite-infected mosquitoes result in infections.\(^{17, 21, 22}\) However in very young children, the number of genotypes encountered is likely to be approximately proportional to, but less than the number of inoculations, since some genotypes will have been encountered more than once. In older children, the repertoire of genotypes will still be strongly correlated with but will be less than proportional to the cumulative inoculations as the child’s repertoire approaches completion.

3) **Cumulative days of blood stage infection since birth.** As in previous reports,\(^{16, 17}\) we approximated the rates of acquisition \( (h) \) and loss \( (r) \) of infection by the catalytic model\(^{23}\)

\[
\frac{dp}{dt} = (1 - p) h - pr,
\]

where \( p \) is the probability that a child has a patent infection and \( t \) refers to the child’s age.

In the companion paper,\(^{17}\) we showed that the force of infection, \( h \), at least at low EIR values, is dependent mainly on the EIR and shows little age dependence, while the rate of recovery from infection, \( r \), is highly age dependent\(^{16}\) but shows little dependence on entomologic exposure. Since congenital malaria is a rare event,\(^{24}\) we assumed the child to be uninfected at birth \( (t = 0) \). We then estimated the probability that the child was infected at age \( t \), as a function of its exposure history, by integrating measure 1 with respect to age. We used exposure specific estimates of \( h \) that varied depending on the two-week period and area of the village,\(^{17}\) and estimates of \( r \) that varied by age.\(^{16}\)

**Data analysis.** The statistical significance of relationships between morbidity and exposure were tested using person-time at risk methods. Children were included in the time at risk either from August 1993 or from birth (whichever was later). Each day until either the end of August 1994 or when the child reached 18 months of age was assigned to the appropriate age group and category for each measure of exposure. Following each episode satisfying the case definition under evaluation, a period of 28 days was excluded from the time at risk and repeat visits to the dispensary during that period were not included in the analyses.

Statistical significance was tested using Poisson regression with the number of days at risk as a rate multiplier,\(^{25}\) and the magnitude of the effects was illustrated by the estimated ratio of incidence rates associated with a 10-fold increase in the EIR. Regression models allowed for the effect of age by including indicator variables for each two-month age group. The effects of exposure variables were tested by including additional terms in log-transformed exposure in the regression model. Analyses of cumulative exposures considered only children more than four months of age, since below that age cumulative exposures are closely related to recent exposures.

**RESULTS**

A total of 424 children who were less than 18 months of age at some time during the study period, and for whom exposures could be calculated, were included in the analyses.

Table 1 shows the total attendance at the dispensary identified using a range of different case definitions. The incidence of nonmalaria fevers was high in children less than two months of age, and reached a maximum in the 2–3-month-old age group. All other measures of morbidity showed a steep increase in incidence over the first few months of life, with the maximum incidence in the 4–6-month-old age group, and a decrease in incidence with subsequent age (Figure 1).

Using the same set of case definitions, Figure 2 shows how the morbidity rates varied with recent exposure to infective mosquitoes. The incidence of clinical malaria mor-
bidity increased with recent exposure irrespective of the case definition. The increases fitted well to Poisson regressions on the logarithm of the recent EIR, with no indication of saturation at high EIRs. However, morbidity rates did not increase in proportion to the EIR: 10-fold increases in the EIR were accompanied by less than two-fold increases in the incidence and no power can be gained by using a case definition (Table 1).

Although case definitions with high parasitemia cut-off values are more specific for clinical malaria in children with substantial exposure, the highest rate ratio in the present analyses was associated with the case definition (Table 1) (fever plus any parasites). This suggests that in contrast to older children, in these young children little additional specificity and no power can be gained by using a case definition of fever plus hyperparasitemia. Subsequent analyses used case definition 2 throughout. Nonmalarial fevers showed no relationship with a recent EIR (Figure 2 and Table 1), suggesting that there is no important confounding of exposures with the propensity to attend the dispensary. Attendance rates for nonmalarial fevers varied considerably over time but the pattern was very different from the temporal patterns, both in terms of incidence of clinical malaria and the EIR (Figure 3). The incidence of clinical malaria was higher during the wet season than in the dry season (meteorologic data are presented elsewhere), but increased before the main rains started, reaching a maximum well before the peak in the EIR (Figure 3).

Because the birth rate was seasonal, the age distribution varied by season. However, age adjustment made little difference to the estimated relationship between the recent EIR and the incidence of clinical malaria (Figure 4a).

The number of sporozoite positive bites since birth increased with age; however, children born at the start of the dry season accumulate exposure to infectious bites at a very different rate from those born at the start of the wet season. Therefore, there was substantial variation in cumulative inoculations at a given age. Because of this variation, it was possible to separate effects associated with cumulative inoculations from those that are simply a function of age. In children with low cumulative exposures to sporozoites, there was an increase in morbidity rate with this measure of exposure (Figure 4b). These are very young children in whom the recent bites constitute a substantial proportion of the total accumulated. There was a slight decrease with cumulative exposure to mosquito bites in children who had already accumulated a considerable number of inoculations, but this was not statistically significant (Poisson regression in children more than four months of age likelihood ratio $\chi^2 = 2.9, P = 0.09$), particularly when adjusted for age (likelihood ratio $\chi^2 = 0.26, P = 0.6$).

The estimated number of months of exposure to blood

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**Table 1**

Recorded morbidity rates and relationships with recent entomologic inoculation rate

<table>
<thead>
<tr>
<th>Case definition</th>
<th>Total episodes*</th>
<th>Child-years at risk</th>
<th>Relative risk†</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 age (months)</td>
<td>0–5 age (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) All attendances at the dispensary‡</td>
<td>311 (4.7)</td>
<td>625 (5.2)</td>
<td>66</td>
<td>120</td>
<td>1.33 (1.15–1.53)</td>
</tr>
<tr>
<td>2) Fever§¶ and <em>Plasmodium falciparum</em> parasite density $&gt;0$</td>
<td>154 (2.0)</td>
<td>411 (3.0)</td>
<td>76</td>
<td>137</td>
<td>1.63 (1.36–1.96)</td>
</tr>
<tr>
<td>3) Fever§¶ and parasite density $&gt;10,000/\mu$L</td>
<td>111 (1.4)</td>
<td>278 (1.9)</td>
<td>79</td>
<td>147</td>
<td>1.57 (1.26–1.96)</td>
</tr>
<tr>
<td>4) Fever§¶ and parasite density $&gt;20,000/\mu$L</td>
<td>94 (1.2)</td>
<td>237 (1.6)</td>
<td>80</td>
<td>150</td>
<td>1.53 (1.21–1.95)</td>
</tr>
<tr>
<td>5) Fever§¶ and parasite density $&gt;40,000/\mu$L</td>
<td>71 (0.9)</td>
<td>193 (1.3)</td>
<td>82</td>
<td>153</td>
<td>1.49 (1.14–1.95)</td>
</tr>
<tr>
<td>6) Fever§¶ and parasite density $=0$</td>
<td>108 (1.5)</td>
<td>138 (1.1)</td>
<td>73</td>
<td>131</td>
<td>1.06 (0.80–1.41)</td>
</tr>
</tbody>
</table>

* Figures in brackets are incidence rates per child-year.
† Rate ratios associated with a 10-fold increase in infectious bites during the previous month estimated from a Poisson regression model. CL = confidence limit.
‡ Time at risk excludes a period of 28 days after each episode.
§ Fever indicates either reported fever during the last 24 hr or a measured axillary temperature of $\geq37.5^\circ{C}$.
¶ Time at risk excludes a period of 28 days after each prescription of anti-malarials at the dispensary.

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**Figure 1.** Incidence of morbidity by age. Different lines correspond to case definitions as described in Table 1. ■ = all attendances at the dispensary; • = fever and *Plasmodium falciparum* parasite density $>0$; -- = fever and parasite density $>10,000/\mu$L; = = fever and parasite density $>20,000/\mu$L; -= = fever and parasite density $>40,000/\mu$L; fever = parasite density $=0$.

**Figure 2.** Incidence of morbidity by entomologic inoculation rate during the previous month. The different lines correspond to case definitions as described in Table 1 and Figure 1.
stages was more closely related to age than was either of the
other measures of exposure. The incidence of malaria
decreased markedly with increased exposure to blood
stages (Figure 4c). However, there was little variability in
cumulative exposure to blood stages within any one age
group (Figure 4c), which makes it difficult to separate effects
of this measure from effects of age per se. However, the fit
of the Poisson regression model for morbidity rates (children
greater than four months of age only) was substantially im-
proved by including a further effect of the logarithm of the
cumulative exposure to blood stages (likelihood ratio $\chi^2 = 24.9$, $P < 0.001$). It might have been anticipated that the
relationship between estimated cumulative exposure to blood
stages and risk would largely be explicable as an age effect.
However, the cumulative exposure effect remained highly
statistically significant even allowing for age (likelihood ra-
tio $\chi^2 = 17.5$, $P < 0.001$), and after adjustment for estimated
cumulative exposure, the morbidity rate actually increased
with age, indicating that cumulative exposure appears to be
a better predictor of acquired protection than age. The over-
all conclusion is that the cumulative exposure to blood
stages, rather than exposure to repeated inoculations per se,
is likely to account for the acquisition of long-term clinical
immunity.

DISCUSSION

The incidence of clinical malaria in children less than 18
months of age in Idete increased with estimated exposure to
sporozoite-carrying mosquitoes during the preceding month.
This relationship was observed over the whole range of EIRs
examined, which includes EIRs higher than those in any
published trial of insecticide-impregnated bed nets. It sug-
gests that even in very high transmission areas, personal pro-
tection against mosquito bites will have a beneficial effect
on morbidity rates in children in this age group. This pro-
tection occurs despite the lack of a relationship between both
the force of infection in infants and the parasite prevalence
in the whole community to changes in transmission inten-
sity within this very high range.

As in comparisons of different communities, we found
that the morbidity rate is not proportional to the number
of recent infectious bites. In our study, this might partly be
because of imprecision in the exposure estimates, which will
tend to lead to underestimation of the strength of the rela-
tionship between morbidity and exposure. However, the
main reduction in the marginal effect of additional inocula-
tions is likely to be a consequence of acquired immunity.

The effects of immunity vary by age. We considered only
the narrow age group with the highest risk of clinical attacks,
and therefore do not have direct evidence of the relationship of clinical malaria to exposure in older children and adults with a greater degree of natural immunity. Some researchers have suggested that reductions in inoculations by individual protection in highly endemic areas might interfere with the development of protective responses, merely postponing clinical episodes. For example, if clinical episodes occur if and only if an individual is infected with a parasite genotype that he or she has not seen before, the lifetime number of clinical malaria episodes depends only on the number of genotypes and is unrelated to the frequency of inoculation with individual genotypes. If this is correct, an increase in morbidity in older individuals might accompany any reduction in inoculations in very young children caused by the use of bed nets.

Antigenic diversity has been widely detected in field studies of *P. falciparum*. This diversity is undoubtedly related to the evasion of immune responses. The pattern of seasonal variation in malaria morbidity in the present study also implies that antigenic diversity in parasites and implicitly genotype-specific immune responses must affect the risk of clinical malaria. There is a sharp increase in malaria morbidity rates at the start of the wet season. This differs from the pattern in older children in southern Tanzania with more immunity, but is similar to the pattern in The Gambia, where the EIR is some two orders of magnitude less than that in Idete, but transmission is also seasonal. Since most children are infected at the start of the rainy season, the number of new merozoites contributed by the additional inoculations must be very small compared with the numbers of existing trophozoites; thus, the additional merozoites can only make a difference in the outcome of infection if qualitative differences of the existing parasites are important. In our recent analyses of the incidence of infection, we found that the force of infection reaches a plateau above an EIR of approximately one per night, although the morbidity rate continues to increase. This also argues that morbidity is not a consequence of additional infections *per se*, but rather that qualitative differences between inocula determine whether a clinical attack will occur.

The evidence of the present study is that effective genotype-specific responses are probably not of long duration, and probably do not account for the build of anti-parasite immunity over time. If clinical immunity is measured by the size of repertoire that has been acquired, then the cumulative number of inoculations should be a good predictor of immune status. Therefore, the absence of a protective effect associated with cumulative exposure to inoculations is evidence against an important role for long-term genotype-specific responses in clinical immunity against *P. falciparum*.

The negative correlation of morbidity with cumulative time exposed to erythrocytic stages suggests that long-term clinical immunity is induced by exposure to blood stages irrespective of the number of different genotypes inoculated. Protection would therefore appear to be associated with the gradual build-up of the immune responses to asexual stage parasites irrespective of their genotypes, not to genetic variation in the inocula. Our result could also be explained if clinical protection depends on responses to epitopes undergoing antigenic switching within a host (e.g., *P. falciparum* erythrocyte membrane protein 1).

If the frequency of inoculations does not account for long-term immunity, then in contrast to chemoprophylaxis, the protection provided by bed nets is unlikely to interfere very much with natural protection because the reduction of mosquito bites has little effect on parasite prevalence. It has also been hypothesized that there might be a reduction in disease in areas of very high exposure because functional immunity might be acquired within the period of passively transferred immunity. Our results do not support this hypothesis because the effect of maternal protection is to reduce exposure to erythrocytic stages during the first few months of life, so that even in the absence of bed nets there is no opportunity to build up substantial immunity to conserved epitopes of asexual parasites before maternal protection wanes.

The higher incidence of cerebral malaria in areas of lower transmission intensity raises the additional concern that in areas of very high transmission, transmission-reducing measures might result in long-term in changes in the severity of clinical malaria or in the presentation of severe malaria. We were not able to address this issue. It was a necessary simplification in the present study to use a single main definition for clinical malaria, although the presentation of the disease and therefore the specificity of our case definitions certainly change as the children acquire immunity. The surveillance system we used missed many mild episodes that were not reported to the dispensary, but incomplete reporting does not appear to have introduced any bias into our estimates of rate ratios since there was no relationship between EIR and the incidence of nonmalarial fevers. Despite these limitations, the present study does provide evidence that personal protection against mosquito bites will be effective in reducing the incidence of clinical malaria in children less than 18 months of age in areas of highest transmission of *P. falciparum*. It also suggests that protection of children in this age range is unlikely to result in any substantial increase in the overall incidence of clinical malaria when they grow older.

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REFERENCES


