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Onset of clinical immunity to *Plasmodium falciparum* among Javanese migrants to Indonesian Papua*

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Onset of clinical immunity to *Plasmodium falciparum* occurred among Javanese migrants to Indonesian Papua. Surveillance of the 243 migrants investigated began on the day of their arrival in Indonesian Papua and continued for 33 months. Asexual parasitaemia without fever constituted objective evidence of clinical immunity. Compared with first infection, the odds ratio (OR) for not having fever at the fourth infection within 24 months was 3.2 [95% confidence interval (CI) = 1.03–10.2; $P = 0.02$]. The corresponding OR with fewer infections within 24 months was not distinguishable from 1.0. The level of the fourth parasitaemia within 24 months ($N = 58$) was classified as 'high' or 'low' in relation to the median count at first infection (840 parasites/μl; $N = 187$). Fourth parasitaemias that were low — but not those that were high (OR = 1.8; CI = 0.6–5.4; $P = 0.35$) — were associated with dramatic protection from fever (OR = 31; CI = 3.5–1348; $P = 0.0001$). Among the adult subjects, the risk of fever with low parasitaemia was significantly higher at the first infection than at the fourth (OR = 12.6; CI = 1.7–530; $P = 0.005$), indicating the development of clinical immunity. A similar but less marked pattern appeared among the children investigated (OR = 6.5; CI = 0.8–285; $P = 0.06$).

Naturally acquired immunity to *Plasmodium falciparum* renders an otherwise debilitating and potentially deadly infection relatively innocuous. Older children and adults exposed to uninterrupted heavy exposure to infection throughout much of sub-Saharan Africa exhibit such immunity. Although nearly continuously infected and often parasitaemic, severe disease only occurs very rarely in these subpopulations (McGregor, 1952). In contrast, infants and very young children in the same region account for the majority of the 1 million–2 million deaths caused annually by *P. falciparum*, and almost all survivors endure debilitating chronic disease until they are aged 3–5 years (Murphy and Breman, 2001). Inoculation with purified IgG from adult Africans has provided clinical immunity to *P. falciparum* malaria in monkeys (Sadun *et al.*, 1966; Diggs *et al.*, 1972), African children (Cohen *et al.*, 1961; Edozien *et al.*, 1962) and Thai adults (Sabchareon *et al.*,

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1991). Taken together, these observations indicate that natural protection from parasitaemia and symptomatic malaria is the result of acquired humoral immunity.

The number and frequency of exposures required to elicit clinical immunity are not known. The results of experiments conducted when malaria was used to provide therapeutic relief from human neurosyphilis (Chernin, 1984; Collins and Jeffery, 1999) demonstrated the onset of clinical immunity after only four to six challenges (Ciuca *et al.*, 1934). In sub-Saharan Africa, however, clinical immunity only appears to develop after 5–15 years of continuous and heavy exposure to infection. The relatively slow onset of clinical immunity in Africans has been attributed to the antigenic repertoire of the parasite and the many heterologous strains of malarial parasite present in Africa (McGregor, 1987; Day and Marsh, 1991; Newbold *et al.*, 1997). The challenge series in neurosyphilis patients was with homologous strains whereas, presumably, a human living in Africa must experience very many heterologous infections to acquire immunological memory of sufficient diversity to suppress any given local strain of the parasite. From this perspective, naturally acquired immunity represents the cumulative product of many years of uninterrupted heavy exposure to infection by *P. falciparum*. This, in part, explains the long-standing reluctance of vaccine developers to examine natural immunity as a model of protection. The results of studies in Indonesian Papua, however, do no support the view that cumulative exposure to *P. falciparum* infection is the primary determinant of clinical immunity to this parasite. Age-dependent clinical immunity to *P. falciparum* malaria developed in non-immune migrants to Indonesian Papua (formerly known as Irian Jaya) within a year or two of their arrival (Baird *et al.*, 1991, 1993). This clinical immunity was apparently the product of recent heavy exposure and intrinsic determinants related to age, independent of a history of chronic exposure (Baird, 1995, 1998). The rapid onset of clinical immunity observed in adult neurosyphilis patients also appeared to occur in the naturally exposed, adult migrants. Malaria-naïve adults may therefore be capable of acquiring clinical immunity within a year or two, after just a few infections.

Those attempting to develop vaccines to prevent the high malaria-attributable mortality and morbidity occurring in sub-Saharan Africa need to know the factors that determine when and if clinical immunity to *P. falciparum* malaria will develop in an individual. They hope to produce vaccines that will evoke, in susceptible children, the naturally acquired protection observed in adults (Miller and Hoffman, 1998), generally assuming that children develop symptomatic malaria simply because they have had insufficient cumulative exposure to malarial antigens (Good *et al.*, 1998; Plebanski and Hill, 2000). Post-infection morbidity, however, may not reflect inadequate experience with malarial antigens but inappropriate immune responses, driven by intrinsic factors linked to the age of the host.

The present, longitudinal study on the onset of clinical immunity to *P. falciparum* malaria was conducted as a follow-up to the cross-sectional studies underpinning the hypothesis of age-dependent, acquired immunity (Baird *et al.*, 1991, 1993). After screening out people who had resided outside of Java or within the few regions of Java with substantial risk of malaria (Baird *et al.*, 1996), a cohort of 243 Javanese migrants was recruited, on the day the migrants arrived in Indonesian Papua, and followed for 33 months. This approach allowed the effects of age and cumulative exposure to infection, upon the onset of clinical immunity to *P. falciparum* malaria, to be separated.

SUBJECTS AND METHODS

Study Site

The study site was the transmigration village designated *Satuan Permukiman* (Settlement Unit) 2 (SP2) by the Indonesian authorities

that built it. The precise location and nature of the study site have been detailed elsewhere (Krisin *et al.*, 2003). In brief, SP2 is located near the Pacific coast in the Bonggo sub-district of north–eastern Papua, Indonesia, about 150 km west of the city of Jayapura. The village, which covers an area of about 4 km^2, consists of 300 identical wood-plank houses with tin roofs, and fields for the cultivation of subsistence and cash crops such as melon, papaya, soy, peanut and maize. At the time of the present study, dense forest surrounded the cleared area of the village.

Subjects

The details of recruitment, enrolment, follow-up and the epidemiology of malaria in the SP2 cohort have been described elsewhere (Krisin *et al.*, 2003). In brief, 243 subjects aged 6–12 years ($N = 97$) or 20–58 years ($N = 146$) were successfully enrolled. After 33 months of follow-up, 159 (69 children and 90 adults) remained under observation. Almost all losses to follow-up were the result of migrants deciding to return to Java.

Follow-up

Each week, the homes of the subjects were visited three times, so that the health status of each subject could be determined. Smears were made of blood samples collected every 2 weeks (without regard to illness) and also immediately from any subject who complained of illness. The smears produced routinely every 2 weeks were not examined immediately but stained, stored and transported to Jakarta for reading.

Classification of Infection

All cases of malaria were microscopically confirmed. A three-tiered classification scheme for parasitaemia was employed. Firstly, the infection sequence was enumerated, separately for *P. falciparum* and *P. vivax* (e.g. 'fourth infection with *P. falciparum*'); a parasitaemia detected $\leqslant 28$ days after another was considered a recrudescence and not counted in the sequence. Secondly, each enumerated new infection was classified as febrile or afebrile. The classification 'febrile' was always supported by a recorded axillary temperature of $> 37.5^{\circ}C$. The classification 'afebrile' was supported by a normal axillary temperature or by the detection of parasitaemia in a routine smear of blood from a subject who appeared healthy and reported no illness. The health workers who collected the routine smears worked under strict orders to bring any ill subject to the study clinic in SP2, for examination. Throughout the study, the same open-ended question — 'Do you have any symptoms?' — was used by the health workers on their home visits, to avoid bias. Finally, each new infection was classified as a low parasitaemia (if the level of parasitaemia was lower than the median first-infection level among all the subjects) or a high parasitaemia.

Statistical Analysis

Results were expressed as frequencies and analysed using Mantel–Haenszel tests or χ^2 tests with Yates' correction. Fisher's exact tests were used when expected cell values were below 5.0. A *P*-value of $\leqslant 0.05$ was considered indicative of statistical significance. All the analyses were carried out using version 9.0 of the SPSS (SPSS Inc., Chicago, IL) or version 6.0 of the Epi Info (Centers for Disease Control and Prevention, Atlanta, GA) software packages. All of the results presented below refer to *P. falciparum* infection.

RESULTS

Exposure Threshold for the Onset of Clinical Immunity

Parasitaemia without fever constituted objective evidence of clinical immunity to *P. falciparum* and the Figure illustrates the odds ratios (OR) for not having fever after experiencing the second, third and fourth infections within 6, 12 or 24 months. As the

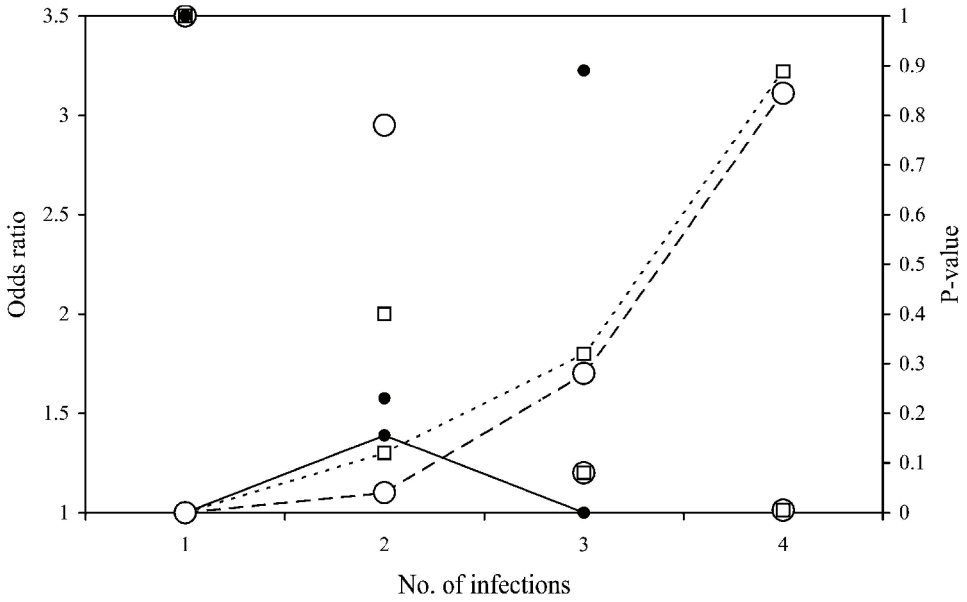

FIG. The lines indicate the odds ratios for fever, relative to the first infection, during the second, third and fourth infections by *P. falciparum* within 6 (●), 12 (□) or 24 (○) months. The additional points show the corresponding *P*-values.

first infection constituted the reference point for the OR in the subsequent infections, the first infection had an OR of 1.0 and a *P*-value of 1.0, by mathematical definition. The second infection within 6, 12 or 24 months was no less likely to cause fever than the first: the OR were $\leqslant 1.4$ and the *P*-values > 0.2. The third infection within 6 months also showed no diminished risk of fever (OR = 1.0; $P = 0.9$), but when the third infection occurred later (within 12 or 24 months), marginally significant protection from fever appeared. The OR for three infections within 12 months was 1.8 ($P = 0.08$) — essentially the same as when the three infections occurred within 24 months (OR = 1.7; $P = 0.08$). When four infections occurred within 12 or 24 months (no subject experienced four within 6 months), the protection from fever markedly improved. The OR for not having fever at fourth infection within 12 and 24 months were 3.2 ($P = 0.02$) and 3.1 ($P < 0.01$), respectively. Onset of clinical immunity thus occurred among members of the SP2 cohort experiencing at least four infections within 12 or 24 months. Three infections in the same interval only provided marginally significant protection and one or two infections failed to show any protection from fever with parasitaemia.

Risk of Fever with Low or High Parasitaemia at First or Fourth Infection

The relationship between risk of fever and density of parasitaemia (stratified by sequence of infection) was investigated (Table 1). At first infection, subjects with low parasitaemia (i.e. with parasitaemias below the median first-infection level of 840 parasites/μl) were at the same risk of fever as the subjects with high parasitaemia (OR = 1.24; $P = 0.916$). In contrast, at fourth infection, the subjects with low parasitaemia were more likely to be afebrile than subjects with high parasitaemia (OR = 21; $P = 0.0004$). When the risk of fever at first or fourth infection was stratified by the density of the concurrent parasitaemia (Table 2), subjects with high parasitaemia were found to be equally likely to be febrile at first and fourth infection

TABLE 1. *Risks of fever, with high-* versus *low-level parasitaemia, at the first and fourth infections of the 58 subjects who had four* Plasmodium falciparum *infections within 24 months*

	No. of subjects:			
	Febrile	Afebrile	Odds ratio and (95% confidence interval)	*P*
FIRST INFECTION				
High-level parasitaemia	21	10	1.24 (0.4–4.2)	0.916*
Low-level parasitaemia	17	10		
FOURTH INFECTION				
High-level parasitaemia	21	18	21 (2.5–916)	0.0004†
Low-level parasitaemia	1	18		

*χ^2 test with Yates' correction.
†Two-tailed Fisher's exact test.

TABLE 2. *Risks of fever with high- and low-level parasitaemias, at first* versus *fourth infection, among the 58 subjects who had four* Plasmodium falciparum *infections within 24 months*

	No. of subjects:			
	Febrile	Afebrile	Odds ratio and (95% confidence interval)	*P*
HIGH-LEVEL PARASITAEMIAS				
First infection	21	10	1.8 (0.6–5.4)	0.350*
Fourth infection	21	18		
LOW-LEVEL PARASITAEMIAS				
First infection	17	10	31 (3.5–1348)	0.0001†
Fourth infection	1	18		

*χ^2 test with Yates' correction.
†Two-tailed Fisher's exact test.

(OR = 1.8; $P = 0.35$) whereas subjects with low parasitaemia were far more likely to be afebrile at the fourth infection than at the first (OR = 31; $P = 0.0001$).

Age-specific Risk of Fever with Low Parasitaemia at First versus Fourth Infection

After febrile subjects with low parasitaemias had been classified as 'sensitive', it became clear that subjects were less likely to be sensitive at their fourth infection than at their first. This trend was more marked among the adult subjects than among the children investigated (Table 3): children were 6.5 times ($P = 0.06$), and adults 13 times ($P = 0.005$), less likely to be sensitive at their fourth *P. falciparum* infection than at their first.

DISCUSSION

In a cohort of 159–243 subjects followed for 33 months, only 58 subjects experienced four infections by *P. falciparum* within any 24-month period. Among these 58 subjects, significant protection from fever was only observed with the fourth parasitaemia (Fig.). In SP2 at least, four infections within 2 years therefore appears to be the minimal threshold for the onset of clinical immunity (as indicated by parasitaemia without fever). Although risk of fever was independent of the level of parasitaemia at first infection, at the fourth infection fever occurred almost exclusively among the subjects with high-level parasitaemias (Table 1). Among the subjects with high-level parasitaemias, no protection from fever occurred at the fourth

TABLE 3. *Risks of fever with low-level parasitaemia, at first* versus *fourth infection, among the child and adult subjects*

	No. at:			
	First infection	Fourth infection	Odds ratio and (95% confidence interval)	*P*
CHILDREN				
Febrile	15	1	6.5 (0.8–285)	0.06*
Afebrile	51	22		
ADULTS				
Febrile	27	1	12.6 (1.9–530)	0.005*
Afebrile	73	34		

*Two-tailed Fisher's exact test.

infection (relative to the first), whereas marked protection appeared among the subjects with low-level parasitaemias (Table 2). If clinical immunity to *P. falciparum* was defined by a low parasitaemia without fever, children appeared slightly less likely than adults to exhibit protection at the fourth infection (Table 3).

Among Javanese migrants to Indonesian Papua, immune responses leading to suppression of parasitaemia are apparently associated with the onset of clinical immunity. The level of parasitaemia appeared to be a primary correlate of fever. Even after four infections within 24 months, subjects with high-level parasitaemias were at the same risk of fever as subjects with such parasitaemias at the first infection. Essentially similar findings were reported from a cohort of adults in holo-endemic northern Ghana: the OR for symptoms with high-level parasitaemia (i.e. >2000 parasites/μl) was 3.2 [95% confidence interval (CI) $= 1.1–9.1$; $P = 0.01$; Owusu-Agyei *et al.*, 2001]. Naturally acquired clinical immunity appears to be associated with anti-parasite rather than anti-disease effectors.

In the present study, the onset of clinical immunity even among the 58 subjects who received sufficient exposure (i.e. four *P. falciparum* infections within 24 months) was not universal: only 18 of the 58 had low parasitaemias without fever at their fourth infection. The other 40 had high parasitaemias without fever ($N = 18$) or were febrile, with high-level ($N = 21$) or low-level parasitaemias ($N = 1$), at their fourth infection (Table 2). Naturally acquired immunity after four infections within 24 months was therefore considered to be, as a crude estimate, 62% effective in conferring protection from fever.

Subtlety marked the differences in the onset of clinical immunity between the children and adults investigated (Table 3). Because the demography of the migrant populations limited the availability of younger subjects, only children aged >5 years at enrolment were investigated. The youngest subjects in the study cohort were approaching 9 years of age when the study ended. Most of the children in the cohort were well into puberty at that time. Kurtis *et al.* (2001) documented the importance of this developmental event as a determinant of susceptibility to *P. falciparum* among African children. The fact that the children investigated in the SP2 cohort were relatively old may have subdued the age-related differences noted in the earlier, cross-sectional studies from similar populations (Baird *et al.*, 1991, 1993).

In summary, protection from fever associated with *P. falciparum* parasitaemia occurred among 62% of Javanese migrants who experienced at least four *P. falciparum* infections within 24 months of arriving in Indonesian Papua. Protection from fever was especially pronounced among subjects with low-level parasitaemias. These findings support the hypothesis that attributes the

onset of clinical immunity to relatively brief exposure and intrinsic factors that are linked to age and independent of lifelong exposure to infection.

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