Development of Clinical Immunity to Malaria in Highland Areas of Low and Unstable Transmission

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Abstract. In highland areas of unstable, low malaria transmission, the extent to which immunity to uncomplicated malaria develops with age and intermittent parasite exposure has not been well characterized. We conducted active surveillance for clinical malaria during April 2003–March 2005 in two highland areas of western Kenya (Kapsisiywa and Kipsamoite). In both sites, annual malaria incidence was significantly lower in persons ≥15 years of age than in persons <5 years of age (Kapsisiywa: incidence = 382.9 cases/1,000 persons among persons <1–4 years of age versus 135.1 cases/1,000 persons among persons ≥15 years of age; Kipsamoite: incidence = 233.0 cases/1,000 persons in persons <1–4 years of age versus 43.3 cases/1,000 persons in persons ≥15 years of age). In Kapsisiywa, among persons with malaria, parasite density and axillary body temperature were also significantly lower in persons ≥15 years of age than in persons <5 years of age. Even in highland areas of unstable and low malaria transmission, age is associated with development of clinical immunity to malaria.

INTRODUCTION

Malaria caused by Plasmodium falciparum is a leading cause of death among children less than five years of age in sub-Saharan Africa.1 Children living where malaria transmission is holo-endemic or hyper-endemic develop immunity and begin to control the level of parasite burden such that, as adults, they are at reduced risk of uncomplicated and severe disease, including death. Parameters of malaria transmission, such as the force of infection, and disease incidence have been shown to correlate with this clinical immunity and the development of overall immune responses to P. falciparum.2–4

Some studies of malaria in areas of low transmission support the premise that the risk of uncomplicated malaria is similar in adults and children, although severe disease and death from malaria in these areas still occurs most frequently in young children.3–5 Studies on age-related risk of malaria in highland areas, which have unstable, seasonal, and low levels of malaria transmission, have produced conflicting results.5–8 Some studies suggest that persons of all ages experience equal risk of malaria, but others, including several historical studies, suggest that adults may develop some degree of immunity and are at a reduced risk for clinical malaria compared with children.3,5,12–14

Most studies of highland malaria and many studies of malaria in low transmission areas have used retrospective chart reviews of hospital data or have studied a limited age range that may not be able to assess nuanced differences in malaria incidence with age. There is also a paucity of recent studies of age-related risk of malaria in low transmission settings. To address this gap in knowledge, we analyzed data from a prospective cohort study of uncomplicated malaria incidence in two highland areas of Kenya that experience very low, seasonal transmission. In this study, we also sought to understand how clinical parameters of immunity, including parasite density and measured temperature during an acute malaria episode, differ with age.

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MATERIALS AND METHODS

Study sites. Two highland sites, comprising multiple villages, were the setting for this analysis. Both sites have low and unstable malaria transmission, as indicated by seasonal peaks in incidence and periodic epidemics, and are located in the Nandi North District of western Kenya: Kapsisiywa (elevation = 1,887–1,982 meters, population in 2003 = 3,412 persons) and Kipsamoite (elevation = 1,950–2,100 meters, population in 2003 = 3,250 persons).15 Most persons in both sites are of the Kalenjin Nandi tribe. A Kenyan Ministry of Health dispensary, capable of microscopy and malaria treatment, is located in each site.

Active surveillance for malaria. Persons from the two cohorts were observed, the first cohort was studied during April–December 2003 and the second cohort was studied during January 2004–March 2005. Written informed consent was obtained from all participants before enrollment in either cohort; parents or guardians of children less than 15 years of age provided consent for children. A literate family member, or other witness, was present during the consent process of illiterate participants to ensure that the details of the study were conveyed effectively.

Enrollment in the first cohort in Kipsamoite was a continuation of a previous study in which persons had been randomly selected from the population to participate in a study of immune response to malaria disease.16 For enrollment into each cohort in Kapsisiywa and the second cohort in Kipsamoite, each site was divided into four geographic subregions of approximately equal population size. Within each subregion, households were randomly selected for recruitment until approximately 15% of the underlying population had been enrolled. Refusal rates were <1% in both communities.

Active surveillance for malaria disease was conducted by trained, local field assistants who made weekly visits to each enrolled household, including the head of the household and all present and enrolled residents. Clinical malaria was detected among study participants in three ways: 1) study participants reported a current illness or illness in the past week during a weekly household visit; 2) study participants contacted a field assistant when ill; or 3) study participants reported
directly to the dispensary when ill. The case definition for suspected clinical malaria was history of fever, chills, headache, or severe malaise in a study participant in the past week. Parents or guardians were asked about illness in infants and young children consistent with the above case definition. The case definition did not differ by age. Field assistants at the participant’s house, or clinical officers at the study dispensaries, took finger prick blood samples and measured axillary body temperature when malaria was suspected. All participants meeting the case definition were referred to the dispensary for blood smear results and treatment. For study participants who attended health facilities outside the study area, axillary temperature and parasite density were not available but data on the results of blood smears were recorded per self-report.

Microscopy testing and treatment of patients with clinical malaria. Clinical malaria was confirmed by microscopy for persons with suspected malaria. Trained microscopists from the Division of Vector-Borne Diseases in the Kenyan Ministry of Health at each of the health dispensaries viewed Giemsa-stained thick and thin blood smears for the presence of Plasmodium species parasites, as described. In brief, slides were considered negative if no asexual parasites were observed after counting microscopic fields containing at least 200 leukocytes. The presence of any asexual Plasmodium parasite indicated a positive blood smear. Parasite densities were estimated per microliter of blood, assuming 8,000 leukocytes/µL of blood. Additionally, the presence and density of gametocytes were assessed in all positive blood smears. Both sites followed the same microscopy protocol for each cohort.

Participants with microscopy-confirmed malaria were treated with sulfadoxine/pyrimethamine according to the Kenyan Ministry of Health treatment guidelines until May 2004; at which time the national treatment guidelines for the first-line antimalarial switched to amodiaquine.

Entomologic surveillance. To estimate the entomologic inoculation rate (EIR) at each site, pyrethrum spray catches (PSC) were performed every two weeks during April 2004–March 2005 in 120 randomly selected households by using standard methods. Collected mosquitoes were identified by species, and the blood-engorged abdomens of anopheline mosquitoes were tested by polymerase chain reaction (PCR) for human blood, and the head and thorax were tested by enzyme-linked immunosorbent assay for P. falciparum circumsporozoite protein (CSP). The EIR per year was calculated as 1.605 × (the human biting rate) × (sporozoite rate) × 365, where the constant 1.605 was used to account for the use of PSC rather than human landing catches. The human biting rate was defined as the number of blood-fed mosquitoes divided by the number of persons who slept in the sampled houses during the night of sampling, and the sporozoite rate was defined as the number of CSP enzyme-linked immunosorbent assay–positive vectors divided by the number of vectors collected.

Statistical analysis. To assess the differences in malaria incidence by age, data were divided into three age groups: 0–4 years old, 5–14 years old, and ≥15 years old. Only microscopy-confirmed cases were considered. All data were summarized by month to ease adjustments for seasonality. Participants who were present for at least one week of the month were counted in the denominator for the respective month. Participants with a malaria diagnosis within 30 days of a prior positive blood smear were considered to have recrudescence disease.

Initial assessment of the age-specific frequency of malaria cases was conducted using the chi-square test. Negative binomial regression was used to model monthly malaria incidence as a function of age group, month, and year of observation. Variability in the seasonal pattern of malaria in both sites indicated that a negative binomial regression was more appropriate for modeling incidence than a typical Poisson model. Indicators for the month and year of observation were included to adjust for seasonal transmission patterns. Clinical characteristics, including parasite density and axillary body temperature (°C), were compared across age groups by using linear regression, adjusting for the study period of observation. Parasite density was log-transformed to preserve normality.

The malaria attributable fraction (MAF), or the proportion of fevers (axillary temperature ≥37.5°C) attributable to malaria, was assessed for each age group in each site. The MAF was estimated by the classic equation for the population attributable risk: MAF = p(RRR − 1)/RR, where p is the prevalence of microscopy parasitemia among those with a fever and RR is the relative risk of having a fever in parasitemic versus non-parasitemic persons. Estimates of asymptomatic parasitemia came from cross-sectional studies conducted in 2002 in Kapsisiywa and Kipsamoite; 7.9% of participants were found to be asymptotically parasitemic during the survey.

Calculation of sensitivity and specificity at specific parasite densities was performed using methods developed by Smith and others. Sensitivity for a specific parasite density was defined as the number of symptomatic persons with parasitemia at that density divided by the total number of persons with parasitemia and measured fever. Specificity for a parasite density was defined as the number of asymptomatic persons without parasitemia at that density divided by the number of asymptomatic persons. Calculations for these results were also made with the inclusion of data on asymptomatic parasitemia from the 2002 parasite prevalence survey. Optimal parasite density was chosen as the density in which the graph of sensitivity and specificity crossed. Stata version 10 (StataCorp LP, College Station, TX) was used for all statistical analyses.

The Institutional Review Boards at Case Western Reserve University (Cleveland, OH), the Centers for Disease Control and Prevention (Atlanta, GA), the Kenya Medical Research Institute (Nairobi, Kenya), and the University of Minnesota (Minneapolis, MN) granted ethical approval for this study.

RESULTS

Study population. In Kapsisiywa, 631 participants (18.5% of the population) were enrolled in the active surveillance cohort in April 2003. In Kipsamoite, a previously established active surveillance cohort of 237 persons (7.3% of the population) was re-enrolled in the present study in April 2003. In January 2004, new cohorts of 549 participants in Kapsisiywa (16.1% of the 2003 population) and 575 participants in Kipsamoite (17.7% of the 2003 population) were enrolled for active malaria surveillance. Active surveillance follow-up rates were high and similar between age groups in Kapsisiywa (average percentage of cohort observed per month, 2003, 0–4 years, 94.7%, 5–14 years, 95.2%, ≥15 years, 91.4%; 2004–2005, 0–4 years, 87.4%, 5–14 years, 82.2%, ≥15 years, 87.1%) and in Kipsamoite (2003, 0–4 years, 96.3%, 5–14 years, 91.2%,
In Kapsisiywa, 1,046 blood smears were obtained during the two study periods, of which 214 (20.5%) were positive for *P. falciparum*. In Kipsamoite, 401 blood smears were obtained and 50 (12.5%) were positive for *P. falciparum*. No smears were positive for *P. malariae*, *P. vivax*, or *P. ovale* in either site.

Visits to outside health facilities comprised 31.1% of all clinic visits by study participants. However, visiting an outside clinic was not associated with age or positivity of a blood smear for *P. falciparum*.

**Entomologic inoculation rate.** Malaria transmission parameters in these two sites were estimated from PSC for 2,651 houses with 9,060 residents during April 2004–March 2005. Eighty-five *Anopheles* mosquitoes were collected (84 *Anopheles gambiae* s.l. and 1 *An. funestus*). Thirty-seven of the *Anopheles* mosquitoes had fed on human blood, and one was positive for *P. falciparum* CSP. Therefore, the estimated EIR for the two areas combined was 0.028 infectious bites per person per year.

**Malaria incidence and age distribution.** Annual incidence of malaria was considerably higher in Kapsisiywa than in Kipsamoite (Figure 1). Incidence of malaria over time was highly seasonal in both sites with peaks during March–August of each year. The distribution of malaria cases differed according to age in both study periods and sites ($P < 0.05$ for all comparisons). In both sites, malaria incidence was highest in children less than five years of age, and more than 50% of malaria incidence in both sites occurred in children less than five years of age, although children in this age group constituted only 15.1% and 12.8% of the study population in Kapsisiywa and 7.6% and 18.8% of the study population in Kipsamoite, in 2003 and 2004–2005, respectively (Table 1). In Kapsisiywa, incidence of malaria was 42% lower (95% confidence interval [CI] = 10–63%) (Table 1) in persons 5–14 years of age compared with those less than five years of age, and 61% lower (95% CI = 40–75%) in persons ≥15 years of age after adjusting for seasonality. In Kipsamoite, malaria incidence was not significantly different for persons less than five years of age and those 5–14 years of age, but was significantly decreased in persons ≥15 years of age compared with those less than five years of age (61% decrease; 95% CI = 21–81%).

In a previous study, PCR testing for *P. falciparum* DNA was performed on blood samples obtained from symptomatic persons from this cohort during 11 months of 2004.

The percentage of malaria incidence in children less than five years of age during these 11 months did not differ significantly between malaria as detected by PCR versus microscopy.
The parasite density of symptomatic clinical malaria varied by age, it may be beneficial to augment the clinical definition of malaria with a parasite density cutoff value for each age group. However, the number of smears obtained for PCR was limited, and therefore the number of PCRs performed was small. The study findings are consistent with studies conducted in other highland areas of low and seasonal transmission. These analyses demonstrate a significant reduction in the incidence of uncomplicated malaria with increasing age in two highland areas of Kenya with seasonal, unstable and low, malaria transmission. In addition, a lower parasitemia density at the time of malaria episode was seen in adults ≥15 years of age compared with children <5 years of age in Kapsisiywa, the area of higher transmission intensity. The study findings provide evidence that even in areas of low malaria transmission, where persons have less than one infectious bite per year, increased age is associated with development of significant clinical immunity to uncomplicated malaria. These findings are important for consideration of interventions and risk prediction during epidemics in highland areas because they suggest that older children and adults are at lower risk than children less than five years of age not only for severe malaria, as shown in earlier studies, but also for mild, uncomplicated disease. The study findings also have implications for what may be expected in areas where malaria transmission is reduced but not eliminated. Adults in our study appear to be at a lower risk for clinical malaria than children even in the presence of low transmission intensity, although, importantly, adults were not found to be free from risk and may still be at a higher risk of malaria than adults from areas with moderate and high transmission intensity.

Our study findings are consistent with studies conducted in areas of low, seasonal malaria transmission in Kenya, South Africa, eastern Sudan, and the highlands of Uganda. Early studies in western Uganda suggested that at high altitudes, the parasite rate in persons less than 20 years of age was nearly twice as high as in those persons ≥20 years of age. Although not in a high altitude region, similar results were found in eastern Sudan where malaria transmission is seasonal but sporadic. In Kenya, a study of highland sites nearby Kapsisiywa and Kipsamoite found that the ratio of the number of hospital admissions for malaria in adults compared with children (the adult-to-child ratio) indicated much greater disease in children. Finally, a range of transmission settings studied in South Africa found that a shift in the pattern of disease towards higher incidence in children was only seen.
once the annual incidence exceeded 3–14 cases/1,000 persons, thresholds that are surpassed in Kapsisiya and Kipsamoite during typical seasonal transmission.\(^3\)\(^2\)\(^3\)\(^6\)

In contrast, results of other studies in the highlands of Uganda, Senegal, Mali, and from passive surveillance in Kipsamoite are not consistent with our findings and have suggested that the risk for uncomplicated malaria may remain constant with age.\(^2\)\(^5\)\(^7\)\(^8\)\(^2\)\(^7\)\(^8\)\(^9\)\(^2\)\(^8\)\(^2\)\(^9\) Because the study in Senegal\(^2\)\(^8\) and a recent systematic review\(^1\) surveyed a much younger age range, only up to 11 years of age, those results are not comparable with the primary findings of our study. Although the study in Mali included adults (through an age of 20 years), the reduced risk of uncomplicated malaria might not be fully realized until 20 or 30 years of age. Thus, studies that exclude middle-age community members may still not be able to detect differences in disease incidence as seen in this study.\(^8\)\(^2\)\(^3\)\(^2\)\(^5\)\(^2\)\(^7\)\(^2\)\(^8\)\(^2\)\(^9\)

When the entire population was observed in Kabale District, Uganda, a site at a similar altitude as Kapsisiya and Kipsamoite, an adult-to-child ratio of 1.5 was calculated and most adult cases (42% of cases in persons \(\geq\) 15 years of age) were among persons 15–29 years of age.\(^2\)\(^7\)

Reduced parasite exposure in older children and adults could explain the lower incidence of clinical malaria seen compared with persons less than five years of age. However, in this study population, we believe that adults and adolescents were likely to have greater, not lower, parasite exposure than children less than five years of age. The two greatest risk factors for exposure, living near or traveling to areas of higher vector density within the sites (e.g., areas near a swamp) and travel outside the sites to areas of higher malaria endemicity are likely to be much more frequent in adults and older children than in children less than five years of age. Use of insecticide-treated bed nets (ITNs) may have also modified parasite exposure. Free ITNs were provided by the Ministry of Health for children less than five years of age and pregnant women. Therefore, despite low reported rates of use at these two sites, children less than five years of age were more likely to sleep under an ITN than older persons.\(^2\)\(^9\) In a concurrent case-control study, ITN use was low, and there was no association between ITN use and malaria.\(^3\)\(^0\) Therefore, it is unlikely that ITN use contributed to protection in older persons, and our estimates of the increased risk seen in children less than five years of age may be conservative.

Furthermore, in these areas, we have found that frequencies and levels of IgG against pre-erythrocytic and blood-stage \(P. falciparum\) antigens and interferon-\(\gamma\) responses to pre-erythrocytic antigens are higher in adults than in children.\(^1\)\(^6\)\(^3\)\(^1\)\(^\) These responses would be seen only in persons exposed to \(P. falciparum\), and support the contention that adults in these highland areas have greater exposure to \(P. falciparum\) than children. Finally, exposure to malaria might have been reduced over the course of the study periods because of enhanced detection and prompt treatment of malaria cases. Although this suggestion might account for decreasing incidence trends over the course of the study, it is unlikely that this reduced exposure would act differentially by age to influence the associations seen in this study. Assessing changes in asymptomatic parasitemia over the course of the observation periods would have aided our estimate of the degree of clinical immunity that exists in this community and how that may change with age. Unfortunately, cross-sectional samples of the cohorts were not conducted during the period of observation.

Earlier studies indicate that immunity to malaria increases with the intensity of transmission and can be related in terms of the number of exposures in a lifetime and the interval of time between exposures.\(^3\)\(^2\)\(^3\)\(^3\) The number of episodes of malaria in an area with unstable, low transmission may be lower during any given year, but the recurrent annual exposure may provide sufficient immune boosting for residents to mount an effective immune response over time. It has been suggested that populations living in low but seasonal transmission zones may be able to mount immunity to severe malaria (e.g., severe malarial anemia) after three malaria episodes, but more data is needed to understand the number and frequency of clinical malaria episodes or malaria exposure required to develop immunity to uncomplicated malaria in areas of unstable transmission.\(^3\)\(^9\) Data from an area of low transmission in Mali (EIR < 1 infectious bite/person/year) demonstrated re-infection rates of 80% in all age groups over a 12-week period during peak seasonal transmission, suggesting that exposure to malaria is nearly universal during a season and may be enough to induce clinical immunity.\(^3\)\(^4\)

When we looked at clinical markers of malaria immunity in Kapsisiya, we found that clinical episodes of malaria and body temperature during a malaria episode decreased when persons reached 5–14 years of age, but parasite density decreased only when persons were \(\geq\) 15 years of age. The findings suggest that in this area, development of clinical immunity may precede development of parasitemia control, a finding consistent with prior research in settings of low transmission.\(^3\) However, this pattern was not seen in Kipsamoite, where relatively small numbers of persons showed development of clinical malaria, which might have hindered our ability to detect meaningful changes in clinical markers of disease with age. Parasite density was significantly higher in the area of higher transmission only in persons \(\geq\) 15 years of age, supporting the idea that control of parasitemia may require immunity that comes with increased age and increased exposure.

In addition, there appears to be an inverse association between age and gametocyte prevalence in the oldest age group. Further studies with larger numbers of study participants and with a \(P. falciparum\) zygote/ookinet surface protein (PIS25) reverse transcription-PCR, which is more sensitive than microscopy for detection of gametocytemia, are required to establish if parasite density and gametocyte prevalence are lower in persons \(\geq\) 15 years of age, supporting the idea that control of parasitemia may require immunity that comes with increased age and increased exposure.

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Using a case definition of malaria that included any parasitemia, we found that the proportion of fevers caused by malaria, or the MAF, was similar to those found in highland Uganda.\(^2\)\(^7\) In the study reported by Lindblade and others\(^2\)\(^7\) and the current study, the MAF was highest among persons 5–14 years of age, further demonstrating that the burden of malaria seems to change with age even in areas with low but seasonal transmission. In areas with higher, more stable transmission (EIR = 10 infectious bites/person/year),
the MAF was much higher (50% overall), and was also found to decrease with age, peaking in persons 5–10 years of age. The optimal parasite density for a maximally sensitive and specific case definition was fairly low among persons with clinical malaria in this study. Several other studies have investigated parasite density thresholds in varying malaria transmission settings and, in general, higher altitudes seem to have a lower parasite density threshold and the threshold also tends to decrease with age, as was seen in this analysis.

Of note from this study, 20% of persons with clinical malaria had a parasitemia less than 100 parasites/μL, the density at which sensitivity for detection of infection is decreased in many rapid diagnostic tests. Furthermore, in a subset of persons in this cohort who had symptoms of malaria, we found that PCR detected many infections not detected by microscopy, and that the MAF increased when assessed by PCR than when assessed by microscopy. Careful study will be required to ensure that field diagnostic systems used in highland areas of low transmission do not miss a small, but significant, proportion of persons with clinical malaria and low-density, or subpatent, parasitemia.

In summary, the present study suggests that in highland areas of unstable and low transmission, significant clinical immunity to uncomplicated malaria seems to develop with age and reduce the burden of disease in adults. This immunity may result from repeated intermittent exposure, changes in the nature of the immune response with age, or, most likely, a combination of these factors. Future studies will continue to investigate immune correlates of protection in these populations. The study findings suggest that interventions that decrease but do not completely eliminate seasonal malaria transmission may still enable development of some immune protection among adults. In addition, the study results support the importance of interventions targeted at young children even in low transmission settings. However, in both Kapsisiywa and Kipsamoite, there remained a substantial proportion of uncomplicated malaria in adults. Thus, although there is clearly a degree of age-related immunity in these populations, it is incomplete and area-wide interventions that include populations of all ages will remain critical to campaigns for malaria elimination and eradication.

Received August 15, 2011. Accepted for publication August 1, 2012.

Acknowledgments: We thank the study participants in Kipsamoite and Kapsisiywa for their participation in this study, the field assistants and David Koech for collecting study data, and Jackson Abuya and the late Livingstone Wanyama for performing microscopy for this study. This paper was published with the permission of the Director of the Kenya Medical Research Institute.

Financial support: This study was supported in part by National Institutes of Allergy and Infectious Diseases grants U01 AI056270, K08 AI01572, and D43 TW008085 to Chandy C. John.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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