SUPPLEMENTAL INFORMATION

Study populations. Children were eligible for inclusion if they were generally healthy and between 1 and 14 years of age at the beginning of the study. The cohort was recruited with a goal of obtaining approximately equal numbers of participants in age groups 1–4, 5–9, and 10–14 years of age. Children were recruited from two areas of western Kenya: 1) Kisumu, which consisted of six villages in this lowland area along the shore of Lake Victoria where malaria transmission is holoendemic, and 2) Nandi, which consisted of seven villages in the highlands approximately 150 km northeast of Kisumu. In Nandi, malaria transmission is hypoendemic, characterized by periodic epidemics interspersed with periods of low transmission. We did not have information about certain potential confounders of the relationship between immune function and parasitemia, including human immunodeficiency virus (HIV) status. During this study a decade ago, HIV testing of Kenyan children was only allowed when clinically warranted. Since this was a village-based study of healthy children, we did not test for HIV infection; however, since the study participants were deemed healthy over the course of the 2-year study, this is less likely to have impacted our findings.

MSP1 antibody levels. Recombinant antigens for the 3D7 and FVO genotypes of the merozoite surface protein 1_{42} ($MSP1_{42}$) antigen were expressed as described elsewhere.[1,2] IgG specific for these antigens were detected using a bio-Plex bead-based assay.[1] One thousand beads of each malaria antigen were placed in wells with plasma from participants and diluted 1:5,000. Included on each plate were negative controls (U.S. residents with no history of malaria) and positive controls (pooled samples from Kisumu residents). Preparation of beads and testing were conducted on all samples at the same time on the same machine to reduce potential variation due to differences in bead preparation and assay. Although antibody results were calculated as mean fluorescence intensity (MFI), slight plate-to-plate variation led to a need to standardize results. Therefore, results are expressed in arbitrary units (AU). For each plate, the participant's AU values were calculated by dividing each participant's MFI antibody response by the negative controls' mean MFI plus three standard deviations (SDs). A positive IgG response was defined as an AU value greater than 1.0.

MSP1 IFN-γ ELISPOT. The same recombinant malaria antigens specific to the $MSP1_{42}$ antigens of 3D7 and FVO *Plasmodium falciparum* strains used for antibody assays described above were used to measure cellular responses determined by enzyme-linked immunosorbent spot assays (ELISPOT) for interferon-gamma (IFN-γ) as described previously.[3,4] In brief, peripheral blood mononuclear cells were incubated for 84 hours with 5 μL $MSP1_{42}$ 3D7 or FVO antigen and the number of spot-forming units in the well was counted. A positive ELISPOT response was defined as a number of spot-forming units that was significantly greater than the number in the negative control well by Fisher's exact test with $P < 0.05$.

MSP1 genotyping. *Plasmodium falciparum* DNA was extracted using Qiagen DNA Mini kits (Qiagen, Valencia, CA) from the blood of participants found to be parasitemic by microscopy. The block 16 section of the MSP1 gene, corresponding to T-cell epitopes in the $MSP1_{33}$ fragment, was then analyzed as previously described using allele-restricted polymerase chain reaction (PCR).[5] In brief, 2 μL of extracted DNA was added to 22.5 μL of PCR Super Mix (Life Technologies, Grand Island, NY) and 400 nM each of forward and reverse primers. The same C3FIR reverse primer (ATTA AGGTAACATATTTTAACTCCTAC) was used for all reactions in conjunction with allele-specific forward primers (M16F forward primer [CCTAATACAATAATATCAAAA TTAATTGA] detects 3D7) and K16F forward primer [CC GTTTTATCTAATTTACTTGATGGAA] detects FVO).[5] Reaction conditions were 45 cycles of 95°C for 30 seconds, then 56°C for 30 seconds, then 72°C for 30 seconds, followed by a 4°C hold. Amplification products were run on a 2% agarose gel yielding expected fragment sizes of 428 base pairs (bp) for 3D7 and 420 bp for FVO. With each reaction, 3D7 and FVO positive controls as well as a negative control (water) were included.

Study population characteristics. Children who dropped out were 1.34 years older than those included in both time points ($P = 0.03$). Observations were available at baseline in 92 participants in Kisumu and 118 in Nandi. In 2004, observations were available for 74 participants in Kisumu and 100 in Nandi. Mean age in February 2003 was 7.9 years (SD = 3.4, range = 1.5–16.1). Ninety-seven participants were male and 113 were female, with no differences in age or sex between sites. All participants were generally healthy, but asymptomatic parasitemia with *P. falciparum* was common.

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SUPPLEMENTAL TABLE 1

Prevalence and magnitude of positive antibody responses in study sites with divergent malaria transmission intensity

		Kisumu			Nandi		Between sites	
		No. positive (%)	AU Median (IQR)*	*P* value†	No. positive (%)	AU Median (IQR)*	*P* value†	*P* value*‡
MSP1 3D7				0.002			0.06	
	February 2003	80 (88)	3.96 (2.73–5.99)		65 (56)	3.84 (2.14–6.52)		0.6
	November 2004	65 (90)	5.53 (3.58–7.29)		54 (54)	2.96 (1.57–5.93)		0.002
MSP1 FVO				0.04			0.3	
	February 2003	55 (60)	2.72 (1.56–3.58)		58 (50)	2.77 (1.65–3.42)		0.98
	November 2004	52 (72)	3.38 (1.95–4.82)		40 (40)	2.27 (1.51–4.25)		0.07

AU = arbitrary unit; ELISPOT = enzyme-linked immunosorbent spot assays; IQR = interquartile range; MSP = merozoite surface protein.
*Measures describing and comparing AU values were reported only for samples with a positive antibody response.
†*P* value for the difference in magnitude of ELISPOT response between years.
‡*P* value for the difference in magnitude of ELISPOT response between sites.

SUPPLEMENTAL TABLE 2

Prevalence and magnitude of positive IFN-γ ELISPOT in each study site

	Kisumu			Nandi		Between sites	
	No. positive (%)	Median (IQR)*	*P* value†	No. positive (%)	Median (IQR)*	*P* value†	*P* value*‡
3D7			0.2			< 0.0005	
February 2003	28 (33)	9 (6.5–16.5)		50 (47)	41 (22–218)		< 0.0005
November 2004	28 (37)	15.5 (8–37.5)		17 (18)	10 (6–16)		0.1
FVO			1			0.0007	
February 2003	4 (5)	5.5 (5–7)		19 (17)	36 (15–836)		0.2
November 2004	2 (3)	14 (8–10)		2 (2)	7 (5–9)		0.4

ELISPOT = enzyme-linked immunosorbent spot assays; IFN = interferon; IQR = interquartile range; MSP = merozoite surface protein.
*Measures describing and comparing AU values were reported only for samples with a positive antibody response.
†*P* value for the difference in magnitude of ELISPOT response between years.
‡*P* value for the difference in magnitude of ELISPOT response between sites.