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Article *in* Infection and immunity · July 2008

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INFECTION AND IMMUNITY, June 2008, p. 2706–2714 Vol. 76, No. 6
0019-9567/08/$08.00+0 doi:10.1128/IAI.01401-06

Differences in Human Antibody Reactivity to *Plasmodium falciparum* Variant Surface Antigens Are Dependent on Age and Malaria Transmission Intensity in Northeastern Tanzania[▽]

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Received 31 August 2006/Returned for modification 7 October 2006/Accepted 24 January 2008

***Plasmodium falciparum* variant surface antigens (VSA) are involved in the pathogenesis of malaria. Immunoglobulin G (IgG) with specificity for VSA (anti-VSA IgG) is therefore considered important for acquired immunity. To better understand the nature and dynamics of variant-specific IgG responses at population level, we conducted an immunoepidemiological study in nearby communities in northeastern Tanzania, situated at different altitudes and therefore exposed to different levels of *P. falciparum* transmission intensity. Samples of plasma and infected red blood cells (IRBC) were collected from 759 individuals aged 0 to 19 years. Plasma levels of IgG with specificity for VSA expressed by a panel of different parasite isolates were measured by flow cytometry, while the ability of plasma to inhibit IRBC adhesion to CD36 was examined in cellular assays. The level and repertoire of the heterologous anti-VSA IgG response developed dramatically in individuals at 1 to 2 years of age in the high-transmission area, reaching a maximum level at around 10 years of age; only a modest further increase was observed among older children and adults. In contrast, at lower levels of malaria transmission, anti-VSA IgG levels were lower and the repertoire was more narrow, and similar age- and transmission-dependent differences were observed with regard to the ability of the plasma samples to inhibit adhesion of IRBC to CD36. These differences indicate a strong and dynamic relationship between malaria exposure and functional characteristics of the variant-specific antibody response, which is likely to be important for protection against malaria.**

In areas where malaria is endemic, the age-specific burden of *Plasmodium falciparum* infection and clinical disease are closely related to the level of malaria transmission. In high-transmission areas the youngest children suffer from high parasite loads and frequent episodes of disease, while older individuals are better able to control parasitemia and in general only suffer from mild malaria episodes. In contrast, in areas with low levels of malaria transmission, the incidence and severity of clinical disease in adults remains similar to that of children (18, 25, 38, 39). This transmission-dependent difference is in agreement with the concept that immunity to malaria is acquired as a result of antigenic stimulation through repeated parasite infections from early childhood onwards (28).

Among immune responses associated with protection against clinical malaria are immunoglobulin G antibodies with specificity for variant surface antigens (VSA) expressed on the surface of *P. falciparum*-infected red blood cells (IRBC) (6, 7, 9, 16, 26, 27). The best-studied VSA, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), mediates the binding of IRBC to endothelial receptors such as CD36 and ICAM-1 (13, 24, 36). This IRBC adhesion enables the parasites to avoid splenic clearance (2, 8, 29). The development of clinical immunity coincides with the gradual acquisition of a broad repertoire of VSA-specific antibodies (6, 20). Each new parasite infection induces a variant-specific immunoglobulin G (IgG) response, with specificity for the VSA expressed by the infecting parasite (23, 33). This response appears to protect the host from future clinical episodes arising from parasites expressing antigenically similar VSA. VSA expressed by parasites isolated from children with severe disease have been found to be more commonly recognized than VSA expressed by parasites isolated from children with nonsevere disease (4, 5, 31). It has been suggested that, in high-transmission areas, infants and young children quickly acquire antibodies and protection against malaria parasites expressing VSA types associated with severe disease outcomes, while in the following years of life individuals gradually expand their anti-VSA IgG repertoire toward parasites expressing VSA associated with uncomplicated malaria (20). According to this hypothesis, the rate of acquisition of IgG repertoires to VSA would also be assumed to be lower in low-transmission areas. To tests these assumptions in order to better understand the dynamics of naturally acquired heterologous anti-VSA IgG responses at the population level, we conducted an immunoepidemiological study among individuals

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▽ Published ahead of print on 4 February 2008.

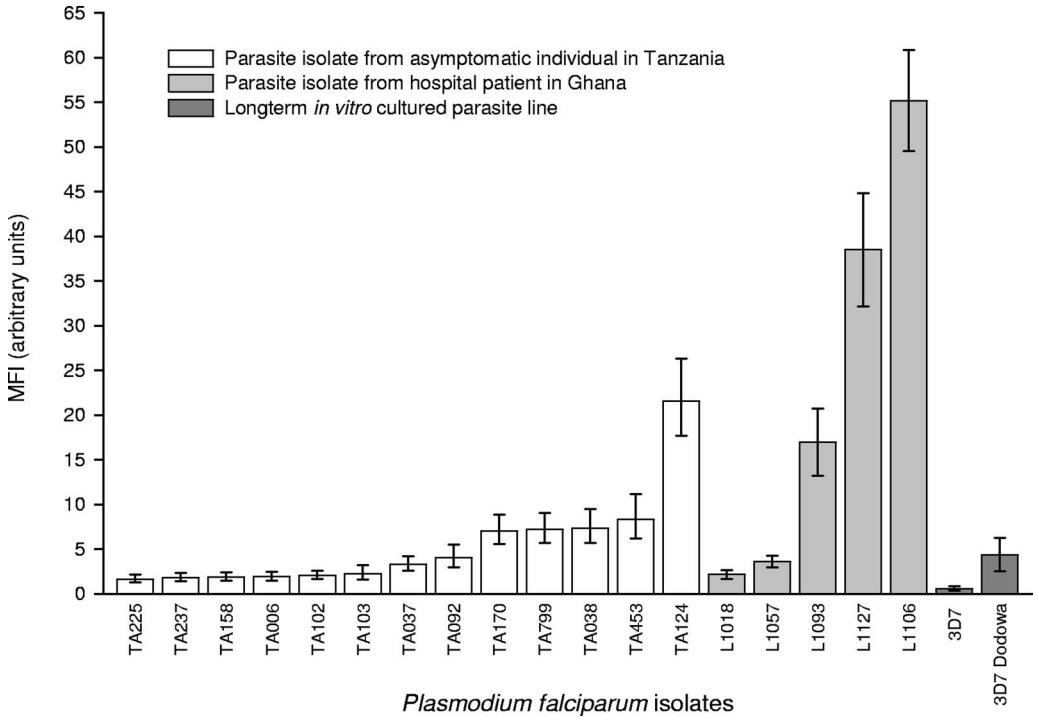

FIG. 1. Levels of anti-VSA IgG in Ghanaian children against *P. falciparum* isolates of different origin. Plasma from 96 healthy children (3 to 8 years old) living in an area of hyperendemic malaria transmission were tested against 13 parasite isolates from asymptomatically infected children in Tanzania, 5 isolates from children suffering from malarial illness in Ghana (31), and 2 laboratory-based parasite isolates, 3D7 and the in vitro-selected 3D7 Dodowa isolate (41), respectively. The vertical bars represent geometric mean MFI values corrected for assay-specific background reactivity defined as the mean reactivity of the negative controls plus two SD. Error bars represent 95% confidence intervals.

living in areas of different altitudes and therefore exposed to different intensities of malaria transmission in northeastern Tanzania (3, 12). By flow cytometry we examined the level and repertoire of anti-VSA antibodies in different age groups, and we measured the adhesion-inhibitory effect of the donor plasma in a CD36-specific adhesion inhibition assay.

MATERIALS AND METHODS

Study sites and populations. The study was conducted in the Tanga region in northeastern Tanzania. This area is characterized by marked variations in intensity of *P. falciparum* transmission related to variations in altitude. Very intense perennial transmission, with reported entomological inoculation rates (EIRs) in the range between 91 and 405 infective bites per person per year, is found in the lowland areas toward the Indian Ocean, with peak seasons following the long rains in May and the short rains in November. Moderate but stable transmission is found at intermediate altitudes of around 1,000 to 1,200 meters above sea level (EIRs in the range 1.8 to 34 infective bites per person per year reported), while very low and unstable transmission is found in highland areas at around 1,600 to 1,800 meters above sea level, with an estimated EIR of only 0.03 infective bites per person per year (3). Three study villages were selected; these were located within short geographical distances but at different altitudes and thus had different malaria transmission intensities: Mgome village at low altitude (200 m), Ubiri village at intermediate altitude (1,100 to 1,200 m), and Magamba village at high altitude (1,600 to 1,700 m). In each village, cohorts of approximately 250 healthy individuals between 0 and 19 years of age were randomly recruited for cross-sectional and longitudinal malariometric studies from April to September 2001; these results are described in detail elsewhere (25). The villages were selected according to predefined criteria to minimize differences in socioeconomic status, ethnicity, seasonal migration, and access to health care, as assessed by village-level socioeconomic surveys. In April and May 2001 the *P. falciparum* parasite prevalence and density in the 0- to 19-year-old age group as a whole was as follows: a point prevalence of 81.1% and a mean parasite density of 771/μl in Mgome, a point prevalence of 40.8% and a mean parasite density of 391/μl in Ubiri, and a point prevalence of 12.1% and a mean parasite density of 127/μl in Magamba. Parasite counts in Mgome showed the typical pattern of a high-transmission village with a particularly high parasite burden in children less than 5 years old, while no such age-specific difference were observed in Ubiri and Magamba, respectively. Thus, malaria transmission levels and levels of acquired malarial immunity differed markedly between the three study villages. Informed written consent was obtained from all study participants or from their parents or guardians. The study protocol was approved by the Ethical Committee of the National Institute for Medical Research and Ministry of Health, Dar es Salaam, Tanzania.

Blood samples. Blood samples were collected from all study individuals during cross-sectional surveys in the three study villages in April 2001 before the peak transmission season. From children under the age of 2 to 3 years, 200- to 300-μl samples of finger prick blood were collected in Eppendorf tubes with EDTA. From older individuals, 5-ml samples of venous blood were collected into Vacutainer tubes with citrate buffer. Hemoglobin concentrations were measured with a HemoCue photometer (Ångelholm, Sweden), and thick and thin malaria blood smears were prepared and examined according to standard procedures. After centrifugation, all blood samples were separated into plasma and red blood cells (RBC); plasma samples were frozen and stored at minus 20°C, while IRBC mixed with sorbitol freezing solution were snap-frozen and stored in liquid nitrogen.

To test how well the parasite isolates obtained from Tanzanian children were recognized by individuals living in another part of the African continent, we also analyzed plasma from 96 asymptomatic children aged 3 to 8 years, obtained as part of previous studies in an area of moderate malaria transmission in Ghana (32).

Parasite isolates and in vitro cultivation. Cryopreserved parasite isolates collected from 13 asymptomatically infected individuals in the high-transmission area were randomly picked, thawed, and successfully adapted to in vitro cultures with O^+ blood and culture medium according to previously described procedures (15). PCR was used to genotype the parasite genes *msp-1* block 2 (primers recognizing allelic variants of MAD20, K1, and RO33) and *msp-2* (primers recognizing allelic variants of FC27 and IC1) of each parasite sample as previously described (37). To compare

TABLE 1. Proportion of positive anti-VSA IgG responders

Village and subject age in yr (*n*)[a]	Proportion (%) of positive anti-VSA IgG responders[b]						
	P. falciparum isolates						Mean
	TA225	TA006	TA170	TA799	TA453	TA124	
Low-transmission village							
<1 (25)	4.0	0.0	0.0	4.0	4.0	0.0	2.0
1–2 (46)	6.5	17.4	28.3	10.9	6.5	15.2	14.1
3–4 (52)	3.8	3.8	19.3	1.9	7.7	7.7	7.3
5–9 (50)	28.0	16.0	26.0	10.0	24.0	40.0	24.0
10–14 (42)	35.7	31.0	19.0	14.3	23.8	28.6	25.4
15–19 (40)	30.0	27.5	12.5	20.0	25.0	27.5	23.8
Total (255)	18.3	16.5	19.0	10.2	16.7	21.2	17.0
Moderate-transmission village							
<1 (23)	52.2	4.3	30.4	21.7	8.7	17.4	22.5
1–2 (46)	78.3	50.0	50.0	45.7	6.5	43.5	45.7
3–4 (30)	70.0	46.6	76.7	70.0	6.7	56.7	54.5
5–9 (67)	70.1	67.2	70.1	83.6	26.9	67.2	64.2
10–14 (48)	100.0	87.5	85.4	93.8	54.2	85.4	84.4
15–19 (36)	100.0	88.9	97.2	97.2	80.6	91.7	92.6
Total (250)	80.0	62.8	70.4	73.2	32.0	64.0	63.7
High-transmission village							
<1 (19)	73.7	10.5	73.6	57.9	15.8	52.6	47.4
1–2 (44)	97.7	90.9	93.2	97.7	52.3	90.9	87.1
3–4 (40)	100.0	100.0	100.0	100.0	90.0	97.5	97.9
5–9 (69)	100.0	100.0	100.0	100.0	97.1	100.0	99.5
10–14 (51)	100.0	100.0	100.0	100.0	96.1	100.0	99.4
15–19 (31)	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total (254)	97.6	91.7	96.9	96.5	82.3	94.5	93.3

[a] *n*, Number of individuals tested.
[b] Anti-VSA IgG above the antibody cutoff is defined as the mean of negative controls plus two SD.

the recognition of the parasites obtained from asymptomatic children in Tanzania with the recognition of parasites from children with strictly defined malaria, five parasite isolates obtained from children admitted to Korle-bu Hospital in Accra in Ghana as part of previous studies (31) were included for analysis. These isolates were selected on the basis of previous knowledge about their VSA expression to represent a spectrum from poorly recognized to well-recognized parasites. Furthermore, the two laboratory lines 3D7 and 3D7 Dodowa were tested, of which 3D7 Dodowa had been selected to express VSA shown to be associated with severe malaria in young children (21, 41).

Analysis of *P. falciparum* anti-VSA IgG by flow cytometry. The anti-VSA plasma IgG levels were measured by flow cytometry as previously described (40). In vitro cultures with the majority of parasites in the late trophozoite and schizont stages and parasitemias of 2 to 3% were enriched to >75% parasitemia by exposure to a strong magnetic field (34, 40). Aliquots of 2×10^5 purified IRBC labeled with ethidium bromide were sequentially incubated for 30 min with 5 μl of human plasma, 0.4 μl of goat anti-human IgG (Dako, Glostrup, Denmark), and 4 μl of fluorescein isothiocyanate-conjugated rabbit anti-goat IgG (Dako). Samples were washed twice in phosphate-buffered saline between each incubation step. A titrated hyperimmune reference plasma pool and plasma from six Danish individuals with no previous exposure to malaria served as positive and negative controls, respectively. A minimum of 5,000 events were recorded for each parasite-plasma combination, measured on a Coulter EPICS XL-MCL flow cytometer (Coulter Electronics, Luton, United Kingdom) and thereafter analyzed with WinList software (version 5.0; Verity Software, Maine). IRBC and RBC were gated according to the ethidium bromide fluorescence, and for each sample the geometric mean fluorescence index (MFI) was recorded as a measure of the amount of anti-VSA IgG present with specificity for that particular parasite isolate. Nonspecific labeling was evaluated by analysis of ethidium bromide-negative RBC. Plasma samples obtained at a particular study site and relating to a particular parasite isolate were processed and analyzed in a single assay. Samples from the three study sites were tested within as few parasite growth cycles as possible (typically one or two) to minimize any interassay variations arising from antigenic variation of the isolate during its cultivation.

A positive anti-VSA IgG response was defined as a MFI value above the mean plus two standard deviations (SD) of negative control samples. To calculate cumulated antibody responses and to be able to compare anti-VSA IgG levels between parasite isolates and plasma samples, we subtracted the mean plus two SD of log MFI values obtained with the six control samples from all test MFI values. To further allow for direct comparisons of antibody recognition of the different parasite isolates, a standardized score was assigned to each parasite-plasma combination. Adjusted test sample MFI values above the MFI of the undiluted (1:1) hyperimmune control pool were assigned an anti-VSA IgG score of 5. Values between the 1:1 and 1:2 dilutions of the positive control pool were assigned an antibody score of 4, values between the 1:2 and 1:4 dilutions of the positive control pool were assigned an antibody score of 3, and so on, until samples with MFI levels below 1:16 dilutions of the positive control pool were assigned an antibody score of 0.

CD36-specific parasite adhesion inhibition assays. Inhibition of CD36-specific cytoadhesion with the donor plasma samples were examined according to the method described by Hasler et al. (19) with some modifications. A 3D7 parasite isolate was selected for CD36 adhesion by panning the isolate in vitro on CD36-transfected Chinese ovarian hamster (CHO) cells (40). The CD36-specific IRBC were radiolabeled by incubating the cultures overnight in the presence of ^{3}H-labeled phenylalanin (1 MBq for a standard culture containing 200 μl of packed IRBC). Prior to the assay, CD36-transfected CHO cells were grown to a monolayer in 96-well microtiter plates (Nunc, Roskilde, Denmark). Wild-type CHO cells were used as negative controls. IRBC with late-stage parasites (100 μl, 10^7 RBC/ml), enriched by gelatin sedimentation (22), were added to the CHO cell monolayer and incubated for 1 h at 37°C before unbound RBC were washed away from the cell monolayer. The number of IRBC remaining in the wells after the washing step was quantified by measuring the ^{3}H activity in the wells by liquid scintillation. The ability of the plasma to inhibit this CD36-specific adhesion was tested in duplicates by the addition of 10 μl of undiluted plasma to the microtiter wells with the CHO-CD36 cell monolayer and IRBC before incubation. To confirm the CD36 specificity of the assay, wells with human immune plasma samples were compared to wells with 5 μl of monoclonal anti-CD36 antibodies or anti-ICAM antibodies, respectively (Dako), which were added in triplicates. Levels of maximum CD36 adhesion were defined as the mean reactivity in wells

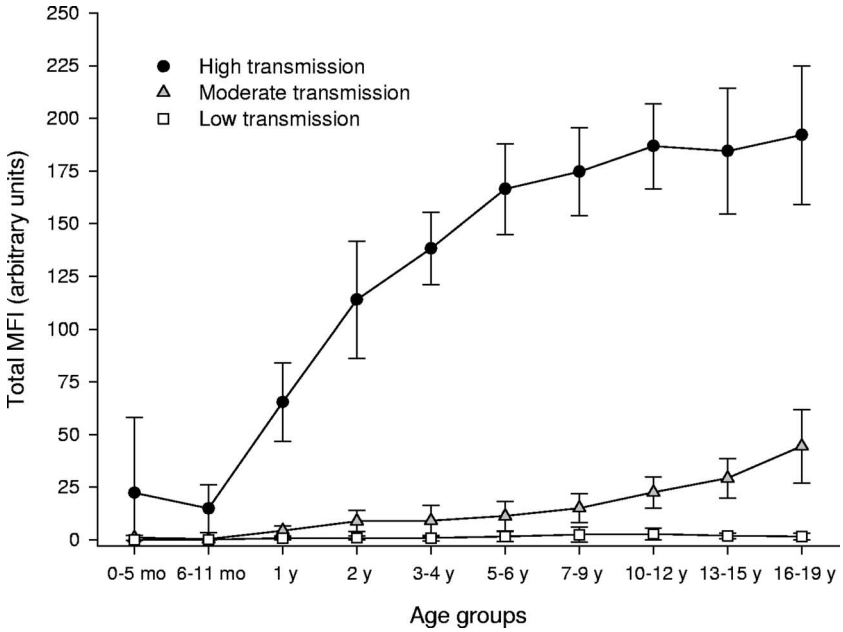

FIG. 2. Cumulative levels of anti-VSA IgG in Tanzanian plasma donors by age and intensity of malaria transmission. The symbols indicate levels of total cumulative anti-VSA IgG against six Tanzanian *P. falciparum* isolates (TA225, TA006, TA170, TA799, TA453, and TA124), where all plasma samples have been tested against each of the six isolates at a single dilution and where the MFI values for each isolate have been adjusted for assay-specific background reactivity before the MFI values for all six isolates were totaled. Plasma samples from all study individuals from each of the three study villages areas with high ($n = 254$), moderate ($n = 250$), and low ($n = 254$) intensities of malaria transmission, respectively, were included and stratified according to age. Error bars indicate 95% confidence intervals. y, years; mo, months.

coated with CHO-CD36 cells and incubated with IRBC and 10 μl of normal human serum from individuals never exposed to malaria.

Statistical analysis. All data were double entered into the Epi-Info database (version 6.04; Centers for Disease Control and Prevention, Atlanta, GA), and statistical analyses were done with STATA version 8 (STATA Corp., Texas). Differences betweens means and proportions were compared by the Student *t* test and χ^2 test, respectively. The Pearson correlation test was used to examine pairwise correlations between cumulated MFI responses of individual plasma samples and the degree of CHO-CD36/IRBC adhesion inhibition. *P* values of <0.05 were considered significant.

RESULTS

Recognition of Tanzanian parasites by plasma antibodies from Ghanaian children. The tested field parasite isolates from Tanzania ($n = 13$) and Ghana ($n = 5$) each had a unique genotype as determined by *msp-1* and *msp-2* PCR analysis. Most parasite samples were polyclonal, with a median clone number of three per sample (minimum, 1; maximum, 5). No correlations between the estimated clone number and the level of antibody recognition were found (data not shown).

The parasites obtained from the asymptomatic carriers in Tanzania were all found to be recognized by plasma antibodies of the asymptomatic Ghanaian children (Fig. 1). The levels of anti-VSA IgG against these parasites were generally low. By comparison, the Ghanaian children had higher plasma antibody levels against the parasites that had been isolated from Ghanaian children admitted to hospital with malaria. The unselected 3D7 parasite line was poorly recognized by plasma antibodies from the asymptomatic Ghanaian children, while the 3D7 Dodowa isolate was more strongly recognized (Fig. 1).

Level and repertoire of anti-VSA IgG in Tanzanian plasma donors. To examine age-specific acquired levels of anti-VSA antibodies at the different levels of transmission, plasma samples from all study individuals ($n = 759$) in each of the three study villages were tested against six of the Tanzanian parasite isolates (TA225, TA006, TA170, TA799, TA453, and TA124). These parasites were chosen on the basis of the initial screening (Fig. 1) to ensure the inclusion of both "well" and "poorly" recognized parasites in this analysis. There were very marked differences between the three villages with respect to the proportion of individuals who had a measurable anti-VSA IgG response to the different parasite isolates (Table 1, the proportions of positive antibody responders are listed for each of the six parasite isolates and for each age group and are further summarized as the mean proportions of responders for the combined collection of parasites across all age groups). In the low-transmission area, the average recognition of the six tested isolates were 2% in children <1 year of age and peaked at 25% in the children aged 10 to 14 years. In the moderate-transmission area, an average parasite was recognized by 92.6% in 15- to 19-year-old individuals, whereas in the high-transmission village this figure was 97.9% in children aged 3 to 4 years.

Similar differences between the three transmission settings were observed with respect to the mean plasma anti-VSA IgG levels at different ages (Fig. 2). In the high-transmission village, the amount of anti-VSA IgG remained constant or dropped slightly during the first year of life but thereafter increased rapidly and reached a plateau around 10 years of age. In the moderate-transmission village, the amounts of anti-VSA IgG increased slowly and did not reach a plateau. In the low-transmission area, anti-VSA IgG levels remained low in all age groups.

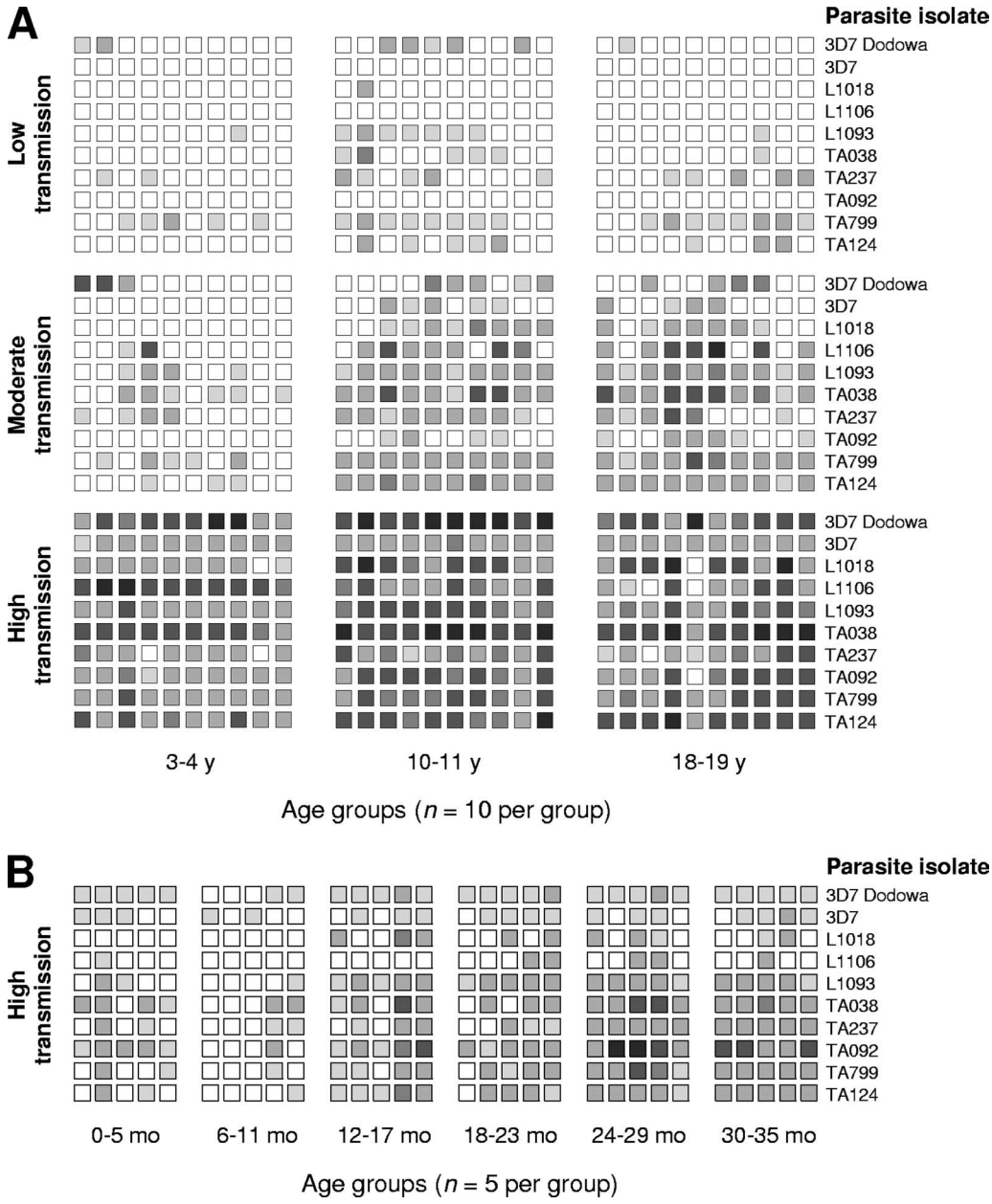

FIG. 3. Repertoires of anti-VSA IgG in Tanzanian plasma donors by age and intensity of malaria transmission. Each square represents one plasma donor, tested against a panel of parasite isolates obtained in Tanzania (TA038, TA237, TA092, and TA124) and Ghana (L1018, L1106, and L1093) and two laboratory isolates (3D7 and 3D7 Dodowa). All values have been adjusted for assay-specific background MFI reactivity defined as the mean of six negative controls plus two SD. To allow for a direct comparisons between the different isolates, a standardized score 0 to 5 was assigned to each parasite-plasma combination, signifying the antibody level of each sample relative to the geometric mean level of MFI values against all of the testes parasite isolates (see the text for further details). Each score were then assigned a color according to its level from white (lowest antibody score) to black (highest antibody score). (A) Antibody scores compared between children and young adults in each of the three study areas with low, moderate, and high transmission of malaria, respectively. (B) Antibody scores compared between infants and young children living in the high-transmission area. y, years; mo, months.

To further examine the age-specific repertoire of anti-VSA IgG acquired by the plasma donors in the three transmission settings, a broader panel of parasites were tested. This panel included five Tanzanian parasite isolates (TA124, TA799, TA092, TA237, and TA038), three Ghanaian isolates (L1093, L1106, and L1018), and the 3D7 and 3D7 Dodowa lines. In these experiments, plasma samples from all three villages were analyzed in the same assay to allow direct comparisons of anti-VSA IgG responses between transmission settings. The number of plasma donors was therefore limited to 30 from each village and comprised 10 randomly selected individuals of three age groups: 3 to 4 years, 10 to 11 years, and 18 to 19 years. As shown in Fig. 3A, the proportion of parasites recognized and the anti-VSA IgG levels increased with the trans-

TABLE 2. Proportion of positive parasite-plasma combinations

Age (yr) of plasma donors	Proportion (%) of positive parasite-plasma combinations[a]			P^b
	Low transmission	Moderate transmission	High transmission	
3–4	10	27	95	<0.001
10–11	27	78	100	<0.001
18–19	19	74	95	<0.001

[a] Anti-VSA IgG tested in samples of plasma from 10 individuals against 10 parasite isolates for each age group.
[b] Determined by Kruskal-Wallis one-way analysis of variance on ranks.

mission intensity. In the high-transmission village, broad repertoires of VSA-specific IgG were already acquired by subjects at the age of 3 to 4 years. In the moderate-transmission village, broad repertoires of antibodies were not acquired until around 10 to 11 years of age, while in the low-transmission village the antibody repertoires remained narrow even in the oldest age group. Proportions of positive parasite-plasma combinations by age and transmission level are listed in Table 2.

Antibody responses to VSA among children younger than 3 years were examined in the high-transmission village (Fig. 3B). The antibody repertoire among 0- to 5-month-old infants appeared to be broader than among the 6- to 11-month-old children (34% versus 10% of tested parasites recognized), although this difference was not statistically significant ($P = 0.08$). From the age of 6 to 11 months, children rapidly developed a broader repertoire of variant-specific antibodies, and in the group of children aged 12 to 17 months the mean number of parasites recognized had increased to 60% ($P = 0.004$ compared to 34% of parasites recognized by children aged 0 to 5 months). Thus, in the high-transmission village, VSA-specific IgG responses developed rapidly and antibody repertoires in 1- to 2-year-old children were comparable to the antibody repertoires in the 10- to 11-year-old children living in the moderate-transmission village (Fig. 3).

Antibody inhibition of parasite adhesion to CD36. The relationship between malaria transmission and the ability of plasma to inhibit adhesion to CD36 was investigated in an assay measuring the adhesion of a CD36-selected parasite line to a monolayer of CD36-transfected CHO cells. In this assay approximately half of the maximal adhesion can be inhibited by anti-CD36 monoclonal antibodies (Fig. 4). We first compared plasma samples from the 10 18- to 19-year-old individuals from each of the three study villages and found that the adhesion-inhibitory effect was highest in plasma samples collected in the high-transmission village (Fig. 4A). Acquisition of the adhesion inhibition capacity by age in this village was investigated by comparing samples obtained from different age groups. The inhibitory effect was higher in the children aged 10 to 11 years than in the group aged 3 to 4 years ($P < 0.004$). In addition, a positive correlation was found between the cumulated MFI level of the individual sample and its capacity to inhibit CD36-specific cytoadhesion (Pearson correlation test, $R = -0.63$, $P < 0.001$) (Fig. 4C).

DISCUSSION

The importance of variant-specific antibodies against *P. falciparum* for protection against malaria is well documented. Antibodies to VSA mediate protection against severe malaria (6, 27, 43), uncomplicated malarial fevers (9, 16), and malaria in pregnancy (11, 35, 42); however, our understanding of the dynamics of naturally acquired anti-VSA IgG responses at the population level remains limited. To elucidate how anti-VSA responses are influenced and shaped by cumulative malaria exposure, we examined the acquisition of anti-VSA IgG in different age groups among populations residing in nearby communities at different altitude levels and therefore at markedly different levels of malaria transmission.

Major differences in the acquisition of VSA-specific antibodies were observed. In the high-transmission village, 3- to 4-year-old children had already acquired antibodies to most parasites and the anti-VSA IgG levels increased rapidly with age and reached a high-level plateau at about the age of 10 years. This suggests that the overall level of anti-VSA IgG saturates and is maintained in an ongoing dynamic process with decay of old and acquisition of new VSA antibodies. In the high-transmission village, most if not all individuals were infected with *P. falciparum* blood-stage parasites, and in children aged 1 to 2 years these were carried at high densities. From age 3 to 10 years the carrier rate and the mean parasite density declined, and this coincided with the drop in malaria fever incidence rate, which decreased markedly after the age of 4 years (25).

In the moderate-transmission setting most parasites were recognized by more than 90% of individuals aged 15 to 19 years, but the levels of antibodies were much lower than in the plasma samples collected in the high-transmission village. In this village about half of the volunteers were infected, and there were no marked differences in the parasite point prevalence between the ages of 1 and 19 years. We do not have age-specific morbidity data from this village, but in a village of coastal Ghana with similar carrier rates, the incidence of febrile malaria declined after 10 years of age (10). It is interesting that the age at which children had acquired a broad anti-VSA response in the moderate- and high-transmission villages largely coincided with the age at which clinical immunity would be predicted to occur. The levels of VSA-specific antibodies were markedly higher in individuals living in the high-transmission village than in individuals from the moderate-transmission village, despite the fact that a high proportion of the individuals in the moderate-transmission village were also infected with blood-stage parasites. This finding suggests that it is the number of new infections, which is determined by the intensity of transmission, rather than the presence of parasites per se, that determines the level of anti-VSA antibodies.

In the low-transmission village, the anti-VSA repertoire was very incomplete, and the level of antibodies to parasites recognized was low. In this village, the carrier rate was ca. 10%. In areas of low and seasonal transmission anti-VSA antibody levels show marked seasonal variations (17), and the measured levels of anti-VSA IgG might have been higher if samples had been collected just after the peak transmission season. In a Sudanese village with a similar parasite point prevalence, we previously found that individuals of all age groups regularly suffer from febrile malaria attacks. However, the malaria incidence rate is lower in adults than in children and adolescent (14), and it is possible that some adults mount efficient and

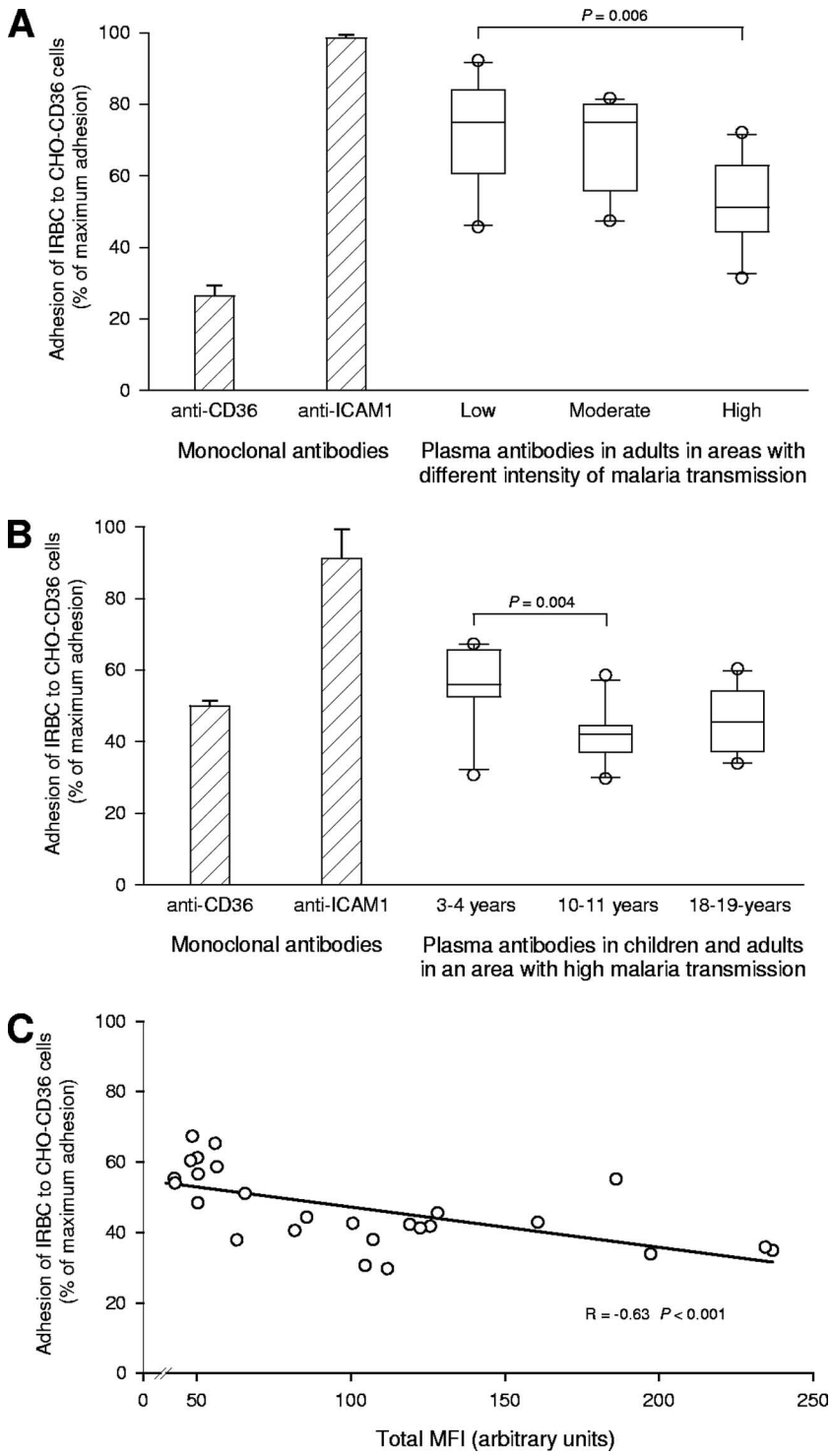

FIG. 4. Inhibition of parasite adhesion to CD36. The adhesion of IRBC to CD36-transfected CHO cells and the adhesion-inhibitory effects of monoclonal antibodies and human plasma antibodies, respectively, are shown. Binding levels are expressed as percentages of maximum binding, measured in wells with IRBC, CHO-CD36 cells, and normal human plasma from negative controls. Vertical bars represent binding of IRBC to CHO-CD36 cells in wells with monoclonal anti-CD36 and anti-ICAM1 antibodies, respectively (means of triplicates with error bars representing standard errors of the mean). The box plots represent human plasma samples ($n = 10$ per group, samples are identical to those shown in Fig. 3A), showing medians, 25th and 75th percentiles, 5th and 95th percentiles, and outliers. (A) Comparison of the adhesion-inhibitory effect of plasma anti-VSA IgG in samples from 18- to 19-year-old individuals living in areas with low, moderate, or high malaria transmission. (B) Comparison of the adhesion-inhibitory effect of plasma anti-VSA IgG in samples from individuals aged 3 to 4 years, 10 to 11 years, and 18 to 19 years, living with high malaria transmission. (C) Correlation between total cumulated MFI to six *P. falciparum* isolates (see the text) of the 30 plasma donors of the high-transmission village also shown in panel B and the adhesion-inhibitory effect of the same samples. Statistical comparisons between groups were performed with the Student *t* test, and the correlation coefficient was calculated with the Pearson correlation test.

protective VSA memory responses even though their plasma anti-VSA IgG levels are low prior to challenge (30).

Interestingly, the differences in plasma anti-VSA IgG between age groups and transmission settings were paralleled by the ability to inhibit parasite adhesion to CD36. "High-transmission" plasma samples were more inhibitory than "low-transmission" plasma, and plasma from older individuals were more inhibitory than plasma from young children in the high-transmission village. Thus, since levels and repertoires of VSA-specific antibodies depend on transmission intensity, so do the functional characteristics of the plasma samples, although our results does not provide direct evidence of an adhesion-inhibitory effect of the anti-VSA IgG antibodies per se.

While the levels of variant-specific IgG were strongly influenced by previous malaria exposure, the geographical origin of the parasites seemed less important. The local "Tanzanian" parasites and the distant "Ghanaian" parasites, respectively, were generally recognized to a similar degree by the Tanzanian children. Thus, the antigenic properties of VSA indeed appear to be largely conserved across large geographical distances, as previously suggested (32), and to be dominated by certain specificities (1). The effective global VSA repertoire may thus be smaller than has often been assumed. Parasites obtained from the severely ill Ghanaian children, however, were still more strongly recognized than parasites from the asymptomatic Tanzanian children, and the phenotypic "severe malaria-type" 3D7 Dodowa parasite was more strongly recognized than the "mild malaria-type" 3D7 parasite. Taken together, these findings seem to reflect the importance of acquiring "severe type" anti-VSA IgG at an early age for protection against severe malarial illness. Identification of "severe type" VSA specificities thus appears as a rational means to develop a VSA-based vaccine to protect against severe malaria in young children.

Overall, our findings indicate a strong and dynamic relationship between the level of *P. falciparum* exposure and VSA-specific antibody responses. Our observations are thus well in agreement with the concept of rapidly evolving antibody responses in the youngest children with specificities for the most common VSA types first, while further acquisition of antibodies with specificities for the less commonly found VSA types happens at a slower speed at older age.

ACKNOWLEDGMENTS

We thank all study participants, village helpers, and health officials in Tanga region, Tanzania, for their contribution to the study. We thank Anne Corfitz, Maiken Christensen, Kirsten Pihl, Jimmy Weng, Juma Akida, Zacharia Savaeli, William Chambo and John Hiza for their excellent technical assistance in Tanzania and/or Denmark. We thank Jørgen Kurtzhals for his contribution to the collection of parasite isolates from malaria patients in Ghana and Andrew Kitua, Chris Drakeley, Ilona Carneiro, and Caroline Jones for their useful advice during the planning of the field work.

This study was conducted under the auspices of the Joint Malaria Programme, a collaborative research initiative between Centre for Medical Parasitology at the University of Copenhagen and Copenhagen University Hospital, Kilimanjaro Christian Medical College, London School of Hygiene and Tropical Medicine, and the Tanzania National Institute for Medical Research. L.S.V. was employed as a Clinical Research Fellow by a grant from The Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark. The study received funding from the ENRECA program of the Danish International Development Agency.

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Editor: J. F. Urban, Jr.