MAJOR ARTICLE

Predominant Influence of Environmental Determinants on the Persistence and Avidity Maturation of Antibody Responses to Vaccines in Infants

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Background. Immune responses are complex traits influenced by genetic and environmental factors. We previously reported that genetic factors control early antibody responses to vaccines in Gambian infants. For the present study, we evaluated the determinants of the memory phase of immunoglobulin G (IgG) responses.

Methods. Antibody responses to tetanus toxoid (TT), measles vaccines, and environmental antigens (total IgG levels) were measured in 210 Gambian twin pairs recruited at birth. Intrapair correlations for monozygous and dizygous pairs were compared to estimate the environmental and genetic components of variations in response.

Results. In contrast to antibody responses measured in infants at age 5 months, 1 month after immunization, no significant contribution of genetic factors to anti-TT antibody and total IgG levels was detected at age 12 months. Genetic factors controlled measles antibody responses in 12-month-old infants, which indicates that the increasing influence of environmental determinants on anti-TT responses was not related to the older age of the children but, rather, to the time elapsed since immunization. Environmental factors also predominantly controlled affinity maturation and the production of high-avidity antibodies to TT.

Conclusions. Genetic determinants control the early phase of the vaccine antibody response in Gambian infants, whereas environmental determinants predominantly influence antibody persistence and avidity maturation.

The immunization of young infants is required to prevent infectious diseases in early life, but its effectiveness is impeded by the immaturity of the infant immune system [1–3]. Young infants produce significantly lower antibody responses to T cell–independent and most T cell–dependent vaccine antigens than do older children or adults [4–9]. Therefore, the induction of protective immunity in early life generally requires the administration of multiple doses of primary vaccine. Unfortunately, antibody responses to vaccines administered during the first year of life are of shorter duration than those elicited in adults, even after several doses of vaccine. This may result in short-term protection and re-

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quire the administration of booster doses of vaccines as soon as the second year of life [10–14].

The capacity of young infants to develop high-avidity antibody responses to vaccines has not yet been fully characterized. Avidity maturation results from the combined effects of somatic hypermutation and a process of antigen-driven selection [15–17]. Somatic mutations in immunoglobulin genes mature during the first year of life, and evidence for selection has been observed only after 6 months of age [18, 19]. After *Neisseria meningitidis* infection, infants produce antibodies of lower avidity than those produced by older children [20]. Similarly, fewer mutations have been observed in immunoglobulin genes of B cells harvested after rotavirus infection in infants, compared with adults [21]. In contrast, high-avidity antibodies are induced by the immunization of infants with polysaccharide conjugate vaccines [22–25], and an adult-like process of avidity maturation to complex vaccine antigens has been demonstrated in murine models of neonatal immunization [26].

Immune responses are inherited as complex quantitative traits, with variation resulting from genetic and environmental factors. Twin studies are a powerful method with which to quantify the relative contribution of genetic and environmental factors to any phenotype. Genetic effects are revealed if the concordance for a phenotype is higher within monozygous (MZ) twin pairs, who are genetically identical, than within dizygous (DZ) twin pairs, who share, on average, 50% of their genes. We previously reported that host genes play a predominant role in the control of early primary antibody responses to vaccine antigens in Gambian infants [27]. The present analysis was undertaken in the same study population, for the evaluation of the respective role of genetic and environmental determinants in (1) long-term persistence and (2) the avidity maturation of antibody responses in infants.

We assessed antibody responses to a protein antigen, tetanus toxoid (TT), in a population of Gambian infant twins immunized at 2, 3, and 4 months of age [27]. We compared titers and the avidity of anti-TT antibodies present during the early (5 months) and late (12 months) phase of the vaccine response in MZ and DZ twins, to define whether these were influenced by mainly genetic or environmental determinants. We selected TT because it is sufficiently immunogenic for antibody responses to persist in most 12-month-old infants, in contrast to diphtheria toxoid (data not shown). In addition, environmental exposure to *Clostridium tetanii* does not influence TT-specific antibodies [28], in contrast to the likely exposure of Gambian infants to pertussis, *Haemophilus influenzae* type b, or hepatitis B virus. Between 5 and 12 months of age, the persistence and avidity maturation of TT-specific antibodies is therefore driven solely by the late development of the primary immune response and/or by exposure to non–TT-specific factors. The antibody response to measles vaccine given at 9 months of age was also assessed when infants were 12 months old, to allow the distinction between the influence of the age at sampling and of the postimmunization period on the factors controlling infant antibody responses. In developing countries, immunization with the live attenuated measles vaccine is recommended at age 9 months, to avoid the inhibitory effect of maternal antibodies [29]. Studies have suggested that immunization during infancy could be associated with a more-rapid waning of anti-measles antibodies and with an increased risk of disease during the teen years [11, 13, 14]. Vaccine failure is observed in 2%–5% of measles-vaccine recipients, and this may involve genetic factors [29, 30]. Finally, we measured total serum IgG levels, to compare the influence of genetic and environmental factors on nonvaccine antigens.

SUBJECTS, MATERIALS, AND METHODS

Study population. The study was conducted in The Gambia and was approved by the Gambian government and the Medical Research Council Ethics Committee. Twin pairs were enrolled at birth at the Royal Victoria Hospital (the referral hospital in the capital, Banjul) or at 1 of 2 health centers (Serrekunda and Fajikunda) in the same district. Exclusion criteria were death of 1 or both twins at or shortly after birth, residence outside the study area, and bacille Calmette-Guérin (BCG) vaccine having been administered to the infants in the hospital before their enrollment in the study. Informed consent was obtained from parents, and demographic data—including ethnicity, family history, and maternal health during pregnancy—were collected by interview. Enrolled twins were examined within 3 days after birth, and birth weight, length, and gestational age [31] were recorded. Twins were monitored monthly until they were 5 months old and then when they were 9 and 12 months old; they were vaccinated in accordance with the Expanded Programme on Immunization schedule (World Health Organisation). BCG vaccine (0.05 mL; Statens Serum Institut) was administered intradermally at birth (or at age 1 month if either twin weighed <2.5 kg). A combination diphtheria, tetanus, and whole-cell pertussis vaccine (Aventis Pasteur) in which *H. influenzae* type b vaccine (ActHIB; Aventis Pasteur) was diluted was administered intramuscularly (im) at age 2, 3, and 4 months. Measles vaccine (Aventis Pasteur) was administered im at age 9 months. Blood samples were collected at birth (umbilical cord blood) and when infants were 2, 5, and 12 months old.

Zygosity determination. The zygosity of same-sex pairs was determined genetically by typing 10 microsatellite markers, as described elsewhere [32]. Twin pairs with identical genotypes for all markers were classified as being MZ.

Antibody assays. Concentrations of total serum IgG and antibody to TT were measured as described elsewhere [27]. The avidity (defined for complex antigens as the antigen binding capacity resulting from the addition of all epitope-specific affinities) of TT-specific IgG antibodies was determined by ELISA

Table 1. Antibody response to tetanus toxoid (TT) and measles vaccine and total IgG concentrations in monozygous (MZ) and dizygous (DZ) twins.

Measurement	Age, months	MZ		DZ		P[b]
		No.[a]	Geometric mean (95% CI)	No.[a]	Geometric mean (95% CI)	
Anti-TT antibody concentration, mIU/mL	5	100	3236 (2575–4065)	320	3329 (2967–3736)	.87
	12	86	465 (375–578)	247	455 (396–523)	.89
IgG antibody concentration, mg/mL	5	100	8.76 (8.19–9.37)	319	8.97 (8.58–9.37)	.66
	12	88	11.36 (10.6–12.2)	258	10.72 (10.3–11.2)	.29
Anti-measles antibody concentration, mIU/mL	12	92	1111 (879–1406)	244	1146 (987–1330)	.86

NOTE. CI, confidence interval.

[a] No. of individual twins in whom antibody responses were successfully measured.

[b] Obtained by linear regression of log-normal antibody response, with zygosity as the explanatory variable, adjusting for clustering of response within twin pairs.

elution using ammonium thiocyanate (NH_4SCN) as a chaotropic agent, as described elsewhere [26]. Results are expressed as the avidity index (AI), the concentration of thiocyanate required to elute 50% of antibodies [26], and as proportions of high-avidity (% HA) antibodies, which was defined for this antigen as the proportion of antibody molecules that remain bound to the plates at NH_4SCN concentrations >3 mol/L. Concentrations of antibody to measles vaccine were determined by hemagglutination-inhibition assay [33]. Antibody concentrations below the assay cutoff were arbitrarily given a value of one-half the cutoff value for the determination of geometric mean titers.

Statistical analysis. Differences in logarithmic antibody responses between MZ and DZ twin pairs were analyzed with Stata software (version 8.1; StataCorp), using linear regression with adjustment for the nonindependence of twin pairs. Intra–twin pair correlations were calculated separately for MZ and DZ twins, using Pearson's correlation coefficient. Heritability (the genetic contribution to the total phenotypic variation in the population) was estimated using structural equation modeling, implemented in Mx GUI (version 1.3.65) [34, 35]. Briefly, the total population variance observed for a given phenotype results from the sum of (1) genetic variance, (2) common environmental variance caused by the effects of environmental factors shared within families, and (3) unique environmental variance specific to each individual. Genetic factors increase correlations within MZ twin pairs, common environmental factors increase intrapair correlations for both MZ and DZ twin pairs, and unique environmental factors will decrease intrapair correlations for both MZ and DZ twin pairs.

We compared models that allowed for additive genetic (A), common environmental (C), and unique environmental (E) contributions to phenotypic variation. The results below present heritability under the ACE model—unless the AE model fitted as well as the ACE model ($P > .1$)—generally reflected in lower DZ correlations, and the CE model fitted significantly worse than the ACE model ($P < .1$). In this case, heritability under the AE model is shown. Similarly, the CE model was the final model if it fitted as well as the ACE model ($P > .1$) and the AE model fitted significantly worse than the ACE model ($P < .1$). Point estimates and 95% confidence intervals (CIs) for heritability under the final model are presented.

RESULTS

A total of 560 twin pairs were identified between March 1998 and May 2000; of these, 345 (62%) were eligible for the study. The reasons for ineligibility were as follows: death of 1 or both twins at or shortly after birth (92), residence outside the study area (101), and BCG vaccine having been administered in the hospital before enrollment (22). Of the 345 eligible pairs, 297 (86%) were enrolled. Reasons for nonenrollment were refusal (22) and no traceable address (26). All twin pairs were breast-fed and lived together for the duration of the study. Of the 297 twin pairs enrolled, zygosity data were available for 217 (59 MZ and 158 DZ). Of these, 179 pairs (43 MZ and 136 DZ) were studied at age 2 months, 210 pairs (50 MZ and 160 DZ) were studied at age 5 months, and 175 pairs (46 MZ and 129 DZ) were studied at age 12 months. There were no significant differences in sex, ethnic group, gestational age, birth weight, or birth center between MZ and DZ twin pairs. Parity was higher in DZ than in MZ twin pairs (4.5 vs. 3.6; $P = .02$).

Environmental determinants influence the persistence of anti-TT antibodies. Figure 1 *(top)* shows the geometric mean anti-TT antibody levels in a subgroup of 259 infant twins who could be tested at ages 2, 5, and 12 months. The administration of TT vaccine at ages 2, 3, and 4 months induced high levels of anti-TT antibodies, as measured at age 5 months. Between ages 5 and 12 months, anti-TT antibody levels decreased 7-fold. The levels of anti-TT antibodies at ages 5 and 12 months were similar in MZ and DZ twins (table 1). Intrapair correlations of anti-TT antibody levels within MZ and DZ twin pairs and heritability are shown in table 2. As reported elsewhere [27], the levels of anti-TT antibodies at age 5 months were significantly more correlated within the MZ twin pairs than within the DZ twin pairs, and the final model included additive genetic and environmental factors. The heritability of anti-TT antibodies at

Table 2. Pairwise correlations and heritabilities for antibody responses to tetanus toxoid (TT) and measles vaccine and total IgG concentrations.

Antibody	Age, months	*r* (no.) MZ	*r* (no.) DZ	Heritability (95% CI), %	Final model
Anti-TT	5	0.83 (50)	0.56 (160)	**45 (18–70)**	ACE
	12	0.67 (43)	0.56 (123)	33 (0–66)	ACE
IgG	5	0.74 (50)	0.54 (159)	**79 (69–86)**	AE[a]
	12	0.66 (44)	0.66 (129)	0	CE[b]
Anti-measles	12	0.74 (45)	0.45 (123)	**62 (27–83)**	ACE

NOTE. Significant heritabilities are given in bold type. ACE, additive genetic plus common environmental plus unique environmental model; AE, additive genetic plus unique environmental model; CE, common environmental plus unique environmental model; CI, confidence interval; DZ, dizygous; MZ, monozygous.

[a] Heritability under the ACE model, 66% (95% CI, 35%–85%).

[b] Heritability under the ACE model, 0% (95% CI, 0%–25%).

age 5 months was statistically significant (45% [95% CI, 18%–70%]) (table 2). At age 12 months, however, the correlation of anti-TT antibody levels had decreased in MZ twin pairs, and no significant influence of genetic determinants was detected. To verify that this difference was not related to the smaller group studied at age 12 months, heritability of antibody titers at age 5 months was estimated in the subgroup of MZ and DZ twin pairs for whom data were available at age 12 months. In this subgroup of infants, anti-TT antibody levels at age 5 months were significantly more correlated within MZ twin pairs ($r = 0.85$) than within DZ twin pairs ($r = 0.61$), which resulted in significant heritability (36% [95% CI, 9%–63%]).

Predominant influence of environmental determinants on total IgG levels at age 12 months. To evaluate whether the predominant influence of environmental determinants in the control of anti-TT antibody responses at age 12 months was specific to TT, the relative influence of genetic versus environmental factors on total IgG concentrations at ages 5 and 12 months was estimated. MZ and DZ twins had similar IgG levels at ages 5 and 12 months (table 1). As expected, total IgG levels had increased between ages 5 and 12 months. As reported elsewhere [27], a high heritability of total IgG levels was detected at age 5 months (table 2). In contrast, correlations of IgG levels were identical in MZ and DZ twin pairs at age 12 months, which resulted in no detectable heritability, because the final model at age 12 months included only common and unique environmental factors. Similar estimates of heritability of IgG levels at ages 5 and 12 months were obtained using the ACE model (table 2). Again, the difference between results at ages 5 and 12 months was not related to the smaller group studied at age 12 months—IgG levels measured at age 5 months in MZ and DZ twin pairs for whom data were available at age 12 months were more correlated in MZ ($r = 0.74$) than in DZ ($r = 0.54$) twin pairs, resulting in significant heritability (36% [95% CI, 3%–65%]). Thus, genetic determinants significantly influence early infant antibody responses, whereas environmental factors play a predominant role at age 12 months.

Predominant role of genetic factors in the primary antibody response to measles vaccine. The predominant role of envi-

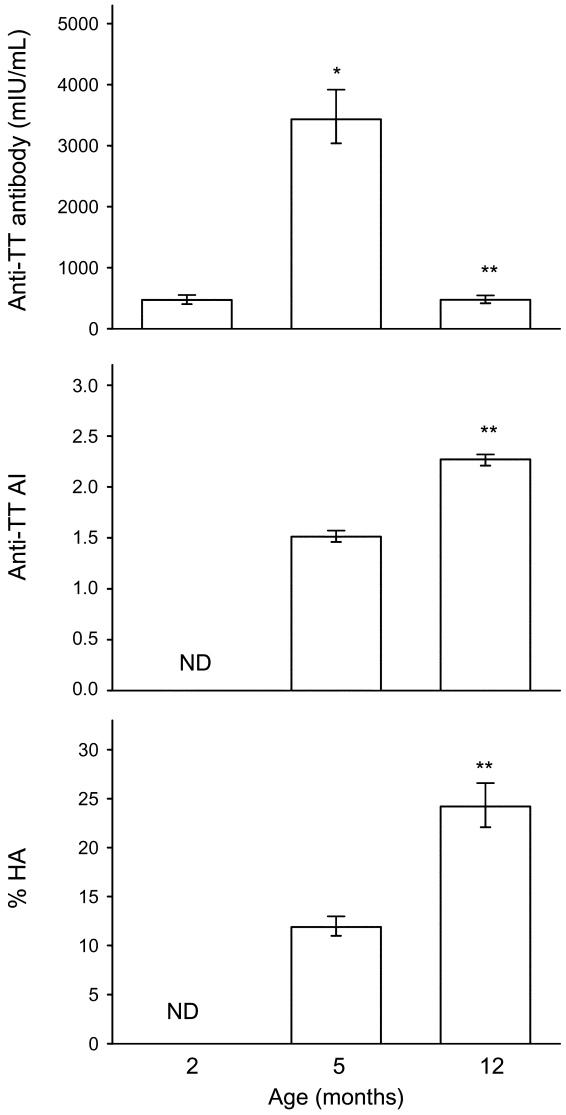

Figure 1. Antibody response to tetanus toxoid (TT) in infants. Infant twins were immunized with TT at 2, 3, and 4 months of age. Anti-TT antibody levels were measured by ELISA when infants were 2, 5, and 12 months old. Antibody avidity was measured when infants were 5 and 12 months old by ELISA elution with thiocyanate as a chaotropic agent. The avidity index (AI) represents the concentration of thiocyanate required to elute 50% of the antibodies, and the percentage of high-avidity (% HA) antibodies is the proportion of antibody molecules bound to the plates at thiocyanate concentrations >3 mol/L. The figure shows geometric mean and 95% confidence intervals of antibody responses measured in a subgroup of infant twin pairs from whom antibody and antibody data were available for 2, 5, and 12 months of age. *$P < .001$ vs. age 2 months; **$P < .001$ vs. age 5 months. ND, not done.

Table 3. Avidity of anti–tetanus toxoid antibodies in monozygous (MZ) and dizygous (DZ) twins.

Avidity parameter	Age, months	MZ		DZ		P^b
		No.[a]	Geometric mean (95% CI)	No.[a]	Geometric mean (95% CI)	
Avidity index	5	100	1.50 (1.44–1.58)	97	1.52 (1.46–1.59)	.77
	12	73	2.22 (2.14–2.30)	203	2.23 (2.18–2.27)	.89
Proportion of high-avidity antibodies	5	98	0.12 (0.11–0.14)	95	0.12 (0.11–0.14)	.97
	12	71	0.22 (0.20–0.26)	202	0.24 (0.23–0.25)	.49

NOTE. CI, confidence interval.

[a] No. of individual twins in whom antibody responses were successfully measured.

[b] Obtained by linear regression of log-normal antibody response with zygosity as the explanatory variable, adjusting for clustering of response within twin pairs.

ronmental determinants in anti-TT and total IgG responses observed at age 12 months could be related to the increasing age of the infants and/or to the time elapsed after immunization. To evaluate the influence of age on the relative role of genetic and environmental factors, we estimated the heritability of the antibody response to measles vaccine, administered at age 9 months, in 12-month-old infants. MZ and DZ twins showed similar antibody responses to measles vaccine (table 1). As shown in table 2, correlations of anti-measles antibody levels were higher within MZ than within DZ twin pairs, resulting in significant heritability (62% [95% CI, 27%–83%]). Thus, genetic factors control the induction of measles antibodies in 12-month-old infants, which suggests that the influence of environmental determinants on anti-TT and total IgG responses at age 12 months does not reflect the age of the child at time of sampling but, rather, the time since immunization.

Predominant role of environmental factors in the avidity maturation of anti-TT antibodies. We next assessed the respective roles of genetic and environmental factors in the avidity maturation of anti-TT antibodies. Protection against tetanus toxin, as assessed by in vitro tests, correlates with the recognition of the toxin-binding site, with a striking influence of antibody affinity. Thus, the maturation of antitoxin responses is essential to reach the affinity threshold that is required for effective toxin neutralization [36]. Figure 1 *(middle* and *bottom)* shows the geometric mean AI and % HA for anti-TT antibodies measured at ages 5 and 12 months in a subgroup of 135 MZ and DZ twin pairs from whom sufficient serum was available. Anti-TT AI and % HA antibodies significantly increased between ages 5 and 12 months, demonstrating efficient avidity maturation. This avidity maturation process was similar in MZ and DZ twin pairs (table 3). Correlations of AI and % HA antibodies measured at ages 5 and 12 months were similar within MZ and DZ twin pairs, resulting in no significant heritability (table 4). The final model for the 12-month AI data included only common and unique environmental factors. Similar estimates of heritability of AI at age 12 months were obtained using the ACE model (table 4). Thus, environmental factors play a predominant role in the control of anti-TT avidity maturation in infants.

DISCUSSION

Immune responses are complex traits influenced by genetic and environmental factors. We previously reported that genetic determinants play a central role in the control of early primary IgG responses to vaccines in young infants [27]. In the present article, we show that the relative role of environmental determinants predominates during the late phase of IgG responses.

The persistence and avidity maturation of TT-specific antibodies in Gambian infants is driven by the late development of the primary immune response and/or by exposure to non–TT-specific factors [28]. Whereas significant heritability of IgG responses to TT was detected in 5-month-old infants immunized at ages 2, 3, and 4 months, we observed that environmental factors predominantly controlled anti-TT IgG levels in infants 12 months old. Although this analysis could not be extended to

Table 4. Pairwise correlations and heritabilities for avidity of anti–tetanus toxoid antibodies.

Avidity parameter	Age, months	*r* (no.)		Heritability (95% CI), %	Final model
		MZ	DZ		
Avidity index	5	0.53 (50)	0.41 (48)	25 (0–67)	ACE
	12	0.35 (36)	0.39 (101)	0	CE[a]
Proportion of high-avidity antibodies	5	0.42 (49)	0.39 (46)	20 (0–64)	ACE
	12	0.58 (35)	0.34 (100)	25 (0–65)	ACE

NOTE. ACE, additive genetic plus common environmental plus unique environmental model; CE, common environmental plus unique environmental model; CI, confidence interval; DZ, dizygous; MZ, monozygous.

[a] Heritability under the ACE model, 0% (95% CI, 0%–39%).

other vaccine antigens, a similar difference was observed with total IgG levels: genetic factors control total IgG titers at age 5 months, whereas environmental factors play a predominant role in the responses to nonvaccine antigens at age 12 months. Unlike exposure to TT, exposure to nonvaccine antigens cannot be controlled, and we cannot formally exclude the possibility that the reduction in the heritability of total IgG levels is partly related to an increased discordance of exposure within twin pairs. However, this possibility is not supported by the fact that twins were raised together for the duration of the study and by the high and identical correlations of total IgG levels measured in MZ and DZ twin pairs at age 12 months (table 2), which indicates an important role of environmental factors.

To our knowledge, this is the first longitudinal study of the determinants of IgG responses in humans. Previously published twin studies of vaccine responses were cross-sectional and therefore could not evaluate the determinants of antibody persistence [30, 37, 38]. The predominant role of environmental determinants in anti-TT responses observed at age 12 months could have been related to the increasing age of the infants or to the time since immunization. The observation that the early IgG response to measles vaccine is predominantly controlled by genetic determinants confirmed that the influence of environmental factors on anti-TT responses at age 12 months reflects the time since immunization. This suggests that distinct factors influence the early-effector and late-memory phases of IgG responses. Among the factors that could be particularly important in the control of early antibody responses are innate immune response genes.

The observation that genetic factors play a predominant role in the control of antibody response to measles vaccine is in keeping with data reported by Tan et al. [30]. The identification of the genes involved may help in the development of more-effective measles vaccines inducing higher early antibody responses [27]. The role of genetic factors in the control of anti-measles antibody persistence and avidity maturation should be examined.

The mechanisms leading to the persistence of antigen-specific antibodies are not fully understood. The postimmunization period is characterized by a rapid decrease in antibody levels that presumably reflects the interruption of antibody production by short-lived antibody-secreting cells. This is followed by a second period, during which antigen-specific antibody levels decrease with slower kinetics. The antibody persistence could result from long-lived plasma cells having reached appropriate survival niches within the bone marrow [39–41]. Alternatively, antibody production may be sustained by antigen-specific or bystander reactivation of memory B cells [42, 43]. A number of environmental factors could influence the persistence of IgG vaccine responses in infants. It is tempting to postulate that exposure to microorganisms plays an important role. Between ages 5 and 12 months, maternal antibodies disappear, and infants are exposed to a large number of microorganisms. These microorganisms could either favor antibody persistence through the bystander activation of memory B cells or favor antibody decline by inducing the differentiation of plasma cells competing for a restricted number of niches within the bone marrow. We cannot exclude that this influence would be more marked in tropical environments. For example, *Plasmodium falciparum* malaria drives immunoglobulin production to high levels, results in a more-rapid turnover of IgG in Gambian adults, and may diminish the persistence of meningococcal vaccine antibodies [14, 44–47]. However, immune responses to TT vaccine are robust even in malaria-infected African children [48], and the shorter duration of infant antibody responses, compared with those in adults, has been observed worldwide [10–12, 14, 49, 50].

In the present study, we also demonstrate that the induction of high-avidity antibodies to TT takes place within a few months after early infant immunization. The detection of high-avidity antibody responses to infant TT immunization are in keeping with our previously published results showing adult-like processes of avidity maturation to TT and other protein antigens in infant mice [23]. These data also complement studies that have indicated that high-avidity antibody responses can be induced by glycoconjugate vaccines in human infants [19, 20, 22, 24]. Thus, the lower affinity of antibodies measured after infant rotavirus [21] or *N. meningitidis* [20] infections, compared with those after adult infections, is likely to reflect differences in the time since first exposure rather than an impaired somatic mutation/antigen-driven B cell selection process. The affinity maturation process of TT-specific antibodies during the first year of life is essentially controlled by nongenetic factors. This could be the consequence of several different mechanisms. Somatic hypermutation is a random phenomenon and, as such, could lead to the generation of a diversity of centrocyte populations that is not primarily programmed by genetic determinants. In addition, the selection of centrocytes producing high-affinity antibodies is mediated by their interactions with follicular dendritic cells (FDCs) bearing antigens and with helper $CD4^+$ follicular T lymphocytes [51]. Microbial antigens could either promote the production of high-affinity antibodies by competing at the level of FDCs for presentation to centrocytes or favor the production of low-affinity antibodies through the bystander activation of centrocytes. Although significant positive correlations were observed between the % HA anti-TT antibodies and total IgG levels at age 12 months ($r = 0.14$ [95% CI, 0.02–0.27]; $P = .02$), in accordance with the hypothesis of a positive competitive influence at the FDC level, further studies will be required to explore this hypothesis and to eventually identify the environmental factors involved. This represents a novel and important objective for the improvement of vaccine efficacy in young children.

THE MEDICAL RESEARCH COUNCIL GAMBIA TWIN STUDY GROUP

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