Immune function in rural Gambian children is not related to season of birth, birth size, or maternal supplementation status[1–4]

Sophie E Moore, Andrew C Collinson, and Andrew M Prentice

ABSTRACT

Background: We previously showed that mortality from infectious diseases among young adults in rural Gambia is strongly correlated with the season of their birth. This suggests that early life insults that involve fetal malnutrition, exposure to natural toxins, or highly seasonal infections affecting the infant or pregnant mother cause permanent damage to the immune system. Excess mortality begins after puberty and has a maximal odds ratio of >10 for deaths between ages 25 and 50 y.

Objective: We investigated the immune function of children according to birth weight, season of birth, and exposure to maternal dietary supplementation during pregnancy.

Design: Immune function was measured in 472 prepubertal children aged 6.5–9.5 y from 28 villages in rural Gambia. The mothers of these children had been randomly assigned to a high-energy prenatal supplementation program, which significantly increased birth weight. This permitted supplementation status, birth weight, and season of birth to be investigated as exposure variables. The outcome variables tested were naive responses to rabies and pneumococcus vaccines, delayed-type hypersensitivity skin reactions, and mucosal defense (secretory immunoglobulin A and dual-sugar permeability).

Results: Immune responses were strongly related to current age and sex, suggesting a high level of sensitivity, but were not consistently related to birth weight, season of birth, or maternal supplementation (control compared with intervention).

Conclusion: Events in early life did not predict a measurable defect in immune response within this cohort of rural Gambian children. It is possible that the early programming of immune function may be mediated through a defect in immunologic memory or early senescence rather than through impairment of early responses. *Am J Clin Nutr* 2001;74:840–7.

KEY WORDS Fetal programming, immune function, maternal supplementation, pregnancy, children, birth weight, rabies vaccine, pneumococcus vaccine, The Gambia

INTRODUCTION

Substantial evidence, mainly from Europe and North America, suggests that events occurring early in life can influence an individual's future susceptibility to certain noncommunicable diseases (1). The fetal origins hypothesis states that cardiovascular disease and type 2 diabetes originate through adaptations that the fetus creates when it is undernourished. These adaptations, which include the slowing of growth, permanently change the structure and function of the body (2).

We have added to the noncommunicable diseases observation by showing that mortality from infectious diseases among young adults is strongly correlated to the season of birth in The Gambia. Individuals born during or shortly after the annual rains (July–December) were found to be >10 times as likely to die prematurely than those born during the dry season (3, 4). The divergence in mortality between the 2 groups occurred after individuals were aged ≈15 y and had a maximal odds ratio of >10 for deaths between ages 25 and 50 y (**Figure 1**). This finding led to the hypothesis that an insult occurring early in life and linked to the season of birth disrupts the immune response to certain infectious diseases in later life, resulting in premature mortality.

In rural Gambia, annual rains coincide with a hungry season when food stocks from the previous year's harvest are running out and the current year's crops have yet to be harvested. The stress of an intensive agricultural workload, in combination with food shortages, force all working adults into a negative energy balance (5). Pregnant and lactating women are not exempt from the farm work and consequently suffer weight loss that represents a mobilization of ≤50% of body fat stores (6). A hungry season deficit occurs in birth weight, with the average hungry season birth weight usually falling by 200–300 g and the prevalence of low birth weight (<2500 g) rising from 11% to 24% (7, 8). An infant born during the annual hungry season in rural Gambia is almost certain to have experienced nutritional deprivation at some stage during late gestation.

[1] From the Medical Research Council (MRC) Keneba, The Gambia; the MRC International Nutrition Group, London School of Hygiene and Tropical Medicine; and the MRC Human Nutrition Research, Cambridge, United Kingdom.

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[4] Address reprint requests to SE Moore, MRC International Nutrition Group, Public Health Nutrition Unit, London School of Hygiene & Tropical Medicine, 49-51 Bedford Square, London WC1B 3DP, United Kingdom. E-mail: sophie.moore@lshtm.ac.uk.

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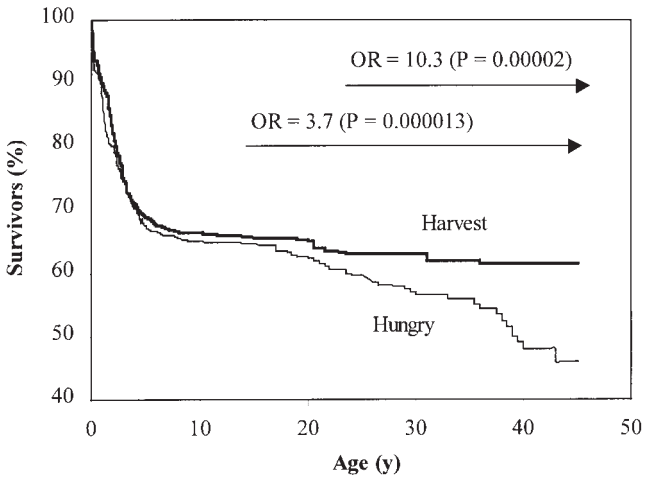

FIGURE 1. Kaplan-Meier survival plots by season of birth. $n = 3162$ (2059 alive and 1103 dead). OR, odds ratio. Reproduced with permission of Oxford University Press from Moore et al (4).

In addition to nutrition, many other factors are influenced by the annual rains, including infections (especially malaria) both during pregnancy and infancy, exposure to contaminated food (including environmental toxins, eg, aflatoxins), and increased physical and psychological stress that could act as mediators of the link between season of birth and premature adult mortality (4). However, the well-documented effects of the annual hungry season on pregnancy weight gains and birth weights, together with evidence from both animal and human studies, generate the primary hypothesis that one or more components of the immune system were permanently damaged by fetal malnutrition. The ontogeny of the immune system begins early during fetal life and continues throughout gestation. Prenatal malnutrition occurring at a critical point in the development of the immune system may therefore compromise postnatal immunity and subsequent survival. The specific effects that intrauterine growth retardation has on immune function have been studied in some detail, and results from some human (9–11) and animal (12, 13) studies suggest that fetal nutrient deprivation impairs immune function later in life. This article explores the relation between current immune function and early-life exposures in a group of rural Gambian children born during a maternal dietary supplementation trial.

SUBJECTS AND METHODS

Study population

The present study was conducted in 28 villages in the rural West Kiang region of The Gambia. In 1989 the Dunn Nutrition Group initiated a 5-y maternal dietary supplementation trial in these villages. During this trial, all pregnant women in the region were randomly assigned by village to daily supplementation with high-energy peanut (*Arachis hypogaea*) biscuits. The biscuits contained roasted peanuts, rice flour, sugar, and peanut oil, and they provided a maximum daily intake (2 biscuits) of 4250 kJ energy, 22 g protein, 56 g fat, 47 mg Ca, and 1.8 mg Fe (8). The supplement was consumed for ≈20 wk before delivery in the intervention group or for ≈20 wk after delivery in the control group. During the study, 2047 live singleton infants were born. Consumption of the supplement significantly increased infant birth weight, particularly during the nutritionally debilitating hungry season, by 201 and 136 g in the hungry season and in the seasons combined, respectively ($P < 0.001$ for both), showing that the hungry season effect on birth weight is primarily mediated through nutrition (8). The children born during the study form an ideal study group because precise records from before birth (maternal details), at birth (including birth size and gestational age), and during infancy and early childhood (anthropometry) are available.

In the present study we followed up on all children born during the first 2 y of the supplementation study who currently live in West Kiang. Meetings were held with the elders from each village and recruitment began after approval. Informed consent was obtained from each participating child and his or her parent or guardian before measurements were taken. The study was conducted for 12 mo beginning in April 1998. No children were recruited into the study (and therefore, no baseline measurements were made) during the month of March. During recruitment, and throughout the course of the study, the principal investigator (SEM) was blinded to the early-life data of the children (including birth weight, season of birth, and supplementation status).

Scientific approval for the study was granted by the Medical Research Council (MRC) The Gambia Scientific Coordinating Committee. Ethical permission for the study was granted by the joint Gambian Government and MRC Ethical Committee.

Study protocol

Each child recruited for participation in the study was seen on 5 occasions over 2 mo (days 0, 2, 16, 30, and 60). During this period, they underwent a series of measures of current immune function including response to vaccination, assessment of delayed-type hypersensitivity, and assessment of some aspects of mucosal immunity [dual-sugar permeability test and salivary secretory immunoglobulin A (sIgA)]. Mucosal biopsy studies have shown that the indirect dual sugar permeability test correlates with histologic evidence for mucosal damage (14). In addition, a series of seasonally varying confounding factors likely to modulate immune function were also measured. These included a measurement of serum aflatoxin-albumin adduct concentrations. Aflatoxins are toxic, secondary fungal metabolites (mycotoxins) produced by various *Aspergillus* species that are commonly found to contaminate many foodstuffs, eg, peanuts, corn, maize, and rice (15). Serum aflatoxin concentrations were measured as a confounding factor because aflatoxin is a powerful acute immunosuppressant (16) that shows a seasonal variation in The Gambia related to the length of time food harvests are stored (17).

At baseline (day 0), the children were brought to the clinic in Keneba after they had fasted overnight. Upon arrival, each child was given a test dose of latulose-mannitol solution for the dual sugar permeability test according to recommendations (18, 19). All urine passed over the next 5 h was collected, the total volume was measured, and a portion was stored for analysis. During this time, weight, height, and midupper arm circumference (MUAC) were measured by using regularly validated, standard equipment. A 6-mL sample of venous blood was drawn and centrifuged at 4°C for 10 min at 1500 × *g*, and plasma and serum aliquots were immediately frozen for later analysis of plasma micronutrients (zinc, vitamin C, and vitamin A and related retinoids), prevaccination antibody titres, and serum aflatoxin-

albumin adduct concentrations. A sample of whole blood was reserved for the assessment of hemoglobin concentrations and a thick film was prepared for the examination of malaria parasites. A saliva sample was also collected by using the Sarstedt Salivette saliva collection device (Sarstedt Inc, Newton, NC) and then frozen until analysis.

Delayed-type hypersensitivity was assessed with a cell-mediated immune response kit (Multitest CMI; Marcel Mérieux, Lyons, France) on the volar surface of the forearm. This multipronged instrument allows 7 recall antigens (proteus, trichophyton, candida, tetanus, diphtheria, streptococcus, and tuberculin) and a glycerin control to be administered simultaneously. Forty-eight hours later (day 2), the perpendicular diameters of induration were measured to the nearest millimeter for each antigen. A positive response was recorded for diameters >2 mm. Failure to respond to any of the 7 test antigens was considered to be indicative of reduced immunocompetence or anergy. All children were then given a single injection of the 23 valent pneumococcal capsular polysaccharide vaccine (Pneumovax 23; Merck and Co Inc, West Point, PA). This vaccine consists of a mixture of highly purified capsular polysaccharides from the 23 most prevalent or invasive pneumococcal types and is indicated for immunization against pneumococcal disease caused by those types included in the vaccine. At the same time, all children also received a preliminary dose of the human diploid cell rabies vaccine (Rabies Vaccine BP; Pasteur-Merieux Connaught, Lyon, France).

Fourteen days after the first vaccination (day 16), a finger-prick blood sample was collected for assessment of antibody response to the first rabies vaccination. Twenty-eight days after the first rabies vaccination (day 30), a second dose was given. At the same visit, a second finger-prick blood sample was obtained for assessment of antibody response to the pneumococcal capsular polysaccharide vaccine. Two months after the initial vaccination (day 60), a final finger-prick blood sample was collected for the measurement of rabies antibodies.

Laboratory analysis

Urinary lactulose and mannitol, salivary sIgA, and plasma zinc, vitamin C, and vitamin A and related retinoid concentrations were measured at the MRC Human Nutrition Research in Cambridge, United Kingdom. Urinary lactulose and mannitol were measured with an automated enzymatic assay by use of the Cobas-Bio centrifugal analyzer (F Hoffmann-La Roche Ltd, Basel, Switzerland), as previously described (20–23). Salivary concentrations of sIgA were measured with an enzyme-linked immunosorbent assay by using an adapted method of the procedure developed for the determination of breast-milk antimicrobial factors (24, 25). Plasma vitamin C concentrations were also measured with the Cobas-Bio analyzer (F Hoffmann-La Roche Ltd), as described elsewhere (26). Plasma zinc concentrations were determined colorimetrically by use of a commercial kit (Wako; Wako Chemicals GmbH, Neuss, Germany). Plasma concentrations of vitamin A and related retinoids were measured by HPLC by use of an assay procedure derived from that of Thurnham et al (27).

Serum aflatoxin-albumin adduct concentrations were measured at the Molecular Epidemiology Unit, University of Leeds, by using the method of Chapot and Wild (28). Antibody titres against the pneumococcal polysaccharide vaccine were measured at the Department of Immunology, Institute of Child Health, London. Antibody titres were tested against 3 capsular polysaccharide components of the vaccine that are usually immunogenic (types 1, 5, and 14) and against 1 component that is less immunogenic (type 23). Antirabies antibody titres were determined at the Central Veterinary Laboratories, Surrey, United Kingdom, by using the rapid-focus fluorescence-inhibition test of the World Health Organization (29).

Statistical analysis

Analysis of variance was used to test the primary hypotheses that season of birth, supplementation status, and birth weight would influence later immune function. All data were adjusted for age, sex, month of study, and current nutritional status (weight-for-age z score). To account for multiple testing, results were considered significant when P values were <0.01 (DATADESK, version 6 for WINDOWS; Data Description Inc, Ithaca, NY). Weight-for-age, height-for-age, and body mass index–for-age z scores were calculated using Cole's LMS method, where L is λ, M is μ, and S is δ, referring to the smoothing of the curve, the mean, and the CV, respectively (30), referenced against height, weight, and body mass index reference curves for the United Kingdom (31, 32). Measurement of the various immune outcomes was 98–100% complete in the 472 subjects recruited for participation in the study. This sample size of 472 subjects permitted the detection of 0.35 SDs for any of the outcomes in the bivariate analysis (eg, hungry compared with harvest season) at 90% power and $P < 0.01$.

RESULTS

The 472 children recruited into the study represent 52% of the infants born during the first 2 y of the supplementation study. Of the remaining 48% of the infants, 266 (29%) had since moved away from the study villages, 82 (9%) had died, 62 (7%) declined to participate in the study, 4 (0.4%) were sick, and the remaining 29 (3%) could not be traced. There were no significant differences in season of birth, supplementation status, and birth weight between the children who were recruited into the study and those who were not. Details of the anthropometry and micronutrient status of the subjects by sex are shown in **Table 1**.

The mean birth weight of the children recruited into the study was 2941 g (range: 1700–4160 g). Although birth weights were higher in the intervention children ($\bar{x}$: 2971 g; range1700–4169 g) than in the control children ($\bar{x}$: 2920 g; range: 1880–4060 g), this was not significant. Furthermore, there was no significant difference in the birth weight of children recruited for participation in the study according to their season of birth. Sixty-three of the children (13.3%) had a birth weight below that of the World Health Organization cutoff for low birth weight of 2500 g (low-birth-weight subgroup). At the time of study, these children had significantly lower current weight-for-age ($P \leq 0.0001$), height-for-age ($P \leq 0.0081$), and body mass index-for-age ($P \leq 0.0001$) z scores than did the children who weighed >2500 g at birth. Gestational age was assessed in 369 (78%) of the children at birth; of these, only 4 (1.1%) were born prematurely (<37 wk gestation).

Analysis of confounders

All measures of immune response showed a marked seasonal variation and were significantly related to the month of measurement, although this variation was not consistent across the different measures (**Figure 2**). An age-related trend was seen in the response to both of the vaccines, with an increased response with increasing age for the pneumococcal vaccine (**Figure 3**); however,

TABLE 1
Anthropometric characteristics of children by sex[1]

	Males (n = 251)	Females (n = 221)
Age (y)	8.01 ± 0.69 (6.69–9.35)	8.00 ± 0.68 (6.65–9.47)
Weight (kg)	20.9 ± 2.7 (14.2–30.7)	20.7 ± 2.7 (14.5–28.5)
Height (cm)	121.7 ± 8.9 (106.9–141.0)	121.6 ± 6.0 (98.9–139.8)
MUAC (mm)	161 ± 12 (129–198)	166 ± 13 (133–211)[2]
Weight-for-age z score	−1.63 ± 1.01 (−4.70–0.91)	−1.53 ± 0.83 (−4.16–0.72)
Height-for-age z score	−1.00 ± 0.86 (−3.28–1.85)	−0.89 ± 0.94 (−5.04–1.35)
BMI-for-age z score	−1.44 ± 0.92 (−5.06–0.78)	−1.42 ± 0.77 (−3.69–0.66)
Vitamin C (μmol/L)	51.0 ± 33.0 (1.0–185.2)	48.8 ± 31.7 (2.2–140.0)
Zinc (μmol/L)	12.4 ± 4.6 (0.7–63.3)	12.7 ± 3.0 (4.0–23.8)
Retinol (μmol/L)	0.84 ± 0.25 (0.31–1.62)	0.85 ± 0.25 (0.28–1.45)
β-Carotene (μmol/L)	0.55 ± 0.51 (0.03–3.45)	0.60 ± 0.69 (0.03–5.50)
Hemoglobin (g/L)	126.8 ± 13.3 (68.0–156.0)	126.5 ± 14.2 (65.0–160.0)

[1] $\bar{x}$ ± SD. Range in parentheses. MUAC, midupper arm circumference.
[2] Significantly different from males, $P < 0.0001$.

this was significant only for the capsular polysaccharide components type 14 and type 23. A decreasing response was observed with increasing age for the rabies vaccine (response after first dose: $P \leq 0.0001$; response after second dose: $P = 0.0124$), for intestinal permeability [lower ratio with increasing age ($P = 0.0005$)], and for salivary concentrations of sIgA [higher concentrations with increasing age ($P = 0.0002$)], but not for the cell-mediated immune response. The response to both vaccinations was significantly higher in female than in male subjects: for the rabies vaccine, $P = 0.005$ for the first dose and $P = 0.0009$ for the second dose; although for the pneumococcal vaccine, $P = 0.044$ for type 1 components and $P = 0.022$ for type 14 components. The cell-mediated immune response was higher in males than in females ($P = 0.0027$). The lactulose-mannitol ratio and the salivary concentrations of sIgA did not differ significantly by sex.

None of the measurements of immune function were related to the serum aflatoxin-albumin adduct concentrations. The only measure of immune response that was related to the presence of malaria parasites was the response to the first dose of the rabies vaccine in which a reduced response was observed in the children who had parasitaemia (response without malaria: 3580 IU/L; response with malaria: 900 IU/L; $P \leq 0.0001$). No consistent associations were found between nutritional status (as assessed by weight-for age z scores, hemoglobin concentrations, and plasma micronutrient status) and any of the measures of immune function.

Immune function by season of birth, supplementation status, and birth weight

Cell-mediated immune response

The total number of positive responses was not related to the season of birth or birth weight of the children and was not significantly different in the subgroup of children born with a low birth weight. This remained the case when the analysis was adjusted for age, sex, month of study, and current weight-for-age z score. However, the cell-mediated immune response was significantly associated with the supplementation status of the children, with more positive responses seen in the intervention children than in the control children. The unadjusted mean total response numbers were 1.17 and 0.83 for the intervention and control children, respectively ($P = 0.007$). The responses adjusted for age, sex, month of study, and current weight-for-age z score were 1.10 and 0.73 for the intervention and control children, respectively ($P = 0.0082$). The analysis was repeated by using the proportion of children who were anergic (no response to any of the test antigens), but this made no substantial change to the main outcome results.

Response to vaccination

The response to the pneumococcal vaccination was not significantly associated with season of birth, supplementation status, or birth weight of the children and was not significantly different in the subgroup of children born with a low birth weight. This remained the case when the analysis was adjusted for age, sex, and month of study. The response to the rabies vaccination was not significantly related to season of birth or to the birth weight of the children (unadjusted or adjusted), but the response to the second dose of the vaccine was significantly higher in the control children than in the intervention children (control group, 11 2500 IU/L; intervention group, 9300 IU/L; $P = 0.01$). This became increasingly significant after adjustment for age, sex, and month of study (control group, 11 480 IU/L; intervention group, 9030 IU/L; $P = 0.0048$).

Mucosal defenses

The lactulose-mannitol ratio was not significantly associated with season of birth, supplement group, or the birth weight of the children and was not significantly different in the subgroup of children born with a low birth weight. This remained the case when the analysis was adjusted for age, sex, and month of study. sIgA concentrations were not significantly related to the supplementation status of the children or to their birth weight (unadjusted or adjusted). When adjusted for age, sex, and month of study, concentrations of sIgA, however, were higher in the children born in the hungry season than in those born in the harvest season, although this was not significant (January–June births, 48.81 μg sIgA/mg protein; July–December births, 54.48 μg sIgA/mg protein; $P = 0.0185$).

DISCUSSION

Most of the data in support of the fetal origins hypothesis come from studies in countries in the developed world where noncommunicable diseases are the major cause of morbidity and mortality. In countries where infectious diseases prevail, the consequence that an insult in early life may have on later disease is

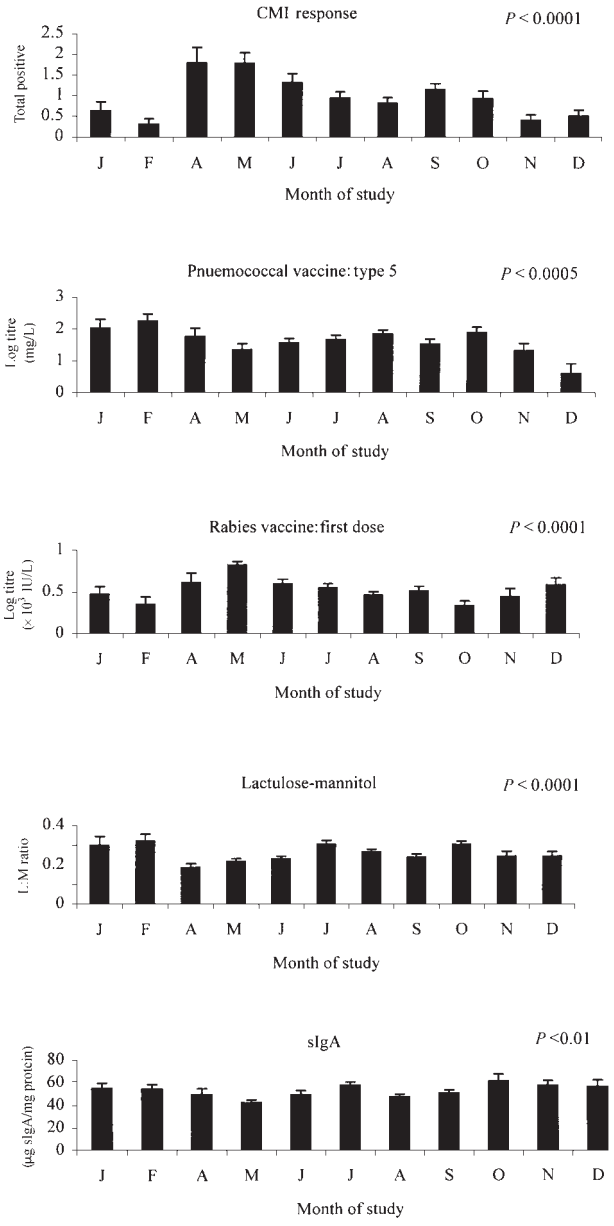

FIGURE 2. Mean (±SEM) immune response by month of study. No baseline measurements were made during the month of March. CMI, cell-mediated immune response; L, lactose; M, mannitol; sIgA, salivary secretory immunoglobulin A.

not as clearly established. Long-term record keeping has not been a high priority in developing countries, which means that the opportunities for follow-up are extremely rare in the very populations in which the hypothesis may have the greatest health effect. There have been some rare opportunities in which birth records and data were available and follow-up was possible. For example, studies in India (33–36) and Jamaica (37, 38) provide some evidence for a relation between maternal nutrition, fetal growth, and future noncommunicable disease risk. A study in The Gambia, however, found no relation among adults who were still following a rural lifestyle (39).

By using the MRC's long-term demographic records for 3 villages in rural Gambia, we showed previously that individuals born during or shortly after the nutritionally debilitating hungry season in rural Gambia were >10 times as likely to die prematurely from infections than were their counterparts born during the harvest season (3). This led to the hypothesis that immune function may be programmed by an insult occurring in early life, correlated with season of birth.

The ontogeny of the immune system begins early during fetal life, and although some areas are fully mature at birth, others continue to develop postnatally. A fetal immune response is not essential for normal fetal growth and development, but becomes absolutely necessary for survival after birth (40). Prenatal malnutrition occurring at a critical point in the development of the immune system may compromise postnatal immunity and subsequent survival. Epidemiologic evidence links low birth weight to an increase in prevalence of mortality during infancy. In Brazil for example, Victora et al (41) found that low-birth-weight infants were 2.0 times more likely to die from diarrhea, 1.9 times more likely to die of respiratory infections, and 5.0 times more likely to die from other infections than were children who weighed ≥2500 g at birth.

Although many studies on the fetal origins hypothesis have focused on the programming of noncommunicable diseases, some evidence, other than our own observations in The Gambia, suggests that certain components of the immune system may indeed be determined by events in early life. Phillips et al (10) found an association between birth size and susceptibility to autoimmune disease in a group of women from Hertfordshire. The proportion of women with thyroglobulin and thyroid peroxidase autoantibodies fell with increasing birth weight. An inverse relation in a group of men and women from Preston between birth size (head circumference but not birth weight) and concentrations of IgE in adult life was also observed (11). This finding suggests a possible relation between the early-life environment and the later development of atopy and allergic disease. The authors of these articles implicate the importance of the possible long-term consequences that fetal growth retardation may have on the structure and function of the thymus gland. The thymus,

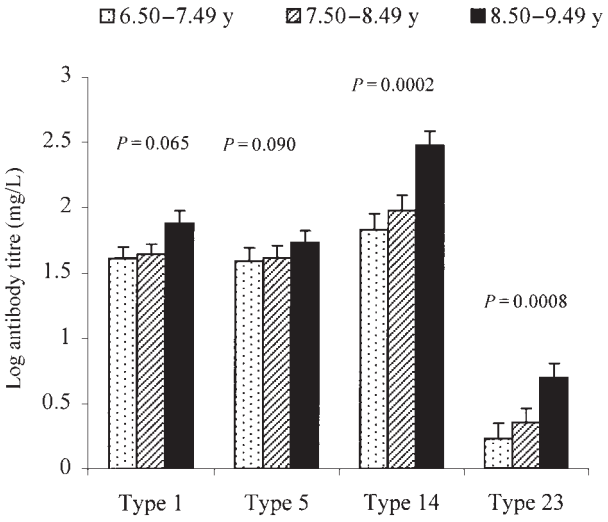

FIGURE 3. Mean (±SEM) response to pneumococcal vaccine by age group.

TABLE 2
Summary of main study findings[1]

Test	Birth weight	Season of birth	Supplementation status
CMI	NS	NS	Higher response in intervention children ($P = 0.006$)[2]
Pneumococcal vaccination	NS	NS	NS
Rabies vaccination	NS	NS	Higher response to second dose in control children ($P = 0.005$)[2]
Intestinal permeability	NS	NS	NS
Salivary sIgA concentrations	NS	Higher response in hungry season births ($P = 0.0185$)[2]	NS

[1] CMI, cell-mediated immune response. sIgA, secretory immunoglobulin A.
[2] Significantly different after adjustment for age, sex, month of study, and current weight-for-age *z* score (ANOVA).

which is fully developed before birth, is the major site of T cell development. In animal models, maternal undernutrition is universally observed to have greater effects on thymic and lymphoid tissue growth than on other organs (42). Development of the microarchitecture of the thymus is also susceptible to nutrient deprivation. The cortex and medulla have an exquisitely detailed microenvironment, which provides a matrix to support developing thymocytes and is critical to optimal function (43). It is therefore possible that periods of stress at critical phases of maturation might have permanent effects on the developing thymus, and hence on T cell populations and immune function.

A significant seasonal variation was observed in all measures of immune function, although the pattern was not consistent between the measures. Such seasonality has been observed in previous studies from areas with a prominent pattern of seasonality, although the precise etiology is unknown (44–46). It is possible that the increase in prevalence of malaria and other diseases common during the rainy season may cause a disruption of immune response. In terms of vaccination, it is possible that this acts as an adjuvant to the vaccine, priming the immune system and thus increasing the response elicited. The increased lactulose-to-mannitol ratio observed during the rainy season may be the result of a transient gut enteropathy as a consequence of increased diarrheal disease and infection because intestinal permeability tends to be elevated during acute diarrhea (47). All analyses were appropriately adjusted to account for this seasonality.

The present study was designed to explore 3 main hypotheses, which were that the season of birth, supplementation status, and birth weight would influence future immune function. Results are summarized in **Table 2**. As indicated, none of the measures of immune function were significantly related to birth weight and were not significantly different in the group of children who were born with a low birth weight. The only measure that correlated with season of birth was the salivary concentrations of sIgA, although this only approached significance. Although the response to the pneumococcal vaccine, intestinal permeability, and salivary sIgA were not significantly associated with supplementation status, the children from the intervention group had a significantly greater cell-mediated immune response than did those from the control group, and those from the control group showed a significantly greater antibody response to the rabies vaccine.

The present study used highly standardized challenges and observed a varied spectrum of tests focusing on T cells, B cells, mucosal barrier mechanisms, and mucosal secretion. The age-related responses observed with both vaccines, intestinal permeability, and the salivary concentrations of sIgA confirm the biological validity of the tests, greatly reducing the likelihood that the negative results are a type II error resulting from imprecision in the outcome measure. Furthermore, vaccine responses were satisfactory in almost all children, indicating that at this age the processes involved in antigen recognition and response are operating effectively.

One possible explanation for the results of the present study relates to the surprising finding that there was no significant effect of maternal supplementation status on infant birth weight. It is possible that the cohort of intervention children selected from the original cohort were nonresponders to the supplement and were therefore not appropriately different from the control children. Note that this does not invalidate the testing of the other 2 main hypotheses in relation to season of birth and birth size. In particular, the range in birth weights (1700–4160 g) should have been large enough to detect any differences in immune function.

The failure to find associations at this particular age does not necessarily negate the main hypothesis that immune function can be programmed during a critical period in early life, as reflected by the Kaplan-Meier survival analysis. In the survival plots, it was only after the age of 15 y that the survival of those born during the hungry season diverged from those born during the harvest season. It may be that the individuals born during or shortly after the hungry season suffer from a premature immunosenescence similar to that observed with aging (48, 49). This premature immunosenescence in those born during the hungry season may occur in many ways. It is possible that immune function may be impaired from early life but the effect may not manifest until the later years when it falls below a functional threshold (ie, the hungry season cohort may have a lower functional reserve that creates earlier susceptibility even if the rate of immunosenescence is the same in all groups). Alternatively, an elevated lymphocyte turnover (as anticipated, but not yet shown in this tropical environment with high disease burden) would accentuate any programmed differences because memory T cells may reach the limit of division early in life (ie, accelerated immunosenescence). Future studies will continue to explore the early programming of immune function hypothesis at later ages with a focus on the possibility that the defect may be in immunologic memory, possibly secondary to early thymic damage, rather than in early immune responses.

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