Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence[1–3]

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ABSTRACT

Background: Recently, researchers have considered the fetal and infant origins of several adult cardiovascular and metabolic diseases, but the implications of early events for immune function and infectious disease are unclear.

Objective: We investigated the association between prenatal undernutrition and immunocompetence in adolescence and hypothesized that intrauterine growth retardation is associated with a lower likelihood of mounting an adequate antibody response later in life.

Design: A subsample of one hundred three 14–15-y-olds was recruited from an ongoing longitudinal study in which data collection began while participants were in utero. A typhoid vaccine was given, and anti-typhoid antibodies were measured 2 wk and 3 mo later as a functional marker of immunocompetence. The likelihood of mounting an adequate antibody response was compared for adolescents who were small for gestational age or appropriate for gestational age at birth while controlling for a range of postnatal exposures.

Results: The predicted probability of mounting a positive antibody response for adolescents who were prenatally and currently undernourished was 0.32, compared with probabilities of 0.49–0.70 for adequately nourished adolescents ($P = 0.023$). Diarrhea in the first year of life ($P = 0.009$) and fast weight gain during the first 6 mo ($P = 0.003$) were also associated with a higher probability of response.

Conclusions: These findings extend the concept of fetal and early infant programming of adult diseases to the immune system and suggest that early environments may have long-term implications for immunocompetence and infectious disease risk, particularly in developing countries. *Am J Clin Nutr* 2001;74:543–8.

KEY WORDS Prenatal exposure, immune system, growth and development, infantile diarrhea, vaccine, nutrition, adolescents

INTRODUCTION

Interest in the fetal and infant origins of many adult chronic, degenerative diseases is currently high, and evidence is mounting for an association between intrauterine growth retardation (IUGR) and adult hypertension, abnormal blood lipid profiles, coronary artery disease, and diabetes (1–3). However, the implications of early events for adult infectious disease risk are unknown (4).

Three convergent lines of evidence suggest that this may be an important area of investigation. First, prenatal and early postnatal undernutrition have been linked to deficits in several aspects of cell-mediated immunity, to involution of lymphoid tissues such as the thymus, and to suppression of antibody responses to vaccination (5–10). These deficits persist for weeks or, in some cases, even years, but their long-term consequences for immune function beyond early childhood have not been reported. Second, murine models have documented impairments in immunity after maternal undernutrition that last through adulthood and into the next generation, despite ad libitum feeding of both F_1 and F_2 generations (11, 12). Last, symptoms of adult atopic and autoimmune disease in humans have been linked to fetal growth (13, 14), and research in the Gambia associated season of birth with infectious disease mortality after the age of 15 y, suggesting an association between prenatal undernutrition, immune function, and adult vulnerability to infectious disease (15, 16). The present study prospectively evaluates the possibility that prenatal and early postnatal environments have long-term implications for immune function in adolescence.

METHODS

Study participants and protocol

Participants were recruited from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing population-based study of maternal and child health in the Philippines that began in 1983 with the recruitment of 3327 pregnant women (17). IUGR is common in the Philippines, as in many countries in the developing world, most likely because of high rates of maternal undernutrition during pregnancy. In the CLHNS, the

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prevalence of IUGR, defined as birth-weight-for-gestational-age below a reference 10th percentile (18), is 20.9% (19).

Home visits were made before birth, immediately after birth, and every 2 mo for 2 y to collect in-depth data on child and maternal health, anthropometric measures, patterns of breast-feeding, dietary intake, rates of diarrhea and respiratory disease, household socioeconomic status and demographics, and environmental quality (20). Follow-up surveys were conducted in 1991, 1994–1995, and 1998–1999. The prospective design of this study, as well as the detailed information collected at multiple time points, provides a unique opportunity to evaluate several variables that may confound, mediate, or moderate the association between IUGR and later immune function.

In 1998–1999, 2089 CLHNS participants—14 or 15 y of age at the time—were contacted for follow-up data collection. From these remaining participants, a subsample of 103 girls and boys was recruited based on the following criteria: full-term birth (37 wk), currently healthy, and small for gestational age (SGA: defined as <10th percentile of birth-weight-for-gestational-age) or appropriate for gestational age (AGA: ≥10th percentile) (18) at birth. By restricting our sample to full-term births, we eliminated the potentially confounding effects of premature delivery and focused on the small size assumed to be related to prenatal undernutrition. Gestational age was determined from maternal recall of the date of her last menstrual period or by clinical (21) assessment of the newborn for those mothers who could not recall their last menstrual period, who had low-birth-weight infants, or who had pregnancy complications. The subsample of SGA-classified adolescents recruited for this study was representative of SGA-classified adolescents in the larger CLHNS cohort, except that the average birth weight of the subsample was significantly lower than the average of 2494 g for all SGA-classified adolescents in the CLHNS ($P < 0.001$).

When adolescents were enrolled in the immune study, <5 mL EDTA-treated plasma was collected and immediately frozen. Also at this time, the participants were vaccinated against typhoid fever with a 25-μg dose of purified Vi cell surface polysaccharide extracted from *Salmonella typhi*, delivered in 500 μL sterile solution via intramuscular injection (Pasteur Merieux, Lyon, France). Additional blood samples were drawn 2 wk and 3 mo later. Participants had not been previously immunized against typhoid. The study protocol was conducted as approved by the University of North Carolina School of Public Health Institutional Review Board for research involving human subjects.

Anti-typhoid antibody enzyme-linked immunosorbent assay

Samples were shipped on dry ice to the United States, and anti-typhoid immunoglobulin G (IgG) antibody titers were analyzed as a functional measure of immunocompetence. Flat-bottomed microtiter plates (Immulon 2; Dynex Technologies, Chantilly, VA) were coated with 0.5 μg Vi antigen per well and washed with phosphate-buffered saline containing 0.05% polysorbate 20, followed by a 10-min soak. Samples were diluted 1:20 in phosphate-buffered saline containing polysorbate 20 and then serially diluted 1:2 to a final concentration of 1:1280. A 100-μL portion of each dilution was added in duplicate, and the plates were incubated at room temperature for 1 h. Positive and negative controls were included with each assay. Plates were washed and 100 μL of a 1:5000 dilution of affinity-purified, alkaline phosphatase–conjugated, goat anti-human IgG (Sigma, St Louis) was added and the plates incubated at room temperature for 1 h. Plates were washed, followed by the addition of 100 μL *p*-nitrophenyl phosphate substrate solution (Sigma) and incubation for 1 h at room temperature. Last, 50 μL of a 3-mol NaOH/L stop solution was added, and absorption values were read at 405 nm on a plate reader spectrophotometer (model MR5000; Dynatech, Chantilly, VA). The highest dilution at which anti-typhoid antibodies were still detectable (>2 SDs from the mean optical density of multiple determinations of seronegative samples) was defined as the endpoint concentration for that adolescent.

Data analysis

Following previous research in which the efficacy of the intramuscular anti-typhoid vaccine was evaluated (22, 23), a ≥4-fold increase in antibody titer from baseline was defined as a positive response to the vaccine challenge. Adolescents with a <4-fold increase were considered nonresponders. By measuring antibody production after vaccination, we attained a functional marker of immunocompetence that mimics the real-world process of pathogen exposure and immune response that is critical in defining resistance to infectious disease. Two persons with elevated baseline titers (indicating recent or ongoing typhoid infection) were removed before analysis. Complete nutritional, anthropometric, morbidity, and sociodemographic data were available for 96 persons.

Maximum likelihood logistic regression (Stata Corporation, College Station, TX) was used to model the likelihood of responding to the vaccine with a ≥4-fold increase in antibody titer. IUGR was the primary independent variable of interest, but aspects of the prenatal environment (maternal nutritional status during pregnancy and parity), postnatal environment (household socioeconomic status, pattern of breast-feeding, pathogen exposure, and infectious morbidity) and growth (length and weight), and current status (pubertal status and nutritional status) were also considered as potential predictors of antibody response. Significance was defined as a P value < 0.05.

We hypothesized that adolescents in the SGA group would be less likely to mount an adequate antibody response than would AGA adolescents. The model-building strategy outlined by Lucas et al (24) was used to increase our confidence in concluding that any association between IUGR and later immune function was due to the quality of the prenatal environment rather than to correlated aspects of postnatal experience. We first evaluated the crude association between SGA status and later immune function. We then added measures of current nutritional status as well as variables representing multiple aspects of postnatal growth and morbidity. We considered interactions between SGA status and these variables where appropriate. If adjustment for postnatal factors was found to attenuate the effect of being SGA, we concluded that postnatal rather than prenatal environments were more likely to be causally related to adolescent immune function. If adjustment for postnatal factors amplified the effect of being SGA, we concluded that both prenatal and postnatal influences were relevant. Significant interactions between SGA status and postnatal factors were assumed to indicate that being SGA modified the effect of later environments.

RESULTS

Basic descriptive statistics for SGA and AGA adolescents are presented in **Table 1**. Proportion of females, gestational age,

TABLE 1
Descriptive statistics for adolescents who were small for gestational age (SGA) or appropriate for gestational age (AGA) at birth

	SGA (n = 55)	AGA (n = 41)
Female (%)	50.9	68.3
Gestational age (wk)	39.4 ± 1.9[1]	40.2 ± 2.0
Birth weight (g)	2376 ± 220	3249 ± 369[2]
Weekly household income (pesos)[3]	203 ± 204	270 ± 213
Maternal BMI at delivery	21.1 ± 2.1	22.2 ± 2.7[4]
First pregnancy (%)	30.9	12.5[4]
Weight gain, first year (kg)	5.0 ± 0.9	4.6 ± 1.1
Length gain, first year (cm)	21.0 ± 2.7	20.3 ± 2.8
Diarrheal episodes, first year[5]	1.4 ± 1.1	1.2 ± 1.0
Duration of exclusive breast-feeding (d)	50.9 ± 34.7	48.6 ± 43.9
Current age (y)	14.5 ± 0.5	14.6 ± 0.5
Current BMI (kg/m^2)	18.0 ± 1.7	18.9 ± 2.7[4]

[1] $\bar{x}$ ± SD.
[2,4] Significantly different from SGA: [2] $P < 0.001$, [4] $P < 0.05$.
[3] One peso ≅ $0.05 in 1983–1984.
[5] Number of times mothers reported diarrhea in the week preceding bimonthly interviews during the infant's first year of life (possible range: 0–6).

household income, duration of exclusive breast-feeding, diarrheal morbidity and growth in the first year of life, and current age did not differ significantly between the 2 groups. As expected, adolescents who were SGA at birth had significantly lower birth weights and lower body mass index (BMI; in kg/m^2) values at age 14–15 y and were significantly more likely to be born to mothers without a prior pregnancy and with lower BMIs at the time of birth.

Overall, 47.9% of the sample responded to the vaccine with a ≥4-fold increase in anti-typhoid antibody titer at 2 wk. No significant bivariate association between birth-weight-for-gestational-age and the likelihood of a positive antibody response was found at 2 wk (positive antibody response in 45.5% and 51.2% of SGA-classified and AGA-classified adolescents, respectively; n = 96) or 3 mo (positive antibody response in 50.0% and 48.7% of SGA-classified and AGA-classified adolescents, respectively; n = 91). Five adolescents were lost to follow-up between the 2-wk and 3-mo follow-up periods, limiting the sample size available for analysis at this time point to 91. The pattern of results was comparable at 2 wk and 3 mo, although fewer statistically significant associations were found at 3 mo. We found no evidence for a delay or shift in the pattern of antibody response among SGA adolescents. For these reasons, we report results for the 2-wk follow-up time point only.

To evaluate the effect of current nutritional status, adolescents were split into lower and higher BMI groups based on sex-specific median values (girls: 18.3; boys: 18.1). The addition of current BMI did not alter the effect of SGA status (β for SGA with BMI in model = 0.18, P = 0.67). However, there was a trend toward an effect of SGA status when an interaction term with BMI was included (β for SGA = 1.0, P = 0.085; β for BMI = 0.85, P = 0.13), and the SGA × BMI interaction term was significant (β = −1.72, P = 0.041).

Next, several postnatal variables were included with SGA status and BMI. With these covariates in the model, the main and interacted effects of SGA status and BMI strengthened considerably. Sex, pubertal status, and diarrheal morbidity and weight velocity in the first year of life were also significantly associated with the likelihood of mounting a positive antibody response (**Table 2**).

Adolescents in the SGA group who currently had low BMIs stood out as being the least likely to respond to the vaccine, whereas adolescents with higher current BMIs, normal birth-weight-for-gestational-age, or both were likely to respond at a frequency higher than the overall sample prevalence. When adolescents in the SGA group with low current BMIs were defined as the reference group, the adolescents in the AGA group with low current BMIs were significantly more likely to respond to the vaccine (β = 2.20, P = 0.007), and there was a trend toward a higher likelihood of response for the SGA, high current BMI group (β = 1.15, P = 0.09). Adolescents in the AGA group with high current BMIs were not significantly more likely to respond (β = 0.94, P = 0.18). The amplification of the SGA effect after adjustment for multiple postnatal variables is consistent with the interpretation that IUGR is causally related to adolescent immune function.

Shown in **Figure 1** are the predicted probabilities of responding to the vaccine for the following groups: SGA, low current BMI; SGA, high current BMI; AGA, low current BMI; and AGA, high current BMI. Predicted probabilities were calculated by using regression coefficients from the full maximum likelihood model and a set of assigned exposures. The variables of interest (in this case birth-weight-for-gestational-age and current BMI) were set to the desired level, individual values were retained for other covariates, and the probability of a positive response was calculated based on the β coefficients from the full model (Table 2). This procedure allowed us to examine the effect of specific variables while controlling for potentially confounding factors. For example, in this sample, the probability of

TABLE 2
Maximum likelihood model with significant predictors of mounting a positive antibody response (≥4-fold increase in antibody titer) to typhoid vaccination[1]

	β	SE	P
Sex (female = 0, male = 1)	0.06	0.52	0.901
Maturational timing (early = 0, late = 1)	1.33	0.55	0.016
Diarrhea (none = 0, ≥1 = 1)	1.62	0.62	0.009
Birth-weight-for-gestational-age (SGA = 0, AGA = 1)	2.20	0.82	0.007
Current BMI (low = 0, high = 1)	1.15	0.69	0.094
Birth-weight-for-gestational-age × current BMI	−2.41	1.06	0.023
Weight velocity (low = 0, high = 1)	2.30	0.78	0.003
Breast-feeding duration (short = 0, long = 1)	1.25	0.76	0.100
Weight velocity × breast-feeding	−2.71	1.07	0.011
Constant	−2.80	1.03	0.007

[1] Log likelihood = −51.73; $P < 0.001$. n = 96. SGA, small for gestational age; AGA, appropriate for gestational age.

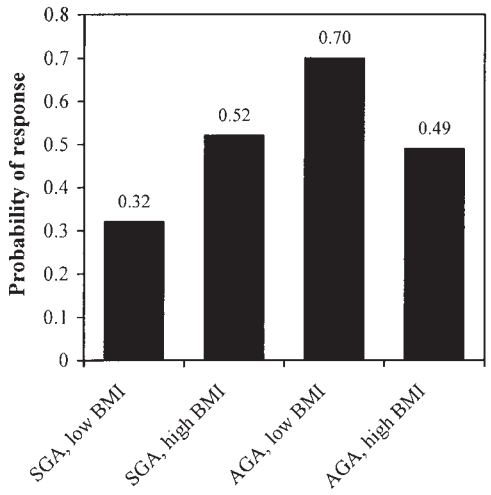

FIGURE 1. Predicted probabilities of mounting a positive antibody response 2 wk after vaccination for adolescents born small for gestational age (SGA) or appropriate for gestational age (AGA) and with lower or higher BMIs at age 14–15 y (n = 96). Predicted probabilities are based on the full logistic regression model in Table 2 and are adjusted for the effects of variables in the model that are not represented in the figure.

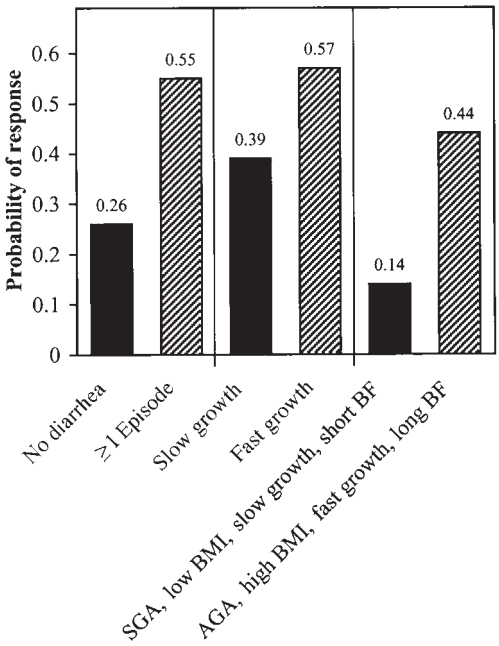

FIGURE 2. Predicted probabilities of mounting an adequate antibody response according to diarrheal morbidity in the first year (0 or ≥1 episode), weight velocity in the first 6 mo (slow growth or fast growth), and small for gestational age (SGA) at birth, low current BMI, slow growth in the first year, and short duration of exclusive breast-feeding (short BF) or appropriate for gestational age (AGA) at birth, high current BMI, fast growth in the first year, and long duration of exclusive breast-feeding (long BF). Predictions are based on the full model presented in Table 2 and are adjusted for potentially confounding variables (n = 96).

mounting a positive antibody response to vaccination was 0.32 for an adolescent in the SGA group with a low current BMI and at the sample mean for other covariates.

Boys and girls did not differ in likelihood of responding to the vaccine, but developmental status was identified as a potential confounder because the timing of puberty may be related to IUGR as well as to current BMI. Boys were grouped into early and late maturing groups on the basis of self-reported pubic hair growth. Girls were grouped according to the relative timing of menarche: girls who reached menarche before the median menarcheal age were labeled early maturers, whereas girls who had yet to reach menarche or who reached menarche after the median age were considered late maturers. Earlier maturing adolescents (those in later stages of puberty) were significantly less likely to respond to the vaccine than were later maturers (Table 2).

Mothers were asked whether their infants had diarrhea in the week preceding each bimonthly visit during the first year of life, and 75% of the sample reported a diarrheal episode at one or more of these intervals. The presence of at least one diarrheal episode during the first year of life was associated with a significantly higher likelihood of antibody response (Table 2). The probability of responding to the vaccine for adolescents lacking this exposure was less than half that of adolescents reporting diarrhea morbidity (**Figure 2**). No significant relation was found for reports of respiratory morbidity.

Postnatal growth rates were also related to adolescent vaccine response. Weight velocity (kg/mo) was calculated from birth to 6 mo, and adolescents were assigned to slow or fast growth groups on the basis of a median weight velocity of 0.63 kg/mo. When this variable was included in the full model, there was a trend toward an effect of growth rate (β = 2.35, P = 0.076). The effect of weight velocity strengthened when considered in interaction with duration of exclusive breast-feeding. Adolescents were classified as being short or long exclusive breast-feeders on the basis of a median duration of exclusive breast-feeding of 50 d (ie, no supplemental foods or liquids of any kind). Without weight velocity in the model, the main effect of exclusive breast-feeding duration was not significant (β = 0.87, P = 0.76). However, the interaction between weight velocity and duration of exclusive breast-feeding was significant, and the main effect of weight velocity was significant when this interaction term was included in the model (Table 2). The probability of responding to the vaccine for slow growers was predicted to be 0.39, compared with 0.57 for fast growers (Figure 2). The effect of weight velocity over the first 12 mo of life was comparable but weaker (β = 1.65, P = 0.025), and no significant effect of 6-mo or 12-mo length velocity (cm/mo) was found.

Last, the combined effects of IUGR, weight velocity, breast-feeding duration, and current BMI were considered to model the best and worst case scenarios for adolescents in the Philippines. The predicted probability of mounting a positive antibody response for adolescents who were SGA, slow growing, and short exclusive breast-feeders and had low current BMIs—representing chronic prenatal and postnatal undernutrition—was 0.14. The probability of responding was more than 3 times higher for adolescents who were AGA, fast growing, and long exclusive breast-feeders and had high current BMIs (Figure 2).

DISCUSSION

Prenatal undernutrition and early postnatal morbidity and growth velocity were significantly associated with typhoid anti-

body responsiveness in Filipino adolescents, possibly due to permanent effects on components of immunity that mature early in life. The effect of IUGR was significant in interaction with current nutritional status and remained significant after adjustment for a range of postnatal exposures. This provides tentative support for our hypothesis that prenatal environments may be causally related to adolescent immune function, although additional research in a larger sample will be required to confirm and elaborate these results. Previous work has emphasized the relevance of programming for chronic, degenerative diseases involving cardiovascular and endocrine systems (1–3); our findings suggest a role for fetal and early infant experience in programming the immune system as well.

Limitations of this study include the relatively small sample size and its associated reduction in statistical power. In future research we hope to take full advantage of the CLHNS cohort, but in this study it is possible that the effects of birth weight are overestimated because the mean birth weight for SGA-classified adolescents in the subsample was 118 g lower than the mean for the remaining SGA-classified adolescents in the cohort. However, this was the only significant difference.

The positive association between diarrheal morbidity and antibody response is consistent with the hypothesis that exposure to pathogens is critical for normal immune development. In effect, the immune system expects to be primed by antigenic experience, and without such experience the system may develop in such a way that diminishes its ability to fight infectious disease later in life (25). However, early morbidity has also been associated with suppressed immune function 3–5 y later (26, 27), suggesting that the timing and/or nature of pathogen exposure, as well as the nature of subsequent exposures, may be important factors in determining whether early morbidity has positive or negative long-term immunologic consequences. In this study, reported diarrheal frequency may serve as a general marker of hygiene, with a higher number of diarrheal episodes indicating a higher level of antigenic exposure in infancy.

IUGR has been linked to immunodeficiency in infancy and early childhood (7–9), and findings from the present study provide evidence for prolonged impairment that lasts at least into adolescence. However, the effect of IUGR was strongest in interaction with current nutritional status, such that adolescents in the SGA group with low current BMIs were most likely to be immunocompromised. As suggested by this pattern of results, prenatal undernutrition may exert its influence by predisposing persons to a higher level of vulnerability to the immunosuppressive effects of poor nutritional status later in life.

Several studies have pointed to the thymus as a potential mediator of the immunologic consequences of undernutrition. Lymphoid tissues are acutely sensitive to undernutrition in infancy and early childhood, and severe malnutrition may lead to nutritional thymectomy, with lasting effects on immunity (28–30). Recent sonographic assessments of thymic volume reported positive associations with birth weight, body length, and breast-feeding in infancy. In additional analysis of our CLHNS subsample, we found that thymopoietin production—a marker of thymic activity—at age 14–15 y is positively associated with growth in length during the first year of life and negatively associated with prenatal undernutrition in interaction with the duration of exclusive breast-feeding (31).

Lymphoid tissues begin to emerge in the second and third month of gestation, and insults early in development may have more serious consequences than those endured later in life (32). The positive association between postnatal weight velocity and adolescent immune response reported here might be a reflection of this process: compared with a fast-growing infant, an infant who gains less weight during this critical period of immune development may also grow a smaller thymus, with potentially lasting implications for immune function. Alternatively, researchers using animal models have documented lasting irregularities in immune function and hypothalamus-pituitary-adrenal function in offspring of mothers stressed during pregnancy, suggesting a possible mechanistic role for glucocorticoids in mediating this effect (33–36).

These findings suggest that intrauterine and early postnatal environments have long-term consequences for infectious disease risk, as well as for risk of other diseases with an immunologic component, including asthma and allergy, autoimmunity, neoplasia, and cardiovascular diseases with potentially infectious origins. In addition, the success of immunization programs that target adolescents and young adults (eg, tetanus and hepatitis B) may be affected, and efforts may be necessary to increase the effectiveness of vaccines in persons with low birth weights. Marginally nourished populations may be particularly vulnerable, but the possibility of fetal programming of immune function will probably have implications for other populations as well. Further research is needed to identify the moderators of early environmental effects, the impact these effects have on specific immune pathways, and the mechanisms that link early environments to immunity later in life.

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