Introduction

Evidence continues to mount in support of the hypothesis that many chronic degenerative diseases in adulthood have prenatal or early postnatal origins, probably due to programming of cardiovascular and endocrine systems early in development. However, the implications of early environments for the development and function of the immune system in adulthood are less well understood (Moore, 1998). This is an important area of enquiry, in that immunological processes are centrally involved in infectious, neoplastic, atopic and autoimmune diseases, and may, in some cases, contribute to cardiovascular disease. This chapter will review a range of research relevant to the immune programming hypothesis, and discuss attempts to investigate the long-term immunological effects of early environments in an ongoing, prospective study in the Philippines (McDade et al., 2001a,b, 2004).

The Early Life Origins of Immune Function

Compelling circumstantial evidence for the relevance of early environments to adult immunocompetence comes from recent research in the Gambia. In this region, the annual cycle of climate introduces marked seasonal variation in nutritional status, with the annual rains contributing to periods of significant hunger. Analysis of demographic records indicates that being born during the hungry season – itself associated with lower birthweight and higher postnatal disease exposure – increases the risk of premature death in young adulthood by a factor of 3.7 after the age of 14.5 years, and by a factor of 10.3 after 25 years (Moore et al., 1999). The majority of these premature deaths were infectious in aetiology, suggesting impairment in some aspect of immune function. Although direct support for this link in human populations is
currently limited, a number of convergent lines of evidence suggest that this is an important area of enquiry.

Early research with murine models documented alterations in offspring immune function following maternal nutritional deficiencies (both macro- and micro-nutrient) that last into adulthood and into the next generation, despite ad libitum feeding of both F1 and F2 generations (Chandra, 1975a; Beach et al., 1982). There is evidence that the hypothalamic-pituitary-adrenal axis may be involved in these associations. Injection of pregnant rat dams with dexamethasone, a cortisol agonist, has been associated with lymphoid atrophy and impaired immunity in offspring (Elshin et al., 1983), while rats born to undernourished mothers demonstrate reduced hypothalamic-pituitary-adrenal (HPA) and tumour necrosis factor (TNF)-α responsiveness to endotoxaemia, compared to offspring of well-nourished mothers (Chisari et al., 2001). A similar pattern of results is emerging from research conducted among non-human primates: gestational stress, as well as injections of adrenocorticotropic hormone (ACTH) or dexamethasone during pregnancy, are associated with lasting irregularities in immune function and HPA activity in juvenile offspring (Coe et al., 1992, 1996; Clarke et al., 1994; Coe and Lubach, 2000).

The immediate effects of protein-energy malnutrition on immune function in infancy and early childhood have been well documented. Postnatal undernutrition is associated with deficits in several components of cell-mediated immunity, involution of the thymus and reduced antibody response to vaccination (Chandra, 1988; Gershwin et al., 2000; Suskind and Tontisirin, 2001). Less intensively investigated are the immunological implications of prenatal undernutrition, although a relatively limited number of studies suggest that there are significant impairments in aspects of immunity following intrauterine growth retardation (IUGR) (Table 13.1) (Chandra, 1975b, 1981; Moscatelli et al., 1976; Chandra et al., 1977; Ferguson, 1978; Saha et al., 1983; Pittard et al., 1984; Musi-Pinhata et al., 1993; Chatrath et al., 1997; Moore et al., 1999). These findings are consistent with the fourfold increase in neonatal mortality risk, and the twofold increase in postneonatal mortality risk, for infants born small for gestational age (SGA, full term, less than 2500 g) compared to infants born appropriate for gestational age (AGA, full term, > 2500 g) (Ashworth, 1998). Similarly, SGA infants have been shown to be at increased risk of infectious morbidity in infancy and early childhood (Kebede and Larson, 1994; Lira et al., 1996). Deficits in immune function following prenatal undernutrition persist for weeks, and in some cases months, but their long-term implications for immunocompetence beyond childhood have not been previously reported.

Gestational age is a potential confounder of the association between low birthweight and immune function, and in a number of studies it is not possible to separate the effects of preterm delivery from fetal undernutrition and growth restriction. This may be an important distinction, since Chandra (1981) has shown that by 3 months of age, pre-term AGA infants (30–34 weeks' gestation) recover normal T lymphocyte percentage and proliferative responsiveness to phytohaemagglutinin (PHA), whereas full-term, SGA infants (37–41 weeks) continue to demonstrate impairment at 12 months. Similarly, SGA infants have reduced thymic hormone activity at 1 month, while pre-term AGA infants do not
<table>
<thead>
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<th>Participants (gestational age)</th>
<th>Age at assessment</th>
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<tr>
<td>26 SGA (29–42 weeks), 26 AGA (not reported)</td>
<td>Birth, 1 month, 3 months</td>
<td>SGA = reduced DTH response at 1 month; reduced lymphocyte response to PHA at birth; reduced % T lymphocytes at birth; 1 month, 3 months; reduced bactericidal capacity at birth; reduced response to polio vaccine</td>
<td>Chandra (1975b)</td>
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<td>19 SGA (38–41 weeks), 30 AGA (not reported)</td>
<td>Birth to 3 months</td>
<td>SGA = reduced % and number of T and B lymphocytes at birth; no difference in lymphocyte response to PHA</td>
<td>Moscatelli et al. (1976)</td>
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<td>50 SGA (not reported)</td>
<td>3 months to 5 years</td>
<td>SGA = reduced % T lymphocyte, DTH response</td>
<td>Chandra et al. (1977)</td>
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<tr>
<td>17 SGA (35–41 weeks), 31 AGA (31–41 weeks)</td>
<td>&lt; 4 days</td>
<td>SGA = reduced DTH response; reduced number total and T lymphocytes; no difference in lymphocyte response to PHA</td>
<td>Ferguson (1978)</td>
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<td>8 children born SGA (not reported), 11 'healthy' age-matched controls (not reported)</td>
<td>1–5 years</td>
<td>SGA = reduced DTH response; reduced % T lymphocytes; reduced lymphocyte response to PHA</td>
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<td>9 SGA (37–41 weeks), 7 AGA (30–34 weeks), 7 AGA (37–41 weeks)</td>
<td>Birth, 1, 3 and 12 months</td>
<td>SGA = reduced thymic hormone activity at 1 month; reduced number T lymphocytes at all ages; reduced PHA response at birth, 3 months, 12 months</td>
<td>Chandra (1987)</td>
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<td>20 AGA (38 ± 1.0), 15 AGA (32.8 ± 1.4), 18 AGA (38.0 ± 0.8)</td>
<td>Birth, 3 and 6 months</td>
<td>SGA = reduced DTH response; reduced IgA and IgM production at 3 and 6 months; reduced number total lymphocytes at 3 months</td>
<td>Saha et al. (1983)</td>
</tr>
<tr>
<td>29 SGA (33–42 weeks), 120 AGA (33–42 weeks)</td>
<td>Birth</td>
<td>No difference in lymphocyte response to PHA, PWM, ConA, SpA</td>
<td>Pittard et al. (1984)</td>
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<tr>
<td>57 SGA (37–41 weeks), 52 AGA (37–41 weeks)</td>
<td>3 months, 6 months or 9 months</td>
<td>No difference in DTH or lymphocyte response to BCG vaccination given 12–14 weeks prior to assessment</td>
<td>Musi-Pinhata et al. (1999)</td>
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<tr>
<td>25 SGA (‘full-term’), 25 AGA (‘full-term’)</td>
<td>Birth</td>
<td>SGA = reduced number and % lymphocytes, reduced CD4:CD8 ratio, reduced IgG and C3</td>
<td>Chatterjee et al. (1997)</td>
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<td>472 children in the Gambian Intervention group: maternal dietary supplementation for 20 weeks before delivery; control group: maternal supplementation for 20 weeks after delivery</td>
<td>6–9 years</td>
<td>Maternal supplementation before delivery associated with improved DTH response; supplementation after delivery associated with improved response to rabies vaccine; no associations with birthweight</td>
<td>Moore et al. (1999)</td>
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AGA, appropriate for gestational age; SGA, small for gestational age; DTH, delayed type hypersensitivity; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; ConA, concanavalin A; SpA, serotype c-specific polysaccharide antigen; BCG, bacille Calmette-Guérin.
differ substantially from healthy full-term infants. However, mortality statistics in a range of populations do not suggest a dramatic difference in neonatal and post-neonatal mortality risk between full-term, SGA infants, and preterm infants of the same birthweight (Ashworth, 1998).

Prior to our research in the Philippines (see below), only two studies had reported an association between fetal growth and a measure of immune activity beyond childhood. In a sample of 280 men and women aged 47–55 years from the UK, a larger head circumference at birth was associated with an increased likelihood of elevated immunoglobulin E (IgE) in adulthood (Godfrey et al., 1994). A larger birthweight was also associated with increased IgE, but this association disappeared when the associations were adjusted for head circumference. In a different UK sample of 305 women aged 60–71 years, lower birthweights were associated with increased likelihood of thyroglobulin and thyroid peroxidase autoantibody production in adults (Phillips et al., 1993). These findings suggest that fetal environments may programme immune function in adulthood, with potentially important implications for the development of allergic and autoimmune disease.

In both these studies, as well as those from the Gambia (Moore et al., 1999, 2001), impaired thymic development has been proposed as a key mediator of the association between early environments and later immunocompetence. The thymus is a primary lymphoid organ critical for normal T-lymphocyte maturation and function, and for the production of thymic hormones with wide-ranging immuno-regulatory properties (Steinman 1986; Schulof et al., 1987). Lymphoid tissues are acutely sensitive to undernutrition in infancy and early childhood, to the extent that severe malnutrition may lead to what has been described as a nutritional ‘thymectomy’, with significant and lasting impairments in cell-mediated immunity (Naeye et al., 1971; Dutz et al., 1976; Dourov, 1986). Recent sonographic analyses indicate that thymic size in infancy is positively associated with birthweight and body length, suggesting that prenatal nutrition or fetal growth influence thymic development (Hasselbalch et al., 1999). Since lymphoid tissues begin to develop in the second and third month of gestation, and since prenatal undernutrition appears to have a disproportionate impact on these tissues, the effects of early nutritional insults may be even greater than those endured postnatally (Xanthou, 1985; Owens et al., 1989).

A parallel line of enquiry has indicated that exposure to infectious disease early in life may have significant long-term consequences for immunity. Findings from Guinea-Bissau suggest that measles infection in early infancy increases the risk of death before 5 years of age by a factor of 3.8 (Aaby et al., 1993), and that measles infection in childhood more than doubles the risk of suppressed cell-mediated immunity 3 years later (Shaheen et al., 1996a). In addition, earlier research has shown that gastroenteritis in the first 6 months of life – but not after – is associated with thymic atrophy and impaired cell-mediated immunity up to 5 years later (Dutz et al., 1976; Ghavami et al., 1979).

Similarly, proponents of the ‘hygiene hypothesis’ suggest that infectious morbidity early in life has long-term consequences for immune function by biasing lymphocyte maturation toward the Th1 phenotype. Atopic immune processes associated with Th2 lymphocytes are therefore reduced, protecting
against the development of atopic disease in adulthood (Shaheen et al., 1996b; Shirakawa et al., 1997; Matricardi et al., 2000; Ili et al., 2001). A relative absence of infectious disease early in life is thereby hypothesized as accounting for the rising rates of allergy and asthma in developed nations (Rook and Stanford, 1998).

In sum, research to date suggests that both prenatal and early postnatal environments are important for immune development and may have long-term implications for infectious, atopic and autoimmune disease. Although the fetal origins hypothesis focuses attention on prenatal conditions, early postnatal environments are also critical in shaping immune development, and should be given serious consideration. Few studies have been able to simultaneously evaluate the effects of prenatal and postnatal environments, even though it is likely that they play independent, and perhaps interactive, roles in the development of immunocompetence. A unique opportunity to explore these issues is provided by a dataset collected from a small cohort in the Philippines. Data from this sample have been collected prospectively from the third trimester of pregnancy through adolescence, and allow examination of the interaction between factors in fetal life, infancy and childhood as determinants of immune development and function.

Early Origins of Immune Function in the Philippines

Research participants and protocol

The early origins of immune function were evaluated in the Cebu Longitudinal Health and Nutrition Study (CLHNS). This ongoing population-based survey of maternal and child health in the Philippines began in 1983 with the recruitment of 3327 pregnant women (Cebu Study Team, 1989). As in many countries in the developing world, IUGR (defined as birthweight for gestational age below a reference 10th percentile; Hoffman et al., 1974) is common in the Philippines, most likely due to high rates of maternal undernutrition during pregnancy associated with poverty. In the CLHNS, the prevalence of IUGR is 20.9% (Adair, 1989).

Home visits were made prior to birth, immediately following birth, and every 2 months for 2 years, to collect in-depth data on child and maternal health, anthropometry, patterns of breastfeeding, dietary intake, rates of diarrhoea and respiratory disease, household socio-economic status and demographics, and environmental quality (Popkin et al., 1990). 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 a number of variables that may confound, mediate or moderate the association between IUGR and later immune function. The immune studies reported here are part of a broader research agenda focused on elucidating the role of prenatal and early postnatal environments on growth, maturation and risk for chronic and infectious disease in childhood and adolescence (Adair, 1999, 2001; Adair et al., 2001; Adair and Cole, 2003; Kuzawa and Adair, 2003).
In 1998–1999, 2089 CLHNS participants, 14–15 years 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 weeks), currently healthy, and small for gestational age (SGA: defined as < 10th percentile of birthweight for gestational age) versus appropriate for gestational age (AGA: ≥ 10th percentile) (Hoffman et al., 1974). By restricting the sample to full-term births, the potentially confounding effects of preterm delivery were eliminated. The study therefore focused upon small size, assumed to be related to fetal undernutrition. The subsample of SGA individuals recruited for this study was representative of all SGA individuals in the larger CLHNS cohort, except that the average birthweight was significantly lower than the average of 2494 g for all SGA individuals in the CLHNS (P < 0.001).

Upon enrollment in the immune study, EDTA plasma was collected and immediately frozen, followed by immunization 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). Two weeks and 3 months later, follow-up blood was drawn. Participants had not been previously immunized against typhoid.

**Measures of immune function**

A range of complementary immune measures was investigated to illuminate the possible role of early environments in shaping resistance to infectious disease, thymic development and function, and risk for atopic disease.

**Anti-typhoid antibody titre**

The humoral-mediated response to vaccination was assessed by comparing anti-typhoid antibody titres in baseline samples to those drawn 2 weeks and 3 months following vaccination. This method provides an in vivo, functional measure of immunocompetence that mimics the real-world process of pathogen exposure and immune response that is critical in defining resistance to infectious disease.

**Thymopoietin**

The thymus produces a range of thymic hormones with important roles in T-lymphocyte maturation and peripheral T-lymphocyte function (Dardenne and Bach, 1988; Goss and Fyfe, 1993). Thymopoietin — a 49-amino-acid polypeptide produced primarily by thymic epithelial cells — is among the best characterized, and is involved in early T-lymphocyte differentiation and the peripheral regulation of mature T-lymphocyte function (Goldstein et al., 1979; Ranges et al., 1982; Singh et al., 1998). Serum thymopoietin concentrations show a pattern of age-related decline that parallels declines in thymic volume: levels are highest at 15–30 years and drop with age (Lewis et al., 1978). Since the thymus may be an important link between early environments and later
immunocompetence, thymopoietin was measured as an indicator of thymic activity.

*Immunoglobulin E*

Asthma, allergy and other atopic diseases are mediated by IgE responses to primarily innocuous, common environmental allergens, and elevated total IgE concentrations are consistently found in atopic individuals (Wittig et al., 1980; Magnusson, 1988). A number of studies have reported significant associations between measures of fetal growth and later risk for allergy, asthma and obstructive airways disease (Barker et al., 1991; Seidman et al., 1991; Godfrey et al., 1994; Fergusson et al., 1997; Gregory et al., 1999; Shaheen et al., 1999; Lopuhaa et al., 2000). IgE is also significant in that early postnatal exposure to infectious disease has been associated with reduced IgE production, and reduced risk for allergy and asthma later in life (Martinez et al., 1995; Shaheen et al., 1996b; Shirakawa et al., 1997; Matricardi et al., 2000; Illi et al., 2001).

**Data analysis**

Maximum likelihood logistic regression (Stata Corporation, College Station, Texas, USA) was used to model the likelihood of responding to the vaccine with at least a fourfold increase in antibody titre, and least squares regression was used to evaluate predictors of log-transformed thymopoietin and of log-transformed IgE. Intrauterine growth retardation was the primary independent variable of interest, but aspects of the prenatal environment (maternal nutritional status during pregnancy, parity and season of birth), postnatal environment (household socio-economic status, pattern of breastfeeding, pathogen exposure and infectious morbidity) and growth (length, weight) and current status (pubertal status, nutritional status) were also considered as potential confounders or covariates in these analyses (see Table 13.2).

It was hypothesized that SGA individuals would differ significantly from AGA individuals in our markers of immune function. The model-building strategy outlined by Lucas et al. (1999) was applied to increase confidence that any association between IUGR and later immune function was due to the quality of the prenatal environment, rather than correlated aspects of postnatal experience. The crude association between SGA and later immune function was first evaluated. Measures of current nutritional status, as well as variables representing multiple aspects of postnatal growth and morbidity, were then added. Interactions between SGA and these variables were considered where appropriate. If adjustment for postnatal factors was found to attenuate the effect of 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 SGA, it was concluded that both prenatal and postnatal influences were relevant. Significant interactions between SGA and postnatal factors were assumed to indicate that SGA modified the effect of later environments.
Prenatal and postnatal influences on adolescent immune function

Characteristics of SGA and AGA individuals in the sample are presented in Table 13.2. As expected, SGA individuals had significantly lower birthweights, and were significantly shorter and lighter in adolescence as well. In addition, SGA individuals were more likely to come from households with lower incomes, and to be born to mothers with lower body mass index (BMI), who were pregnant for the first time.

Table 13.3 presents the mean unadjusted differences in the three measures of immunity. It was immediately apparent that prenatal undernutrition did not have a dramatic main effect on immune function. Although differences were in

<table>
<thead>
<tr>
<th>Table 13.2. Descriptive statistics for small-for-gestational age (SGA) and appropriate-for-gestational age (AGA) girls and boys. Mean (sd) values are presented for continuous variables.</th>
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<tr>
<td>SGA (N = 55)</td>
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<td>AGA (N = 48)</td>
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<tr>
<td>Female (%)</td>
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<td>Female (%)</td>
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<td>Prenatal environment</td>
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<td>Gestational age (weeks)</td>
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<td>Birthweight (g)</td>
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<td>Ponderal Index</td>
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<td>Maternal BMI (kg/m²)</td>
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<td>First pregnancy (%)</td>
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<td>Born in wet season (%)</td>
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<td>Weekly HH income (pesos)</td>
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<td>Birth to 1 year</td>
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<td>Growth in length (cm)</td>
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<td>Weight gain, 1st year (kg)</td>
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<td>Duration of exclusive breastfeeding (days)</td>
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<td>Episodes of diarrhoea</td>
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<td>Episodes of respiratory infection</td>
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<td>Unsanitary food storage area (%)</td>
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<td>Adequate excreta disposal (%)</td>
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<td>Crowding, persons/room</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>Age 14 or 15 years</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>Weekly HH income (pesos)</td>
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<td>Unsanitary food storage area (%)</td>
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*As assessed at six bimonthly intervals. Scores can range from 0 to 6.
*P < 0.05 (Student’s t test for continuous variables); **P < 0.01; ***P < 0.001.

BMI, body mass index; HH, household.
the hypothesized direction, they were not statistically significant. However, multivariate analyses revealed that prenatal undernutrition was significantly associated with each immune marker when considered in interaction with aspects of the postnatal environment. Table 13.4 demonstrates the importance of considering such interactions.

**Predictors of antibody response to typhoid vaccination**

Current nutritional status is an important factor in shaping one's ability to mount an adequate immune response to vaccination, but in this sample responders and non-responders did not differ significantly in BMI (18.4 and 18.5 kg/m², respectively, $P = 0.80$ for Student’s $t$-test). However, current nutritional status interacted significantly with prenatal undernutrition to predict antibody

| Table 13.3. Measures of immune function for adolescents born small-for-gestational age (SGA) and appropriate-for-gestational age (AGA). |
|---------------------------------|------------------|------------------|
|                                  | SGA   | AGA   | $P$ value$^a$ |
| Adequate vaccine response        | 45.5% | 51.2% | 0.78           |
| (N = 55)                        |       | (N = 41) |           |
| Thymopoietin (pmol/l)           | 31.1  (33.5) | 35.5  (32.0) | 0.31           |
| (N = 48)                        |       | (N = 45) |           |
| IgE (IU/ml)                     | 146.5 (219.1) | 99.0  (117.7) | 0.47           |
| (N = 55)                        |       | (N = 48) |           |

$^a$Student’s $t$-tests were used to evaluate the difference in log-transformed thymopoietin and IgE concentrations for SGA and AGA individuals. The $\chi^2$ test for independence of categorical variables was used to evaluate vaccine responsiveness.

| Table 13.4. Measures of immune function for adolescents born small-for-gestational age (SGA) and appropriate-for-gestational age (AGA) in interaction with significant postnatal factors. |
|---------------------------------|------------------|------------------|
|                                  | SGA   | AGA   | $P$ value$^a$ |
| Probability of adequate vaccine response$^b$ | BMI:  | < sex-specific median | 0.32  | 0.71  | < 0.05 |
|                                   | $\geq$ sex-specific median | 0.52  | 0.49  |       |
| Thymopoietin (log pmol/l)$^c$    | Exclusive breastfeeding: < 51 days | 1.59  | 1.54  | < 0.01 |
|                                   | $\geq$ 51 days | 1.53  | 1.77  |       |
| IgE (log IU/ml)$^d$              | Household hygiene: poor | 2.11  | 1.88  | < 0.01 |
|                                   | good | 1.88  | 1.90  |       |

$^a$Significance level for the interaction term with birthweight-for-gestational-age.

$^b$Adjusted for sex, pubertal status, duration of exclusive breastfeeding and diarrhoeal morbidity in first year.

$^c$Adjusted for sex.

$^d$Adjusted for sex and current household income.

BMI, body mass index.
responsiveness (Table 13.4). Adolescents born SGA, and who were currently below the sex-specific median value for BMI, stood out as those who were least likely to respond to the vaccination. With other covariates held constant, the probability of an adequate response was 0.32 for SGA/low current BMI adolescents, compared to a range of 0.49–0.70 for adolescents born AGA, with high current BMI or both (McDade et al., 2001b).

Aspects of the early postnatal environment were also found to be significant predictors of adolescent immune function. The presence of at least one episode of diarrhoea in the first year of life (as assessed at six bimonthly home visits) more than doubled the likelihood of an adequate antibody response, while rapid weight gain in the first 6 months and prolonged exclusive breastfeeding were also associated with improved responsiveness. The combined effects of significant prenatal and postnatal exposures is presented in Fig. 13.1. In the best-case scenario – AGA, high current BMI, rapid weight gain in the first 6 months and prolonged breastfeeding – the predicted probability of mounting an adequate antibody response to vaccination in adolescence was more than three times greater than in the worst-case scenario of SGA, low current BMI, slow postnatal weight gain and abbreviated breastfeeding.

Recent work on cardiovascular disease (CVD) risk factors has revealed sex differences in relationships with prenatal nutrition or birth outcome (Adair et al., 2001; Adair and Cole, 2003; Kuzawa and Adair, 2003). Poor maternal energy status during pregnancy (low adiposity) is a stronger predictor of future CVD risk.

![Fig. 13.1. Probability of mounting an adequate antibody response in relation to significant prenatal and postnatal exposures: diarrhoea in the first year of life, rate of weight gain in the first 6 months, and the combined effects of birthweight for gestational age, current body mass index (BMI), rate of weight gain and duration of exclusive breastfeeding. The ‘worst-case’ scenario represents adolescents born small for gestational age (SGA), low current BMI, slow weight gain and short duration of breastfeeding. (Data from McDade et al., 2001b.)](image-url)
than is low birthweight, and this association is independent of birthweight status. Similar relationships with immune function were considered in the CLHNS cohort. There was a significant sex-by-SGA interaction in logistic regression models predicting antibody response to typhoid vaccination (Fig. 13.2). Males who were SGA had a higher response rate than males who were AGA, while the opposite trend by birth-outcome status was apparent among females. Building from these models, maternal third-trimester arm fat area was modestly positively related to vaccine responsiveness, and both associations were strengthened to borderline significance \(P < 0.10\) once the child's own adiposity was added to the model (not shown). In contrast to the association with SGA, there were no sex differences in the relationship between maternal pregnancy adiposity and offspring immunity.

**Predictors of thymopoietin concentration**

Individuals born SGA were found to have lower concentrations of thymopoietin in adolescence than AGA individuals, but this difference was only statistically significant when prenatal undernutrition was considered in interaction with the duration of exclusive breastfeeding. Individuals were classified as short or long breastfeeders if they were below or above the median duration of exclusive breastfeeding in this population (51 days). Thymopoietin production was reduced in adolescents born SGA, regardless of the duration of breastfeeding. For AGA individuals, short breastfeeding was associated with comparably low concentrations, while thymopoietin was significantly elevated in long breastfeeders (Table 13.4) (McDade et al., 2001a).

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**Fig. 13.2.** Interaction between male sex and birthweight for gestational age in predicting the likelihood of an adequate antibody response to typhoid vaccination. Parameters from logistic regression model including maturational status, male sex, small for gestational age (SGA) and male \(\times\) SGA; odds ratio (95% confidence interval): male 0.15 (0.03, 0.67) \(P < 0.02\); SGA, 0.29 (0.10, 0.90) \(P < 0.04\); male \(\times\) SGA 18.7 (2.86, 122.1) \(P < 0.002\).
Postnatal growth in the first year of life was an equally important predictor of adolescent thymopoietin production: adolescents one standard deviation above the mean in first-year length increment (23.4 cm) had thymopoietin concentrations that were 1.5 times higher than those one standard deviation below the mean (18.0 cm). Growth during the first 6 months appeared to be particularly important, showing a stronger positive association with adolescent thymopoietin than growth between 6 and 12 months. Sex was also an important factor, with boys having median thymopoietin concentrations (35.8 pmol/l) that were more than twice those for girls (14.9 pmol/l).

Predictors of total IgE

Consistent with previous work suggesting an association between impaired fetal growth and subsequent IgE production, individuals born SGA had elevated IgE concentrations in adolescence, although this elevation was not statistically significant. However, a significant effect of IUGR emerged in interaction with the pathogenicity of the postnatal environment. For both boys and girls born SGA, adolescent IgE production was positively associated with a relatively unsanitary household environment in the first year of life (presence of domestic animals under the house, unhygienic food storage area, unsanitary infant food preparation practices) (Table 13.4). For AGA girls, a similar, but weaker association was found, whereas the opposite pattern was found for AGA boys: unsanitary conditions in the first year were associated with slightly reduced levels of adolescent IgE (McDade et al., 2004).

In addition, consistent with previous reports of a protective effect of early infection on later risk for atopy, respiratory and/or diarrhoeal disease in the first 6 months of life was associated with reduced IgE production in adolescence. For adolescents who had only one reported episode of morbidity in early infancy (recorded at 2 months, 4 months and 6 months of age), average IgE concentrations were nearly four times higher than those of adolescents who had four morbidity episodes (114.8 IU/ml versus 32.4 IU/ml). Similar associations were found when respiratory and diarrhoeal morbidity were considered separately, and infectious morbidity in the second 6 months of life was not significantly associated with IgE. Weight velocity in the first 6 months was positively associated with adolescent IgE, and current household income was negatively related to IgE. Lastly, as with thymopoietin, boys were found to have significantly higher IgE concentrations than girls (72.8 and 43.2 IU/ml, respectively).

Associations between immune measures

Since it was hypothesized that IUGR would be associated with reduced antibody responsiveness to vaccination, reduced thymopoietin concentration and increased IgE concentration, it was anticipated that vaccine responsiveness and thymopoietin should be positively related, and that IgE should be negatively related to both these measures. Physiologically, it is reasonable to expect these
relationships to be at least in part causal – rather than merely correlational – since the thymus plays an important role in processes of T-cell development and function that have implications for IgE production and antibody responsiveness to pathogen challenge.

Since thymopoietin and IgE concentrations differed significantly for boys and girls, separate analyses were performed for each. No significant associations among the immune measures were found, although there was a trend toward reduced IgE in boys who did not respond to typhoid vaccination. The opposite pattern was found in girls, although this difference was not statistically significant. In addition, for girls, a trend toward the expected negative association between thymopoietin and IgE (Pearson $R = -0.22, P = 0.09, N = 57$) was noted. A weak positive association was found for boys (Pearson $R = 0.23, P = 0.18, N = 36$). The small sample size – particularly with boys – was an obvious limitation here, and these associations should be interpreted with caution.

**Leptin**

Immune defences – particularly antigen-specific defences – are energetically expensive, and a growing number of investigators are applying an evolutionary, cost–benefit approach to the study of immunology (McDade and Withman, 1999; Buttger et al., 2000; Read and Allen, 2000). Scaffolded in an adaptationist framework, this perspective recognizes that organisms have limited energy, and that investment in immune defences consumes energy that cannot be used for other purposes. The physiological mechanisms through which these trade-offs are mediated are far from clear, but leptin is a likely candidate.

Leptin is similar in structure to IL-2, and is produced primarily by adipocytes. Its role as an endocrine indicator of energy status has been probed intensively, and a number of studies with animal models have indicated that leptin is critical to normal cell-mediated immune processes (Howard et al., 1999; Lord, 2002), although a recent study of Gambian children did not detect an in vivo association (Moore et al., 2002). In the Filipino sample, prenatal undernutrition was not related to leptin concentrations independent of adiposity. However, because leptin signals energy reserves, it was assessed as a potential mediator of the long-term effects of early environments on later immune competence. Since leptin concentrations showed the expected sexual dimorphism, with levels considerably higher in females compared to males, separate analyses were performed for each. Prenatal undernutrition was not associated with leptin concentration in adolescence. However, consistent with leptin’s role as an indicator of energy status, current BMI was positively associated with leptin for both boys (Pearson $R = 0.58, P < 0.001$) and girls (Pearson $R = 0.50, P < 0.001$).

Antibody responsiveness to typhoid vaccination was not associated with leptin; for both boys and girls, responders and non-responders did not differ in their mean leptin concentrations (boys: 0.22 versus 0.20 log ng/ml; girls: 0.59 versus 0.59 log ng/ml). Controlling for current BMI did not alter this pattern of associations.
In contrast, a positive association between leptin and thymopoietin was noted in girls (Fig. 13.3). The crude association between these measures was weak (Pearson $R = 0.20$, $P = 0.15$, $N = 55$), but was strengthened considerably when the effect of current BMI was taken into account (partial $R = 0.30$, $P = 0.029$). A positive association between leptin and thymopoietin makes sense from an energetic perspective, and it is consistent with previous research in starved mice, indicating that leptin administration protects against cell-mediated immune suppression and thymic involution that would normally follow undernutrition (Howard et al., 1999). However, this finding needs to be interpreted with caution given the small sample size, and the absence of a significant association between leptin and thymopoietin in boys (partial $R = -0.12$, $P = 0.50$, $N = 32$).

For both boys and girls, lower concentrations of leptin were associated with increased IgE production (Fig. 13.4). Partial correlations – controlling again

![Figure 13.3](image1.png)

**Fig. 13.3.** The partial correlation between thymopoietin and leptin in adolescent girls ($N = 55$), controlling for current body mass index (BMI).

![Figure 13.4](image2.png)

**Fig. 13.4.** The partial correlation between IgE and leptin in adolescent girls ($N = 57$) and boys ($N = 40$), controlling for current body mass index (BMI).
for current BMI – approached statistical significance (boys: partial $R = -0.23$, $P = 0.16$, $N = 40$; girls: partial $R = -0.19$, $P = 0.17$, $N = 57$). However, for the entire sample, the negative relationship between leptin and IgE was significant (controlling for BMI and sex, partial $R = -0.21$, $P = 0.037$, $N = 97$). Although negative associations between leptin and IgE have not been reported, this pattern is consistent with previous research in mice indicating that leptin promotes activity associated with Th1 lymphocytes, rather than Th2 activity linked to IgE production (Matarese et al., 2001; Lord, 2002).

For the most part, the Filipino study found a pattern of associations among leptin, thymopoietin and IgE that was consistent with current research in this area. However, these analyses must be regarded as preliminary, and suggestive for future research into the potential mechanisms associated with immune programming.

Current Perspectives on the Programming of Immunity

Research on the programming of immune function is in its early stages, but it is clear that this is an important area of enquiry. Future work with animal models and clinical human samples will be necessary to clarify the physiological processes through which early environments exert long-term immunological effects. Complementing this approach, additional population-based research will be necessary to explore the ecological contexts within which these processes operate, and the factors that moderate their expression. More specifically, future research should consider the following: definitions of prenatal environmental quality, interactions between pre- and postnatal environments, physiological mechanisms and clinical implications.

In a sample of adolescents from the Philippines, prenatal undernutrition was associated with reduced thymopoietin production, reduced antibody response to typhoid vaccination and elevated total IgE. We now have consistent evidence in this population, using three different parameters of immunity, that provides support for the hypothesis that human immune function is, at least in part, programmed by early environments. These findings underscore the importance of accounting for interactions between prenatal and postnatal environments when studying early-life influences on immune function. For each immune marker, the main effect of prenatal undernutrition was not statistically significant, but emerged only in interaction with aspects of postnatal experience: The duration of exclusive breastfeeding was found to moderate the effect of prenatal undernutrition on adolescent thymopoietin concentration; adolescents born SGA were less likely to respond to typhoid vaccination only if they were also below the median for BMI at the time of inoculation; and a relatively unhygienic household environment in the first year of life was associated with significant elevations in IgE only for adolescents born SGA. This pattern of results suggests that the long-term immunological effects of prenatal environments are not over-determined, and may in fact be largely contingent.

While the prenatal nutritional environment is an important predictor of later immune function, the Filipino findings also underscore the role of the early
postnatal environment in shaping immune development. Vaccine responsiveness, thymopoietin concentration and IgE concentration in adolescence were all positively associated with growth in length and/or weight during the first 6 months of life. Early episodes of infectious morbidity were associated with reduced IgE and increased vaccine responsiveness in adolescence. Since early infancy is a critical period for immune development in general, and the thymus in particular, it is not surprising that postnatal factors programme immunity in significant ways (Lewis and Wilson, 1995; Hasselbalch et al., 1999).

It is likely that significant correlations exist between prenatal and postnatal environments, thereby increasing the challenges associated with separating their relative contributions to immune development. For example, in the Filipino study population, prenatal undernutrition reduced antibody responsiveness to vaccination in adolescence, whereas diarrhoeal morbidity in the first year of life increased antibody responsiveness. Since SGA infants are more likely to suffer from diarrhoea in the first year of life (Table 13.2) (Ashworth, 1998), the long-term immunological effects of prenatal undernutrition may in part be attenuated by postnatal infectious disease experience. This situation, as well as the failure to explicitly consider interactions between pre- and postnatal environments, may obscure significant long-term effects of prenatal undernutrition and lead to an underestimation of their importance.

Birthweight alone is a poor indicator of the prenatal nutritional environment, and prior research emphasizes the importance of distinguishing between infants born small for gestational age and those born preterm, but with a comparable birthweight. For the former, immunological impairment in infancy may be more severe, whereas preterm AGA infants demonstrate recovery (Chandra, 1981). Similarly, disproportionate fetal growth (as indicated by an increase in head circumference) has been shown to be a better predictor of adult IgE concentration than birthweight, perhaps revealing an impoverished prenatal environment that favours brain growth at a cost to the development of lymphoid tissues (Godfrey et al., 1994).

Moreover, because birthweight follows a normal distribution among even well-nourished and healthy populations, size at birth per se may not always be a reliable indicator of prenatal nutritional sufficiency (Chard et al., 1993). The limitations of birthweight as a marker of relevant prenatal exposures are illustrated by research into the early life predictors of adolescent blood pressure and cholesterol metabolism, both of which relate most strongly to maternal energy status during pregnancy and are independent of variation in birthweight (Adair et al., 2001; Kuzawa and Adair, 2003). Observation of a borderline positive association between third-trimester maternal energy status and offspring antibody response that was independent of SGA status is consistent with these findings. Thus, as has been demonstrated for CVD risk factors, using a birth outcome such as birthweight as the sole marker of fetal nutrition is likely to miss important associations between the prenatal environment and postnatal immune function.

It is also interesting that measures of current environmental quality do not appear to be significantly related to measures of adolescent immunity. Exceptions include a negative association between current household income and IgE, and a moderating role for current BMI with respect to vaccine
responsiveness. Measures of pre- and postnatal environmental quality were the primary predictors of adolescent immunity, despite the wide range of measures of childhood and adolescent environmental quality available in the CLHNS dataset. The relative importance of early environments in setting the long-term trajectory of immune development is further evidence in support of the programming hypothesis.

The long-term immunological effects of early environments may, however, be largely contingent upon conditions experienced after birth, and interactions between prenatal, postnatal and current environments should be considered explicitly. Research to date indicates that large main effects of prenatal undernutrition on immune function may not be evident beyond infancy and early childhood. Findings such as ours suggest that effects may emerge only in a subset of individuals who are exposed to specific combinations of prenatal and postnatal environments.

The physiological mechanisms linking early experience to later immunocompetence require further elucidation. Likely candidates that have been explored in previous research include impaired development of lymphoid tissues (the thymus in particular), and lasting irregularities in hypothalamus–pituitary–adrenal function. Preliminary findings from our research also suggest that leptin – as an indicator of energetic status with immunoregulatory properties – may be an important mediator of early environments. In addition, we have found a number of sex differences in immune outcomes, and instances suggestive of sex differences in relationships between early exposures and later immune function, perhaps indicating a role for other hormones, such as sex steroids. It is likely that a range of experimental, clinical and population-level research designs will be necessary to reveal the mechanisms associated with immune programming.

The health implications of immune programming remain to be explored. Prior research suggests that prenatal undernutrition increases the risk of premature death from infectious disease (Moore et al., 1999), as well as the risk of atopic and autoimmune disease in adulthood (Phillips et al., 1993; Godfrey et al., 1994; Moore et al., 1999), and that early exposure to infectious disease reduces adult risk of atopy (Shaheen et al., 1996b; Shirakawa et al., 1997; Maticardi et al., 2000; Illi et al., 2001). With respect to infectious disease morbidity and mortality, the impact of immune programming is likely to be most evident in impoverished settings, where pathogen exposure is relatively high. In addition, to the extent that inflammatory processes are related to the pathophysiology of cardiovascular disease, cancers and other chronic conditions (Kalavoglou et al., 2002; Ryu, 2003), immune programming may be a critical factor mediating the association between prenatal undernutrition and adult chronic disease processes that has been reported in a large range of populations, from both developed and developing nations (Yajnik, 2000; Gill, 2001).

References


