Hornstra G, Uauy R, Yang X (eds): The Impact of Maternal Nutrition on the Offspring.
Nestlé Nutrition Workshop Series Pediatric Program, vol 55, pp 153–167,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2005.

Maternal Malnutrition and the Risk of Infection in Later Life

Sophie E. Moore, Andrew C. Collinson, Pa Tamba N'Gom, and Andrew M. Prentice

MRC International Nutrition Group, Nutrition and Public Health Intervention Research Unit, London School of Hygiene and Tropical Medicine, London, UK, and MRC Keneba, The Gambia

Introduction

Considerable evidence now exists to suggest that events during early life can influence future susceptibility to certain non-communicable diseases (NCDs). The fetal origins hypothesis states that cardiovascular disease and non-insulin-dependent diabetes originate through adaptations that the fetus makes when it is undernourished. These adaptations, which include slowing of growth, permanently change the structure and function of the body [1]. We have now added to the NCD observation with evidence from rural Gambia that we have found that an individual's susceptibility to infectious diseases may also be programmed by events early in life, particularly maternal undernutrition. In this review, we describe the existing evidence to support the hypothesis that immune function may be permanently programmed by nutritional status early in life, and describe the findings from our ongoing program of work in this area.

Background Evidence

In rural Gambia, existence on subsistence farming is heavily influenced by the annual rainy season (July to October). This coincides with a 'hungry' season when food crops from the previous year's harvest become depleted, and adults are engaged in heavy agricultural labor prior to the next harvest. As a consequence, a chronic negative energy balance is observed in all adults, including pregnant women [2]. Birth weights are around 200 g lower during the hungry season, a deficit that can be reversed by maternal dietary

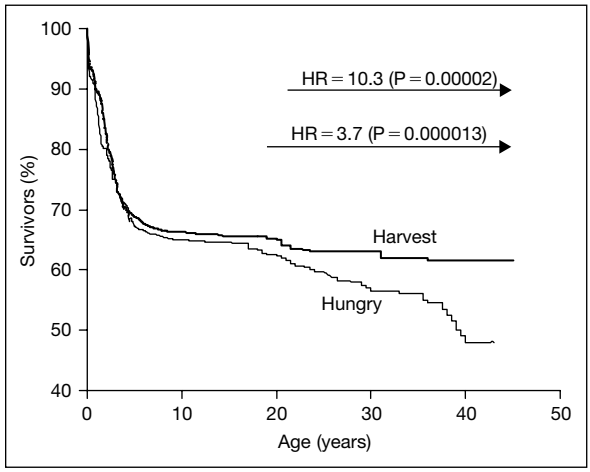

Fig. 1. Kaplan-Meier survival plots by season of birth. n = 3,162 (2,059 alive and 1,103 dead). OR = Odds ratio. Adapted from Moore et al. [7].

supplementation [3, 4]. Most maternal and infant diseases also peak during the hungry season, especially malaria [5] and diarrhea [6]. The season of birth can therefore be used as an indicator of fetal and early infant exposure to malnutrition and infectious diseases.

Using a demographic record dating back to 1949, a survival analysis according to season of birth found a profound bias in adult mortality in individuals born during or shortly after the hungry season, with a maximal hazard ratio of 10.3 for deaths between 25 and 50 years of age (fig. 1) [7]. Since the majority of these premature adult deaths were from infections or infection-related diseases (e.g. hepatoma as a late outcome of hepatitis B infection) [8], this finding led to the hypothesis that an insult occurring in early life and linked to season of birth is disrupting the immune response resulting in increased susceptibility to infections and premature mortality.

The hypothesis that immune function may be programmed by events early in life is supported by several pieces of evidence from the literature. The principle components of the human immune system develop in fetal life [9], and it is therefore plausible that fetal nutrient deprivation could lead to a more permanent immunological insult than a similar degree of undernutrition experienced in postnatal life. Furthermore, maternal malnutrition has been observed to have greater effects on thymic and lymphoid tissue development than on other organs [10–12] presumably reflecting a physiological mechanism to protect the growth and development of other specific organs, such as the brain. There is also evidence to demonstrate that such deficits in organ growth and development occurring in utero are more serious and longer-lasting than those caused by later malnutrition [13]. But do such physiological

changes in relation to early life nutrition permanently alter later immune function and risk of infectious disease? There is evidence that low birth weight babies may have sustained impairment of immune competence as infants and children when assessed by various in vitro methods [14–17], though such findings are not universal [18]. Increased susceptibility, following intrauterine growth restriction (IUGR), to infections in childhood is also well known [19], with hazards ratios for infectious deaths rising as high as 5.0 in Brazil [17].

Evidence that certain components of the adult immune system are 'set' by events in early life comes from data on a limited number of published studies. An association has been observed between birth size and susceptibility to autoimmune disease in a cohort of adult women, aged 60–71 years, from the UK with the proportion of women with thyroglobulin and thyroid peroxidase autoantibodies falling with increasing birth weight [20]. In a comparable cohort study also from the UK, Phillips et al. [21] found higher serum IgE concentrations in adults who had a large head circumference in relation to trunk and limb length at birth, suggesting a possible relationship between the early-life environment and the later development of atopy and allergic disease. The authors of both these studies speculated that the fetal thymus is a target for programming influences related to fetal undernutrition. This hypothesis has been further supported by a study comparing thymopoietin production in 103 Filipino adolescents who were appropriate for gestational age or small for gestational age at birth [22]. No association was found with birth weight alone, but when the duration of breast-feeding was added to the analysis, the interaction of these two factors emerged as a significant predictor of adolescent thymopoietin concentration.

Despite such evidence, mechanisms to explain any of these observations have not been described. We are therefore attempting to define the biological mechanisms underlying the early-life programming of immune function through a series of ongoing studies. The wide variety of seasonal exposures that could be responsible for this early insult combined with the complexity of the human immune system, mean that no single study will be able to identify the casual mechanism(s). For this reason we have initiated a number of studies in different age groups and using different investigative tools in the Gambia and in other seasonally affected populations. The evidence obtained so far from these studies is presented below.

Infant Immune Development

The major components of the human immune system develop in fetal life, and then undergo important maturational changes in infancy, dependent on influences such as antigenic and cytokine exposures, nutritional status, and breast-feeding. The study of infant immune development in relation to these

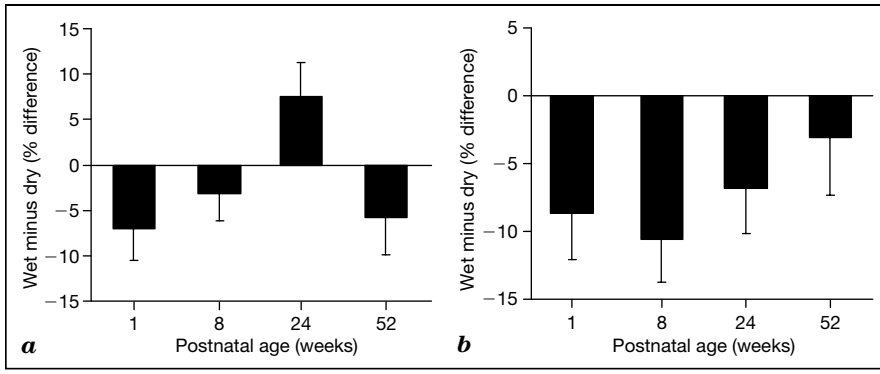

Fig. 2. Percentage (standard error) difference in mean thymic index between hungry and harvest season births (***a***), and hungry and harvest season measurements (***b***), adjusted for gender, gestation and current weight. Adapted from Collinson et al. [24].

environmental variants is therefore key to determining mechanisms of immune programming. In a prospective birth cohort study in rural Gambia, we have demonstrated initial evidence to link early life exposures to alterations in the development of the infant immune system.

Over a complete calendar year, 138 singleton infants were recruited antenatally into this study from 5 villages in the West Kiang region of the Gambia. Infants were seen at birth, when anthropometry and gestational age were measured, and then followed until 52 weeks of age. At 1, 8, 24 and 52 weeks of age thymic size was measured sonographically using a validated method in which the transverse diameter of the thymus and the sagittal area of its largest lobe are multiplied to give a volume-related thymic index. This index has been shown to correlate with thymus weight at autopsy [23]. At 1, 8, 16 and 52 weeks of age, venous blood (infant) and breast milk samples were collected. Blood samples were used to investigate lymphocyte counts, micronutrient levels, infant antibody responses to vaccination (tetanus toxoid, diphtheria, hepatitis B) and in vitro cytokine responses (interferon-γ (IFN-γ), interleukin (IL)-5) and rates of cellular proliferation. Growth (monthly) and morbidity (twice monthly) estimates were additionally made. The key findings from this study are detailed below.

Thymic Size and Breast Milk IL-7 Levels

Mean thymic index increased dramatically up to 24 weeks of age, and then decreased to the measurement taken at 52 weeks. At all ages, the thymic index was strongly associated with current weight ($p \leq 0.001$). The thymic index at 1 and 8 weeks was associated with birth weight, but this did not persist after adjusting for current weight. Figure 2 demonstrates a seasonal effect on thymic size, with the smallest thymuses, from week 1 onwards, when

measured in the hungry season after adjustment for infant weight [24]. In addition, there is evidence that this tracks within individuals up to the end of the 1st year of life.

These results demonstrate that the size of the thymus is closely related to body weight throughout infancy. The results also show that infants have a characteristic thymic index, with tracking of thymic growth that is at least partially distinct from the postnatal effects of season and body weight [24]. Of particular interest, this difference in thymic size between the harvest and hungry season babies is greatest at 8 weeks of age, an age at which infants in this community are exclusively breast-fed, have good weight, and have a minimal incidence of active infections. This observation could suggest that breast milk has a specific trophic effect on the thymus. Although it is universally accepted that breast milk supports passive immunity, the extent to which trophic and immune factors in breast milk influence adaptive immune function remains to be established. Breast-feeding may promote thymic growth, and it has been suggested that this is mediated by the transfer of immunological or trophic factors [25, 26]. A detailed study of breast milk antimicrobial factors in rural Gambian women found that in comparison with dry (harvest) season samples, breast milk collected in the late rainy (hungry) season contained 35% less IgA and IgG, and 20% less secretory component and lysozyme [27]. A slight fall in milk production during the rainy season compounded the decrease in daily production of these factors. A more recent study has confirmed the seasonality of breast-milk IgA levels in this community [28].

Breast milk may also be a medium for hormonal or cytokine signals. These may exert direct trophic effects on the thymus, or act indirectly via specific cells or cytokine networks of the infant immune system. Many such candidate factors have been identified in breast milk, including leptin, epidermal growth factor, transforming growth factors α and β, interleukin-1 (IL-1), IL-6, and other cytokines [29–31]. Of novel interest is the cytokine IL-7. IL-7 is known to be essential for normal thymocyte development, and for the proliferation and survival of precursor T cells [32, 33]. There is also evidence that IL-7 reduces the rate of thymocyte apoptosis at the $CD3^-CD4^-CD8^-$ triple-negative stage. An increase in apoptosis of triple-negative thymocytes has been implicated in the age-related decline in thymopoesis [34]. Animal experiments have shown that cytokines can retain biological activity during passage through the gastrointestinal tract and may be taken up into the circulation [35–37]. However, the IL-7 content of human breast milk and its potential function in thymic development during early infancy are not known.

IL-7 levels were therefore measured in frozen samples of breast milk collected at weeks 1 and 8 from this Gambian study and kept at $-80°C$ until use. Despite considerable monthly variation, IL-7 levels in week-1 breast milk samples from hungry season mothers were significantly lower than harvest season mothers (79 vs. 100 pg/ml, $p = 0.02$) [38]. A similar trend existed in

the samples collected 8 weeks postpartum, although this did not reach statistical significance. This observation suggests that improved maternal nutrition during the harvest season could increase certain factors in breast milk, with the consequent improvement in thymic size and function.

Thymic Function

The key question in relation to the thymic size data is whether the size of the thymus is correlated to its function? Of particular relevance, a longitudinal study of 278 infants in Guinea Bissau found that a small thymus at birth predicted increased infant mortality independent of birth weight, and especially in the 2nd year of life [39]. All 45 deaths in this cohort were attributed to infectious disease, suggesting that thymic size is an important indicator of immune capacity.

To specifically assess whether the observed changes in thymic size are of relevance to thymic function, we looked at T-lymphocyte populations and assessed thymic output by measuring T-cell receptor rearrangement excision circles (TRECs). The $CD4^+/CD8^+$ ratio averaged over the 1st year of life was significantly lower for infants born in the hungry season and this difference was already apparent in cord blood where an unusually high level of double-positive CD4+CD8+ T cells might indicate a premature release of thymocytes in response to an environmental stress [40]. Using blood from infants at 8 weeks of age, analysis of TREC levels demonstrated considerable monthly variation, but those born in the harvest season had significantly higher levels than those born in the hungry season (2.12 versus 0.92 TRECs/100 T cells, $p = 0.006$) [38]. Ongoing work in a prospective birth cohort from Matlab, Bangladesh, is attempting to replicate this finding in a larger cohort of infants.

Placental Function

Maternal undernutrition may also influence placental development and physiology, and this may in turn constitute a common pathway for putative programming influences on the developing immune system. This hypothesis is supported by several observations in the literature. During normal pregnancy, a predominance of Th2-type cytokines exist and are considered to protect the fetus. Animal experiments suggest that an increase of Th1-type cytokines may instead have deleterious effects. A recent study from Hahn-Zoric et al. [41] has shown that placentas from Swedish infants born with IUGR have significantly higher IL-8 and significantly reduced IL-10 mRNA than normal infants. It is therefore possible that reduced IL-10 in the placenta is involved in the pathogenesis of IUGR. Indeed, ongoing work has confirmed this observation in placentas from IUGR deliveries in a study in Pakistan (Prof. LÅ Hanson, personal communication). If such abnormalities then lead to immune defects beyond infancy, this could explain the link between early

undernutrition and later immune dysfunction. Further work is required to confirm the long-term effects of these observed defects.

Evidence for the Early-Life Nutritional Programming of Long-Term Immune Function

Although the results from our study of infant immune development support the hypothesis that the early programming of immune function is mediated by effects on the thymus and T-cell lineage, few consistent functional defects were seen in relation to either nutritional status at birth or to birth season, using in vitro cytokine (IFN-γ and IL-5) production or antibody response to vaccination (tetanus, diphtheria or HBV). [40] Indeed, these negative findings parallel those from a study of immune function in a cohort of older Gambian children (n = 472). [42] In this study, immune function was measured by delayed-type hypersensitivity responses (Merieux Multitest Cell Mediated Immunity kit), response to T-cell-mediated (Human Diploid Cell Rabies vaccine) and B-cell-mediated vaccination (Pneumovax® 23 valent pneumococcal capsular polysaccharide vaccine), intestinal permeability (lactulose-mannitol test), and levels of salivary secretory IgA. Seasonally varying confounding factors also measured included anthropometry, micronutrient status (plasma zinc, vitamin A, vitamin C and hemoglobin levels), malaria parasitemia, and serum aflatoxin-albumin adduct levels.

Table 1 details the key results from this study. None of the measures of immune function were related to the birth weight of the children, and they were not significantly different in the group of children who were born of a low birth weight (<2.5 kg). In addition, there were no consistent associations between prenatal supplementation status or season of birth and immune function. However, such negative findings do not necessarily negate the main hypothesis that immune function can be programmed during a critical period in early life. From the original Kaplan-Meier survival plots (fig. 1) it is only after the age of 15 years that the survival of those born during the hungry season diverged from those born during the harvest season. It is therefore possible that immune function may be impaired from early life but the effect may not manifest until later in life. The use of retrospective birth cohorts is therefore critical to determining later life immune function in relation to early life events. An ongoing study of immune function in relation to early life in a cohort of young Gambian adults hopes to explore this in the very setting that the original finding was made. In addition, we have investigated the association between size at birth and response to vaccination in a cohort of 257 adults (mean age 29.4 years; 146 males) born in an urban slum in Lahore, Pakistan, during 1964–1978 [43].

A single dose of purified Vi surface polysaccharide extracted from *Salmonella typhi* and two doses of rabies vaccine were given to each subject

Table 1. Immune function in relation to birth weight, season of birth and maternal supplementation status in 6- to 10-year-old Gambian children

Measure	Birth weight	Season of birth	Supplementation status[1]
CMI	NS	NS	Increased response in intervention children (p = 0.006)*
Pneumococcal vaccination	NS	NS	NS
Rabies vaccination	NS	NS	Increased response in control children (1st dose p = 0.024, 2nd dose p = 0.005)*
Intestinal permeability	NS	NS	NS
Salivary sIgA levels	NS	Increased response in hungry season births (p = 0.0018)	NS

CMI = Cell-mediated immune response; sIgA = secretory immunoglobulin A. Adapted from Moore et al. [42].

*Significantly different after adjustment for age, sex, month of study, and current weight-for-age z score.

[1]Maternal dietary supplement during pregnancy (intervention) or during lactation (control).

[44]. Antibody titers were measured on pre-vaccination serum samples (Vi) and post-vaccination samples (Vi and rabies). The mean weight at birth of the subjects was 3.24 kg and 14% had a birth weight of <2.5 kg. Vaccine responses were not consistently associated with contemporary variables (month of study, gender, current age, indicators of wealth). The response to typhoid vaccination was positively related to birth weight (anti-Vi IgG p = 0.031; anti-Vi IgM p = 0.034). The response to the rabies vaccine, however, was not associated with birth weight. The contrasting effects on typhoid and rabies responses observed in this study seem to suggest that the antibody generation to polysaccharide antigens, which has greater B-cell involvement, has been compromised by fetal growth retardation. Of added interest, this is not the first study to show such a relationship with the Vi vaccine. A study of Filipino adolescents participating in an ongoing longitudinal study has shown that prenatal undernutrition is significantly associated with reduced thymopoietin production, and growth in length during the first year of life was shown to be positively associated with adolescent thymopoietin production [22]. In the same cohort, the predicted probability of mounting an adequate antibody response to a typhoid vaccine was lower in adolescents who were prenatally and currently undernourished (probability = 0.32) compared to adequately nourished adolescents (probability = 0.49–0.70; p value for difference = 0.023) [45].

Polysaccharide vaccines are not as effective in infants and young children as in older individuals. Generally children respond with more IgG1 antibodies and adults with more IgG2 antibodies to bacterial polysaccharides [46, 47]. In children below 18 months of age there is a high proportion of non-responders with IgG2 antibodies [48, 49]. The detailed background of these age differences is not really understood, but further work in this area may also help explain the lower response to the typhoid vaccine in subjects born small for gestational age. A follow-up study in this same cohort of Pakistani adults and using a broader range of vaccines is ongoing to help understand the specific mechanisms involved.

Concluding Remarks

Our initial observation linking infectious disease mortality to season of birth in the Gambia has initiated a new program of work exploring the relationship between the early life environment and later immune function. The preliminary findings from these studies indicate that infant thymic development is impaired by seasonally dependent early-life exposures. This could be mediated through seasonal variations in breast milk trophic factors, with IL-7 identified as a strong candidate. Measures of lymphocyte numbers and of TREC levels in this same cohort of infants suggests that thymic function is also impaired as a consequence of this early insult. The long-term consequence of this early defect in thymic development is under investigation. Our data from Pakistani adults indicate that one long-term consequence of small size at birth is an impaired antibody generation to a polysaccharide typhoid vaccine. Elucidating the mechanism for this potential defect will be critical in determining specific components of the immune system that are sensitive to early nutritional deficiencies.

All the key studies within this area of research have so far focused on a limited number of cohorts from specific countries where infectious diseases still prevail as the leading cause of mortality. However, if true, then this hypothesis clearly has relevance for many more sectors of society, and demonstrates the necessity for continued research into the key factors that impact on the development of the human immune system during fetal and early-postnatal life.

Acknowledgements

The work presented in this review is the product of a dedicated team working within the MRC's Nutrition Program in the Gambia, and at our collaborative field sites around the world. We specifically acknowledge Prof. Fehmida Jalil and Prof. Lars Hanson for their leadership in the Lahore studies. This work was primarily funded by the UK Medical Research Council (MRC), with additional support from the Nestlé Foundation. Ethical permission for the Gambian studies was granted by the joint

MRC/Gambian Government Ethics Committee. For work in Pakistan, approval was given by the Medical Ethics Committee for Research, King Edward, Medical College, Lahore, Pakistan, and by the Ethics Committee of Göteborg University, Sweden. All studies were conducted with informed consent from all subjects and/or their parents/guardians.

References

1 Barker DJP: Early growth and cardiovascular disease. Arch Dis Child 1999;80:305–310.
2 Prentice AM, Whitehead RG, Roberts SB, Paul AA: Long-term energy balance in childbearing Gambian women. Am J Clin Nutr 1981;34:2790–2799.
3 Prentice AM, Cole TJ, Foord FA, et al: Increased birthweight after prenatal dietary supplementation of rural African women. Am J Clin Nutr 1987;46:912–925.
4 Ceesay SM, Prentice AM, Cole TJ, et al: Effects on birthweight and perinatal mortality of maternal dietary supplementation in a primary health care setting in rural Gambia. BMJ 1997;315:786–790.
5 Greenwood BM, Bradley AK, Greenwood AM, et al: Mortality and morbidity from malaria among children in a rural area of the Gambia, West Africa. Trans R Soc Trop Med Hyg 1987;81:478–486.
6 Rowland MGM, Cole TJ, Whitehead RG: A quantitative study into the role of infection in determining nutritional status in Gambian village children. Br J Nutr 1977;37:441–450.
7 Moore SE, Cole TJ, Poskitt EME, et al: Season of birth predicts mortality in rural Gambia. Nature 1997;388:434.
8 Moore SE, Cole TJ, Collinson AC, et al: Prenatal or early postnatal events predict infectious deaths in young adulthood in rural Africa. Int J Epidemiol 1999;28:1088–1095.
9 Hayward AR: Development of immune responsiveness; in Falkner F, Tanner JM (eds): Human Growth. 1. Principles and Prenatal Growth. New York, Plenum Press, 1978, pp 593–607.
10 Winick M, Noble A: Cellular response in rats during malnutrition at various ages. J Nutr 1966;89:300–306.
11 Owens JA, Owens PC: Experimental fetal growth retardation: Metabolic and endocrine aspects; in Gluckman PD, Johnston BM, Nathanielsz PW (eds): Advances in Fetal Physiology. Ithaca, Perinatology Press, 1989, pp 263–286.
12 Chandra RK: Interactions between early nutrition and the immune system. Ciba Found Symp 1991;156:77–89.
13 Beach RS, Gershwin ME, Hurley LS: Gestational zinc deprivation in mice: Persistence of immunodeficiency for three generations. Science 1982;218:469–471.
14 Chandra RK: Immunocompetence in low-birth-weight infants after intrauterine malnutrition. Lancet 1974;ii:1393–1394.
15 Chandra RK: Fetal malnutrition and postnatal immunocompetence. Am J Dis Child 1975;129:450–454.
16 Ferguson AC, Lawlor GJ, Neuman GG, et al: Decreased rosette forming lymphocytes in malnutrition and intra-uterine growth retardation. J Pediatr 1974;85:717–723.
17 Victora CG, Smith PG, Vaughan JP: Influence of birth weight on mortality from infectious diseases: A case control study. Pediatrics 1988;81:807–811.
18 Pittard WB, Miller K, Sorensen RU: Normal lymphocyte responses to mitogens in term and premature neonates following normal and abnormal intrauterine growth. Clin Immunol Immunopathol 1984;30:178–187.
19 Ashworth A: Effects of intrauterine growth retardation on mortality and morbidity in infants and young children. Eur J Clin Nutr 1998;52:S34–S42.
20 Godfrey KM, Barker DJP, Osmond C: Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994;24:641–648.
21 Phillips DIW, Cooper C, Fall C, et al: Fetal growth and autoimmune thyroid disease. Q J Med 1993;86:247–253.
22 McDade TW, Beck MA, Kuzawa CW, Adair LS: Prenatal undernutrition and postnatal growth are associated with adolescent thymic function. J Nutr 2001;131:1225–1231.

23 Hasselbalch H, Nielsen MB, Jeppsen D, et al: Sonographic measurement of the thymus in infants. Eur Radiol 1996;6:700–703.
24 Collinson AC, Moore SE, Cole TJ, Prentice AM: Birth season and environmental influences on patterns of thymic growth in rural Gambian infants. Acta Paediatr 2003;92:1014–1020.
25 Hasselbalch H, Jeppesen DL, Engelmann MDM, et al: Decreased thymus size in formula-fed infants compared with breastfed infants. Acta Paediatr 1996;85:1029–1032.
26 Prentice AM, Collinson AC: Does breast feeding increase thymic size? Acta Paediatr Scand 2000; 89:8–12.
27 Prentice A, Watkinson M, Prentice AM, et al: Breast-milk antimicrobial factors of rural Gambian mothers. II. Influence of season and prevalence of infection. Acta Paediatr Scand 1984; 73: 803–809.
28 Weaver LT, Arthur HML, Bunn JEG, Thomas JE: Human milk IgA concentrations during the first year of lactation. Arch Dis Child 1998;78:235–239.
29 Houseknecht KL, McGuire MK, Portocarrero CP, et al: Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. Biochem Biophys Res Commun 1997;26:742–747.
30 Garofalo RP, Goldman AS: Cytokines, chemokines, and colony-stimulating factors in human milk: The 1997 update. Biol Neonate 1998;74:134–142.
31 Hawkes JS, Bryan DL, James MJ, Gibson RA: Cytokines (Il-1beta, Il-6, TNF-alpha, TGF-beta1, and TGF-beta2) and prostaglandin E2 in human milk during the first three months postpartum. Pediatr Res 1999;46:194–199.
32 Suda T, Zlotnik A: IL-7 maintains the T cell precursor potential of CD3-CD4-CD8- thymocytes. J Immunol 1991;146:3068–3073.
33 Morrissey PJ, McKenna H, Widmer MB, et al: Steel factor (c-kit ligand) stimulates the in vitro growth of immature CD3-/CD4-/CD8- thymocytes: Synergy with IL-7. Cell Immunol 1994;157: 118–131.
34 Andrew D, Aspinall R: IL-7 and not stem cell factor reverses both the increase in apoptosis and the decline in thymopoiesis seen in aged mice. J Immunol 2001;166:1524–1530.
35 Hamosh M, Peterson JA, Henderson TR, et al: Protective function of human milk: The milk fat globule. Semin Perinatol 1999;23:242–249.
36 Kaiserlian D, Etchart N: Entry sites for oral vaccines and drugs: A role for M cells, enterocytes and dendritic cells? Semin Immunol 1999;11:217–224.
37 Mowat AM, Viney JL: The anatomical basis of intestinal immunity. Immunol Rev 1997;156: 145–166.
38 N'Gom PT, Collinson AC, Pido-Lopez J, et al: Improved thymic function in exclusively breastfed babies is associated with higher breast milk IL-7. Am J Clin Nutr 2004, in press.
39 Aaby P, Marx C, Trautner S, et al: Thymus size at birth is associated with infant mortality: A community study from Guinea-Bissau. Acta Paediatr 2002;91:698–703.
40 Collinson AC: Early Nutritional and Environmental Influences on Immune Function in Rural Gambian Infants; thesis, University of Bristol, 2002.
41 Hahn-Zoric M, Hagberg H, Kjellmer I, et al: Aberrations in placental cytokine mRNA related to intrauterine growth retardation. Pediatr Res 2002;51:201–206.
42 Moore SE, Collinson AC, Prentice AM: Immune function in rural Gambian children is not related to season of birth, birth size, or maternal supplementation status. Am J Clin Nutr 2001;74: 840–847.
43 Jalil F, Karlberg J, Hanson LA, Lindbland BS: Growth disturbances in an urban area of Lahore, Pakistan related to feeding patterns, infections and age, sex, socio-economic factors and seasons. Acta Paediatr Scand Suppl 1989;1989:44–54.
44 Moore SE, Jalil F, Ashraf R, et al: Birth weight predicts response to vaccination in adults born in an urban slum in Lahore, Pakistan. Am J Clin Nutr 2004, in press.
45 McDade TW, Beck MA, Kuzawa C, Adair LS: Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. Am J Clin Nutr 2001;74:543–548.
46 Lottenbach KR, Mink CM, Barenkamp SJ, et al: Age-associated differences in immunoglobulin G1 (IgG1) and IgG2 subclass antibodies to pneumococcal polysaccharides following vaccination. Infect Immun 1999;67:4935–4938.
47 Herrmann DJ, Hamilton RG, Barington T, et al: Quantitation of human IgG subclass antibodies to *Haemophilus influenzae* type b capsular polysaccharide. Results of an international collaborative study using enzyme immunoassay methodology. J Immunol Methods 1992;148:101–114.

48 Shackelford PG, Granoff DM, Nelson SJ, et al: Subclass distribution of human antibodies to *Haemophilus influenzae* type b capsular polysaccharide. J Immunol 1987;138:587–592.
49 Trollfors B, Lagergard T, Claesson BA, et al: Characterization of the serum antibody response to the capsular polysaccharide of *Haemophilus influenzae* type b in children with invasive infections. J Infect Dis 1992;166:1335–1339.

Discussion

Dr. Uauy: In looking at the response to vaccines, did you try the weaker antigens because some of the vaccines are probably more demanding in the immune system?

Dr. Moore: The problem with many commercial vaccines is that they are designed to generate a good antibody response in all recipients, so therefore perhaps you might not expect to see a difference. When we started these studies we were looking for clues, and since the immune system is so complex, this was difficult. Certainly the data that I have presented from Lahore have given us certain clues as to which components of the vaccine response we should be looking at and it is interesting that it was the polysaccharide vaccine as opposed to the protein vaccine where we have seen this differential response. Now the response to polysaccharide vaccines are primarily B-cell-mediated and we know that young infants, for example, don't respond very well to such vaccines. So we are doing some continued analysis on the serum samples from these cohorts specifically looking at IgG1 and IgG2 subclasses to try to look into this. But I do agree that we need to try vaccines that would generate a low response, and indeed we are going to revaccinate all those adults with another selection of vaccines.

Dr. Kramer: Infectious disease mortality in most developing countries is higher in childhood than in adulthood. I wonder if you could speculate why? If this is an effect of season of birth on long-term immune function, why should you see no differences in mortality until late adolescence or early adulthood?

Dr. Moore: Interesting question and one we have thought about in some detail. In fact it is not completely true that there was no difference in survival in the early years. We had such an enormous number of deaths in the early years that we were able to break them down by season of birth into individual diseases. What we actually found is that although for malarial deaths there was no difference in terms of season of birth; for deaths from gastroenteritis and diarrheal disease, infants born in the hungry season were actually more susceptible to death than infants born in the harvest season. So it is possible that in a larger cohort we would detect differences for other diseases, but for malaria, which isn't very selective, we can't pick up a differential response. In fact that is another reason why we wanted to try and replicate our findings in other cohorts across the globe because our cohort in the Gambia is pretty small, although it has been studied intensively for a long period of time. Whereas we have recently done a survival analysis using a demographic data set from Matlab in Bangladesh, from a huge demographic surveillance program, and we do have cause of death data for all those individuals as well. So maybe by analyzing those data we will be able to elucidate in more detail whether infant death could indeed be programmed. For example malaria isn't predominant in that area and we may pick up differences in other specific details.

Dr. Kramer: Is there any evidence from the supplementation trial, for example, that some of these differences you were seeing in diarrheal mortality early on were reduced by the intervention?

Dr. Moore: No, we haven't looked at that. The supplementation study was only in just over 2,000 individuals and to date they are only between 10 and 15 years of age. Although mortality is still comparatively high in this area of the Gambia, there haven't been that many deaths from the data set of 2,000. We would be able to look at specific diseases according to seasons, but I don't think the power will be strong enough.

Dr. Waller: I wanted to comment on your original observation: you showed a very large difference in survival between infants born in the hungry and the harvest season. I am wondering if that was controlled for socioeconomic status, that large predictor of mortality? I wonder if you could have considered that possibly what you saw could have been due to differences across the social classes in a number of social aspects such as timing of birth, marriages, family planning, and migration of men in and out of the home, different seasons, frequency of sexual intercourse at different seasons?

Dr. Moore: Certainly when we first made this finding, we wondered what could be responsible for this profound difference in survival, and did consider factors such as socioeconomic status, timing of marriages. We don't have detailed socioeconomic status data for these populations, but I am not entirely sure that, even if we did, we would have a profound enough spectrum of differences in socioeconomic status for us to find differences. For example, nobody has running water in the village, education seems to be fairly similar between different families. There doesn't seem to be a seasonal association in terms of timing of marriage, There is, however, a profound seasonality in terms of fertility, and we don't really know for sure what the reason for this is, and whether it has to do with increased physical activity at certain times of the year, the men and women are simply exhausted, or whether there is greater fetal wastage at particular times of the year, we don't know the reason for that. And yes, it is possible that some other reasons that you mentioned could be responsible for our initial observations.

Dr. Waller: If you see a large difference in the number of births with the season of the year, then women who are conceiving in a period where you are less likely to conceive would be relatively well-nourished and advantaged to begin with, so there would be a selection bias there.

Dr. Moore: A survival of the fittest kind of hypothesis. If that was the case we would need to see a crossing of the survival curves, and we don't see that.

Dr. Waller: In which season do you have most of the births?

Dr. Moore: Most births are early in the harvest season, January-February.

Dr. Waller: They are generally conceived when?

Dr. Moore: Nine months before that, so some are in the harvest season.

Dr. Waller: This brings me to the second point, I was wondering if you have considered redoing your analysis looking at an estimated date of conception. I don't know how interested you are in the effect of nutrition during pregnancy versus nutrition after birth, but if you are interested in the effect of nutrition during pregnancy your two periods of time are each 6-month periods and they have got a lot of misclassification in them. The babies born at the beginning of the harvest period were largely in utero second and third trimester during the hungry period; the babies born at the end of the harvest period would be in utero second and third trimester during the harvest period, so you have a lot of misclassification in that analysis if you are looking at nutrition in pregnancy.

Dr. Moore: I completely agree and in fact when we did the initial analysis we tried various seasonal divisions, not only by 6-month periods, but also shifting it by 3-month periods, 4-month periods, single-month periods, and this was the only classification where we saw such profound divergence. It is possible that it is not a nutritional insult. It is also possible that it is a nutritional insult early during fetal growth as opposed to late during fetal growth. With this cohort of individuals we don't have enough power to really explore this information in much detail. So this is why we want bigger data sets where we might have more detailed data on nutritional status. For example in Bangladesh they have a narrower pattern of seasonality in terms of the difference in infant death and the difference in nutritional status, and maybe that will help unravel some of the relationship.

Dr. Yang: In your studies you show that the cytokine IL-7 is different in the various seasons. Did you get breast milk samples in the different seasons, and did you measure the other cytokines in the different seasons?

Dr. Moore: No, answering the final part of your question first. We haven't measured different cytokines, partly because we did think about this retrospectively after we had collected the breast milk samples, and then analyzed the thymic index data, and we then thought this is strange why we are seeing the biggest difference at 8 weeks of age when infants are all being exclusively breast fed, and therefore maybe it is some factor in breast milk that is influencing these differences. We don't have very large samples of stored breast milk from this study, and therefore we wanted to do what we thought was the best candidate factor and that is why we started with IL-7 because it is so involved in thymic growth and thymocyte development. We are now collecting more breast milk samples in an ongoing study in Matlab, Bangladesh, and we do hope to explore other cytokines and other immune factors in breast milk. Sorry, I forgot the first part of your question.

Dr. Yang: Did you measure the other cytokines because you just mentioned that IL-7 is different?

Dr. Moore: We could, but we haven't yet. But we will now that we have found this difference in IL-7. We are considering what other cytokines and what other immune factors in breast milk could be responsible. So we will be looking at more factors in more detail in another cohort.

Dr. Butte: Did you measure the thymic size in the infants born in the supplementation study trying to look at the dramatic decrease that you saw in mortality in that study?

Dr. Moore: We wanted to and indeed we got ethical approval to do so, but the size of the thymus is changing a lot. When we visited the supplementation children, they were between 6.5 and 9.5 years, and at that age the thymus has evolved quite a lot compared to infancy, and it is therefore harder to measure. Also using the probes that are designed for this type of measurement, it is difficult to get an echo because of the thickness of the bone. So we couldn't measure it, which is very unfortunate. But perhaps we would not detect a difference because the infants who were most susceptible were those who had already died, and they would have been excluded from the cohort of the supplementation study.

Dr. Cai: In the same cohort of mothers, did you look for the cytokine differences in breast milk and also the lymphocyte differences in cord blood?

Dr. Moore: Yes, it was the same cohort of mothers. Again it was quite a small cohort, it was only 138 mothers. These mothers and their babies were very intensively studied, which limited the number we could recruit. But all the measurements I presented from the infant cohort were from the same group of infants and the same group of mothers.

Dr. Cai: Did you look at the cytokine profile of the mothers?

Dr. Moore: We didn't measure cytokines in the mothers at all.

Dr. Hornstra: I am interested to know whether by any chance you had two infants from the same mother in your cohort, one being born during the harvest season and the other during the wet season, and if that could take away some of the problems Dr. Waller was alluding to?

Dr. Moore: Yes, we certainly would have had more than 1 child from individual mothers. We did look at whether the effect was due to birth ordering and we had enough power to do that, and there was no difference whether the child was a first born or a second or a third or a fourth born in terms of survival, that didn't differentiate effects. I am not sure whether we have looked in enough detail at individual mothers, and whether they are conceiving at the same time for all their children. So therefore it will certainly be interesting to look at that and we will be able to look at that more successfully in our Bangladesh cohort because it is so much larger.

Dr. Uauy: Have you looked at other cohorts from developed countries? For example the Dutch famine survivors I am sure that they have perfect data for the last years on that same cohort. Could there be clues that come from the developed countries?

Dr. Moore: The answer is no, we haven't looked at developed countries, but I agree with you we could find some important evidence. In terms of our survival assays, you may remember that we looked at survival in a historical cohort of adults from Finland (unpublished data), for the very reason that we wanted to select a country without malaria to try to eliminate the early effects of malaria. Unfortunately we haven't been able to detect any differences in terms of birth and survival in the Finnish cohort, but now I would be very keen to get access to other cohorts in developed countries to explore my hypothesis. For example, cohorts such as those of Dr. Lucas from the Institute of Child Health because, as Dr. Hornstra mentioned earlier, they manipulated a cohort of preterms and intrauterine growth-restricted infants to different infant formulas or breast milk. I think that they are revisiting the children now, and I would encourage them to look at immune function, atopy, allergy, and so on.

Dr. Bleker: In the Dutch famine cohort so far, at age of 50, we found no relationship between mortality and the time of exposure to the famine, but they were only 50. It may change in the future.

Dr. Waller: Approximately how many individuals did you have in the survival curves you showed?

Dr. Moore: Approximately 3,000 in total, but we only had about 60 adult deaths. In fact it is amazing that the significance was so great considering it was just a small number, but that is why we want to use larger cohorts.

Dr. Uauy: The situation where I think there could be useful data is that of refugees. I am not sure there would be data on birth weight, but the mortality rates that you see under refugee situations (because of the burden of infectious disease is quite high) is that they have very high death rates, so some of those refugee populations are in fact chronic refugees. So if you have control data you could actually see the outcome of babies under refugee conditions (based on weight at birth) since they have high mortality rates?

Dr. Moore: A very good point, particularly as in many cases we would also have an indication of the mothers' nutritional status during pregnancy if their food intake is controlled. A good suggestion.