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Author

Cripps, AW, Otczyk, DC, Barker, J, Lehmann, D, Alpers, MP

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The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea

ALLAN W. CRIPPS¹, DIANA C. OTCZYK¹, JANE BARKER^{2,3}, DEBORAH LEHMANN^{2,4} AND MICHAEL P. ALPERS^{2,5}

School of Medicine, Griffith University, Gold Coast, Queensland, Australia, Papua New Guinea Institute of Medical Research, Goroka, Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, Perth and Curtin University of Technology, Perth, Western Australia

SUMMARY

Malnutrition is a significant risk factor for childhood infectious diseases in developing countries, including Papua New Guinea (PNG). Whilst the mechanisms are not fully understood there is little doubt that impairment of immune function is a major contributing factor in enhancing disease susceptibility in malnourished children. This susceptibility has been clearly shown for pneumonia in PNG. The aim of this study was to examine the effect of undernutrition on the humoral immune profile in children less than 60 months of age with pneumonia. The study was cross-sectional with measurements of nutritional status and parameters of the immune response being assessed simultaneously. The children were grouped according to age for the purpose of comparative analysis. The children were from the Goroka region of the Eastern Highlands Province of PNG and had been admitted to hospital with moderate-severe pneumonia. They were classified as undernourished (less than 80% weight for age) or nourished (greater than or equal to 80% weight for age). Serum albumin, IgG, IgA and IgM and salivary albumin and IgA were measured. Antibodies to nontypeable *Haemophilus influenzae* outer membrane protein and *Escherichia coli* O antigen were also determined in serum and saliva. Undernourished children aged less than 49 months had lower levels of serum albumin than nourished children throughout this age range. Lower values of salivary IgA were observed in infants (less than 13 months of age) than in older children, with a larger proportion of younger children having no detectable IgA. The age-related immunological profile was similar in undernourished and nourished children. At different age intervals the concentration of immunoglobulins in serum and saliva from undernourished children was generally found to be less than or the same as that from nourished children. In most cases undernourished children had lower levels of specific antibodies than nourished children but for some antibodies in some age groups the levels in the undernourished were higher. In conclusion, undernutrition was associated with hypoalbuminaemia and reduced humoral immune responses in children with pneumonia but its immunological effects varied with age in an unpredictable way.

¹ School of Medicine, Griffith Health, Gold Coast Campus, Griffith University, Queensland 4222, Australia
allan.cripps@griffith.edu.au

² Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, Eastern Highlands Province 441, Papua New Guinea

³ Present address: Taylors Road, Eureka, New South Wales 2480, Australia

⁴ Present address: Division of Population Sciences, Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, PO Box 855, West Perth, Western Australia 6872, Australia

⁵ Centre for International Health, ABCRC, Shenton Park Campus, Curtin University of Technology, GPO Box U1987, Western Australia 6845, Australia

Introduction

Maternal and child undernutrition accounts for 35% of deaths in children younger than 5 years and 11% of the total global disease burden (1). Most of this burden is borne by developing countries. Intrauterine growth restriction and child malnutrition together lead to 2.2 million deaths and 21% of disability-adjusted life-years (DALYs) in children under 5 years (1). Malnutrition impacts on the body's ability to function properly, with particularly deleterious effects in young children. For many malnourished children, inadequate food intake is secondary to endemic chronic infectious diseases. Diseases such as pneumonia, malaria, tuberculosis, diarrhoea and parasitic infections constitute the main burden of ill health among under-five children in developing countries (2). Significantly, nearly half of the deaths due to these diseases each year are associated with malnutrition (3). Indeed, the relationship between malnutrition and a higher risk of respiratory and enteric infections has been well established (4). What is becoming most evident is that the profound interactions between the pathogenicity of malnutrition, disturbances in the ontogeny of the immune system and chronic antigenic exposure from infections may have a more significant impact on child death than the direct sequelae of the nutritional deficiencies themselves.

Malnutrition is an important cause of impaired immune competence. The effect of undernutrition on the ontogeny of the immune system of the fetus during gestation and neonatal maturation may lead to deficits in immunity in infancy and early childhood. The impairments have been reported in several aspects of immunity such as cell-mediated immune responses (5-10), the complement system (5,6,10-12) and phagocytic function (13). When the immature and compromised immune system of an undernourished child is challenged by chronic and repeated infections, there is a further weakening of the immune response as evidenced by altered immune cell populations (14-18) and a generalized increase in pro-inflammatory cytokines (19). The overall effects might include altered mucosal immune defences and compromised barrier function against invasion by pathogens (20). The results concerning immunoglobulin levels and antibody synthesis to common pathogens have been variable, with depressed, normal or elevated levels being reported

(9,10,12,18,21-27).

Most of the previously published studies have involved severely malnourished children. Whilst the highest risk of mortality is associated with the most severely underweight children, in developing countries children with mild to moderate underweight status constitute the greater burden of disease because of their high prevalence (3). Whilst there is little doubt that severe nutritional stress impairs immune function, the evidence that mild to moderate malnutrition does so remains controversial. Papua New Guinea (PNG) has a significant number of children below 80% weight for age, who are classified as undernourished (28). In PNG, malnutrition accounts for 34% of deaths in children under 5 years of age whilst very low birthweight from intrauterine growth restriction is responsible for more than half of neonatal mortality (29). Pneumonia is the leading cause of death (29), with low birthweight (30), undernutrition (31-33), poor infant feeding practices (34) and depressed cell-mediated immunity (35) as significant contributing factors. Previous studies have shown that, as elsewhere in the world, *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most significant bacterial pathogens in pneumonia (29,33,36,37). In PNG, children acquire *S. pneumoniae* and *H. influenzae* at an extremely early age, with all children being colonized by 3 months of age (38). A previous study suggested that early colonization may result in a 'high zone' tolerance to infection by *H. influenzae* (39). The present study was conducted to examine age-related profiles of the humoral immune responses in children with pneumonia from the Goroka area in the Eastern Highlands Province (EHP) of PNG, where heavy bacterial colonization is the norm (38). All children included in the study had moderate-severe pneumonia. The relationship between undernutrition and humoral immune status was examined in these children.

Methods

Subjects

238 children aged 6 weeks to 60 months who were admitted to the Goroka Base Hospital, EHP, PNG with a clinical diagnosis of moderate or severe pneumonia were enrolled in the study. The study children were grouped into 6 age intervals: 6 weeks-6 months, 7-12 months, 13-24 months, 25-36

months, 37-48 months and 49-60 months.

Nutritional assessment

On admission to hospital the children were weighed with minimal clothing. The children were classified as undernourished (less than 80% weight for age) or nourished (greater than or equal to 80% weight for age) (28). This is the standard method of assessing and following the nutritional status of children in PNG, though in research studies the Waterlow classification is now more commonly used (40,41).

Sample collection

Saliva (1 ml) was collected by gentle suction and blood (3 ml) by venepuncture. The samples were transported to the laboratory and serum was prepared from the blood sample. The saliva and serum were stored frozen until assayed.

Quantitation of serum albumin, IgG, IgA and IgM and salivary albumin and IgA

Serum albumin, IgG, IgA and IgM were measured by rate nephelometry. Salivary albumin and IgA were measured by electroimmunodiffusion (42). Contamination of saliva samples with breastmilk was identified by immunoelectrophoresis. Any sample containing breastmilk was excluded from the analysis.

Measurement of specific antibodies in serum and saliva

Isotype-specific antibodies to nontypeable *H. influenzae* (NTHi) outer membrane protein (OMP) and *Escherichia coli* O antigen were determined by enzyme-linked immunosorbent assay (ELISA) (39,43).

Statistical analysis

For the purpose of analysis the children were grouped into 6 age groups: ≤ 6 months, 7-12 months, 13-24 months, 25-36 months, 37-48 months and 49-60 months. The saliva data contained numerous values that were below the detectable limit of the assays. Hence, for saliva, the medians and interquartile ranges of positive values in each age group are presented, as well as the percentage of 'zero' IgA values. Statistical differences between the values in serum and saliva for the two groups were assessed by

the Mann-Whitney test or the Kruskal-Wallis test for more than two groups.

Ethical approval

The study was approved by the Medical Research Advisory Committee of Papua New Guinea through the pneumonia research program of the PNG Institute of Medical Research.

Results

Serum albumin, immunoglobulins and specific antibodies

The median levels of albumin, immunoglobulins, NTHi OMP antibodies and *E. coli* antibodies are presented in Table 1.

Serum albumin

Undernourished children less than 49 months of age had lower levels of serum albumin than nourished children (≤ 6 months, 7-12 months and 13-24 months, $p < 0.001$; 25-36 months and 37-48 months, $p < 0.05$). The serum albumin levels were the same in undernourished and nourished children in the 49-60 month age group.

Serum immunoglobulins

IgA: The IgA level in serum was significantly lower in undernourished children aged 7-12 months than in nourished children of the same age ($p < 0.001$). In the other age groups there was no difference between the levels in the nourished and the undernourished children.

IgG: At 25-36 months of age the level of serum IgG in undernourished children was lower than that observed in nourished children ($p < 0.05$). The differences between the levels in the other age groups were not considered to be significant.

IgM: The level of IgM in the serum of undernourished children was lower than in nourished children at the ages of ≤ 6 months ($p < 0.05$), 25-36 months ($p < 0.05$) and 37-48 months ($p < 0.001$).

Serum NTHi OMP and E. coli antibodies

IgA: The level of serum NTHi OMP-specific IgA antibody was higher in undernourished children 49-60 months of age than in nourished children ($p < 0.05$). The differences

between the levels in the other age groups were not considered to be significant. At 7-12 months of age the level of serum *E. coli*-specific IgA antibody was lower in undernourished than in nourished children ($p < 0.05$).

IgG: At 7-12 months and 37-48 months of age the levels of serum NTHi OMP-specific IgG antibody were lower in undernourished than in nourished children ($p < 0.05$ and $p < 0.002$, respectively) and in the other age groups the differences were not considered to be significant. Similar results were found with *E. coli*-specific IgG antibody: at 7-12 months and 37-48 months of age the levels of antibody were lower in undernourished than in nourished children ($p < 0.05$).

IgM: The level of NTHi OMP-specific IgM antibody was higher in undernourished than in nourished children at 7-12 months of age ($p < 0.01$). Serum *E. coli*-specific IgM antibody was lower in undernourished than in nourished children aged 13-24 months ($p < 0.05$), 37-48 months ($p < 0.05$) and 49-60 months ($p < 0.01$). However, at 25-36 months of age the level of *E. coli*-specific IgM antibody was higher in the undernourished children ($p < 0.05$).

Salivary albumin, IgA and IgA-specific antibodies

The median levels of positive values of albumin, IgA, NTHi OMP-specific IgA antibody and *E. coli*-specific IgA antibody and the proportion with no detectable amounts of IgA in saliva are presented in Table 2.

No significant differences were observed between undernourished and nourished children in the levels of albumin in saliva. However, at ≤ 6 months of age the level of albumin in saliva in both undernourished and nourished children was lower than in older age groups.

The level of IgA in positive samples of saliva was lower in undernourished than in nourished children at ≤ 6 months ($p < 0.05$) and 49-60 months ($p < 0.02$) of age. The level of salivary IgA in both nourished and undernourished children increased from ≤ 6 months of age to 13-24 months ($p < 0.05$) and then tended to increase with age in the nourished children and decrease in the undernourished. At ≤ 6 months of age approximately 80% of the children irrespective

of nutritional status had no detectable salivary IgA. This proportion decreased with age in both groups of children. However, after 6 months of age, there was always a greater proportion of undernourished children with no detectable salivary IgA.

The level of *E. coli*-specific IgA antibody in saliva was significantly lower in undernourished than in nourished children at 7-12 months of age ($p < 0.005$). There was an increase in *E. coli*-specific IgA antibody in the nourished children between the ages of ≤ 6 months and 7-12 months ($p < 0.05$) followed by a decline at 13-24 months ($p = 0.002$).

Discussion

It is generally accepted that undernourished children are more susceptible to infections than nourished children; this is mediated in large by an impaired immune function. The results concerning immunoglobulin and antibody synthesis, however, have been highly variable and nonspecific (9,10,12,18,21-27), with the majority of these investigations dealing with the severe forms of malnutrition, ie, marasmus and kwashiorkor (9,12,18,21,25-27). The few studies that have looked at mild malnutrition and its impact on humoral immune function have been less than convincing (10,22-24). These inconsistencies may be associated with inadequately characterized population groups. There has been some suggestion that various aspects of the immune response are affected in different ways depending on the severity and type of malnutrition (7,8), the presence of micronutrient deficiencies (44,45), the age of the children studied (27) and the existence and type of infection (9,10,15,16,17,46).

The mucosal immune system begins to develop very shortly after conception; however, human beings are born with a structurally complete but functionally immature and inexperienced mucosal immune system (47). Environmental influences such as nutrition (48,49), feeding practices (50) and intestinal colonization with microflora (51,52) play an important role in influencing the developing human immune system. The immunological abnormalities resulting from intrauterine malnutrition may persist for several months after birth (48) and may compromise postnatal immunity and host resistance to infection. The risk of

TABLE 1

MEDIAN CONCENTRATION OF SERUM ALBUMIN, IgA, IgG, IgM, NTHI OMP-SPECIFIC ANTIBODY (IgA, IgG, IgM) AND E. COLI-SPECIFIC ANTIBODY (IgA, IgG, IgM) IN NOURISHED AND UNDERNOURISHED CHILDREN AT DIFFERENT AGES

Serum	≤6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months
	n	n	n	n	n	n
Albumin (mg/l)						
Nourished	34.5 ^a (30.0-41.5)	39.0 ^a (29.0-46.0)	28.5 ^a (20.8-37.3)	39.0 ^d (34.5-46.0)	42.0 ^d (22.5-42.5)	34.0 (28.5-42.5)
Undernourished	25.0 ^a (17.0-34.3)	27.0 ^a (22.5-34.5)	16.5 ^a (15.0-19.8)	30.0 ^d (23.5-38.0)	20.0 ^d (17.0-26.5)	34.0 (30.3-39.5)
IgA (mg/l)						
Nourished	1.0 (0.6-1.6)	1.2 ^a (0.8-1.8)	0.6 (0.4-1.0)	1.0 (0.6-1.2)	1.3 (0.7-2.0)	1.8 (1.4-2.0)
Undernourished	1.1 (0.9-1.2)	0.7 ^a (0.4-0.9)	0.8 (0.5-1.1)	1.1 (0.8-1.6)	1.6 (1.0-2.3)	1.2 (1.0-2.1)
IgG (mg/l)						
Nourished	11.1 (8.6-15.8)	13.9 (8.0-14.8)	15.1 (9.5-21.1)	16.8 ^d (12.6-20.1)	9.3 (7.2-11.0)	17.4 (12.6-25.5)
Undernourished	11.1 (7.2-15.2)	12.1 (8.5-14.5)	10.8 (7.2-19.0)	12.6 ^d (11.1-15.0)	13.5 (9.5-17.4)	12.5 (9.0-15.9)
IgM (mg/l)						
Nourished	1.4 ^d (0.8-1.7)	1.9 (1.4-2.4)	0.7 (0.6-0.8)	1.4 ^d (1.1-1.9)	0.5 ^a (0.4-0.9)	1.1 (0.8-1.5)
Undernourished	0.8 ^d (0.6-1.1)	1.4 (1.0-3.0)	0.8 (0.5-2.1)	0.8 ^d (0.7-1.7)	0.2 ^a (0.2-0.3)	0.8 (0.6-0.8)
NTHI OMP-specific antibody (EU/ml)						
Nourished	66.8 (30.0-128.6)	90.0 (50.6-240.4)	53.3 (24.0-210.8)	127.5 (51.8-211.5)	684.0 (63.0-911.3)	87.0 ^d (54.0-111.0)
Undernourished	75.0 (49.5-155.8)	73.2 (48.8-106.5)	87.0 (62.3-301.5)	90.0 (39.0-204.0)	60.0 (40.5-199.5)	195.0 ^d (126.0-483.0)

IgG	Nourished	19,291 (11,386-34,696)	21,997 ^d (13,182-48,903)	19	14,420 (10,746-31,444)	20	7131 (6,255-11,733)	18	12,383 ^b (6,220-20,768)	10	20,737 (10,085-61,782)	18
	Undernourished	17,018 (14,365-23,736)	12,454 ^d (7,669-18,934)	19	21,287 (15,672-26,148)	20	10,385 (7,055-18,405)	20	5775 ^b (5,118-6,182)	9	14,022 (11,560-27,861)	6
IgM	Nourished	2224 (1439-4948)	1438 ^c (843-2234)	20	2843 (1732-5590)	19	2425 (1237-4883)	10	nd	nd	8460 (2030-12,861)	18
	Undernourished	1946 (1455-2484)	2910 ^c (1791-5494)	19	1604 (852-3432)	19	3072 (1319-6264)	20	nd	nd	nd	nd
E. coli-specific antibody (EU/ml)												
IgA	Nourished	34.8 (10.2-60.0)	64.5 ^d (29.1-116.4)	20	40.2 (26.3-69.6)	20	61.5 (17.7-120.0)	20	nd	nd	43.2 (13.2-118.2)	18
	Undernourished	17.0 (8.9-56.1)	20.4 ^d (10.8-49.4)	20	43.4 (24.2-63.0)	20	37.2 (18.0-120.0)	19	20.4 (18.9-39.3)	9	nd	nd
IgG	Nourished	199.0 (106.0-346.3)	635.5 ^e (232.4-1310.0)	16	245.6 (117.3-689.5)	15	208.5 (156.1-405.6)	16	562.6 ^d (375.4-845.9)	8	396.7 (177.1-977.0)	13
	Undernourished	144.5 (57.5-235.3)	283.7 ^d (130.4-472.4)	17	538.0 (178.3-817.0)	14	564.6 (147.3-918.2)	17	117.1 ^d (82.0-428.1)	9	492.2 (227.2-1402.5)	4
IgM	Nourished	204.0 (105.6-297.3)	265.3 (188.6-382.2)	20	330.3 ^d (254.6-455.7)	20	303.4 ^d (157.1-495.0)	19	466.8 ^d (182.5-710.7)	10	1141.2 ^c (691.8-1494.0)	17
	Undernourished	129.0 (97.0-192.3)	320.9 (167.8-414.5)	19	165.5 ^d (77.1-407.6)	20	657.6 ^d (238.7-1080.8)	19	160.6 ^d (114.3-280.0)	9	398.1 ^c (100.7-685.1)	6

Median values are reported with the interquartile range in parenthesis
 nd = no assays were conducted or insufficient data for analysis
 NTHi = nontypeable *Haemophilus influenzae*
 OMP = outer membrane protein
 EU = ELISA units

Difference between nourished and undernourished children for respective values within an age group:
^a p <0.001; ^b p <0.002; ^c p <0.01; ^d p <0.05

TABLE 2

MEDIAN POSITIVE VALUES OF SALIVARY ALBUMIN, IgA, NTHI OMP-SPECIFIC IgA ANTIBODY, E. COLI-SPECIFIC IgA ANTIBODY AND THE PERCENTAGE OF ZERO IgA VALUES IN NOURISHED AND UNDERNOURISHED CHILDREN AT DIFFERENT AGES

	Saliva	≤6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months
		n	n	n	n	n	n
Albumin (mg/l)	Nourished	3.0* (2.0-15.0)	25.0* (21.0-36.5)	13 (6.8-24.8)	10 (12.5-42.0)	13 (15.0-20.5)	9 (17.3-31.3)
	Undernourished	2.0* (1.0-12.0)	21.0* (6.0-25.0)	7 (10.0-36.0)	10 (15.0-23.0)	9 (14.8-26.0)	10 (12.0-36.0)
IgA (mg/l)	Nourished	12.0 ^{a*} (8.8-20.8)	11.0 ^{b*} (5.0-23.0)	9 (20.0-31.0)	9 (12.0-38.3)	8 (24.5-64.0)	9 (21.5-52.0)
	Undernourished	4.0 ^{c**} (3.3-7.5)	16.0* (6.0-21.0)	3 (21.5-45.0)	5 (16.0-37.5)	8 (11.0-54.0)	8 (6.5-25.0)
% zero IgA value	Nourished	81.3	55.0	11/20	27.3	3/11	10.0
	Undernourished	78.3	84.2	16/19	57.9	11/19	33.3
NTHI OMP antibody (EU/ml)	Nourished	50.6 (13.0-163.9)	59.9 (42.8-206.2)	14 (12.9-71.3)	7 (14.6-222.3)	5	4 (11.6-30.1)
	Undernourished	35.3 (7.8-56.8)	54.6 (43.7-70.4)	7	31.9 (14.1-84.8)	12	6 (10.9-32.4)
E. coli IgA antibody (EU/ml)	Nourished	116.1* (44.7-157.7)	268.6 ^{a**} (94.7-471.7)	10 (12.9-54.6)	8 (17.0-56.6)	5	7 (27.7-50.1)
	Undernourished	nd	44.4 ^b (18.3-63.1)	5	40.7 (10.9-64.2)	9	5 (10.6-93.9)

Median values are reported with the interquartile range in parenthesis
 nd = no assays were conducted or insufficient data for analysis
 NTHI = nontypeable *Haemophilus influenzae*
 OMP = outer membrane protein
 EU = ELISA units
 Difference between nourished and undernourished children for respective values within an age group:
^a p < 0.005; ^b p < 0.02; ^c p < 0.05
 Difference between age groups for respective values within nourished or undernourished children:
 * p < 0.05; † p < 0.005; ‡ p = 0.002

undernourishment is heightened in children where breastfeeding practices are suboptimal (53), with an associated increased risk of infections, particularly diarrhoea (54) and pneumonia (34). However, in the population under study exclusive and prolonged breastfeeding was the norm.

Our results provide some insight into the effects of infection and undernutrition on the humoral immune responses in a population of undernourished children with acute lower respiratory infection compared to similarly infected children who maintained normal nutritional status before contracting pneumonia and during their disease. Albumin, immunoglobulin and specific antibody levels in serum and saliva were contrasted between nourished infected children and undernourished infected children. Any differences observed were therefore related, directly or indirectly, to their nutritional condition.

This was a cross-sectional study with measurements of nutritional status and parameters of the immune response being assessed simultaneously. The children were divided according to their weight for age into two nutritional groups, nourished and undernourished. The children within each nutritional group were stratified into age groups for the purpose of comparative analysis. The results of this study showed that undernourished children less than 49 months of age have significantly lower levels of albumin in serum than nourished children. These findings are consistent with previous studies which have shown an association of hypoalbuminaemia with protein malnutrition (9,10,24,25,55) and more specifically with pleural effusion in children with pneumonia (56,57). Previous studies investigating the more severe forms of malnutrition have demonstrated IgG and IgM levels in serum to be decreased (7) or increased (9,12,22) or show no change (10,24,26), while the level of IgA has been more consistently demonstrated to be elevated (7,8,12,22,24,27). Serum immunoglobulin levels in mild to moderately undernourished children have generally been shown to be not significantly affected (10,23,24). This study demonstrated that in different age groups the concentration of immunoglobulins in serum and saliva in children who were undernourished was in most cases lower than or no different from that in nourished children. Undernourished children at 7-12 months had

significantly decreased levels of serum IgA in comparison with nourished children. Decreases in the level of IgM in the undernourished were observed at the age of ≤ 6 months and from 25 to 48 months. In children aged 25-36 months the IgG levels in serum were lower in the undernourished. What was most apparent in our study population was that the level of immunoglobulin present at any given time was highly dependent on the age of the child. This observation may offer some insight into the inconsistencies between previous studies, where the age range of children was anywhere from birth to 7 years of age.

In saliva, it was demonstrated that albumin does not appear to be affected by nutritional status, which is consistent with previous observations (22). The immune system at mucosal surfaces has been shown to be suppressed in severe to moderate malnutrition, with decreased levels of IgA observed in nasopharyngeal fluid, tears, saliva and duodenal fluid (21,22,25,27); in the case of mild malnutrition there was no change in salivary IgA (23). Our results support these observations, with total IgA levels in saliva being lower in undernourished children aged ≤ 6 months and 49-60 months. The age profiles in both groups up to 24 months of age show a development pattern similar to that previously described by Gleeson et al. (50) with lower values of salivary IgA observed in infants and young children and a larger proportion of younger than older children having no detectable IgA. The proportion of children with no detectable salivary IgA decreased with age from 80% at ≤ 6 months to approximately 25% at 49-60 months. However, after 6 months of age, the proportion of children with no detectable IgA in saliva was always greater in the undernourished group. Also, in the undernourished children, after the age of 24 months, and in contrast to the nourished children, the levels of salivary IgA decreased with age.

Antigenic exposure is important to the ontogeny of IgA responses to common enteric and respiratory organisms. Because of the heavy early exposure to *E. coli* and NTHi, antibodies to *E. coli* O antigen and NTHi OMP were investigated in this study as markers of the immune response to bacterial exposure. PNG children have an early bacterial colonization (29,38) characterized by a rapid development of serum and salivary IgA and

specific IgA antibody, which was nevertheless lower than that in Australian children of the same age with considerably less antigenic exposure (39,58). This study has confirmed that the salivary IgA and specific IgA antibody levels in PNG children are lower than those previously reported in Australian children (39,50). This observation provides further support for the hypothesis that early colonization may result in a 'high zone' tolerance to antigens that children are exposed to early in life.

Our data showed that undernourished children had significantly lower serum levels of NTHi OMP and *E. coli* antibodies than nourished children in 8 results distributed across the age groups (Table 1). In the remainder, in 21 cases there was no difference attributable to nutritional status. There were 3 exceptions where the levels were increased in the serum of undernourished children: for NTHi OMP-specific IgA antibody at 49-60 months and IgM antibody at 7-12 months, and for *E. coli*-specific IgM antibody at 25-36 months of age.

In saliva, IgA levels remain relatively constant until there is exposure to increased antigenic loads (43). The age profiles for both NTHi OMP and *E. coli* antibody in nourished children (Table 2) were similar to each other but different from that observed for IgA levels. In this group of children both specific antibody levels in saliva peaked at 7-12 months; however, only with the *E. coli*-specific IgA antibody was the peak level significantly higher. The level of *E. coli*-specific IgA antibody was significantly higher in nourished children than in undernourished children at 7-12 months. Interestingly, at 7-12 months there was a dramatic rise in the proportion of children with detectable *E. coli*-specific IgA antibodies in both undernourished and nourished groups with virtually all children having detectable antibody in their saliva (data not shown) with a gradual decline in these numbers with age. The decline in concentration of NTHi OMP- and *E. coli*-specific IgA antibody levels after 7-12 months may follow the action of immune control mechanisms, with suppression of an initial cooperative interaction between T and B lymphocytes, in response to differing bacterial colonization patterns.

In severely malnourished children with respiratory and enteric infections, it has been demonstrated that they have a lower

proportion of peripheral blood B cells (10,16) and decreased number of IgA-containing cells in the jejunal mucosa (59,60) compared with well-nourished children with infection. It is therefore not unreasonable to assume that the ontogeny of humoral immunity may also be compromised. This study is consistent with these observations since, in a number of age intervals studied, lower levels of IgG, IgM, IgA and specific antibody were observed in undernourished children. It is indeed this failure in malnourished children to increase the proportion of B lymphocytes during infection (16) that may offer some explanation for the observation in our study that at age 7-12 months, despite a greater than two-fold increase in the proportion of undernourished and nourished children with detectable antibody to *E. coli* (data not shown), the nourished children were able to mount an antibody response that was significantly greater than that in the undernourished children.

This is the most comprehensive study undertaken that examines undernourished children at specific ages from 6 weeks to 60 months. These observations, while generally suggesting that undernutrition may compromise both mucosal and systemic immunity in children with pneumonia, are not fully consistent between age groups. Clearly, the effect of undernutrition on the immune system is complex and modified by the age of the child, whether or not infection is present, the history of recurrent infections of different kinds, and the degree of malnutrition.

The influence of nutritional status on the outcome of pneumonia is profound (32). This study confirms that the mechanisms of this influence involve perturbations of mucosal and systemic humoral immunity. However, the evidence from both the literature and this study leads to the conclusion that these mechanisms are not to be found simply in the cross-sectional levels of humoral immune factors.

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