Immune status of the neonate maintained on total parenteral nutrition

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SUMMARY Immunological studies were carried out on 7 neonates maintained on total parenteral nutrition for periods ranging from 14 to 31 days after surgery for correction of gastrointestinal anomalies. IgM and IgA levels were high in all patients but IgG levels were low, compared with normal values. Total leucocyte counts, absolute lymphocyte counts, and T- and B-lymphocytes in all patients were similar to those of healthy controls. Neonates maintained on adequate total parenteral nutrition showed no evidence of impairment of immune function.

In early postnatal life calorie restriction impedes cell division, organ growth, and differentiation, and may result in permanent stunting and retardation of brain growth. Later in life, under-nutrition affects cell size and composition, but this may be reversible after nutritional recovery.¹

The most prominent association of nutritional deficiency is with infection.² It is known that under-nutrition in late fetal and early postnatal life impairs the ability of the infant to respond to infections. Several aspects of immunocompetence—such as T-cell number, cell-mediated immunity, polymorphonuclear cell function, antibody responses, and complement components—are diminished in human undernutrition.³⁻⁵ Similar defects are seen in infants of low birthweights who also have intrauterine malnutrition.⁶

Malnutrition readily develops if there is failure of gastrointestinal function after surgical correction of severe gastrointestinal malformations, after prolonged gastroenteritis, and in very low birthweight preterm infants. The neonate with its relatively high metabolic rate, its requirements for growth, and its limited calorie stores is particularly susceptible to the effects of starvation.⁷ With the advent of parenteral nutrition it has become possible to provide metabolic support to those infants who would otherwise have suffered from prolonged periods of malnutrition because of temporary, but total, gastrointestinal malfunction.

Although the technical and metabolic aspects of total parenteral nutrition (TPN) and its complications have been studied, the immune status of the neonate maintained on TPN has not. The purpose

of this study was to assess immune function in neonates maintained on long-term parenteral nutrition and to compare this with the immune status of healthy neonates.

Material and methods

Patients. Seven neonates (4 boys and 3 girls) receiving total parenteral nutrition for periods ranging from 14 to 31 days were evaluated for immunoglobulins and complement levels, total leucocyte and absolute lymphocyte counts, and T- and B-lymphocyte subpopulations. Their mean gestational age was 39.5 (range 38–41) weeks and their mean birthweight 2.9 (range 2.1 to 3.9) kg. The indications for TPN were gastroschisis (2), massive small-bowel resection (2), anastomotic leak after repair of tracheo-oesophageal fistula (2), and reoperation for anastomotic leak in jejunal atresia (1). The age at which TPN was begun ranged from 1 to 6 (with a mean of 3) days.

The regimen used for TPN was similar to that reported previously.⁸ The infusate consisted of four basic solutions: (1) Vamin, composed of amino-acids, glucose, and electrolytes, (2) 20% Intralipid, containing the fat emulsion, (3) 10% glucose, and (4) 4.3% glucose in 0.18% saline. This regimen provided a daily calorie intake of between 100 and 120 calories/kg and a daily water intake of 150 ml/kg. Daily requirements of vitamins and minerals were added to 10% glucose. Plasma was given once a week to provide trace elements.

Controls. Thirty term neonates were evaluated for total leucocytes and absolute lymphocyte counts,

and T- and B-lymphocyte subpopulations. After determining the parent's willingness to participate in the study, samples of venous blood were obtained from the neonate on day 1, day 5, and day 20 after birth. The cumulative mean values obtained on the 3 separate days of testing are described as the normal neonatal control values. Cord blood samples from 25 healthy term neonates were estimated for IgG, IgA, IgM, and C3 and C4 levels.

Immunological studies.

Immunoglobulins and complement components

IgG, IgA, IgM, and C3 (β_1C/β_1A), and C4 (β_1E) levels were determined quantitatively by the automated immunoprecipitation technique from Technicon Corp. Tarrytown, NY, using PEG 6000 diluent for maximum sensitivity.⁹

Total leucocyte and absolute lymphocyte counts

Leucocyte counts were made on an electronic cell counter (Coulter Counter Model ZF) and the differential cell counts were made on blood smears using Giemsa stain.

T- and B- lymphocytes

Spontaneous erythrocyte rosette-forming lympho-

cytes (T-cells) were estimated after they had been incubated first at 37°C for 10 minutes and then at 4°C for 18 hours. SmIg-positive lymphocytes (B-cells) were evaluated using fluorescein-conjugated antiserum to whole immunoglobulin as described previously.¹⁰

Statistical method. Significance of difference between mean patient and control values for immunoglobulin levels based on log-transformed data was calculated using Student's *t* test for two independent observations.¹¹

Results

All 7 infants survived the period of parenteral nutrition. There was no septicaemia nor was there any other major infection during the period of observation. All infants showed a satisfactory weight gain while on TPN.

Immunoglobulins. The levels of IgA and IgM were significantly ($P < 0.001$) raised throughout the period of TPN compared with normal neonatal values (Table 1). However, the mean IgG levels were

Table 1 IgG, IgA, and IgM in neonates on total parenteral nutrition

Day of TPN		IgG (IU/ml)	P	IgA (IU/ml)	P	IgM (IU/ml)	P
Prefeeding (n=7)	+ 2 SD	108.56		43.46		118.08	
	Mean	61.18	<0.001	9.54	<0.001	16.18	NS
	- 2 SD	34.38		2.09		2.21	
	Range	42-92		5-30		5-66	
Day 7 (n=7)	+ 2 SD	103.64		36.58		173.1	
	Mean	62.26	<0.001	9.95	<0.001	51.89	<0.001
	- 2 SD	37.40		2.70		15.55	
	Range	46-84		5-21		19-111	
Day 14 (n=7)	+ 2 SD	94.75		42.30		117.92	
	Mean	60.68	<0.01	11.95	<0.001	55.73	<0.001
	- 2 SD	38.87		3.38		26.34	
	Range	38-74		5-23		40-92	
Day 20 (n=5)	+ 2 SD	147.62		29.84		96.94	
	Mean	62.83	<0.001	15.11	<0.001	53.49	<0.001
	- 2 SD	26.74		7.65		29.51	
	Range	33-100		10-21		36-79	
Controls (cord blood) (n=25)	+ 2 SD	140		—		13	
	Mean	105		0.52		8	
	- 2 SD	79		—		4	
	Range	73-130		0-2		5-12	

NS=not significant ($P > 0.01$).

Table 2 C3 and C4 levels in neonates on total parenteral nutrition

Day of TPN	C3 (g/l)			C4 (g/l)		
	Range	Mean $\pm$ 1 SD	P	Range	Mean $\pm$ 1 SD	P
Prefeeding (n=7)	0.52-1.55	1.01 $\pm$ 0.13	NS	0.11-0.28	0.16 $\pm$ 0.02	NS
Day 7 (n=7)	0.73-1.33	1.06 $\pm$ 0.08	NS	0.10-0.33	0.14 $\pm$ 0.03	NS
Day 14 (n=7)	0.67-1.90	1.33 $\pm$ 0.16	NS	0.09-0.34	0.19 $\pm$ 0.03	NS
Day 20 (n=5)	0.86-1.50	1.09 $\pm$ 0.11	NS	0.14-0.26	0.16 $\pm$ 0.02	NS
Controls (cord blood) (n=25)	0.55-1.36	0.93 $\pm$ 0.22		0.05-0.13	0.10 $\pm$ 0.04	

NS=not significant ($P > 0.01$).

Table 3 Total leucocyte and absolute lymphocyte counts in neonates maintained on total parenteral nutrition

Cases	Duration of TPN (days)	Total leucocyte count ($\times 10^9/l$)		Absolute lymphocyte count ($\times 10^9/l$)	
		Range	Mean ± 1 SD	Range	Mean ± 1 SD
1	27	4.5-24.6	14.0 $\pm$ 4.3	3.4- 8.1	5.3 $\pm$ 1.0
2	21	7.9-16.6	12.0 $\pm$ 1.6	3.0- 5.8	4.3 $\pm$ 0.5
3	31	10.2-32.8	19.0 $\pm$ 4.6	2.2- 5.9	4.1 $\pm$ 0.7
4	26	13.0-25.9	16.7 $\pm$ 2.3	3.8-10.9	5.3 $\pm$ 1.4
5	17	9.6-12.2	12.0 $\pm$ 1.0	4.0- 8.4	5.3 $\pm$ 0.8
6	14	14.9-21.8	17.9 $\pm$ 2.0	4.1- 7.4	5.7 $\pm$ 1.0
Controls (neonates)		8.1-47.0	19.4 $\pm$ 5.0	2.4-15.0	6.8 $\pm$ 0.3

Table 4 Absolute numbers of E-binding lymphocytes (T-cells) and SmIg-positive lymphocytes (B-cells) in neonates on total parenteral nutrition

Cases	Duration of TPN (days)	T-cells ($\times 10^9/l$)		B-cells ($\times 10^9/l$)	
		Range	Mean ± 1 SD	Range	Mean ± 1 SD
1	27	2.3-5.4	3.7 $\pm$ 0.6	0.57-0.77	0.70 $\pm$ 0.06
2	21	2.0-4.1	2.7 $\pm$ 0.4	0.39-0.83	0.64 $\pm$ 0.1
3	31	1.1-3.2	2.2 $\pm$ 0.3	0.54-1.30	0.89 $\pm$ 0.19
4	26	1.0-9.3	3.8 $\pm$ 1.1	0.54-2.17	1.05 $\pm$ 0.28
5	17	1.8-5.3	2.8 $\pm$ 0.7	0.91-1.67	1.16 $\pm$ 0.13
6	14	3.1-5.6	4.1 $\pm$ 0.7	0.21-1.00	0.73 $\pm$ 0.26
Controls (neonates)		0.9-8.7	4.0 $\pm$ 0.2	0.49-3.59	1.49 $\pm$ 0.18

persistently low ($P < 0.001$) in all 7 infants during the period of study.

Complement. The mean levels of complement C3 and C4 components were not significantly different from normal mean neonatal values ($P > 0.01$) (Table 2).

Total leucocyte and absolute lymphocyte counts. The mean total leucocyte count showed a wide variation in number both in infants on TPN (range 12.0 ± 1.0 to $19.0 \pm 4.6 \times 10^9/l$) and in normal healthy neonates ($19.4 \pm 5.0 \times 10^9/l$) (Table 3). The mean absolute lymphocyte count in neonates on TPN (range 4.1 ± 0.7 to $5.7 \pm 1.0 \times 10^9/l$) was lower, but not significantly so, than the normal neonatal values ($6.8 \pm 0.3 \times 10^9/l$).

T- and B-lymphocytes. The Figure shows the percentage of T- and B-lymphocytes in infants on TPN. The percentage of T- and B-cells remained within or slightly above the determined range (mean ± 1 SD) for healthy normal neonates. The lower levels for percentage of T-cells observed in the controls during the early neonatal period were also seen in infants on TPN during the early neonatal period. The absolute numbers of E-rosettes (T-cells) in all the infants were similar to the normal neonatal values while the numbers of B-cells were slightly diminished (Table 4).

Figure Percentage of T-cells and B-cells in neonates during maintenance on total parenteral nutrition. Vertical lines represent normal neonatal values ± 1 SD on day 1, day 5, and day 20.

Discussion

Neonates, particularly after surgery, have an increased susceptibility to overwhelming infection from opportunistic organisms. Moreover, several

studies have confirmed the long-held clinical impression that infectious diseases adversely affect nutritional status,^{4,12} and infection *per se* can impair immune function.⁵ Therefore the relationship between nutrition, immunity, and infection—particularly in infants—is important since the neonate is unique in its vulnerability to nutrient insufficiency during illness.

Current regimens for TPN in the neonate are based on maintaining satisfactory weight gain and normal biochemical values, and on providing sufficient calories to meet the daily requirements of the developing infant. All infants in our study showed adequate weight gain, normal growth and development, as well as normal wound healing.

Our results suggest that infants adequately maintained on TPN are capable of antibody synthesis in response to antigenic stimulus as evident from increased IgM and IgA levels. The raised IgM levels suggest an active subclinical infective process, since IgM is the initial antibody produced in the circulation in response to a primary infection. This was especially so in this group of infants who all underwent surgery for correction of congenital abnormalities. The raised IgA levels are probably related to the gastrointestinal origin of the primary anomalies in the neonates. The fall in IgG values which in the neonate are largely maternally derived may further reflect ongoing subclinical infection since such passively-acquired maternal IgG used during infective processes is not immediately replenished in the developing neonate.

Further evidence of a functional and active immune system in neonates maintained on TPN is seen in the results of lymphocyte subpopulation studies. The proportions of both T-lymphocyte and B-lymphocyte levels reflect a normal developing lymphocyte mediated immune system. The total leucocyte count and absolute count were also similar to those of normal healthy neonates. However, it is important to note that the neonate is undergoing rapid changes in peripheral blood leucocyte numbers and polymorphonuclear leucocyte-lymphocyte ratio as a consequence of the sudden change of environment after birth,¹³ and this

fact must be considered when assessing immune function in the neonate.

While these results do not represent a comprehensive investigation of the complex relationship that exists between nutritional status and the immune capacity of the developing neonate, there was no evidence to suggest impairment of immune function in the neonate adequately maintained on TPN.

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Received 4 December 1979