Serum complement and immunoconglutinin in malnutrition*

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Chandra, R. K. (1975). *Archives of Disease in Childhood*, 50, 225. **Serum complement and immunoconglutinin in malnutrition.** Serum haemolytic complement activity and C3 were significantly decreased in 35 malnourished children. The changes were more pronounced in those with infection. Electrophoretically altered forms of complement C3 were detected in 14. There was an inverse correlation between C3 levels and immunoconglutinin titres. Nutritional rehabilitation and eradication of infection reversed the abnormalities. It is suggested that reduced complement function in malnutrition is the combined result of impaired synthesis, complement activation *in vivo*, and changes in plasma volume, and that it may contribute to an increased susceptibility to infection in undernourished individuals.

Malnutrition and infection, singly and in combination, contribute significantly to morbidity and mortality of infants and children in the developing countries (Ramalingaswami and Ramalingaswami, 1973). The clinical impression that nutritional deficiency predisposes to frequent and severe infections is supported by epidemiological data and experimental studies in laboratory animals (Scrimshaw, Taylor and Gordon, 1968). Several parameters of immunocompetence, including T-cell number, cell-mediated immunity, polymorph function, and antibody response to some antigens, are impaired in human malnutrition (Harland, 1965; Antia, McFarlane, and Soothill, 1968; Smythe *et al.*, 1971; Chandra, 1972; Seth and Chandra, 1972; Sellmeyer *et al.*, 1972; Selvaraj and Bhat, 1972; Chandra, 1974a, c). Similar defects are seen in low birthweight infants with intrauterine malnutrition (Chandra, 1974b, 1975). We have previously reported a decrease in serum complement C3 in marasmus (Chandra, 1972). This paper describes the levels of serum complement and immunoconglutinin (IK) in malnourished children, their relation to coexisting infection, the evidence for complement activation *in vivo*, and the effect of dietary rehabilitation.

Material and methods

Patients. 35 infants and children, aged 6 months

to 4 years, were diagnosed as having primary protein-calorie malnutrition on the basis of a history of deficient nutrient intake, loss of subcutaneous tissue, skin and hair changes, and a weight and height less than 80% of the 50th centile for age on Boston growth charts. 5 patients showed pedal oedema. In 12 children there was clinical and microbiological or radiological evidence of systemic infection.

Controls. 20 healthy children matched for age and sex attending the Well Baby Clinic, who were bled routinely for haemogram and serum protein estimations, provided the control samples.

Haemolytic complement activity. Haemolytic complement was assayed by the method of Mayer (1961) using sheep red blood cells stored at 4°C in Alsever's solution up to 2 weeks, and rabbit haemolytic serum. Incubation of test serum and sensitized sheep cells was carried out for 90 min. Anticomplementary activity was looked for by mixing the test serum with serum from a healthy subject in different proportions and then titrating the mixture for complement.

Complement C3. Complement component C3 was estimated immunochemically by the radial immunodiffusion method (Mancini, Carbonara, and Heremans, 1965) using monospecific antiserum against human C3 raised in goat.

Immunoconglutinin (IK). Method IIb of Coombs, Coombs, and Ingram (1961) was employed, using positive, negative, and specificity controls in each run as described by Ngu and Soothill (1969). Serial dilutions of the test sera, in diluted inactivated horse serum, were tested for their ability to agglutinate sheep

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TABLE I

Haemolytic complement, C3, and immunoconglutinin (IK) in healthy and malnourished children, with or without infection

	Well nourished		Undernourished	
	No infection	With infection	No infection	With infection
No.	20	10	23	12
CH ₅₀ (units/ml)	58 ± 13	105 ± 21	39 ± 15	28 ± 11
C3 (mg/100 ml)	132 ± 18	245 ± 57	89 ± 23	57 ± 19
Altered C3 on IEP (no. positive)	0	6	9	5
IK (-log _e)	<1	5	3	4
(range)		(2-8)	(<1-5)	<1-8)

Note: Mean ± standard deviation are given.
IEP, immunoelectrophoresis.

erythrocytes sensitized with bovine serum containing naturally occurring Forssman antibody and alexinated with horse complement.

Electrophoretically altered C components. Blood was collected in EDTA and the presence of electrophoretically distinct metabolic conversion products of complement C3 was detected by immunoelectrophoresis and Laurell's cross-over electrophoresis into antibody containing gel, using barbitone buffer pH 8.6 and monospecific anti-C3 antiserum (goat).

Nitroblue tetrazolium test. Spontaneous *in vitro* reduction of nitroblue tetrazolium was employed as one of the indices of concurrent bacterial infection, using a normogram derived from discriminant analysis (Feigin *et al.*, 1971).

Nutritional rehabilitation. All patients were either given or advised to take dietary supplements to raise the daily intake to more than 100 calories/kg of expected body weight. Those with infection were treated with appropriate antibiotics. 3-8 weeks later a second sample of blood was drawn from 10 children who were available for re-examination and who were no longer undernourished.

Results

The total haemolytic complement activity and C3 concentration are shown in Table I and Fig. 1. There was a wide scatter of values in individual children but the mean levels in different groups were significantly different from each other. The mean concentration of C3 in malnourished children was lower than that of healthy controls ($P < 0.01$). 25 patients had C3 values lower than 2 SD below the mean for the healthy group. The reduction was more marked in those with infection. There was a significant positive correlation between C3 concentration and CH₅₀ activity ($r = 0.7131$).

The sera of 5 infected patients showed significant anticomplementary activity. In 3, the titre was

FIG. 1.—Serum complement C3 levels in malnourished children with (●) or without (○) overt infection. The bar indicates the mean ± 2 SD for healthy controls.

1:10, and in 2, 1:5. Electrophoretically altered C3 fractions were detected in a significant proportion of healthy infected children and malnourished children with or without infection (Table I). There was a significant inverse correlation between levels of C3 and IK (Fig. 2).

After nutritional therapy and control of infection there was a consistent rise in CH₅₀ and C3. IK fell in most instances, though to a variable extent. In 2 children (Cases 4 and 10), IK titre was higher in the convalescent sample, indicating a possible lag in the development and decay of IK.

Discussion

The complement cascade has a complex biological role in man. It is an important nonspecific

FIG. 2.—Relation between C₃ level and IK titre.

mechanism involved in host defence and a deficiency of some complement components may be associated with recurrent infections and autoimmune phenomena. Alper *et al.* (1970) found reduced levels of complement and impaired C-dependent phagocytosis associated with hypercatabolism and spontaneous activation of the alternate pathway in a patient with a life-long history of infections. Miller and Nilsson (1970) described recurrent Gram-negative bacteraemia in an 18-month-old infant whose serum failed to enhance C-dependent phagocytosis, chemotactic factor generation, or bacteriolysis. There was clinical improvement with plasma transfusion and a dysfunction of C₅ was postulated. Lachmann, Day, and Leddy (1972) observed an increased incidence of autoimmune phenomena with C₂ deficiency.

In our study the low level of complement in undernourished children and its increase to the normal range after dietary treatment suggests that the nutritional status of the individual can influence the complement system. This defect could be responsible in part for the increased susceptibility of malnourished subjects to infection. Several hypotheses can be invoked to explain the reduction in C₃ levels in malnutrition.

Low complement activity in nutritional deficiency states may reflect reduced protein synthesis, which is the hallmark of kwashiorkor. The data of Sirisinha *et al.* (1973) suggested a correlation between the degree of complement depletion and the severity of depletion of other proteins. Moreover, the limited body resources may be mobilized to form more useful proteins such as antibodies to the

invading pathogen, a process illustrated dramatically by measles leading to hypoalbuminaemia (R. K. Chandra, unpublished data). Alternatively, reduced C₃ synthesis may be a corollary of liver damage. The liver is the main site of C₃ synthesis, and hepatocytic injury as in hepatitis and cirrhosis is associated with a significant reduction in serum C₃ concentration (Chandra, 1970). In protein-calorie malnutrition, the liver is invariably involved (Ramalingaswami, 1964) and hepatic failure is closely linked with mortality (Garrow and Pike, 1967). In orthotopic transplantation of human liver, C₃ levels parallel functional liver mass (Torisu, Kohler, and Yokoyama, 1972).

We observed a greater reduction in complement levels in malnourished children with infection compared with noninfected ones. In nutritionally normal subjects, infection was associated with high C₃ levels (Table I). The more profound disturbance of complement seen in infected undernourished patients may be the result of at least two factors. One, antibody synthesis and cell division may get priority over C synthesis in the face of limited nutrient resources of the host. Secondly, infection may be associated with complement consumption, as has been shown in patients of bacterial endocarditis with renal complications (Williams and Kunkel, 1962), septicaemia in patients with ventriculoatrial shunt tubes (Stickler, *et al.* 1968) and ECHO virus type 9 infection (Yuceoglu, Berkovich, and Minkowitz, 1966). That this phenomenon was operating in our patients is suggested by the presence of electrophoretically altered forms of C₃ and raised levels of IK. Even in those without apparent infection, a subclinical process cannot be excluded, since the sera of 9 out of 23 such patients showed altered C₃ and raised IK. This mimics the changes observed in acute glomerulonephritis (Soothill, 1967; West *et al.*, 1967; Ngu and Soothill, 1969), and suggests the participation of complement in an immunological reaction. Although IK is established to be an antibody to reacted C₃ and C₄ (Lachmann and Coombs, 1965; Lachmann, 1966), its relation to the altered forms seen on immunoelectrophoresis is not clear. There is often a lag between the decrease in C₃ and the rise in IK (Marks and Coombs, 1957; Ngu and Soothill, 1969), which would explain the findings in Cases 4 and 10 (Table II). The failure to show electrophoretically altered C₃ components in some children with raised IK may be due to the limiting factor of C₃ concentration needed for clear delineation of the precipitin arcs.

Other possible explanations of low complement activity need mention, though they are not signifi-

TABLE II

Effect of nutritional supplements and antibiotic therapy on complement and immunoconglutinin (IK)

Case no.	On admission				After therapy			
	CH ₅₀ (units/ml)	C3 (mg/100 ml)	Altered C3 on IEP	IK (-log ₂)	CH ₅₀ (units/ml)	C3 (mg/100 ml)	Altered C3 on IEP	IK (-log ₂)
1	24	55	+	3	50	124	—	1
2	32	60	+	5	56	210	—	2
3	36	90	—	1	72	150	—	<1
4	50	112	—	<1	60	156	—	2
5	50	75	—	2	50	240	—	2
6*	4	12	—	6	44	180	—	2
7*	16	32	+	5	36	124	—	3
8*	24	40	+	4	50	150	—	1
9*	24	64	+	5	64	136	—	<1
10*	60	105	—	<1	72	188	—	2

*Cases 6 to 10 had systemic infection at the time of first examination.
IEP, immunoelectrophoresis.

cant factors. Changes in blood vascular compartment, especially plasma volume, may alter the concentration of C3, since in malnutrition there is an expansion of this compartment as measured directly (Gollan, 1948; Gomez *et al.*, 1950) and suggested in addition by the presence of hyponatraemia (Garrow and Pike, 1967) possibly due to an inappropriate secretion of antidiuretic hormone. Loss through the gastrointestinal tract is another possible avenue of complement depletion; in protein-calorie malnutrition, the intestinal mucosa is flattened (Ramalingaswami, 1964) which may lead to protein-losing gastroenteropathy.

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