Clin. exp. Immunol. (1978) 34, 248-252.

Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition

L. HALLER*, R. H. ZUBLER† & P. H. LAMBERT† * *Nestlé Foundation, Research Centre Adiopodoumé, Ivory Coast.* † *WHO-Immunology Research and Training Centre, Geneva, Switzerland*

(*Received* 27 *April* 1978)

SUMMARY

The plasma levels of complement haemolytic activity (CH50) of some complement components and of C3d, a C3 breakdown product, were measured in fifty-nine African children with various types of protein-energy malnutrition (PEM) including kwashiorkor, before and during recovery. A significant decrease of CH50, C3, C9 and factor B was observed in PEM without a concomitant decrease of C4 and C5. Increased plasma levels of C3d were also found in PEM patients. Two mechanisms seem to be involved in the impairment of the complement system in PEM: (1) a decreased synthesis of at least C3 and C9 as suggested by a significant correlation between C3 or C9, levels and those of albumin and cholinesterase; (2) an increased catabolism of C3 possibly due to an activation of the alternative complement pathway, as suggested by the increased level of C3d and the decreased level of factor B which are significantly correlated with C3 levels but not with albumin levels.

These data support the possible role of a relative complement deficiency upon the decreased resistance to infections observed in malnourished children.

INTRODUCTION

There is good evidence that malnutrition favours the development of diseases, particularly in children. It has been shown that up to 50% of children with protein-energy malnutrition (PEM) may suffer from a variety of severe and often fatal infections (Philipps & Wharton, 1968; Scrimshaw, Taylor & Gordon, 1968). This effect of malnutrition has been related to alterations of the endocrine system (Hadde n 1967; Whitehead, Coward & Lunn, 1973), impaired enzymatic function (Metcoff *et al.*, 1966; Waterlow & Stephen, 1969) hepatic disorders (Antener *et al.*, 1977) and impaired immune responses (humoral and cell-mediated) (Brown & Katz, 1966; Douglas & Schopfer, 1974; Neumann *et al.*, 1975; Schopfer & Douglas, 1976a, b; Smythe *et al.*, 1971). Decreased complement activity (Suskind *et al.*, 1972) and a reduced serum concentration of some complement components has been observed (Sirisinha *et al.*, 1973). These changes may result from a deficient protein synthesis or from an increased catabolism possibly related to concomitant infections (Chandra, 1975; Olusi *et al.*, 1976).

In the present study, the complement haemolytic activity, the serum level of various complement components and the concentration of a complement breakdown product, C3d, have been measured in fifty-nine African children with PEM. In addition, these data were correlated with other immunological parameters and with biochemical features of liver dysfunction. The serum levels of immunoglobulins, cholinesterase, albumin, prealbumin and transferrin were determined. The results were compared to those of two control groups of age-matched children without signs of malnutrition, one group suffering from various infectious diseases, the other group being apparently healthy.

Correspondence: Dr L. Haller, Nestlé Foundation, P.O. Box 1051, 1001-Lausanne, Switzerland

0099-9104/78/0110-0248$02.00

MATERIAL AND METHODS

Sampling. Fifty-nine children aged 15 to 25 months admitted for severe malnutrition to the CHU-Treichville Hospital in Abidjan were examined. They were divided into three groups according to the McLaren scoring system (McLaren, Pellett & Read, 1967): marasmus ($n = 10$), marasmic-kwashiorkor ($n = 18$) and kwashiorkor ($n = 31$). Furthermore, the kwashiorkor group was subdivided into two groups depending on the serum cholinesterase level group 1: cholinesterase < 1000 IU; group 2: cholinesterase > 1000 IU. Some of the children were re-examined during recovery at 7, 15 and 30 days after admission. The patients were compared with two control groups of ten age-matched children children each with a weight/age ratio > 90% of the Harvard standard: one group of healthy children and one group of children admitted for serious bacterial infections (meningitis, pneumonia, pleurisy, enteritis, and osteomyelitis).

Blood samples were taken from the femoral vein. For the collection of serum, blood was allowed to clot at ambient temperature for 1–2 hr, then centrifuged at 1500 *g* for 15 min. To obtain plasma, blood was collected in plastic tubes with EDTA (20 mM final concentration) and centrifuged at 1500 *g* for 15 min. Both serum and plasma were stored in liquid nitrogen at −190°C until determination.

Immunochemical studies. Pooled plasma from Ivorian adults served as standard for complement estimations and determinations of the complement haemolytic activity (CH50). Transferrin, prealbumin, fibrinogen, IgG, IgA, IgM, the complement components: C4, factor B, C3, C5, C9 and Cl-Inactivator were measured by single radial immunodiffusion using specific antisera from Behringwerke (Marburg, Germany).

In a first step, for the determination of C3d, native C3 and high molecular weight fragments C3b and C3c were precipitated with polyethylene-glycol (PEG). In a second step, the C3d fragment was measured in the PEG supernatant by single radial immunodiffusion using specific anti-C3d antiserum (Perrin, Lambert & Miescher, 1974). The haemolytic activity of complement (CH50) was measured according to Nydegger *et al.*, (1972).

Biochemical studies. The cholinesterase (E.C. 3.1.1.8) activity was measured with the iodide-acetylthiocholine method of Ellman (kits no. 15.984 from Boehringer, Mannheim). Lysozyme (E.C. 3.2.1.17) concentration in serum was determined by turbidimetry after lysis of *Micrococcus lysodeicticus* (kit no. 15.351 from Boehringer, Mannheim).

RESULTS

Complement studies (Table 1) show a decreased complement haemolytic activity (CH50) for all types of PEM with the lowest values in severe kwashiorkor cases. Such a decrease is not generally observed in

TABLE 1. Complement components and complement haemolytic activity in various types of PEM. (Values expressed in percentage of the controls C = 100%.)

	N	C4	Factor B	C3	C5	C9	Cl-I	CH50
Kwashiorkor I	24	97±33	58*±19	46*±22	105±20	44*±25	94±26	57*±19
Kwashiorkor II	7	100±49	59*±37	62‡±32	95±13	44*±23	102±45	63‡±37
Marasmic kwashiorkor	18	108±38	64*±26	72‡±28	109±17	73±39	100±27	70†±26
Marasmus	10	101±35	65*±14	76‡±28	111±13	82±36	117±39	75†±14
Infections	10	102±53	87±20	123±36	125±17	118±25	100±17	98±20
Controls (100%)	10	100±49	100±19	100±26	100±14	100±31	100±17	100±16

Mean±1 s.d. * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$.

children hospitalized only for infections without malnutrition. The plasma level of complement components is not uniformly influenced by malnutrition. On one hand, C3, C9 and factor B are significantly lower in the various types of PEM than in age-matched control children. The decrease of C3 and C9 appears to be correlated with the severity of PEM, which is not the case for factor B levels. On the other hand, the levels of C4, C5 and of Cl-Inactivator fall within normal range.

The plasma concentration of C3d, one of C3 breakdown product, is significantly increased ($P < 0.01$) in patients with PEM as compared to non-malnourished-infected patients and to normal children (Table 2). In addition, the C3d/C3 ratio appears higher in all PEM groups than in the control population. Sequential studies during recovery of PEM indicate a progressive normalization of all complement values (Fig.1), as well as a decrease of the C3d/C3 ratio (Fig. 2).

TABLE 2. C3d level and C3d/C3 ratio in various types of PEM

	N	C3d (mg%)	C3d/C3
Kwashiorkor I	13	1·7±0·5	5·1*±2·1
Kwashiorkor II	6	2·2±0·9	8·4±7·0
Marasmic kwashiorkor	9	2·4±1·1	5·1±4·5
Marasmus	8	2·4±1·1	4·1‡±3·5
Mean PEM	36	2·1†±0·7	5·4*±1·8
Infections	8	1·8±0·6	2·1±0·9
Controls (100%)	8	1·7±0·2	2·3±0·5

Mean±1 s.d. * $P < 0{\cdot}001$, †: $P < 0{\cdot}01$, ‡ $P < 0{\cdot}05$.

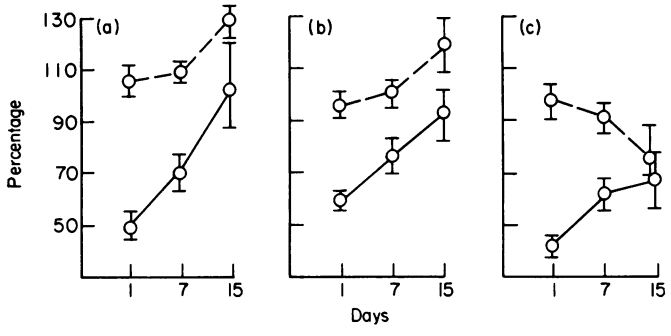

FIG. 1. Complement components during recovery in protein-energy malnutrition (percentage of controls ±s.e.m.). (a) (——) C3; (– – –) C5; (b) (——) Factor B; (– – –) Cl-Inactivator; and (c) (——) C9; (– – –) C4.

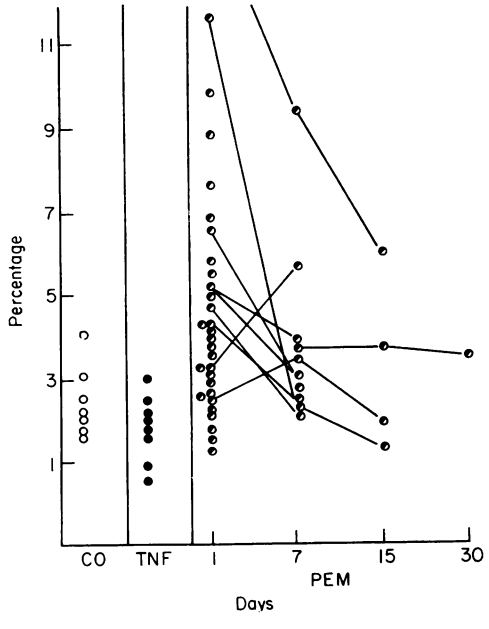

FIG. 2. C3d/C3 ratio in controls infectious diseases and protein-energy malnutrition during recovery.

TABLE 3. Albumin, transferrin, prealbumin, cholinesterase and lysozyme levels in various types of malnutrition

	N	Albumin (g/100 ml)	Transferrin (mg/100 ml)	Prealbumin (mg/100 ml)	Cholinesterase (UI)	Lysozyme (UI)
Kwashiorkor I	24	1·9*±0·4	78*±54	6·8*±3·3	670*±192	84±31
Kwashiorkor II	7	2·1*±0·4	102*±60	10·6±4·0	1375*±274	94±29
Marasmic kwashiorkor	18	2·4*±0·6	141*±66	8·0†±3·8	941±478	119±86
Marasmus	10	3·3*±0·6	148†±91	7·2†±4·0	1842†±942	135±60
Infections	10	2·9*±0·6	187±79	7·4†±3·0	1646†±1035	100±30
Controls	10	4·5±0·4	282±67	12·5±2·7	3160±785	100±27
Normal values at about 2 years of age	—	3·6±0·3	253±31	17·9±1·9	2900±900	

Mean±1 s.d. * $P < 0·001$, † $P < 0·01$.

A correlation study performed on malnourished children showed a significant correlation coefficient between C3 level and those of: C9 ($r = 0·37$; $P < 0·01$), factor B ($r = 0·51$; $P < 0·001$) and CH50 ($r = 0·67$; $P < 0·001$), but not with C4 or C5.

As expected, the PEM patients exhibit normal or increased serum levels of IgA, IgG and IgM, while there was a progressive decrease of albumin, transferrin, and cholinesterase levels, but not of lysozyme according to the severity of PEM (Table 3). Correlation studies show some significant correlations between the level of albumin and those of C3 ($P < 0·001$) and C9 ($P < 0·05$), but not with factor B nor C5. Similar correlations are observed between cholinesterase level and those of C3 and C9.

DISCUSSION

The present study confirms the occurrence of major changes in the activity of the complement system in malnourished children. The striking decrease of CH50 observed in the various forms of kwashiorkor is probably dependent on the very low levels of C3 associated with PEM, considering the good correlation between C3 levels and CH50. The relative influence of a decreased C3 synthesis and an increased catabolism of C3 in the modification of C3 levels in PEM has to be considered.

The significant correlation between the plasma levels of albumin or cholinesterase and those of C3 suggest a major role of a decreased protein synthesis in the liver. This hypothesis is supported by the concomitant decrease of C9 since both C3 and C9 seem to be mainly synthesized in liver cells (Ruddy, Sigli & Austen, 1972). It is likely, however, that an increased catabolism of C3 is also involved in the decrease of C3 levels since an increase of the plasma level of C3d, one breakdown product of C3, is associated with the decrease of the absolute plasma concentration of C3 in all types of PEM. This suggests that an activation of the complement system also occurs during PEM which may further decrease its residual activity. The possible mechanism of such an activation process should be questioned. The fact that normal levels of C4 are observed in PEM, while decreased levels of factor B appear to be correlated with C3 levels but not with albumin or cholinesterase levels, tends to indicate that an activation of the alternative complement pathway may be responsible for an increased complement consumption. This activation may be triggered directly by infectious agents or by immune complexes, but one cannot exclude the possibility that PEM could be associated with a decreased activity of factors modulating the activation of the pathway, such as C3b Inactivator of β1H.

The significance of the alteration of the complement system and particularly of the decrease of factor B levels during PEM should be considered in relation to the high incidence of a variety of infections in this condition. Severe complement deficiencies such as C3 deficiency, and relative deficiencies of the alternative complement pathway have been shown to be associated also with a decreased resistance against infections. Therefore, considering the normal levels of antibodies observed in malnourished children, a decreased efficiency of a potent effector mechanism such, as the complement system, may be involved in

the decreased resistance to infection in those children. In addition, it should be noted that the CH50 level seems to reflect the degree of malnutrition and may be used as a good immunological indicator of PEM since it is not decreased in infected but well-nourished children, unlike the serum levels of albumin or cholinesterase.

We are much indebted to Prof. Essoh Nomel for making case material available to us, and wish to thank Mrs C. G. Meyer and K. Schmocker for excellent technical assistance, as well as Mrs. C. Meylan for her help in preparing the manuscript.

This work of the Nestlé Foundation was also supported by WHO and the Swiss National Foundation (grant no. 371771)

REFERENCES

ANTENER, I., VERWILGHEN, A.M., VAN GEERT, C. & MAURON, J. (1977) Etude biochimique de la nutrition. I. Situation avant traitement. *Bull. Schweiz. Ges. Klin. Chemie*, **2**, 17.

BROWN, R.E. & KATZ, M. (1966) Failure of antibody production to yellow fever vaccine in children with kwashiorkor. *Trop. Geogr. Med.* **18**, 125.

CHANDRA, R.K. (1975) Serum complement and immunoconglutinin in malnutrition. *Arch. Dis. Childhd.* **50**, 3, 225.

DOUGLAS, S.D. & SCHOPFER, K. (1974) Phagocyte function in protein-calorie malnutrition. *Clin. exp. Immunol.* **17**, 121.

HADDEN, D.R. (1967) Glucose, free fatty acid, and insulin interrelations in kwashiorkor and marasmus. *Lancet*, **ii**, 589.

MCLAREN, D.S., PELLETT, P.L. & READ, W.W.C. (1967) A simple scoring system for classifying the severe forms of protein-calorie malnutrition of early childhood. *Lancet*, **i**, 533.

METCOFF, J., FRENK, S., YOSHIDA, T., TORRES PINEDO, R., KAISER, E. & HANSEN, J.D.L. (1966) Cell composition and metabolism in kwashiorkor (severe protein-calorie malnutrition in children). *Medicine*, **45**, 5, 365.

NEUMANN, C.G., LAWLOR, G.J., STIEHM, E.R., SWENDSEID, M.E., NEWTON, C., HERBERT, J., AMMANN, A.J. & JACOB, M. (1975) Immunologic responses in malnourished children. *Amer. J. Clin. Nutr.* **28**, 89.

NYDEGGER, U.E., ACKERMANN, L.M., LAMBERT, P.H. & MIESCHER, P.A. (1972) A simple automated method for complement estimation in a continuous flow system. *J. Immunol.* **109**, 4, 909.

OLUSI, S.O., MCFARLANE, H., ADE-SERRANO, M., OSUNKOYA, B.O. & ADESINA, H. (1976) Complement components in children with protein-calorie malnutrition. *Trop. Geogr. Med.* **28**, 4, 323.

PERRIN, L.H., LAMBERT, P.H. & MIESCHER, P.A. (1975) Complement breakdown products in plasma from patients with systemic lupus erythematosus and patients with membranoproliferative or other glomerulonephritis. *J. clin. Invest.* **56**, 165.

PHILLIPS, I. & WHARTON, B. (1968) Acute bacterial infection in kwashiorkor and marasmus. *Brit. Med. J.* **i**, 407.

RUDDY, S., GIGLI, I. & AUSTEN, K.F. (1972) The complement system of man. *New Engl. J. Med.* **11**, 545.

SCHOPFER, K. & DOUGLAS, S.D. (1976a) Neutrophil function in children with kwashiorkor. *J. Lab. clin. Med.* **88**, 3, 450.

SCHOPFER, K. & DOUGLAS, S.D. (1976b) *In vitro* studies of lymphocytes from children with kwashiorkor. *Clin. Immunol. Immunopath.* **5**, 21.

SCRIMSHAW, N.S., TAYLOR, C.E. & GORDON, J.E. (1968) Interactions of nutrition and infection. *WHO Monograph Series*, no. 57.

SIRISINHA, S., SUSKIND, R., EDELMAN, R., CHARUPATANA, C. & OLSON, R.E. (1973) Complement and C3-proactivator levels in children with protein-calorie malnutrition and effect of dietary treatment. *Lancet*, **i**, 1016.

SMYTHE, P.M., SCHONLAND, M., BRERETON-STILES, G.G., COOVADIA, H.M., GRACE, H.J., LOENING, W.E.K., MAFOYANE, A., PARENT, M.A. & VOS, G.H. (1971) Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. *Lancet*, **ii**, 939.

SUSKIND, R., EDELMAN, R., KULAPONGS, P., PARIYANONDA, A. & SIRISINHA, S. (1972) Complement activity in children with protein-calorie malnutrition. *Amer. J. Clin. Nutr.* **25**, 166.

WATERLOW, J.C. & STEPHEN, J.M.L. (1969) Enzymes and the assessment of protein nutrition. *Proc. Nutr. Soc.* **28**, 234.

WHITEHEAD, R.G., COWARD, W.A. & LUNN, P.G. (1973) Serum-albumin concentration and the onset of kwashiorkor. *Lancet*, **i**, 63.