Journal Pediatrics 105:434-436, 1984.

Impairment of hemolytic complement activation by both classical and alternative pathways in serum from patients with kwashiorkor

Gerald T. Keusch, M.D., Benjamin Torun, M.D., Ph.D., Richard B. Johnston, Jr., M.D., and Juan J. Urrutia, M.D.
Boston, Massachusetts, Guatemala City, Guatemala, and Denver, Colorado

MANY DIVERSE DEFECTS in host defense mechanisms have been described in children with severe protein-energy malnutrition.[1-3] A consistent finding has been a reduction in classical pathway hemolytic complement activity,[1,2] diminished concentration of all classical pathway components except C4,[4] and evidence of in vivo complement activation (including the demonstration of C3 breakdown products and immunoconglutinin, an antibody to activated C423 complexes, in serum.[5] Much less has been reported about alternative pathway activity in PEM,[6,7] although this mechanism may be of considerable importance in defense against gram-negative bacteria, a common cause of terminal sepsis in PEM.[8]

We assessed both pathways of complement activation and serum opsonic activity in patients with acute PEM and observed changes with nutritional rehabilitation.

METHODS

Seven boys, aged 19 to 36 months, with acute kwashiorkor-type PEM, were referred by cooperating local hospitals. Parental consent for all studies was obtained, and the children were admitted to the clinical research ward of the Institute of Nutrition of Central America and Panama. PEM was carefully defined by anthropometric (weight/height index, arm circumference, and skinfold thickness) and biochemical criteria (creatinine height index, total serum protein). All seven children were severely malnourished. All improved during 28 days of optimal dietary therapy.

Five milliliters blood was obtained on admission and on days 3, 7, 14, and 28. Serum was removed and kept frozen at −70° C until thawed for assay of complement and opsonins. Complement activity was measured by a kinetic hemolytic assay for classical[9] and alternative pathways.[10] Data are reported as the ratio of the half-lysis time (Lt ½) of patient/control serum × 100. Opsonic activity was measured as the serum-dependent uptake of radioactive bacteria by normal neutrophils at limiting concentrations of serum.[11] Opsonic activity is reported as a percent of a standard serum used in each assay as reported.

PEM	Protein-energy malnutrition

RESULTS

Nutritional status indicators improved during the study (e.g., admission and day 28 mean values: weight/height deficit 25.5% and 12.8%, respectively; total serum protein concentration 5.0 and 7.0 gm/dl), with duration of catch-up growth averaging 22.7 days and mean weight gain of 8.2 gm/kg/day. During hospitalization three patients had urinary tract infections, two had varicella, one developed hepatitis, one had shigellosis, and one had bacteremia with a non-group A β-hemolytic streptococcus; one or more intestinal parasites were found in four children.

Prolongation of Lt ½ >50% control was present in five patients each for the classical and alternative pathways and for both pathways in four patients (Table I). Opsonic activity was <50% of standard for *Staphylococcus aureus* in one patient, for *Esherichia coli* 286 in two, and for *E. coli* ON-2 in three. Although there was no direct correlation between opsonic activity and Lt½, in four of five patients with decreased opsonic activity there was a significant prolongation of Lt½, in contrast to one of nine patients with higher opsonic activity.

There was relatively little change in complement and

From the Division of Geographic Medicine, Tufts-New England Medical Center; the Institute of Nutrition of Central America and Panama; and the Department of Pediatrics, National Jewish Hospital and Research Center.

Supported by grants HD 11844 from The National Institute of Child Health and Human Development and AI 14148 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and by a grant in Geographic Medicine from the Rockefeller Foundation.

Submitted for publication Dec. 5, 1983; accepted March 10, 1984.

Reprint requests: Gerald T. Keusch, M.D., Division of Geographic Medicine, Department of Medicine, Tufts-New England Medical Center, 136 Harrison Ave., Boston, MA 02111.

Table I. Hemolytic complement and opsonic activity at admission

Patient	Complement* (Lt½ % control)		Opsonins (% standard serum)		
	Classical	Alternative	S aureus	E coli 286	E coli ON-2
1	140	140	63	124	31
2	570	270	65	118	122
3	160	140	81	65	92
4	170	160	53	96	65
5	580	350	39	23	19
6	450	370	65	38	12
7	130	210	82	149	119

*In the classical pathway, no Lt½ values in 22 normal sera exceeded 149% of the value obtained with standard normal serum used in these experiments In the alternative pathway, no Lt½ values for 30 normal sera exceeded 132% of the value obtained with the standard normal serum used in these experiments

Table II. Serial complement and opsonin assays in seven children with acute protein-energy malnutrition

Hospital day	Complement activity (Lt½, % control)		Opsonin activity (% standard serum)		
	Classical	Alternative	S aureus	E coli 286	E coli ON-2
Admission	313 ± 38	235 ± 36	65 ± 18	81 ± 15	65 ± 18
3	253 ± 60	213 ± 41	72 ± 4	81 ± 12	64 ± 16
7	293 ± 59	201 ± 18	80 ± 5	87 ± 13	79 ± 13
14	226 ± 64	144 ± 19	70 ± 4	98 ± 13	98 ± 10
28	96 ± 15	101 ± 15	74 ± 5	101 ± 16	95 ± 10

opsonic activity during the first week, but steady improvement thereafter, with normalization of complement activity and opsonins for the two *E coli* strains by day 28. However, there was little change in opsonic activity for *S aureus* (Table II)

DISCUSSION

Our data confirm a reversible decrease in classical pathway complement as well as opsonic activity in some children with PEM[1,2,4,5,12] and clearly document an accompanying abnormality in the alternative pathway Of note is the lack of significant change during the first week of hospitalization, when malnourished children are at risk of lethal gram-negative sepsis [8] Although there was a good correlation between mean complement and opsonic activities, there was marked disparity in individual sera in which complement was low but opsonins were high This disparity may be explainable by noncomplement opsonins in some patients, however, we did not assay for heat-stable opsonic activity

In PEM, the opsonic defect for the two *E coli* strains resembles previous data in patient populations at risk for or with gram-negative sepsis [13] Based on the susceptibility to gram-negative infections of subjects with genetic and acquired defects in the complement system, such as sickle cell disease or β-thalassemia major,[14] it is likely that decreased complement in PEM contributes to opsonic defects of clinical importance

The complement and opsonic defects were present in some but not all of our patients with PEM, who were all similarly malnourished The explanation for such heterogeneity is uncertain but may be related to variability in the duration of PEM prior to hospitalization, the recent history of infectious disease in our patients, complement or opsonic consumption in some, or secondary abnormalities in specific vitamins or minerals If the last mentioned is true, rapid correction of these deficiencies might improve host defense in the critical first week of hospitalization Lacking such information, we are proceeding to a trial of opsonic replacement therapy

REFERENCES

1 Keusch GT Nutrition as a determinant of host response to infection and the metabolic sequellae of infectious diseases Semin Infect Dis 2.265, 1979
2 Watson RR, McMurray DN The effects of malnutrition on secretory and cellular immune processes CRC Crit Rev Food Sci Nutr 12.113, 1979
3 Keusch GT, Wilson CS, Waksal SD Nutrition, host defenses, and the lymphoid system *In* Gallin JI, Fauci AS, editors Advances in host defense mechanisms, vol 2, New York, 1983, Raven Press, pp 275-359
4 Sirisinha S, Suskind R, Edelman R, Charupatana C, Olsen RE Complement and C3 proactivator levels in children with protein-calorie malnutrition and the effect of dietary treatment Lancet 1 1016, 1973
5 Chandra RK Serum complement and immunoconglutinin in malnutrition Arch Dis Child 50 225, 1975
6 Sakamoto M The sequence of recovery of the complement

systems and phytohemagglutinin skin reactivity in malnutrition Nutr Res 2.137, 1983

7. Gelfand J, Torun B, Keusch GT, Cruz JR Profound depression of the alternative pathway in protein-energy malnutrition A reversible defect of host defenses (abstract) Proceedings of the 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, DC, 1982, p 157
8 Parent MA, Loening WEK, Coovadia HM, Smythe PM Pattern of biochemical and immune recovery in protein-calorie malnutrition S Afr Med J 48 1375, 1974
9 Johnston RB Jr, Altenberger KM, Atkinson AW Jr, Curry RH Complement in the newborn infant Pediatrics 64 696, 1979
10 Polhill RB Jr, Pruitt KM, Johnston RB Jr Kinetic assessment of alternative complement pathway activity in a hemolytic system I Experimental and mathematical analysis J Immunol 121:363, 1978
11 Keusch GT, Ambinder EP, Kovacs I, Goldberg JD, Phillips D, Holland JT. Role of opsonins in clinical response to granulocyte transfusion in granulocytopenic patients. Am J Med 73:552, 1982
12 Keusch GT, Urrutia JJ, Guerrero O, Castaneda G, Smith H Jr Serum opsonic activity activity in acute protein-energy malnutrition Bull WHO 59 923, 1981
13 Weinstein RJ, Young LS Neutrophil function in gram-negative rod bacteremia The interaction between phagocytic cells, infecting organisms, and humoral factors J Clin Invest 58 190, 1976
14 Corry JM, Marshall WC, Guthrie LA, Peerless AG, Johnston RB Jr Deficient activity of the alternative pathway of complement in β-thalassemia major Am J Dis Child 135.529, 1981