Some Observations on the Properties of Precipitating Antibody to *Staphylococcus Aureus* in Children with Kwashiorkor

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Summary

Using three relatively sensitive immunochemical assay procedures, we were unable to detect either precipitating or hemagglutinating antibodies to food antigens in sera from normal and kwashiorkor Nigerian children.

However, antibody to *Staphylococcus aureus*, a common pathogenic bacteria in the tropics, could be detected in the sera of 28 of 30 control children but in only 7 of 30 kwashiorkor cases, suggesting that there is an impairment to specific antibody production in children with malnutrition.

Comparative biologic and physicochemical studies carried out on precipitating antibodies to *S. aureus* isolated from normal and kwashiorkor subjects showed that the antibody from malnourished individuals fixed complement less readily and was deficient in some essential amino acids.

These defects might in part explain why children with protein-energy malnutrition are more susceptible to infection in spite of the fact that they have normal serum immunoglobulin concentrations.

Speculation

Protein-energy malnutrition may cause some qualitative alterations in the molecule of antibody produced against specific antigens.

Although it has been established that humoral antibody production to some antigens may be depressed in malnutrition (4, 6, 15, 16), there is not sufficient information on the properties of antibodies in children with protein energy malnutrition (PEM). This study is therefore concerned with some aspects of the biologic and physicochemical properties of antibody to *S. aureus* in children with kwashiorkor.

MATERIALS AND METHODS

PATIENTS

Studies were conducted on 30 children suffering from kwashiorkor between 12 and 72 months old. The children were grossly underweight, the extent of weight deficit when compared to Harvard standards was 40-60%. All of the children had varying degrees of edema, mental changes, hypoalbuminemia, hypoprealbuminemia, and hypotransferrinemia. Thirty apparently normal children of a similar age group and belonging to the same socioeconomic status were studied as controls. Sera obtained from these children were stored at -20° until ready for antibody testing.

The first part of the study involved the estimations of the serum concentrations of the immunoglobulins in control children and children with kwashiorkor. This was done by the automated immunonephelometric assay technique, according to our previous publication (12).

The second part involved the detection of antibodies against a variety of food, bacterial, and parasitic antigens in the sera of normal and kwashiorkor cases.

TEST ANTIGENS

Saline extracts of cassava and corn flour were prepared by us. These extracts were considered to possess antigenic properties because of their ability to stimulate antibody production when immunized into rabbits or rats. Cassava and corn flour were used because they form part of the staple diet in Nigeria.

Other test antigens, human serum albumin, bovine serum albumin, lactalbumin, casein, gluten, egg white, whole egg, *S. aureus*, *Streptococcus viridans*, *Haemophilus influenzae*, *Salmonella typhi*, *Entamoeba histolytica*, and *Ascaris lumbricoides*, were obtained from Bencard and Wellcome Laboratories, United Kingdom.

ANTIBODY TESTS

Sera obtained from children with kwashiorkor and controls were tested against the above antigens by the immunodiffusion method (13), counterimmunoelectrophoresis (7), and by the tanned sheep erythrocyte hemagglutination method of Boyden (1).

Double Immunodiffusion. Purified Noble agar was prepared as a 1.5% solution in phosphate-buffered saline (pH 7.0, ionic strength 0.05 M), with sodium azide as preservative.

Counterimmunoelectrophoresis. The method for counterimmunoelectrophoresis described by Kohn (7) was modified for use in the detection of antibodies to the various antigens. This method, with its greater sensitivity than ordinary double immunodiffusion was particularly suitable to the detection of small quantities of antibodies in the serum. The serum specimens were used as the antibody-containing solutions and were placed in wells toward the anode side. The various antigens were placed in wells on the cathode side of the agar gel. Positive specimens were those which gave clearly visible, well demarcated precipitin lines completely separated from both the cathode and the anode wells. Barbitone buffer (pH 8.6, ionic strength 0.05 M, and a current of 5 mA/8.1 × 8.1 cm agar plate were routinely used.

Hemagglutination Method. This was carried out according to the method of Boyden (1).

Since antibody against *S. aureus* could be detected in both control and some kwashiorkor sera, the third part of the study involved the isolation, purification, and determination of the biologic and physicochemical properties of antibodies from normal children and children with kwashiorkor. For this purpose all positive kwashiorkor sera were separately pooled.

Isolation of Precipitating Antibodies. Precipitating antibodies to *S. aureus* from pooled normal and pooled kwashiorkor sera were separated according to the method described by Margni and Binaghi (9) using *S. aureus* as antigen. In each case, the solution

containing the antibody was dialysed extensively against phosphate buffer (0.01 M, pH 7.6) and passed through a DEAE-cellulose column equilibrated with the same buffer. Using this procedure, only one protein peak was obtained from each effluent.

DETERMINATION OF IMMUNOGLOBULIN CLASS OF ANTIBODY

The immunoglobulin class of each isolated precipitating antibody was established as follows: Part of the solution containing the antibody was treated with 0.1 M 2-mercaptoethanol for 2 hr at room temperature and then dialysed according to the method of Dixon *et al.* (3). Antibody inactivated by this treatment was considered to be of the 19S immunoglobulin class, while that which resisted this treatment was considered to be of the 7S, IgG, or IgA type.

IMMUNOELECTROPHORESIS OF ANTIBODY

Immunoelectrophoretic analysis was carried out according to the method of Graber and Burtin (5). Noble agar (1.5%) in barbitone buffer (pH 8.6, ionic strength 0.05 M) was used.

COMPLEMENT FIXATION

This was ascertained by reading 100% of lysis using approximately 100 µg/ml of each antibody and different concentrations of *S. aureus* antigen according to the method of Lelchuk *et al.* (8).

ANALYSIS OF FAB AND FC FRAGMENTS

After hydrolysis with papain according to the method of Porter (14), the Fab and Fc fragments from both normal and kwashiorkor antibodies were examined by immunoelectrophoresis and by double immunodiffusion in agar gel using monospecific antisera to human Fab and Fc fragments obtained from Wellcome Laboratories.

HEAVY AND LIGHT CHAINS

After treating each antibody with 0.1 M 2-mercaptoethanol at 60° for 30 min, its heavy and light chains were examined by the polyacrylamide disc gel electrophoretic technique of Neville (11). The electrophoresis was performed with a Shandon apparatus. The gels were stained in 0.2% Coomassie Blue and destained with a solution containing 50% methanol, 7% acetic acid, and 43%

water. The upper reservoir buffer contained 0.04 M boric acid and 0.041 M Tris with sodium dodecyl sulfate (pH 8.64). The staking gel buffer was 0.0267 M H₂SO₄ and 0.0541 M Tris (pH 6.1). The separating gel and lower reservoir buffer contained 0.0308 M HCl and 0.4244 M Tris (pH 9.18). The separating gel was polymerized with 10% acrylamide and 0.026% *N,N'*-methylenebisacrylamide with 0.05% ammonium sulfate and 0.0005% *N,N,N',N'*-tetramethylethylenediamine as a catalyst.

AMINO ACID ANALYSIS

This was carried out by the automation (17) of the classical procedure of Moore and Stein (10). Briefly, the method involved the chromatographic separation of acid (6 N HCl) hydrolysates of each antibody on columns of ion-exchange resins. The effluents were monitored by reacting with ninhydrin in the presence of a reducing agent. The amino acids were eluted from the ion-exchange resins successively, their concentration being a function of the peak area from which the molar ratios of the constituent amino acids were calculated.

RESULTS

IMMUNOGLOBULIN CONCENTRATIONS

The mean serum IgG and IgA concentrations in control children were 17 ± 3.6 g/liter and 1.5 ± 0.2 g/liter, respectively. The corresponding values in children with kwashiorkor were 18 ± 4.2 g/liter and 1.3 ± 0.25 g/liter, respectively. Thus, there were no significant differences ($P > 0.1$) in serum IgG and IgA concentrations between normal and malnourished children.

PRECIPITATING ANTIBODIES

Using the above highly sensitive immunochemical assay methods, no precipitating antibodies to antigenic extracts of cassava, corn flour, or milk products could be detected in the serum of either the control or the children with kwashiorkor from Nigeria. By the double immunodiffusion in the agar gel method, antibody to *S. aureus* could be detected in the sera from 19 of 24 normal children (Fig. 1A), but in only 3 of 24 children with kwashiorkor (Fig. 1B). Using the method of counterimmunoelectrophoresis, antibody to *S. aureus* could be detected in 27 of 30 control sera, but in only 6 of 30 kwashiorkor cases. The age ranges of kwashi-

Fig. 1. A. Double immunodiffusion plate. The central wells contained *S. aureus* antigen. The peripheral wells contained sera from control children. This shows that most of the control children had antibody to this antigen in their serum. B. The central wells contained *S. aureus* antigen while the peripheral wells contained sera from children with kwashiorkor. This shows that most of the malnourished children have no antibody to this antigen.