Serum opsonic activity in acute protein-energy malnutrition

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Phagocytic host defence mechanisms require both normally functioning cells and humoral factors. For example, activated complement components and/or specific immunoglobulin are essential for effective ingestion and killing of bacteria by neutrophils, and complement is especially important early in infection, before specific antibody has been produced. Abnormalities of serum complement have previously been reported in malnutrition, and the present study investigated the levels of serum opsonins in children with protein-energy malnutrition (PEM).

Opsonic activity for Escherichia coli and Staphylococcus aureus was depressed in acute PEM patients, but recovered to higher levels with treatment. This depression was detected only when low concentrations of serum (10 – 20 ml/litre) were used. Marked and persistent opsonin deficiencies were associated with poor clinical response. Reduced opsonic activity may adversely affect host defence mechanisms and contribute to morbidity and mortality from pyogenic infections in PEM. Replacement therapy with fresh or fresh frozen plasma might restore opsonic activity in these patients and reduce the risk of septicaemia and its attendant high mortality.

Protein-energy malnutrition (PEM) is a serious public health problem in many parts of the world (1), and whatever its cause, is always closely linked with infectious disease. Malnourished individuals appear to be more susceptible to infection, and to suffer more severe illness than do persons who are well nourished. In turn, infection itself results in deterioration of the patient’s nutritional status (2), thus precipitating an adverse cyclic interaction of malnutrition and infection which has been labelled synergistic by Scrimshaw (3).

Recent investigations have probed the mechanisms by which malnutrition alters the host response in infection, and have reported significant and profound changes in cell-mediated immune reactions and in complement levels (4, 5). In contrast, observed abnormalities of phagocytic cell function and intracellular bactericidal activity have ranged from profound to mild (6). Generally, impairment of polymorphonuclear (PMN) leukocyte bactericidal activity in malnutrition does not appear to play a major role in impaired host defences in vivo (6 – 8). Nevertheless, pyogenic bacterial infections, which are usually dealt with by leukocyte phagocytic mechanisms, are common in these patients (3, 9).

Effective phagocytosis requires both normally functioning PMN leukocytes and complement-derived humoral factors that mediate the inflammatory response, chemotaxis (PMN leukocyte migration), and opsonization (facilitation of bacterial uptake by PMN leukocytes and mononuclear phagocytes). Complement-dependent opsonization may be crucial to the host’s response early in infection, before specific antibody has been formed (10).

In view of the documented alterations in complement activity in PEM (5, 11, 12), it is surprising that most investigators report normal serum opsonic activity in these patients (13 – 15). We have previously shown that titration of serum of PEM patients reveals opsonic defects at low concentrations of serum, which are not observed when higher concentrations are used (7). These assays measured serum-dependent intracellular killing of *Escherichia coli* or *Staphylococcus aureus* by normal
The study group comprised eight Guatemalan children, ranging in age from 1 year 4 months to 9 years 6 months (Table 1), who had been admitted to the Department of Pediatrics of the Roosevelt Hospital in Guatemala City with severe oedematous (kwashiorkor type) PEM and diarrhoea. The signs and symptoms had been present or developing for 1–12 weeks before admission to hospital. PEM was diagnosed and treated by the house physicians and attending staff of the department, who used nutritional rehabilitation and antibiotics when indicated. There were no deaths in this series. The anthropometric status of the patients did not improve during their stay in hospital (Table 2), probably because of the short period of hospitalization and the compensatory loss of oedema.

The control group comprised 14 children, aged between 25 and 47 months, who had been admitted to a study ward in the Clinical Research Center, INCAP with severe oedematous PEM, and had undergone nutritional rehabilitation for three months. Anthropometric data for these children at the time of study are also shown in Table 2 and indi-
cate their satisfactory nutritional status. These data serve as baseline values for the Guatemalan paediatric population under study.

With permission, umbilical cord sera from 8 full-term, adequate weight newborns and sera from 8 healthy adult volunteers were obtained in New York to determine the pattern of serum opsonic activity for the three test organisms employed in these studies.

**Experimental techniques**

With the approval of the Human Experimentation Committees of both the Roosevelt Hospital and the Institute of Nutrition of Central America and Panama, a 4-ml sample of blood was obtained from 7 children in the study group on admission. A sample was taken from 4 patients at 1 week, and from 4 others just prior to discharge from the hospital. A single blood sample was taken from members of the control group at the time of discharge.

The blood was allowed to clot, the serum was separated rapidly, and maintained at −70 °C until assayed for opsonic activity. Opsonins were determined by a modification of the method of Root et al. (16) as described by Keusch et al. (17). Normal human peripheral blood PMN leukocytes, obtained by dextran sedimentation of heparinized venous blood, were suspended in Hanks' balanced salt solution (HBSS), pH 7.2, at a concentration of 10^7 cells/ml. *S. aureus* 876, *E. coli* 286 (17), and *E. coli* ON-2 (18) were grown overnight in 10 ml of trypticase soy broth containing 0.925 MBq (25 μCi) of [14C]-amino acid mix. The radioactive bacteria were heat-killed at 100 °C for 30 min, washed three times in HBSS, and resuspended in 10 ml of HBSS.

The assay system consisted of 0.5 ml of the PMN leukocyte suspension and 0.4 ml of serum diluted in HBSS (10–80 ml of serum per litre) in a 10 x 75-mm polypropylene tube. An aliquot of 0.1 ml of the bacterial suspension was added to start the assay. The final volume of 1 ml thus contained 5 x 10^6 PMN leukocytes and approximately 2.5 x 10^9 bacteria. The tubes were tightly stoppered and incubated with end-over-end rotation at 37 °C for 20 min. The experiment was terminated by adding 4 ml of ice-cold stop solution (0.2 mol/litre NaF in Ca++/Mg++-free HBSS containing 100 ml of heat-inactivated fetal calf serum per litre) and plunging the tube into ice. For the baseline (zero time) sample, cells and serum were pre-chilled in iced tubes to which [14C]-bacteria and chilled stop solution were added in rapid succession.

All samples were then centrifuged at 40 g for 5 min and washed three times in chilled stop solution. Evidence that this procedure separates ingested from adherent bacteria will be presented elsewhere. Washed pellets were solubilized in 0.8 ml of NCS solubilizer, placed in a toluene-based scintillation mixture, and counted in a Beckman LS-200 liquid scintillation spectrometer. The radioactivity of the bacterial inoculum was determined by solubilizing 0.1 ml of the bacterial suspension in NCS and scintillation mixture. These data were quench-corrected using an external standard, and the results were calculated as percentage phagocytosis:

\[
\text{sample radioactivity} - \text{zero time radioactivity} \times 100
\]

\[
\text{inoculum radioactivity}
\]

In order to make comparisons between patients, the percentage phagocytosis was standardized against a standard serum, maintained in small volumes at −70°C, and used in each assay. Results are reported as percentage of the standard.

The data were analysed statistically using a least-squares technique, with analysis of variance, linear contrast, t test, and F test, to compare opsonin activity on the three sampling occasions.

**RESULTS**

*Normal sera*

Normal adult and umbilical cord sera from healthy full-term newborns opsonized the three test bacteria differently (Table 3). In comparison with the adult

Table 3. Opsonic activity of normal adult and umbilical cord sera

<table>
<thead>
<tr>
<th>Organism</th>
<th>Serum concentration (ml/litre)</th>
<th>Opsonic activity (percentage phagocytosis)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> 876</td>
<td>80</td>
<td>47.5 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>37.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32.2 ± 4.4</td>
</tr>
<tr>
<td><em>E. coli</em> 286</td>
<td>80</td>
<td>31.8 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>36.6 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>25.0 ± 2.4</td>
</tr>
<tr>
<td><em>E. coli</em> ON-2</td>
<td>80</td>
<td>40.7 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>31.6 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4.5 ± 1.8</td>
</tr>
</tbody>
</table>

* Mean ± SE.

b Significantly less than adult value (P < 0.01).

* New England Nuclear, Boston, MA, USA.
sera, the cord samples were deficient in opsonins for E. coli, particularly the ON-2 strain. Differential chelation experiments, performed by the method of Bjorksten et al. (18), demonstrated that serum-dependent phagocytosis of the three bacteria opsonized in 80 ml/litre normal adult serum was inhibited by 10 mmol/litre EDTA, whereas 10 mmol/litre Mg-EDTA, which preferentially chelates Ca²⁺ and not Mg²⁺, inhibited opsonization of S. aureus and E. coli 286, but not of E. coli ON-2 (data not shown). Because of the marked deficiency of factor B of the alternative pathway in newborn sera (19) and its requirement for Mg²⁺ but not Ca²⁺ chelation (20), these data suggest that both S. aureus and E. coli 286 are opsonized in this system, at least in part, via the classical activating pathway of complement. In contrast, E. coli ON-2 can be opsonized via the alternative activating pathway of complement, confirming the results of Bjorksten et al. (18), although this may be less efficient than opsonization via the classical pathway or both pathways simultaneously (21). However, the concentration of opsonizing serum may also influence the pathway involved. Opsonic activity for both E. coli strains was destroyed by heating sera at 56 °C for 30 min. Most, but not all of the opsonin for S. aureus was heat labile (data not shown), indicating the role of heat stable factors in normal sera, presumably immunoglobulin, in the opsonization of this organism.

**Malnourished children**

Results obtained in the acutely ill and recovered PEM children are shown in Table 4. No opsonic deficiency was seen in the acutely ill patients on admission at the higher serum concentration tested, but at the lowest concentrations, opsonins for S. aureus and E. coli ON-2 were clearly depressed (P < 0.05).

There was a significant drop in mean serum opsonic activity against all three organisms during the first week of hospitalization (S. aureus, t = 4.06, P < 0.001; E. coli 286, t = 3.01, P < 0.005; E. coli ON-2, t = 5.12, P < 0.001). This drop was most dramatic in patients no. 3 and 8, who had persistent illness throughout hospitalization. Opsonic activity recovered in 3 of 4 patients studied 2–5 weeks after admission, rising to a level equal to or greater than that observed in the nutritionally rehabilitated patient group. The single exception was patient 7, who experienced a continuous febrile illness, persistent cough, and progressive anaemia to a haemoglobin level of 45 g/litre and haematocrit of 0.13. He was treated with antibiotics during this entire period and failed to show any improvement in serum

### Table 4. Opsonic activity of sera from children admitted to hospital with acute malnutrition and control group

<table>
<thead>
<tr>
<th>Organism</th>
<th>Serum concentration (ml/litre)</th>
<th>Acute PEM sera</th>
<th>Controls* (14 sera)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>On admission (7 sera)</td>
<td>1 week (4 sera)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>80</td>
<td>75.4 ± 6.6</td>
<td>60.2 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>45.5 ± 7.6</td>
<td>33.6 ± 7.0</td>
</tr>
<tr>
<td>E. coli 286</td>
<td>80</td>
<td>89.7 ± 6.2</td>
<td>75.0 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86.3 ± 8.8</td>
<td>62.2 ± 21.5</td>
</tr>
<tr>
<td>E. coli ON-2</td>
<td>80</td>
<td>92.0 ± 8.2</td>
<td>68.8 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>44.6 ± 16.3</td>
<td>21.9 ± 11.2</td>
</tr>
</tbody>
</table>

*a Mean ± SE.*

*b Sera from children after uncomplicated clinical recovery from PEM.*

### Table 5. Serum opsonic activity in serum from a patient with poor clinical response to therapy for acute protein-energy malnutrition

<table>
<thead>
<tr>
<th>Serum concentration (ml/litre)</th>
<th>Opsonic activity (percentage of standard)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>On admission</td>
<td>On discharge</td>
</tr>
<tr>
<td>80</td>
<td>67</td>
</tr>
<tr>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
</tr>
</tbody>
</table>

*a ND = Not done.*
opsonic activity (Table 5) between admission and discharge, against advice, on day 21.

DISCUSSION

These studies have demonstrated a quantitative defect in serum opsonins in patients with acute protein-energy malnutrition, which recovers during nutritional rehabilitation. Whereas immunoglobulin levels are normal or elevated in PEM, complement activity is low initially, increasing to normal after a few weeks of nutritional rehabilitation (4, 5, 12). Although we did not specifically separate heat-labile from heat-stable opsonic factors, the previously reported time course of recovery of complement is similar to that seen in the present study. Decreased levels of opsonins have previously been reported in experimental malnutrition in animals (18, 22) and in malnourished children (8, 23), but other investigations of malnourished human subjects have not noted any defect (14-16). However, these latter studies have employed high concentrations of serum (80-100 ml/litre) which may not be rate-limiting in the opsonin assay, as demonstrated for E. coli 286 and S. aureus 876 in the present study (Table 3). It is possible that sufficient opsonin for maximum activity circulates within the intravascular compartment in the malnourished child, as this represents the equivalent of 100% serum in the in vitro assay. This would explain the considerable in vitro bactericidal activity of phagocytic cells in whole blood of children with PEM (8).

It is difficult to assess the clinical significance of diminished opsonin activity at low concentrations of serum, as demonstrated in this study. In assessing the in vivo host response to infection, tissue opsonin levels are clearly more relevant than serum opsonins. A few studies have shown that extravascular complement activity in suction blister fluids, lymph, and inflammatory exudates is lower than the level of activity in simultaneous serum samples (24-29). Such data are not at present available for children with PEM. On the other hand, this study, and another in which clinical response to granulocyte transfusion in infected granulocytopenic patients was found to be related to the recipient's serum opsonic activity (30), indicate a relationship between serum opsonin levels and clinical response to infection.

The present demonstration of decreased opsonic activity in PEM is consistent with decreased complement activation by both pathways in these patients. Opsonin deficiency could increase susceptibility and impair host response to infection (30). Certainly, children with acute PEM are often infected when admitted to hospital and sepsis is often the immediate cause of death.

In some human immunodeficiencies, replacement of the missing factor has become possible in recent years, for example by leukocyte transfusion in granulocytopenic patients (32). If it were possible to replace opsonins in PEM children on admission, an immediate and significant improvement in host defence responses might result. For example, it is already known that plasma infusion in a patient with type I hypercatabolism of C3 (C3b-inactivator deficiency) will promptly restore complement levels and the biological functions of C3 (33). We suggest that further work is needed to investigate the specific nature and importance of the opsonin defect in acute PEM and to consider the therapeutic efficacy of opsonin replacement with either complement or specific immunoglobulin or both.

ACKNOWLEDGEMENTS

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RÉSUMÉ

ACTIVITÉ OPSONIQUE DU SÉRUM DANS LA MALNUTRITION PROTÉINO-ÉNERGÉTIQUE AIGUÉ

Les défenses normales de l'hôte contre les bactéries pathogènes exigent une réponse intégrée des cellules phagocytagères et des facteurs humoraux qui se trouvent dans le sérum. Par exemple, des facteurs opsoniques du sérum dérivés d'une immunoglobuline spécifique et/ou de produits activés du complément sont nécessaires pour l'ingestion efficace des microorganismes, précédant leur destruction intracellulaire. Les opsonines dérivant du complément peuvent avoir un rôle particulièrement important au début de l'infection, avant que l'immmunoglobuline

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spécifique ne soit produite. Du fait que, chez les enfants souffrant d’une grave malnutrition protéino-énergétique aiguë, on signale un déficit de l’activité complémentaire et une grande fréquence de bactériémies à germes Gram-négatifs, nous avons mesuré l’activité opsonique du sérum chez 8 enfants atteints de cette affection afin de déterminer les relations fonctionnelles possibles avec la réponse de l’hôte à l’infection.

La méthode consistait à mesurer l’ingestion dépendante du sérum de bactéries radiomarquées tuées par la chaleur, par des neutrophiles humains adultes normaux. L’activité opsonique s’est révélée être seulement de 45% du niveau témoin dans le cas de Escherichia coli ON-2 ou de Staphylococcus aureus 876 pour de faibles concentrations de sérum (10 – 20 ml/litre) d’enfants atteints de malnutrition. L’activité opsonique n’était pas supérieure, mais semblait même encore plus basse (22 – 34% de la valeur témoin) lorsqu’elle a été mesurée chez 4 sujets une semaine plus tard, alors que chez 4 malades étudiés 2 à 5 semaines après l’admission, les valeurs étaient semblables à celles des témoins normaux (75 à 135%). Au cours de l’observation ultérieure, l’activité opsonique la plus faible a été notée dans les échantillons de 3 enfants atteints d’infection, de fièvre, ou d’anémie persistantes ou progressives.

Ces études ont mis en évidence l’abaissement de la fonction opsonique pour de faibles concentrations de sérum de malnutrition protéino-énergétique in vitro, mais ce n’était pas le cas à des concentrations supérieures, sans doute en raison du grand excès d’activité opsonique ordinaire présent. Ce déficit se corrige avec le temps au cours de la convalescence et se trouve en corrélation avec l’état clinique. Selon ces résultats, l’opsonine pourrait être un déterminant de la réponse clinique dans la malnutrition protéino-énergétique et le traitement destiné à restaurer les opsonines pourrait améliorer l’évolution chez les sujets gravement atteints. Il convient de poursuivre les études sur l’activité opsonique chez les malades atteints de malnutrition protéino-énergétique, sur la relation quantitative entre cette activité et les taux du complément ainsi que sur l’efficacité clinique du remplacement de l’opsonine.

REFERENCES


