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Inflammatory mediators in children with protein-energy malnutrition

Robert W Sauerwein, Janet A Mulder, Lambertus Mulder, Brett Lowe, Norbert Peslu, Pierre NM Demacker, Jos WM van der Meer, and Kevin Marsh

ABSTRACT

Edema is a typical sign in kwashiorkor, which is present in a subset of patients with protein-energy-malnutrition (PEM). The pathophysiology of this edema is not well established. One of the abnormalities found in kwashiorkor is reduced concentrations of antioxidants, suggesting a compromised capacity to neutralize free radicals, which are known to induce tissue damage. We have studied plasma concentrations of several mediators of the inflammatory cascade. Concentrations of interleukin 6 (IL-6), C-reactive protein, and the soluble receptors of tumor necrosis factor α (sTNFR-p55 and sTNFR-p75) are greater in children with PEM, particularly in those with kwashiorkor, whereas soluble receptors of IL-6 (sIL6R-gp80) and IL-1 receptor antagonist concentrations are not significantly different from those of healthy children. In addition, concentrations of IL-6, sTNFR-p55, and sTNFR-p75 are greater in kwashiorkor patients irrespective of the presence of infection. Antioxidant status, as determined by plasma concentrations of glutathione and vitamin E, is significantly reduced in kwashiorkor patients. These data support the notion that children with edematous malnutrition show increased inflammatory activity that may contribute to edema formation.

KEY WORDS

Protein-energy malnutrition, cytokines, free radicals, antioxidants, infection, children, interleukin 6, C-reactive protein, inflammation

INTRODUCTION

In children with protein-energy malnutrition (PEM), the presence of edema is one of the hallmarks of the subgroup with kwashiorkor. The pathogenesis of edema in PEM is currently unresolved and most likely multifactorial. Hypalbuminemia and electrolyte imbalances have been put forward as possible causes (1). Although low serum albumin is probably a necessary condition, it is certainly not always a sufficient explanation (1).

The “radical theory” advanced by Golden and Ramdath (2) postulate that the imbalance between the production of free radicals and their neutralization by scavengers plays an important role in the development of the kwashiorkor syndrome. These radicals, which are products of the inflammatory response, generate peroxides, particularly in cell membranes. It is hypothesized that unscavenged radicals damage tissues and induce vascular leakage in kwashiorkor (2). Prostaglandin E2 and cysteinyl leukotrienes, which are powerful agents in the inflammatory response, increase in PEM upon in vitro stimulation (3, 4). In addition, the concentrations of several molecules that protect against free radical damage are reduced in PEM, particularly in kwashiorkor. These include glutathione (GSH), vitamin E, zinc, and the selenium-containing enzyme glutathione peroxidase (2). These findings suggest that an uncontrolled inflammatory response contributes to the clinical syndrome of kwashiorkor.

The aim of the present study was to determine the concentrations of pro- and antiinflammatory mediators upstream in the cascade of the inflammatory response in children with PEM and to find out whether there was an association with the clinical presentation of PEM.

PATIENTS AND METHODS

Patients

Children with PEM were recruited prospectively at the District Hospital and the Family Life Centre in Kilifi, Kenya. Inclusion criteria were weight-for-age < 70% and weight-for-height < 80% of National Center for Health Statistics (NCHS) standards (5). Kwashiorkor patients were defined as those with the typical clinical syndrome of edematous malnutrition with hair loss, hair discolorization, or both, and flaky skin. Marasmus patients were defined as children with nonedematous malnutrition. PEM patients with obvious signs of clinical infections, weight < 5 kg, and a hemoglobin concentration < 7 g/L, or who were in a clinical condition that required intravenous treatment with fluids or medication were excluded from the study. As the control group, healthy children from the same community and preferentially the same family were recruited who had weight-for-age and weight-for-height values

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2 Supported by KEMRI, The Welcome Trust (031342), and the Academic Hospital, Nijmegen. RWS received the Merck Sharp & Dohme stipend of the Infectious Disease Society of the Netherlands and Flanders in 1992. KM is a Welcome Trust Senior Fellow in Clinical Sciences (031342).

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Measurements of antiinflammatory variables

Blood was collected with heparin on study entry after immediate centrifugation for 10 min at 1250 × g at room temperature plasma was stored at −20 °C. Interleukin 6 (IL-6) and soluble IL-6 receptor (sIL-6R) (6), IL-1 receptor antagonist (IL-1Ra) (7), soluble tumor necrosis factor receptors (sTNFR)-p55, and sTNFR-p75 (8) were tested as described previously. C-reactive protein (CRP) was measured by turbidimetry (Behringwerke AG, Marburg, Germany). Vitamin E was measured by HPLC (9). Total plasma cholesterol was determined by enzymatic analysis on a Hitachi 747 analyzer with reagents (Behringwerke AG, Marburg, Germany). Vitamin E was measured against the CDC/Abell-Kendall (Bacillus sp. or Staphylococcus epidermidis), or 5) positive urine culture. Possible infection was defined as the presence of one major or two minor criteria; likely infection was defined as the presence of at least one major plus one minor criterion. Severity of edema was classified as mild if swelling was limited to feet or ankles, moderate if both lower and upper extremities were affected, and severe if there was orbital edema as well as edema of lower and upper extremities.

Statistical analysis

Data were analyzed with SPSS statistical software (SPSS Inc, Chicago). Differences in group means were analyzed according to distribution by one-way analysis of variance (ANOVA), t test, or nonparametric test (Mann-Whitney U test). A multifactorial analysis by stepwise-linear regression on the effect of infection, kwashiorkor, or both was performed with IL-6, CRP, sTNFR-p55, and sTNFR-p75 as independent variables and with edema as a dependent variable.

RESULTS

Forty-six children with PEM were studied, 30 of whom had kwashiorkor and 16 had marasmus; 39 healthy children were recruited as control subjects (Table 1). Severe edema was present in 10 (33%) of the kwashiorkor patients whereas edema was of medium severity in 11 (37%) children; edema was mild in 9 children (30%). In PEM patients, midupper arm circumferences (MUACs) were significantly lower than in control subjects but showed no difference between kwashiorkor and marasmus patients. Albumin concentrations were different among all study groups and were lowest in kwashiorkor patients (Table 1).

Plasma concentrations of the antioxidants GSH and vitamin E were significantly lower in the kwashiorkor group than in both the marasmic and the control groups (Figure 1). Marasmus patients only differed from the control subjects in concentrations of vitamin E, whereas plasma GSH concentrations were not different. Plasma cholesterol needs to be taken into consideration for a proper interpretation of the vitamin E status (12). Cholesterol concentrations were also lower in the kwashiorkor group than in the marasmic or control groups (Figure 1). The ratio of vitamin E to cholesterol in these patients was significantly lower (P < 0.003) than in the control group.

Plasma concentrations of IL-6, sTNFR-p55, and sTNFR-p75 as well as the acute phase protein CRP were greater in PEM patients than in the control subjects and greater in kwashiorkor than in marasmus patients (Figure 2). IL-1Ra and sIL-6R concentrations in PEM were not significantly different from the values obtained in the control subjects. Immunoreactive TNF concentrations were < 100 ng/mL in all groups (data not shown).

The presence of infection was likely in five children with kwashiorkor and two children with marasmus and possible in four children with kwashiorkor and four with marasmus. In the control subjects there was a probable infection in four children

TABLE 1
Demographic and laboratory data of study groups

<table>
<thead>
<tr>
<th></th>
<th>Kwashiorkor (n = 15 M, 15 F)</th>
<th>Marasmus (n = 10 M, 6 F)</th>
<th>Control (n = 27 M, 12 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>3.1 ± 1.5 [30]</td>
<td>2.5 ± 1.1 [16]</td>
<td>3.4 ± 1.3 [39]</td>
</tr>
<tr>
<td>Midupper arm circumference (cm)</td>
<td>11.6 ± 1.5 [29]</td>
<td>11.2 ± 1.4 [16]</td>
<td>15.7 ± 0.9 [39]</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>19.2 ± 7.2 [30]</td>
<td>31.0 ± 8.7 [16]</td>
<td>41.0 ± 5.2 [35]</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>8.4 ± 1.7 [28]</td>
<td>8.2 ± 2.6 [13]</td>
<td>11 ± 2.2 [36]</td>
</tr>
<tr>
<td>White blood cells (× 109/L)</td>
<td>11.9 ± 4.5 [27]</td>
<td>10.5 ± 4.8 [13]</td>
<td>10.1 ± 3.8 [36]</td>
</tr>
</tbody>
</table>

* x ± SD; n in brackets.
* b Significantly different from control (t test); a P = 0.02, d P = 0.0001, d P = 0.001.
* c Significantly different from kwashiorkor, P = 0.0001 (t test).
and a possible infection in one child. Kwashiorkor patients without possible or likely infections had significantly higher concentrations of IL-6, sTNFR-p55, and sTNFR-p75 in their circulation than did noninfected patients of the control group (Figure 3). Infected patients in both study groups had higher concentrations of inflammatory mediators than did patients without infection, but these differences were not significant except for sTNFR-p55 in kwashiorkor patients \( P = 0.03 \). In kwashiorkor patients edema was an independent risk factor for increased concentrations of sTNFR-p55 \( P < 0.001 \), sTNFR-p75 \( P = 0.005 \), and IL-6 \( P = 0.05 \) but not for CRP.

**DISCUSSION**

The principle finding of the present study was that circulating concentrations of IL-6, sTNFR-p55, sTNFR-p75, and CRP are increased markedly in patients with kwashiorkor and to a lesser extent in patients with marasmus. The increase in these inflammatory indexes may be explained by concomitant infections, which are frequently found in PEM patients [14]. In fact, infections have been postulated to be a trigger for edema formation in kwashiorkor [2]. Patients with obvious infections were deliberately excluded from the study, but those with positive criteria for infections showed increased concentrations of IL-6, IL-1Ra, sTNFR, and CRP (data not shown). More importantly, sTNFR-p55, sTNFR-p75, and IL-6 concentrations were greater in kwashiorkor patients independent of the presence of possible or likely infections.
Increased plasma concentrations of sTNFR are found in patients with renal failure (13), but there was no indication of such abnormalities in our study population. Plasma creatinine concentrations were lower in the PI:EM patients, reflecting their reduced muscle mass and suggesting that kidney functions were not grossly abnormal. Moreover,
We thank J Gulani, M Amir, A Omar, and the clinical staff of KDH, KEMRI, for clinical and technical support; C Kambi and the staff of FLMC for collaboration; D Forster for support with data handling; and J van de Ven-Jongekrijg for laboratory assistance.

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11. Nijhoff WA, Groen GM, Peters WHM. Induction of rat hepatic and normal and marasmus (2, 6, 8, 22, 23). Plasma concentrations of vitamin E are diminished in marasmus and particularly in kwashiorkor (2); vitamin E is transported by cholesterol-rich lipoproteins, and plasma cholesterol is also reduced in PEM (24). The ratio of vitamin E to cholesterol was significantly reduced in our patient population, which may have resulted in a diminished resistance to oxidative stress.

One can speculate that the inflammatory response in PEM originated because 1) the prevalence of infections was increased and possibly the result of the compromised immune status or the increased microbial pressure from the overgrown small intestine (25), and 2) patients with PEM may be unable to adequately neutralize endotoxemia, which occurs frequently, particularly in kwashiorkor (2). Lipoproteins form complexes with lipopolysaccharides (LPSs) and LPS in these LPS-lipoprotein complexes is biologically inactive (26). Lipoprotein concentrations are low in kwashiorkor (24), which may result in a reduced capacity to neutralize LPS, and 3) changes in hormones may modulate cytokine responses (27). However, hormonal changes, which indeed can be found in PEM patients (1), are more likely to be secondary to cytokine responses.

In conclusion, our data further support the notion that inflammation may play a critical role in the pathogenesis of edematous malnutrition. The primary trigger may be invasion by microorganisms, translocation of bacterial products, or other as yet unidentified stimuli interacting with an environment that under nutritional stress is unable to control the initiated inflammatory response.