Inflammatory mediators in children with protein-energy malnutrition

Robert W Sauerwein, Janet A Mulder, Lambertus Mulder, Brett Lowe, Norbert Pesu, 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 reactivity that may contribute to edema formation. Am J Clin Nutr 1997;65:1534–9.

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. Hypoalbuminemia 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 unsavaged radicals damage tissues and induce vascular leakage in kwashiorkor (2). Prostaglandin E2 and cysteinyi 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

---

1 From the Departments of Medical Microbiology and Internal Medicine, University Hospital Nijmegen, Nijmegen, Netherlands, and the Kenya Medical Research Institute, Clinical Research Centre, Kilifi Unit, Kilifi, Kenya.

2 Supported by KEMRI, The Welcome Trust (040313), 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 Science (031342).

3 Address reprint requests to RW Sauerwein, Department of Medical Microbiology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, Netherlands. E-mail: R.Sauerwein@MMB.AZN.NL.
Diarrhea was classified as mild if swelling was limited to feet or ankles and as 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.

**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 (sIL-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 enzymatically on a Hitachi 747 analyzer with reagents from Boehringer (both from Boehringer, Mannheim, Germany). Results were checked regularly against control values with assigned values determined against the CDC/Abell-Kendall reference method (10). Plasma glutathione was quantified by HPLC after reaction with monobromobimane as described previously (11).

### Tables

**Table 1**

<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><strong>Age (y)</strong></td>
<td>3.1 ± 1.5 [30]</td>
<td>2.5 ± 1.2 [16]</td>
<td>3.4 ± 1.3 [39]</td>
</tr>
<tr>
<td><strong>Midupper arm circumference (cm)</strong></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><strong>Albumin (g/L)</strong></td>
<td>19.2 ± 7.2 [30]</td>
<td>31.6 ± 8.7 [16]</td>
<td>41.0 ± 5.2 [35]</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/L)</strong></td>
<td>8.4 ± 1.7 [28]</td>
<td>8.2 ± 2.6 [13]</td>
<td>11 ± 2.2 [36]</td>
</tr>
<tr>
<td><strong>White blood cells (× 10³/L)</strong></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>

*Significantly different from control (t test): \* P = 0.02, \* P = 0.001. \* P = 0.001.*
result in a significant increase 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 PEM patients, reflecting their reduced muscle mass and suggesting that kidney functions were not grossly abnormal. Moreover,
concentrations of sIL-6R, which are also increased in renal failure (JTM Frieling, H van Hamersvelt, J Wijdenes, T Hendriks, RW Sauerwein, CJ van der Linden, unpublished observations, 1996), were reduced in the kwashiorkor group. Changes in the other inflammatory indexes also support the notion that there was an inflammatory reaction in the PEM group. Both sTNFRs have been found to be elevated in human immunodeficiency virus (HIV)–infected patients (15), but only indeterminate serologic results for HIV were found in five PEM patients (data not shown).

Published data on cytokines in PEM are limited and generally show that the capacity of WBCs to produce cytokines is diminished. Production of TNF-α, IL-6, and IL-1-like activity by stimulated blood cells in vitro is reduced in malnourished children (16, 17; RW Sauerwein, K Marsh, unpublished observations, 1996). In rats IL-1 and TNF production of peritoneal macrophages, Kupffer cells, or alveolar macrophages is not different between animals with PEM and controls (3, 18). Diminished production of endogenous pyrogens has been shown previously in animals with PEM and PEM patients (18–20). The combination of increased plasma cytokine concentrations and reduced ex vivo production has been found previously in the acute stage of clinical infections (8, 21).

Our data corroborate earlier studies that showed that concentrations of red blood cell GSH are reduced in kwashiorkor and normal in 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 endotoxin, 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.

This paper is published with the permission of the Director of KEMRI. 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 FLC for collaboration; D Forster for support with data handling; and J van de Ven-Jongeckrijg for laboratory assistance.

REFERENCES


PROTEIN-ENERGY MALNUTRITION AND INFLAMMATION


