Inflammatory mediators in children with protein-energy malnutrition[1–3]

Robert W Sauerwein, Janet A Mulder, Lambertus Mulder, Brett Lowe, Norbert Peshu, 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 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 $\leq$ 70% and weight-for-height $\leq$ 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 $\leq$ 5 kg, and a hemoglobin concentration $\leq$ z 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 Sciences (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.

≥ 90% of the NCHS standards. Informed consent was obtained from at least one of the parents or grandparents. This study was approved by the Kenya National Ethical Committee.

A history was taken followed by a physical examination. The following laboratory tests were performed: full blood count including white blood cell (WBC) differential, thick and thin smear for detection of blood parasites, albumin concentration, and one blood culture for bacteremia. One urine and three fecal samples were collected for microscopy and culture and a chest X-ray was taken. Major criteria for possible or likely infection included the following: *1*) axillary temperature > 37.5 °C, *2*) abnormalities on chest X-ray consistent with an infectious process, *3*) a positive blood culture with pathogenic microorganisms, and *4*) a positive urine culture with the presence of WBCs. Minor criteria for infection were as follows: *1*) a history of fever in the past 3 d, *2*) a positive blood slide for malaria parasites > 50/100 WBCs, *3*) a WBC count > 17.5×10^9/L or < 2.5×10^9/L (ie, > 2 × SEM of the control group), *4*) a positive blood culture with possible contaminant (*Bacillus sp.* or *Staphylococcus epidermidis*), or *5*) positive urine culture. Possible infection was defined by 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.

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 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).

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/L 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[1]

	Kwashiorkor (n = 15 M, 15 F)	Marasmus (n = 10 M, 6 F)	Control (n = 27 M, 12 F)
Age (y)	3.1 ± 1.5 [30]	2.5 ± 1.1[2] [16]	3.4 ± 1.3 [39]
Midupper arm circumference (cm)	11.6 ± 1.5[4] [29]	11.2 ± 1.4[4] [16]	15.7 ± 0.9 [39]
Albumin (g/L)	19.2 ± 7.2[3] [30]	31.6 ± 8.7[4,5] [16]	41.0 ± 5.2 [35]
Hemoglobin (g/L)	8.4 ± 1.7 [28][4]	8.2 ± 2.6 [13][4]	11 ± 2.2 [36]
White blood cells (× 10^9/L)	11.9 ± 4.5 [27]	10.5 ± 4.8 [13]	10.1 ± 3.8 [36]

[1] $\bar{x}$ ± SD; n in brackets.
[2–4] Significantly different from control (*t* test): [2] $P = 0.02$, [3] $P = 0.0001$, [4] $P = 0.001$.
[5] Significantly different from kwashiorkor, $P = 0.0001$ (*t* test).

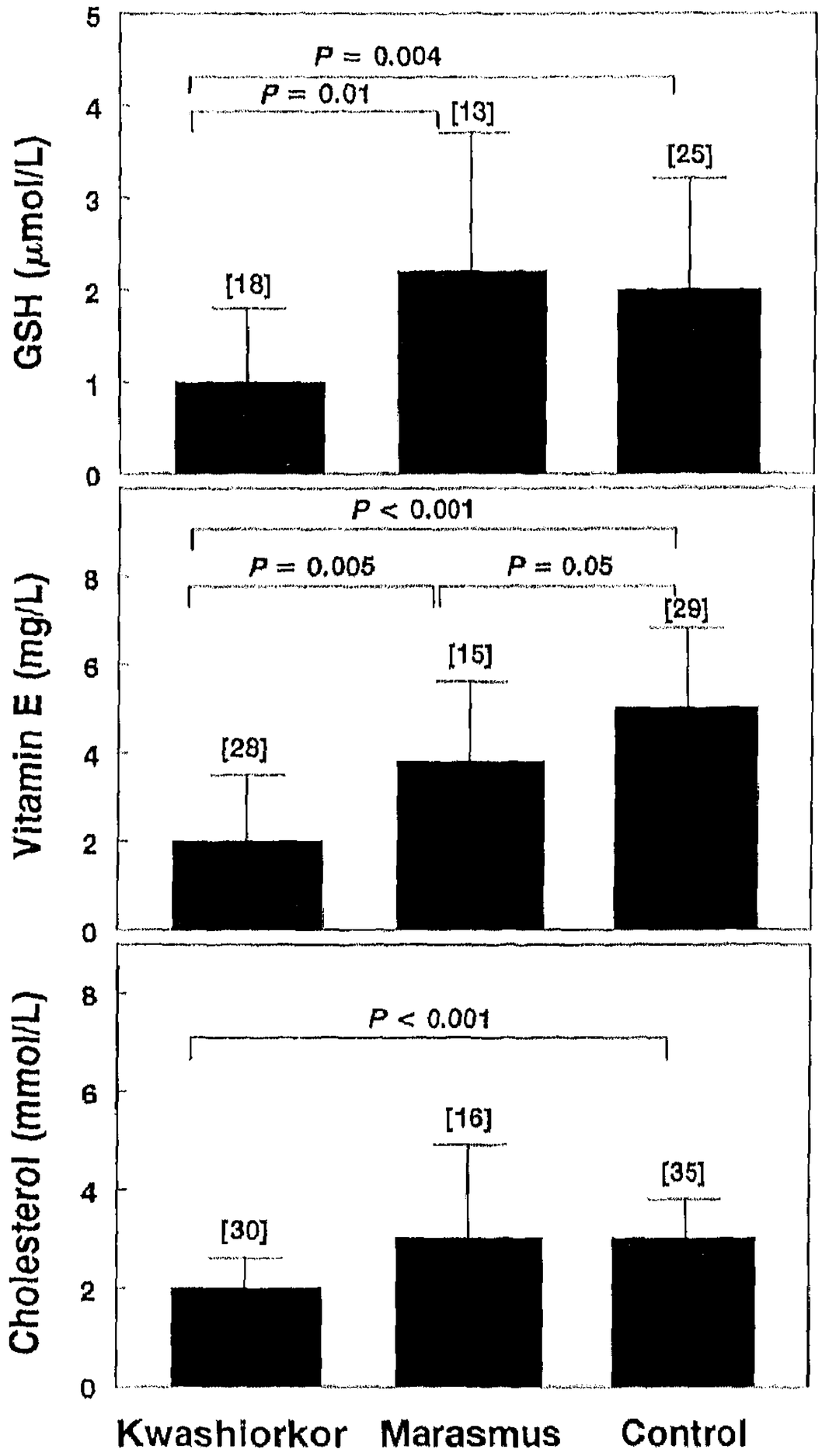

FIGURE 1. Plasma concentrations of the antioxidants glutathione (GSH) and vitamin E and of cholesterol. The mean (± SD) ratio of vitamin E to cholesterol for kwashiorkor was 1.1 ± 0.7, for marasmus was 1.4 ± 0.8, and for the control group was 1.6 ± 0.3. Ratio is significantly different between children with kwashiorkor and control children, $P < 0.003$. *n* in brackets over bar.

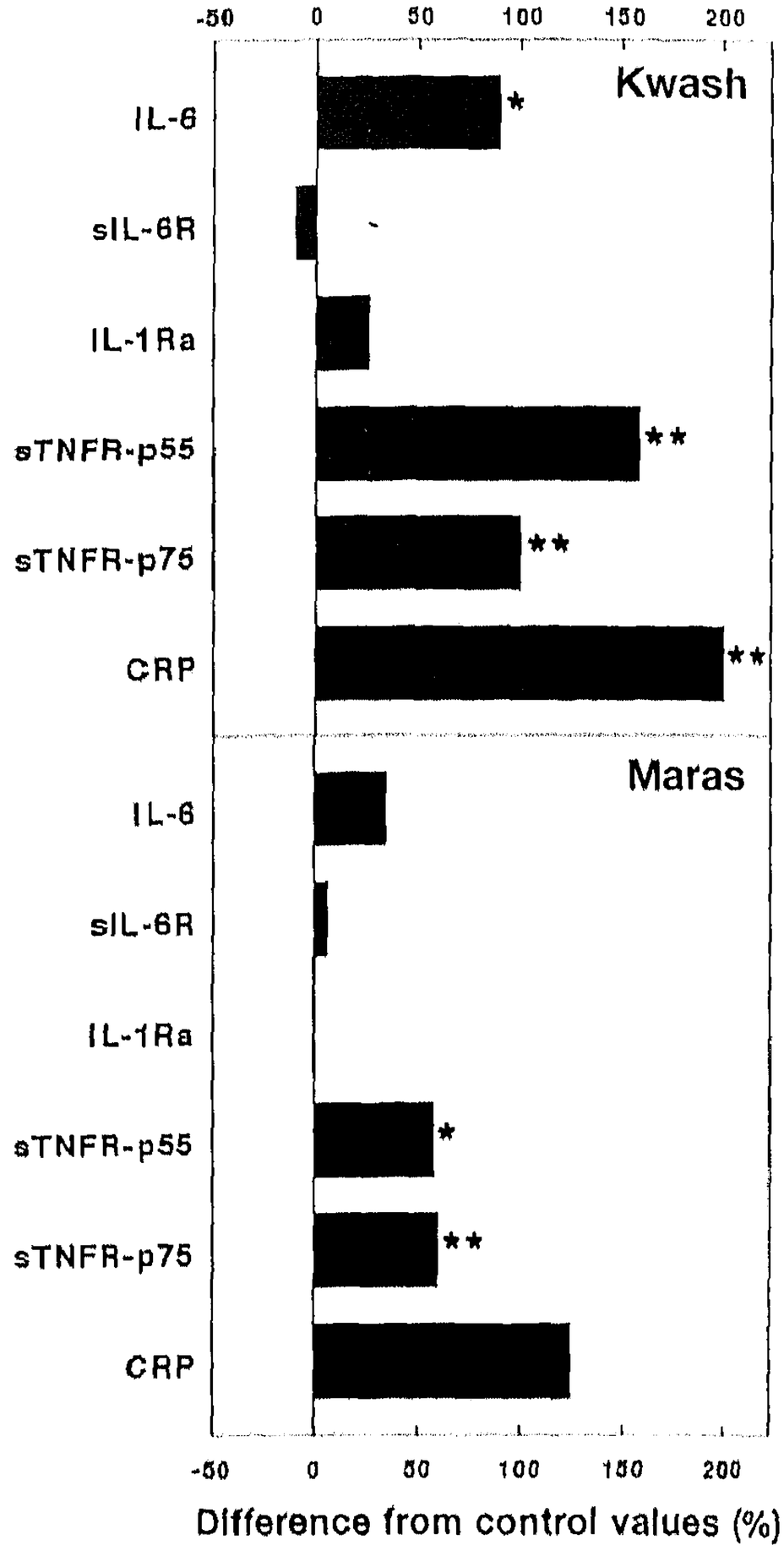

FIGURE 2. Percentage difference from mean values of plasma concentrations of inflammatory mediators in children with kwashiorkor (Kwash) or marasmus (Maras). The values of the control children were as follows: interleukin 6 (IL-6), 46 ng/L (range: 20–400 ng/L); soluble receptor of IL-6 (sIL-6R), 81 ng/L (range: 53–121 µg/L); IL-1 receptor agonist (IL-1Ra), 1.5 µg/L (range: 1–6.3 µg/L); soluble tumor necrosis factor receptor (sTNFR)p55, 2.9 µg/L (range: 1.4–10.1 µg/L); sTNFR-p75, 8.1 µg/L (2.7–22.9 µg/L); and C-reactive protein (CRP), 12 mg/L (range: 5–62 mg/L). For mean control values $n = 38$ except for CRP, where $n = 37$. For Kwash $n = 30$ except for sTNFR-p55 and sTNFR-p75, where $n = 29$; for Maras $n = 16$ except for sTNFR-p75, where $n = 15$. Significantly different from control subjects: * $P < 0.05$, ** $P < 0.005$.

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.

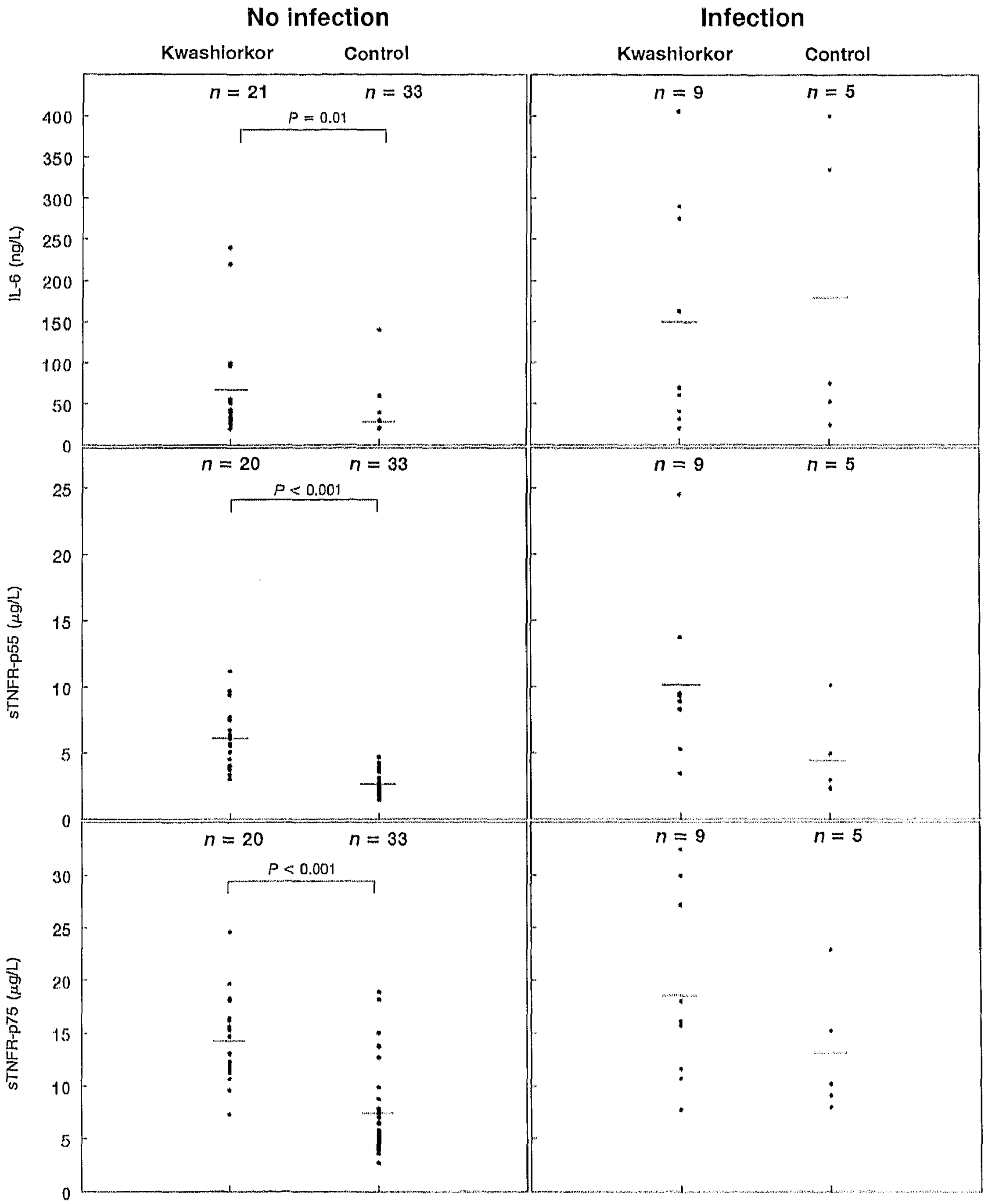

FIGURE 3. Plasma concentrations of interleukin 6 (IL-6), soluble tumor necrosis factor receptor (sTNFR)p55, and sTNFR-p75 in kwashiorkor patients and control children without infections or with likely or possible infections. There were significant differences between kwashiorkor patients and control children without infection, but not between those with infections. In kwashiorkor patients the sTNFR-p55 concentrations were significantly higher in infected patients than in noninfected patients ($P = 0.03$). Horizontal lines represent the mean of each group.

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 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.

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-Jongekrijg for laboratory assistance.

REFERENCES

1. Waterlow JC. Causes of edema and its relation to kwashiorkor. In: Waterlow JC, ed. Protein energy malnutrition. London: Edward Arnold, 1992.
2. Golden MHN, Ramdath D. Free radicals in the pathogenesis of kwashiorkor. Proc Nutr Soc 1987;46:53–68.
3. Skerett SJ, Henderson WR, Martin TR. Alveolar macrophage function in rats with severe protein calorie malnutrition. Arachidonic acid metabolism, cytokine release and antimicrobial activity. J Immunol 1990;144:1052–61.
4. Mayatepek E, Becker K, Gana L, Hoffmann GF, Leichsenring M. Leukotrienes in the pathophysiology of kwashiorkor. Lancet 1993;342:958–60.
5. Stuart HC, Stevenson SS. Growth and development. In: Behrman RE, Vaughan VS, eds. Textbook of pediatrics. Philadelphia: WB Saunders, 1987:6–35.
6. Frieling JTM, Sauerwein RW, Wijdenes J, et al. Soluble-interleukin 6 receptor in biological fluids of human origin. Cytokine 1994;6: 376–81.
7. Drenth JPH, Van Uum SHM, Van Deuren M, Pesman GJ, et al. Endurance run increases circulating IL-6 and IL1ra but downregulates ex vivo TNF-α and IL-1 β production. J Appl Physiol; 79:1497–503.
8. Van Deuren M, Van der Ven-Jongekrijg J, Demacker PNM, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. J Infect Dis 1994;169:157–61.
9. De Graaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Stalenhoef AFH. Enhanced susceptibility to in vitro oxidation of the low density lipoprotein subfraction in healthy subjects. Atheroscler Thromb 1991;11:298–306.
10. Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952;195:357–62.
11. Nijhoff WA, Groen GM, Peters WHM. Induction of rat hepatic and intestinal glutathione S-transferase and glutathione by dietary occurring anticarcinogens. Int J Oncol 1993;3:1131–9.
12. Horwitt MK, Harvey CC, Dahm Jr CH, Searcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann N Y Acad Sci 1972;203:223–36.
13. Brockhaus M, Bar-Khayim Y, Ciurwiez S, et al. Plasma tumor necrosis factor soluble receptors in chronic renal failure. Kidney Int 1992;42:663–7.
14. Scrimshaw NS, Taylor CE, Gordon JE. Interactions of nutrition and infection. World Health Organ Monogr Ser 1968;57.
15. Godfried MH, van der Poll T, Weverling GJ, et al. Soluble receptors for tumor necrosis factor as predictors of progression to AIDS in asymptomatic HIV-type I infection. J Infect Dis 1994;169:739–45.
16. Bhaskaram P, Sivakumar B. Interleukin-1 in malnutrition. Arch Dis Child 1986;61:182–5.
17. Doherty JF, Golden MHN, Remick DG, Griffin GE. Production of interleukin-6 and tumor necrosis factor-alpha in vitro is reduced in whole blood of severely malnourished children. Clin Sci 1994;86:347–51.
18. Bradley SF, Vibhagool A, Kunkel SL, Kauffman CA. Monokine secretion in aging and protein malnutrition. J Leukoc Biol 1989;45: 510–4.
19. Hoffman-Goetz L, McFarlane D, Bistrian BR, Blackburn GL. Febrile and plasma iron responses of rabbits injected with endogenous pyrogen from malnourished patients. Am J Clin Nutr 1981;34:1109–16.
20. Kauffman CA, Jones PG, Kluger MJ. Fever and malnutrition: endogenous pyrogen/interleukin-1 in malnourished patients. Am J Clin Nutr 1986;44:449–52.
21. Keuter M, Dharmana E, Hussein Gasem M, et al. Patterns of proinflammatory cytokines and inhibitors during typhoid fever. J Infect Dis 1994;169:1306–11.

22. Sive AA, Subotzky EF, Dempster WS, De V, Heese H. Red blood cell anti-oxidant concentrations in kwashiorkor and marasmus. Ann Trop Paediatr 1993;13:33–8.
23. Jackson AA. Blood gluthathione in severe malnutrition in childhood. Trans R Soc Trop Med Hyg 1986;80:911–3.
24. Feillet F, Parra H-J, Kamian K, Bard J-M, Fruchart J-C, Vidailhet M. Lipoprotein metabolism in marasmic children of Northern Mauritania. Am J Clin Nutr 1993;58:484–8.
25. Chandra RK. Nutrition, immunity and infection: present knowledge and future directions. Lancet 1983;1:688–91.
26. Munford RS, Hall CL, Dietschy JM. Binding of *S. typhimurium* lipopolysacharides to rat high density lipoproteins. Infect Immun 1981;34:835–43.
27. Nobuko S, Sakurai A, Haranaka K. Relationship of hypoglycemia to tumor necrosis factor production and antitumor activity: role of glucose, insulin and macrophages. J Natl Cancer Inst 1985;74:1255–60.