Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats

Pei-Ra Ling, Robert J. Smith, Susanne Kie, Patricia Boyce, and Bruce R. Bistrian

1Nutrition/Infection Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215; and 2Division of Endocrinology, Brown University Medical School, Providence, Rhode Island 02908

Submitted 15 December 2003; accepted in final form 21 May 2004

Ling, Pei-Ra, Robert J. Smith, Susanne Kie, Patricia Boyce, and Bruce R. Bistrian. Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats. Am J Physiol Regul Integr Comp Physiol 287: R801–R808, 2004; 10.1152/ajpregu.00715.2003.—This study examines the effects of malnutrition on IL-6 signaling pathways of rats fed 2% vs. 20% casein diets for 14 days. Effects of malnutrition on the abundance and IL-6-stimulated phosphorylation of signaling proteins in the JAK-STAT and MAP kinase pathways were examined in the liver. Changes of the acute-phase response as reflected by serum α1-acid glycoprotein (AG), TNF-α (TNF), and IL-1β (IL-1) were compared in two dietary groups at 0, 4, 8, 16, and 24 h after IL-6 administration. Under basal conditions, the abundance of the IL-6 receptor, gp130, JAK1, STAT1, and STAT3 proteins and levels of phosphorylation of ERK1/2 and p38 were significantly increased in the liver in the 2% casein group compared with the 20% casein group. With IL-6 stimulation, the increased phosphorylation per unit of protein of these signaling proteins was not different in the liver between the two groups. Before IL-6 stimulation, serum levels of TNF, IL-1, IL-6, and AG were significantly higher in the 2% casein group than in the 20% casein group. After bolus injection of IL-6, changes in IL-1 and AG were similar in the two dietary groups, although a slight decline in AG level was noted after 8 h of IL-6 administration in the 2% protein group. These data demonstrate that protein malnutrition produces changes in inflammation-related proteins characteristic of a low-grade systemic inflammatory response and, thus, can serve as an inflammatory stimulus. The capacity for response to IL-6 is preserved, suggesting adaptive preservation of acute-phase responsiveness during malnutrition.

protein-calorie malnutrition; α1-acid glycoprotein; tumor necrosis factor-α; interleukin-1β; immune function; acute phase response

PROTEIN-CALORIE MALNUTRITION (PCM) is commonly observed in hospitalized patients suffering from critical illness or chronic diseases (5–8). During PCM, an attenuated acute-phase response and reduced production of acute-phase proteins and cytokines have been proposed as mechanisms for impairment of immune function contributing to increased mortality and morbidity due to infections (1, 9, 10). However, it remains unclear whether the activation and responsiveness to individual cytokines also are affected by PCM.

Our previous studies have shown that the in vivo capacity of IL-1β (IL-1) production, as reflected by changes in trace metal levels, was attenuated in malnourished guinea pigs in response to a challenge of endotoxin. However, these animals were able to maintain an appropriate profile of trace metal response after a bolus dose of exogenous IL-1 (24). Moreover, administration of human IL-1 improved the clearance of Pseudomonas from the circulation in these malnourished animals (24). These findings suggest that the capacity for responsiveness to individual cytokines may be maintained during malnutrition, despite an overall reduction in cytokine production in response to complex external stimuli and despite the depletion of structural and other constitutive tissue proteins.

The cytokine IL-6 represents a distal element of the inflammatory cascade (14a, 37). Among three principal and proximal cytokines, TNF-α (TNF), IL-1, and IL-6, IL-6 can have anti- and proinflammatory functions, and the circulating levels of IL-6 are directly and closely correlated with the severity of disease (11). In the liver, IL-6, after its binding to the IL-6 receptors on the cell surface, stimulates hepatocytes to produce acute-phase proteins as well as cytokines through multiple signaling pathways (15, 16). In one signaling pathway, IL-6 binding leads to the rapid association of the transmembrane IL-6 receptors and intracellular gp130 followed by the activation of receptor-associated Janus-activated kinases (JAKs). The activated JAKs induce self-phosphorylation on tyrosine residues and the tyrosine phosphorylation of the IL-6 receptor, gp130, and a family of transcription factors termed signal transducers and activators of transcription (STATs). The activated STAT proteins, mainly STAT1 and STAT3, then dimerize, translocate to the nucleus, and play an important role in inducing or modulating the transcription of multiple genes, including those encoding acute-phase proteins that are primarily anti-inflammatory. α1-Acid glycoprotein (AG) is one of the major acute-phase proteins in rats. Its serum concentration increases proportionately in response to systemic inflammation or infection and is controlled by the cytokine network involving TNF, IL-1, and IL-6 (13). Activation of the IL-6 receptor also stimulates a second pathway, which involves various members of the mitogen-activated protein (MAP) kinase family, including extracellular signal-regulated kinase (ERK) 1, ERK2, and p38 stress-activated protein kinase (p38). The activation of these MAP kinases also is induced by many other extracellular stimuli and leads to cellular responses, including secretion of cytokines, such as TNF and IL-1, which tend to be proinflammatory. The balance between these anti- and proinflammatory pathways presumably has the potential to influence clinical responses. Recent studies have demonstrated that inhibition of the IL-6-mediated JAK/STATs in the absence of MAP kinase pathway inhibition in the liver can enhance a proinflammatory response (2, 18, 22). In this context, it is important to understand the effects of PCM on IL-6-mediated...
IL-6 feeding a 2% casein diet for 14 days. Food intake is reduced in the acute-phase response.

We have established a malnutrition rat model by ad libitum feeding a 2% casein diet for 14 days. Other investigators have demonstrated that protein-malnourished rats are at increased risk of infection or are more susceptible to a microbial challenge (3, 26, 28, 38). Using this model, we examined the abundance and activation of IL-6 signaling proteins in the liver. Changes in the acute-phase response as reflected by serum AG, TNF, and IL-1 were determined during a 24-h period after the administration of exogenous IL-6.

**MATERIALS AND METHODS**

**IL-6**

Recombinant mouse IL-6 was used in this study, because the availability of rat IL-6 was limited. Mouse and rat IL-6 contain 211 amino acids, and the sequences exhibit 85% amino acid identity and 92% homology (including identical amino acid residues and conserved amino acid sequences). Our previous studies have shown that both types of recombinant IL-6 induce similar responses in rats (22). Recombinant mouse IL-6 containing <0.1 ng of endotoxin per microgram of cytokine was obtained from R & D Systems (Minneapolis, MN). Lyophilized mouse IL-6 was stored at −80°C and freshly made in saline with 0.1% human albumin before the experiments.

**Animals**

Pathogen-free male Sprague-Dawley rats (220–250 g) were purchased from Taconic Farms (Germantown, NY) and maintained on a diet of AIN-76 purified diets containing 20% or 2% casein (Dyets, Bethlehem, PA) ad libitum for 14 days. These two diets contained the same amounts of mineral and vitamins, and similar energy density was achieved by addition of more cornstarch and sucrose to the 2% casein diet. During the feeding period, body weight and food intake were recorded every other day.

**Experiment 1.** On the day of the experiment, eight rats from each dietary group (20% and 2% casein) were further divided into two subgroups that received IL-6 (20 μg/kg) by bolus injection through the portal vein under anesthesia or an equivalent volume of saline injection. The portal route was chosen (22). After 5 min, the animals were decapitated, and liver tissue was rapidly removed, frozen in liquid nitrogen, and stored at −80°C for subsequent analysis.

**Experiment 2.** On the day of the experiment, 50 rats from each dietary group received IL-6 (20 μg/kg) by bolus injection through the tail vein under anesthesia and were killed 0, 4, 8, 16, and 24 h (n = 10 at each time point) after IL-6 administration. Mixed arterial and venous blood was collected, and the whole liver, lungs, and heart were removed and weighed. A portion of the liver, rectus abdominis muscle, lung, and whole heart were weighed, desiccated in a vacuum dryer, and reweighed. The weight changes of each piece of tissue or organ were used to calculate water content. Blood was used to determine the levels of serum total protein, albumin, glucose, AG, IL-1, IL-6, and TNF. A portion of liver tissue also was used to determine the content of total glutathione.

**Analytic Methods**

**Immunoprecipitation and immunoblotting.** Liver samples (~1 g) were pulverized in a liquid nitrogen-cooled stainless steel mortar and pestle. The powdered tissue was transferred to a tube containing 6 ml of buffer consisting of 20 mM Tris, pH 7.6, 120 mM NaCl, 1% NP-40, 10% glycerol, 2 mM sodium vanadate, 10 mM sodium pyrophosphate, 1 mM PMSF, 40 μg/ml leupeptin, and 100 μM sodium fluoride. After homogenization in an ice bath for 45 s at maximum speed with a Polytron (Brinkman, Westbury, NY), the samples were mixed for 45 min at 4°C by end-over-end rotation and centrifuged at 200,000 g for 1 h. The clear supernatant was removed with care to avoid the overlying fat layer and stored in aliquots at −80°C for later analysis. Protein content was determined using a protein assay (Bio-Rad, Hercules, CA).

Activated IL-6 receptor and JAK1 were determined by sequential immunoprecipitation and immunoblotting. Brieﬂy, 8 mg of solubilized tissue protein were incubated overnight at 4°C on a rocking platform with protein A-conjugated 4G10 phosphoantibody. The 4G10 immunocomplexes then were recovered by centrifugation (13,000 g for 1 min) and washed three times with buffer containing 10 mM Tris-HCl, pH 7.4, 1% NP-40, 150 mM NaCl, 1 mM sodium orthovanadate, and 1 mM PMSF. The resulting pellets were dissolved in Laemmli buffer (0.25 M Tris, 8% SDS, and 100 mM dithiothreitol), heated for 5 min in a boiling water bath, and cleared by centrifugation. The immunoprecipitated proteins were separated on 7% SDS-polyacrylamide gels, electroblotted onto nitrocellulose membranes (0.2 μm; BA 83, Schleicher & Schuell, Keene, NH), and incubated in 5% bovine serum albumin for 2 h at 37°C to block nonspecific binding. The membranes then were further blotted with IL-6 receptor or JAK1 antibodies. The resulting protein bands were detected by enhanced chemiluminescence according to the manufacturer’s recommendations (Amersham Pharmacia Biotech, Piscataway, NJ), identified by molecular weight, and quantitated using the Imagej program provided by the National Institutes of Health.

Activated STAT1, STAT3, ERK1, ERK2, and p38 were determined by direct immunoblotting using phosphospecific (activation-specific) antibodies. Specific protein bands were detected by enhanced chemiluminescence and quantitated using the Imagej program. The abundance of each signaling protein in the liver extracts also was determined by direct immunoblotting using individual specific antibodies that recognize active and inactive forms of the proteins. To make possible the pooling of data from multiple immunoblots, the relative density of each band was normalized against an internal standard analyzed on each blot. The data from the 20% casein group with saline injection were expressed as 1, and the relative changes in other groups were calculated against these controls.

**Assays.** Serum IL-1, IL-6, and TNF were determined with ELISA kits (Biosource International, Camarillo, CA). Serum AG concentration was determined using the rat α1-AG plate kit (Cardiotech Services, Louisville, KY). Serum total protein and albumin levels were...
determined using an albumin reagent kit (Sigma Diagnostics, St. Louis, MO). Glucose levels were determined in tail vein whole blood using a glucometer (Glucometer Elite XL, Bayer, Elkhart, IN).

The total glutathione (GSH + GSSG) content in liver tissue was determined with a colorimetric determination kit (Oxis Health Products, Portland, OR). The amount of total glutathione in the liver tissue was expressed per unit of tissue protein.

Statistical Analysis

Values are means ± SE. Results were analyzed by two-way ANOVA (diet and time) with Fisher’s least significant difference test for individual comparisons of means among groups after IL-6 administration. In addition, Student’s t-test was used for comparisons of the effects of 2% and 20% casein diets on serum levels of IL-1, IL-6, TNF, glucose, AG, and total glutathione in the liver at each time point after IL-6 administration. Significance for all analyses was defined as \( P \leq 0.05 \).

RESULTS:

Food Intake and Body Weight

Rats fed the 20% casein diet consumed more food than those fed the 2% casein diet, as expected on the basis of previous studies with this experimental model (30–32). When expressed per unit of body weight, protein intake was 90% lower in the 2% casein group than in the 20% casein group, and energy intake was 20% lower. After 14 days of feeding, animals fed the 2% casein diet had lost 16% of their initial body weight, whereas those fed the 20% casein diet had gained an average 72.1 ± 6.8 g, a 24% increase from their original weight. As a result, the average final body weight of malnourished animals was 25–30% below that of the well-nourished control animals. Weights of the liver, heart, and lungs were lower in the malnourished rats. However, the ratios of organ weight to total body weight were not significantly different between the two dietary groups (data not shown). Water content of the liver, heart, lung, and muscle was also not significantly different between the two dietary groups (data not shown), suggesting that this experimental malnutrition model does not completely mirror this feature of kwashiorkor as seen in human patients (27).

Effects of PCM on Signaling Protein Abundance and IL-6-Mediated Signaling (Basal and Stimulated) in the Liver

IL-6 receptor and JAK-STAT pathway. In rats fed the 2% casein diet for 14 days, abundance of the JAK1 protein was increased by ~50% in the liver (\( P < 0.05 \)) relative to total extract protein compared with that in rats fed the 20% casein diet (Fig. 1). In the basal state, phosphorylation of JAK1 was detectable in both dietary groups. The mean level of tyrosine phosphorylation of JAK1 was higher in the 2% casein group than in the 20% casein group, but this did not reach statistical significance as a consequence of high interanimal variability. With IL-6 stimulation, JAK1 tyrosine phosphorylation significantly increased from basal in the 20% casein group (2-fold, \( P < 0.05 \)) but did not change in the 2% casein group, such that the level of phosphorylated JAK1 per unit of JAK1 protein appeared to be comparable in both dietary groups.

The abundance of IL-6 receptors in the liver was increased approximately twofold (\( P < 0.05 \)) in the 2% casein group vs. the 20% casein group (Fig. 2). However, basal and IL-6-stimulated receptor phosphorylation per unit of IL-6 receptor protein was equivalent in the two dietary groups. The abundance of gp130 also increased (3-fold) in the 2% casein group compared with the 20% casein group (\( P < 0.05 \); data not shown). The limited assay sensitivity did not permit detection of the phosphorylation of gp130 after IL-6 injection in either group of animals.

The abundance of STAT1 and STAT3 as a fraction of total extract protein was significantly higher (2.3- and 6.4-fold, respectively) in the malnourished animals than in the controls (Fig. 3). STAT1 or STAT3 tyrosine phosphorylation was undetectable in the basal state, and injection of IL-6 rapidly increased tyrosine phosphorylation of STAT1 and STAT3 proteins in the liver in both dietary groups. The extent of

Fig. 1. Effects of feeding a low-protein diet for 14 days on JAK1 abundance and tyrosine phosphorylation in rat liver in vivo. Liver extracts were directly blotted with JAK1 antibody (A) or subjected to immunoprecipitation with phosphotyrosine antibody and sequential immunoblotting with JAK1 antibody (B). Each lane in blots corresponds to an individual representative animal in the indicated group. Results of quantitative analysis of immunoblots are means ± SE for 4 rats per group expressed as relative changes in arbitrary densitometry units normalized against an internal standard. 2%, 2% casein diet; 20%, 20% casein diet; −IL-6, saline injection (i.e., no IL-6); +IL-6, 5 min after IL-6 injection. *\( P < 0.05 \) vs. all other conditions. **\( P < 0.05 \) vs. 2%.
IL-6-stimulated STAT1 and STAT3 activation was significantly higher in malnourished animals than in controls: 38.7 ± 8.8 vs. 26.7 ± 1.5 arbitrary units (P < 0.05) and 34.8 ± 3.3 vs. 13.5 ± 2.0 arbitrary units (P < 0.001), respectively. Because of the higher abundance of STAT1 and STAT3 in the malnourished animals, when averaged per unit protein of STAT1 or STAT3, the IL-6-activated tyrosine-phosphorylated STAT1 or STAT3 was comparable in both nutritional conditions.

Fig. 2. Effects of feeding a low-protein diet for 14 days on IL-6 receptor abundance and tyrosine phosphorylation in rat liver in vivo. Liver extracts were directly blotted with IL-6 receptor antibody (A) or immunoprecipitated with phosphotyrosine antibody and sequentially immunoblotted with IL-6 receptor antibody (B). Each lane in blots corresponds to an individual representative animal in the indicated group. Results of quantitative analysis of immunoblots are means ± SE for 4 rats per group expressed as relative changes in arbitrary densitometry units normalized against an internal standard. 2%, 2% casein diet; 20%, 20% casein diet; ±IL-6, saline injection (i.e., no IL-6); +IL-6, 5 min after IL-6 injection. *P < 0.001 vs. +IL-6. **P < 0.05 vs. 2%.

Fig. 3. Effects of feeding a low-protein diet for 14 days on STAT1 and STAT3 abundance and tyrosine phosphorylation in rat liver in vivo. Liver extracts were directly blotted with anti-STAT1 antibody or immunoblotted with antiphospho-STAT1 antibody (A) and anti-STAT3 antibody or immunoblotted with antiphospho-STAT3 antibody (B). Each lane in blots corresponds to an individual representative animal in the indicated group. Results of quantitative analysis of immunoblots are means ± SE for 4 rats per group expressed as relative changes in arbitrary densitometry units normalized against an internal standard. 2%, 2% casein diet; 20%, 20% casein diet; ±IL-6, saline injection (i.e., no IL-6); +IL-6, 5 min after IL-6 injection. *P < 0.001 vs. +IL-6. **P < 0.05; ***P < 0.01 vs. 2%.
MAP kinase pathway. In the liver, the abundance of ERK1, ERK2, and p38 was not changed by malnutrition (Fig. 4A). However, in the basal state, all three MAP kinase proteins were activated in animals fed the 2% protein diet compared with those fed the 20% diet (Fig. 4B). IL-6 administration markedly increased ERK1, ERK2, and p38 activation in the control group to an extent comparable to that seen in the basal state in the malnourished animals. IL-6 administration did not cause a further increase in phosphorylation (activation) of these three MAP kinase proteins above the already elevated basal level in malnourished animals.

Effects of PCM and IL-6 Administration on Nutritional Status and Inflammatory Response Indicators

Significant reductions of serum albumin (20% decrease, \( P < 0.001 \); Fig. 5), total protein (21% decrease, \( P < 0.001 \); Fig. 5), glucose (19% decrease: 96 ± 3 vs. 118 ± 2 mg/dl, \( P < 0.05 \)), and total glutathione in the liver (65% decrease: 10.5 ± 0.7 vs. 29.4 ± 2.5 \( \mu \)mol/mg, \( P < 0.01 \)) were observed in animals after 14 days of 2% protein diet feeding compared with those fed the 20% diet feeding. In contrast, serum levels of IL-1 (23% increase, \( P < 0.01 \); Fig. 6A), TNF (36% increase: 7.3 ± 0.4 vs. 12.0 ± 0.8 pg/ml, \( P < 0.01 \)), IL-6 (50% increase: 74.4 ± 9.2 vs. 158.0 ± 22.3 pg/ml, \( P < 0.005 \)), and AG (28% increase, \( P < 0.05 \); Fig. 6B) were significantly higher in the malnourished animals than in the controls.

After IL-6 injection, the levels of serum albumin and total protein gradually declined from the basal level, reached a significantly lower level at 8 h, and then further decreased to a nadir (40% decrease from basal level) 16 h after IL-6 administration in control animals (Fig. 5). A subsequent increase in levels of albumin and total protein was observed 24 h after IL-6 injection, although the levels remained below the initial basal values. In malnourished animals, the levels of serum total protein and albumin did not further decrease below the initial low basal level over the 24 h after IL-6 administration (Fig. 5). Serum glucose and total glutathione in the liver were maintained at their basal levels during the 24-h study period after IL-6 administration in both dietary groups (data not shown).

Before IL-6 administration, the basal levels of TNF and IL-6 were significantly higher in the 2% casein group than in the 20% casein group. IL-6 administration did not result in differences in TNF during the study period between the two dietary groups (data not shown). Because a supramaximal dose of IL-6 was administered, changes of IL-6 levels in response to IL-6 were not examined. IL-6 injection caused a steady increment of serum IL-1 from their respective basal levels in both groups, reached a maximum at 16 h, and remained at the higher level for at least another 8 h in both dietary groups. Although the changes in serum IL-1 were similar during 24 h after IL-6 injection in the two dietary groups, higher levels of IL-1 were observed at each time point (\( P < 0.05 \)) in the 20% casein group than in the 20% casein group (Fig. 6A). However, the net changes from basal at 16–24 h were less in the 2% casein group than in the 20% casein group.

IL-6 administration resulted in significantly increased serum AG in both dietary groups, with maximum levels 16–24 h after IL-6 (Fig. 6B). A slightly but significantly higher level of AG

---

**Fig. 4.** Effects of feeding a low-protein diet for 14 days on ERK1, ERK2, and p38 abundance (A) and phosphorylation (B) in rat liver in vivo. Liver extracts were immunoblotted with anti-ERK1, -ERK2, or -p38 antibody or antiphospho-ERK1, -ERK2, or -p38 antibody. Each lane in blots corresponds to an individual representative animal in the indicated group. Results of quantitative analysis of immunoblots are means ± SE for 4 rats per group expressed as relative changes in arbitrary densitometry units normalized against an internal standard. 2%, 2% casein diet; 20%, 20% casein diet; –IL-6, saline injection (i.e., no IL-6); +IL-6, 5 min after IL-6 injection. *\( P < 0.05 \) vs. all other conditions.
was observed in malnourished animals than in controls before IL-6 administration, and then the levels of AG were slightly lower in malnourished animals 16–24 h after IL-6. The area under the curve over 24 h after IL-6 administration, indicating the total amount of AG in serum, was not significantly different between these two dietary groups.

**DISCUSSION**

In this study, we have shown that protein malnutrition induces a low-grade inflammatory state in rats, as evidenced by MAP kinase activation in the liver, elevated serum levels of TNF, IL-1, IL-6, and AG, and reduced serum levels of albumin. MAP kinase signaling pathways are known to play key roles in cytoplasmic-nuclear signaling transmission in response to various extracellular stimuli, resulting in the production of cytokines, tissue damage, and cell death (21, 25). In primary cultured hepatocytes, hyposmotic stress activates ERK1/2 and p38 phosphorylation (20). Repeated fasting as a stress causes activation of ERKs in rat liver (29). Previously, we also showed that endotoxin administration activates ERK1/2 and p38 phosphorylation in rat liver, and these activations are accompanied by the increased cytokine levels in plasma (22). Thus dietary protein depletion alone without another exogenous stimulus is now identified as one of a number of other established stimuli for systemic inflammation. This is not counterintuitive to the increased susceptibility characteristic of malnourished animals, because a protein-malnourished animal with associated inflammation would likely be immunosuppressed compared with a well-nourished animal without inflammation.

The present results demonstrated that body weight, liver size, and final body nitrogen stores were significantly reduced after 14 days of dietary protein depletion. Total glutathione content in the liver was 65% less in malnourished than in well-nourished rats. These changes are similar to responses observed during inflammatory states. Hepatic glutathione levels have been shown to inversely correlate with the levels of NF-κB activation in the liver, leading to increased transcription of IL-1β and TNF-α (35). Glutathione depletion also increases lipid peroxidation in the liver (34, 36) and impairs the hepatic capacity to inactivate reactive oxygen species, which are prominent stimulators of cytokine production (35). In the present study, higher levels of IL-1, IL-6, TNF, and AG and lower levels of albumin in serum were found in malnourished than in well-nourished rats. Thus it is reasonable to consider that the hepatic glutathione reduction induced by dietary protein depletion may be one of the important contributing factors in the activation of a systemic inflammatory response. However, because glutathione depletion can also result from cytokine activation, it is difficult to differentiate between primary and secondary changes in glutathione. Whatever their relative contributions, it is well established that reactive oxygen species...
can activate NF-κB, which then elicits IL-1, IL-6, and TNF production. The lower levels of serum albumin observed in malnourished and well-nourished animals receiving IL-6 may also suggest that systemic inflammation due to protein deficiency and cytokine administration can contribute to the development of hypoalbuminemia (14, 17).

The present results also demonstrated an increase in the abundance of IL-6 receptors, JAK1, STAT1, and STAT3 as a fraction of total soluble protein from liver tissue of malnourished compared with control rats. IL-6-stimulated phosphorylation of the IL-6 receptor, STAT1, and STAT3 is at least as great in the malnourished animals as in the controls, possibly as a consequence of the increases in abundance of the proteins. JAK1 phosphorylation is increased in the basal state in the malnourished rats, and it also is equivalent to the controls after IL-6 stimulation. Even though “labile” and structural proteins undoubtedly were severely depleted in the malnourished animals, there appears to have been a preservation of protein levels and signaling in the IL-6-JAK-STAT pathway. Moreover, AG levels were significantly increased from different basal levels in both dietary groups, and the total change in AG over 24 h after IL-6 administration was not different between the two groups. Because many acute-phase proteins are protease inhibitors (4), these observed changes might represent anti-inflammatory actions mediated by IL-6.

The administration of IL-6 also increased serum IL-1 in both dietary groups, with greater levels of IL-1 observed at each time point in the 2% casein group than in the 20% casein group. As a result, total IL-1 in the circulation was greater in the 2% casein group than in the 20% casein group. Thus IL-6 may also elicit a proinflammatory component of the acute-phase response under both nutritional conditions, but total IL-1 in the circulation was greater in the 2% casein group than in the 20% casein group. The activation of MAP kinase proteins, which are characteristic of a stress response, can increase production of the proximate cytokines, IL-1, TNF, and IL-6, thus enhancing the proinflammatory response. In contrast to well-nourished animals, phosphorylation of MAP kinase proteins in the liver was high in the basal state and not further stimulated by IL-6 in malnourished animals. This may indicate that maximal MAP kinase activation had already occurred in response to PCM, and this level was maintained after IL-6 stimulation. Interestingly, serum TNF and albumin also were maintained at their basal levels during 24 h after IL-6 administration in malnourished animals. The levels of TNF were higher and albumin was lower than in well-nourished animals. These results suggest an augmented systemic inflammatory response before and after IL-6 administration in malnourished animals. This may be the product of selective maintenance of the protein components of the acute-phase response during PCM.

The present results are not consistent with the concept that the acute-phase response to infection or inflammation is reduced in malnutrition. For instance, malnourished humans generate only a partial acute-phase protein response to infection (33). After LPS stimulation, the in vitro production of proinflammatory cytokines such as TNF and IL-6 in whole blood from severely malnourished children is reduced (12). An attenuated synthesis and/or release of IL-1 after bacterial infection also is characteristic of malnourished animals (24). The differences from the experimental model in the present study may result, at least in part, from the use of a single cytokine, whereas infection or LPS administration induces a broad spectrum of cytokines. It also is important to consider a distinction between the innate immune response, which is primarily induced by external stimuli such as endotoxin or infectious agents and mediated by cytokines, and the endogenous production of cytokines as constitutive mediators of basic processes such as mobilization of body fuels. It is reasonable to speculate that these latter roles of cytokines might be better preserved in PCM.

As another important consideration, IL-6 synthesized and released into the circulation is a later event in the systemic inflammatory response resulting from TNF and IL-1 stimulation (14a, 19). The IL-6 actions in induction of the acute-phase response were only examined during a 24-h period in this study. If additional doses of IL-6 were studied over a longer time period, a decrease in the response to IL-6 may have become more evident in malnourished animals, because the maximum IL-1 and AG response to IL-6 appeared to be lessening at 16–24 h in the 2% casein group compared with the 20% casein group. Thus the role of nutrient availability in regulation of the acute-phase response may become more prominent at later time points.

In summary, we have demonstrated that protein malnutrition induces a low-grade systemic inflammatory response, particularly in the proinflammatory component. Despite severe protein depletion, there appears to be preservation, at least initially, of the capacity to manifest a normal systemic inflammatory response in terms of signaling responsiveness as well as cytokine and acute-phase protein production in response to the cytokine IL-6. Further studies are needed to identify specific mechanisms in regulation of the acute-phase response altered by PCM, particularly the roles of reactive oxygen species and antioxidant molecules such as glutathione. The distinction between inducible and constitutive production of cytokines and the mechanisms underlying their activation may be of value in understanding the consequences of malnutrition.

GRANTS

This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-50411 (R. J. Smith, P.-R. Ling, and B. R. Bistrian) and DK-43038 (R. J. Smith).

REFERENCES
