Induction and Modulation of Acute-Phase Response by Protein Malnutrition in Rats: Comparative Effect of Systemic and Localized Inflammation on Interleukin-6 and Acute-Phase Protein Synthesis

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ABSTRACT The acute-phase protein (APP) response is regulated by cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor (TNF), but may also be influenced by malnutrition. The aims of this study were as follows: 1) to determine in rats the effect of a protein-deficient diet on IL-6 mRNA expression in intestine, liver and peripheral blood mononuclear cells (PBMC), and on α-1 acid glycoprotein (AGP) and α-2 macroglobulin (A2M) serum levels and hepatic mRNA expression; 2) to compare, in protein-deficient rats, the IL-6 and APP responses after a turpentine (TO)- or a lipopolysaccharide (LPS)-induced inflammation; and 3) to determine the effect of a protein malnutrition on IL-6 mRNA expression in rat PBMC treated ex vivo with LPS. Interleukin-6 mRNA was present in intestine and PBMC but not in the liver of malnourished rats, and was absent in any tissue or cells of controls. A2M was present in the serum from malnourished rats but not after refeeding. AGP mRNA expression was not influenced by protein malnutrition. In malnourished rats, IL-6 serum level peaked later than in controls after TO and LPS treatment. In malnourished TO-treated rats, A2M mRNA increased earlier than in controls and remained detectable later than in controls. AGP mRNA expression after TO was not influenced by protein malnutrition. In PBMC of malnourished rats, LPS-induced IL-6 mRNA expression occurred earlier and lasted longer than in controls. Our results indicate that protein malnutrition by itself induces IL-6 and A2M expression, and that it modulates the APP response to inflammation. J. Nutr. 128: 166–174, 1998.

KEY WORDS: • acute phase response • malnutrition • systemic inflammation • localized inflammation • rats

In response to tissue injury or acute inflammation, many plasma proteins synthesized by the liver exhibit quantitative changes that become apparent at a variable time after onset of the acute-phase response. Proteins with a transient increase in synthesis and plasma concentration are called positive acute-phase proteins (APP), whereas proteins whose synthesis decreases are referred to as negative APP (Koj 1985, Lebreton et al. 1979). In all mammalian species, the regulation of expression of the APP genes is mediated mainly by inflammatory cytokines that include mainly interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor (TNF). The effects of cytokines on the pattern of liver gene expressions during the inflammatory process enable us to distinguish the following two APP classes: class I in humans includes haptoglobin, C-reactive protein (CRP), serum amyloid A (SAA), α-1 acid glycoprotein (AGP) and hemopexin. It is regulated mainly by IL-1 or combinations of IL-1 + IL-6 and glucocorticoids. Class 2 includes fibrinogen, α-1 antichymotrypsin and α-1 antitrypsin and is regulated by IL-6 and glucocorticoids exclusively (Koj 1985, Kushner and Mackiewicz 1993). Similarly, in rats, the gene for AGP (class 1) is induced by IL-6, IL-1 and glucocorticoids, whereas the gene for α-2 macroglobulin (A2M) (class 2) responds to IL-6 or leukemia inhibitory factor and glucocorticoids (Gabay et al. 1996, Hocke et al. 1993, Koj 1985). Two types of injury are commonly used for eliciting an acute inflammatory reaction in animals. The first is a localized inflammation induced by the formation of a sterile abscess after


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subcutaneous injection of turpentine oil (TO). The second is a systemic inflammation produced by intravenous injection of lipopolysaccharide (LPS) (Geisterfer et al. 1993, Scotte et al. 1996).

Nutritional status may affect the acute-phase protein response to TO-induced inflammation because IL-6 and A2M responses are delayed and reduced in protein-deficient rats (Jennings and Elia 1994). In contrast, protein-depleted rats exhibited higher basal levels of A2M before the challenge with TO, whereas serum IL6 levels were not affected (Jennings et al. 1992a and 1992b). Recently, experiments in mice with disruption of the IL-6 gene showed that the induction of acute-phase proteins is dramatically reduced after TO-induced localized inflammation, whereas the protein response is only slightly modified after LPS-induced systemic inflammation (Fattori et al. 1994). These results indicate that IL-6 is an important mediator of the APP response after tissue damage but not after systemic inflammation. Thus the acute-phase response may differ according to the inducing agent employed. However, the influence of malnutrition on the APP response to LPS in rats has not been investigated. This question is of clinical importance, because malnutrition is a frequent complication during inflammatory diseases such as enterocolitis, e.g., Crohn’s disease. Indeed, it has been suggested that malnutrition and nutritional support may affect AGP levels in Crohn’s disease patients (Carlson et al. 1994), as well as CRP levels in malnourished children (Doherty et al. 1993). However, the influence of malnutrition on APP response in humans remains largely unknown.

To achieve better documentation of the effect of protein malnutrition on the synthesis of APP and cytokines, the aims of this study were as follows: 1) to determine in rats the effect of a protein-deficient diet on IL-6 mRNA expression in intestine, peripheral blood mononuclear cells (PBMC) and liver, and on AGP and A2M serum levels as well as on hepatic A2M mRNA expression; 2) to compare, in protein-deficient rats, the AP response to a systemic induction of inflammation with LPS to that induced by a localized abscess obtained with TO treatment; and 3) to study, ex vivo, the effects of LPS on IL-6 synthesis by PBMC obtained from rats fed a protein-deficient diet.

MATERIALS AND METHODS

**Chemicals.** Lipopolysaccharide (LPS) (from Escherichia coli, serotype 055:B5) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical (Saint Louis, MO). TO was purchased from Cooperative Pharmaceutics (Paris, France). Recombinant human IL-6 (rhl-6) was purchased from Boehringer-Mannheim (Meylan, France).

**Experimental protocols.** Six-week-old male Sprague-Dawley rats (~300 g) (Charles River, Cleon, France) were housed with a 12-h light:dark cycle and received a 2.5% protein diet (23 g casein/100 g synthetic diet; UAR, Epinay sur Orge, France) and water. After an acclimation period of at least 7 d, rats were housed in individual metabolic cages and given free access to the control diet or an isocaloric protein-poor (0% casein) diet over 14 d. The composition of the diets is summarized in Table 1. Weight, oral intakes and urinary losses were monitored daily. After 14 d of either diet treatment, an acute-phase reaction was induced by LPS or TO injection. LPS was dissolved in sterile 9 g/L NaCl and injected intravenously via the saphenous vein (600 μg/kg body weight) (Geisterfer et al. 1993). TO was injected at a single subcutaneous site in the back (5 mL/kg) (Geisterfer et al. 1993). In an additional set of rats, the 14-d period of protein-restricted diet was followed by a 14-d refeeding period with the normal 23% casein diet. Rats were killed while under ether anesthesia. Blood was collected via the vena cava and centri-

### Table 1

**Composition of control and protein-deficient diets**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal diet (23% casein)</th>
<th>Protein deficient diet (0% casein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td>230 g</td>
<td>0 g</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>580 g</td>
<td>800 (+ starch)</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td>50 g</td>
<td>60 g</td>
</tr>
<tr>
<td><strong>Cellulose</strong></td>
<td>60 g</td>
<td>60 g</td>
</tr>
<tr>
<td><strong>Mineral salts</strong></td>
<td>70 g</td>
<td>70 g</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td>10 g</td>
<td>10 g</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1000 g</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

1 The energy density of each diet was 18 kJ/g. In the protein-deficient diet group, energy was replaced with isocaloric quantities of carbohydrates (glucose + starch in equal amounts).

2 Containing (mg/kg): phosphorus, 7750; calcium, 10,000; potassium, 6000; sodium, 4000; magnesium, 1000; manganese, 80; iron, 300; copper, 12.5; zinc, 45; cobalt, 0.08; and iodine, 0.49.

3 Containing (μg/kg): retinyl acetate, 19800; cholecalciferol, 6000; and (mg/kg): thiamin, 20; riboflavin, 15; α-pantothenic acid, 70; pyridoxine, 10; inositol, 150; cyanocobalamin, 0.05; ascorbic acid; 800; dl-α-tocopherol acetate, 170; menadione sodium bisulfite, 40; nicotinic acid, 100; choline, 1930; folic acid, 5; biotin, 0.3. (ND, not detectable).

fuged at 1000 × g for 15 min; the serum was stored at −20°C. The liver was rinsed with sterile 9 g/L NaCl, removed and quickly frozen in liquid nitrogen. The intestines were removed, thoroughly rinsed with sterile 9 g/L NaCl, and either scraped with a glass blade or left unscraped before freezing in liquid nitrogen. All experiments were performed according to the guiding principles in the care and use of animals of the French Ministry of Agriculture.

**Assays**

**IL-6 activity.** Serum IL-6 activity was assayed by measuring the IL-6–dependent proliferation of the B9 murine plasmacytoma cell line, as described (Aarden et al. 1987, Helle et al. 1988, Mosmann 1983). Briefly, IL-6 activities were determined spectrophotometrically by assessing the 72-h proliferation of cell line B9 by using serial dilution of plasma and MTT. The cells were incubated at 35°C for an additional 4-h period. The supernatants were removed and 100 μL isopropanol-HCl was added to cells that were incubated in the dark at room temperature overnight. The absorbance was read at 550 nm. A standard curve with 2 × 10³ units/L of rhl-6 was prepared by plotting absorbancy values vs. log concentration of the standard. Values were determined by comparison with the standard curve. A unit of activity is defined as the dilution that gives half-maximal B9 cell growth in 72 h and corresponds to ~2 ng/L rhl-6.

**Immunoelectrophoresis.** The amounts of serum A2M and AGP were determined by rocket immunoelectrophoresis as described (Laurrel 1966). Albumin and AGP were expressed in grams per liter with purified rat albumin and AGP as a standard. In the absence of standard, A2M was expressed as a percentage of the maximal response obtained at 48 h post-TO injection. The level of detection was 0.1% for serum A2M and 2.5 mg/L for serum AGP and serum albumin.

**Nitrogen balance.** After 14 d of either diet treatment, urine was collected before LPS or TO treatment at d 0, and at 1, 3 and 7 d
after TO or LPS injection. Urine was subsequently stored at −20°C before nitrogen assay with the use of the Antek pyrochemiluminescence technique (Antek Instruments, Houston, TX) as described (Ward et al. 1980). An approximation of the nitrogen balance was made by calculating the difference between input and urinary excretion.

**Isolation and handling of PBMC.** Blood was collected from the inferior vena cava in the presence of anticoagulant and incubated ex-vivo with LPS (10 mg/L) for 45 min at 37°C at 800 × g for 15 min; then, PBMC were isolated by ficoll density gradient centrifugation and were incubated for an additional 1, 3, 6, 9 or 24 h. Finally, cells were harvested and frozen at −20°C for mRNA extraction.

**mRNA preparation, Northern-blot analysis and RT-PCR**

**mRNA extraction.** Rat tissues or cells were homogenized at room temperature in 4 mol/L guanidinium thiocyanate, 0.1 mol/L 2-mercaptoethanol and 20 g/L Na-laurylsarcosine. Total RNA were obtained by centrifugation at 120,000 × g for 16 h at 20°C through a 5.7 mol/L CaCl2 layer (Chirgwin et al. 1979). The RNA pellets were dissolved in 5 g/L SDS and 0.01 mol/L Tris-HCl (pH 7.5) and precipitated twice with ethanol. The RNA pellets were diluted in 100 μL of diethylpyrocarbonate-treated water. The purity and yield of total RNA were determined spectrophotometrically. The 260/280 ratio considered acceptable for the purity of RNA preparation was 1.8–2.0. RNA integrity was checked by agarose gel electrophoresis.

**Northern-blot analysis.** Total liver RNA (20 μg), was pooled from five rats as described (Verma et al. 1992), electrophoresed in 10 g/L agarose gel containing formaldehyde and transferred to nylon membrane Hybond-N+ (Amersham, Les Ulis, France) by capillary action. Each cDNA probe was labeled with 0.2–0.4 mCi (7.4–14.8 MBq) of [α-32P]dCTP (3000 Ci/mmol) (1.1 × 108 GBq/mmol) (Isotop-Pharmacia, France), and 100 pmol/L of each specific primer sequence, in a 13.6 μL reaction buffer (Tris-HCl, KCl, 2.5 mmol/L MgCl2) containing 8 pmol/L hexamer random primer pd(N6) (Pharmacia, Orsay, France), 1 μg prokaryotic chloramphenicol acetyl transferase (CAT) RNA as an exogenous internal standard (Jean et al. 1996), 10 mmol/L of each dNTP (Pharmacia), 100 units RNAse (Promega, Charbonnières, France), 50 units murine Moloney leukemia virus (MMLV) reverse transcriptase (Promega). First-strand cDNAs were obtained after 1 h at 37°C.

**Reverse transcription.** Reverse transcription (RT) was conducted with the use of 1 μg of total RNA in a total volume of 20 μL reaction buffer (Tris-HCl, KCl, 2.5 mmol/L MgCl2) containing 8 pmol/L hexanucleotide random primer pd(N6) (Pharmacia, Orsay, France), 1 μg prokaryotic chloramphenicol acetyl transferase (CAT) RNA as an exogenous internal standard (Jean et al. 1996), 10 mmol/L of each dNTP (Pharmacia), 100 units RNAse (Promega, Charbonnières, France), 50 units murine Moloney leukemia virus (MMLV) reverse transcriptase (Promega). First-strand cDNAs were obtained after 1 h at 37°C.

**Polymerase chain reaction.** The sets of primers (Bioprobe, Montreuil-ss-Bois, France) used are summarized in Table 2. Each oligonucleotide was localized on different exons of the gene under study. The guanine + cytosine content of each set of primers was selected to achieve a high melting temperature (Tm). Polymerase chain reaction (PCR) was conducted in a final volume of 50 μL with 10 μL of the RT mixture, 2.5 units of Taq polymerase (Promega), 200 mmol/L of each dNTP, 0.2–0.4 μCi (7.4–14.8 Mbp) [α-32P]dATP (Isotop-Pharmacia), 100 pmol/L of each of 3` and 5` specific primer sequence, in a buffer consisting of 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, and 2.5 mmol/L MgCl2. The samples were first denatured at 94°C for 3 min; amplification was performed with denaturation at 94°C for 45 s, annealing at the appropriate Tm for 45 s and extension at 72°C for 60 s, for 20–30 cycles as appropriate. The final cycle was followed by a 10-min extension step at 72°C. The absence of contaminants was routinely checked by RT-PCR of negative control samples in which the RNA samples were replaced with sterile water or MMLV RT was omitted.

**Quantitative analysis of amplified products.** Six microliters of each RT-PCR sample was resolved on a 7.5% polyacrylamide gel. The gels were exposed to Kodak film. The levels of amplified product were then measured by quantitative scanning densitometry of autoradiograms. They were normalized to constant amounts of glyceraldehyde-3-phosphate dehydrogenase, CAT, chloramphenicol acetyl transferase.

**Statistical analysis.** Results are presented as means and SD. For serum and liver assays, values were obtained from five rats at each time point in each group. Differences between diet groups were tested using ANOVA followed by the Fisher’s Protected Least Significant Difference test when appropriate. Each group, changes over time were evaluated using ANOVA for repeated measures. P < 0.05 was considered the critical limit.

**RESULTS**

**Variations of food intake, body weight, nitrogen balance and serum albumin level.** After 14 d of protein malnutrition alone, the body weight at d 0 was significantly lower in protein-malnourished rats than in controls (P < 0.001) (Fig. 2A). After TO or LPS injection at d 0, the body weight of malnourished rats decreased further compared with d 0. Injection of TO and LPS had similar effects on food intake and body weight. After induction of inflammation with TO or LPS in control rats, weight did not fluctuate again until d 7 (Fig. 2A) and remained significantly higher than in malnourished rats (P < 0.001). These weight changes were accounted for by a marked decrease in food intake during the 2 d after inflammation in both malnourished and control rats compared with the average daily food intake during the 14 d before inflammation: 13.6 ± 6.3 vs. 23.2 ± 4.6 g/d and 16.7 ± 2.8 vs. 25.1 ± 2.9 g/d (P < 0.01) in the malnourished and control rats, respectively. After d 4 of inflammation, food intake returned to the pre-inflammation level: 26.8 ± 4.6 and 28 ± 3.1 g/d, respectively.

**TABLE 2**

<table>
<thead>
<tr>
<th>cDNA amplified and</th>
<th>Size</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers 1</td>
<td>5<code> to 3</code></td>
<td>bp</td>
</tr>
<tr>
<td>A2M: Upper</td>
<td>CGTGCGGCTGCTTCTCCAGCT</td>
<td>330</td>
</tr>
<tr>
<td>A2M: Lower</td>
<td>GTCGCAACGGCCTGTATCA</td>
<td>330</td>
</tr>
<tr>
<td>IL-6: Upper</td>
<td>AGCAGAGTCTTTCAGAAGSAG</td>
<td>325</td>
</tr>
<tr>
<td>IL-6: Lower</td>
<td>TTTCYCTRAGAGAAACACAT</td>
<td>56</td>
</tr>
<tr>
<td>Actin: Upper</td>
<td>CAGAGCAGAGAGACCTATC</td>
<td>535</td>
</tr>
<tr>
<td>Actin: Lower</td>
<td>CTCCTCTAGTGGTCGACCGG</td>
<td>60</td>
</tr>
<tr>
<td>GAPDH: Upper</td>
<td>COATGACACCTTGGCCATC</td>
<td>262</td>
</tr>
<tr>
<td>GAPDH: Lower</td>
<td>ATGTCACTCCACAAGCAG</td>
<td>60</td>
</tr>
<tr>
<td>CAT: Upper</td>
<td>CAGGCCACATCTTGGCGAATA</td>
<td>385</td>
</tr>
<tr>
<td>CAT: Lower</td>
<td>TCGCCAGGCGCGTGGTTATCA</td>
<td>64</td>
</tr>
</tbody>
</table>

1 In each set of primers, the oligonucleotides were localized on different exons of the gene to avoid genomic DNA contamination. A2M, α-2 microglobulin; IL-6, interleukin-6; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CAT, chloramphenicol acetyl transferase.
it remained negative in protein-restricted rats, whereas it returned to the pre-inflammation level at d 7 in control rats ($P < 0.001$).

A significant reduction of the serum-albumin level was already observed at d 0 in protein-restricted rats ($P < 0.05$), and the level remained significantly lower than in controls after inflammation (Fig. 2C). Albumin level was significantly decreased at d 2 in both groups; only those rats given the control diet had a normal level of serum albumin at d 7 post-inflammation ($P < 0.01$).

**Effects of a protein-deficient diet alone or after a localized abscess on serum levels and hepatic mRNA amounts of A2M and AGP.** After 14 d of protein-deficient diet, rats fed that diet had A2M present in serum, whereas it was not present in controls (see d 0, Fig. 3A); in contrast, the serum level of AGP was not affected by protein deprivation (Fig. 3B, d 0). After TO injection, the A2M serum level showed a maximum at 48 h in control rats; in contrast, in malnourished rats, A2M levels were not significantly different than controls from d 1 through 3, but the A2M level was higher than in controls at d 7 (168 h) ($P < 0.05$, Fig. 3A). In both groups, the serum AGP level was maximal at d 2, with no significant difference between groups. However, a greater AGP level was still observed at d 7 in the rats fed the protein-deficient diet vs. controls ($P < 0.05$, Fig. 3B).

The hepatic mRNA for A2M was detectable after 14 d of protein-deficient diet consumption, whereas it was absent in controls (Fig. 4). In malnourished TO-treated rats, the early 6- to 9-h increase of A2M mRNA post-TO injection was much more pronounced than in controls. Moreover, A2M mRNA was still detectable at 168 h post-treatment in malnourished rats. In contrast, after normalization with ribosomal 18S-RNA standard, there was no significant difference for AGP mRNAs between the two groups. AGP mRNA expres-

**FIGURE 1** Determination of the amplification cycle numbers. For evaluating α2-macroglobulin (A2M) mRNA levels, glyceraldehyde-3 phosphate dehydrogenase (GAPDH) and chloramphenicol acetyl transferase (CAT) (data not shown) standards were used, and the number of polymerase chain reaction (PCR) cycles was chosen within the linear phase. The levels of amplified product were measured by quantitative scanning densitometry of autoradiograms.

**FIGURE 2** Effects of a protein-deficient diet on the body weight (panel A), nitrogen balance (panel B) and serum albumin concentration (panel C) of rats after induction of inflammation. Rats received either a 23% or a 0% casein diet 14 d before receiving an injection of turpentine oil (TO) (5 mL/kg) or lipopolysaccharide (LPS) (600 μg/kg) at d 0. Data are means ± SD, n = 5. Differences between diet groups at a time: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Changes over time: panel A: a, $P < 0.05$ vs. d 14; panel B: a, $P < 0.05$ vs. d 0 (23%); panel C: a, $P < 0.05$ vs. d 0 (23%); b, $P < 0.05$ vs. d 0 (0%).
Effects of an protein-deficient diet on α-2 macroglobulin (A2M) and α-1 acid glycoprotein (AGP) serum levels after a turpentine oil (TO)-induced inflammation in rats. Rats received either (23%) or (0%) casein diet for 14 d before injection of TO (5 mL/kg) at time zero. In the absence of standard, A2M was expressed as a percentage of the maximal response obtained at 48 h post-TO injection. The level of detection for serum A2M was 0.1% and for serum AGP was 2.5 mg/L. Data are means ± SD, n = 5. Differences between diet groups: * P < 0.05. Changes over time in each diet group: a, P < 0.05 vs. h 0; b, P < 0.05 vs. h 9; c, P < 0.05 vs. h 24; d, P < 0.05 vs. h 48; e, P < 0.05 vs. h 72.

Effects of an protein-deficient diet alone or after an LPS-induced systemic inflammation on serum levels of A2M and AGP. As indicated in Figure 5, the maximum 48-h increase of serum A2M and AGP levels in well- and malnourished rats was less marked after LPS- than after TO-induced inflammation (P < 0.001). After LPS injection, the increase in serum level for A2M did not differ between groups until after d 1. Thereafter, the increase of A2M was significantly greater in malnourished rats from 48 to 168 h (Fig. 5A). In contrast, there was no difference in serum AGP between groups at any time (Fig. 5B).

Effects of refeeding after a period of protein-deficient diet on A2M serum levels. After refeeding, previously malnourished rats returned to their basal body weight between day +4 and +7 and continued to gain weight until the end of the experiment (Fig. 6). In contrast, the serum A2M level was highest at d 0 (after the period of protein-deficient diet intake) and lowest at d +14 after reintroduction of the normal protein diet.

Effects of an protein-deficient diet alone on IL-6 mRNA expression in liver, intestine and PBMC. In the absence of inflammation, IL-6 mRNA was absent in any organ or cell of well-nourished rats as analyzed by RT-PCR (Fig. 7, 23% lanes). In contrast, in malnourished rats (0% lanes), IL-6 mRNA was present in intestine and PBMC, but absent in the liver and in mucosal scrapings of intestine.

Effects of an protein-deficient diet on IL-6 serum levels after LPS- vs. TO-induced inflammation. In the absence of inflammation, no serum IL-6 activity could be measured in either controls or malnourished rats (data not shown). The maximal responses of serum IL-6 activity at chosen times were not significantly different between the well- and malnourished rats, during both LPS- and TO-induced inflammation (Fig. 8). However, there was a delay in the kinetics of IL-6 synthesis in the malnourished compared with well-nourished rats. Indeed, in malnourished rats, serum IL-6 level peaked at 9 and 24 h in LPS- and TO-treated rats, respectively, in contrast with 6 and 9 h, respectively, in controls (P < 0.001, Fig. 8).

Effects of ex vivo LPS stimulation on IL-6 synthesis by PBMC. PBMC from protein-deficient and control rats were stimulated ex vivo by LPS for the periods of time indicated in Figure 9. Interleukin-6 mRNA was maximal at 3 h and none was present at 24 h in cells from well-nourished rats,
PROTEIN-DEFICIENT DIET AND ACUTE-PHASE RESPONSE

FIGURE 5  Effects of a protein-deficient diet on α-2 macroglobulin (A2M) (panel A) and α-1 acid glycoprotein (AGP) (panel B) serum concentrations after a lipopolysaccharide (LPS)-induced systemic inflammation compared with turpentine oil (TO)–induced localized inflammation in rats. Rats received either a 23% or a 0% casein diet for 14 d before injection of LPS (600 μg/kg) at time zero. In the absence of standard, A2M was expressed as a percentage of the maximal response obtained at 48 h post-TO injection. The level of detection for serum A2M was 0.1% and for serum AGP was 2.5 mg/L. Data are given as means ± so, n = 5. Differences between diet groups: *P < 0.05, **P < 0.001. Changes over time in each diet group: a, P < 0.05 vs. h 0; b, P < 0.05 vs. h 9; c, P < 0.05 vs. h 24; d, P < 0.05 vs. h 48; e, P < 0.05 vs. h 72.

whereas it was maximal at 6 h and still present at 24 h in cells from malnourished rats.

DISCUSSION

Severe protein malnutrition influences the ability of the body to face an infectious or inflammatory challenge. Some experimental and clinical studies have suggested that malnutrition may affect cytokine and acute-phase response (Carlson et al. 1994, Doherty et al. 1993, Jennings et al. 1992b). Liver A2M synthesis is reduced in rats fed a protein-deficient diet during a localized inflammation (Jennings et al. 1992b). Because IL-6 is the predominant proinflammatory cytokine in the control of hepatic APP production (Gabay et al. 1996, Koj 1985, Kushner and Mackiewicz 1993), we investigated whether synthesis of A2M and IL-6 could be altered after a severe protein deficiency. This study has shown that A2M mRNA was expressed in the liver and that its protein was present in the serum of malnourished rats. In addition, we have shown that IL-6 mRNA was present in PBMC and intestine of these rats, but not in control rats. However, no serum IL-6

FIGURE 6  Effects of refeeding rats a normal diet (23% casein diet) after a period of protein-deficient diet (0% casein diet) on body weight and α-2 macroglobulin (A2M) serum concentration. Rats were given a protein-deficient diet for 14 d. At d 0, rats received a normal protein diet. In the absence of standard, A2M was expressed as a percentage of the maximal response obtained at 48 h post-turpentine oil (TO) injection. The level of detection for serum A2M was 0.1%.

FIGURE 7  Effects of a protein-deficient diet alone on interleukin-6 (IL-6) mRNA expression in liver (L), peripheral blood mononuclear cells (M), intestine (I) and scraped intestine (si). The levels of amplified product were measured by quantitative scanning densitometry of autoradiograms. Control (C) is IL-6 mRNA obtained from PBMC treated for 1 h with LPS (see Materials and Methods). Data represent three separate experiments performed with tissues or cells pooled from 5 rats.
liver that was observed after a severe protein-deficient diet. The source of IL-6 production in intestine could be epithelial cells (Isaacs et al. 1992) or equally, the mononuclear cells of the lamina propria (Stevens et al. 1992). In this study, the fact that IL-6 expression was observed in the whole intestine but not in the mucosal scraping suggests that the site of IL-6 production was mainly the mononuclear cells of the lamina propria. However, some contribution of epithelial cells and of mononuclear cells of mesenteric lymph nodes cannot be completely ruled out.

Of particular interest is the finding that A2M synthesis was suppressed when rats were refed after the period of protein deprivation. This result suggests that the phenomenon of IL-6 synthesis by PBMC of malnourished rats is reversible and that it is tightly associated with the amount of protein in the diet.

In contrast to the increase in A2M synthesis seen in malnourished rats, we did not observe any changes in serum AGP levels. Although the synthesis of A2M is controlled by a unique cytokine (IL-6), regulation of AGP is under the control of a complex set of cytokines (Kushner and Mackiewicz, 1993) that may be influenced in different ways by protein malnutrition. Finally, because a basal production of AGP already takes place in the liver in physiologic conditions (Hocke et al. 1993), weak modifications in AGP synthesis may be more difficult to detect.

After LPS- as well as TO-induced inflammation, A2M mRNA expression increased earlier and remained more pronounced at d 7 in malnourished rats than in controls. Despite this early mRNA expression, serum A2M levels differed significantly from controls only after 24 h in malnourished rats receiving LPS and after 72 h in TO-treated rats. This cannot be explained by some late increase in serum IL-6 activity, because no residual IL-6 activity could be detected after 48 h (data not shown). Alternatively, the sustained level of A2M at late time points may be accounted for by an increased mRNA stability and/or transcription activity. Finally, the reorientation of the flow of amino acids provided by muscular proteolysis toward the liver during the inflammatory process may allow an adequate synthesis of A2M in the first days.
Despite severe protein malnutrition (Ballmer et al. 1991). Surprisingly, studies have shown that the maximal response of AGP, among other APP, was overexpressed after TO-compared with LPS-induced inflammation, despite similar IL-6 activity levels in LPS- and TO-treated rats (Geisterfer et al. 1993). Similarly, our results (Fig. 5) demonstrate that A2M belongs to this group of overexpressed proteins and that its overexpression was not abolished by protein depletion. To date, this differential expression of APP remains unexplained.

At present, the source of IL-6 expression in intestine is unclear. Although IL-6 mRNA is barely detectable in lamina propria monoclonal cells from normal intestine, it is strongly detected in cells from ulcerative colitis and Crohn’s disease patients (McCabe et al. 1993). Infiltrating leukocytes have also been identified as sources of IL-6 in inflammatory diseases (Stevens et al. 1992). In other studies, IL-6 was detected by immunofluorescence in enterocytes of patients with inflammatory bowel disease as well as in rats after hemorrhagic shock (Jones et al. 1993, Shiroma et al. 1990, Tamion et al. 1997). In our study, IL-6 mRNA was present in the whole intestine, but not in the mucosal scraping; it suggests that the main site for IL-6 synthesis is located in the lamina propria and is probably accounted for by mononuclear cells, in accordance with our findings in PBMC. As a consequence of prolonged protein malnutrition, disruption of the gut mucosal barrier may have allowed small amounts of endotoxin or intestinal bacteria to reach the subepithelial region and elicit IL-6 synthesis in submucosal monoclonal cells. However, the mechanisms and significance of bacterial translocation remain controversial (Schlichting et al. 1995).

Our results indicate that, in rats, the acute-phase response after either TO- or LPS-induced inflammation is only marginally attenuated even after a severe protein malnutrition. This is at variance with observations made in humans with severe undernutrition. In humans, protein deprivation results in a diminished production of IL-1 by blood monocytes (Kauffman et al. 1986, Keenan et al. 1982). Indeed, IL-1, obtained from blood monocytes of patients suffering from a wasting malnutrition, induced an attenuated fever in adult rabbits (Hoffman-Goetz et al. 1981). In severely malnourished children, it has been reported that the response of both CRP and SAA to diphtheria-pertussis-tetanus vaccine was impaired; accordingly, after LPS stimulation, the in vitro production of proinflammatory cytokines such as TNF-α and IL-6 in whole blood from these children was reduced (Doherty et al. 1994). Finally, it has been reported that the responses of serum IL-1 and granulocyte-macrophage-stimulating factor to acute-phase infection of kwashiorkor patients were defective, and that nutritional-repletion therapies restored the production of these cytokines (Aslan et al. 1996).

Our results indicate that a protein-deficient diet by itself induces IL-6 and A2M mRNA expression, and also influences the kinetics of serum IL-6 activities during an LPS-induced systemic inflammation in rats. This study extends former results obtained in TO-treated malnourished rats and supports the need for further evaluation of the clinical relevance of the modulation of inflammatory response by nutritional status.

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