Bacterial Infection Affects Protein Synthesis in Primary Lymphoid Tissues and Circulating Lymphocytes of Rats

Isabelle Papet, Benoît Ruot, Denis Breuille, Stéphane Walrand, Marie-Chantal Farges, Marie-Paule Vasson and Christiane Obled

ABSTRACT Bacterial infection alters whole-body protein homeostasis. Although immune cells are of prime importance for host defense, the effect of sepsis on their protein synthesis rates is poorly documented. We analyzed protein synthesis rates in rat primary lymphoid tissues and circulating lymphocytes after infection. Male Sprague-Dawley rats were studied 1, 2, 6 or 10 d after an intravenous injection of live Escherichia coli. Control healthy rats consumed food ad libitum (d 0) or were pair-fed to infected rats. Protein synthesis was quantified using a flooding dose of L-(4,4,4-2H3)valine. Sepsis induced a delayed increase in total blood leukocytes and a rapid and persistent inversion of the counts. Basal fractional rates of protein synthesis (ks) were 117, 73 and 29%/d in bone marrow, thymus and circulating lymphocytes, respectively. Pair-feeding strongly depressed the absolute protein synthesis rates (ASR) of bone marrow (d 2 and 10) and thymus (d 2–10). The infection per se increased bone marrow, thymus and circulating lymphocyte ks but at various postinfection times. It decreased bone marrow (d 1) and thymus (d 1 and 2) ASR but increased lymphocyte (d 2 and 10) and bone marrow (d 10) ASR. Our results reflect the deleterious effect of anorexia on primary lymphoid tissues. The host defense against bacterial infection exhibited time- and tissue-dependent modifications of protein synthesis, indicating that blood lymphocyte protein data are not representative of the immune system as a whole. Optimization of nutritional supports would be facilitated by including protein synthesis measurements of the immune system.

KEY WORDS: rats • sepsis • protein synthesis • lymphoid tissues • circulating lymphocytes

The hypermetabolic response to bacterial infection alters whole-body protein homeostasis, leading to a negative nitrogen balance and a redistribution of body proteins (1). Indeed, in skeletal muscle, sustained protein wasting occurs mainly via a lasting increase in protein degradation (2,3). On the other hand, protein anabolism takes place in the liver (4), which develops the acute phase response by increasing the synthesis of both resident and exported proteins, essentially the acute phase proteins (4–6). The contribution of the liver to whole-body protein synthesis was doubled in infected compared with pair-fed rats (4).

Despite the fact that the immune system is of vital importance in infection, the extent to which sepsis modifies protein synthesis in immune cells is uncertain. Accurate measurement of the protein synthesis of the whole immune system, which has been estimated to account for roughly 8% of the whole-body protein synthesis in humans (7), is a problem in itself because immune cells are present everywhere. Nevertheless, it is possible to quantify protein synthesis in primary and secondary lymphoid organs and in circulating cells. Protein synthesis has already been measured in lymphocytes from humans (8–10) and sheep (11) and shown to be sensitive to stress factors such as surgery, acquired immune deficiency syndrome, combined stress hormone infusion and lipopolysaccharide challenge. In addition, a huge increase in protein synthesis was reported previously in the spleen of infected rats (12).

This paper describes sepsis-induced alterations in protein synthesis in bone marrow and thymus (primary lymphoid organs) and in blood lymphocytes (80–85% of blood leukocytes in rats) using our rat model of long-lasting sepsis (13). It consists in an intravenous (i.v.) injection of live bacteria and reproduces a sustained and reversible hypercatabolic state as observed in patients. Measurements were performed at various postinfection times as follows: d 1 and 2 correspond to the important reduction of both food intake and body weight, d 6 is characteristic of the chronic phase and d 10 corresponds to the beginning of the recovery period. Protein synthesis is dependent on food intake. Healthy control rats consumed food ad libitum (d 0) or were pair-fed to the intake of infected rats (d 1, 2, 6 and 10) to establish whether protein synthesis changes were specific to the infection per se or to the concomitant anorexia. In addition, we were able to determine whether lymphocyte protein data could be representative of that of lymphoid organs. The albumin synthesis rates of these rats were reported (6,14).
MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (n = 54; Ifta Credo, L’Arbresle, France) were housed individually in wire-bottomed cages in a temperature-controlled room (22°C) with a 12-h light/12-h dark cycle. They were fed a purified diet containing 12% protein, distributed by an automatic device (15) and had free access to water. Body weight and food intake were recorded daily. At the end of the 6-d accretion period, rats weighed ~300 g. They were divided into 9 groups (n = 6) of equal mean initial body weights. The first group consisted of healthy control rats consuming food ad libitum and injected i.v. with Sb(0) (10 mg/kg). The second group consisted of healthy control rats consuming food ad libitum and injected i.v. with Sb(0) (10 mg/kg), but were pair-fed to the mean intake of infected rats. Infected and pair-fed rats were studied at 1, 2, 6, or 10 d postinjection. Four extra rats were used to determine the basal enrichment of the tracer amino acid required for protein synthesis measurements. Experiments complied with the NIH guidelines (16).

Leukocyte subpopulation counts. Blood was withdrawn into EDTA-containing tubes from a lateral tail vein. Absolute counts of leukocyte, lymphocyte and polymorphonuclear neutrophil (PMN) were subpopulations were determined on a flow cytometer (Epics XL, Beckman Coulter, Villepinte, France) equipped with an argon laser (488 nm emission) and interfaced to System II software. Whole blood was stained by immunoreaction with a fluorochrome conjugated monoclonal antibody anti-CD45 (Beckman Coulter); then an equal volume of fluorospheres (Flow-Count spheres, Beckman Coulter) was added. After 15 min of incubation at room temperature, the sample was prepared using the Q-Prep Epics immunology work station (Beckman Coulter). The cells were recognized on the basis of their size and the CD45 staining, which permitted identification of leukocytes and exclusion of other cell types (a few contaminating erythrocytes), debris and aggregates from the analysis. The absolute count of the cells (cells/L) was normalized on the basis of the number of fluorospheres counted. Intra- and interassay CV were <2% using a suspension of fluorospheres (Flow-Check, Beckman Coulter) uniform in size and fluorescence intensity (17).

Measurement of protein synthesis. Rates of protein synthesis were quantified in vivo by using the flooding dose method (18,19), which reduces uncertainty over the labeling of the tracer amino acid in the precursor pool for protein synthesis, and which is suitable when exported proteins and high turnover proteins are issues. Rats received an i.v. bolus injection of [4,4,4-2H3]valine (150 μmol/100 g body weight) in 60 atom % excess (massTrace, Woburn, MA), 0.5 mL/100 g). Within 1 min of the injection, Sb(t) was recorded for 45 min by synchronized gas chromatography/mass spectrometry (quadrupole mass spectrometer quadrupole (Helwett-Packard, Paris, France). The fractional rate of protein synthesis ks (%/d) was calculated as [Sb(t) − Sb(0)]×100/AUC, where Sb(0) (atom % excess) is the mean basal enrichment of valine from protein at the end of the incorporation period and AUC (atom % excess × d) is the area under the curve of the enrichment of tissue free valine during the incorporation period. For the circulating lymphocytes, the enrichment of plasma free valine was used because the amount of free valine extracted from lymphocytes was too low to measure its enrichment. Protein content was measured using the bichromatic acid procedure (BCA Protein Assay Kit; Pierce Chemical, Rockford, IL). The absolute rate of protein synthesis (ASR) was calculated by multiplying ks by the total amount of protein and expressed in milligrams of protein synthesized per day. For lymphocytes, ASR could not be expressed for the whole body because blood volume was not determined. The protein content of isolated lymphocytes was independent of the group and the mean was 58.7 ± 3.3 (n = 22) μg/106 cells. Lymphocyte ASR was calculated by multiplying ks by lymphocyte protein content and lymphocyte count and was expressed as follows: mg protein synthesized/(d × L blood).

Statistics. Data are presented as means ± SEM, n = 6. The significance of differences was analyzed by one-way ANOVA and subsequent Fisher’s test when appropriate. P-values < 0.05 were considered significant. All statistical analyses were performed using StatView for Windows, version 5 software (SAS Institute, Cary, NC).

RESULTS

Animal characteristics. Food intake and body weight data were reported earlier (6,14). Briefly, food intake of infected rats was only 5–10% of the control during the early phase (d 1–3 of infection). This anorexia led to considerable body weight loss until d 4, which was more pronounced in infected rats than in their respective pair-fed rats (52 and 37 g maximum cumulative loss, respectively). Thereafter, rats regained weight because food intake increased progressively and reached the initial value 9 d after the bacterial injection. At d 10, infected rats were 15 g lighter and pair-fed rats 10 g heavier than before the injection.

Leukocyte counts. Leukocyte absolute count was ~10–11 × 10^9/L in control and pair-fed rats (Fig. 1). The number of leukocytes was significantly higher in infected rats than in pair-fed rats at d 6 and 10. The leukocyte counts, 83 to 88% of lymphocytes, did not differ between control and pair-fed rats. Bacterial infection induced a rapid and marked inversion of the counts because PMN were 65% of leukocytes at d 1 in infected rats. This percentage decreased thereafter but was not normalized by d 10 (28% in infected vs. 11% in control rats).

Bone marrow and thymus protein contents. At d 0 in rats consuming food ad libitum, protein contents of bone marrow from two tibiae and thymus were 7.7 and 49.3 mg, respectively (Table 1). Pair-feeding increased protein content in bone marrow at d 2 and 6, but decreased it in thymus at d 2, 6 and 10. Infected rats had lower protein contents in both bone marrow samples (except for d 10) and thymus than their pair-fed counterparts.

Protein synthesis. In control rats consuming food ad libitum (d 0), ks was highest in the bone marrow, intermediate in the thymus and lowest in circulating lymphocytes (Table 1). The effects of the pair-feeding and of the infection per se depended on the time postinfection and were specific to each immune compartment. The bone marrow ks was lower in all pair-fed rats consuming food ad libitum. By contrast, neither thymus nor circulating lymphocyte ks was affected by pair-feeding. The bone marrow ks was 149 and 86% higher in infected rats than in the respective pair-fed rats at d
corresponding pair-fed rats, respectively.

PMN, polymorphonuclear neutrophils. Bars are means ± SEM for n = 6.

Different from the group of control rats consuming food ad libitum, *P < 0.05 vs. control rats consuming food ad libitum.

FIGURE 1 Effect of bacterial infection on leukocyte counts in rats. PMN, polymorphonuclear neutrophils. Bars are means ± SEM for n = 6.

**DIFFERENT FROM THE CORRESPONDING GROUP OF PAIR-FED RATS, P < 0.05.**

Different from the corresponding group of pair-fed rats, †P < 0.05.

N, number; ASR, absolute synthesis rate; †Different from the corresponding group of pair-fed rats, P < 0.05.

TABLE 1

<table>
<thead>
<tr>
<th>Effect of bacterial infection on protein synthesis in bone marrow, thymus and blood lymphocytes in rats</th>
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<tr>
<td><strong>Day 0</strong></td>
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<td><strong>Bone marrow</strong></td>
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<td>ASR, mg/(L · d)</td>
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1 Values are means ± SEM; n = 6. ks, fractional synthesis rate; ASR, absolute synthesis rate.

2 Bone marrow was collected from the 2 posterior tibiae.

3 Different from the group of control rats consuming food ad libitum, ASR, absolute synthesis rate.

4 Different from the corresponding group of pair-fed rats, P < 0.05.

5 Different from the respective pair-fed controls at d 2 and 10.

6 The infection induced a decrease of 45% in thymus ASR at d 1 and 2 only compared with the respective pair-fed rats. Blood lymphocyte ASR was greater in infected rats than in their respective pair-fed controls at d 2 and 10.

DISCUSSION

Understanding pathophysiology in humans is of primary importance in providing effective nutritional support to patients. Compared with other animal models, our rat model of long-lasting sepsis more accurately reproduces the sustained and reversible hypercatabolic state observed in patients (13). Basically, muscle stores are mobilized in infected rats (2) as in patients (21), and amino acids are used to support crucial functions, especially acute phase protein synthesis (4,5). Changes in immunologic function occurring in hypercatabolic situations could also increase the utilization of amino acids.

This paper presents the first quantification of protein synthesis in rat primary lymphoid tissues and circulating lymphocytes after infection. The use of the flooding dose technique for immune cells is appropriate because their half-life ranges from 6 h to several days and their resident time in any compartment is much longer than the short time of tracer incorporation. In healthy well-nourished rats, ks decreased progressively in bone marrow, which is composed of stem cells, then in thymus, which is the site of maturation, followed by the circulating mature cells. This is consistent with their replicative and metabolic activities. Primary lymphoid tissues exhibit higher ks than the intestine (71%/d) and the liver (60%/d), two other metabolically active organs (12). Circulating lymphocyte ks (29%/d) was similar to that of spleen (32%/d) and lungs (24%/d) obtained from similar rats (12). In humans, lymphocyte ks (7–14%/d) (8–10,20,22,23) was lower than in growing rats as described also for muscle proteins and albumin (5,12,14,24).

In the present experiment, pair-fed rats exhibited considerable thymus atrophy, which is a dramatic feature of protein-energy malnutrition (25). Blood leukocyte counts and bone...
marrow protein content were affected only slightly by food restriction. By contrast, food restriction had a dramatic and long-lasting effect on bone marrow and thymus protein synthesis rates. Although the mechanisms are different, ASR of bone marrow and thymus were profoundly depressed in pair-fed rats at d 10 compared with control values. This result was unexpected because ASR was normalized on d 10 in all other tissues studied previously (12). Our data reflect the dramatic effect of undernutrition on immune function resulting from a reduction in the production and the maturation of immune cells and leading to an impairment of their cellular activities (17,25,26). The measurement of protein synthesis rates appears to be a valuable index for analysis of these alterations. Our results demonstrate the importance of avoiding periods of food restriction in maintaining an efficient immune system, especially the primary lymphoid tissues.

Because of the accompanying anorexia, the host response to infection is a result of both food restriction and infection per se. An interaction between these two factors cannot be ruled out for certain; however, the infection-induced changes in immune cells are discussed here in the context of pair-feeding as the most appropriate control. The bacterial infection induced a generalized inflammatory response (acute phase response), through an immunoinflammatory cascade activation involving the cytokine network and other mediators (27). The major modifications in circulating pools of leukocytes observed in the present experiment are in agreement with the perturbations reported in rats after a single injection of endotoxin (28–30). The increase in PMN, which are usually the first phagocytic cells at the site of infection, is caused by both an increase in myelopoiesis and a greater rate of release of granulocytes from the bone marrow. Indeed, the protein content of bone marrow was inversely correlated with the PMN count ($r^2 = 0.4, P < 0.05$). The low bone marrow ASR at d 1 likely resulted from a high release of PMN, whereas intense myelopoiesis probably contributed to the high value observed 10 d after the infection.

The host defense against bacterial infection exhibited time- and tissue-dependent modifications of protein synthesis, which probably reflect the specific role of each component of the immune system in protecting the host. Lymphocyte ks increased markedly 1 and 2 d after the infection, likely due to such activation. Similar observations have been reported in humans 24 h after moderately stressful surgery (8), in AIDS patients compared with either HIV-positive or healthy volunteers (10) and in sheep after an 18-h lipopolysaccharide challenge (11). By contrast, in healthy volunteers, protein synthesis of circulating T lymphocytes did not respond to a cortisol challenge (23) and decreased after a combined stress hormone infusion (9).

Infected rats exhibited considerable and persistent atrophy of the thymus. The acute phase response stimulates the hypothalamic-pituitary-adrenal axis responsible for thymic apoptosis (31). Thymic apoptosis contributes to cellular immunodeficiency but represents a beneficial temporary switch from the adaptive immune response to a less specific but more intense reaction. This eliminates potentially harmful autoreactive T lymphocytes (31,32). Our results indicate that thymic apoptosis would be associated with low ASR and that protein synthesis likely plays a role in thymic atrophy induced by infection.

It is tempting to estimate the contribution of the immune system to whole-body protein synthesis and determine whether circulating cells would be a good indicator. Due to the diversity of immune cells and the fact that they exhibit specific behavior, total protein synthesis of all immune cells cannot be estimated from lymphocyte data. On the basis of whole-body protein synthesis previously reported in similar rats [~6 g/d (4)], primary lymphoid tissues plus blood lymphocytes would represent a very low percentage of the whole-body protein synthesis (~1%) in rats whether infected or not. Immune cells invade the secondary lymphoid organs during infection. Spleen ASR has been shown to comprise 0.7 and 2.5% of the whole-body protein synthesis in pair-fed and infected rats, respectively, studied at d 2 (12). Similarly, the ASR of whole intestine (immune cells represent ~25% of mucosa cells) increased by 20% 2 and 6 d after infection (12). From a quantitative point of view, secondary lymphoid organs contribute much more to whole-body protein synthesis than primary lymphoid tissues plus blood lymphocytes. Thus, ASR of the secondary lymphoid organs should be considered when quantitative assessment of protein synthesis and amino acid requirement are concerned.

In conclusion, the time course of protein synthesis quantified in primary lymphoid tissues and blood lymphocytes is consistent with the known activation of immune function after a bacterial infection. Unfortunately, circulating lymphocyte protein metabolism did not reflect that of the immune system as a whole. Our results emphasize the need to avoid any excessive food restriction in humans to preserve an efficient immune system and to provide nutrients to critically ill patients. Further investigations are required to optimize the nutrient supply and ensure an efficient immune defense; one important aspect would be to include protein synthesis measurements of the immune system.

**LITERATURE CITED**


Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. Am. J. Physiol. 279: E244–E251.


