Cytokines, Muscle Proteolysis, and the Catabolic Response to Infection and Inflammation (43423)

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Apart from fever, the most striking characteristic of infection to the layman and the professional alike are the development of anorexia and associated weight loss. Although the unintentional weight loss is composed of both fat and lean tissue, it is the loss of the lean tissue representing protein (nitrogen) that has the greatest clinical impact. The relationship among inflammation and infection, protein metabolism, and endogenous mediators has been both a general research interest for others and a personal one of this laboratory for some time.

Early studies demonstrated a catabolic nitrogen response to fever and infection (1, 2). Still other investigators reported that factors derived from white blood cells, which were at that time collectively termed leukocyte endogenous mediator (LEM), could be identified and postulated their role in these responses (3, 4). LEM was considered to be a group of low molecular weight, heat-labile proteins that were synthesized and released by circulating and fixed monocytes of the reticuloendothelial system in response to inflammation and infection (4, 5). In the study of the role of these factors in the protein metabolic response to inflammation and injury, initial work employed the amino acid analogs aminoisobutyric acid and 1-aminocyclopentanecarboxylic acid to show a direct hepatic effect of LEM to take up, transport, and incorporate amino acids into protein, specifically acute phase globulin (6–8). Our initial experimental work with LEM and its effect on protein metabolism employed a relatively crude product produced by intraperitoneal injection of shellfish glycogen into rabbits and harvesting a cell-free supernatant (9). This study compared the effect of saline, heat-treated LEM, and LEM infused for 30 h in fasted rats on whole body protein kinetics, oxidation, and protein synthesis rates in liver and skeletal muscle. Endogenous amino acid oxidation was found to be increased to nearly twice the control values in the animals receiving LEM. Nonsecretory protein synthesis in the liver was also approximately doubled in the rats receiving LEM, whereas skeletal protein synthesis rates were unchanged. The urinary excretions of N^{+}-methylhistidine and hydroxyproline were used to estimate the rates of skeletal muscle and collagen protein breakdown, which rose by 30% and 42%, respectively. These results employing dynamic measurements of protein metabolism were consistent with the hypothesis, which has been largely unchanged up to the present, that there is a redistribution of body protein following injury or inflammation, with mobilization of peripheral protein (skeletal muscle and collagen) to support increased hepatic protein anabolism in both secretory and nonsecretory components while whole body amino acid oxidation is also enhanced. A corollary is that LEM is the proximate endogenous mediator that is largely responsible for this activity. Subsequent study has sought (i) to further elucidate details of this process using recombinant monokines (interleukin [IL] 1-α and -β, and tumor necrosis factor-α) that are the active components of LEM, either singly or in combination; (ii) to use more sophisticated modeling to allow assessment of individual tissue protein breakdown rates; and (iii) to explore the effects of a series of perturbations, such as malnutrition, feeding, and major organ dysfunction, specific drugs, such as nonsteroidal anti-inflammatory agents, and specific nutrients, such as fish oil, on the integrated and presumed beneficial components of the metabolic response to injury-inflammation.

Improvement in Understanding the Metabolic Role of Cytokines in Protein Wasting

The method we used to assess protein metabolism dynamically was generally the continuous infusion method. In laboratory animals wtih the availability of direct tissue sampling, estimates of protein synthesis

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were calculated based on the incorporation of the tracer from the tissue free amino acid pool into the protein-bound pool (10–13). Protein breakdown was determined from the dilution of specific radioactivity in the tissue free amino acid pool relative to the plasma pool (11, 12). The algebraic sum of these isotopic estimates of protein synthesis and breakdown rates is in general agreement with independent assessment of tissue growth.

The next advance was in the investigation of the individual and combined effects of the various monokines made available by recombinant technology. The biological response of rats to infusion of recombinant murine IL-1α was examined over nearly a 2 log dose range. Fever, hypozincemia, and leukocytosis were produced, but there were no changes in whole body leucine kinetics or in protein synthetic rates in the rectus abdominis or in the hepatic nonsecretory protein fraction (14). This data provided strong evidence that this murine recombinant IL-1 was not the same as monocytic derived IL-1, the biological activities of which had been well characterized previously. The other major recombinant IL-1, IL-1β, and recombinant tumor necrosis factor (TNF)-α, a component of monocytic-derived IL-1, alone and in combination with IL-1β, were subsequently investigated for their effects on protein metabolism (15). TNF has overlapping biological activities with IL-1, and it had been hypothesized that muscle catabolism was mediated by this factor (16). Using the tracer model previously described that allows simultaneous estimation of protein synthesis and breakdown in different tissues as well as investigating the effect of repeated cytokine infusions on urinary nitrogen excretion, we characterized the effect of several doses (20 μg/kg and 100 μg/kg) on leucine turnover and 2, 2, 20, and 200 μ/kg) on nitrogen balance of IL-1β, TNF-α, or the combination of both. Muscle proteolysis was significantly increased by TNF-α and synergistically increased when combined with IL-1β. More prolonged infusion of TNF-α alone or with IL-1β also significantly increased nitrogen excretion. Changes in the liver protein parameters were less dramatic, but did confirm a net anabolic effect of TNF on the liver. These results supported the hypothesis that the redistribution of body cell mass occurring with inflammation and infection characterized by increased net protein oxidation, skeletal muscle proteolysis, and net hepatic anabolism in both secretory and nonsecretory compartments can be reproduced by TNF-α or more relevant to the clinical situation synergistically by IL-1β and TNF-α co-infusion.

Certain issues are unresolved. The putative mechanism for increased proteolysis in skeletal muscle was via production of prostaglandin E_2 stimulated by IL-1 (17). In an *in vitro* model used in this study the monocytic-derived IL-1 of Dinarello was shown to increase prostaglandin E_2 secretion and muscle protein breakdown and to be prevented by indomethacin. However, attempts to block proteolysis by indomethacin were unsuccessful in burned or septic rats (18, 19). Similarly ibuprofen failed to alter changes in whole body protein metabolism induced by monocytic-derived IL-1 (13). However, indomethacin, with its wider spectrum of enzyme inhibition, was able to reduce leucine oxidation and urinary nitrogen excretion while enhancing the acute phase protein response to monocytic-derived IL-1 in guinea pigs (20). One hypothesis that could explain this disparate data is that a lyosomal pathway of protein degradation which does not involve arachidonic acid metabolites may be operative, since indomethacin does have additional anticatabolic activity (18).

A second unresolved issue is the seeming lack of effect of recombinant IL-1 or TNF *in vitro* on skeletal muscle proteolysis. Goldberg *et al.* (21) were unable to detect a catabolic effect of TNF-α, IL-1α or IL-1β singly or together, as well as a number of other cytokines, on proteolysis after incubation with muscle *in vitro*, although a monocyte-derived IL-1 as well as several serum factors isolated from infected animals, including the fraction continuing IL-1, were confirmed effective (21). In an accompanying study, these authors also showed that repeated single daily doses for 5 days *in vivo* in rats caused fever but did not alter prostaglandin E_2 or increase protein breakdown in muscle tissue examined *ex vivo* (22). Using similar methods, Moldawer *et al.* (23) also found that TNF *in vitro* incubation with recombinant products did not regulate protein metabolism in muscle, although monocyte-derived IL-1 was active. Antibody to IL-1 eliminated the lymphocyte activation, but not the skeletal protein degration induced by monocyte-derived IL-1. Furthermore, IL-1α and IL-1β, as well as TNF-α were able to increase prostaglandin E_2 production despite a lack of activity on skeletal protein degradation, leading these investigators to conclude that prostaglandin E_2 levels do not regulate skeletal protein balance *in vitro.* Although the *in vitro* method of assessing skeletal protein degradation is inherently catabolic (15, 21, 22) and thus, perhaps, less able to detect protein degradation induced by other agents (15), a distinct possibility remains that some other agent or the proper mix of IL-1 and TNF may be required to produce changes *in vitro.* This, of course, does not diminish the strong and repeated finding that TNF-α and the combination, as well as monocyte-derived IL-1 containing them both, are very potent proximate causes for skeletal protein degradation when provided *in vivo* by continuous infusion. This latter method has been consistently used by our group in order to simulate more closely the effects of infection or inflammation.

Recently, we conducted a study looking at the effect of the chronic (7-day) infusion of IL-1α, TNF-α,

or the combination compared with saline and pair-fed controls (J. Schwartz and B. Bistrian, unpublished observations). Each cytokine and the combination produced anorexia and weight loss, but not greater than the equivalent degree of semistarvation. However, only the chronic IL-1-TNF group had a significantly cumulative negative nitrogen balance differing from all other groups. Protein kinetics measured on the last day of infusion revealed a decrease in whole body protein flux in all cytokine groups. Total liver protein and weight were dramatically increased in both TNF groups despite a reduction in fractional synthetic rate due primarily to an even greater reduction in the breakdown rate. Only the IL-1-TNF group had a reduction in the total skeletal protein synthesis rate. Increased skeletal protein breakdown was seen in all cytokine groups, with the catabolic ratio greatest in the TNF groups. Thus, it appears that despite some diminution of effect with time, due perhaps in part to some tachyphylaxis and in part to reduced body protein stores, the overall process that serves to increase hepatic protein mass at the expense of skeletal and collagen protein is maintained. The principal mechanism appears to be an increased catabolic rate in muscle and a reduced catabolic rate in the liver.

Other Variables (Malnutrition, Feeding, Diet Composition, and Liver Function) that Can Alter Cytokine Effects

The principal focus of this review has been on the effect of cytokines on protein metabolism in normal animals. A much smaller body of data exists on the effects of common physiologic and pathophysiologic conditions on cytokine action. We assessed the ability of crude monocyte-derived IL-1 from malnourished patients of the marasmic or hypoalbuminemic malnutrition type to induce fever and hypoferremia in a rabbit (24). There was a diminished ability in patients with hypoalbuminemic malnutrition that was restored by 7 days of adequate feeding by total parenteral nutrition, but no impairment was seen initially or after feeding in marasmus. These observations suggested that cytokine production is affected by prolonged stress plus malnutrition, but not by malnutrition alone, and is rapidly restored by feeding. In a second clinical study, only patients with hypoalbuminemic malnutrition were once again assessed before and after feeding. There was a reduced capacity to synthesize monocytic-derived IL-1 that, when restorable by adequate feeding, was associated with an improved clinical outcome (25).

The effect of feeding on the host response to monocyte-derived IL-1 has been explored in animal studies (26). Protein-depleted guinea pigs failed to exhibit fever, granulocytosis, or characteristic changes in amino acid oxidation or acute phase protein synthesis in response to monocyte-derived IL-1 or endotoxin, although trace mineral (iron, zinc) depression was seen after IL-1 but not endotoxin. Short-term (1 day) administration of total parenteral nutrition did not restore responsiveness. It was concluded that both an inability to produce interleukin 1 endogenously or to respond to exogenous IL-1 occurs with protein malnutrition, which is not corrected by short-term substrate provision.

Diet composition may also be an important variable. The influence of dietary lipid intake as either menhaden or safflower oil on changes in protein metabolism in rats receiving recombinat IL-1β, TNF-α, or the combination was studied. Animals fed menhaden oil, which is high in omega 3 fatty acids, had lower rates of leucine oxidation and significantly larger livers. Liver weight was even greater after TNF-α or the combination. Thus, a diet high in omega 3 fatty acid appeared to modulate in a beneficial manner some of the effect of monokines to increase liver size and protein oxidation.

Finally, the liver has a pivotal role in the protein metabolic response to infection or inflammation, since the increase in liver size and activity facilitates the increase in acute phase protein synthesis and gluconeogenesis. A portacaval shunt in rats that diminishes liver size and function was employed as an experimental model to test the effect of monocyte-derived IL-1 (20). IL-1 increased protein turnover, an acute phase protein, α1-acid glycoprotein, and urinary excretion of nitrogen and urea in sham-operated animals, but not in the shunted group. These findings support the concept of the essential role of the liver in the acute phase response.

The clinical implications of these studies principally conducted in animals are, of course, speculative. The protein metabolic response to infection and inflammation is generally considered to be beneficial over the short term of 7–10 days in previously well-nourished individuals, although some of the consequences, such as anorexia and net skeletal protein catabolism, are potentially harmful, with the development of malnutrition and immunoincompetence over time. These studies would be consistent with the now common practice of treating significant malnutrition before elective injury, such as major surgery in order to restore the full responsiveness of the acute phase response. In a similar manner patients already stressed and malnourished (i.e., beyond 7–10 days without feeding) would probably benefit in terms of restored responsiveness. Finally, a new era has been heralded with the recent evidence that substrates such as fish oil may modulate the infection-injury response by reducing some of the adverse consequences of inflammation while enhancing the immune component (27).

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