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CACHECTIN: Its Impact on Metabolism and Nutritional Status

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INTRODUCTION

Bacterial, viral, and parasitic infections, as well as trauma and many forms of neoplastic disease, elicit a well-characterized series of changes in host metabolism. These alterations include anorexia, loss of carcass protein and fat, changes in blood leukocyte patterns, altered hepatic secretory protein synthesis, trace mineral redistribution, and frequently, variations in energy expenditure and in carbohydrate and fat utilization. It is now generally recognized that these acute changes in host physiology often promote recovery in the host, by increasing resistance to the pathogen, by supporting the anabolic needs of immune tissues, and by reducing the degree of tissue destruction (15, 29, 70). However, these alterations in normal homeostasis, when prolonged, may lead to the depletion of host protein and energy stores and ultimately result in tissue damage and organ system failure (15, 94).

These changes in host metabolism, once ascribed to the direct actions of the pathogen or its products on host tissues, are now recognized as being a series of characteristic endogenous host responses. Many if not all of the biological responses to infection, injury, or cancer can be ascribed to the host production of endogenous mediators and particularly to the release of cytokines. This "new found" appreciation for the essential role of endogenous inflammatory cytokines has provided a focus for renewed efforts to block the catabolic changes occurring in infectious disease, as well as to promote those host responses leading to wound healing and improved bactericidal responses.

Although leukocyte-derived mediators of the host response to inflammation have been investigated for almost a century, the isolation, characterization, and demonstration that cachectin is one of these key endogenous mediators has only occurred since 1980. In the past three years, however, the cloning of the human and mouse proteins and the development of specific polyclonal and monoclonal antibodies to human cachectin have permitted a more rapid and detailed analysis of this protein's function during inflammation. Information obtained to date confirms that cachectin plays a central and proximal role in determining how the infected or injured organism responds to an inflammatory stress.

BRIEF HISTORY OF CACHECTIN AND THE HOST RESPONSE TO INFLAMMATION

The role that endogenous hormonal factors play in the wasting of some tissue proteins during inflammation, as well as many of the anabolic changes that occur simultaneously in some organs, has been the subject of considerable investigation during the last 40 years. Prior to the isolation and characterization of cachectin and interleukin 1 in the late 1970s and early 1980s, there was

considerable evidence that products of the immune response were responsible for many of the changes in somatic tissue that occur during inflammation. In the early 1950s, Bennett & Beeson revealed that a product of "activated" leukocytes produced fever when administered to a healthy animal (10). Later studies by Atkins and Bodel at Yale identified this host mediator as an endogenous pyrogen and showed that it was a product of the immune system and not due to the direct action of bacterial cell products (reviewed in 4). This latter finding was important, since it redirected investigation away from the earlier perceived notion that bacterial cell products in themselves were toxic to tissues. Rather, these initial studies highlighted a new approach to investigating the underlying mechanisms that regulate host responses to inflammation.

In the late 1960s and early 1970s, two principal groups of investigators sought to delineate the multiple functions of these newly described leukocyte products. Kampschmidt and Upchurch at the Noble Foundation (51–53) and Wannamacher and Beisel at Fort Detrick (8, 86, 129, 130), among others, undertook several studies clearly demonstrating that protein products of elicited cell exudates were responsible for the leukocytic, trace mineral, and hepatic protein changes that occur during inflammation. These investigators isolated what they believed to be at the time a single or, at most, two separate classes of heat-labile proteins, which were originally termed "leukocyte endogenous mediators." For the first time, such studies confirmed that endogenously produced products of the immune system were directly responsible for many of the changes in somatic tissue metabolism that occurred during inflammation. In the late 1970s, Dinarello and Murphy (42, 77, 99), as well as others, independently showed that these leukocyte endogenous mediators were similar to the previously described proteins "endogenous pyrogen" and "lymphocyte-activating factor." At the time, consensus was developing that the variety of host responses to inflammation, including fever, trace mineral, leukocyte, and hepatic protein changes, were the result of a single peptide family, interleukin 1.

Simultaneously, during the late 1970s, Cerami and colleagues at Rockefeller University were studying a novel macrophage-derived cytokine, which they had termed "cachectin" (54). These investigators had sought to determine the underlying mechanisms behind cachexia, a chronic wasting diathesis common to a variety of invasive disease states of varying etiology. They observed, initially in rabbits infected with *Trypanosoma brucei,* that considerable wasting of body protein and fat occurred despite relatively light parasite burdens (reviewed in 13). Paradoxically, during the final weeks of life, the infected rabbits became severely anorexic and exhibited a striking plasma lipidemia. In later studies, the authors demonstrated that the elevated plasma lipids were due to hypertriglyceridemia associated with very low

density lipoproteins, VLDL (47). The defect in plasma lipid clearance appeared to result, at least in part, from a profound systemic suppression of the enzyme lipoprotein lipase (triacylglyceroprotein acyl hydrolase EC 3.1.1.34).

A comparable model of suppressed lipoprotein lipase activity was also noted in mice treated with bacterial endotoxin. Utilizing the endotoxin-sensitive C3H/HeN and -resistant C3H/HeJ strains of mice, Kawakami & Cerami (54) demonstrated that a serum factor from endotoxin-sensitive mice could produce lipoprotein lipase suppression in endotoxin-resistant animals. Thus demonstrating that a humoral factor conferring this effect was produced in endotoxin-sensitive animals, Beutler and Cerami went on to isolate this protein, cachectin, as a product of tissue macrophages. In subsequent studies, Beutler and Cerami purified to homogeneity cachectin from the endotoxin-stimulated murine macrophage cell line, RAW 264.7, and characterized it as the 17-kilodalton protein responsible for lipoprotein lipase inhibition (17).

Immediately thereafter, Beutler & Cerami (15) demonstrated that the amino terminal sequence of purified murine cachectin was highly homologous to human tumor necrosis factor. In addition, the two peptides shared a spectrum of bioactivities and immunological cross-reactivity. Subsequent genetic analysis soon confirmed that cachectin and tumor necrosis factor were indeed identical, which suggests an underlying unity between the pathologic sequelae of cachectin and the therapeutic potential of tumor necrosis factor (14, 23).

The relationship between cachectin, interleukin 1, and the earlier described proteins, endogenous pyrogen and leukocyte endogenous mediator, has only recently begun to resolve (61). Since the purification and cloning of cachectin (87, 88) and two interleukin 1 species, alpha and beta (65), it is now recognized that these two families of protein, interleukin 1 and cachectin, share a number of important biologic functions once attributed to leukocyte endogenous mediator or endogenous pyrogen. Indeed, there is now sufficient evidence to suggest that interferon B_2 (also termed hepatocyte-stimulating factor or interleukin 6), another product of stimulated tissue macrophages and fibroblasts, shares considerable biologic activity with cachectin and interleukin 1 (7, 59, 97, 123, 134). However, more importantly, evidence is accumulating that many biologic functions originally attributed to interleukin 1 can now be ascribed to cachectin. The synergistic as well as unique actions of cachectin have clearly placed it in a central and proximal position for influencing how the host responds to invasive disease. Only a few years ago it was generally assumed that many components of the host response to inflammation could be attributed to the synthesis and release of a single class of peptides, thought to be interleukin 1, but it is now clear that the response is not mediated by an individual protein, but is an orchestrated cytokine response.

The evidence for cachectin's role as a central mediator of the nutritional consequences of infections, trauma, and malignancy currently rests on two lines of evidence. The first and perhaps most convincing is the data recently obtained by studies with recombinant-derived protein and anticachectin antibodies. Either by directly administering recombinant cachectin to otherwise healthy animals, or by adding these pure preparations to tissue cultures, large amounts of data have been obtained that delineate the biological actions of this protein. In many cases, such data have revealed that many, if not all, of the catabolic changes occurring in infections and disease can be reproduced by administration of purified protein. Such information has provided evidence that cachectin acts on a wide variety of tissue types, in a classic hormonal fashion.

Administering cachectin antibodies to infected or injured animals has also revealed a number of key functions that this monokine plays during inflammation and disease processes (18, 41, 115). This approach in some regards offers unique advantages over the administration of recombinant protein to healthy animals. The removal of cachectin from the hormonal and paracrine milieu present during inflammation provides additional information regarding the unique functions that this peptide elicits. Such data cannot be easily extrapolated from studies in which recombinant protein is given to healthy animals.

The second approach has been to measure cachectin production directly in various disease processes. Sensitive assays for cachectin now permit a more precise determination of the magnitude and time course of cachectin production in critically ill patients and in experimental models of inflammation. Although these newer techniques have revealed a much greater frequency of cachectin production in such clinical circumstances than originally observed, more precise techniques are required to assess in vivo tissue sources and local production rates. Recent evidence with direct immunocytochemical and molecular hybridization techniques have revealed tissue cachectin production occurring in the absence of detectable serum concentrations (63). Future in vivo studies will include the importance of cachectin as a locally produced paracrine agent as well as current efforts to delineate its systemic endocrine functions.

STRUCTURE OF CACHECTIN AND ITS REGULATION IN DISEASE

Protein Structure and Gene Sequence

During the past three years, the primary structures of human, murine, and rabbit cachectin have been described (49, 87, 88). Based upon the sequence of cachectin's cDNA, human cachectin is composed of a 233-amino-acid

prosequence divided into a 157-amino-acid mature peptide following a 76-amino-acid residue signal peptide. In comparison to murine cachectin, the human peptide has one additional amino acid, histidine, at position 73. Human cachectin also possesses one disulfide linkage (between positions 69 and 101), although such a bond does not appear necessary for either the tertiary structure or the biological activity of the protein. The signal sequence of cachectin is atypical for most secretory proteins, being almost twice as long as seen with many other peptides. However, like other secretory proteins, the signal sequence contains a hydrophobic-rich sequence in the middle and potential trypsin-sensitive cleavage doublets.

The human cachectin gene also contains three intervening sequences (introns) that share considerable homology among rabbits, mouse, and human proteins. In addition, the nontranslated 5' and 3' regions are also conserved, which leads some to speculate that these sequences may regulate the expression of the protein. One such untranslated sequence has recently attracted attention. The spliced mRNA transcript of human cachectin contains a 33-nucleotide 3' untranslated sequence composed entirely of adenosine (A) and uridine (U) residues featuring repeated, overlapping copies of the octamer UUAUUUAU. The sequence also appears in the mRNA coding for other inflammatory mediators including interleukin 1, lymphotoxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferons, and several acute-phase proteins. Recent studies have suggested that this nontranslated sequence determines the relative half-life of the mRNA transcript. Shaw and Kamen have shown that normally long-lived B-globin mRNA transcripts are rapidly degraded by insertion of the 3' untranslated sequence from GM-CSF (104). It is presumed that the presence of such a sequence in the cachectin transcript would thus contribute to the relatively short half-life of cachectin mRNA.

The human cachectin gene has been identified on the short arm of chromosome 6, adjacent to the lymphotoxin locus (80). The gene also lies between the HLA-DR and HLA-A loci, linking them to the major histocompatibility complex. The consequences of the fact that cachectin is located so close to the major histocompatibility complex are unknown, but cachectin does influence the expression of several surface antigens (95).

Sequence homology among human, rabbit, and murine species is nearly 80%. Biologically derived cachectin reveals an association into dimeric and higher oligomeric units in biological fluids or culture medium. Although these associations do not appear to be covalently linked, biological activity has been ascribed to both the monomeric and polymeric forms. Cachectin is also relatively stable over a wide pH range of 5.5 or 10, but can be irreversibly denatured outside that range (43). In addition, the protein loses bioactivity at 100°C and is destroyed by trypsin, elastase, and alpha-chymotrypsin.

Regulation of Synthesis

Historically, endotoxin has been shown to be the most potent in vitro agent capable of eliciting cachectin production by tissue macrophages. Although blood monocytes and tissue macrophages appear to be the principal sources for cachectin, recent studies suggest that natural killer cells, cytotoxic cells, and bone-marrow-derived mast cells are all capable of synthesizing cachectin (13).

In addition, it is now recognized that other agents induce cachectin biosynthesis. For example, Hotez et al (47) reported that trypansome or plasmodium cell lysates induce peritoneal exudate cells to synthesize cachectin. Wong & Goeddal (135) also reported that a variety of viral particles, including the construct poly I–poly C, stimulate cultured blood monocytes to increase their pool of cachectin mRNA and synthesize the protein (135). Dinarello has observed that toxic shock exotoxin-1 is a potent inducer of cachectin biosynthesis (48). In addition, Vlassara et al (126) recently reported that phagocytosis of aged erythrocytes is in itself a sufficient stimulus to induce an in vitro cachectin response. In general, the results confirm that cachectin can be elicited by a variety of both exogenous and endogenous mechanisms, arguing a more generalized induction than one limited to endotoxin or gram-negative bacteria.

Beutler and Cerami have examined the regulation of cachectin secretion in peritoneal macrophages in response to in vitro induction with endotoxin (16). Rather surprisingly, unstimulated macrophages express low but detectable levels of cachectin mRNA; however, there was no evidence that the transcripts are being translated. These findings have been confirmed in vivo. Lonnroth et al (63) recently reported a constitutive cachectin mRNA production from spleen and liver of healthy mice. The presence of a pool of cryptic mRNA is thought to permit a more rapid translation and synthesis of the protein in response to stimulation.

Exposure to endotoxin increases the rate of cachectin mRNA transcription as well as inducing an efficient translation of the message (16). Prior treatment of macrophages with dexamethasone suppresses cachectin production in response to endotoxin at the levels of both transcriptional and posttranscriptional regulation. Conversely, gamma-interferon exerts a permissive effect on macrophage synthesis of cachectin (25, 100). Although not an inducer of cachectin synthesis directly, gamma-interferon appears to augment endotoxin-induced cachectin secretion. This permissive influence is also achieved at the level of gene transcription and posttranscription; one explanation is that gamma-interferon suppresses the synthesis of short-lived repressors of cachectin transcription (25).

Once synthesized and released, cachectin has a short half-life in vivo. In the rabbit, radiolabeled cachectin is cleared from the circulation with a

half-life of approximately six minutes (19), a value reasonably similar to that seen for another monokine, interleukin 1. The majority of protein is degraded in the liver, kidney, and skin.

Most cell types possess specific high-affinity receptors for cachectin despite the fact that only certain tissues appear responsive to the protein. Indeed, cells responsive to cachectin have always been found to bind protein, but binding, internalization, and degradation can also occur in cell types for which no biologic response can be ascribed. Studies of binding kinetics for cachectin receptors have demonstrated that most cell types possess between 500 and 5000 receptors per cell (2). These receptors have a high affinity, such that at least 50% are occupied by concentrations of cachectin within the physiologic range. In addition, more recent studies have shown that only a low level of occupancy (5%) is required to elicit a response (119). These receptors share a similar affinity for the lymphokine lymphotoxin. Because they share considerable homology (30%), it has been suggested that these two peptides were the products of tandem gene duplication (88). The sharing of a receptor for cachectin and lymphotoxin can explain many of the similar in vivo actions of these two cytokines.

The number of cell surface receptors can be regulated by several factors. Gamma-interferon strongly upregulates the number of receptors, although their affinity is unchanged (2, 119, 120). In contrast, interleukin 1 and phorbal-myristate-acetate appear to downregulate the number of cachectin receptors, at least in macrophages, which suggests a close interrelationship between the effects and production of these two hormones (46).

Similarities to Interleukin 1 and Other Cytokines

One of the most perplexing questions facing investigators is the remarkable duplicity in biological actions between cachectin and interleukin 1 (61). These two peptides, both of which are synthesized and released by blood monocytes and tissue macrophages, share little if any sequence homology and act through distinct receptors, yet they overlap significantly in their biologic actions. Whereas both proteins regulate many of the nonspecific host responses to inflammation, cachectin does not possess significant lymphocyte proliferative activity and interleukin 1 does not appear to induce irreversible shock and organ failure.

Otherwise, both interleukin 1 and cachectin are induced by similar stimuli. Peritoneal macrophages commonly synthesize both proteins simultaneously, although the time course for stimulus-response varies considerably. Gamma-interferon also upregulates the synthesis of interleukin 1 as it does cachectin, and dexamethasone inhibits interleukin 1 genes' transcription and translation (109). Nevertheless, apparent differences in synthesis of these two monokines do exist. Endotoxin is a potent inducer of both cachectin and interleukin 1

production by blood monocytes, whereas heat-killed *Staphylococcus albus* will elicit principally an interleukin 1 synthesis. In addition, the historical technique of eliciting "leukocyte endogenous mediator" production by shellfish-glycogen-induced rabbit peritoneal exudate results in predominantly an interleukin 1 response, with little cachectin production (L. L. Moldawer and D. Bornstein, unpublished observations). Conversely, the murine macrophage cell line RAW 264.7 stimulated with endotoxin produces copious amounts of cachectin but little if any interleukin 1. Another interesting finding is that cachectin is a strong inducer of both interleukin 1 (30, 79) and interferon B_2 (58) synthesis, although the converse has not been shown.

What remains unresolved is why two such seemingly similar monokines exist. It is possible that these functions are so essential that duplication is required for the host's continued survival. Conversely, sufficient differences in biologic responses may exist for the molecules to survive evolutionary pressures independently. Regardless, the two proteins' existence raises an interesting question about their unique positions in the host's response to invasive stimuli.

BIOLOGICAL RESPONSES TO CACHECTIN

Effects on Food Intake and Weight Loss

Anorexia and weight loss are almost universal findings during inflammation, infection, injury, and many forms of neoplastic disease. In previously healthy individuals with sepsis, lean tissue losses can exceed half a pound per day (56); in experimental animals following endotoxemia, anorexia occurs, skeletal protein synthesis is lessened, and protein content is lost (50). The mechanisms behind this pattern of altered food intake and tissue loss have not been fully resolved but an endogenous cachectin response has been proposed to be at least one partial explanation (15).

Early studies suggested that cachectin did not produce anorexia or weight loss when administered in healthy animals (82), whereas interleukin 1 was a potent anorexia-producing agent (66). In most cases, however, this failure to observe anorexia or weight loss with cachectin could be explained by the low quantities of recombinant human protein administered to rodents. Species specificity between human and murine protein has been reported (107), and the quantities of human protein required to elicit an equivalent response in other species have not been fully defined.

However, in endotoxin-resistant C3H/HeJ mice, 50 μg/kg body weight of human recombinant cachectin produces a transient 10–15% reduction in food intake (73). At higher intakes, greater reductions are observed. In rats receiving 250 μg/kg body weight twice daily, 40% reductions in food intake are observed (74, 118). However, continued intraperitoneal administration often

results in tachyphylaxis, such that after five days, almost 2 mg/kg body weight/day are required to maintain a 40% reduction in food intake.

Loss of body weight and changes in body composition have also been observed with cachectin administration. These changes cannot be fully explained by the associated anorexia. In a phase 1 trial of cachectin as an antineoplastic agent in patients with metastatic disease, 100 $\mu g/m^2$ of human recombinant protein resulted in weight loss (131). In rats given recombinant human cachectin, Tracey et al (118) reported that such rats lose significantly more body protein and water while, despite a negative energy balance, they preserve body fat relative to comparably pair-fed controls. Such changes in body composition are often similar to those seen in some chronic inflammatory states.

Skeletal Muscle Protein Metabolism

Several independent lines of evidence suggest that cachectin can regulate both energy-substrate and protein metabolism in skeletal muscle. Only recently though has there been an appreciation of the important role that monokines play in protein homeostasis in skeletal muscle. Historically, protein wasting in skeletal muscle has been considered in terms of macrohormonal and substrate metabolism. Prior to 1983, the losses of skeletal protein observed in patients with sepsis, trauma, or surgical injury were generally explained in terms of alterations in the substrate-hormonal milieu (8, 56, 133). Insulin resistance and altered levels of growth hormone, cortisol, and epinephrine present in inflammatory states were thought to largely explain many of the primary catabolic changes occurring in skeletal muscle. More recent studies have confirmed that loss of muscle amino acids can be induced in otherwise healthy individuals by pharmacologically raising the circulating levels of cortisol and epinephrine, although the magnitude of such an increase is generally less than that observed in acute catabolic states (12, 132).

In 1983, studies by Baracos et al (6), Clowes et al (24, 31), Yang et al (136), and Sobrado et al (108) demonstrated that partially purified preparations of activated blood monocytes could increase skeletal protein breakdown. Thought to be mediated in part by prostaglandin E_2, through both a direct action on skeletal muscle and the induction of fever, monocyte products were proposed to increase skeletal protein catabolism through a macrohormonal-independent process (40).

Although more recent studies have questioned whether protein balance in skeletal muscle is regulated by prostaglandin levels (36, 44, 71, 81), the question of whether cachectin in particular, and monokines in general, regulate protein balance remains unresolved. More recent findings with recombinant human cachectin and interleukin 1α and 1β have shown that both preparations increase prostaglandin E_2 production in murine extensor dig-

itorum longus preparations (71), but do not increase muscle protein degradation or decrease muscle protein balance (44, 71, 112).

Although most investigators have been unable to demonstrate a direct tissue effect of cachectin (or interleukin 1) on skeletal muscle protein dynamics, evidence exists that skeletal protein kinetics in vivo can be influenced by cachectin. In a recent report, Warren and colleagues (131) observed that recombinant human cachectin administration significantly increases the release of amino acids from the legs of patients with cancer. In addition, rats receiving recombinant human cachectin also lose total body protein at rates higher than similar pair-fed controls (118). Pomposelli and colleagues (93) also observed, using isotopic dilution techniques, that recombinant human cachectin increases skeletal protein degradation in vivo when given at levels less than required to induce shock and hemodynamic changes.

The underlying mechanism by which cachectin administration may alter muscle protein balance in vivo is still unknown, but one focus of recent investigation has been the role of adrenocortical hormones as secondary mediators. Both in vivo and in vitro studies have shown that cachectin stimulates a direct release of ACTH (11), and both patients and dogs given high doses of cachectin have elevated circulating levels of corticosterone (117, 131). Interleukin 1 is also known to stimulate glucocorticoid release, through both a hypothalamic process (11) and directly in the adrenal glands (98), which suggests that synergistic influences of the two monokines during inflammatory disease may lead to a heightened glucocorticoid response and subsequent effects on skeletal muscle.

In addition, there is evidence that cachectin can directly influence the energy state of skeletal muscle. Administration of recombinant human cachectin to rats in quantities sufficient to induce anorexia, weight loss, and skeletal protein net catabolism is sufficient to induce a net reduction of skeletal muscle resting transmembrane potential difference (E_m); this depression appears to be both dose-dependent and inhibited by a monoclonal antibody (116). Earlier investigators suggested that this reduced membrane potential may in part explain the sequestration of intracellular sodium and water accompanying sepsis. In addition, in vitro studies with cultured myocytes demonstrate that cachectin also increases muscle glycogenolysis and lactate production (62). Increased uptake of glucose into muscle cells appears to be facilitated by a cachectin-mediated increase in the synthesis of hexose transporters on the plasma membrane. As a result, cachectin appears to participate in the regulation of skeletal muscle membrane function and the subsequent utilization of energy stores.

Adipose Tissue and Fat Metabolism

It was not merely fortuitous that cachectin was originally identified by its in vivo and in vitro effects on lipid metabolism. Much of the initial work with

cachectin highlighted its anti-anabolic and acutely catabolic effects on lipid metabolism. Reductions in serum lipoprotein lipase activity have also been observed in a variety of inflammatory states, ranging from bacterial and parasitic infections, to sepsis and many neoplastic states.

Much of the work regarding the impact of cachectin on lipid metabolism has been performed on cultured cell lines. For example, the adipocyte cell line, TA1, expresses several adipocyte-specific mRNAs that are not expressed by preadipocytes. Addition of cachectin does not affect cell viability, growth, or differentiation, but completely ablates the expression of adipose-specific genes (14, 113). In addition, cachectin completely blocked the deposition of lipids in differentiating adipocytes and also caused mature adipocytes to lose previously accumulated triglycerides (85). Patton and colleagues (84) also reported that recombinant human cachectin specifically inhibits fatty acid biosynthesis in cultured 3T3-L1 adipocytes by decreasing the upregulation of those genes responsible for acetate incorporation into fatty acid synthesis. In addition, cachectin stimulates lipolysis directly (85).

In rats and dogs given sublethal quantities of cachectin, levels of serum triglycerides and free fatty acids are often increased (117, 118). Such a cachectin-induced inhibition of lipogenesis and accelerated lipolysis may promote a mobilization of lipid reserves to meet the acute metabolic demands of inflammatory responses for host defense. However, a continuous secretion would also lead to exhaustion of nonprotein energy reserves and death. Indeed, in a recent report, Vlassara and colleagues (125) demonstrated in a population of hospitalized patients with cancer that those exhibiting the greatest weight loss are those with the most depressed serum lipoprotein lipase activity.

Liver

Despite a significant loss of whole-body and skeletal protein during inflammatory states, the liver often undergoes a net gain of protein. Increased hepatic protein, DNA, and RNA content have often been reported in experimental animals with trauma, infection, and cancer (50, 56, 69). Yet the changes are not uniform and the pattern of protein and RNA synthesis varies considerably. Hepatic structural protein synthesis and total RNA content are often increased (50). In addition, the pattern of secretory protein synthesis also shifts, with decreases in the transcriptional and translational regulation of albumin synthesis and a corresponding increase in the transcription and translation of the "acute-phase reactant" proteins. As early as 1975 it was known that leukocytic products could directly upregulate the synthesis of several of these positive acute-phase reactants, including fibrinogen, orosmucoid, and haptoglobin (129, 130).

Many of the gross changes in hepatic protein, RNA, and DNA content seen

during inflammatory states have now been reproduced by cachectin administration. A dose of 50 μg/kg body weight/day of human cachectin given to C3H/HeJ mice increases liver protein, RNA, and DNA contents by 22, 31, and 27%, respectively (73). The magnitude of increase is identical to that seen with comparable amounts of human interleukin 1α or 1β.

Although not fully resolved at present, several monokines, independently and together, appear to regulate the synthesis of several acute-phase reactants. Cachectin, interleukin 1, and interferon B_2 (hepatocyte-stimulating factor or interleukin 6) all appear to regulate several components of hepatocyte secretary protein synthesis (26, 89, 90, 96, 134). The quantitative importance of these three monokines remains controversial, as conflicting information has been obtained depending upon the cell lines employed and the secretory proteins examined. However, it appears that cachectin plays a principal role in the downregulation of the albumin gene as well as reducing the rate of albumin mRNA translation (90). This trait is shared by interleukin 1 and by interferon B_2. In mice given as little as 50 μg/kg human cachectin/day, serum albumin concentrations decrease by 45%, similar to the decrease seen with administration of human interleukin 1 alpha (73). In murine hepatocytes and the human hepatocyte cell line Hep2, cachectin also increases the synthesis of several minor acute-phase reactants, including Factor B and properdin (90). However, several lines of evidence suggest that cachectin is not a principal mediator of fibrinogen, alpha$_1$-antiprotease inhibitor, or haptoglobin biosynthesis; these acute-phase reactants appear to be under regulation of the other monokines, interleukin 1 and interferon B_2 (26, 59, 134).

Leukocyte Populations and Erythropoiesis

It is now generally recognized that many putative beneficial responses of cachectin are directed at improving the antimicrobial actions of leukocyte populations. In this regard, some biological actions previously attributed to interleukin 1 have now been ascribed to cachectin. For example, cachectin, and not interleukin 1, is directly responsible for neutrophil degranulation, superoxide production, and lysozyme release (34, 38, 57, 60, 68, 103).

Other investigators have reported that cachectin, like interleukin 1, promotes a release of neutrophils from bone marrow, resulting in neutrophilia (121). However, cachectin also promotes the margination and activation of neutrophils, which is thought to explain the neutropenia secondary to sepsis and bacteremia. Although interleukin 1 produces similar effects, it appears to induce this margination through a prostanoid-dependent pathway; the effects of cachectin are prostaglandin independent (121).

Cachectin also regulates monocyte differentiation and activation, a clear example of autocrine regulation. Cachectin appears to induce the differentiation of myelogenous cell lines along a monocyte/macrophage pathway (76).

Therefore, macrophages responding to invasive stimuli would recruit the differentiation of additional cells from precursor populations. In addition, cachectin is capable of transforming macrophages into an activated state (91, 122), which thereby inhibits viral and parasitic replication intracellularly (67, 135). Cachectin-activated macrophages also exhibit increased cytotoxicity against transformed and virally infected cells (67, 83, 91, 106, 135).

Anemia remains one of the hallmarks of infectious disease, and recent evidence has suggested that cachectin may be one such mediator involved. Tracey et al (118) observed a 40% reduction in total red cell mass in rats treated with sublethal quantities of cachectin. Although they did not study the underlying mechanisms, these authors reported an absence of reticulocytes in the circulation and an extrahematopoietic erythopoiesis in liver and spleen from such animals.

Red cell homeostasis can be altered in at least two different ways, through reductions in the rate of erythropoiesis and by increases in the degradation of circulating red cells. It now appears that cachectin acts directly on both pathways to induce a loss of red cell mass. Moldawer et al (74) observed in rats receiving cachectin treatment a 42% reduction in red cell synthesis, which could explain 90% of the resulting anemia.

In addition to evidence for a reduced erythropoiesis, cachectin also appears to increase the clearance of red cells from the circulation. Vlassara has recently reported that cachectin upregulates the appearance of macrophage receptors specific for aged red cells (personal communication, manuscript in preparation). The authors have shown that elicited macrophages incubated with cachectin accelerated their rate of red cell phagocytosis. Moldawer observed that "aged" red cells from rats treated with cachectin or interleukin 1 have significantly shorter half-lives than those from untreated rats (74). However, significant increases in the clearance of total red cell population by cachectin administration was not observed.

Wound Healing and Tissue Repair

Recent evidence suggests that cachectin may also play an important role in wound healing and tissue repair. Local production of cachectin by infiltrating monocytes has been suggested as a chemotactic signal for neutrophil emigration and activation (68). However, supplementary functions have also been proposed. For example, the discovery that cachectin is a potent angiogenic factor suggests a possible role for the remodeling of local vasculature for healing tissue (35). Cachectin also augments the growth of normal diploid fibroblasts from several tissue sources (124) and is proposed to be an essential growth factor for granulating tissue.

In addition, cachectin has been shown to promote cartilage and bone remodeling processes typical of abscess formation and resorption of cartilage

and bone (27, 101, 110, 111). In bone, this is achieved by accelerating the actions of osteoclasts while suppressing osteoblast formation (110). Cachectin-mediated increases in proteolysis of cartilage and decreases in proteoglycan synthesis are thought to regulate new bone and collagen biosynthesis. Under controlled conditions, such a remodeling is essential for wound healing. However, like many of cachectin's other responses, excessive or continued production produces excessive remodeling, as occurs in chronic inflammatory diseases such as rheumatoid arthritis.

Shock

In the past two years, our knowledge regarding the proximal role that cachectin plays in the pathogenesis of septic shock has increased considerably. Such studies were predicated on the observation of Tracey et al (114) that the tissue responses to high doses of human recombinant cachectin are remarkably similar to those derangements seen in response to endotoxin. Demonstrated first in rats (114) and subsequently in dogs (117), these experiments reveal that infusion of cachectin in physiologically relevant quantities is sufficient to reproduce many if not all of the pathologic sequelae typical of lethal endotoxemia. In rats, high doses of cachectin (700 μg/kg body weight) produce lethargy, piloerection without chills, bloody diarrhea, and tachynpea within minutes. The animals expire with severe metabolic acidosis and respiratory arrest. Postmortem examination reveals significant inflammatory damage to vital organs. Lungs are diffusely hyperemic with histopathologic evidence of focal hemorrhagic areas. Furthermore, there is evidence of arterial occlusion by polymorphonuclear leukocytic thrombi and margination of polymorphonuclear leukocytes through the walls of the pulmonary vessels, accompanied by severe interstitial and peribronchiolar pneumonitis. Segmental ischemia and regional hemorrhage and necrosis of the bowel are also present. In addition, congestion of the kidneys attributable to acute tubular necrosis is seen.

In subsequent studies, Tracey et al (117) examined the dose-dependent hemodynamic and metabolic responses to infused human cachectin in dogs. Sublethal quantities of cachectin (10 μg/kg body weight/day) produce transient hemodynamic and nonsignificant counterregulatory hormonal responses, whereas lethal doses induce irreversible hypotension associated with decreased cardiac function requiring hemodynamic support. Despite fluid resuscitation and a significant counterregulatory hormone response (epinephrine, norepinephrine, and cortisol), 100 μg/kg body weight of human cachectin is uniformly lethal, with shock, organ system failure, and death occurring within three hours.

Investigations have demonstrated that these potent effects are not shared by the other principal monokine, interleukin 1. Administration of recombinant

human interleukin 1β to rabbits produces a transient decline in hemodynamic performance, but the changes are reversible and do not lead to tissue damage or shock (C. Dinarello, personal communication). However, like cachectin, interleukin 1 also has the potential to produce lung damage, through a prostaglandin-mediated margination of neutrophils in the pulmonary epithelium (39). Not surprisingly, a combination of the two monokines, cachectin and interleukin 1, results in severe lung damage at doses far lower than observed when each monokine is given separately (C. Dinarello and J. Van der Meer, unpublished observations).

Goldberg and colleagues reported that cachectin-induced shock is completely reversible by prostaglandin synthesis inhibitors (55). Since cachectin and interleukin 1 are potent inducers of prostanoid synthesis (3, 27, 28, 32, 110), one putative mechanism of cachectin-mediated shock is thought to be excessive production of prostaglandin E_2 and thromboxane B_2, which together lead to vasoconstriction and neutrophil margination. Another contributing factor may be a cachectin-mediated alteration in procoagulant activity by vascular endothelium (20, 78, 92). Such alterations are thought to promote a hypercoagulopathy that can be aggravated by interleukin 1 (20).

Many of the pathobiologic responses to overwhelming invasive stimuli can be directly attributed to the cellular effects of cachectin. Early passive immunization of mice with heterologous anticachectin sera prevents death following injection with lethal quantities of endotoxin (18). This suggests that cachectin is a necessary mediator of the lethal host responses to experimental endotoxemia, and that by preventing the systemic exposure to cachectin, death might be prevented in cases of bacteremia and sepsis. These findings have been carried one step further. Pretreatment of baboons with a monoclonal antibody directed against human cachectin results in survival and hemodynamic stability following a lethal challenge with gram-negative bacteria (115).

CACHECTIN PRODUCTION IN DISEASE STATES

In the past two years, several reports have appeared relating elevated cachectin levels to specific human disease states. Much of this has been attributed to the development of specific enzyme-linked immunoabsorbant assays (ELISA) utilizing monoclonal antibodies specific to human protein. In many cases, the sensitivity of the assay is as low as 15–20 pg/ml, almost 15 times more sensitive than the L-929 cytotoxicity assay when applied to human sera or plasma (250–500 pg/ml).

The first such report utilizing the newer specific ELISA assays demonstrated elevated cachectin levels in a large proportion of patients with such parasitic diseases as visceral leishmaniasis or malaria (102). In addition, other

studies have shown that patients with metastatic cancer frequently have elevated levels of cachectin in their serum (5). This latter finding confirms the earlier in vitro observation of Aderka et al (1) that blood mononuclear cells from patients with a variety of malignancies frequently produce cachectin spontaneously, and when stimulated with endotoxin, produce quantitites greater than seen from cells of individuals without cancer.

In a series of more recent studies, cachectin appearance has been evaluated in critically ill patients with bacterial infections, trauma, or thermal injury (64, 127, 128). Waage et al (128) in a series of studies evaluated a cross section of bacterially infected patients and found detectable cachectin levels in 29% of the patients. This number is remarkably similar to our own observations in patients hospitalized for major thermal injury (24%) (64). However, in a subset of Waage's patient population, detectable serum cachectin levels have been detected in 17 of 18 patients with meningococcal septic shock. In addition, patients with serum cachectin levels in excess of 440 pg/ml invariably die (127).

The frequency with which cachectin has been detected in the circulation of critically ill patients appears to be significantly less than for another monokine, interleukin 1. Interleukin 1 is detected in serum from septic patients (9, 21, 31) and in mice after ultraviolet irradiation (37), a terpentine abscess, cecal ligation, and endotoxemia (72, 109), where cachectin has not been frequently found (72). In addition, interleukin 1 is also seen in the circulation of ovulating women (21), in nonathletes following moderate exercise (22, 33), and even in well-trained athletes at rest (33). In a series of rodent studies, cachectin can only be detected in animals experiencing a lethal endotoxemia and is not routinely observed in animals suffering a mild endotoxemia, terpentine abscess, or cecal ligation (72). As observed by Waage in the clinical studies, the appearance of cachectin in the circulation is generally associated with a poor prognosis (127); such a correlation is not observed with interleukin 1.

The failure to detect cachectin uniformly in a variety of inflammatory states may be attributed to the kinetics of cachectin production. Early studies by Beutler et al (16) revealed a cryptic pool of cachectin mRNA in peritoneal macrophages that can be rapidly translated upon appropriate stimulation. In rabbits exposed to endotoxin, detectable levels of cachectin are observed in the circulation within minutes, and peak concentrations appear within two hours (19). Hesse et al (45) report a monophasic appearance of cachectin, peaking within 120 min and disappearing from the circulation within four hours in baboons subjected to a lethal gram-negative infection (45). Despite the fact that these animals remain bacteremic for almost 18 hours, there is evidence only for a single monophasic appearance curve. Such studies imply that a transient cachectin appearance may be sufficient in itself to initiate and

regulate a chain of events leading to irreversible shock. An inability to detect cachectin in the serum of critically ill patients may reflect an inadequate sampling interval.

An additional difficulty with emphasizing circulating concentrations is that local tissue production of cachectin may be more important than the systemic appearance. Renal cortical cells, Kupffer cells and aveolar macrophages are all capable of synthesizing cachectin, albeit at a lower rate than peritoneal macrophages (D. Hesse and M. Silen, unpublished observations). Nevertheless, since fixed tissue macrophages account for almost 90% of total body macrophages, the quantitative contribution these organs make to whole-body production is sizable. For example, Lonnroth et al (63) report that spleens and tumors of cachectic mice bearing a methylcholanthrene-induced sarcoma are spontaneously producing cachectin and interleukin 1, despite the fact that detectable levels are not observed in the serum. This tumor production is proposed to explain in part the profound cachexia these animals develop (75).

CONCLUSIONS

The diversity of tissue responses to cachectin suggests a key role for this peptide during inflammation. Cachectin must now be considered a primary and pleiotropic inflammatory mediator with both acute and chronic effects on cellular and tissue processes. As an early mediator of the host response to inflammation, cachectin initiates a series of tissue responses that, when taken in toto, promote recovery and survival in the organism suffering an invasive stimuli. By promoting organism-specific and -nonspecific host immunity, directing immune processes at the sites of tissue damage, modulating whole-body protein precursors and energy-substrate availability, and providing the stimulus for initiating a macrohormonal environment that fosters a redistribution of body constituents, cachectin serves to initiate and integrate the multiple changes in host physiology during inflammation.

However, when cachectin release is excessive, or continues for extended periods of time, an exaggerated or continued inflammatory response can in itself induce significant pathology. The continued loss of skeletal protein and body fat, altered substrate utilization and increased energy demands, all mediated in part by cachectin, can lead to tissue wasting and organ dysfunction. Similarly, excessive acute production of cachectin, as seen during endotoxemia or overwhelming gram-negative infections, can result in significant hemodynamic alterations, disseminated hypercoagulopathy, organ damage, and ultimately death.

The challenge for the future rests in a better understanding of the regulation of cachectin's biosynthesis and its mechanism of actions on end tissues. Therapeutic efforts will be directed at potentiating the beneficial host re-

sponses to cachectin, especially during those conditions where the host response to inflammation is insufficient. In addition, efforts to block an inappropriate or excessive production of cachectin may offer an alternative approach to reduce the pathology associated with shock or chronic inflammatory diseases, such as cancer cachexia or parasitic diseases. Through a better understanding of cachectin's actions, a greater insight into the metabolic response to invasive stimuli will be achieved.

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