Biochemistry

 Volume 27, Number 20 October 4, 1988

Perspectives in Biochemistry

The History, Properties, and Biological Effects of Cachectin

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Received May 23, 1988; Revised Manuscript Received July 19, 1988

Implicit in the study of immune function lies the assumption that immune mechanisms work to the benefit of the host. Yet, a species is fashioned by evolution at many levels, and not all mutations that survive selection favor the individual. The finding that certain aspects of the immune response act to the detriment of the host might therefore come as no surprise.

Even the inflammatory response, surely the most primitive immune mechanism of all, may inflict injury or death if it is sufficiently intense or prolonged. Inflammation may be attended by a number of metabolic changes, both local and systemic, and by derangements of host physiology, including fever, hypotension, and organ failure.

Several terminal mediators of inflammation have been identified in recent decades, among them products of the arachidonic acid cascade, bradykinin, platelet activating factor (PAF), and others. Moreover, it has become apparent that the inflammatory response is governed by a relatively small number of polypeptide hormones (collectively termed "cytokines" for want of a better descriptor).

Most remarkable is the fact that individual agents have been implicated as mediators of disease processes that, superficially, seem scarcely related. Thus, the macrophage hormone known as cachectin, or tumor necrosis factor, has been implicated as a factor essential in the pathogenesis of shock in sepsis (Beutler et al., 1985c; Tracey et al., 1986a,b, 1987a,b), wasting in chronic disease, cerebral malaria (Grau et al., 1987; Clark et al., 1987), and other disparate pathologic states. The remainder of this review will deal with the history, structure, biological activity, and biosynthetic control of this hormone, which undoubtedly protects the host, yet often injures it as well.

"Mediated" Disease Processes

It was once widely assumed that invasive pathogens, whether infectious or neoplastic, damaged host tissues directly. Through a mass effect, or through the elaboration of toxins, tumors and microbial invaders were believed to modulate metabolic activities of the host, leading to cachexia, shock, and death. While many believed that humoral mediators were responsible for cachexia in the setting of cancer, few suspected that a connection might exist between this chronic process and the acute disturbances observed in bacterial infections. The concept of immune-mediated disease was seldom invoked to explain either phenomenon. It was well-known that many tumors were capable of secreting hormones that might disturb host metabolism and that specific bacterial toxins [e.g., endotoxin [lipopolysaccharide (LPS)] and various exotoxins] would reproduce much of the symptomatology of shock as it occurred in sepsis.

However, for over a decade, it has been clear that LPS works its effects indirectly. LPS is not toxic to most cultured cells. Moreover, the endotoxin-resistant C3H/HeJ mouse is rendered sensitive to LPS if irradiated and transplanted with hematopoietic progenitor cells derived from mice of endotoxin-sensitive strains (e.g., C3H/HeN). Thus, a cell of hematopoietic origin, or a product produced by such a cell, confers the lethal effect of LPS (Michalek et al., 1980).

The identity of this cell, and the mediator(s) involved, remained unclear until recently. Much suspicion centered on the macrophage, since various facultative intracellular pathogens (e.g., *Bacillus Calmette-Guerin* and *Mycobacterium lepraemurium*) that infect macrophages also greatly sensitive animals to the lethal effect of LPS (Ha et al., 1983; Vogel et al., 1980). Moreover, a factor produced by cultured macrophages in response to LPS was shown to be lethal to C3H/HeJ mice (Cerami et al., 1985).

Perhaps surprisingly, it is now known that a single protein mediator is required for, and can by itself elicit, most of the changes observed in endotoxic shock. The isolation of this mediator was accomplished by two groups of investigators, both engaged in the study of LPS-related phenomena. On the one hand, the protein was isolated as "cachectin", a mediator

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0006-2960/88/0427-7575$01.50/0

implicated in the pathogenesis of wasting and responsible for the elevation of plasma triglyceride seen following injection of LPS. On the other hand, the protein was isolated as "tumor necrosis factor", a cytotoxic mediator responsible for the induction of tumor necrosis following endotoxin challenge. The story of each of these lines of investigation is presented briefly below.

Cachectin

Cachectin was originally conceived as a mediator of wasting in chronic disease. Trypanosome-infected rabbits often display a low-parasite burden yet become anorectic, severely emaciated during the terminal stages of the disease (Guy, 1975). Interestingly, such animals often develop a marked hypertriglyceridemia, associated with deficient expression of lipoprotein lipase (LPL) (Rouzer & Cerami, 1980). Presumably, LPL suppression led to inadequate clearance of plasma triglyceride and, concomitantly, denied adipocytes access to the pool of circulating lipid.

Kawakami and Cerami (1981) showed that mice, like trypanosome-infected rabbits, developed LPL suppression and hypertriglyceridemia following challenge with LPS. This metabolic effect of LPS was shown to be mediated by a serum factor, apparently produced by macrophages (Kawakami & Cerami, 1981). The agent responsible was also capable of suppressing LPL expression in cultured adipocytes (3T3-L1 cells) (Kawakami et al., 1982) and was produced in considerable abundance by the mouse macrophage cell line RAW 264.7 (Mahoney et al., 1985; Beutler et al., 1985b). These findings were utilized for purifying cachectin, which was shown to be a protein with a subunit size of approximately 17 kDa (Beutler et al., 1985b).

Cachectin was entirely inducible by endotoxin and, to a lesser extent, by other invasive stimuli (Kawakami et al., 1984). It was not produced by quiescent cells (Mahoney et al., 1985). A plasma membrane receptor for cachectin was identified on 3T3-L1 cells, C2 myotubules, and a variety of other cells and tissues (Beutler et al., 1985b). The kinetics of cachectin production were studied, and it was found that the hormone was secreted within minutes following intravenous injection of LPS; plasma levels peaked between 90 min and 2 h following injection and then declined rapidly (Beutler et al., 1985d). Large amounts of cachectin were produced both in vitro and in vivo; in response to LPS, cachectin was secreted in quantities sufficient to comprise approximately 1% of the total protein in serum-free conditioned medium (Beutler et al., 1985b). Correspondingly, high nanomolar concentrations were achieved in plasma in rabbits (Abe et al., 1985). Thus, individual animals appeared capable of producing milligram quantities of the protein in response to challenge with a lethal dose of LPS.

Following interaction with its receptor, cachectin was rapidly degraded (Beutler et al., 1985d). This mode of clearance was found to be the principal route by which the hormone was metabolized. It remains to be established whether internalization of the hormone is essential to its biological actions.

Cachectin was found to suppress the expression of a number of mRNA molecules specific for mature adipose tissue, acting at the level of transcription (Torti et al., 1985). Among these mRNAs were those encoding glycerol-phosphate dehydrogenase, the fatty acid binding protein, and others yet to be determined. Other "household" mRNAs, such as actin mRNA, were not affected. Cachectin also suppressed the expression of fatty acid synthetase and acetyl-CoA carboxylase, thereby limiting de novo synthesis of triglyceride (Pekala et al., 1983). Enhanced release of glycerol from fat cells was also observed, suggesting activation of the hormone-sensitive lipase, and differentiation of adipocytes was prevented by cachectin in vitro (Pekala et al., 1984). All of these findings supported the view that cachectin might play a role in the pathogenesis of wasting during chronic disease.

When the amino-terminal sequence of cachectin was determined, a striking homology was noted between the primary structure of this hormone and that of the macrophage-derived hormone tumor necrosis factor (TNF), which had recently been isolated, partially sequenced, and cloned from cells of human origin (Beutler et al., 1985a). Moreover, in reciprocal bioassays, cachectin and TNF were both shown to be highly active in suppressing LPL expression and in lysing susceptible transformed cells. The identity of cachectin and TNF was confirmed when mouse TNF cDNA, cloned with a probe based on the DNA sequence of human TNF, was shown to exactly predict the amino acid sequence of mouse cachectin (Caput et al., 1986). The identity of cachectin and TNF raised many interesting questions concerning the actions of this hormone, as discussed below.

Tumor Necrosis Factor

An entirely different LPS-related phenomenon served to motivate the isolation of tumor necrosis factor. No less dramatic (but surely less common) than shock or cachexia is the hemorrhagic necrosis of certain tumors, as it occurs in the context of bacterial infection. On occasion, sepsis in a tumor-bearing animal or patient is accompanied by hemorrhage within the vascular bed of the tumor, tumor necrosis, and involution. Histologic studies (Algire et al., 1952) suggest that such necrosis is primarily caused by ischemia.

This phenomenon has long been of interest to clinicians, since it offers a potential chemotherapeutic approach: if hemorrhagic necrosis of tumors could be reliably induced by bacterial products, or by endogenous mediators produced in response to them, an effective means of treatment might be at hand. Thus, clinical investigators initially sought to induce tumor necrosis by infusion of bacterial products in crude form (Coley, 1893) and in purified form (LPS) (Shear et al., 1943). The efficacy of this approach was limited by the marked toxicity of the preparations used.

In the early 1960s, it became clear that the necrotizing reaction observed following injection of LPS was due to a transferrable factor produced by the host (O'Malley et al., 1962). This factor, later identified in the serum of *Bacillus Calmette-Guerin* pretreated, endotoxin-injected mice (Carswell et al., 1975) and shown to exert a direct cytotoxic effect on tumor cells, was termed "tumor necrosis factor" (TNF). As had been the case with cachectin, TNF was found to be a product of LPS-activated macrophages (Mannel et al., 1980; Satomi et al., 1981; Fisch & Gifford, 1983; Matthews, 1981). It was purified by following its ability to cause lysis of mouse fibrosarcoma (L-929) cells.

Far from being a selective tumoricidal drug, TNF proved to have marked toxicities of its own, mirroring those of LPS in many respects. Indeed, cachectin/TNF proved to be one of the central mediators of LPS action.

Cachectin as a Mediator of the Effects of LPS

Given its catabolic character, its production in large quantities following LPS challenge, and its strictly hematopoietic source, cachectin seemed a candidate mediator of endotoxicity. This possibility seemed still more likely when it was realized that two very disparate effects of LPS (LPL suppression and tumor necrosis) were both dependent upon the elaboration of this hormone. Perhaps, it was felt, many

of the effects of LPS (including the lethal effect) were mediated by cachectin.

In an attempt to examine this possibility, mice were passively immunized against cachectin prior to challenge with varying doses of LPS (Beutler et al., 1985c). Passive immunization was shown to exert a partial but highly significant protective effect against the lethal effect of LPS, suggesting that cachectin was, indeed, involved in mediating the shock state that ensues following LPS administration. Additional experiments, employing both polyclonal and monoclonal reagents, have since supported this conclusion (Tracey et al., 1987b; Mathison et al., 1988).

Soon thereafter, it was shown that cachectin could induce a shock state very similar to that elicited by LPS. When injected into rats, cachectin caused hypotension, tachypnea, diarrhea, hematuria, hemoconcentration, metabolic acidosis, and an initial phase of hyperglycemia followed by hypoglycemia (Tracey et al., 1986a,b). A variety of inflammatory lesions were observed in these animals, including severe pulmonary leukostasis (which in many instances led to respiratory arrest), necrosis of the gastrointestinal tract, and acute renal tubular necrosis. In dogs, similar changes were observed, together with a generalized stress response marked by elevated plasma cortisol, catecholamine, and insulin levels (Tracey et al., 1987a). In rabbits, the hormone was found to be pyrogenic, causing fever both by a direct central effect and by stimulating production of a second endogenous pyrogen, interleukin 1 (IL-1) (Dinarello et al., 1986).

In most respects, the changes observed following infusion of cachectin in vivo were very similar to those caused by LPS. Moreover, chemical agents known to sensitize animals to the lethal effect of LPS (e.g., D-galactosamine, which lowers the mean lethal dose of LPS by a factor of 10^5) sensitize them to cachectin as well (Freudenberg et al., 1986; Lehmann et al., 1987), and agents that protect against LPS (e.g., glucocorticoids; see below) strongly inhibit cachectin biosynthesis (Beutler et al., 1986a). Thus, a large body of evidence suggests that cachectin is a central endogenous mediator of LPS action.

Cachectin as a Mediator of Wasting

Though it was originally isolated as a mediator of wasting in chronic disease, the chronic effects of cachectin have only recently been studied in detail in vivo. One of the major obstacles to such studies appears to have been the need for slow, constant hormone administration over a period of months. This difficulty was overcome (Oliff et al., 1987) through the design of a novel method for infusion of biologically active material. When genetically modified by transfection with a vector that causes them to secrete recombinant human cachectin, Chinese hamster ovary (CHO) cells produce small nonmetastatic tumors in nude (*nu/nu*) mice which, unlike control tumors containing an empty vector, lead to severe wasting and early mortality. In large part, wasting appears to be attributable to anorexia and, at least superficially, is strongly reminiscent of cachexia observed in association with malignant disease.

In separate studies, rats treated chronically with cachectin were shown to undergo transient weight loss and to develop anemia, related to underproduction of erythrocytes and to their accelerated destruction (Tracey et al., 1988). For reasons that remain unclear, a tachyphylactic response may be observed when cachectin is administered to animals intermittantly (Patton et al., 1987). The mechanisms responsible for resistance to the effects of the hormone are not related to the formation of humoral antibodies and presumably occur at a tissue level. Thus, particularly when cachectin is administered on a daily basis rather than continuously, weight gain may be observed after the initial phase of loss. Other studies (Michie et al., 1987) suggest that a part of this gain is attributable to fluid retention.

Very recently, a number of physiologic studies designed to determine the basis of cytokine-induced anorexia have been carried out, utilizing both cachectin and interleukin 1 (Rothwell, 1988a,b; Coombes et al., 1987). The data suggest that intracerebroventricular administration of cachectin in minute doses causes marked appetite suppression. In addition, central or peripheral administration of the hormone markedly increases net caloric expenditure.

With the improvement of techniques for detection of cachectin in human biological fluids, the hormone has been measured in plasma derived from human cancer patients (Balkwill et al., 1987), as well as patients with meningococcal sepsis (Waage et al., 1987). Consistent with the rapid kinetics of hormone clearance, levels are far lower in the former case, although they may be within the nanomolar range in the latter.

No correlation between wasting and cachectin levels was noted in the studies performed by Balkwill et al.; however, no experiments have yet been designed to assess cachectin levels as related to metabolic demand or anorexia in cancer.

Moreover, it must be emphasized that, in many instances, it is unnecessary to invoke the action of a mediator like cachectin to explain the anorexia and wasting to which patients with neoplastic diseases are subject. Undoubtedly, enteric obstruction, pain, anxiety, and anorexia produced by cytotoxic antineoplastic drugs are sufficient to explain weight loss in many individual cases.

Lymphotoxin

A second hormone, structurally and biologically related to cachectin/TNF and genetically linked to it, is produced by T-lymphocytes and B-lymphoblastoid cell lines in response to activating stimuli that are either cell or tissue specific (e.g., specific antigenic or nonspecific mitogenic challenge). Known as lymphotoxin, this hormone is not produced by macrophages and is not produced in response to LPS. It is elaborated in smaller quantities than cachectin and more slowly with respect to the activating stimulus (Li et al., 1987).

Lymphotoxin was originally discovered as a cytolytic protein presumed to play a role in cell-mediated hypersensitivity reactions (Ruddle & Waksman, 1967, 1968a–c; Ruddle, 1978, 1979a,b). Following its purification, it was noted to be a glycoprotein with significant sequence homology to cachectin/TNF and was shown to bind with high affinity to the same receptor as the latter. It exerts a spectrum of biological activities that is highly concordant with that of the macrophage hormone, although perhaps not identical (Broudy et al., 1987).

Redundancy of the type seen in the lymphotoxin/cachectin "family" is not unknown. A large number of loci encode interferons that display modest structural differences, bind to the same receptors, and exert similar, if not identical, biological effects. IL-1 α and IL-1 β provide additional examples of such "duplication of effort." However, the tissue-specific production of lymphotoxin and cachectin is of considerable basic interest, since each hormone represents the end product of a distinct signaling system and each may be called into service selectively, depending upon the nature of the stimulus. In certain situations, one hormone may be produced to the exclusion of the other, yielding a similar clinical response to different infectious agents.

Alternate Tissue Sources of Cachectin

Since its isolation as a macrophage product, alternate tissue sources of cachectin have been sought. It is now clear that

cachectin is produced in considerable quantities by lymphocytes following activation by simultaneous exposure to phorbol myristate acetate and the calcium ionophore A23187 (Cuturi et al., 1987). Production in response to more natural stimuli capable of activating lymphocytes has not yet been documented. Natural killer (NK) cells have also been shown to produce cachectin albeit in far smaller quantities. Cachectin is not solely responsible for the cytotoxic action of these cells but appears to comprise natural killer cell colony inhibiting activity (NK-CIA) (Degliantoni et al., 1985). Finally, various malignantly transformed cells reportedly produce cachectin when rendered resistant to the cytolytic effect of this hormone or lymphotoxin (Rubin et al., 1986; Spriggs et al., 1987, 1988). The quantity of protein produced by such cells is very small compared to that elaborated by macrophages; however, the significance of this phenomenon as it may occur in vivo cannot be discounted.

The Structure of Cachectin

Intensive structural studies of cachectin have culminated in the production of high-quality rhombohedral crystals, which have now been employed in crystallographic work (Eck et al., 1988). The hormone is clearly trimeric in structure and consists largely of β-pleated sheet, with little or no α-helix. This may account for its relative fragility to mechanical shearing and the variable potency of different preparations of recombinant material in vivo.

The primary structure of cachectin has now been established in four species (human, mouse, rabbit, and cow) (Goeddel et al., 1986). In these, it is 154–157 residues in length. It is anticipated that, when details of the tertiary structure are known, it will be possible to assign functional importance to various parts of the molecule, on the basis of a knowledge of amino acid conservation.

Cachectin contains a disulfide bridge linking two cysteine residues within the protein (Aggarwal et al., 1985); while conserved among all species yet studied, this feature does not appear to be essential for biological activity, since reduced preparations of the hormone are still active in vitro and since lymphotoxin lacks one of these cysteines (Gray et al., 1984) and yet retains biological activity.

Cachectin is initially produced as a prohormone, containing 79 (mouse) or 76 (human) extra amino acids appended at the amino terminus. Interestingly, the propeptide is more highly conserved among species than is the mature hormone, a fact which may suggest that it fulfils a separate function. Recent work has suggested that, at times, the entire prohormone may exist as an integral membrane protein, expressed at the monocyte surface (Kriegler et al., 1988).

The intact prohormone, produced by recombinant means, may shed light on the nature of the receptor binding site, since it may be shielded or distorted prior to processing; indeed, it may also prevent aggregation of the monomers to form the mature, trimeric molecule.

The Cellular Actions of Cachectin

Cachectin not only exerts a direct influence on the metabolic activities of many cells and tissues but also induces the release of a variety of secondary cytokine mediators and low molecular weight inflammatory molecules (e.g., prostanoids, PAF, and leukotrienes). The nature of the signal transduced by cachectin following association with its plasma membrane receptor is not known, and details concerning the structure of the receptor are scarce (Vitt et al., 1987; Smith et al., 1986). Indeed, it remains to be seen whether different signals might explain the effects observed in various tissues. Some of cachectin's effects are produced very rapidly (e.g., augmentation of the adhesive properties of neutrophils in their interaction with vascular endothelial cells) (Gamble et al., 1985). Other effects, including the cytolytic effect and LPL suppressing effect, take several hours to develop, perhaps because they depend upon depletion of a target protein or upon suppression of transcription.

About one-third of transformed cell lines are susceptible to the growth inhibitory or cytolytic action of cachectin (TNF) (Sugarman et al., 1985). The mechanism of growth inhibition appears to be quite different from that which brings about cell lysis (Ruggiero et al., 1987). However, neither process is fully understood in molecular terms.

Well before lysis occurs, the target cell DNA is fragmented into pieces of regular incremental size, suggesting that cleavage may be related to nucleosome spacing (Schmid et al., 1986, 1987). It is possible, although as yet unproved, that DNA fragmentation leads to activation of an ADP-ribosyltransferase activity, which effects cell death by consuming NAD^+ or by irreversibly modifying a critical protein (Carson et al., 1986). It has been noticed as well that, in response to cachectin, certain cells initiate synthesis of proteins of unknown function that may serve to protect the cell from the hormone's cytolytic action (Stolpen et al., 1986; Ruggiero et al., 1987). This may explain the marked sensitization to which both normal cells and tumor cells are subject if treated with transcriptional or translational inhibitors concurrent with cachectin.

The majority of cachectin's biological effects are catabolic. In adipose tissue, for example, cachectin inhibits lipoprotein lipase synthesis (Beutler et al., 1985b; Kawakami et al., 1987), as well as the synthesis of acetyl-CoA carboxylase, fatty acid synthetase (Pekala et al., 1983), fatty acid binding protein, and glycerol-phosphate dehydrogenase (Torti et al., 1985), all of which are involved in the biosynthesis of triglyceride. It also prompts triglyceride release from adipocytes by activating the hormone-sensitive lipase (Pekala et al., 1984). In bone, cachectin serves to activate osteoclasts, leading to resorption (Bertolini et al., 1986). Lymphotoxin also shows this activity, and its release from myeloma cells presents a possible explanation for the osteolytic and hypercalcemic features associated with this disease (Garrett et al., 1987). In cartilage, cachectin promotes the degradation of proteoglycan and inhibits its biosynthesis (Saklatvala, 1986). And when applied to synovial cells, cachectin (like IL-1) triggers the release of collagenase and PGE_2, which both play a role in the destruction and remodeling of tissues at a local level (Dayer et al., 1985).

Cachectin has gained recognition as a mediator of the acute-phase response to inflammatory stimuli (Perlmutter et al., 1986), although it is perhaps not the major mediator responsible for changes monitored in the plasma (Sipe et al., 1987; Koj et al., 1987). By diminishing hepatocyte albumin synthesis and increasing production of several other proteins, cachectin may contribute to the redistribution of biosynthetic resources available to the organism during infection, perhaps abetting the immune response. Cachectin also depresses cytochrome P-450 dependent microsomal drug metabolism in mice and, as such, may later host response to a variety of drugs and metabolites (Ghezzi et al., 1986).

While it is not cytotoxic to most normal tissues, cachectin inhibits the division of cells of the amnion and, as such, when secreted by cells of the decidua, may play a role in the pathogenesis of preterm labor (Casey et al., 1988). Cachectin also inhibits erythropoiesis and myelopoiesis in vitro assay systems (Broxmeyer et al., 1986) and, when chronically administered to animals, leads to the development of a moderate

anemic state (Tracey et al., 1988). It is not known whether this effect is attributable to a direct action of cachectin on stem cells or whether a second mediator, produced by stromal cells, may be involved.

Surprisingly, cachectin is actually growth stimulatory to certain transformed fibroblasts (Sugarman et al., 1985; Vilcek et al., 1986). The mechanism by which replication is augmented remains unclear.

Among the most important effects of cachectin are those that it exerts on circulating leukocytes (particularly neutrophils) and vascular endothelial cells. Many of the gross pathophysiologic effects of the hormone may be explained on the basis of its effects on these cells. Cachectin activates human neutrophils according to a number of criteria. Neutrophil adhesion (Shalaby et al., 1985; Gamble et al., 1985; Klebanoff et al., 1986), phagocytic activity (Shalaby et al., 1985; Gamble et al., 1985), and ability to kill *Candida* organisms (Djeu et al., 1986) are all augmented by cachectin. Cachectin also triggers degranulation, as well as the oxidative burst (Klebanoff et al., 1986; Shalaby et al., 1987), and is reportedly chemotactic for neutrophils and monocytes (Ming et al., 1987). Neutrophil complement receptors are up regulated by cachectin (Berger et al., 1988). Other leukocytes are also affected by the hormone. Eosinophils have been shoown to kill schistosomula more effectively following exposure to cachectin (Silberstein & David, 1986), and monocytes have been shown to release IL-1 in response to the hormone (Bachwich et al., 1986). Cachectin also enhances the response of T-lymphocytes to activating stimuli (Scheurich et al., 1987a,b) and inhibits B-cell differentiation (Kashiwa et al., 1987).

Vascular endothelial cells are affected by cachectin in a variety of important ways. Cachectin promotes the production of IL-1 by vascular endothelial cells (Nawroth et al., 1986; Libby et al., 1986). IL-1 and TNF both act to down regulate the expression of thrombomodulin on endothelial surfaces and cause the production of a procoagulant activity (Stern & Nawroth, 1986; Bevilacqua et al., 1986; Stern et al., 1985). Both of these effects favor coagulation and may contribute to disseminated intravascular coagulation as it occurs in sepsis and in various neoplastic diseases. Cachectin also modulates the expression of certain endothelial antigens (Pober et al., 1986as,b, 1987; Collins et al., 1986; Pohlman et al., 1986), alters endothelial cell morphology and growth viability (Stolpen et al., 1986; Sato et al., 1986; Schweigerer et al., 1987), and favors the adhesion of neutrophils through a direct effect on endothelial cells (Gamble et al., 1985). It is likely that the combined effect of cachectin on endothelial cells and on neutrophils leads to the cell margination and systemic inflammatory changes that are seen in endotoxic shock.

Cachectin has a direct effect on hypothalamic neurons, causing them to produce PGE_2, thereby eliciting fever (Dinarello et al., 1986). A secondary febrile response is also generated as the result of induction of peripheral IL-1 synthesis. Muscle cells are depolarized by cachectin (Tracey et al., 1986a–c); the generality of this phenomenon has not been established; however, it may relate to fluid sequestration as it occurs in shock.

It is anticipated that, since the cachectin receptor is expressed by the majority of somatic cells, future work will reveal that hormone release has other physiologic consequences as well.

Control of Cachectin Biosynthesis

The toxicity of cachectin necessitates strict control of hormone biosynthesis. Cachectin biosynthesis does not occur in unstimulated cells, and with the most sensitive assays available, cachectin is undetectable in the plasma of normal individuals. However, following macrophage activation by LPS or other stimuli, abundant quantities of the hormone are produced within a short period of time (Mahoney et al., 1985; Beutler et al., 1985d; Watanabe et al., 1984). Detectable amounts of cachectin are secreted by cultured macrophages within 15–20 min following induction.

The changes that occur following activation of quiescent cells are complex; however, it is clear that biosynthetic control operates at multiple levels. The cachectin gene is transcribed in resting macrophages (Beutler et al., 1986a), but only low levels of cachectin mRNA are detected within the cytoplasm prior to activation. Moreover, this mRNA is ineffectively translated, since none of the hormone may be detected within the cell or culture medium. Following induction, cachectin gene transcription is augmented some 3-fold (Beutler et al., 1986a); however, cachectin mRNA accumulates to very high levels within the cell, exceeding base-line concentrations by a factor of 50 or more.

At this point, under normal circumstances, the mRNA is efficiently translated to yield secretable protein. However, in macrophages obtained from the LPS-resistant C3H/HeJ mouse or in macrophages pretreated with dexamethasone, little cachectin synthesis occurs despite the presence of cachectin mRNA (Beutler et al., 1986a). Moreover, if LPS is removed from normal macrophages at various times following induction, cachectin biosynthesis ceases immediately, despite the fact that considerable amounts of the mRNA must remain within the cells (Gifford & Flick, 1987).

Thus, LPS influences macrophage synthesis of cachectin at a minimum of two levels. Through a mechanism that is not yet clear, LPS exerts a permissive influence on cachectin biosynthesis, acting at a translational level. It also prompts accelerated transcription of the cachectin gene. This latter effect may itself represent "derepression", since cycloheximide seems to accelerate transcription (Collart et al., 1986).

Dexamethasone counters both of these effects of LPS. It down regulates cachectin gene transcription when added to cells at any time with respect to the addition of LPS. However, it only appears to block cachectin mRNA translation if added in advance of LPS. This may explain the strictly prophylactic effect of glucocorticoid hormones in animals challenged with LPS: both in vivo (Mustafa, 1988) and in vitro (Beutler et al., 1986a), cachectin production is only attenuated if dexamethasone is administered prior to LPS.

Interestingly, other cytokines (notably interferon γ and transforming growth factor β) influence production of cachectin. Interferon γ augments both the transcriptional and posttranscriptional responses to LPS (Beutler et al., 1986b; Collart et al., 1986) while TGF-β inhibits TNF biosynthesis through a mechanism that has yet to be elucidated. Such cytokines may be involved in endotoxin "tolerance" or "priming".

In some measure, the posttranscriptional regulation of cachectin gene expression, like that of many other cytokines and certain protooncogenes, may be explained by the presence of an unusual 3′-untranslated structure (the "TTATTTAT" sequence) that is found in cDNAs that encode them. This sequence was first identified in the 3′-untranslated region of cachectin cDNA, when it was noted that a 33-np AT-exclusive segment was conserved in toto between human and murine forms and that similar sequences were present in mRNAs encoding lymphotoxin and a variety of other biologically active proteins (Caput et al., 1986). Subsequently, it became clear that the sequence conferred instability, as well as superindu-

cibility, upon mRNAs containing it (Shaw & Kamen, 1986).

Presumably, ribonuclease(s) present in macrophages and other tissues recognize(s) the UUAUUUAU sequence with high selectivity and eventuate(s) destruction of mRNAs containing it. Mouse peritoneal macrophages have been shown to contain a ribonuclease that selectively destabilizes mRNA containing the cachectin-derived UUAUUUAU sequence (Beutler et al., 1988). It remains to be determined whether the enzyme is actively regulated. Such ribonucleases may prove to be central elements in the control of cachectin gene expression, as well as the expression of other cytokines and certain protooncogenes (e.g., c-*fos* and c-*myc*) that contain the instability sequence.

Future Directions

As mediators of shock, inflammation, and tissue catabolism, cachectin and lymphotoxin are hormones of major importance in many disease states. Yet, the precise mechanism by which they operate, the full range of stimuli required to elicit their production, the molecular details of their regulation, and, perhaps most important of all, the beneficial effects that have justified their conservation throughout mammalian evolution remain unknown.

Work in several laboratories is directed toward the elucidation of each of these questions. There is reason to hope that means of preventing cachectin biosynthesis, or inhibiting the activity of this hormone, will one day be found. Conceivably, when an understanding of the tumorolytic action of cachectin has been gained, novel chemotherapeutic strategies may present themselves.

Among the conclusions to be drawn from the story of cachectin, none is more important than the fact that immunoregulatory hormones are pluripotent in their effects and that a single hormone may contribute to the pathogenesis of a surprising array of disease states. The availability of cachectin (and other proinflammatory cytokines) in pure form thus poses an inviting challenge to students of inflammatory biology.

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