Immunol. Res. 5: 281–293 (1986)

© 1986 S. Karger AG, Basel
0257–277X/86/0054–0281$2.75/0

Cachectin/Tumor Necrosis Factor: An Endogenous Mediator of Shock and Inflammation

Bruce Beutler[a], *Anthony Cerami*[b]

[a] Howard Hughes Medical Institute, University of Texas Health Science Center, Dallas, Tex.;
[b] Laboratory of Medical Biochemistry, Rockefeller University, New York, N.Y., USA

Introduction

The inflammatory response is the most primitive and universal aspect of immune function, represented in some form among all phyla of the animal kingdom. The protective effect of inflammation is undoubtedly very great, since pharmacologic or genetic lesions that hamper the inflammatory response impact negatively upon survival during the course of infection. Yet, when unchecked, the inflammatory response may also prove injurious, leading to widespread tissue injury, shock and death.

One of the most striking examples in which inflammation acts to the detriment of the host may be witnessed in gram-negative septicemia, or following endotoxin (lipopolysaccharide; LPS) administration [1, 2]. The lesions present in the lungs, kidneys and gastrointestinal tract result largely from the infiltration of tissues by polymorphonuclear leukocytes and from inappropriate hemostasis. Often, the diffuse tissue injury wrought by LPS, along with severe derangements of glucose metabolism, acidosis and hypotension, will lead to the demise of the organism.

For many years, it was supposed that LPS possessed cytotoxic properties responsible for its deleterious effects following infusion. Recently, it has become clear that it does not. In fact, the lethal effects of LPS are mediated by cells of the host. On contact with LPS, mononuclear phagocytes elaborate a series of cytokines capable of initiating inflammatory changes. When secreted in sufficient quantity, these effectors may prove lethal. The principal endogenous mediator of endotoxicity is a molecule known widely as 'cachectin', or as 'tumor necrosis factor' (TNF).

An Endogenous Mediator Causes Endotoxic Shock

In 1968, Sultzer [3] reported that the C3H/HeJ mouse failed to develop a normal leukocytic infiltrate in response to intraperitoneal administration of LPS. Moreover, the mouse was highly resistant to the lethal effect of LPS, whereas the closely related strain A/HeJ was normally sensitive [3]. It was

soon found that isolated C3H/HeJ lymphocytes failed to exhibit a normal mitogenic response to LPS. C3H/HeJ macrophages failed to produce lymphocyte-activating factor (LAF) activity [4], colony-stimulating factor activity [5] and interferon [6] in response to LPS. C3H/HeJ fibroblasts similarly failed to respond to LPS with accelerated glucose uptake [7]. Hence, all tissues examined appeared unresponsive to LPS. The exact cause of LPS resistance remains unknown; however, it is conferred by a codominant allele (lps^d) carried on the fourth chromosome in mice [8].

Michalek et al. [9] utilized mice of the C3H/HeJ strain in adoptive transfer experiments, designed to determine whether LPS sensitivity was conferred by cells of hematopoietic origin. When marrow obtained from sensitive (C3H/HeN) mice transplanted into lethally irradiated resistant (C3H/HeJ) mice, the resulting radiation chimeras were found to be LPS-sensitive. Conversely, C3H/HeN mice transplanted with C3H/HeJ marrow were rendered resistant to LPS. The concordance of LPS sensitivity and donor phenotype suggested that indeed, the lethal effect of LPS was conferred by cells of hematopoietic origin.

The identity of the cell involved could not be resolved by genetic means. However, the macrophage was suspected as the mediator of endotoxicity, since facultative intracellular bacteria capable of eliciting reticuloendothelial hyperplasia are known to sensitize animals to the lethal effect of LPS. Moreover, macrophages obtained from sensitive strains of mice, when stimulated with LPS in vitro, were shown to be capable of producing a factor toxic or lethal to LPS-resistant animals [10, 11]. The isolation of this macrophage factor, which conferred LPS toxicity, was accomplished by two groups of investigators studying widely differing aspects of the host response to LPS.

Cachectin and TNF: History and Isolation

Among the many phenomena induced by LPS in the mammalian host, hemorrhagic necrosis of tumors has generated the greatest excitement among clinical audiences. As first described nearly 100 years ago be Coley [12], hemorrhagic necrosis of a tumor may follow administration of bacterial toxins to tumor-bearing animals. Shear [13] succeeded, in collaboration with Andervont [14] and others [15–17], in isolating the bacterial product responsible for inducing necrosis, now known as lipopolysaccharide. Since these studies and the report of Coley [12], LPS has been viewed as a potential chemotherapeutic agent, albeit a very toxic one.

As early as 1962, O'Malley et al. [18] noted that shock serum obtained from mice very soon after LPS injection was capable of inducing hemorrhagic necrosis of transplantable tumors in recipient animals. These investigators also noted that after several hours had elapsed, the necrotizing principle was no longer present in the serum, and that a second injection of LPS was not effective in inducing its production. This important work went largely unnoticed.

In 1975, Carswell et al. [19] reported that hemorrhagic necrosis could be induced in tumor-bearing mice by injection of LPS-induced shock sera derived from animals previously 'primed' by administration of Calmette-Guérin bacillus. The serum factor responsible for eliciting hemorrhagic necrosis was termed 'TNF'. Subsequently, several investigators showed that TNF was pri-

marily a product of monocytes or macrophages. It was also shown that TNF was capable of lysing certain tumor cells in vitro [19–21].

The mechanism of hemorrhagic necrosis has never been fully clarified, although it seems probable that a vascular mechanism underlies the phenomenon. Algire et al. [22] pointed to the fact that mechanical vaso-occlusion could effectively reproduce the histologic appearance of hemorrhagic necrosis. More recently, it has been shown that hemorrhagic necrosis of certain tumors can proceed in vivo, although cells derived from these tumors are insensitive to TNF in vitro. Moreover, the anatomical location of a tumor appears to determine its sensitivity, in so far as certain tumors grown in skin appear far more vulnerable to the effect of the hormone than tumors grown at visceral sites [I. Fidler, pers. communication]. Thus, the mechanism by which hemorrhagic necrosis proceeds is likely distinct from the mechanism by which tumorolysis occurs in vitro, and the ability of a common mediator to elicit both phenomena is probably fortuitous.

The factor responsible for tumor necrosis in vivo and tumorolysis in vitro was isolated by Aggarwal et al. [23] from the cultured human monocyte line HL-60. Subsequently, TNF was cloned and expressed in *Escherichia coli,* and the cDNA [24–26] and genomic [27] sequences were established. Human TNF was a 17,000-dalton polypeptide containing two cysteine residues linked by a disulfide bond [23]. The molecule was noted to bear approximately 30% primary structural homology to lymphotoxin [24], a lymphocyte-derived polypeptide hormone with a similar spectrum of activities, which was closely linked to TNF on chromosome 6 [27].

While the isolation of TNF was in progress, several investigators were concerned with the pathogenesis of cachexia (wasting) in chronic disease. It was noted that a paradoxical hypertriglyceridemia occurred in the course of certain wasting infectious diseases, notably trypanosomiasis in rabbits [28, 29], despite a loss of body fat and diminished caloric intake. This hypertriglyceridemia was also noted in endotoxin-poisoned mice [30], and was associated with systemic suppression of lipoprotein lipase (LPL) activity [30]. A macrophage factor capable of suppressing LPL in vivo, and capable of suppressing LPL expression by adipocytes in vitro, was identified by Kawakami et al. [31] and purified to homogeneity by Beutler et al. [32]. This factor was termed cachectin, in view of its possible involvement in the pathogenesis of cachexia. Cachectin was found to be produced in great quantity by LPS-activated macrophages. Approximately 1–2% of the total secretory product of RAW 264.7 (mouse macrophage) cells was found to be cachectin [32], and cachectin was later found to comprise a similar percentage of the protein elaborated by thioglycollate-elicited peritoneal macrophages. Estimates of the quantity of the protein produced in vivo in response to LPS challenge [33, 34] were correspondingly large; rabbits, for example, generate plasma concentrations of cachectin that approach the micromolar range, suggesting that milligram quantities of cachectin may be released per kilogram of body weight.

When the amino-terminal sequence of mouse cachectin was determined, a high degree of homology to the human TNF sequence was noted [35], suggesting that cachectin and TNF were in fact identical proteins. Comparative bioactivity studies and

immunological studies supported this conclusion, which was confirmed when the cDNA sequence of mouse TNF was shown to exactly predict the protein sequence established for mouse cachectin [36–38]. Thus, a single polypeptide hormone was shown to possess both LPL-suppressing activity and 'TNF' activity, in vitro and in vivo.

Cachectin/TNF as a Mediator of Endotoxicity

The fact that this single protein was capable of mediating two disparate effects of LPS suggested that many of the effects of LPS, including the lethal effect, might operate through a common mediator, e.g. cachectin/TNF. This appeared to be a particularly plausible hypothesis, in view of the time course of cachectin induction, the great quantity in which the hormone was produced, and the catabolic character of its effects.

Beutler et al. [39] sought to determine whether cachectin might be involved in the host response to LPS, contributing to the lethal effect of endotoxin. Passive immunization of mice against cachectin significantly protected them against subsequent challenge with LPS administered at varying doses. Thus, it seemed that cachectin was indeed involved in the production of endotoxicity.

More recently, it has been shown that cachectin alone is capable of reproducing many of the physiologic derangements associated with LPS administration, including hypotension, metabolic acidosis, transient hyperglycemia followed by a hypoglycemic state, hemoconcentration, diffuse tissue injury and death [40]. Acute renal tubular necrosis and ischemic necrosis of the gastrointestinal tract occur following intravenous administration of cachectin, along with a severe (and often fatal) interstitial pneumonitis. Thus, it would appear that this single mediator plays a prominent role in the pathogenesis of endotoxin-induced shock. The precise mechanism by which cachectin evokes the severe inflammatory response that it does is under active investigation. It would seem likely that this protein represents a proximal mediator of endotoxic shock and that other mediators, including platelet-activating factor (PAF) [41, 42] and leukotrienes [43–45], are the final effectors.

Biological Effects of Cachectin on Specific Target Cells

Cachectin was first isolated by virtue of its ability to suppress the biosynthesis of lipoprotein lipase in cultured adipocytes [32]. Suppression of lipoprotein lipase activity in 3T3-L1 cells is also caused by interleukin-1 [46] and by interferon-gamma [47]. However, the predominant suppressor produced by the macrophage is cachectin [32, 48]. It has been shown that cachectin is also capable of suppressing the expression of several differentiation-specific mRNA molecules in adipose tissue [49].

In nonadipose tissue, cachectin induces the synthesis and/or secretion of a number of proteins, also acting (in at least some cases) at the level of transcription. Class-I MHC antigen expression (and mRNA levels) are increased in fibroblasts and endothelial cells following addition of TNF [50]. Certain other antigenic determinants, as yet uncharacterized, are also induced on endothelial cells by TNF [51], an effect which is blocked by inhibitors of protein or RNA synthesis.

A number of cytokines, including IL-1 [52–54] and beta-2-interferon [55], are induced by TNF. In the case of IL-1, it is clear that cachectin prompts both an increase in the level of endothelial cell IL-1 mRNA [56], and causes IL-1 secretion [52–54].

Kirstein and Baglioni [57] have proposed that the cellular response to TNF includes, in many instances, the production of certain proteins that protect the target cell from lysis. Hence, actinomycin-D and cycloheximide, which (respectively) inhibit RNA and protein synthesis, potentiate the cytotoxic effect of TNF. These investigators have noted that certain proteins are induced by TNF in fibroblasts [57] and speculate that these or other proteins like them might play a homeostatic role, protecting the cell against the hormone's cytotoxic effect. As yet, however, these proteins have not been isolated and their function remains unclear.

Cachectin has numerous effects on hematopoietic cells and their progenitors. Among these, one of the most important in terms of its physiologic consequences is the capacity of cachectin (TNF) to activate neutrophils [58, 59]. Within 5 min following addition of the hormone to neutrophil preparations, the target cells show an increased tendency to adhere to endothelial surfaces [58] and increased phagocytic activity [59]. Neutrophil activation, occurring in the setting of endotoxemia, is responsible for many of the inflammatory changes that eventuate tissue damage. As such, neutrophil activation by cachectin may represent one of the most important means by which the cytokine causes injury.

Like neutrophils, macrohages appear to be activated by cachectin (TNF), which augments the killing of intracellular *Trypanosoma cruzi* parasites [60]. Macrophages produce IL-1 in response to cachectin [52, 54]. Cachectin (TNF) suppresses the induction of granulocyte/monocyte colony-forming units, acting by itself or in synergy with interferon-gamma [61], yet also appears to serve as a myeloid differentiation-inducing factor (DIF) [62, 63]. TNF appears to be responsible for the colony-inhibiting activity produced by natural killer (NK) cells; however, very small amounts of TNF are actually produced in NK cell cultures, and it is unclear whether NK cells comprise a major source of the protein [64, 65]. Eosinophils, like macrophages, appear to possess TNF receptors, since in the presence of dilute concentrations of the hormone, they show enhanced cytotoxicity toward schistosomula in vitro [66].

Cachectin (TNF) exhibits a number of dramatic effects on vascular endothelial cells. TNF has been shown to effect structural rearrangement of endothelial cells grown in culture [67, 68]. This effect may represent an in vitro model of the vascular changes responsible for the hemorrhagic necrosis of tumors, as well as for many of the vascular changes that accompany endotoxemia. The administration of TNF to endothelial cells also increases neutrophil adherence by a mechanism distinct from the direct effect on neutrophils themselves [58]. This mechanism appears to involve the production of endothelial cell surface factor(s) that increases neutrophil adherence [69]. Endothelial cells produce a procoagulant activity in response to cachectin (TNF) [70, 71] and downregulate their expression of thrombomodulin [71]. Both of these effects of the hormone tend to favor hemostasis and possibly contribute to the clotting abnormalities associated with endotoxemia. TNF-induced IL-1 production [52, 53, 56] may potentiate many of the primary effects of TNF.

Cachectin (TNF) exerts a catabolic effect on bone and cartilage, causing the release of $^{45}Ca^{2+}$ from the former [72], and the degradation of proteoglycan in the latter tissue [73]. Cachectin also causes the release of collagenase and PGE_2 from human synovial cells and dermal fibroblasts [74]. All of these effects suggest that cachectin may be involved in acute and chronic inflammatory processes.

Cachectin is an endogenous pyrogen, acting to produce fever by a direct effect upon hypothalamic neurons, in which PGE_2 production is stimulated, and indirectly by eliciting the release of IL-1 from peripheral sources. Thus, a biphasic fever curve occurs in rabbits given recombinant, pyrogen-free TNF by an intravenous route [54].

TNF appears to act on a wide variety of cell types to inhibit viral proliferation. This interferon-like effect appears to be independent of endogenous production of interferon-beta-2 [55]. The mechanism of action of TNF in preventing viral cytolysis remains unknown. This phenomenon may be of considerable physiologic importance, however, and may even comprise the beneficial effect responsible for the evolutionary conservation of cachectin throughout mammalian evolution.

Control of Cachectin Biosynthesis

Since cachectin is an inflammatory mediator capable of exerting highly toxic effects, its biosynthesis is strictly regulated. Endogenous mediators capable of inducing cachectin production have not been identified as yet. However, a variety of stimuli simulating host invasion are capable of eliciting cachectin production by peritoneal macrophages in vitro. These agents include Sendai virus [75] and influenza virus [76] as well as LPS. In cultured macrophage-derived cell lines, other agents, including phorbol esters and staphylococcal enterotoxin B, are known to be effective inducers [77]. Certain agents capable of inducing IL-1 production, notably the staphylococcal exotoxin TSST-1 [78], are quite incapable of eliciting cachectin biosynthesis [unpubl. data]. Hence, macrophage activation appears to be a selective process rather than a strictly 'all-or-none' phenomenon.

Within quiescent peritoneal macrophages, cachectin mRNA may frequently be found in an untranslated form [79]. Following activation of the macrophages by LPS, cachectin gene transcription is enhanced and both nacent and preformed messages are translated to produce active protein.

These events are influenced by internal (genetic) and external (pharmacologic) factors. In the C3H/HeJ mouse, a mutational event (the *lps*^{d} allele) blocks transcriptional and posttranscriptional activation of cachectin biosynthesis. Except in the presence of very high concentrations of LPS, cachectin mRNA synthesis is not accelerated [79]. Moreover, the mRNA that is produced is not actively translated [79]. Similarly, LPS-responsive macrophages, when pretreated with dexamethasone, fail to produce cachectin mRNA in normal quantity and fail to translate the message that is present [79].

On the other hand, interferon-gamma augments LPS-induced cachectin biosynthesis, acting at both transcriptional and posttranscriptional levels [80]. As such, C3H/HeJ macrophages show partial correction of the *lps*^{d} phenotype when pretreated with interferon-γ [80]. It is possible that the 'priming' effect exerted by facultative intra-

cellular bacteria, whereby infected animals are rendered hypersensitive to LPS, may depend upon endogenous production of interferon-gamma or a related cytokine. Moreover, the remarkable sensitivity of adrenalectomized animals to the lethal effect of LPS may result from the loss of biosynthetic control normally afforded by glucocorticoid hormones.

The transcriptional and posttranscriptional events required for the production of active cachectin remain incompletely understood. Presumably, cachectin gene transcription is controlled by a genetic element upstream of the coding sequence.

The nature of the posttranscriptional (presumably translational) control also remains unknown. It has recently been observed that the 3′-untranslated region of genes encoding cachectin, lymphotoxin, IL-1, interferons, GM-CSF and other cytokines contains a conserved sequence consisting of a repeating and overlapping AT-exclusive octamer (TTATTTAT) [36]. This octameric sequence was considered to be a potential recognition site through which translation of these messages might be governed [36, 79]. Shaw and Kamen [81] have studied the AT-exclusive region associated with the GM-CSF gene and have demonstrated that this sequence markedly destabilizes mRNA with which it is associated. Moreover, it appears to confer superinducibility upon those messages that contain it [81]. Thus, the sequence may indeed govern cachectin translation, although the precise mechanism by which it is recognized and its mode of action remain unknown.

The range of pathologic processes in which cachectin is produced remains unknown. The evidence gathered to date would suggest that a fixed amount of cachectin can be produced by the macrophage in response to a given invasive stimulus. Thereafter, production ceases, despite continued presence of the stimulus [82]. Nonetheless, it is possible that cachectin, or the closely related protein lymphotoxin, plays a role in many inflammatory disease states.

The natural circumstances under which lymphotoxin is produced include specific antigenic challenge [83]. Thus, one might expect it to be produced in chronic (and particularly in cyclical) infectious diseases. It is anticipated that with improving assay techniques, it will be possible to closely analyze the conditions under which each of these cytokines is released in vivo.

Receptor and Postreceptor Response

The existence of a specific, high-affinity receptor for cachectin on diverse tissues has been reported [32] and widely confirmed [84–89]. It has been suggested that more than one class of receptor may exist, since Scatchard plots of binding over a wide range of concentration are frequently curvilinear [32, 47]. It has been shown that lymphotoxin is capable of competing with TNF for binding sites present on cultured cells, suggesting that despite their structural disparity, both proteins utilize a common receptor [86].

Initial studies of TNF receptor structure have suggested that the hormone interacts with a protein of approximately 75 kilodaltons in size [89]. The receptor is trypsin-sensitive and is very likely a glycoprotein, since concanavalin A (Con A) and wheat germ agglutinin strongly inhibit hormone binding, though they increase the apparent receptor number [90]. The effect of Con A on TNF bioactivity is fully inhibited by the addition

of alpha-methylmannoside and has been reported to occur in other systems as well, notably in the case of diphtheria toxin.

The Biological Role of Cachectin

Cachectin (TNF) and lymphotoxin are encoded by genes that arose from an ancestral locus hundreds of millions of years ago. Since the occurrence of the duplication event that generated these genes, mutational events have altered their structure such that only 30% identity remains at the amino acid level [27]. It is unclear whether duplication of the ancestral gene antedated the differentiation of lymphocytes and macrophages; however, it is clear that cachectin and lymphotoxin are expressed in a strictly tissue-specific manner and in response to different stimuli. Yet, with few exceptions [64], the proteins elicit identical patterns of biological response.

Throughout mammalian evolution, the cachectin (TNF-alpha) gene has changed little; nearly 80% homology remains between the amino acid sequences of mouse, human, and rabbit proteins [34, 36–38, 91–93]. In addition to the deleterious effect that accompanies the release of massive quantities of cachectin into the circulation, it would seem likely that cachectin, produced in smaller amounts, confers a survival advantage upon the individual.

The administration of sublethal doses of cachectin to mice is highly protective against subsequent challenge with murine malaria [L. Schofield, pers. communication]. Cachectin also possesses an antiviral activity [G. Wong, pers. communication] that may serve to protect the host, particularly as protein synthesis is known to be induced by certain viruses [75, 76].

It remains to be seen whether cachectin possesses a similar protective value when administered in small quantities to animals prior to challenge with gram-negative bacteria. However, cachectin may act to check the spread of these and a wide variety of other pathogens in vivo.

Conclusion

During the past 3 years, cachectin (TNF) has come to be regarded as a proximal mediator of inflammation, which exerts powerful effects on the metabolic activities of host tissues during infection. In the future, it is anticipated that a growing understanding of cachectin and its role in mammalian physiology will yield important advances in basic and clinical science.

A precise knowledge of the hormone's mechanism of action may shed light on the causes of inflammation, its benefits and its liabilities. The unusual ability of cachectin to lyse tumor cells in vitro may ultimately lead to the design of effective antineolastic agents. Finally, the molecular events involved that govern the induction of cachectin gene expression by LPS may prove to be very general. Thus, studies of cachectin gene expression may lead to a broader understanding of the central mechanisms by which transcription and translation are effected in mammalian cells.

Acknowledgements

We wish to express our gratitude to Chiron Corporation (Emeryville, Calif., USA) for supporting our work on cachectin/TNF. We also acknowledge the funding provided by NIH grant No. AI 21359 and AM 1314.

References

1 Morgan, H.R.: Pathologic changes produced in rabbits by a toxic somatic antigen derived from *Eberthella typhosa.* Am. J. Path. *19:* 135–145 (1942).
2 Brunson, J.G.; Gamble, C.N.; Thomas, L.: Morphologic changes in rabbits following the intravenous administration of meningicoccal toxin. I. The effects produced in young and in mature animals by a single injection. Am. J. Path. *31:* 489–499 (1955).
3 Sultzer, B.M.: Genetic control of leucocyte responses to endotoxin. Nature *219:* 1253–1254 (1968).
4 Rosenstreich, D.L.; Vogel, S.N.; Jacques, A.R.; Wahl, L.M.; Oppenheim, J.J.: Macrophage sensitivity to endotoxin. Genetic control by a single codominant gene. J. Immun. *121:* 1664–1670 (1978).
5 Apte, R.N.; Pluznik, D.H.: Genetic control of lipopolysaccharide induced generation of serum colony stimulating factor and proliferation of splenic granulocyte/macrophage precursor cells. J. cell. Physiol. *89:* 313–323 (1976).
6 Apte, R.N.; Ascher, O.; Pluznik, D.H.: Genetic analysis of generation of serum interferon by bacterial lipopolysaccharide. J. Immun. *119:* 1898 (1977).
7 Ryan, J.L.; McAdam, K.P.W.J.: Genetic nonresponsiveness of murine fibroblasts to bacterial endotoxin. Nature *269:* 153–155 (1977).
8 Watson, J.; Kelly, K.; Largen, M.; Taylor, B.A.: The genetic mapping of a defective LPS response gene in C3H/HeJ mice. J. Immun. *120:* 422–424 (1978).
9 Michalek, S.M.; Moore, R.N.; McGhee, J.R.; Rosenstreich, D.L.; Mergenhagen, S.E.: The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxin. J. infect. Dis. *141:* 55–63 (1980).
10 Moore, R.N.; Goodrum, K.J.; Couch, R.E., Jr.; Berry, L.J.: Elicitation of endotoxemic effects in C3H/HeJ mice with glucocorticoid antagonizing factor and partial characterization of the factor. Infect. Immunity *19:* 79–86 (1978).
11 Cerami, A.; Ikeda, Y.; Le Trang, N.; Hotez, P.J.; Beutler, B.: Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumor necrosis factor). Immunol. Lett. *11:* 173–177 (1985).
12 Coley, W.B.: The treatment of malignant tumors by repeated inoculations of erysipelas; with a report of ten original cases. Am. J. med. Sci. *105:* 487–511 (1983).
13 Shear, M.J.: Chemical treatment of tumors. IX. Reactions of mice with primary subcutaneous tumors to injection of a hemorrhage-producing bacterial polysaccharide. J. natn. Cancer Inst. *4:* 461–476 (1944).
14 Shear, M.J.; Andervont, H.B.: Chemical treatment of tumors. III. Separation of hemorrhage-producing fraction of *E. coli* filtrate. Proc. Soc. exp. Biol. med. *34:* 323–325 (1936).
15 Shear, M.J.; Kahler, H.; Hartwell, J.L.: Chemical treatment of tumors. VIII. Ultracentrifugal and electrophoretic analysis of the hemorrhage-producing fraction from *Serratia marcescens* (bacillus prodigiosus) culture filtrate. J. natn. Cancer Inst. *4:* 123–129 (1943).
16 Shear, M.J.; Hartwell, J.L.; Adams, J.R., Jr.: Chemical treatment of tumors. VII. Nature of the hemorrhage-producing fraction from *Serratia marcescens* (bacillus prodigiosus) culture filtrate. J. natn. Cancer Inst. *4:* 107–122 (1943).
17 Shear, M.J.; Turner, F.C.; Perrault, A.; Shovelton, J.: Chemical treatment of tumors. V. Isolation of the hemorrhage-producing fraction from *Serratia marcescens* (bacillus prodigiosus) culture filtrate. J. natn. Cancer Inst. *4:* 81–97 (1943).
18 O'Malley, W.E.; Achinstein, B.; Shear, M.J.: Action of bacterial polysaccharide on tumors. II. Damage of sarcoma 37 by serum of mice treated with *Serratia marcescens* polysaccharide, and induced tolerance. J. natn. Cancer Inst. *29:* 1169–1175 (1962).
19 Carswell, E.A.; Old, L.J.; Kassel, R.L.; Green, S.; Fiore, N.; Williamson, B.: An endotoxin-induced serum factor that causes necrosis of tumors. Proc. natn. Acad. Sci. USA *72:* 3666–3670 (1975).
20 Helson, L.; Green, S.; Carswell, E.; Old, L.J.: Effect of tumour necrosis factor on cultured human melanoma cells. Nature *258:* 731–732 (1975).
21 Sugarman, B.J.; Aggarwal, B.B.; Hass, P.E.; Figari, I.S.; Palladino, M.A., Jr.; Shepard, H.M.: Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science *230:* 943–945 (1985).
22 Algire, G.H.; Legallais, F.Y.; Anderson, B.F.: Vascular reactions of normal and malignant tissues in vivo. V. The role of hypotension in the action of a

bacterial polysaccharide on tumors. J. natn. Cancer Inst. *12:* 1279–1295 (1952).

23 Aggarwal, B.B.; Kohr, W.J.; Hass, P.E.; Moffat, B.; Spencer, S.A.; Henzel, W.J.; Bringman, T.S.; Nedwin, G.E.; Goeddel, D.V.; Harkins, R.N.: Human tumor necrosis factor. Production, purification, and characterization. J. biol. Chem. *260:* 2345–2354 (1985).

24 Pennica, D.; Nedwin, G.E.; Hayflick, J.S.; Seeburg, P.H.; Derynck, R.; Palladino, M.A.; Kohr, W.J.; Aggarwal, B.B.; Goeddel, D.V.: Human tumor necrosis factor: precursors structure, expression and homology to lymphotoxin. Nature *312:* 724–729 (1984).

25 Wang, A.M.; Creasey, A.A.; Ladner, M.B.; Lin, L.S.; Strickler, J.; Van Arsdell, J.N.; Yamamoto, R.; Mark, D.F.: Molecular cloning of the complementary DNA for human tumor necrosis factor. Science *228:* 149–154 (1985).

26 Shirai, T.; Yamaguchi, H.; Ito, H.; Todd, C.W.; Wallace, R.B.: Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor. Nature *313:* 803–806 (1985).

27 Nedwin, G.E.; Naylor, S.L.; Sakaguchi, A.Y.; Smith, D.; Jarrett-Nedwin, J.; Pennica, D.; Goeddel, D.V.; Gray, P.W.: Human lymphotoxin and tumor necrosis factor genes: structure homology and chromosomal localization. Nucl. Acids Res. *13:* 6361–6373 (1985).

28 Rouzer, C.A.; Cerami, A.: Hypertriglyceridemia associated with *Trypanosoma brucei* infection in rabbits: role of defective triglyceride removal. Molec. biochem. Parasitol. *2:* 31–38 (1980).

29 Guy, M.W.: Serum and tissue fluid lipids in rabbits experimentally infected with *Trypanosoma brucei.* Trans. R. Soc. trop. Med. Hyg. *69:* 429 (1975).

30 Kawakami, M.; Cerami, A.: Studies of endotoxin-induced decrease in lipoprotein lipase activity. J. exp. Med. *154:* 631–639 (1981).

31 Kawakami, M.; Pekala, P.H.; Lane, M.D.; Cerami, A.: Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. Proc. natn. Acad. Sci. USA *79:* 912–916 (1982).

32 Beutler, B.; Mahoney, J.; Le Trang, N.; Pekala, P.; Cerami, A.: Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. J. exp. Med. *161:* 984–995 (1985).

33 Beutler, B.; Milsark, I.W.; Cerami, A.: Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. J. Immun. *135:* 3972–3977 (1985).

34 Abe, S.; Gatanaga, T.; Yamazaki, M.; Soma, G.; Mizuno, D.: Purification of rabbit tumor necrosis factor. FEBS Lett. *180:* 203–206 (1985).

35 Beutler, B.; Greenwald, D.; Hulmes, J.D.; Chang, M.; Pan, Y.-C.E.; Mathison, J.; Ulevitch, R.; Cerami, A.: Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature *316:* 552–554 (1985).

36 Caput, D.; Beutler, B.; Hartog, K.; Brown-Shimer, S.; Cerami, A.: Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. natn. Acad. Sci. USA *83:* 1670–1674 (1986).

37 Pennica, D.; Hayflick, J.S.; Bringman, T.S.; Palladino, M.A.; Goeddel, D.V.: Cloning and expression in *Escherichia coli* of the cDNA for murine tumor necrosis factor. Proc. natn. Acad. Sci. USA *82:* 6060–6064 (1985).

38 Fransen, L.; Müller, R.; Marmenout, A.; Tavernier, J.; Heyden, J. van der; Kawashima, E.; Chollet, A.; Tizard, R.; Heuverswyn, H. van; Vliet, A. van; Ruysschaert, M.-R.; Fiers, W.: Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. Nucl. Acids Res. *13:* 4417–4429 (1985).

39 Beutler, B.; Milsark, I.W.; Cerami, A.: Passive immunization against cachectin/tumor necrosis factor (TNF) protects mice from the lethal effect of endotoxin. Science *229:* 869–871 (1985).

40 Tracey, K.J.; Beutler, B.; Lowry, S.F.; Merryweather, J.; Wolpe, S.; Milsark, I.W.; Hariri, R.J.; Fahey, T.J., III.; Zentella, A.; Albert, J.D.; Shires, G.T.; Cerami, A.: Shock and tissue injury induced by recombinant human cachectin. Science *234:* 470–474 (1986).

41 Terashita, Z.Y.; Imura, Y.; Nishikawa, K.; Sumida, S.: Is platelet activating factor (PAF) a mediator of endotoxin shock? Eur. J. Pharmacol. *109:* 257–261 (1985).

42 Doebber, T.W.; Wu, M.S.; Robbins, J.C.; Choy, B.M.; Chang, M.N.; Shen, T.Y.: Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist kadsurenone. Biochem. biophys, Res. Commun. *127:* 799–808 (1985).

43 Dahlen, S.-E.; Bjork, J.; Hedqvist, P.; Arfors, K.-E.; Hammarstrom, S.; Lindgren, J.-A.; Samuelsson, B.: Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc. natn. Acad. Sci. USA *78:* 3887–3891 (1981).
44 Bremm, K.D.; Konig, W.; Spur, B.; Crea, A.; Galanos, C.: Generation of slow-reacting substance (leukotrienes) by endotoxin and lipid A from human polymorphonuclear granulocytes. Immunology *53:* 299–305 (1984).
45 Hagmann, W.; Keppler, D.: Leukotriene antagonists prevent endotoxin lethality. Naturwissenschaften *69:* 594–595 (1982).
46 Beutler, B.; Cerami, A.: Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. J. Immun. *135:* 3969–3971 (1985).
47 Patton, J.S.; Shepard, H.M.; Wilking, H.; Lewis, G.; Aggarwal, B.B.; Eessalu, T.E.; Gavin, L.A.; Grunfeld, C.: Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. Proc. natn. Acad. Sci. USA (in press, 1986).
48 Beutler, B.; Mahoney, J.; Pekala, P.; Le Trang, N.; Cerami, A.: The major endotoxin-inducible secretory product of cultured macrophages is a novel lipolytic hormone. Blood *64:* 65a (1984).
49 Torti, F.M.; Dieckmann, B.; Beutler, B.; Cerami, A.; Ringold, G.M.: A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. Science *229:* 867–869 (1985).
50 Collins, T.; Lapierre, L.A.; Fiers, W.; Strominger, J.L.; Pober, J.S.: Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular endothelial cells and dermal fibroblasts in vitro. Proc. natn. Acad. Sci. USA *83:* 446–450 (1986).
51 Pober, J.S.; Bevilacqua, M.P.; Mendrick, D.L.; Lapierre, L.A.; Fiers, W.; Gimbrone, M.A., Jr.: Two distinct monokines, interleukin-1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J. Immun. *136:* 1680–1687 (1986).
52 Bachwich, P.R.; Chensue, S.W.; Larrick, J.W.; Kunkel, S.L.: Tumor necrosis factor stimulates interleukin-1 and prostaglandin E2 production in resting macrophages. Biochem. biophys. Res. Commun. *136:* 94–101 (1986).
53 Nawroth, P.; Bank, I.; Handley, D.; Cassimeris, J.; Chess, L.; Stern, D.: Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin-1. J. exp. Med. *163:* 1363–1375 (1986).
54 Dinarello, C.A.; Cannon, J.G.; Wolff, S.M.; Bernheim, H.A.; Beutler, B.; Cerami, A.; Palladino, M.A.; O'Connor, J.V.: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin-1. J. exp. Med. *163:* 1433–1450 (1986).
55 Kohase, M.; Henriksen DeStefano, D.; May, L.T.; Vilcek, J.; Sehgal, P.B.: Induction of beta 2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell *45:* 659–666 (1986).
56 Libby, P.; Ordovas, J.M.; Auger, K.R.; Robbins, A.H.; Birinyi, L.K.; Dinarello, C.A.: Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am. J. Path. *124:* 179–185 (1986).
57 Kirstein, M.; Baglioni, C.: Tumor necrosis factor induces synthesis of two proteins in human fibroblasts. J. biol. Chem. *261:* 9565–9567 (1986).
58 Gamble, J.R.; Harlan, J.M.; Klebanoff, S.J.; Lopez, A.F.; Vadas, M.A.: Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc. natn. Acad. Sci. USA *82:* 8667–8671 (1985).
59 Shalaby, M.R.; Aggarwal, B.B.; Rinderknecht, E.; Svedersky, L.P.; Finkle, B.S.; Palladino, M.A., Jr.: Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. J. Immun. *135:* 2069–2073 (1985).
60 De Titto, E.H.; Catterall, J.R.; Remington, J.S.: Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. J. Immun. *137:* 1342–1345 (1986).
61 Murphy, M.; Loudon, R.; Kobayashi, M.; Trinchieri, G.: Gamma interferon and lymphotoxin, released by activated T cells, synergize to inhibit granulocyte/monocyte colony formation. J. exp. Med. *164:* 263–279 (1986).
62 Takeda, K.; Iwamoto, S.; Sugimoto, H.; Takuma, T.; Kawatani, N.; Noda, M.; Masaki, A.; Morise, H.; Arimura, H.; Konno, K.: Identity of differentiation inducing factor and tumour necrosis factor. Nature *323:* 338–340 (1986).

63 Trinchieri, G.; Kobayashi, M.; Rosen, M.; Loudon, R.; Murphy, M.; Perussia, B.: Tumor necrosis factor and lymphotoxin induce differentiation of human myeloid cell lines in synergy with immune interferon. J. exp. Med. *164:* 1206–1225 (1986).

64 Degliantoni, G.; Murphy, M.; Kobayashi, M.; Francis, M.K.; Perussia, B.; Trinchieri, G.: Natural killer (NK) cell-derived hematopoietic colony-inhibiting activity and NK cytotoxic factor. Relationship with tumor necrosis factor and synergism with immune interferon. J. exp. Med. *162:* 1512–1530 (1985).

65 Ortaldo, J.R.; Mason, L.H.; Mathieson, B.J.; Liang, S.M.; Flick, D.A.; Herberman, R.B.: Mediation of mouse natural cytotoxic activity by tumour necrosis factor. Nature *321:* 700–702 (1986).

66 Silberstein, D.S.; David, J.R.: Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. Proc. natn. Acad. Sci. USA *83:* 1055–1059 (1986).

67 Stolpen, A.H.; Guinan, E.C.; Fiers, W.; Pober, J.S.: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am. J. Path. *123:* 16–24 (1986).

68 Sato, N.; Goto, T.; Haranaka, K.; Satomi, N.; Nariuchi, H.; Mano Hirano, Y.: Actions of tumor necrosis factor on cultured vascular endothelial cells: morphologic modulation, growth inhibition, and cytotoxicity. J. natn. Cancer Inst. *76:* 1113–1121 (1986).

69 Pohlman, T.H.; Stanness, K.A.; Beatty, P.G.; Ochs, H.D.; Harlan, J.M.: An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin-1, and tumor necrosis factor-alpha increases neutrophil adherence by a CDw18-dependent mechanism. J. Immun. *136:* 4548–4553 (1986).

70 Bevilacqua, M.P.; Pober, J.S.; Majeau, G.R.; Fiers, W.; Cotran, R.S.; Gimbrone, M.A., Jr.: Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin-1. Proc. natn. Acad. Sci. USA *83:* 4533–4537 (1986).

71 Stern, D.M.; Nawroth, P.P.: Modulation of endothelial hemostatic properties by tumor necrosis factor. J. exp. Med. *163:* 740–745 (1986).

72 Bertolini, D.R.; Nedwin, G.; Bringman, T.; Smith, D.; Mundy, G.R.: Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factor. Nature *319:* 516–518 (1986).

73 Saklatvala, J.: Tumour necrosis factor-α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature *322:* 547–549 (1986).

74 Dayer, J.-M.; Beutler, B.; Cerami, A.: Cachectin/tumor necrosis factor (TNF) stimulates collagenase and PGE_2 production by human synovial cells and dermal fibroblasts. J. exp. Med. *162:* 2163–2168 (1985).

75 Aderka, D.; Holtmann, H.; Toker, L.; Hahn, T.; Wallach, D.: Tumor necrosis factor induction by Sendai virus. J. Immun. *136:* 2938–2942 (1986).

76 Beutler, B.; Krochin, N.; Milsark, I.W.; Goldberg, A.; Cerami, A.: Induction of cachectin (tumor necrosis factor) synthesis by influenza virus: deficient production by endotoxin-resistant (C3H/HeJ) macrophages. Clin. Res. *34:* 491a (1986).

77 Nedwin, G.E.; Svedersky, L.P.; Bringman, T.S.; Palladino, M.A.; Goeddel, D.V.: Effect of interleukin-2, interferon-gamma, and mitogens on the production of tumor necrosis factors alpha and beta. J. Immun. *135:* 2492–2497 (1985).

78 Ikejima, T.; Minami, M.; Gill, D.M.; Dinarello, C.A.: Human, rabbit and murine interleukin-1 production in response to toxic shock syndrome toxin-1 (submitted, 1985).

79 Beutler, B.; Krochin, N.; Milsark, I.W.; Luedke, C.; Cerami, A.: Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science *232:* 977–980 (1986).

80 Beutler, B.; Tkacenko, V.; Milsark, I.W.; Krochin, N.; Cerami, A.: The effect of interferon-gamma on cachectin expression by mononuclear phagocytes: reversal of the lps-d (endotoxin resistance) phenotype. J. exp. Med. (in press, 1986).

81 Shaw, G.; Kamen, R.: A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell *46:* 659–667 (1986).

82 Gifford, G.E.; Lohmann-Matthes, M.L.: Requirement for the continual presence of lipopolysaccharide for production of tumor necrosis factor by thioglycollate-induced peritoneal murine macrophages. Int. J. Cancer *38:* 135–137 (1986).

83 Conta, B.S.; Powell, M.B.; Ruddle, N.H.: Activa-

tion of LyT-1+ and LyT-2+ T cell cloned lines: stimulation of proliferation, lymphokine production, and self-destruction. J. Immun. *134:* 2185–2190 (1985).

84 Tsujimoto, M.; Vilcek, J.: Tumor necrosis factor receptors in HeLa cells and their regulation by interferon-gamma. J. biol. Chem. *261:* 5384–5388 (1986).

85 Lehmann, V.; Droge, W.: Demonstration of membrane receptors for human natural and recombinant 125I-labeled tumor necrosis factor on HeLa cell clones and their role in tumor cell sensitivity. Eur. J. Biochem. *158:* 1–5 (1986).

86 Aggarwal, B.B.; Eessalu, T.E.; Hass, P.E.: Characterization of receptors for human tumour necrosis factor and their regulation by gamma-interferon. Nature *318:* 665–667 (1985).

87 Tsujimoto, M.; Yip, Y.K.; Vilcek, J.: Tumor necrosis factor: Specific binding and internalizating in sensitive and resistant cells. Proc. natn. Acad. Sci. USA *82:* 7626–7630 (1985).

88 Ruggiero, V.; Tavernier, J.; Fiers, W.; Baglioni, C.: Induction of the synthesis of tumor necrosis factor receptors by interferon-gamma. J. Immun. (in press, 1986).

89 Kull, F.C., Jr.; Jacobs, S.; Cuatrecasas, P.: Cellular receptor for ^{125}I-labeled tumor necrosis factor: specific binding, affinity labeling, and relationship to sensitivity. Proc. natn. Acad. Sci. USA *82:* 5756–5760 (1985).

90 Aggarwal, B.B.; Traquina, P.R.; Eessalu, T.E.: Modulation of receptors and cytotoxic response of tumor necrosis factor-alpha by various lectins. J. biol. Chem. *262:* 1–5 (1986).

91 Ito, H.; Yamamoto, S.; Kuroda, S.; Sakamoto, H.; Kajihara, J.; Kiyota, T.; Hayashi, H.; Kato, M.; Seko, M.: Molecular cloning and expression in *Escherichia coli* of the cDNA coding for rabbit tumor necrosis factor. DNA *5:* 149–156 (1986).

92 Ito, H.; Shirai, T.; Yamamoto, S.; Akira, M.; Kawahara, S.; Todd, C.W.; Wallace, R.B.: Molecular cloning of the gene encoding rabbit tumor necrosis factor. DNA *5:* 157–165 (1986).

93 Haranaka, K.; Satomi, N.; Sakurai, A.; Nariuchi, H.: Purification and partial amino acid sequence of rabbit tumor necrosis factor. Int. J. Cancer *36:* 395–400 (1985).

Dr. Bruce Beutler,
Howard Hughes Medical Institute,
University of Texas Health Science Center at Dallas,
5323 Harry Hines Blvd.,
Dallas, TX 75235 (USA)