

CACHECTIN/TUMOR NECROSIS FACTOR STIMULATES  
COLLAGENASE AND PROSTAGLANDIN E<sub>2</sub> PRODUCTION  
BY HUMAN SYNOVIAL CELLS AND DERMAL  
FIBROBLASTS

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When threatened by pathogenic organisms or neoplastic disease, the mammalian host responds with a complex repertoire of metabolic alterations (1–3). During acute infection, accelerated protein and lipid catabolism are often observed, as are fever, hypoglycemia, and shock. In chronic neoplastic or infectious disease states, unabated depletion of energy stores may lead to cachexia, and ultimately, to the demise of the host. These processes appear to be governed by the immune system, which, in part, exerts its influence over other somatic tissues through the action of various cytokines.

Recently (4), we reported the isolation of cachectin, a murine macrophage hormone capable of suppressing the expression of anabolic enzymes in adipocytes, thus preventing the uptake and storage of triglyceride. Subsequently, cachectin was shown (5) to be identical to tumor necrosis factor (TNF), a monokine known for its ability to selectively lyse malignantly transformed cells (6–8). Although this latter property has been the most studied, we have suggested that the biological function of this monokine is far broader. Cachectin/TNF is secreted in large quantities by endotoxin-induced macrophages, or macrophage-derived cells (4, 9). Upon interaction with a specific plasma membrane receptor on the adipocyte (4), it is capable of altering cellular metabolism by selectively suppressing the expression of mRNA encoding specific anabolic proteins (10). Cachectin/TNF has been implicated both as the primary mediator of shock in the setting of gram-negative septicemia (11), and as the agent responsible for cachexia in chronic infectious disease states (12). Thus, it seems that cachectin plays a major role in the mammalian inflammatory response. As such, it is functionally related to several other inflammatory cytokines, including the interferons (IFN), interleukins (IL), and granulocyte-macrophage colony stimulating factors.

Inflammation depends, in part, upon regional production of proteolytic enzymes that participate in the destruction and remodelling of tissues. It has

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previously been shown that IL-1 elicits collagenase secretion and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis by human synovial cells (13–15) and dermal fibroblasts (15). Collagenase acts to disrupt the extracellular collagen matrix in inflamed tissues (16). PGE<sub>2</sub> is believed to be an important stimulus for the production of intracellular proteases (17, 18), and is also known to trigger bone resorption by osteoclasts in the course of inflammatory disease (19). Herein, we observe that cachectin/TNF also evokes the production of collagenase and PGE<sub>2</sub> by synovial cells and dermal fibroblasts.

### Materials and Methods

Purified mouse cachectin/TNF was prepared as previously described (5). In the preparations used for these experiments, IL-1 (leukocyte activating factor [LAF]) activity was assayed (14) and found to be absent. Human mononuclear cell factor (MCF/IL-1) was isolated by the method of Dayer et al. (20), and was free of IL-2 and IFN- $\gamma$  activities. LAF activity was measured in this preparation as previously described (14). Human IFN- $\alpha$  was obtained from Hoffmann-La Roche, Inc. (Basel, Switzerland). Homogeneously pure mouse recombinant (r) IL-1 was obtained from P. Lomedico of Hoffmann-La Roche, Inc. Lipopolysaccharide (LPS) (*E. coli* strain 055:B5) was obtained from Difco Laboratories (Detroit, MI).

Human synovial cells were isolated by proteolytic dispersion (13) from surgical synovectomy specimens, obtained from patients with rheumatoid arthritis. Cultures were established and cells were passaged by methods detailed previously (15). The adherent synovial cells (ASC) obtained from the primary cultures were plated in 96-well flat-bottom plates (Costar, Cambridge, MA) at a density of  $2 \times 10^4$  cells/well, using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). ASC were used as targets for the action of the above mediators 3–6 d after plating. For assay of collagenase and PGE<sub>2</sub> production, the cells were incubated in DMEM supplemented with 10% FBS, as well as 5  $\mu$ g/ml polymixin B (Sigma Chemical Co., St. Louis, MO) (polymixin B was omitted from wells used for the assay of LPS bioactivity). Cytokines were added to the final concentrations indicated. After 3 d, the medium was harvested, and determinations of collagenase and PGE<sub>2</sub> levels were made.

Human foreskin fibroblasts were similarly isolated from tissue specimens by protease treatment (13), and plated at near-confluence ( $2 \times 10^4$  cells in 200  $\mu$ l medium) in 96-well plates (Costar) using DMEM supplemented with 10% FBS. After 48 h of culture, fresh medium containing cytokines to be tested for collagenase or PGE<sub>2</sub> induction was added to the culture.

Collagenase activity was assayed as previously described (15) using [<sup>14</sup>C]acetylated rat tail collagen as a substrate. PGE<sub>2</sub> was measured in culture media by radioimmunoassay using an antibody obtained from L. Levine (Brandeis University, Waltham, MA).

All bioassays were performed in triplicate.

### Results and Discussion

Addition of purified mouse cachectin/TNF to cultures of human synovial cells stimulated collagenase and PGE<sub>2</sub> production in a dose-related manner (Table I). Significant stimulation of both collagenase and PGE<sub>2</sub> production was apparent when cachectin/TNF concentrations exceeded 0.3 nM. The rise in both activities occurred in a parallel fashion over the range of concentrations tested. A saturating concentration of cachectin/TNF (e.g., that concentration required to produce maximal effects) was not reached in the experiments performed. Murine rIL-1 and highly purified human MCF/IL-1 also elicited a rise in collagenase and PGE<sub>2</sub> production, but it appeared that higher concentrations of these

TABLE I  
*Effect of Cachectin/TNF and Other Mediators on PGE<sub>2</sub> and  
 Collagenase Production by Human Synovial Cells*

Cytokine	PGE <sub>2</sub>	Collagenase*
	<i>µg/ml</i>	<i>U/ml</i>
Control	17 ± 6	<0.01
LPS 5 µg/ml	16 ± 2	<0.01
Cachectin/TNF (nM)		
0.03	27 ± 5	<0.01
0.3	87 ± 12	0.21 ± 0.03
1.5	494 ± 36	0.25 ± 0.06
3.0	650 ± 49	0.46 ± 0.08
Mouse rIL-1 20 nM <sup>‡</sup>	503 ± 45	0.15 ± 0.02
MCF/IL-1 5 nM <sup>‡</sup>	772 ± 28	0.30 ± 0.05
rIFN-α (U/ml)		
10	37 ± 3	<0.01
100	26 ± 12	<0.01
1,000	29 ± 10	<0.01

\* Mean ± SEM.

<sup>‡</sup> Molar concentration of IL-1 is based on the specific activity estimate of Lomedico et al. (26): 6 × 10<sup>5</sup> U/mg (LAF assay).

proteins were required to produce a response of the magnitude observed using cachectin/TNF. For example, a 20 nM concentration of rIL-1 (250 LAF U/ml) was necessary to promote stimulation comparable to that observed using 1.5 nM cachectin/TNF.

rIFN-α caused no demonstrable increase in collagenase activity, and only slightly stimulated the production of PGE<sub>2</sub>. The addition of LPS alone to the cultures, in the absence of polymixin B, did not stimulate either collagenase or PGE<sub>2</sub> production.

Dermal fibroblasts (Table II) were also stimulated by cachectin/TNF to produce collagenase and PGE<sub>2</sub>. In these experiments, dermal fibroblasts appeared relatively less sensitive to cachectin/TNF and relatively more sensitive to IL-1. Neither IFN-α nor LPS had a significant effect on collagenase or PGE<sub>2</sub> production.

Previous studies (13, 14, 16, 20) have implicated IL-1 as the principal mediator for the induction of the proteolysis noted in rheumatoid arthritis and in other inflammatory joint diseases. The enhanced secretion of collagenase and PGE<sub>2</sub> by synovial cells are believed to contribute to the destructive process that is clinically observed. From the data presented above, however, it is apparent that cachectin/TNF, a structurally distinct monokine, is a very potent stimulator of collagenase and PGE<sub>2</sub> production by synovial cells. Moreover, cachectin/TNF is, under some circumstances, produced in far larger quantities than IL-1 in vivo. In rabbits, data suggest (21, 22) that several milligrams are produced per kilogram body mass in response to intravenous injection of LPS.

The effect of cachectin/TNF on dermal fibroblasts is particularly interesting in view of the large quantities of the hormone that are bound by skin following injection of the radiolabeled hormone into mice (21). While the role of the

TABLE II  
*Effect of Cachectin and Other Mediators on PGE<sub>2</sub> and Collagenase  
 Production by Human Dermal Fibroblasts*

Cytokine	PGE <sub>2</sub>	Collagenase
	<i>μg/ml</i>	<i>U/ml</i>
Control	5 ± 1	<0.01
LPS 5 μg/ml	6 ± 2	<0.01
Cachectin/TNF (nM)		
0.03	6 ± 1	<0.01
0.3	15 ± 3	<0.01
1.5	27 ± 5	<0.01
3.0	54 ± 3	0.11 ± 0.02
Mouse rIL-1 20 nM	105 ± 17	0.36 ± 0.05
MCF/IL-1 5 nM	109 ± 29	0.27 ± 0.06
rIFN-α (U/ml)		
10	6 ± 2	<0.01
100	3 ± 2	<0.01
1,000	2 ± 2	<0.01

Concentration values reported as in Table I.

hormone in skin is unclear, it may evoke some of the changes observed in this organ in response to infection, e.g., vasoconstriction and increased capillary fragility. The fact that keratinocytes are known to produce an IL-1-like substance (23) raises the question of whether they, like macrophages, are a potential source of cachectin/TNF.

The different sensitivities of synovial cells and dermal fibroblasts to cachectin/TNF and IL-1 indicate that cachectin/TNF may exert a dominant effect in some tissues, whereas IL-1 may exert a dominant effect in others. In addition, although IL-1 and cachectin/TNF are both produced by mononuclear phagocytic cells, the nature of the stimuli that evoke production of IL-1 differ substantially from those that evoke production of cachectin/TNF. For example, IL-1 is produced in comparable amounts, whether induced by T lymphocyte products, LPS, concanavalin A, or phorbol ester (24), whereas cachectin/TNF production is induced far more strongly by LPS than by concanavalin A or phorbol ester (25). Thus, the selective inducibility of these two monokines, and the graded sensitivity of their target tissues, may allow for the broad variability of the biological effects that occur when different invasive agents challenge the integrity of the host.

It is important to note that cachectin/TNF is capable of binding via a specific receptor to a wide range of tissues (4, 21), with biological effects that have yet to be determined. Given its potency in eliciting collagenase and PGE<sub>2</sub> production by human synovial cells, cachectin/TNF (rather than IL-1) may represent the major mediator of this phenomenon in vivo. Similarly, many of the other bioactivities once thought to be mediated exclusively by IL-1 (e.g., muscle proteolysis, bone resorption, fever) might actually be attributable to cachectin/TNF. A careful reappraisal of these activities seems appropriate.

### Summary

Cachectin/TNF (tumor necrosis factor), an endotoxin-induced murine macrophage hormone implicated in the pathogenesis of cachexia and shock, has been found capable of stimulating collagenase and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production by isolated human synovial cells and dermal fibroblasts. This bioactivity associated with cachectin is comparable to that observed with the monokine interleukin 1 (IL-1), previously suggested as the major mediator of proteolysis. The ability of cachectin/TNF to stimulate collagenase and PGE<sub>2</sub> production suggests that it may play a role in tissue destruction and remodelling, as these processes occur in inflammatory diseases.

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