Brief Definitive Report

EFFECT OF γ INTERFERON ON CACHECTIN EXPRESSION BY MONONUCLEAR PHAGOCYTES

Reversal of the *lps*^{d} (Endotoxin Resistance) Phenotype

BY BRUCE BEUTLER, VERONICA TKACENKO, IAN MILSARK, NADIA KROCHIN, AND ANTHONY CERAMI

From the Laboratory of Medical Biochemistry, The Rockefeller University, New York 10021

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023

Endotoxin (LPS) can evoke a shock state in mammals, characterized by fever, hypotension, diarrhea, leukopenia, disseminated intravascular coagulation, and multiple end-organ damage. This syndrome does not result from the action of the LPS per se; on the contrary, endotoxin evokes the production of toxic endogenous mediators, which in turn cause widespread tissue injury (1).

The most prominent mediator of the lethal effect of LPS appears to be cachectin (tumor necrosis factor; TNF) (2, 3), a macrophage hormone produced in milligram quantities by animals treated with LPS (4, 5). Cachectin binds to receptors widely distributed throughout the body (4), causing severe metabolic acidosis, a biphasic change in plasma glucose levels, and inflammatory lesions in the lungs, gastrointestinal tract, kidneys, adrenals, and pancreas (6), often with fatal consequences.

Recently (7), we showed that endotoxin-resistant (C3H/HeJ) mouse macrophages, bearing the *lps*^{d} mutation, fail to produce cachectin in significant quantities in response to LPS, as a result of a dual biosynthetic lesion. First, the cells fail to exhibit a normal transcriptional response to low concentrations of endotoxin, and second, the cachectin mRNA that is produced is not translated to protein. Probably as a result of these defects, the mice are capable of surviving the administration of large amounts of LPS.

IFN-γ, like LPS, is capable of activating human monocytes/macrophages (8). Increased phagocytic activity and increased generation of H_2O_2 may be observed in these cells after addition of IFN-γ. We wished to determine whether IFN-γ was also capable of eliciting cachectin production, and if so, whether macrophages obtained from the endotoxin-resistant C3H/HeJ mouse would respond to this mediator.

In the present report, we show that IFN-γ by itself is not capable of stimulating cachectin production by isolated mouse peritoneal macrophages. However, IFN-γ appears to augment cachectin biosynthesis in response to LPS, acting at both transcriptional and posttranscriptional levels. It can also overcome the blockade imposed by the *lps*^{d} mutation in C3H/HeJ (endotoxin-resistant) macrophages and permit the biosynthesis of cachectin in response to LPS.

This work was supported in part by grants AM-01314 and AI-21359 from the National Institutes of Health, Bethesda, MD. Address correspondence to B. Beutler, The Howard Hughes Medical Institute, The University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

J. EXP. MED. © The Rockefeller University Press · 0022-1007/86/11/1791/6 $1.00 1791
Volume 164 November 1986 1791–1796

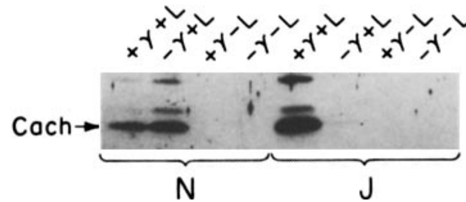

FIGURE 1. Production of cachectin (*Cach*) by peritoneal macrophages obtained from C3H/HeN (*N*) and C3H/HeJ (*J*) mice. Cells were treated with (+) or without (−) endotoxin (*L*) or IFN-γ (γ), each at a concentration of 1 μg/ml. Medium was harvested after 16 h and analyzed by immunoconcentration and immunoblotting.

Materials and Methods

Recombinant mouse IFN-γ was obtained from Genentech Corporation (South San Francisco, CA). *E. coli* (strain 0127:B8) LPS was obtained from Difco Laboratories (Detroit, MI).

Female C3H/HeJ mice (20-g size) were obtained from The Jackson Laboratory (Bar Harbor, ME), and female C3H/HeN mice (20-g size) were obtained from the Charles River Breeding Co. (Wilmington, MA). Animals were kept in the Rockefeller University Laboratory Research Center before use in these experiments. Mice were injected i.p. with 3 ml of sterile Brewer's thioglycollate medium. 3–5 d later, macrophages were harvested by peritoneal lavage using HBSS, sedimented at 1,000 *g*, resuspended in RPMI 1640 medium containing 10% FCS, and plated at confluent density in 24-well Linbro plates (Flow Laboratories, Inc., McLean, VA). After 1 h, nonadherent cells were washed away with serum-free RPMI 1640 medium, and adherent monolayers were covered with the same medium. Cells were then induced with varying concentrations of IFN-γ and/or LPS at the times noted.

Medium was harvested from monocyte or macrophage cultures after incubation with the above mediators, and cachectin was detected by means of an immunoconcentration/immunoblot assay described previously (7). Cell monolayers were lysed, and total cellular RNA was isolated in order to quantitate cachectin mRNA. To prepare RNA for electrophoresis, a standard procedure involving ribonuclease inactivation by guanidinium isothiocyanate and selective precipitation of RNA by 3 M LiCl (9) was used. After electrophoresis of the RNA from each monolayer, cachectin mRNA was measured by blot hybridization using a nick-translated probe complementary to mouse cachectin mRNA (10), or in some instances, using a synthetic RNA probe (11).

Results

As described previously (7), both the mature hormone and processing intermediates are readily visualized on immunoblots of macrophage medium (Fig. 1). Macrophages obtained from C3H/HeN (endotoxin-sensitive) mice produce copious quantities of cachectin in response to LPS, whether or not they have been treated with IFN-γ. Moreover, IFN-γ is incapable of eliciting cachectin production in these cells in the absence of LPS.

C3H/HeJ (endotoxin-resistant) mouse macrophages produce little or no cachectin in response to LPS at 1 μg/ml, or in response to IFN-γ alone; however, normal quantities of cachectin are produced if the cells are treated with IFN-γ before LPS induction (Fig. 1). Thus, the effect of the lps^d mutation is mitigated by exposure to IFN-γ. As seen in Fig. 2*a*, relatively high concentrations of LPS are required to induce cachectin secretion in IFN-γ-treated C3H/HeJ macrophages. Very little cachectin is produced by C3H/HeJ cells exposed to LPS at a concentration below 1 μg/ml.

IFN-γ appears to increase the cellular content of cachectin mRNA in LPS-

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023

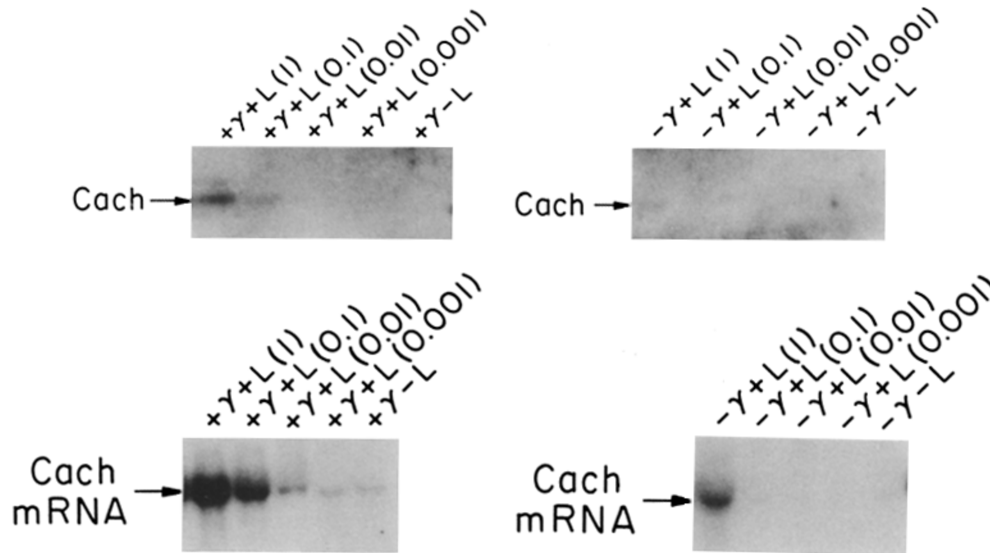

FIGURE 2. Induction of cachectin mRNA and protein biosynthesis in C3H/HeJ macrophages treated with varying concentrations of endotoxin, with or without IFN-γ. *a* and *b*, immunoreactive cachectin (*Cach*) production in cells exposed to LPS at the indicated concentrations (μg/ml) after treatment (*a* and *b*, respectively) with or without IFN-γ. *c* and *d*, cachectin mRNA production by cells exposed to LPS at the indicated concentrations after treatment (*c* and *d*, respectively) with or without IFN-γ.

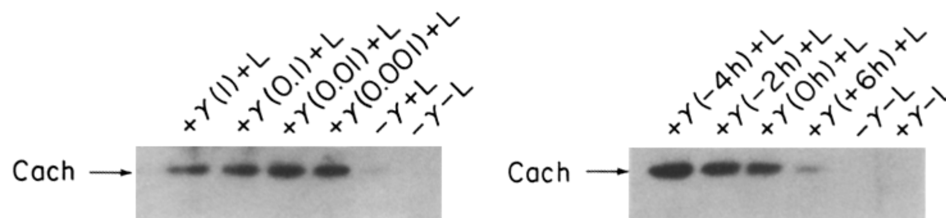

FIGURE 3. *a*, production of cachectin (*Cach*) by C3H/HeJ macrophages treated with 1 μg/ml LPS in the presence of varying concentrations (μg/ml) of recombinant mouse IFN-γ. *b*, production of cachectin by C3H/HeJ macrophages treated with 1 μg/ml LPS, with 1 μg/ml IFN-γ added at varying times relative to the addition of LPS. In both instances, medium was harvested 16 h after the addition of LPS, and cachectin concentration estimated by immunoconcentration and immunoblotting.

treated C3H/HeJ macrophages (Fig. 2*c*). In addition, it appears to facilitate endotoxin-induced cachectin biosynthesis at a posttranscriptional level, since cells containing high levels of cachectin mRNA (Fig. 2*d*) (for example, those treated with 1 μg/ml of LPS) fail to produce normal quantities of immunoreactive protein in the absence of IFN-γ (Fig. 2*b*).

IFN-γ was added to C3H/HeJ macrophages over a wide range of concentrations to determine whether the observed effect occurred at a high dilution, consistent with a receptor-mediated phenomenon (Fig. 3*a*). Full activation of endotoxin-induced cachectin biosynthesis was observed in the presence of 1 ng/ml IFN-γ. It was also noted (Fig. 3*b*) that cells treated with IFN-γ before addition of LPS produced greater quantities of cachectin than cells treated with both agents simultaneously, or cells treated with LPS followed by IFN-γ.

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023

Discussion

Cachectin is a highly toxic protein that appears to act as the primary mediator of LPS-induced shock (3, 6, 7, 12). Systemic release of cachectin leads, among other things, to fever, hypotension, metabolic acidosis, and inflammatory lesions of the lungs, bowel, adrenals, and kidneys. As such, cachectin biosynthesis must be strictly regulated (7). While cachectin mRNA synthesis occurs constitutively, and is augmented by a number of agents, the protein is not translated under normal circumstances. Endotoxin has been shown (7) to greatly increase the rate of cachectin gene transcription, and to allow the posttranscriptional phase of biosynthesis to proceed. In the present report, we have studied the effect of IFN-γ on cachectin expression at both levels.

A variety of chemical and biological agents, including LPS and IFN-γ, activate macrophages, as assessed by morphologic criteria, secretion of plasminogen activator, phagocytosis of opsonized erythrocytes, suppression of 5′-nucleotidase activity, production of oxidative metabolites, and destruction of intracellular pathogens (13, 14). However, with respect to the production of cachectin, macrophage activation induced by IFN-γ differs from activation induced by LPS. IFN-γ appears to prime murine peritoneal macrophages, allowing them to produce cachectin in response to LPS. However, IFN-γ does not evoke cachectin production by itself.

This permissive effect has been noticed by other investigators. Pace, et al. (15, 16) reported that IFN-γ allowed the expression of tumoricidal activity by mouse macrophages after LPS stimulation. More recently, Nedwin, et al. (17) showed that IFN-γ augmented the LPS-induced production of TNF activity by human peripheral blood monocytes. Here, we have demonstrated that the permissive effect of IFN-γ involves both transcriptional and posttranscriptional phases (7) of the LPS-induced response.

In macrophages obtained from the C3H/HeJ mouse, cachectin biosynthesis is defective because a proximal lesion prevents both LPS-induced cachectin gene transcription and cachectin mRNA translation. IFN-γ appears to partially correct the lps^d phenotype, allowing normal expression of cachectin by these cells in response to relatively low concentrations LPS.

Vogel and her associates (18) have reported that macrophages derived from C3H/HeJ mice fail to develop a normal capacity for Fc receptor–mediated phagocytosis in vitro. This defect can be corrected by treatment with conditioned medium obtained from Con A–stimulated lymphocytes (19), or by direct treatment with recombinant interferons (20).

It has been suggested (21) that the abnormality manifested by C3H/HeJ macrophages might reflect a differentiation defect, consequent to insufficient production of interferon, since anti–interferon α/β prevented the normal development of Fc receptor–mediated phagocytosis in cultures of C3H/HeN cells. As mentioned above, it has been noted (17) that human peripheral blood monocytes produce far larger quantities of TNF in response to LPS if first primed with IFN-γ. Our own unpublished studies confirm this observation, and suggest that the enhancement of monocyte cachectin biosynthesis results from augmented cachectin gene transcription and cachectin mRNA translation. The phenotypic resemblance of human peripheral blood monocytes and C3H/HeJ macrophages

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023

(with respect to cachectin production) may be taken to imply that the defect manifested by *lps*^{d}-type cells is, indeed, of a developmental nature.

The mechanism by which IFN-γ augments the normal transcriptional and posttranscriptional responses to endotoxin peripheral blood monocytes and in C3H/HeJ macrophages (thus mitigating the effect of the *lps*^{d} allele) remains unclear. In view of the fact that pretreatment of the macrophages with IFN-γ maximizes cachectin production in response to endotoxin, it may be that protein synthesis is required for IFN-γ to exert its effect.

The identification of IFN-γ as a modulator of cachectin production in response to LPS may be of assistance in defining the molecular events that occur after the macrophage has been exposed to this agent. Moreover, this effect of IFN-γ may have significant consequences in vivo. IFN-γ may act to increase the sensitivity of recipient animals to endotoxin, by facilitating cachectin production in response to LPS. Indeed, it is possible that the priming effect exerted by facultative intracellular bacterial infection may depend upon IFN-γ production, or the elaboration of a related cytokine.

Summary

IFN-γ permits the endotoxin-induced production of cachectin by C3H/HeJ (endotoxin resistant) macrophages, apparently by facilitating endotoxin-induced cachectin biosynthesis at both transcriptional and posttranscriptional levels. IFN-γ cannot induce cachectin biosynthesis by itself, nor does it markedly enhance cachectin production by endotoxin-induced peritoneal macrophages obtained from endotoxin-responsive mice. Elucidation of the precise mechanism through which IFN-γ influences cachectin biosynthesis may permit a better understanding of the molecular events that follow endotoxin-induced activation of macrophages. Moreover, the permissive effect of IFN-γ on cachectin biosynthesis might elicit enhanced endotoxin sensitivity in vivo.

We thank the Genentech Corporation of South San Francisco, CA for providing the mouse IFN-γ used in these studies. Cachectin was provided by Chiron Corporation of Emeryville, CA.

Received for publication 16 July 1986.

References

1. Michalek, S. M., R. N. Moore, J. R. McGhee, D. L. Rosenstreich, and S. E. Mergenhagen. 1980. The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxin. *J. Infect. Dis.* 141:55.
2. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* 161:984.
3. Beutler, B., and A. Cerami. 1986. Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature (Lond.).* 320:584.
4. Beutler, B., I. W. Milsark, and A. Cerami. 1985. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J. Immunol.* 135:3972.
5. Abe, S., T. Gatanaga, M. Yamazaki, G. Soma, and D. Mizuno. 1985. Purification of rabbit tumor necrosis factor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 180:203.
6. Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R.

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023

J. Hariri, T. J. Fahey, III, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami. 1986. Shock and tissue injury induced by recombinant human cachectin. Science (*Wash. DC*). In press.

7. Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* (*Wash. DC*). 232:977.
8. Nathan, C. F., H. W. Murray, M. E. Wiebe, and B. Y. Rubin. 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* 158:670.
9. Cathala, G., J.-F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial, and J. D. Baxter. 1983. A method for isolation of intact, translationally active ribonucleic acid. *DNA* (*NY*). 2:329.
10. Caput, D., B. Beutler, K. Hartog, S. Brown-Shimer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA.* 83:1670.
11. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promotor. *Nucleic Acids Res.* 12:7035.
12. Beutler, B., I. W. Milsark, and A. Cerami. 1985. Passive immunization against cachectin/tumor necrosis factor (TNF) protects mice from the lethal effect of endotoxin. *Science* (*Wash. DC*). 229:869.
13. Johnson, W. J., and D. O. Adams. 1984. Activation of mononuclear phagocytes for tumor cytolysis: analysis of inductive and regulatory signals. *In* Mononuclear Phagocyte Biology. A. Volkman, editor. Marcel Dekker, Inc., New York. 279.
14. Nathan, C. F., T. J. Prendergast, M. E. Wiebe, E. R. Stanley, E. Platzer, H. G. Remold, K. Welte, B. Y. Rubin, and H. W. Murray. 1984. Activation of human macrophages. Comparison of other cytokines with interferon-γ. *J. Exp. Med.* 160:600.
15. Pace, J. L., S. W. Russell, R. D. Schreiber, A. Altman, and D. H. Katz. 1983. Macrophage activation: priming activity from a T-cell hybridoma is attributable to interferon-γ. *Proc. Natl. Acad. Sci. USA.* 80:3782.
16. Pace, J. L., S. W. Russell, B. A. Torres, H. M. Johnson, and P. W. Gray. 1983. Recombinant mouse gamma interferon induces the priming step in macrophage activation for tumor cell killing. *J. Immunol.* 130:2011.
17. Nedwin, G. E., L. P. Svedersky, T. S. Bringman, M. A. Palladino, and D. V. Goeddel. 1985. Effect of interleukin 2, interferon-γ, and mitogens on the production of tumor necrosis factors α and β. *J. Immunol.* 135:2492.
18. Vogel, S. N., and D. L. Rosenstreich. 1979. Defective Fc receptor-mediated phagocytosis in C3H/HeJ macrophages. I. Correction by lymphokine-induced stimulation. *J. Immunol.* 123:2842.
19. Vogel, S. N., L. L. Weedon, R. N. Moore, and D. L. Rosenstreich. 1982. Correction of defective macrophage differentiation in C3H/HeJ mice by an interferon-like molecule. *J. Immunol.* 128:380.
20. Fertsch, D., and S. N. Vogel. 1984. Recombinant interferons increase macrophage Fc receptor capacity. *J. Immunol.* 132:2436.
21. Vogel, S. N., and D. Fertsch. 1984. Endogenous interferon production by endotoxin-responsive macrophages provides an autostimulatory differentiation signal. *Infect. Immun.* 45:417.

Downloaded from http://rup.silverchair.com/jem/article-pdf/164/5/1791/1096628/1791.pdf by guest on 28 March 2023