Dissociation of Transcription from Translation of Human IL-1β: Induction of Steady State mRNA by Adherence or Recombinant C5a in the Absence of Translation (43425)

CHARLES A. DINARELLO[1]

Department of Medicine, Tufts University School of Medicine and New England Medical Center, Boston, Massachusetts 02111

Abstract. **Interleukin (IL) 1 is an important mediator of local and systemic disease. Blocking IL-1 using the IL-1 receptor antagonist has reduced the severity of disease in animal models of septic shock, diabetes, graft-vs-host disease, inflammatory bowel disease, and the spontaneous proliferation of leukemia cells. Blocking IL-1 and reduction in the synthesis of IL-1 are important strategies for reducing the progression of inflammatory disease and autoimmune diseases. Nature, however, maintains control over the synthesis of IL-1 by dissociating transcription for translation. In this paper, the basis for the dissociation of IL-1β synthesis of mRNA from synthesis of the IL-1β protein is reviewed.** [P.S.E.B.M. 1992, Vol 200]

The basis for these studies began about 20 years ago, during which time we produced endogenous pyrogen from human blood monocytes. Because the bioassay for endogenous pyrogens is the rabbit fever response, contamination of small quantities of bacterial endotoxin(s) (lipopolysaccharide [LPS]) in any preparation was a major concern. The rabbit will respond to 5–10 ng/kg of LPS with a monophasic fever, very similar to, albeit not identical with, the fever induced by true endogenous pyrogens. The presence of albumin enhances the monophasic fever in rabbits such that 0.1–1 ng/kg would also produce fever. Although the type of LPS found in most "water" microbial contaminants, for example, Pseudomonas or Aerobacter species, is far less potent a pyrogen than LPS produced by Salmonella or Escherichia, we were highly vigilant about the conditions of our cultures of human cells and particularly the choice of stimulants. Otto Westphal, who is associated with the discovery that Lipid A contains the toxic moiety of LPS, often criticized the actual existence of endogenous pyrogen by claiming that Lipid A bound to small molecular weight protein would produce fever indistinguishable from what was characterized as endogenous pyrogen. Westphal's protests did not exactly encourage research on endogenous pyrogen, since his claims were difficult to disprove. Despite these difficulties, we were confident of our methods because products of unstimulated human cells did not produce fever in rabbits.

We had become highly aware of the potential for LPS contamination and thus did not use LPS as a stimulator of endogenous pyrogen synthesis; instead, we used boiled or autoclaved suspensions of *Staphylococcus epidermidis*. This stimulant had been introduced by fever researchers Phillis Bodel, Elisha Atkins, and Sheldon Wolff. Thus, we were able to purify human endogenous pyrogen in the absence of added LPS. All columns and materials were tested for the presence of LPS and were run aseptically. Sterility tests were routinely done.

Why discuss this issue? In the following pages, it will become clear that one cannot study transcription or translation of interleukin (IL) 1 (or tumor necrosis factor [TNF] for that matter) unless the experimental design considers the sensitivity of the cells for very low concentrations of LPS. For example, it is not unusual

[1] To whom requests for reprints should be addressed at Department of Medicine, Tufts University School of Medicine, New England Medical Center, Boston, MA 02111.

0037-9727/92/2002-0228$3.00/0

to observe IL-1β and TNF production by 1 pg/ml of LPS when the responding cell is the human blood monocyte. Assuming a molecular weight of LPS of 1,000,000 and about 600,000 monocytes required to detect IL-1 or TNF by enzym-linked immunosorbent assay or radioimmunoassay, 1 pg/600,000 monocytes is approximately one LPS molecule per monocyte. This is sufficient to trigger not only detectable transcription, but also translation of 1–5 ng/600,000 monocytes of immunoreactive IL-1β and TNF.

Since nearly all commercially available tissue culture media, lymphocyte separation preparations, and fetal or bovine sera contain from at least 5 to 500 pg/ml of LPS, most reports on IL-1 transcription from human blood monocytes are in need of reinterpretation. For example, it is not difficult to find in the published literature the use of 10 μg/ml or greater LPS concentrations to stimulate the production of IL-1 over control values. Indeed, some experimentalists claim that there is "spontaneous" production of IL-1 by human blood cells. Others have reduced the stringency of their cDNA probes to have a "negative" cell control over that of stimulated cells. Thus, the issue of transcriptional and translational signals being distinct cellular events for IL-1 (or TNF) has been overlooked. The biological phenomenon of "priming" for cytokine synthesis has similarly escaped investigation due to the ubiquitous presence of LPS in most experiments. An example of misinformation is the data published on the induction of IL-1 by the human immunodeficiency virus. Several papers have suggested that this virus induces IL-1 and, moreover, that the induction of IL-1 is due to the 120 glycoprotein of the viral envelope. However, when these same experiments are done in the strict absence of LPS, there is no induction of IL-1β (1–4). These studies have been confirmed by others using care not to induce LPS into the cultures. Of interest is the observation that human immunodeficiency virus will prime the monocyte to make more IL-1.

Experiments in an LPS-Free Environment

The best method for removing LPS and other microbial products is ultrafiltration using either a hollow fiber filter device or a spun filter. Both methods require positive pressure or a pump to accomplish ultrafiltration. In addition, the choice of ultrafiltration membrane is also important. We have published details concerning these methods (5–8). Water used to dissolve Ficoll or dilute Hypaque requires ultrafiltration. After adding antibiotics and amino acids, tissue culture media should be subjected to ultrafiltration. Finally, a small amount of human serum is used which provides LPS binding protein; it is collected and processed under LPS-free conditions. A single AB donor can be used for all experiments; furthermore, the use of not more than 2% (v/v) for 2.5 million human peripheral blood mononuclear cells (PBMC) is recommended. Baked (180°C $\times$ 4 hr) glass or plastics formed by high heat are LPS-free.

Perhaps one of the most frustrating and time-consuming aspects of our cloning of the cDNA for human IL-1β (9) was the presence of mRNA from unstimulated cells that translated into IL-1β protein in a rabbit reticulocyte or frog oocyte system. We observed this despite the absence of biologically active IL-1 in the cells from which the RNA was extracted. Although there appeared to be more poly(A) mRNA coding for IL-1β in cells stimulated by LPS or *S. epidermidis*, poly(A) mRNA from unstimulated cells adhering to glass was nearly the same. In fact, this observation retarded our clonal selection for several months. It was not until a poly(A) preparation was made from *non-adherent* PBMC that we observed an absence of poly(A) coding for IL-1β.

For the experimental conditions of inducing poly(A) mRNA coding for IL-1β in the absence of exogenous stimuli, two conditions must be met: (i) the adherence surface and culture condition must be LPS-free and (ii) the measurement of both intracellular and extracellular pools of IL-1 must be made by assessing total synthesis, not biological activity. Clearly, for IL-1β, the latter becomes an important issue, since the IL-1β precursor is 100-fold less active (10) than the mature, processed 17.5-kDa IL-1 in biological assays (reviewed in Ref. 11). Recent evidence demonstrates that commercially available enzyme-linked immunosorbent assay kits that are based on monoclonal antibodies to the mature 17.5-kDa IL-1β mature protein fail to detect over 90% of the IL-1β precursor (12) and, hence, total synthesis of IL-1β requires an immunoreactive assay that detects both the precursor as well as the mature form. We use an radioimmunoassay for human IL-1β that detects the precursor and mature forms. We also have used radioimmunoprecipitation to detect total synthesis (13). Using a high dose of IL-2 as a stimulant, the radioimmunoassay also detects intracellular IL-1β (14). The conditions for obtaining cell-associated (cytosolic and membrane) fractions have been optimized (15).

Major Effect of LPS Is on Translation

Using these conditions, we observed that the adherence of PBMC to glass surfaces is a signal for transcription only, but not for synthesis into IL-1β protein. An aliquot of PBMC from the same adhering cell population but stimulated by LPS (1 ng/ml) produces levels of mRNA over that of non-LPS-treated cells; the ratio of increase in mRNA coding for IL-1β after LPS compared with adherence alone ranges from 2- to 10-fold. Table I illustrates this in the cells of five human donors.

mRNA from adhering cells was not incapable of

Table I. Comparison of IL-1β mRNA and IL-1β Protein

	IL-1β mRNA			IL-1β protein[a]	
	Adherence vs LPS		Ratio	Adherence vs LPS	Ratio
1	3568	13276	3.7	<80[b]	3700
2	1391	13209	9.4	<50	12600
3	524	7434	14.2	<40	2500
4	4877	11377	2.3	<40	5500
5	5041	19553	3.8	<40	8300
Mean (± SE) Stimulation Index	6.7 ± 2.2			141 ± 39	

[a] Based on radioimmunoassay in pg/2.5 million PBMC.
[b] For calculation of the ratio, we used the limit of detection, although the amount of IL-1β was below the detection limit.

translating IL-1β; the addition of LPS to PBMC adhering to a surface yielded significantly more IL-1β than cells stimulated by LPS without a previous step of adherence (13). Furthermore, we observed the enhanced synthesis of IL-1β at all time points examined (4, 8, 12, and 24 hr). The half-life of the IL-1β mRNA induced by adherence was not significantly shorter than that for LPS-induced mRNA. Thus, we conclude that: (i) there is no significant mRNA transcribed in nonadherent PBMC from healthy donors; (ii) within 1 hr, there are increases in the levels of steady state mRNA as a result of surface contact; and (iii) the effect of LPS appears to be primarily at the translational level.

The best example for showing that transcription is an isolated event from translation is the C3H/HeJ mouse. We have observed significant transcription in the splenic and peritoneal macrophages of these mice using LPS or toxic shock syndrome toxin; however, unlike the parent strain A/HeJ, there is no or very little translation of this mRNA into IL-1 protein (16). It is tempting to speculate that LPS induces a transcriptional signal as well as a translational trigger in the cells of the C3H/HeJ mouse, but that there are high levels of translational blockers in the cells of these animals. In normal mice, this blockade is overcome by LPS. The 3′-sequences TTATTTAT common to many LPS-induced cytokines may play a role in preventing translation (17). It has been speculated that these sequences impart mRNA instability to several cytokines, whereas LPS stabilizes the mRNA, allowing it to be translated. Recently, it was shown that attaching these sequences to the 3′-end of hemoglobulin results in hemoglobin mRNA instability and markedly reduced hemoglobin synthesis. It is interesting to speculate that a repressor of translation is present in most cells and that this is overcome by LPS, but that the C3H/HeJ mouse lacks either the mechanism to overcome this repression protein or produces excessive amounts. The induction of an enzyme that hydrolyzes such a translational repressor protein may explain the effect of LPS on translation.

Although adherence to glass is a strong signal for transcription, we have also observed transcription in polypropylene tubes, to which PBMC do not adhere. Since the induction of mRNA in polypropylene was reduced when these cells were rotated (13, 18), we conclude that cell to cell interaction also serves as a stimulus. We have even taken whole heparinized human blood and placed it in a closed circuit of LPS-free tubing (polyethylene and silicon used in blood pumps such as renal dialysis or blood oxygenators) and circulated this blood at 250 ml/min at 37°C. This blood was then removed and separated by Ficoll-Hypaque and total nucleic acids were prepared. There was no evidence of IL-1 gene expression in blood subjected to pumping in this circuit (19).

Translation is Stimulus Specific

We compared gene expression for IL-1β with translation rates using either LPS or boiled *S. epidermidis* as a stimulant. LPS and Staphylococcus induce nearly comparable amounts of mRNA from IL-1β at all time points examined (2, 4, 8, and 24 hr) (13). The ratios of stimulation LPS versus Staphylococcus was 2.2-fold in one donor and 0.48-fold in another. However, when IL-1β protein was determined, the amount of IL-1β protein was 50-fold greater in cells stimulated with Staphylococcus. We also observed a similar dissociation of transcription to translation when we measured TNF. There is another example of the separate signals provided by stimulants. LPS and toxic shock syndrome toxin induce the same amount of mRNA for IL-1 in mouse macrophages; however, the amount of IL-1 protein synthesized in response to the toxic shock toxin is significantly greater by a factor of 10. It appears that the exogenous stimulants which trigger translation involve cellular events distinctly different from those which trigger transcription. Although the second messengers are unknown, as discussed below, these do not include elevations in cytosolic calcium, generation of reactive oxygen radicals, or phosphatidylinositol phospholipase C.

Recombinant, Unglycosylated C5a Is Only a Transcriptional Signal for IL-1β

We have extended our studies to demonstrate the separation of transcription and translation of IL-1β

using recombinant human C5a. We reported that purified, natural C5a induced IL-1β, IL-1α, and TNF synthesis (20, 21). Recombinant C5a lacks the glycosylation side chains of the carboxyl terminus, although the N-terminal amino acids of the recombinant molecule bind to the C5a receptor on neutrophils and monocytes and transduce the signals associated with its biological properties. For example, recombinant C5a induces reactive oxygen radicals, cytosolic calcium, chemotaxis, and granule release. However, we have not observed synthesis of IL-1 or TNF with these events.

Using recombinant C5a from 10 to 500 ng/ml on human PBMC, we have observed significant transcription of IL-1β and TNF (22). Similar to the situation in adherent monocytes, this mRNA in cells primed with C5a translates more IL-1β protein with additional LPS than cells stimulated with LPS alone. Since C5a induces prostaglandin E and prostaglandin E suppresses the translation of IL-1β (23), our experiments were carried out in the presence of concentrations of indomethacin that block all prostaglandin E production.

One interesting aspect to these studies is that fact that recombinant C5a not only primes cells for translation induced by low, femtomolar concentrations of LPS (24), but also translation induced by IL-1 itself (18, 22, 24). Since IL-1 induces its own gene expression as well as its own translation, the binding of IL-1 to its receptor is sufficient to trigger both events. Recently, it has been shown that there are two IL-1 receptors: a 68-kDa glycoprotein found on neutrophils, macrophages, and B cells (1), and an 80-kDa glycoprotein found on T cells, hepatocytes, endothelial and smooth muscle cells, and fibroblasts (reviewed in Ref. 11). Triggering of the p68 IL-1R is sufficient to stimulate both transcriptional events.

Cellular Messengers for IL-1 Transcription and Translation

Why does glycosylated C5a trigger both transcription and translation of IL-1? The N-terminus of C5a contains the amino acids associated with induction of superoxide, chemotaxis, and increased cytosolic calcium. Formylmethionine leucine phenylalanine (f-met-leu-phe) also triggers these same cellular events, but f-met-leu-phe does not trigger IL-1 gene expression. Hence, these events per se are *unrelated* to transcription of IL-1. What, then, is the signal for transcription?

Are Two Receptors Involved in Triggering Translation for IL-1β?

This question is presently unanswerable, but we propose the following as a working hypothesis. Since glycosylated C5a is triggering an event on the cell membrane clearly distinct from the events triggered by the C5a receptor, another receptor appears to be involved, and this apparently requires the ligand's glycosylated side chains. These side chains extend from the carboxyl-terminal and are spatially distinct from the rest of the molecule. The cross-linking of a second receptor by glycosylated C5a might provide the signal for translation that is missing from the C5a receptor, which only triggers transcription.

Why does IL-1 itself accomplish both transcription and translation? If the proposed model of C5a action is correct, then IL-1 must also bring about the cross-linking of two peptides during cell activation. Actually, there is now a good case for several cytokines triggering cellular events by a cross-linking mechanism. Examples include IL-2, IL-4, IL-6, and TNF. The case for the TNF receptor being a two-polypeptide complex is now quite strong. The exquisite and far-reaching work of Engelmann *et al.* (25) now provides evidence that the TNF trimer cross-links two distinct polypeptide membrane receptors. Cross-linking by anti-TNF receptors is sufficient to induce a biological response in the absence of TNF.

We and others also have reported the existence of a third IL-1R with a molecular mass of 29–30 kDa (26, 27). Given the present information on the existence of the p68 IL-1R on macrophages and B cells (1, 2), we speculate that it is this receptor and possibly a 30-kDa protein which is cross-linked by IL-1.

These studies are supported by National Institutes of Health Grant AI 15614. The author thanks Drs. Jeffrey A. Gelfand, Stanley Shaldon, and Ralf Schindler for their contributions to these studies.

1. Horuk R, Huang JJ, Covington M, Newton RC. A biochemical and kinetic analysis of the interleukin-1 receptor. Evidence for differences in molecular properties of IL-1 receptor. J Biol Chem **262**:16275–16283, 1988.
2. Ghiara P, Scapigliati G, Tagliabue A, Boraschi D. Characterization of receptors for IL-1 alpha and IL-1 beta in lymphoid and non-lymphoid cells. J Immunol Res **1**:13–16, 1989.
3. Molina J-M, Schindler R, Ferriani R, Sakaguchi M, Vannier E, Dinarello CA, Groopman JE. Production of cytokines by peripheral blood monocytes/macrophages infected with human immunodeficiency virus type 1 (HIV-1). J Infect Dis **161**:888–893, 1990.
4. Molina J-M, Scadden DT, Amirault C, Woon A, Vannier E, Dinarello CA, Groopman JE. Human immunodeficiency virus does not induce interleukin-1, interleukin-6, or tumor necrosis factor in mononuclear cells. J Virol **64**:2901–2906, 1990.
5. Dinarello CA, O'Connor JV, LoPreste G, Swift RL. Human leukocyte pyrogen test to detect pyrogenic material in growth hormone from recombinant *E. coli*. J Clin Microbiol **20**:323–329, 1984.
6. Dinarello CA, Lonnemann G, Maxwell R, Shaldon S. Ultrafiltration to reject human interleukin-1-inducing substances derived from bacterial cultures. J Clin Microbiol **25**:1233–1238, 1987.
7. Schindler R, Dinarello CA. A method for removing interleukin-1- and tumor necrosis factor-inducing substances from bacterial cultures by ultrafiltration with polysulfone. J Immunol Methods **116**:159–165, 1989.
8. Schindler R, Dinarello CA. Ultrafiltration to remove endotoxins

and other cytokine-inducing materials from tissue culture media and parenteral fluids. Biotechniques **8**:408–413, 1990.
9. Auron PE, Webb AC, Rosenwasser LJ, Lucci SF, Rich A, Wolff SM, Dinarello CA. Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. Proc Natl Acad Sci USA **81**:7907–7911, 1984.
10. Rosenwasser LJ, Webb AC, Clark BD, Irie S, Chang L, Dinarello CA, Gehrke L, Wolff SM, Rich A, Auron PE. Expression of biologically active human interleukin 1 subpeptides by transfected simian COS cells. Proc Natl Acad Sci USA **83**:5243–5246. 1986.
11. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood **77**:1–26, 1991.
12. Herzyk DJ, Berger AE, Allen JN, Wewers MD. Conformational differences between mature, 17.5 kDA IL-1β and the carboxyterminus of 35 kDa pro-IL-1β identified by immunodetection with western blot and ELISA. J Immunol Methods (in press).
13. Schindler R, Clark BD, Dinarello CA. Dissociation between interleukin-1 beta mRNA and protein synthesis in human peripheral blood mononuclear cells. J Biol Chem **265**:10232–10237, 1990.
14. Numerof RP, Kotick AN, Dinarello CA, Mier JW. Pro-interleukin-1β production by a subpopulation of human T cells, but not NK cells, in response to interleukin-2. Cell Immunol **130**:118–128, 1990.
15. Endres S, Ghorbani R, Lonnemann G, van der Meer JWM, Dinarello CA. Measurement of immunoreactive interleukin-1 beta from human mononuclear cells: Optimization of recovery, intrasubject consistency and comparison with interleukin-1 alpha and tumor necrosis factor. Clin Exp Immunopathol **49**:424–438, 1988.
16. Ikejima T, Orencole SF, Saijo T, Webb AC, Auron PE, Dinarello CA. Endotoxin resistance in mice is related to IL-1 production by toxic shock syndrome Toxin-1 [Abstract]. Lymphokine Res **6**:1110, 1987.
17. Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. Proc Natl Acad Sci USA **83**:1670–1674, 1986.
18. Dinarello CA, Okejima T, Warner SJC, Orencole SF, Lonnemann G, Cannon JG, Libby P. Interleukin-1 induces interleukin-1. I: Induction of circulating interleukin-1 in rabbits *in vivo* and in human mononuclear cells *in vitro*. J Immunol **139**:1902–1910, 1987.
19. Schindler R, Lonnemann G, Shaldon S, Koch KM, Dinarello CA. Transcription, not synthesis, of interleukin-1 and tumor necrosis factor by complement. Kidney Int **37**:85–93, 1990.
20. Okusawa S, Yancey KB, van der Meer JWM, Endres S, Lonnemann G, Hefter K, Frank MM, Burke JF, Dinarello CA, Gelfand JA. C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro: Comparison with secretion of interleukin-1 beta and interleukin-1 alpha. J Exp Med **168**:443–448, 1988.
21. Okusawa S, Dinarello CA, Yancey KB, Endres S, Lawley TJ, Frank MM, Burke JF, Gelfand JA. C5a induction of human IL-1: Synergistic effect with endotoxin or inlerferon-gamma. J Immunol **139**:2635–2640, 1987.
22. Schindler R, Gelfand JA, Dinarello CA. Recombinant C5a stimulates transcription rather than translation of interleukin-1 (IL-1) and tumor necrosis factor: Translational signal provided by lipopolysaccharide or IL-1 itself. Blood **76**:1631–1638, 1990.
23. Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol **137**:3189–3194, 1986.
24. Morin M, Schindler R, Wakabayashi G, Dinarello CA, Gelfand JA. Recombinant human C5a amplifies the synthesis of IL-1b and TNFa by human peripheral blood mononuclear cells exposed to femtomolar concentrations of endotoxin. Eur Cytokine Network **2**:27–30, 1991.
25. Engelmann H, Aderka D, Holtmann C, Brakebusch C, Nophar Y, D. N, Kemper O, Shermer-Avni Y, Sarov I, Wallach D. Soluble forms of the TNF receptors, purified from human urine, inhibit TNF effects. Antibodies against the soluble receptors induce TNF-like effect [Abstract]. Cytokine **1**:146, 1989.
26. Savage N, Puren AJ, Orencole SF, Ikejima T, Clark BD, Dinarello CA. Studies on IL-1 receptors on D10S T-helper cells: Demonstration of two molecularly and antigenically distinct IL-1 binding protein. Cytokine **1**:23–35, 1989.
27. Kroggel R, Martin M, Pingoud V, Dayer J-M, Resch K. Two-chain structure of the interleukin-1 receptor. FEBS Lett **229**:59–65, 1988.