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Interleukin 1 Regulates Human Metallothionein Gene Expression

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Incubation of cultured human cells with interleukin 1 leads to increased expression of the human metallothionein-II$_A$ gene. Recently, metallothionein has been shown to be an efficient free radical scavenger, and induction by interleukin 1 may be part of a protective response to minimize damage by hydroxyl radicals.

Interleukin (IL-1), also known as leukocytic pyrogen, leukocytic endogenous mediator, and lymphocyte-activating factor, is a 13,000-dalton polypeptide hormone produced by activated macrophages (6, 24). IL-1 is responsible for the initiation and modulation of a broad range of immune and inflammatory responses (6). Among the IL-1-controlled responses is the acute-phase response, during which the biosynthesis of a number of liver proteins, such as c-reactive protein, serum amyloid, fibrinogen, α_1-antitrypsin, α_1-acid glycoprotein, and others, is induced many fold (23, 26, 31).

Acute stress induced, for example, by the injection of lipopolysaccharide also leads to increased biosynthesis of the metallothioneins (MTs) (8, 25), a group of low-molecular-weight, cysteine-rich, heavy metal-binding proteins (14). The increased production of MTs during stress is due to an increased level of MT mRNA (30). While the exact role of these proteins is not clear, they are thought to serve as a protective system against heavy metal toxicity. This hypothesis is supported by their rapid transcriptional activation by heavy metal ions (9) and by the fact that increased expression of MTs in cultured mammalian cells confers resistance to heavy metal ions (4, 16). Another function suggested for these genes is the regulation of trace metal metabolism, especially the maintenance of zinc homeostasis (14). Recently, it was demonstrated in vitro that MTs are extremely efficient scavengers of free hydroxyl (· OH) radicals (34), and, as discussed later, this same function is likely to occur in vivo.

In addition to being induced by heavy metal ions (Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, etc.), transcription of MT genes is regulated by glucocorticoid hormones (12, 17, 18). Some time ago, a previous report suggested that glucocorticoid hormones could be the possible mediators of the stress-related induction of MT (19), since the blood levels of glucocorticoids are elevated during acute stress. In this communication, we report that, in addition to glucocorticoids, MT gene expression also is induced by IL-1, another mediator of the acute response.

To obtain a human tissue culture system for investigating the response of the MT genes to IL-1, a variety of human cell lines and primary cultures grown in the presence of IL-1 (affinity-purified material purchased from Genzyme, Inc., Boston, Mass.) were screened by RNA blot hybridization (33) with an MT-II cDNA probe (20). Three of the cell lines examined exhibited significant induction of MT mRNA in response to IL-1: Hep G2, a hepatoma line (1); HL-60, a promyelocytic cell line (7); and primary human fibroblasts isolated from foreskin. The reason for the lack of responsiveness of many of the other cell lines examined is not clear but could be due, among other factors, to a lack of cell surface receptors to IL-1. MT mRNA induction by IL-1 in a positive cell line, Hep G2, and a negative line, HA251, is shown in Fig. 1. As a control, we have examined the levels of both actin and tubulin mRNAs in these cell lines, and in no case was their level affected by the treatment (data not shown; see Table 1).

Next, we examined the time course of the response to IL-1; the response of Hep G2 cells is relatively fast (Fig. 2A). Maximal levels of MT mRNA were achieved at 12 h postinduction, and the kinetics of the response is similar to MT mRNA induction by glucocorticoids and heavy metals (15, 22). In contrast, the response in HL-60 cells is slower, with maximal levels of MT mRNA obtained 24 h after the addition of inducer. The basis for these differences is currently under investigation.

Since several functional human MT genes hybridize to the MT-II coding region probe, we have used the S1 nuclease protection assay to determine which of the MT genes responds to IL-1 (5). Specific hybridization probes were derived from the hMT-II$_A$ (21) or the hMT-I$_A$ (27) genes. These probes hybridize to the 5′ untranslated region of MT mRNA,

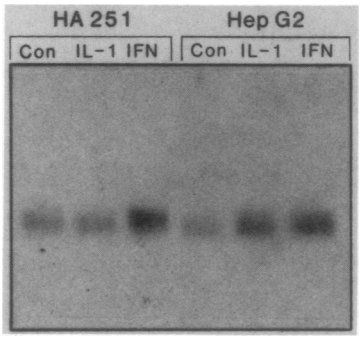

FIG. 1. Differential response of cultured human cells to IL-1 and interferon (IFN). Confluent cultures of a kidney tumor cell line, HA251, and a liver tumor cell line, Hep G2, were incubated for 12 h in normal growth medium (Dulbecco modified Eagle medium plus 10% fetal calf serum) in the absence of any added inducers (lanes labeled Con) or in the presence of 10 U of IL-1 (IL-1-labeled lanes) per ml or 20% of a supernatant from phytohemagglutinin-stimulated lymphocytes, a source of IFN-α and IFN-γ. Total cytoplasmic RNA was prepared by established procedures (17), separated on 1.5% agarose gels, transferred to nitrocellulose filters (33), and probed with an MT-II coding region probe. This probe hybridizes to all of the human MT genes (21). HA251 is representative of cell lines which respond to IFN but not IL-1, and Hep G2 is representative of cell lines which respond to both agents (similar responses are observed also with human fibroblasts and HL-60 cells).

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a region which shows a high degree of divergence among the different genes (27). Only the hMT-II_A gene exhibited detectable induction with IL-1 (Fig. 3). The S1 protection assay also indicates that MT-II mRNA induced by IL-1 is initiated at the same start site as the heavy metal- and glucocorticoid-induced mRNA's. The hMT-II_A gene now has been shown to respond to four different inducers: heavy metal ions, glucocorticoids, interferon (10), and IL-1.

Affinity-purified protein preparations still may contain trace amounts of contaminants, and the possibility exists that the induction of MT mRNA we observe is not due to IL-1 but is a response to some rare contaminant. The cDNA for murine IL-1 has been cloned and expressed in *Escherichia coli* (24). The material produced by the bacteria should be free of the contaminants likely to be present in IL-1 prepared from human plasma.

In Table 1 we compare the dose-response relationships between human IL-1 and recombinant murine IL-1 with respect to induction of MT mRNA. Both IL-1 preparations lead to significant (four- to fivefold) induction of MT mRNA in the Hep G2 cell line. However, the recombinant murine IL-1 seems to be required in somewhat higher doses than the human IL-1 to achieve an equivalent induction of MT mRNA. We do not know whether those differences are due to intrinsic differences between human and murine IL-1 in their reactivity toward human cells, to some variation in the determination of IL-1 activity units for the two different preparations, or to the absence of some posttranslational modifications on the recombinant IL-1 which might affect its activity or stability.

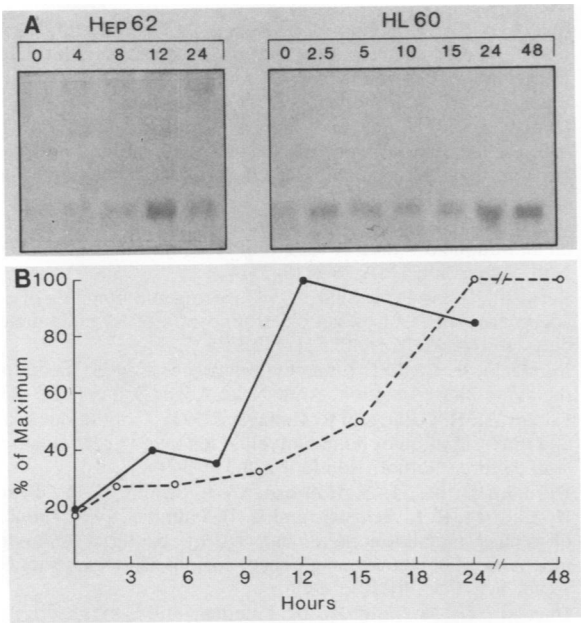

FIG. 2. Time course of MT mRNA induction by IL-1. Panel A, Confluent cultures of Hep G2 cells or suspension of 10^7 HL-60 cells per ml were incubated for the indicated time periods in the presence of 10 U of IL-1 per ml. RNA was extracted and analyzed by using the coding region probe as described in the legend to Fig. 1. Panel B, Quantitation of the induction kinetics of MT mRNA by IL-1. Two different exposures (within the linear range) of the autoradiograms shown in panel A were quantitated by densitometry. The optical densities of the MT-specific bands were normalized relative to the optical density of an actin mRNA band, as detected by hybridization of the same blots to an actin specific probe (27).

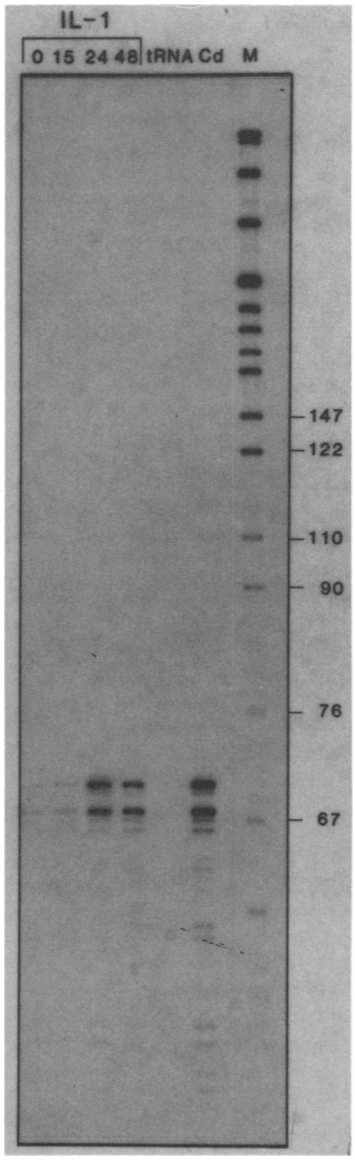

FIG. 3. Mapping of the start sites of IL-1-induced MT-II mRNA. Fifty micrograms of total cytoplasmic RNA samples from HL-60 cells incubated for the indicated times with 10 U of IL-1 per ml or for 8 h with 5×10^{-6} M $CdCl_2$ was hybridized to 50,000 cpm (specific activity, 10^6 cpm/pmol) of a single-stranded *Taq*I-*Bam*HI fragment, labeled at the *Bam*HI site, which contains the 5′ untranslated region of the hMT-II_A gene (21). The hybrids were digested with 300 U of S1 nuclease per ml, and the S1-resistant products were separated on an 8% polyacrylamide–urea gel. As a negative control, the same amount of probe was hybridized to 50 μg of yeast tRNA. The markers shown on the right-hand side of the figure are labeled *Hpa*II restriction fragments of pBR322.

As far as we know, this study represents the first clear demonstration of induction of a known mRNA species by pure IL-1 in cultured human cell lines. Presently, we do not know whether IL-1 elicits a pleiotropic response in the hepatoma cell line Hep G2, which would be reflective of the complete spectrum of induced proteins observed in vivo (23, 26, 31). Recently, crude preparations, containing some IL-1 as well as several other factors, from culture supernatants of human keratinocytes induced only α_1 anti-chymotrypsin mRNA in that cell line, whereas the synthesis of several

TABLE 1. Dose response of MT mRNA induction by IL-1[a]

Concn of IL-1 (U/ml)	Fold induction of MT mRNA	
	Human IL-1	Recombinant murine IL-1
0	1	1
1	1.3	1.2
3	2.0	—
6	3.7	—
10	4.0	2.3
100	5.0	3.4
1,000	—	4.0
10,000	—	4.1

[a] Confluent cultures of Hep G2 cells were treated for 12 h with the indicated concentrations of IL-1, and the relative levels of MT mRNA were determined by RNA blot hybridization and densitometry and normalized relative to the optical density of an α-tubulin mRNA band. —, Not tested.

other acute-phase proteins was not stimulated (3). However, the supernatants that were used as a source of stimulatory activity are crude preparations and may contain only trace amounts of IL-1 or inhibitors of IL-1 activity.

The acute-phase response is species specific with regard to the spectrum of proteins induced. For example, α_2-macroglobulin is produced in rats, but not in humans, as an acute-phase reactant (23). In rodents, the induction of MT seems to be part of the acute-phase response (25, 30). Therefore, our results are important in suggesting that MT production may be part of this response in humans as well as in mice.

What is the role of MT in the acute-phase response? The exact answer to this question is not yet known; however, we can speculate that perhaps MT, in addition to being capable of binding heavy metal ions, also acts as a free radical scavenger, and its induction serves a protective role to prevent tissue injury. The activation of macrophages and neutrophils by IL-1 during the acute-phase response results in massive release of oxygen-free radicals (13, 28, 32). While these free radicals are required for destruction of invading bacteria and parasites, they also cause a great deal of nonspecific damage to the host. One effective countermeasure is the existence of free radical scavengers such as superoxide dismutase. Recently, Thornalley and Vasak (34) have demonstrated that MT is a very efficient scavenger of free hydroxyl (· OH) radical in vitro but is relatively inefficient towards the superoxide radical. Superoxide dismutase, on the other hand, is effective mostly against superoxide anions (34). Therefore, MT and superoxide dismutase complement each other's activity and together may confer a wider range of protection against the two common active oxygen species.

In addition to IL-1, hMT-II mRNA is also induced by another lymphokine, interferon (10). Since interferon is also involved in the onset of various inflammatory and immunological reactions, it is likely that the induction of MT by this class of lymphokines serves a role similar to its induction by IL-1. It will be interesting to determine whether interferon and IL-1 induce MT by a common pathway. However, at this time we believe that at least the early phases of both responses, such as binding to a cell surface receptor, are different. Whereas all of the human cell lines we have examined responded to incubation with interferon by induction of MT mRNA, in only three of the cell lines was MT mRNA induced by IL-1.

Increased expression of MT also increases the resistance of mouse L cells to radiation damage (2), suggesting that MT could be part of a generalized protective system in mammalian cells, similar to the SOS system in bacteria. In fact, Herrlich and co-workers recently found that MT mRNA is induced both by UV light and by a peptide hormone known as EPIF, an elicitor of the UV response in cultured human fibroblasts (29; P. Herrlich, personal communication).

The induction of MT mRNA by IL-1 in cultured human cells appears to reflect a normal physiological response, as based on dose response, induction kinetics, and the responsive cell types. While the exact role of MT in the acute-phase response is not clear and requires further investigation, its induction by IL-1 offers the opportunity to investigate the molecular basis for the action of this important hormone.

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