Interleukin-1β Induces Production of Granulocyte Colony-Stimulating Factor in Human Hepatoma Cells

By Chun-Fai Lai and Heinz Baumann

Interleukin-1 (IL-1) is a proinflammatory cytokine that participates in the activation of the acute-phase plasma protein genes in hepatic cells during infection and injury. In human hepatoma HepG2 and Hep3B cells, IL-1β induced production of the granulocyte colony-stimulating factor (G-CSF) in a dose-dependent manner. Activation of G-CSF gene expression was an early and transient response. In HepG2 cells, G-CSF mRNA was strongly upregulated 2 hours after IL-1β treatment and returned to the pretreatment level by 6 hours. The secreted G-CSF was biologically active, as shown by the induction of gene transcription through the G-CSF receptor. Maximal G-CSF activity released to culture medium occurred after 8 hours. Previous studies have shown that liver expression of G-CSF was augmented in mice challenged by inflammatory stimuli. Our data suggest that IL-1β mediates, at least in part, this cytokine activation program in parenchymal cells and that liver-derived G-CSF may contribute to the regulation of hematopoiesis during the acute-phase response.

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MATERIALS AND METHODS

Cells and treatments. Human hepatoma HepG2 and Hep3B cells were cultured in minimal essential medium (MEM), whereas rat hepatoma H-35 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) media supplemented with 10% fetal calf serum, respectively. For cytokine treatments, cells were incubated with 10 ng/mL IL-1β and 100 ng/mL G-CSF (Immunex Corp, Seattle, WA) or COS cell-derived IL-6 (Genetics Institute, Boston, MA), unless indicated otherwise.

Plasmid constructs. The expression vector for the truncated form of human G-CSFR, G-CSFR(96), which contains membrane-proximal 96 amino acids of the cytoplasmic domain, has been described previously. The CAT-reporter gene construct, pHpIL-6RE-CAT, was cloned by inserting 5 copies of the oligonucleotides with sequence of the IL-6RE from rat haptoglobin (Hp) gene promoter. To monitor the transfection efficiency, the expression vector for the major urinary protein under the control of the immediately-early promoter of adenovirus was cotransfected into the cells.

Transfection. HepG2 cells were transfected by calcium phosphate-DNA precipitate, whereas H-35 cells were transfected using diethyl aminoethyl (DEAE)-dextran-DNA complex. Cells were transfected on 10-cm dishes and, after a recovery period of 16 hours, were redistributed equally into 6-well cluster plates. Twenty-four hours later, cells were treated with cytokines or conditioned media in the presence of 1 μmol/L dexamethasone for 16 hours. CAT activity was determined by incubating cell extracts with 14C-chloroamphenicol and acetyl-CoA. Reaction products were separated by thin-layer chromatography and quantitated by PhosphorImager (Molecular Dynamics, Inc, Sunnyvale, CA).

Preparation of conditioned media and assay for G-CSF. HepG2 and Hep3B cells were cultured on 10-cm dishes and grown to confluence. Cells were treated with IL-1β alone in 10 mL serum-free MEM medium at the indicated dose and the culture media were collected after 1 to 24 hours. The amounts of G-CSF in the condi-
tioned media were determined by enzyme-linked immunosorbent assay (ELISA; R & D Systems, Inc, Minneapolis, MN).

Northern blot analysis. Total RNA from HepG2 and Hep3B cells was prepared by guanidine thiocyanate extraction and cesium chloride gradient. Equal amounts of RNA were fractionated on 1% agarose gel and transferred to Nytran+ membrane (Schleicher & Schuell, Keene, NH). The membrane was hybridized with a 32P-labeled cDNA probe for human G-CSF (kindly provided by Dr. D. Cosman, Immunex Corp).

RESULTS

Detection of G-CSF production by HepG2 and Hep3B cells. Hepatic cells do not express G-CSFR; hence, these cells were used in previous studies to reconstitute the function of G-CSFR and to characterize G-CSF action on gene transcription. The human hepatoma HepG2 cells transiently transfected with expression vector for G-CSFR responded to G-CSF treatment by a prominent induction of transcription similar to that mediated by the endogenous IL-6R. Surprisingly, G-CSFR-transfected cells also gained an IL-1-mediated regulation of the IL-6RE-CAT construct. To determine whether the IL-1 response was mediated by the transfected G-CSFR, the same combination of DNA was also introduced into the rat hepatoma H-35 cells, a cell line that is known for its prominent IL-1 response. The induction of the CAT construct by IL-6 and G-CSF in the G-CSFR-expressing cells was confirmed (Fig 1A, right panel), but no response to IL-1 treatment was observed. We conclude from these results that in HepG2 cells, but not H-35 cells, IL-1 induces the production of a stimulatory activity that acts on the transfected G-CSFR leading to the gene regulation via IL-6RE.

To determine whether this activity is secretory, conditioned media were prepared from control and IL-1β-treated untransfected HepG2 cells and used to treat H-35 cells that had been cotransfected with pHIL-6RE-CAT and G-CSFR. Only conditioned medium from the IL-1β-treated HepG2 cells did induce CAT activity in the tested H-35 cells (Fig 1B). However, the same medium had no stimulatory effect on H-35 cells lacking transfected G-CSFR. To investigate whether it is a phenomenon peculiar to HepG2 cells, we also examined another human hepatoma cell line, Hep3B. Similarly, conditioned medium from unstimulated cells did not yield any detectable induction of CAT activity (Fig 1C). However, conditioned medium from cells treated with IL-1β activated the CAT-reporter gene in cells transfected with G-CSFR. Taken together, these data indicate that the activity from HepG2 and Hep3B cells is highly specific to G-CSFR and likely represents G-CSF.

IL-1β induces G-CSF gene expression. The suspected induction of G-CSF by IL-1β could be determined by the autocrine effect via G-CSFR. Moreover, the action could be quantitated by comparing it with the G-CSF added exogenously. Therefore, we cotransfected the CAT construct with increasing amounts of expression vector for G-CSFR into HepG2 cells. A dose-dependent increase in the CAT gene induction was observed with G-CSF and IL-1β (Fig 2). The IL-1β effect differed from that of G-CSF only by a lower magnitude of CAT gene regulation, suggesting different concentrations of ligands present. In the same transfected cells, the IL-6 response remained relatively constant. The IL-1 response was undetectable at 0.1 µg/mL of cotransfected G-CSFR and improved by 120-fold when the amount of the cotransfected receptor was increased to 10 µg/mL.

Characterization of the G-CSF production. The amounts of G-CSF produced by HepG2 cells in response to IL-1β were determined by ELISA. G-CSF activity was detected in conditioned medium from HepG2 cells treated with IL-1β as low as 0.1 ng/mL (Fig 3). Maximal amounts of G-CSF (1.550 ± 100 pg/10⁶ cells) were produced by cells treated with 1 ng/mL IL-1β. The amounts of G-CSF were also measured by the induction of the CAT gene on G-CSFR-transfected cells and were determined to be 18.8 ± 3.5 ng/mL.

The time course of G-CSF accumulation in conditioned media of HepG2 cells indicated no detectable G-CSF within 1 hour of IL-1β treatment (Fig 4). Prominent increase in G-CSF activity occurred afterward and peaked at 8 hours. The level of G-CSF declined after 8 hours and became stable from 16 to 24 hours. In Hep3B cells, a comparable amount of G-CSF was detected at 6 hours after IL-1β treatment and this level was maintained to 24 hours.

IL-1β regulates G-CSF mRNA expression. The induction of G-CSF expression was studied by Northern blot analysis of RNA from HepG2 cells treated with IL-1β for various times (Fig 5). G-CSF mRNA was undetectable in untreated cells. After 2 hours of IL-1β treatment, a significant increase in G-CSF mRNA was observed and the expression declined to the basal level after 6 hours. The induction of G-CSF mRNA was also detected in Hep3B cells treated with IL-1β for 6 hours. The data show that the increase in G-CSF mRNA accounted for the elevated G-CSF activity in the culture supernatant.

DISCUSSION

IL-1 is an important proinflammatory cytokine that, in concert with other cytokines, modulates the acute-phase response in liver. Together with IL-6 and glucocorticoids, IL-1 plays an essential role in liver to stimulate the synthesis of APPs, which perform protective functions for the host organism. A subset of the APP genes, including α1-acid glycoprotein, Hp, hemopexin, complement C3, and serum amyloid A, require IL-1 for maximal transcription. IL-1 acts additively or synergistically with IL-6 and glucocorticoids in coordinating the expression of the APPs. Whereas the synthesis of APPs is well documented as a major function of liver in the acute-phase response, the potential of liver in cytokine production is largely unrecognized. Previous studies have shown that intraperitoneal injection of LPS or intrapulmonary inoculation of Escherichia coli would induce the expression of G-CSF in liver. However, the cell type expressing G-CSF and the mechanism responsible for the induction have yet to be defined. Our present study showed that IL-1β mediates the induction of G-CSF in HepG2 and Hep3B cells, cell types that closely resemble the parenchymal liver cells based on the pattern of plasma protein expression. Because the production of IL-1 is promi-
IL-1β induces the secretion of G-CSFR-activating activity by human hepatoma cells. (A) HepG2 cells (left panel) or H-35 cells (right panel) were transfected with pHpIL-6RE-CAT alone or with G-CSFR. Transfected cells were treated with medium containing dexamethasone alone (-), or with IL-1β (1), IL-6 (6), or G-CSF (G). CAT activities in each experimental series were determined and expressed as fold induction over control cells indicated above the autoradiogram. (B) H-35 cells were transfected as in (A) but treated with conditioned media from HepG2 cells that had been incubated with medium alone or medium containing 10 ng/mL IL-1β for 24 hours. (C) Similar to (B), transfected H-35 cells were treated with conditioned media from Hep3B cells with or without IL-1β treatment.

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sites have been identified. Moreover, NF-κB p65 and NF-IL6 have been shown to bind cooperatively to this region. Based on these observations, p65 and NF-IL6 are postulated to be the transcription factors controlling the regulation of G-CSF promoter. In mice with NF-IL6 gene disrupted by gene targeting, the induction of G-CSF in fibroblasts and macrophages by LPS injection is severely impaired. Intriguingly, the expression of G-CSF in liver remains normal. These results indicate that the G-CSF production in hepatic cells is controlled by a mechanism distinct from other cell types and NF-IL6 appears dispensable in this signaling pathway. Because the NF-κB family of proteins are also involved in IL-1 signaling, it remains to be determined whether they serve as the mediators for regulating the G-CSF promoter in hepatic cells. It would be of interest to analyze the effects on the hepatic expression of G-CSF in the p65 gene knockout mice. Evidently, the contribution of tissue-specific factors to the IL-1 response cannot be ruled out.

It is noteworthy that the secretion of G-CSF in HepG2 cells was not inhibited by dexamethasone (Fig 1). The presence of dexamethasone neither delayed the activation kinetics nor reduced the magnitude of the response (data not shown). Dexamethasone has been shown to potently suppress the expression of a number of cytokine genes, including IL-1, IL-2, IL-3, IL-6, interferon-γ, TNF-α, and GM-CSF. The inhibition of cytokine production is believed to be an important mechanism responsible for immunosuppression. The independence of the glucocorticoids regulation in hepatic cells may allow the expression of G-CSF gene at an increased level of glucocorticoids during the late stage of acute phase. Nevertheless, its significance in vivo still awaits further exploration.

During infection, neutrophils are recruited into the inflammation sites. Depending on the extent of recruitment, an appreciable decrease in the number of circulating neutrophils in blood is expected and replenishment from the progenitors cells in bone marrow is required. Thus, an elevation of G-CSF production by an organ as massive as liver seems to be critical for host defense. Newborn mice with bacterial infection were found to be more susceptible to neutropenia and prone to death than adults. It correlates with the observation that upregulation of G-CSF mRNA by infection is deficient in the liver of newborn mice. In light of these studies, one has to conclude that liver has a broader func-
tional role in the acute-phase response than merely the synthesis of APPs. Obviously, the production of G-CSF would contribute beneficially to the control of the activities of cells involved in the inflammatory response at sites distant to liver. In conclusion, our results suggest a potential physiologic link between hepatic cells and hematoipoiesis in the context of acute-phase response.

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