Vol. 193, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 28, 1993 Pages 297-302

EFFECT OF INTERLEUKIN-11 ON THE LEVELS OF mRNAs ENCODING HEME OXYGENASE AND HAPTOGLOBIN IN HUMAN HEPG2 HEPATOMA CELLS

Yoshiaki Fukuda and Shigeru Sassa

The Rockefeller University, New York, N.Y. 10021

Received April 6, 1993

Effect of recombinant human interleukin-11 (rhIL-11) on the expression of transcripts encoding microsomal heme oxygenase (HO), the rate-limiting enzyme in heme catabolism, and haptoglobin (Hpt), a major acute-phase protein, were examined in human HepG2 hepatoma cells. Treatment of HepG2 cells with rhIL-11 elicited an increase in HO mRNA in a dose- and a time-dependent fashion. The dose response curve, its magnitude of response and its time course were similar to those observed with recombinant human interleukin-6 (rhIL-6). In contrast, rhIL-11 had a far smaller effect on the level of Hpt mRNA than did rhIL-6. These findings demonstrate that the two cytokines are similar in regulating heme catabolism, while markedly different in inducing certain acute-phase proteins.

Human interleukin-11 is a novel cytokine isolated from bone marrow derived stromal fibroblasts (1). It has a multifunctional activity in the lymphohemopoietic system, and shares various important physiological and pathological roles with recombinant human interleukin-6 (rhIL-6) (1,2). Recombinant human interleukin-11 (rhIL-11), similar to rhIL-6, is a synergistic factor for rhIL-3-dependent proliferation of primitive progenitors (3). Both rhIL-11 and rhIL-6 stimulate megakaryopoiesis and increase peripheral platelets in animals and in man (4-10). rhIL-6 and erythropoietin support the growth of macrophages and neutrophil/macrophage colonies, while rhIL-11 and erythropoietin support the growth of only macrophage colonies (3). These findings suggest that rhIL-6 and rhIL-11 interact with overlapping but different subsets of progenitor cells, and that the effect of rhIL-11 is preferentially directed to macrophage progenitor populations.

The major role of rhIL-6 is the induction of an acute-phase reaction (11) which is characterized by hepatic synthesis of 'positive' acute-phase reactants such as haptoglobin (Hpt), C-reactive protein (CRP), fibrinogen, α_1-acid glycoprotein, α_1-proteinase inhibitor and ceruloplasmin with a concomitant repression of 'negative' acute-phase proteins such as albumin and transferrin (12). Induction of the acute-phase reaction by endotoxin treatment in animals is known to elicit a marked increase in the activity of microsomal heme oxygenase (HO), the rate-

0006-291X/93 $4.00

limiting enzyme in heme catabolism, and reduction in the level of cytochrome P450 (13). Our previous studies demonstrated that rhIL-6 induces HO mRNA (14,15) and decreases mRNA levels for cytochrome P450*IA1*, *IA2* and *IIIA3* in human hepatoma cells in culture (16). Since rhIL-11 has been shown to increase certain acute-phase proteins similar to that induced by rhIL-6 (2), we examined the effect of rhIL-11 on the level of mRNAs encoding HO and Hpt in human HepG2 hepatoma cells. Our results indicate that rhIL-11 induces HO mRNA to a similar level as does rhIL-6, while it has a significantly weaker effect in eliciting the induction of Hpt mRNA than rhIL-6.

MATERIALS AND METHODS

Cell culture and treatment with cytokines: HepG2 cells were cultivated as described previously (17). To treat cells with the cytokines, cells were replenished with a minimum essential medium containing Earle's salts, 25mM HEPES buffer (pH 7.4), 2mM L-glutamine (GIBCO Laboratories, Grand Island, N.Y.) and 10%[v/v] defined calf serum (HyClone Laboratories, Logan, UT), and cytokines were added as indicated in each experiment. Cells were then incubated up to 48 h. Northern blot analysis of HO and Hpt mRNAs: Total RNA was isolated from cultured cells according to the method of Cathala et al. (18). Northern blot analysis was performed using 15 μg total RNA as described previously (17). mRNA levels were determined by densitometry using a Ultroscan XL enhanced laser densitometer (Pharmacia LKB Biotech., Inc., Piscataway, NJ). Probes: Human HO (pHHO1) cDNA (19) and human Hpt cDNA (20) were inserted into pGEM-4Z vector (Promega Corp., Madison, WI) as described previously (17). Radioactive RNA probes were prepared according to the method of Melton et al. (21). Human ß-actin cDNA was obtained from CLONTECH Laboratories, Inc., Palo Alto, CA and labeled by nick translation.

RESULTS

Dose response effects of rhIL-11 on the levels of HO and Hpt mRNAs: Effects of rhIL-11 on the levels of mRNAs encoding HO and Hpt in HepG2 cells were examined by incubating cells with various concentrations of rhIL-11 for 48 h. HO mRNA levels increased in a dose-dependent manner, with a maximal effect occurring at 50U/ml (Fig. 1). At this concentration, an increase of HO mRNA by 2.2-fold over the untreated level was observed. Hpt mRNA also increased in a dose-dependent manner, with a maximal effect (2.5-fold) also occurring at 50U/ml (Fig. 1). These findings were significant, since results were normalized to the level of ß-actin mRNA (Fig. 1).

Changes in the level of HO mRNA: Changes in HO mRNA levels in HepG2 cells after treatment with rhIL-11 are shown in Fig. 2. The level of HO mRNA increased (1.5-fold over the untreated control) within 2 h after treatment with rhIL-11 (100U/ml), and reached a maximum (2.0-fold over the untreated control) at 24 h.

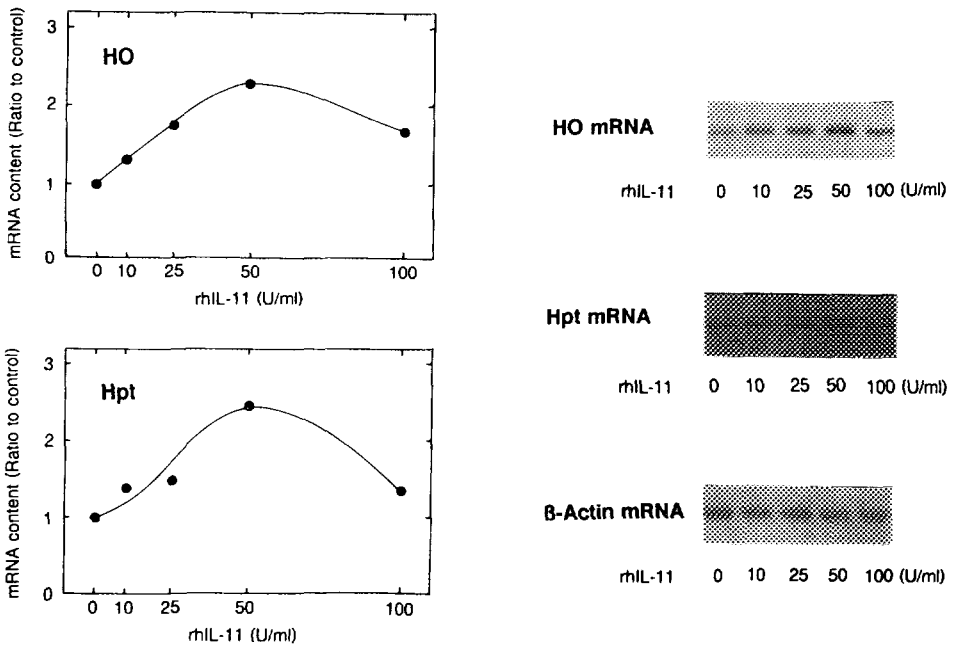

Figure 1. The dose response curves of increases in mRNA levels for HO and Hpt after treatment of HepG2 cells with rhIL-11. Cells were incubated for 48 h. Total RNA was isolated and subjected to Northern blot analysis as described in MATERIALS AND METHODS. β-Actin mRNA levels were determined as a loading control.

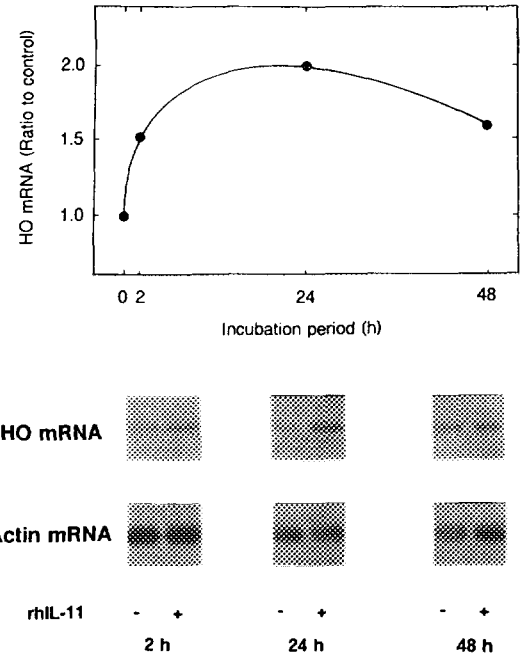

Figure 2. Changes in the level of HO mRNA in HepG2 cells after rhIL-11 treatment. Cells were treated with 100U/ml of rhIL-11. β-Actin mRNA levels were determined as a loading control.

Comparison of the effect of rhIL-11 and those of rhIL-6 on the levels of HO mRNA and Hpt mRNA: Our previous findings demonstrated that rhIL-6 induces HO mRNA in a dose- and a time-dependent manner (15). The effects of rhIL-11 and rhIL-6 were examined using the concentration at which each cytokine induced a maximal response. The levels of increases in HO mRNA by rhIL-11 (50U/ml) and rhIL-6 (100U/ml) were similar (2.3-fold and 2.7-fold, respectively) (Fig. 3). In contrast, rhIL-6 markedly increased Hpt mRNA (27-fold), while rhIL-11 had only a small effect (2.4-fold) (Fig. 3). The results with rhIL-6 are consistent with our earlier findings (15).

DISCUSSION

Hematopoietic cytokines exhibit a wide variety of responses which may overlap extensively. It is known that rhIL-11 and rhIL-6 share many overlapping but some distinct functions in eliciting their biological responses. Our results in this study demonstrate that rhIL-11 increases mRNA for HO, the rate-limiting enzyme for heme catabolism, in a dose- and a time-dependent manner. The dose response curve, the magnitude of its response, and the time course of increases in HO mRNA after rhIL-11 treatment are similar to those elicited by rhIL-6 in

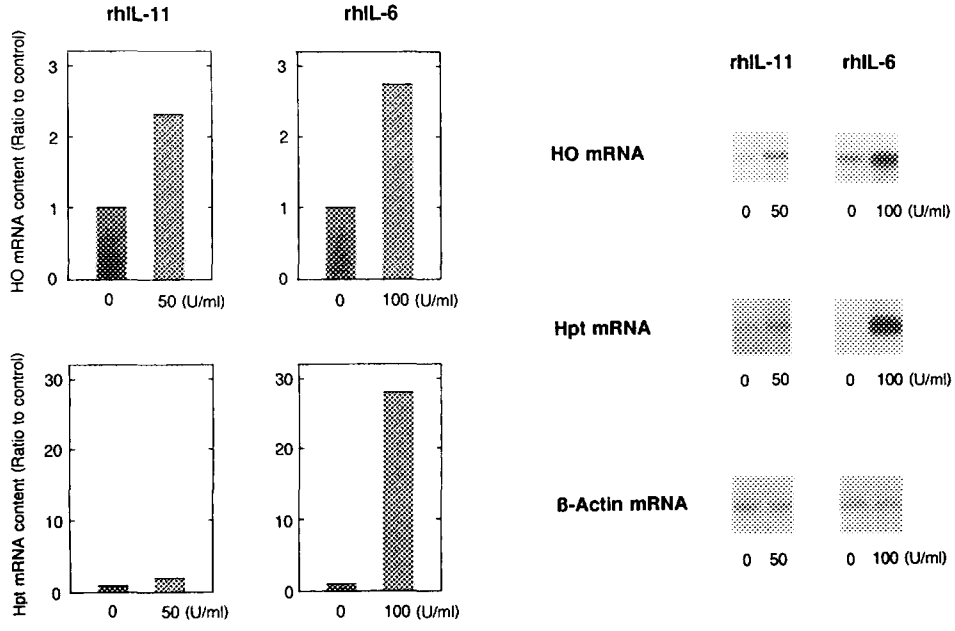

Figure 3. Comparison of the effects of rhIL-11 and rhIL-6 on mRNA levels for HO and Hpt. Cells were incubated with rhIL-11 (50U/ml), or rhIL-6 (100U/ml). These concentrations are those which elicit the maximal effect by each cytokine. β-Actin mRNA levels were determined as a loading control.

human hepatoma cells (15). These findings indicate that, although they are structurally unrelated (1,22), both rhIL-11 and rhIL-6 elicit similar induction responses of HO mRNA. It has been suggested that these two cytokines may share a common receptor subunits (22,23), and that biological responses induced by rhIL-11 may be mediated through the IL-6 signal transducer, gp130 (23). Our findings in this study suggest that rhIL-11 may also upregulate the human HO gene via the gp130-dependent signal transduction pathway.

We have previously shown that HO is a novel positive acute-phase reactant (15). The reasons for this conclusion are based on the findings that rhIL-6 treatment increases the level of HO mRNA in human hepatoma cells (14,15), there are three consensus sequences for the IL-6 responsive element (IL-6RE) in the human HO gene (19), and there is a nuclear factor that binds specifically to one of these sequences (15). Thus HO induction may be important not only in heme catabolism, but also in protecting the host from oxidative stimuli (24), since it reduces the concentration of heme, a pro-oxidant, and produces bile pigments, anti-oxidants (25). It is not however known whether rhIL-11 activates the HO gene via an inducible *trans*-acting factor in a manner similar to IL-6DBP which is necessary for IL-6 mediated gene expression (26). A further study is needed to clarify this question. Our findings however do suggest that rhIL-11, similar to rhIL-6, may also participate in the host defense reaction against oxidative stimuli.

In contrast to the similar increases in HO mRNA produced by both cytokines, there was a marked difference in the level of Hpt mRNA induced by rhIL-11 and by rhIL-6. Specifically rhIL-6 was a potent inducer of Hpt mRNA (Fig. 3) (15), whereas rhIL-11 was a very weak inducer in this respect. Thus it can be concluded that, while the two cytokines elicit a similar induction response of the HO gene, they differ significantly in the induction of other acute-phase genes.

Acknowledgments - We are grateful to Dr. Barbara B. Knowles (Wistar Institute of Anatomy and Biology, Philadelphia, PA), Dr. Shigeki Shibahara (Tohoku University, Sendai, Japan) and Dr. Riccardo Cortese (European Molecular Biology Institute, Heidelberg, Germany) for supplying us with HepG2 cells, human HO cDNA and Hpt cDNA, respectively. We are also grateful to Dr. James A. Kaye, Genetics Institute, Boston, MA and Dr. Takamoto Suzuki, KIRIN Brewery Co., Ltd., Maebashi, Gunma, Japan, for providing us with rhIL-11 and rhIL-6, respectively. This work was supported in part by grants from U.S.P.H.S. DK-39264 and the Suntory Fund for Biomedical Research. We are grateful to Drs. George S. Drummond and Toshihiro Nakanishi for their helpful comments. The excellent technical assistance of Ms. Luba Garbaczewski is gratefully acknowledged.

REFERENCES

1. Paul, S.R., Bennett, F., Calvetti, J.A., Kelleher, K., Wood, C.R., OHara, R.M.,Jr., Leary, A.C., Sibley, B., Clark, S.C., Williams, D.A., and et al., (1990) Proc. Natl. Acad. Sci. U. S. A.87, 7512-7516.

2. Baumann, H. and Schendel, P. (1991) J. Biol. Chem.266, 20424-20427.
3. Musashi, M., Yang, Y.C., Paul, S.R., Clark, S.C., Sudo, T., and Ogawa, M. (1991) Proc. Natl. Acad. Sci. U. S. A.88, 765-769.
4. Neben, T.Y., Loebelenz, J., Hayes, L., McCarthy, K., Stoudemire, J., Schaub, R., and Goldman, S.J. (1993) Blood81, 901-908.
5. Kobayashi, S., Teramura, M., Sugawara, I., Oshimi, K., and Mizoguchi, H. (1993) Blood81, 889-893.
6. Burstein, S.A., Mei, R.L., Henthorn, J., Friese, P., and Turner, K. (1992) J. Cell Physiol.153, 305-312.
7. Teramura, M., Kobayashi, S., Hoshino, S., Oshimi, K., and Mizoguchi, H. (1992) Blood79, 327-331.
8. Bruno, E., Briddell, R.A., Cooper, R.J., and Hoffman, R. (1991) Exp. Hematol.19, 378-381.
9. Ishibashi, T., Kimura, H., Shikama, Y., Uchida, T., Kariyone, S., Hirano, T., Kishimoto, T., Takatsuki, F., and Akiyama, Y. (1989) Blood74, 1241-1244.
10. Bruno, E. and Hoffman, R. (1989) Exp. Hematol.17, 1038-1043.
11. Akira, S. and Kishimoto, T. (1992) Immunol. Rev.127, 25-50.
12. Koj, A., Magielska-Zero, D., Kurdowska, A., Bereta, J., Rokita, H., and Dubin, A. (1988) Adv. Clin. Enzymol.6, 143-151.
13. Bissell, D.M. and Hammaker, L.E. (1976) Arch. Biochem. Biophys.176, 91-102.
14. Sassa, S., Fujita, H., Mitani, K., Shibahara, S., Bishop, T.R., Yoshinaga, T., de Verneuil, H., Romeo, P-H., and Kappas, A. (1990) In Molecular Biology of Haematopoiesis (L. Sachs, N.G. Abraham, C.J. Wiedermann, A.S. Levine and G. Konwalinka, Eds.), pp. 413-424. Intercept, Ltd., Andover, Hampshire, U.K.
15. Mitani, K., Fujita, H., Kappas, A., and Sassa, S. (1992) Blood79, 1255-1259.
16. Fukuda, Y., Ishida, N., Noguchi, T., Kappas, A., and Sassa, S. (1992) Biochem. Biophys. Res. Commun.184, 960-965.
17. Mitani, K., Fujita, H., Sassa, S., and Kappas, A. (1989) Biochem. Biophys. Res. Commun.165, 437-441.
18. Cathala, G., Savouret, J.-F., Mendez, B., West, B.L., Karlin, M., Martial, J.A., and Baxter, J.D. (1983) DNA2, 329-335.
19. Shibahara, S., Sato, M., Muller, R.M., and Yoshida, T. (1989) Eur. J Biochem.179, 557-563.
20. Raugei, G., Bensi, G., Colantuoni, V., Romano, V., Santoro, C., Costanzo, F., and Cortese, R. (1983) Nucleic Acids Res.11, 5811-5819.
21. Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K., and Green, M.R. (1984) Nucl. Acids Res.12, 7035-7056.
22. Paul, S.R. and Schendel, P. (1992) Int. J. Cell Cloning10, 135-143.
23. Yin, T., Taga, T., Tsang, M.L.-S., Yasukawa, K., Kishimoto, T., and Yang, Y.-C. (1992) Blood80, No. 594.(Abstract)
24. Stocker, R. (1990) Free Radical Research Communications9, 101-112.
25. Stocker, R., Yamamoto, Y., McDonagh, A.F., Glazer, A.N., and Ames, B.N. (1987) Science235, 1043-1046.
26. Poli, V., Mancini, F.P., and Cortese, R. (1990) Cell63, 643-653.