Volume 249, number 1, 27–30 FEB 07156 May 1989

# Regulation of interleukin-6 receptor expression in human monocytes and hepatocytes

Joachim Bauer°+, Gabriella Lengyel+, Tilman Martin Bauer°, George Acs+ and Wolfgang Gerok°

°*Medizinische Universitätsklinik Freiburg, D-7800 Freiburg, FRG* and +*Mount Sinai Medical Center, New York City, NY 10029, USA*

Received 30 March 1989

Human blood monocytes normally express the interleukin-6 receptor. Treatment of cultured monocytes with endotoxin, interleukin-1$\beta$, or interleukin-6 results in a decrease of interleukin-6 receptor mRNA levels. Glucocorticoids also cause a drop in monocytic interleukin-6 receptor mRNA levels. We also found interleukin-6 receptor expression in cultured human hepatocytes, but in contrast to monocytes, where interleukin-6 receptor mRNA is repressed by the ligand and by interleukin-1, treatment of hepatocytes with interleukin-6 or interleukin-1 resulted in increased interleukin-6 receptor mRNA levels. Induction of interleukin-6 receptor mRNA in hepatocytes was less pronounced when glucocorticoids were omitted from the culture medium. We conclude that during noninflammatory homeostasis, blood monocytes are involved in binding of trace amounts of circulating interleukin-6. During inflammatory events, the main tissue target of interleukin-6 may be changed from the monocytic population not only to activated B-cells, but also to the hepatocytes.

Interleukin-6; Receptor; Monocyte; Hepatocyte; Inflammation

## 1. INTRODUCTION

Interleukin-6, previously investigated under the designations interferon $\beta 2$ (IFN$\beta 2$), 26 kDa protein, B-cell stimulatory factor 2 (BSF-2), hybridoma growth factor (HGF), and hepatocyte-stimulating factor (HSF), is synthesized under inflammatory conditions primarily in stimulated peripheral blood monocytes [1–4], fibroblasts [5–15] and endothelial cells [16–18]. The main physiological functions of interleukin-6 (IL-6) are: the induction of the hepatic acute phase response [19–27], which is characterized by increased expression of acute phase proteins like C-reactive protein (CRP); decreased synthesis of albumin and the stimulation of preactivated B-cells for immunoglobulin secretion [28–30].

In order to understand more fully the role of IL-6 in the context of inflammatory mediators, it is necessary to study the interaction of this molecule with its target cells. Since the interleukin-6 receptor (IL-6R) has recently been cloned [31], it became possible to examine which tissues express this receptor and how its expression is regulated. Here, we report expression of the interleukin-6 receptor in primary cultures of human monocytes and hepatocytes.

*Correspondence address:* J. Bauer, Medizinische Universitätsklinik, D-7800 Freiburg, FRG

## 2. MATERIALS AND METHODS

Endotoxin (*Salmonella abortus equi*) was a gift of C. Galanos, Max-Planck-Institut für Immunbiologie (Freiburg, FRG). Human recombinant interleukin-1$\beta$ was from Biogen (Geneva, Switzerland). Human recombinant interleukin-6 was either BSF-2 (obtained from T. Kishimoto, University of Osaka, Japan) or HGF (obtained from L.A. Aarden, Netherlands Red Cross). Interleukin-6 cDNA (pBSF2-38.1) [29] and interleukin-6 receptor cDNA (pBSF2R.236) [31] was provided by T. Kishimoto. C-reactive protein and albumin cDNA was from G. Ciliberto and R. Cortese (EMBL Heidelberg, FRG).

Human blood monocytes were prepared and cultivated as described in detail previously by the authors [4]. For the experiments, cells were kept in RPMI 1640 medium containing 5% human AB serum. Endotoxin-free conditions during

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/89/$3.50 © 1989 Federation of European Biochemical Societies

preparation and culture of the cells were strictly observed. All media and sera used were tested for trace amounts of endotoxins according to [32]. Primary hepatocyte cultures were prepared from fetal liver cells of human fetuses after legal abortion at 20 weeks of gestation. The cultures were prepared by J.B. in G.A.'s laboratory in New York City according to a procedure previously described by this laboratory [33]. Hepatocytes used in this study were kept in Ham's F12 medium containing 2% human AB serum, $10^{-6}$ dexamethasone, $10^{-7}$ insulin and $10^{-8}$ glucagon.

RNA was extracted according to the protocol of Chomczynski and Sacchi [34]. Poly(A)$^+$ RNA was prepared by poly(U)-Sepharose chromatography. For Northern analysis, as indicated, either total or poly(A)$^+$ RNA was electrophoresed on 1.2% agarose-formaldehyde gels and transferred to nitrocellulose filters in 20 × SSC. Standardization was done with respect to 28 S and 18 S RNA as well as by control hybridizations with cDNA coding for the housekeeping protein glyceraldehyde-3-phosphate dehydrogenase [35]. Hybridizations were performed for 24 h with $^{32}$P labeled specific cDNAs using $2 \times 10^6$ cpm of labeled probe per ml hybridization buffer (6 × SSC, 50% formamide, 0.02 Denhardt's solution). Filters were exposed to Kodak XAR5 films at −70°C.

## 3. RESULTS

Freshly prepared human blood monocytes express the interleukin-6 receptor (IL-6R). If monocytic RNA was subjected to Northern analysis, a strong signal for interleukin-6 receptor mRNA (IL-6R mRNA) could be detected (fig.1A, lane 1; fig.2, lane 1). In addition, we were able to show the presence of IL-6R molecules on the monocyte surface by fluorescence activated cell sorter (FACS) analysis using a monoclonal antibody directed against the IL-6R (not shown; Bauer, J. and Kishimoto, T., in preparation). If the monocytes were stimulated with endotoxin/LPS, a marked and rapid drop in IL-6R mRNA levels could be observed (fig.1A, lanes 2–4), concomitant with a strong induction of interleukin-6 (IL-6) mRNA (fig.1B, lanes 1–4). Human recombinant IL-6 could substitute for LPS in reducing the interleukin-6 receptor mRNA levels in monocytes (fig.2), indicating a repression of the monocytic IL-6R synthesis by its ligand. Besides IL-6, human recombinant IL-1$\beta$ was also able to repress IL-6R mRNA in monocytes (not shown). Repression of IL-6R mRNA by LPS, IL-6 and IL-1 was time- and dose-dependent. Maximal effects were observed with 100 ng LPS/ml, 200 U IL-6 (BSF-2 units)/ml, or 100 U IL-1$\beta$, respectively.

Nonstimulated cultured human hepatocytes do not express detectable amounts of IL-6R mRNA (fig.3A, lanes 1,4). However, the presence of functioning receptor molecules on the surface of unstimulated hepatocytes is indicated by the fact that these cells respond to interleukin-6 treatment by strong induction of C-reactive protein (CRP)

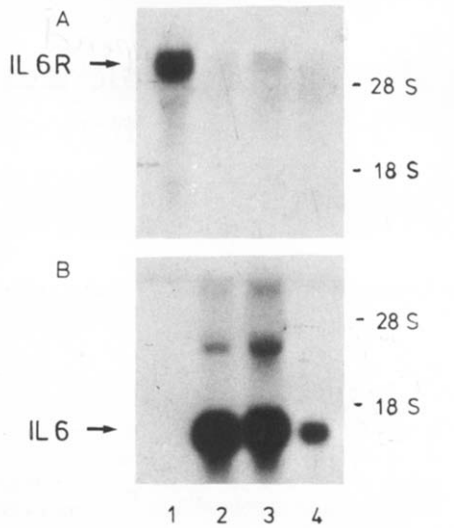


Fig.1. RNA was extracted from either unstimulated human blood monocytes (lane 1) or from cells after different times of endotoxin (100 ng/ml) treatment: 4 h (lane 2); 8 h (lane 3); 16 h (lane 4). 20 $\mu$g of total RNA/lane were subjected to agarose-formaldehyde electrophoresis, transferred to nitrocellulose and hybridized to radiolabeled interleukin-6 receptor (IL 6R) cDNA (A) or interleukin-6 (IL 6) cDNA (B). The filter was exposed to the film for 3 days (A) and 15 h (B), respectively.

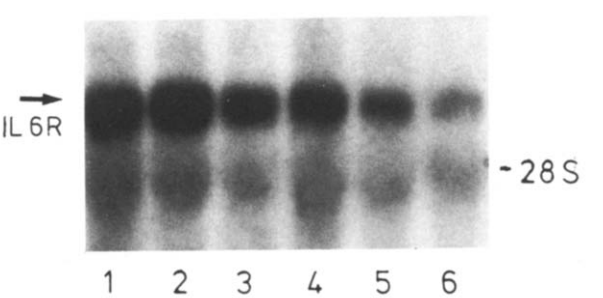


Fig.2. Monocytes were treated with human recombinant interleukin-6 (10000 HGF U/ml, equivalent to 50 BSF-2 U/ml) for different times: 1 h (lane 2); 3 h (lane 3); 6 h (lane 4); 9 h (lane 5); and 18 h (lane 6); control (lane 1). RNA was extracted, 20 $\mu$g of total RNA/lane were electrophoresed through an agarose-formaldehyde gel, transferred to nitrocellulose and hybridized to radiolabeled interleukin-6 receptor (IL 6R) cDNA. Autoradiographic exposure time was 5 days.

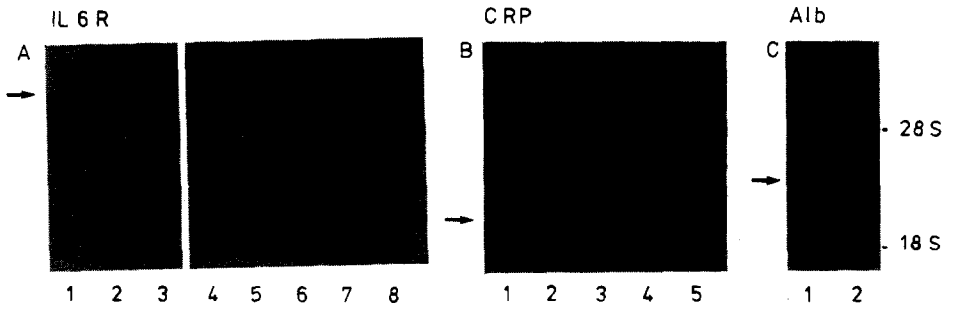


Fig.3. Primary human fetal hepatocytes were kept in culture for four days. Poly(A)$^+$ RNA was prepared, subjected to agarose-formaldehyde electrophoresis (2.5 $\mu$g RNA/lane), blotted onto nitrocellulose, and hybridized to radiolabeled interleukin-6 receptor (IL 6R) cDNA (A), CRP cDNA (B) or albumin cDNA (C). (A) Before harvesting, cells were treated with human recombinant interleukin-6 (100 BSF-2 U/ml) for 12 h (lane 2) or 24 h (lane 3); controls are shown in lanes 1 and 4. Alternatively, cells were treated for 24 h with 10 BSF-2 U/ml (lane 5), or 100 BSF-2 U/ml (lane 6); lane 7 represents the experiment of lane 6, except that glucocorticoids were omitted from the culture medium; lane 8, treatment with 100 U interleukin-1$\beta$/ml for 24 h. (B) Lanes 1–5 represent the experiments of the right part of panel A (panel A, lanes 4–8). (C) Control (lane 1); 24 h of interleukin-6 treatment (100 BSF-2 U/ml) (lane 2).

mRNA (fig.3B) and by repression of albumin mRNA (fig.3C). Upon treatment with IL-6, the mRNA levels of IL-6R in hepatocytes increase (fig.3A, lanes 1–6). If glucocorticoids were omitted from the culture medium, induction of IL-6R mRNA by IL-6 was slightly reduced (fig.3A, lane 7). Besides IL-6, also IL-1$\beta$ could induce IL-6R mRNA in hepatocytes (fig.3A, lane 8).

## 4. DISCUSSION

The interleukin-6 receptor is an 80 kDa molecule, which upon binding of the ligand associates with a second membrane protein of 130 kDa, resulting in signal transduction into the cell (Kishimoto, T., personal communication). Until now, expression of interleukin-6 receptor mRNA has only been shown in a number of leukemic cells [31]. Since several leukemic cell lines express both interleukin-6 and its receptor, it has been presumed that this simultaneous expression may support an autocrine growth mechanism resulting in tumor cell growth [36,37]. The finding that monocytes, the main site of IL-6 synthesis, also express the IL-6 receptor was therefore rather unexpected. However, simultaneous repression of the receptor under conditions when interleukin-6 expression is induced (fig.1) may indicate the normal biological evasion of such an autocrine loop.

In contrast to interleukin-6 receptor expression in monocytes, expression in hepatocytes was expected since it has been shown that intravenously administered interleukin-6 is cleared primarily by the liver [38]. Hepatocytes show an inverse pattern of regulation compared to the situation in monocytes. Not only is the interleukin-6 receptor in these two cell types subjected to contrary regulation by the ligand, glucocorticoids also had an opposite effect: in hepatocytes, dexamethasone augmented IL-6R mRNA induction by the ligand (fig.3A); in monocytes, treatment with dexamethasone led to a rapid and marked drop in IL-6R mRNA levels (not shown).

In conclusion, our data indicate that under conditions of noninflammatory homeostasis monocytes strongly express the interleukin-6 receptor and may be involved in binding trace amounts of circulating IL-6. As soon as an inflammatory event develops, the tissue target for interleukin-6 appears to change from the monocytic population to that of the hepatocytes.

*Acknowledgements:* The authors wish to thank Dr T. Kishimoto and Dr T. Hirano for providing the interleukin-6 receptor cDNA. We thank Dr Barry Drees for critical reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB Leber, Teilprojekt C8). J.B. is a recipient of a DFG research fellowship. T.M.B. was supported by a Fritz Thyssen Stipend.

## REFERENCES

[1] Aarden, L.A., Lansdorp, P.M. and De Groot, E.R. (1985) Lymphokines 10, 175–185.

[2] Aarden, L.A., De Groot, E.R., Schaap, O.L. and Lansdorp, P.M. (1987) Eur. J. Immunol. 17, 1411–1416.
[3] Horii, Y., Maraguchi, A., Suematsu, S., Matsuda, T., Yoshizaki, K., Hirano, T. and Kishimoto, T. (1988) J. Immunol. 141, 1529–1535.
[4] Bauer, J., Ganter, U., Geiger, T., Jacobshagen, U., Hirano, T., Matsuda, T., Kishimoto, T., Andus, T., Acs, G., Gerok, W. and Ciliberto, G. (1988) Blood 72, 1134–1140.
[5] Sehgal, P.B. and Sagar, A.D. (1980) Nature 288, 95–97.
[6] Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P. and Revel, M. (1980) Proc. Natl. Acad. Sci. USA 77, 7152–7156.
[7] Content, J., De Wit, L., Pierard, D., Derynck, R., De Clercq, E. and Fiers, W. (1982) Proc. Natl. Acad. Sci. USA 79, 2768–2772.
[8] Poupart, P., De Wit, L. and Content, J. (1984) Eur. J. Biochem. 143, 15–21.
[9] Content, J., De Wit, L., Poupart, P., Opdenakker, G., Van Damme, J. and Billiau, A. (1985) Eur. J. Biochem. 152, 253–257.
[10] Haegemann, G., Content, J., Volckaert, G., Derynck, R., Tavernier, J. and Fiers, W. (1986) Eur. J. Biochem. 159, 625–632.
[11] Zilberstein, A., Ruggieri, R., Korn, J.H. and Revel, M. (1986) EMBO J. 5, 2529–2537.
[12] Van Damme, J., Cayphas, S., Opdenakker, G., Billiau, A. and Van Snick, J. (1987) Eur. J. Immunol. 17, 1–7.
[13] Kohase, M., May, L.T., Tamm, I., Vilcek, J. and Sehgal, P.B. (1987) Mol. Cell. Biol. 45, 273–280.
[14] Defilippi, P., Poupart, P., Tavernier, J., Fiers, W. and Content, J. (1987) Proc. Natl. Acad. Sci. USA 84, 4557–4561.
[15] Van Damme, J., Schaafsma, M.R., Fibbe, W.E., Falkenburg, J.H.F., Opdenakker, G. and Billiau, A. (1989) Eur. J. Immunol. 19, 163–168.
[16] Norioka, K., Hara, M., Harigai, M., Kitani, A., Hirose, T., Suzuku, K., Kawakami, M., Tabata, H., Kawagoe, M. and Nakamura, H. (1988) Biochem. Biophys. Res. Commun. 153, 1045–1050.
[17] Sironi, M., Breviario, F., Proserpio, P., Biondi, A., Vecchi, A., Van Damme, J., Dejana, E. and Mantovani, A. (1989) J. Immunol. 142, 549–553.
[18] Jirik, F.R., Podor, T.J., Hirano, T., Kishimoto, T., Loskutoff, D.J., Carson, D.A. and Lotz, M. (1989) J. Immunol. 142, 144–147.
[19] Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. and Baumann, H. (1987) Proc. Natl. Acad. Sci. USA 84, 7251–7255.
[20] Andus, T., Geiger, T., Hirano, T., Northoff, H., Ganter, U., Bauer, J., Kishimoto, T. and Heinrich, P.C. (1987) FEBS Lett. 221, 18–22.
[21] Geiger, T., Andus, T., Klapproth, J., Hirano, T., Kishimoto, T. and Heinrich, P.C. (1988) Eur. J. Immunol. 18, 717–721.
[22] Andus, T., Geiger, T., Hirano, T., Kishimoto, T. and Heinrich, P.C. (1988) Eur. J. Immunol. 18, 739–746.
[23] Andus, T., Geiger, T., Hirano, T., Kishimoto, T., Tran-Thi, T.A., Decker, K. and Heinrich, P.C. (1988) Eur. J. Biochem. 173, 287–289.
[24] Ramadori, G., Van Damme, J., Rieder, H. and Meyer zum Büschenfelde, K.H. (1988) Eur. J. Immunol. 18, 1259–1264.
[25] Moshage, H.J., Roelofs, H.M.J., Van Pelt, J.F., Hazenberg, B.P.C., Van Leeuwen, M.A., Limburg, P.C., Aarden, L.A. and Yap, S.H. (1988) Biochem. Biophys. Res. Commun. 155, 112–117.
[26] Castell, J.V., Gomez-Lechon, M.J., David, M., Hirano, T., Kishimoto, T. and Heinrich, P.C. (1988) FEBS Lett. 232, 347–350.
[27] Castell, J.V., Gomez-Lechon, M.J., David, M., Andus, T., Geiger, T., Trullenque, R., Fabra, R. and Heinrich, P.C. (1989) FEBS Lett. 242, 237–239.
[28] Hirano, T., Taga, T., Nakano, N., Yasukawa, K., Kashiwamura, S., Shimizu, K., Nakajima, K., Pyun, K.H. and Kishimoto, T. (1985) Proc. Natl. Acad. Sci. USA 82, 5490–5494.
[29] Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T. and Kishimoto, T. (1986) Nature 324, 73–76.
[30] Maraguchi, A., Hirano, T., Tang, B., Matsuda, T., Horii, Y., Nakajima, K. and Kishimoto, T. (1988) J. Exp. Med. 167, 332–344.
[31] Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T. and Kishimoto, T. (1988) Science 241, 825–828.
[32] Northoff, H., Kabelitz, D. and Galanos, C. (1986) Immunol. Today 7, 126.
[33] Sells, M.A., Chernoff, J., Cerda, A., Bowers, C., Shafritz, D.A., Kase, N., Christman, J.K. and Acs, G. (1984) In Vitro Cell Dev. Biol. 21, 216–220.
[34] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[35] Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. and Blanchard, J.M. (1985) Nucleic Acids Res. 13, 1431–1442.
[36] Kishimoto, T. (1987) J. Clin. Immunol. 7, 343–355.
[37] Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A. and Kishimoto, T. (1988) Nature 332, 83–85.
[38] Castell, J.V., Geiger, T., Gross, V., Andus, T., Walter, E., Hirano, T., Kishimoto, T. and Heinrich, P.C. (1988) Eur. J. Biochem. 177, 357–361.