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Stimulation of hepatic T-kininogen production by interferon

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T-kininogen is known to be an acute-phase reactant as well as a kininogen in rat plasma. Three kinds of cytokines, interleukin-1, tumor necrosis factor and interferon, were assayed for their abilities to stimulate hepatic production of T-kininogen. Of these cytokines, interferon was able to stimulate hepatic production of T-kininogen, but few effects were observed for interleukin-1 and tumor necrosis factor. In addition, the stimulatory effect of interferon was inhibited by tumor necrosis factor. Our data suggest that interferon is a candidate for the leukocyte-derived factor mediating the acute-phase response of T-kininogen.

T-kininogen; Acute-phase reactant; Interleukin-1; Tumor necrosis factor; Interferon; Cytokine

1. INTRODUCTION

Tissue injury caused by chemical agents, bacterial infections, rheumatoid arthritis, tissue infarction and neoplasia results in biochemical and physiological changes known as the acute-phase response [1,2]. One of these changes includes elevated levels of plasma proteins designated acute-phase reactants [3]. It has been found that activated monocytes, tissue macrophages and keratinocytes synthesize and release factors responsible for stimulating the hepatic production of acute-phase reactants [4–6]. Some of these factors have been identified to be IL-1, TNF and HSF [7].

Rat plasma contains a unique kininogen, T-kininogen, which releases isoleucyl-seryl-bradykinin (T-kinin) upon trypsinization [8]. T-kininogen has also been identified as an acute-phase reactant [9,10] and has subsequently been demonstrated to be a cysteine proteinase inhibitor present in rat plasma [11]. In the previous study, we demonstrated that macrophages collected from rats receiving inflammatory stimuli triggered T-kininogen production in vivo and in vitro [12]. In order to identify the macrophage-derived factor(s) responsible for stimulating the hepatic production of T-kininogen, we have investigated the in vitro and in vivo effects of IL-1, TNF and IFN in rats.

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Abbreviations: IL-1, interleukin-1; TNF, tumor necrosis factor; IFN, interferon; HSF, hepatocyte-stimulating factor

2. MATERIALS AND METHODS

2.1. *Materials*

Recombinant human IL-1α (2×10^7 units/mg protein) and TNF (3×10^6 units/mg protein) were kindly supplied by Dainippon Pharmaceutical Co., Ltd (Osaka, Japan). Rat IFN (1.9×10^6 units/mg protein) and antibody against rat IFN were purchased from Lee Biomolecular Research Laboratories, Inc. (San Diego, CA).

2.2. *In vivo experiments*

Rat hepatocytes were prepared from male Fisher-344 rats as described [12], and the cells (1.8×10^5) were plated in 24-well culture plates in 1 ml of William's E medium supplemented with insulin (1×10^{-9} M), dexamethasone (1×10^{-9} M), gen-

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tamicin (50 μg/ml) and fetal calf serum (5%) (basal medium) at 37°C in 5% CO_2–95% air, as described [12]. Following culture for 48 h, the medium was changed to 0.5 ml of fresh medium containing one of several concentrations of IL-1, TNF or IFN. 48 h after culture, the medium was collected and subjected to T-kininogen radioimmunoassay as described in [13]. Amounts of rat albumin secreted from hepatocytes were also determined by radioimmunoassay using ^{125}I-labeled rat albumin and rabbit antiserum for rat albumin.

2.3. *In vivo experiments*

IL-1 (2×10^4 units), TNF (5×10^4 units) and IFN (500 units), made up in saline, were injected intraperitoneally into male Fisher-344 rats, weighing 100–120 g. Immediately before and at different times after the treatments, the tail-tip of each animal was cut with a razor and 100 μl of blood was collected into an EDTA-treated capillary tube. The tube was centrifuged and the plasma obtained was subjected to T-kininogen radioimmunoassay.

2.4. *Statistical analysis*

Data were expressed as the mean ± SD of 4 experiments. Statistical analysis was carried out using Student's *t*-test.

3. RESULTS

Fig.1 shows effects of human IL-1, TNF and rat IFN on the amount of T-kininogen secreted from cultured rat hepatocytes. IL-1 had no effect on T-kininogen secretion at concentrations up to 10000 units/ml, but TNF caused a slight increase in T-kininogen secretion at high concentrations (10000 units/ml). IFN exhibited dose-dependent stimulation of T-kininogen secretion in the range from 16 to 500 units/ml. The stimulatory activity of IFN was neutralized by an antibody against rat IFN

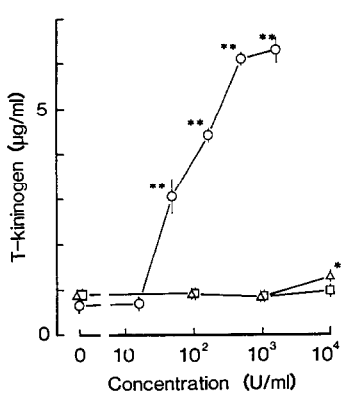

Fig.1. Effects of interleukin-1, tumor necrosis factor and interferon on the secretion of T-kininogen from cultured hepatocytes. Rat hepatocytes were cultured in 0.5 ml of basal medium supplemented with one of several concentrations of human interleukin-1 (□), human tumor necrosis factor (△) and rat interferon (○) for 48 h, and then the secreted amounts of T-kininogen were assayed. Significantly different from basal secretion: $*p < 0.05$, $**p < 0.001$.

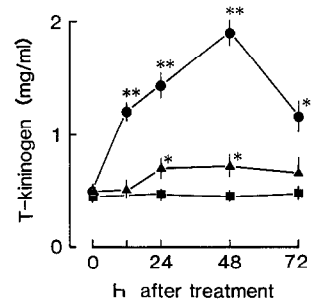

Fig.2. Effects of intraperitoneal administration of interleukin-1, tumor necrosis factor and interferon on plasma level of T-kininogen. Following i.p. administration of human interleukin-1 (2×10^4 units; ■) and tumor necrosis factor (5×10^4 units; ▲) and rat interferon (500 units; ●), plasma was collected at different times and T-kininogen concentration was assayed. Significantly different from control (0 h): $*p < 0.05$, $**p < 0.001$.

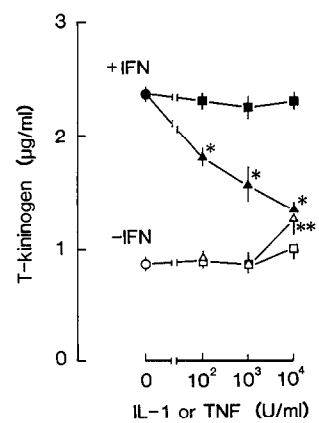

Fig.3. Effects of interleukin-1 and tumor necrosis factor on interferon-induced stimulation of T-kininogen secretion from cultured hepatocytes. Rat hepatocytes were cultured for 48 h in the presence or absence of rat interferon (50 units/ml) with one of several concentrations of human interleukin-1 or tumor necrosis factor. (○) Non-addition; (●) interferon alone; (□) interleukin-1 alone; (△) tumor necrosis factor alone; (■) interferon plus interleukin-1; (▲) interferon plus tumor necrosis factor. * Significantly different ($p < 0.001$) from interferon alone. ** Significantly different ($p < 0.05$) from non-addition.

(not shown). At the maximum response, T-kininogen secretion was increased approx. 10-fold over the basal secretion. On the other hand, neither cytokine affected the secretion of albumin at concentrations used in this study (not shown).

In order to ascertain the effect of IFN in vivo, two monokines and IFN were injected into rats, and plasma T-kininogen levels were measured. As shown in fig.2, IFN caused a significant elevation of the plasma T-kininogen level, and an approx. 4-fold elevation was observed at 48 h. On the other hand, IL-1 had no effect and TNF caused a slight elevation of the plasma T-kininogen level.

Since synergism between TNF and IFN has been reported with regard to their cytostatic or cytotoxic effect [14–16], it was examined in vitro whether the stimulatory effect of IFN on hepatic production of T-kininogen was modified by the presence of TNF or IL-1. As shown in fig.3, the IFN-induced increase of T-kininogen secretion from hepatocytes was inhibited by the presence of TNF in a dose-dependent manner. However, IL-1 did not affect the IFN-induced stimulation.

4. DISCUSSION

In the present study, we demonstrated that IFN had the ability to stimulate the hepatic production of T-kininogen. Three kinds of monokines, i.e., IL-1, TNF and HSF, have been identified to be mediators responsible for the induction of acute-phase reactants. Recently, Baumann et al. [7] demonstrated the existence of at least two types of HSF and showed that a combination of the two HSFs had the ability to induce overall acute-phase reactants, while the abilities of IL-1 and TNF were limited to only a few sets of acute-phase reactants. In addition, it was recently reported that human B-cell stimulatory factor 2, which was identical to IFN-β_2, had the ability to increase or decrease the hepatic mRNA level of fibrinogen or albumin, respectively [17]. From evidence that an antibody against B-cell stimulatory factor 2 inhibited this stimulatory activity in monocyte-conditioned medium, the authors suggested that B-cell stimulatory factor 2 or IFN-β_2 were identical to HSF [17]. These findings suggest a possibility that IFN-β_2 which is possibly identical to HSF, in the preparation of rat IFN used in this study, is a factor responsible for stimulating the hepatic production of T-kininogen. However, in the present study, albumin secretion from hepatocytes was not affected by rat IFN suggesting that IFN-β_2 in the preparation of rat IFN may not be responsible for the stimulatory effect of T-kininogen production. Since, in addition to the species specificity of IFN, rat IFN has not been extensively investigated, it is difficult to identify the kind if IFN which is responsible for the induction of T-kininogen.

IFN has been shown to promote the cytostatic or cytotoxic activity of TNF in different types of transformed cells [14–16]. In contrast to the cytotoxic synergism between IFN and TNF, the stimulatory activity of IFN on the hepatic production of T-kininogen was inhibited by TNF. A similar antagonism has been reported in the growth of fibroblasts, in which the stimulatory activity of TNF was inhibited by IFN [18]. The mechanism responsible for the inhibition of hepatic production of T-kininogen by TNF remains to be elucidated.

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REFERENCES

[1] Beisel, W.R. (1980) Fed. Proc. 39, 3105–3108.
[2] Kushner, I. (1982) Ann. NY Acad. Sci. 389, 39–48.
[3] Koji, A. (1985) in: The Acute Phase Response to Injury and Infection (Gordon, A.H. and Koj, A. eds) pp.145–151, Elsevier, Amsterdam.
[4] Richie, D.G. and Fuller, G.M. (1983) Ann. NY Acad. Sci. 408, 490–502.
[5] Koj, A., Gauldie, J., Regoeczi, E., Sauder, D.N. and Sweeney, G.D. (1984) Biochem. J. 224, 505–514.
[6] Baumann, H., Jahreis, G.P., Sauder, D.N. and Koj, A. (1984) J. Biol. Chem. 259, 7331–7342.
[7] Baumann, H., Onorato, V., Gauldie, J. and Jahreis, G.P. (1987) J. Biol. Chem. 262, 9756–9768.
[8] Okamoto, H. and Greenbaum, L.M. (1983) Biochem. Biophys. Res. Commun. 112, 701–708.
[9] Barlas, A., Okamoto, H. and Greenbaum, L.M. (1985) Biochem. Biophys. Res. Commun. 129, 280–286.
[10] Kageyama, R., Kitamura, N., Ohkubo, H. and Nakanishi, S. (1985) J. Biol. Chem. 260, 12060–12064.
[11] Sueyoshi, T., Enjyoji, K., Shimada, T., Kato, H., Iwanaga, S., Bando, Y., Kominami, E. and Katunuma, N. (1985) FEBS Lett. 182, 193–195.

[12] Itoh, N., Toyohama, T., Okamoto, H., Kawano, H., Mayumi, T. and Hama, T. (1987) Inflammation 11, 345–352.
[13] Okamoto, H., Itoh, N. and Uwani, M. (1987) Biochem. Pharmacol. 36, 2979–2984.
[14] William, T.W. and Bellanti, J.A. (1983) J. Immunol. 130, 518–520.
[15] Williamson, B.D., Carswell, E.A., Rubin, B.Y., Prendergast, J.S. and Old, L.J. (1983) Proc. Natl. Acad. Sci. USA 80, 5397–5401.
[16] Lee, S.H., Aggarwal, B.B., Rinderknecht, E., Assisi, F. and Chiu, H. (1984) J. Immunol. 133, 1083–1086.
[17] Andus, T., Geiger, T., Hirano, T., Northoff, H., Ganter, U., Bauer, J., Kishimoto, T. and Heinrich, P.C. (1987) FEBS Lett. 221, 18–22.
[18] Vilcek, J., Palombella, V.J., Henriksen-DeStefano, D., Swenson, C., Feinman, R., Hirai, M. and Tsujimoto, M. (1986) J. Exp. Med. 163, 632–643.