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Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-β2) regulates β-fibrinogen and albumin mRNA levels in Fao-9 cells

Tilo Andus, Thomas Geiger, Toshio Hirano*, Hinnak Northoff+, Ursula Ganter°, Joachim Bauer°, Tadamitsu Kishimoto* and Peter C. Heinrich

*Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freibrug, FRG, *Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan, +Hinnak Northoff, Blutspendezentrale des DRK, Oberer Eselsberg, D-7900 Ulm* and *°Medizinische Universitätsklinik, Hugstetter Str. 55, D-7800 Freiburg, FRG*

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Conditioned medium from human monocytes contains a partially characterized hepatocyte-stimulating factor that simultaneously elevates the mRNA levels of the acute-phase protein β-fibrinogen and decreases albumin mRNA in rat hepatoma cells. We demonstrate that recombinant human B-cell stimulatory factor 2, which is identical to interferon-β2/26 kDa protein and interleukin-HP1, exhibits the same activity as hepatocyte-stimulating factor. Furthermore, a specific antibody against B-cell stimulatory factor 2 was able to inhibit hepatocyte-stimulating factor in conditioned medium from human monocytes. Our data show that hepatocyte-stimulating factor and B-cell stimulatory factor 2 are functionally and immunologically related proteins.

Acute-phase protein; Albumin; BSF-2; β-Fibrinogen; Hepatocyte-stimulating factor; Interferon-β2

1. INTRODUCTION

In many species disturbances of the physiological homeostasis lead to a complex series of local and systemic reactions, designated as the acute-phase reponse [1]. The systemic reaction is characterized by drastic changes in the levels of various plasma proteins, the so called acute-phase proteins [2]. The major site of acute-phase protein synthesis is the liver. The increases in plasma levels of acute-phase proteins are preceded by increases in hepatic mRNA levels ([3,4], review [1]). These changes are generated by mediators secreted by mononuclear phagocytes [5-8]. With respect to acute-phase protein regulation HSF [9-12], interleukin-1 [13-16] and tumor necrosis factor α/cachectin [15,17] are the most thoroughly investigated of these monokines. Recently, the mediator responsible for induction of α_2-macroglobulin was discriminated from interleukin-1 and tumor necrosis factor α by ourselves [18,19] and others [20-22]. BSF-2 has been studied as a factor involved in the final differentiation of activated B cells into antibody-secreting cells [23]. The cDNA for BSF-2 was cloned and the results indicate that BSF-2 is iden-

Correspondence address: P.C. Heinrich, Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG

Abbreviations: BSF-2, B-cell differentiation factor; FCS, fetal calf serum; HSF, hepatocyte-stimulating factor; rhIFN-β2, recombinant human interferon-β2

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tical to IFN-β2, 26 kDa protein and interleukin HP1. Here, we describe that recombinant human BSF-2 acts as an acute-phase mediator on rat hepatoma cells.

2. MATERIALS AND METHODS

2.1. *Chemicals*

Deoxycytidine 5'-[α-^{32}P]triphosphate (110 TBq/mmol) was purchased from Amersham Buchler (Braunschweig). FCS (lot 85F-0541) and RPMI 1640 medium were from Sigma (Munich). Purified IFN-α (3×10^6 IU/ml; ch-B.: 11205008) and IFN-β (2×10^7 IU/mg protein), rhIFN-β1 (1.5×10^8 IU/mg protein; ch-B.: 221.T1001) and rhIFN-γ (1.5×10^7 IU/mg protein; ch-B.: 321.01003) were kindly supplied by Dr Obert (Bioferon, Laupheim, FRG). BSF-2 (5×10^6 U/mg protein) was prepared as described in [24]. Polyclonal anti-BSF-2 (rabbit IgG fraction, 1 mg/ml) inhibited 5 U of BSF-2 activity per ml at a dilution of 1:200. Albumin cDNA was supplied by Dr A. Alonso (DKFZ, Heidelberg) and β-fibrinogen cDNA by Dr A. Mitchell (Parkville, Australia). Dr F. Wiebel (GSF, Munich) provided the Fao-9 cells.

2.2. *Hepatoma cell culture*

Fao-9 cells were grown in RPMI 1640 medium containing 5% FCS, 100 mg/l streptomycin and 65 mg/l penicillin and passaged once per week by trypsinization. For most experiments the cells were plated on a 24-well dish (Falcon, no. 3047) 2 days earlier. About 5×10^5 cells per well were used for experiments.

2.3. *Cytoblot and mRNA hybridization*

Cells (5×10^5 per well) were lysed in 10 mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA and 0.5% Nonidet P40, nuclei removed by centrifugation, cytoplasmic RNA denatured in the presence of formaldehyde essentially as in [25] and RNA blotted to a Gene Screen membrane (NEN) using a Manifold dot-blot apparatus (Schleicher and Schüll). After baking the filters at 80°C for 2 h and prehybridization for 6 h, the filters were hybridized to ^{32}P-labeled cDNA probes [26]. The cDNAs were radiolabeled using the random primer technique in [27]. Specificity of hybridization was controlled by Northern blot analysis (not shown).

2.4. *Conditioned media from human monocytes and macrophages*

These were prepared as described in [18,19].

3. RESULTS AND DISCUSSION

A well-differentiated rat hepatoma cell line (Fao-9) was used for studying alterations in β-fibrinogen and albumin mRNA levels as an in vitro system to measure the influence of mediators on acute-phase protein synthesis. Human purified IFN-α and purified IFN-β, rhIFN-β1 and rhIFN-γ were added to Fao-9 cells in increasing amounts. As shown in fig.1 only purified IFN-β elevated β-fibrinogen mRNA (A) and simultaneously lowered albumin mRNA levels (B). All other IFNs used – including rhIFN-β1 – had no effect. Conditioned media from human monocytes (cm-mo) and human macrophages (cm-Mϕ) served as positive controls. Since very high concentrations of purified huIFN-β were needed (50% increase in β-

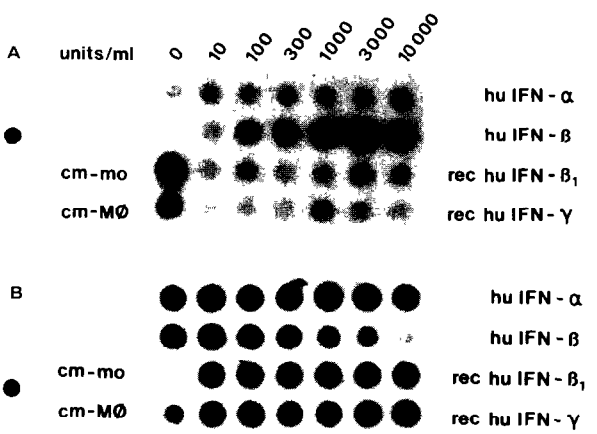

Fig.1. Induction of rat β-fibrinogen mRNA by human interferon-β. Fao-9 cells (5×10^5 cells/well) were incubated with purified human interferon-α (huIFN-α), purified human interferon-β (huIFN-β), recombinant human interferon-β1 (rec huIFN-β1) and recombinant human interferon-γ (rec huIFN-γ) at the indicated concentrations or with conditioned media from human monocytes (cm-mo) or macrophages (cm-Mϕ) in the presence of 10^{-6} M dexamethasone for 12 h. The different IFN concentrations were obtained by dilution in RPMI 1640 medium containing 5% FCS and 10^{-6} M dexamethasone shortly before addition to the cells. The cells were lysed, the cytoplasmic RNA extract blotted to a Gene Screen membrane and hybridized with ^{32}P-labeled β-fibrinogen cDNA (A) or albumin cDNA (B).

fibrinogen mRNA at 700 IU/ml), and huIFN-β1 had no effect, we concluded that a protein similar to human IFN-β1, but still different from it, may be an acute-phase mediator. Recently, Gauldie reported that BSF-2 was able to act as an acute-phase mediator in HepG2 cells, Hep3B2 cells and rat hepatocyte primary cultures (personal communication, Cold Spring Harbor Symposium on Regulation of Liver Gene Expression, 1987). From data obtained in several laboratories it became evident that BSF-2 is identical to interferon-β2, the 26 kDa protein [28–32] and the hybridoma/plasmocytoma growth factor (interleukin-HP1) [33]. Therefore, we added rhBSF-2 to Fao-9 cultures. rhBSF-2 induced a 5.1-fold increase in β-fibrinogen mRNA levels (fig.2, filled triangles). At the same time albumin mRNA decreased 3.9-fold (closed circles). Only 4 U/ml of BSF-2 led to a half-maximal induction of β-fibrinogen mRNA. Assuming an M_r of 21 000 for BSF-2, this half-maximal induction is obtained at an rhBSF-2 con-

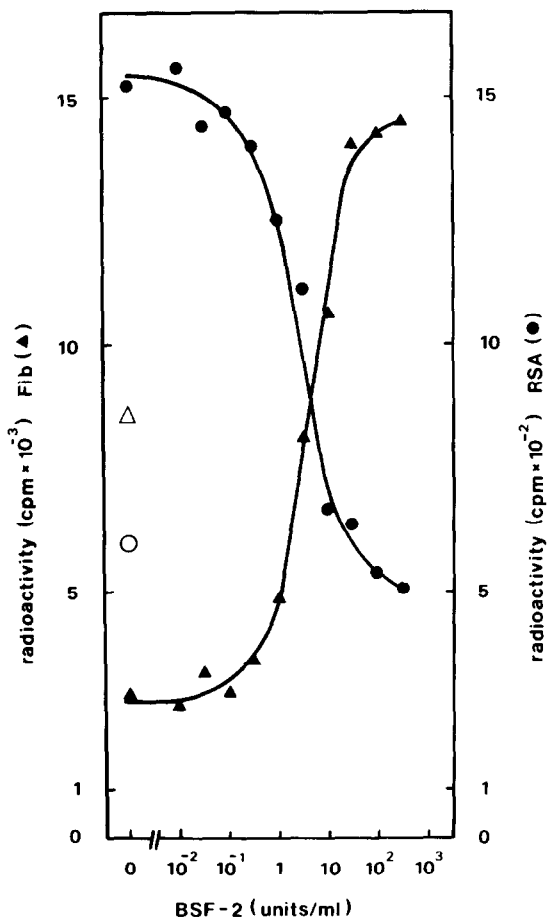

Fig.2. Regulation of β-fibrinogen and albumin mRNA levels by recombinant human B cell stimulatory factor 2 (BSF-2). Fao-9 cells (5×10^5 cells/well) were incubated with rec huBSF-2 at the indicated concentrations (▲,●) or with conditioned media from human monocytes (△,○) in the presence of 10^{-6} M dexamethasone for 12 h. The dilution of rec huBSF-2 and hybridization of mRNA with ^{32}P-labeled β-fibrinogen cDNA (▲,△) or albumin cDNA (●,○) were carried out as described in the legend to fig.1. For quantification the dots on the Gene Screen membrane were counted in a liquid scintillation counter.

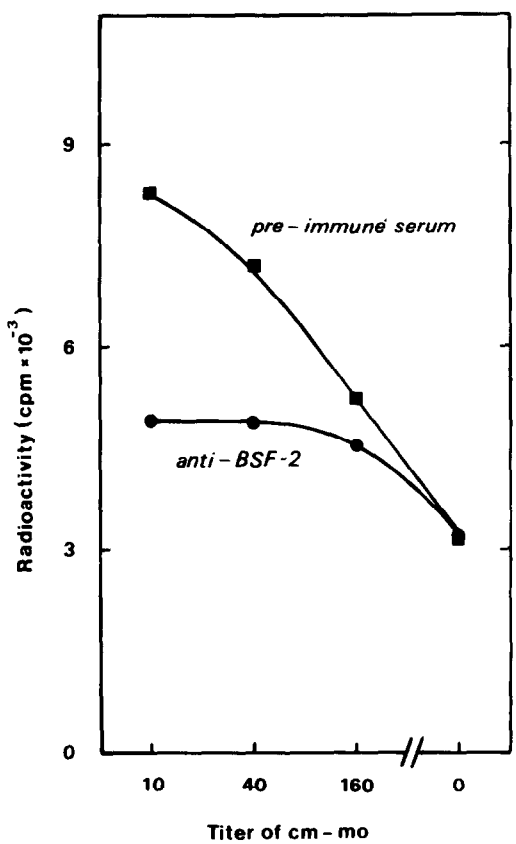

Fig.3. Inhibition of hepatocyte-stimulating factor in conditioned medium from human monocytes by anti-BSF-2. 3 vols conditioned medium from human monocytes were pre-incubated with 1 vol. anti-BSF-2 (●) or pre-immune serum (■) at 25°C for 30 min. This pretreated conditioned medium was added to the culture medium of Fao-9 cells at the indicated dilutions. β-Fibrinogen mRNA induction was measured as described in section 2.

centration of about 40 pM. Interestingly, pure rhBSF-2 was a more effective stimulant of β-fibrinogen mRNA synthesis than conditioned medium from human monocytes (open triangles).

After having demonstrated that BSF-2 stimulates β-fibrinogen mRNA synthesis, we further investigated the relationship between the T-cell derived BSF-2 and the monocyte-derived HSF. Therefore, we pre-incubated conditioned medium with anti-BSF-2 or with pre-immune serum. Fig.3 shows that anti-BSF-2 neutralized about 70% of the hepatocyte-stimulating activity in conditioned medium from human monocytes (filled circles), whereas the pre-immune serum (filled squares) had no effect. The activity remaining after the addition of anti-BSF-2 could either be due to an incomplete neutralizing capacity of the antibody or to the presence of other acute-phase mediators in conditioned medium of monocytes. HSF in conditioned medium from human macrophages derived from monocytes by in vitro maturation, however, was totally blocked by anti-BSF-2 (not shown).

Our data have shown a strong functional and immunological relationship between BSF-2/IFN-β2 and HSF. Experiments are underway to prove finally a possible identity by sequence data. Since the interferon activity of IFN-β2/BSF-2 is presently questioned [32], the stimulation of acute-phase protein synthesis in hepatocytes may be one of the major functions of IFN-β2/BSF-2.

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