ELSEVIER

FEBS Letters 337 (1994) 99–102

FEBS 13502

FEBS LETTERS

Both type I and type II interferons down-regulate human tumor necrosis factor receptors in human hepatocellular carcinoma cell line Hep G2

Role of protein kinase C

Bharat B. Aggarwal*, Raj Pandita

Cytokine Research Laboratory, Department of Clinical Immunology and Biological Therapy, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA

Received 18 October 1993; revised version received 22 November 1993

Abstract

It is well known that interferon-γ (IFN-γ; type II) potentiates various responses of human tumor necrosis factor (TNF) in a wide variety of cells and that this potentiation is accompanied by the up-regulation of TNF receptor synthesis. In the present studies we examined the regulation of TNF receptors by type I and type II IFNs in a hepatocellular carcinoma cell line, HEP G2. Exposure of these cells to IFN-γ led to a decrease in TNF receptor number (4029 vs. 2719 sites/cell) without any change in the receptor affinity (0.96 nM vs. 1.1 nM). The effect was time and dose-dependent. Like IFN-γ, IFN-α and IFN-β (type I) down-modulated the TNF receptors on these cells. The effect of IFNs on the TNF receptors was inhibited by staurosporin, a protein kinase C (PK-C) inhibitor. Furthermore, by the use of receptor-specific antibodies, we found that the IFN-dependent decrease was primarily due to the p60 form of the TNF receptor. Our results presented are the first to demonstrate that IFNs can also down-modulate TNF receptors in certain cells and that this effect is mediated through PK-C.

Key words: Tumor necrosis factor; Interferon; TNF receptor; Protein kinase

1. Introduction

Soon after human tumor necrosis factor (TNF) was isolated to homogeneity, it was found to synergize with interferon-γ (IFN-γ) in inducing antiproliferative effects against a wide variety of tumor cells [1–5]. Very quickly, demonstration of this synergistic action was extended to several other effects of TNF [1]. Our laboratory and several others have reported that IFN-γ increases TNF receptor numbers on a wide variety of cells, which perhaps accounts for the synergistic effects in at least some systems [6–11].

We have recently reported that IFNs, both type I (IFN-α and IFN-β) and type II (IFN-γ), upregulate both p60 and p80 forms of the TNF receptors in a wide variety of cells [12]. In the present report, we demonstrate that in Hep G2 cells the IFNs down-modulate TNF receptors and that this effect is mediated through the activation of a protein kinase-C. Further, we show that it is the p60 receptor that is down-modulated.

*Corresponding author. Fax: (1) (713) 794 1613.

Abbreviations: TNF, tumor necrosis factor; IFN, interferon; p60, also referred to as p55, TNF receptor I, or TNF receptor type B; p80, also referred to as p75, TNF receptor II, or TNF receptor type A; FCS, fetal calf serum; PK-C, protein kinase C.

2. Materials and methods

2.1. Materials

Bacteria-derived recombinant human TNF-α, IFN-α, and IFN-γ purified to homogeneity with a specific activity of 50×10^6, 100×10^6 and 10×10^6 units/mg, respectively, were kindly provided by Genentech, Inc., South San Francisco, CA. Recombinant human IFN-β with a specific activity of 10×10^6 units/mg protein was a kind gift of Toray Ltd., Tokyo, Japan. Polyclonal antibodies against each type of soluble receptor were raised in rabbits and purified by receptor-affinity chromatography.

2.2. Cell lines

The human hepatocellular carcinoma cell line HepG2 (ATCC No. HB-8065) was grown in DMEM supplemented with fetal calf serum (FCS) (10%) and gentamicin (50 μg/ml). The cells were seeded at a density of 1×10^5 cells/ml in T-25 flasks (Falcon 3013, Becton Dickinson Labware, Lincoln Park, NJ) containing 10 ml of medium and grown at 37°C in an atmosphere of 95% air and 5% CO_2. Cell cultures were split every 3 or 4 days.

2.3. Receptor-binding assay

Recombinant human TNF was labeled with $Na^{125}I$ using the Iodogen procedure as described previously [6]. The specific activity of labeled TNF was 30 μCi/μg. Binding assays were performed in 12-well plates as described [6]. Briefly, cells (0.2×10^6 cells/well) were incubated in binding buffer (DMEM supplemented with 10% FCS) with ^{125}I-labeled TNF (0.2×10^6 cpm) with or without 250 nM TNF in a total final volume of 0.5 ml for 1 h at 4°C. Thereafter, cells were washed three times with 0.5 ml of ice-cold washing buffer (PBS containing 0.1% BSA). Cell-bound radioactivity was then determined by a Packard gamma counter (model CD 5010).

The binding of TNF to p60 or p80 receptor was determined by preincubating the cells at 37°C for 1 h with 0.6–0.8 μg/ml of either preimmune or anti-p60 or anti-p80 antibodies before initiating the

SSDI 0014-5793(93)E1419-M

ligand binding. The amount of p60 or p80 receptor was calculated by subtracting TNF-specific binding in the absence of antibody from that in the presence of either anti-p80 or anti-p60 receptor antibodies. All determinations were made in triplicate and expressed as mean ± S.E.

3. Results

3.1. Interferon-γ down-modulates TNF receptors

We first examined the effect of pretreatment of IFN-γ on TNF receptors in Hep G2 cells. For this, cells were treated with different concentrations of IFN-γ for 18 h at 37°C, washed, and examined for TNF receptors. The results shown in Fig. 1a demonstrate that IFN-γ produces a dose-dependent decrease in TNF receptors. A maximum decrease was observed at 100 ng/ml of IFN. The decrease in TNF receptors by IFN-γ under these conditions was not due to its effect on cell viability as determined by Trypan blue exclusion and also by tritiated thymidine incorporation (data not shown). We also examined the time-course of down-modulation of TNF receptors by 100 ng/ml of IFN. The results of this experiment (Fig. 1b) indicate that receptor decreased in a time-dependent manner reaching maximum levels at 18 h. To determine whether the decrease in binding of TNF to the cells was due to receptor number or receptor affinity, we carried out competitive TNF receptor binding and performed Scatchard analysis. The results of these experiments (Fig. 2a,b) demonstrate that TNF receptor number decreased but receptor affinity did not.

3.2. TNF receptors are also down-modulated by IFN-α and IFN-β

Since the biological responses of IFN-α and IFN-β are mediated through a receptor distinct from that of IFN-γ [13], we examined the effect of the type I IFNs on the TNF receptors in HepG2 cells. As shown in Table 1, all three IFNs down-modulated the binding of TNF to these cells. The effect appeared to be very comparable, although IFN-α seemed somewhat less effective than the other IFNs.

3.3. Staurosporin inhibits down-modulation of IFN-induced TNF receptors

Staurosporin is a well-known inhibitor of protein kinase C (PK-C) [14]. Since it has been reported that some of the effects of IFNs are PK-C mediated [15–17], we examined the effect of staurosporin on the interferon-induced down-modulation of TNF receptors. The results of this experiment, shown in Table 1, demonstrate that though the PK-C inhibitor had no effect by itself on TNF receptors, it completely abolished the down-modulation of the receptor by IFN-γ. Staurosporin was also effective against IFN-α and IFN-β but not to the same extent as against IFN-γ. These results suggest that the down-modulation of TNF receptors by IFNs may be PK-C-mediated.

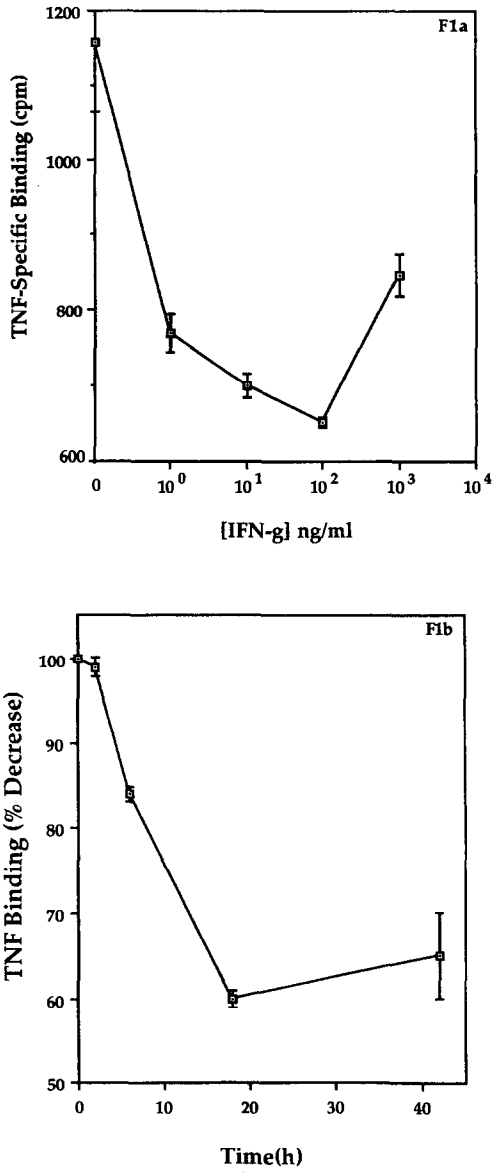

Fig. 1. Dose-dependency (upper panel) and time course (lower panel) of down-modulation of TNF receptor by IFN-γ. Cells (2×10^5) were incubated with different concentrations of IFN-γ for 18 h at 37°C (upper panel) or with 100 ng/ml IFN-γ for different times at 37°C (lower panel), washed, and tested for TNF receptors as described in Section 2.

3.4. The p60 form of the TNF receptor is modulated by IFN

We examined which of the TNF receptors, referred to as type I or p60 and type II or p80 was modulated by IFN-γ. We used antibodies specific to each receptor to block ligand binding. The results in Table 2 indicate that HepG2 cells essentially expressed only the p60 form of

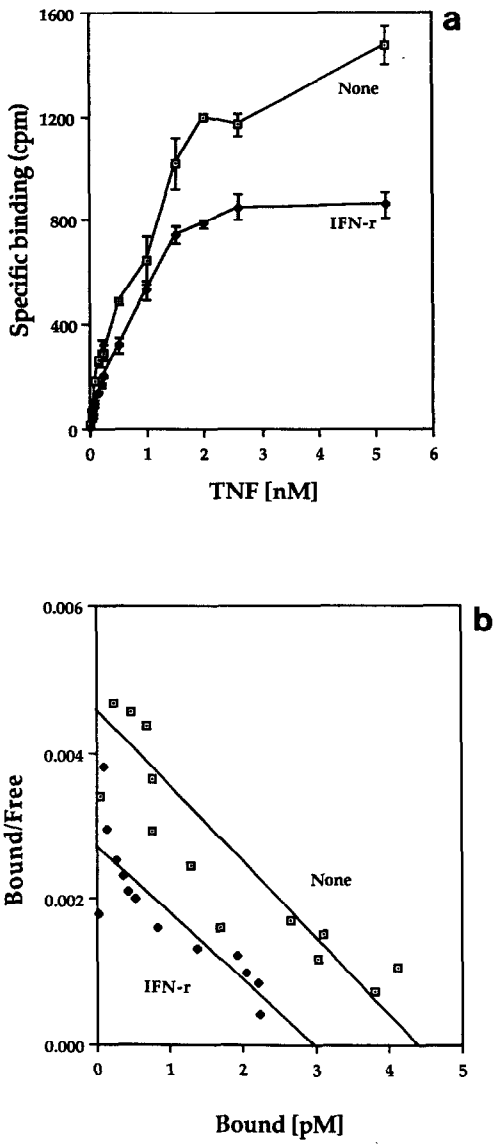

Fig. 2. Competitive TNF receptor binding analysis (upper panel) and Scatchard analysis (lower panel) of TNF binding to untreated HepG2 cells and HepG2 cells treated with IFN-γ. Cells (2×10^5) were incubated with 100 ng/ml IFN-γ for 18 h at 37°C, washed, and tested for TNF receptors as described in Section 2.

the TNF receptor, and it is this receptor that was down-modulated by IFN-γ.

4. Discussion

Several previous reports have indicated that TNF receptors are up-regulated by treatment of cells with IFN-γ [6–11]. In the present report, however, we demonstrate that treatment of Hep G2 cells with IFN-γ down-modulates the TNF receptors. Similar to IFN-γ, IFN-α and IFN-β also caused a decrease in the receptors. IFNs induced a decrease in TNF receptor number without any effect on the affinity.

Table 1

Effect of staurosporine on interferons-induced TNF-receptor down-regulation in HepG2 cells

Treatment	Specific binding (cpm)	
	Control	Staurosporin treatment
None	929 ± 80	957 ± 63
IFN-γ	651 ± 90	948 ± 55
IFN-α	742 ± 14	803 ± 69
IFN-β	640 ± 50	711 ± 95

0.2×10^6 cells were incubated overnight with each interferon (100 ng/ml) and without or with staurosporine (3 nM) at 37°C for 18 h and then washed off and examined for TNF receptors as indicated in Section 2. All determinations were made in truplicate.

How IFNs mediate a decrease in TNF receptors is not clear. Since the down-modulation of TNF receptors by IFNs could be prevented by staurosporin, a PK-C inhibitor, IFNs may activate a PK-C that in turn decreases the number of TNF receptors. IFN-α and IFN-β bind to a common receptor that is distinct from that of IFN-γ [13]. Even though both IFN receptors lack intrinsic protein kinase activity, they do, however, activate PK-C which has been shown to be critical for the transcriptional activity of the IFNs [15–17]. A novel staurosporin-sensitive kinase has been shown to be required for the induction of a transcriptional factor ISGF3 needed for the signal transduction of IFN-a [15]. Therefore, it is possible that the same staurosporin-sensitive kinase is involved in the downmodulation of TNF receptors.

Our results on the downmodulation of TNF receptors through the PK-C pathway are consistent with previous reports indicating that treatment of cells with phorbol esters, which are activators of PK-C, and okadaic acid, an inhibitor of serine-threonine phosphatase, also down-modulate TNF receptors [18,19].

Our results also indicate that among the two types of TNF receptors, the p60 form is primarily expressed by HepG2, and it is this receptor that is down-modulated

Table 2

Down-modulation of tumor necrosis factor receptors by IFN-γ on HepG2 cells

Antibody	Specific TNF binding (cpm)	
	Control	IFN-γ
None	613 ± 1	442 ± 7
Preimmune	522 ± 120	433 ± 6
p60	93 ± 6	60 ± 3
p80	359 ± 34	447 ± 80

0.2×10^6 cells were incubated with IFN-γ (100 ng/ml) overnight and then washed. Cells were then preincubated with either pre-immune or anti-p60 or anti-p80 for 1 h at 37°C and then examined for remaining TNF receptors.

by IFNs. In contrast to the p80 receptor, the p60 form of the TNF receptor has been implicated in a wide variety of biological effects [20]. Therefore, it is possible that the modulation of the p60 receptor by IFNs has a direct effect on the biological response to TNF.

Acknowledgements: This research was conducted, in part, by The Clayton Foundation for Research and was supported, in part, by new program development funds from The University of Texas M.D. Anderson Cancer Center.

References

[1] Aggarwal, B.B. and Vilcek, J. (Eds.) (1992) Tumor Necrosis Factor: Structure, Function and Mechanism of Action, Marcel Dekker, New York.
[2] Lee, S.H., Aggarwal, B.B., Rinderknecht, E., Assisi, F. and Chiu, H. (1984) J. Immunol. 133, 1083–1086.
[3] Stone-Wolff, D.S., Yip, Y.K., Kelker, H., Le, J., DeStefano, D.H., Rubin, B., Rinderknecht, E., Aggarwal, B.B. and Vilcek, J. (1984) J. Exp. Med. 159, 828–843.
[4] 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.
[5] Sugarman, B.J., Aggarwal, B.B., Hass, P.E., Figari, I.S., Palladino, M.A. and Shepard, H.M. (1985) Science 230, 943–945.
[6] Aggarwal, B.B., Eessalu, T.E. and Hass, P.E. (1985) Nature 318, 665–667.
[7] Tsujimoto, M. and Vilcek, J. (1986) J. Biol. Chem. 261, 5384–5388.
[8] Tsujimoto, M., Yip, Y.K. and Vilcek, J. (1986) J. Immunol. 136, 2441–2444.
[9] Ruggiero, V. and Baglioni, C. (1987) J. Immunol. 138, 661–663.
[10] Michishita, M., Yoshida, Y., Uchino, H. and Nagata, K. (1989) J. Biol. Chem. 265, 8751–8759.
[11] Israel, S., Hahn, T., Holtman, H. and Wallach, D. (1986) Immunol. Lett. 12, 217–224.
[12] Pandita, R., Pocsik, E. and Aggarwal, B.B. (1992) FEBS Lett. 312, 87–90.
[13] Branca, A.A. and Baglioni, C. (1981) Nature 294, 768–770.
[14] Ruegg, U.T. and Burgess, G.M. (1989) Trends Pharmacol. Sci. 10, 218–220.
[15] Kessler, D.S. and Levy, D.E. (1991) J. Biol. Chem. 266, 23471–23476.
[16] Fan, X.-D., Goldberg, M. and Bloom, B.R. (1988) Proc. Natl. Acad. Sci. USA 85, 5122–5125.
[17] Pfeffer, L.M., Eisenkraft, B.L., Reich, N.C., Improta, T., Baxter, G., Daniel-Issakani, S. and Strulovici, B. (1991) Proc. Natl. Acad. Sci. USA 88, 7988–7992.
[18] Aggarwal, B.B. and Eessalu, T.E. (1987) J. Biol. Chem. 262, 16450–16455.
[19] Higuchi, M. and Aggarwal, B.B. (1993) J. Biol. Chem. 268, 5624–5631.
[20] Tartaglia, L.A. and Goeddel, D.V. (1992) Immunol. Today 13, 151–153.