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Interferon-γ induces cell surface expression for both types of tumor necrosis factor receptors

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Interferons are known to potentiate various biological effects of tumor necrosis factor (TNF). Recently, two different types of TNF receptors with molecular masses of 60 kDa (p60) and 80 kDa (p80), primarily expressed by epithelial cells and myeloid cells, respectively, have been identified. In the present report, we examined the effect of interferon-γ (IFN-γ) on each type of TNF receptor. Our results indicate that IFN-γ induces TNF receptors on both myeloid (e.g. HL-60) and epithelial cells (e.g. HeLa). Furthermore, by using antibodies specific to each type of receptor, we demonstrate that both TNF receptors are equally inducible by IFN-α, IFN-β and IFN-γ. Thus, the increase in TNF receptors by interferons may play a role in their synergistic cellular response.

Tumor necrosis factor; Interferon; TNF receptor

1. INTRODUCTION

Human tumor necrosis factor (TNF) exhibits a wide variety of biological activities in vitro including antiproliferation of tumor cells, proliferation of normal human fibroblasts, B cells, and thymocytes, antiviral effects, and induction of various genes (see [1]). Several of these effects of TNF have been shown to be potentiated by interferons [2–7]. The receptors for TNF have been reported to be induced by IFN-γ [8–13], a process that may play a role in their synergistic biological response.

Recently the cDNA for two different TNF receptors with approximate molecular masses of 60 kDa (p60) and 80 kDa (p80) have been isolated [14–17]. It has been shown that the p60 form of the receptor is primarily expressed on epithelial cells whereas the p80 form is displayed by myeloid cells [18]. Furthermore, a recent report suggests that these receptors mediate distinct signals [19]. How IFN-γ affects each type of TNF receptor is not known. Therefore, we investigated the effect of IFN-γ on the cell surface expression of TNF receptors in epithelial and myeloid cells by using antibodies specific to each type of receptor.

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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.

2. MATERIALS AND METHODS

2.1. *Materials*

Bacteria-derived recombinant human TNF-α and IFN-γ, purified to homogeneity with specific activities of 5×10^7 units/mg and 1×10^7 units/mg, respectively, were kindly provided by Genentech, Inc., South San Francisco, CA. The highly purified form of the recombinant extracellular domains of TNF receptors p60 (TNF receptor I) and p80 (TNF receptor II) with molecular masses of 30 kDa and 40 kDa, respectively, were kindly provided by Dr. Tadahiko Kohno of Synergen, Boulder, CO. The soluble forms of these receptors have been previously characterized [17]. Polyclonal antibodies in rabbits were raised against each type of receptor and purified by receptor-affinity chromatography.

2.2. *Cell lines*

All cell lines employed in these studies were obtained from the American Type Cell Culture Collection (Rockville, MD). Primary human foreskin fibroblasts at early passages were kindly supplied by Dr. Olivia Smith of Baylor College of Medicine, Houston, TX. The myeloid cell lines, KG 1 and KG 1a were kindly provided by Dr. P. Pantazis of the Stehlin Foundation, Houston, TX. Cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and gentamycin (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 [8]. The specific activity of labeled TNF was 30 μCi/μg. Binding assays were performed using the 96-well method as described [21]. Briefly, cells ($0.2–1 \times 10^6$ cells/well) were incubated in a binding buffer (RPMI 1640 supplemented with 10% FCS) in either 12-well plates or 12 mm × 75 mm polypropylene tubes with ^{125}I-labeled ligand and with or without a 100-fold excess of unlabeled ligand for 2 h at 4°C in a total volume of 0.1–0.5 ml. 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).

3. RESULTS

3.1. *Induction of cell-surface expression of TNF receptors by IFN-γ*

We first examined the effect of different concentrations of IFN-γ on the cell surface expression of TNF receptors on HeLa, an epithelial tumor cell line. IFN-γ induced TNF receptors in a dose-dependent manner, and the optimum induction occured at a 100 ng/ml concentration of the cytokine after 6 h incubation (Fig. 1). The induction of TNF receptors in HeLa cells was further analyzed for receptor-density and -affinity by Scatchard analysis. There was approximately a two-fold induction in TNF receptor number without any significant change in the receptor affinity (Fig. 2).

3.2. *Induction of TNF receptors by IFN-γ occurs on both epithelial and myeloid cells*

It has been shown that the p60 form of the TNF receptor is mainly expressed by epithelial cells, whereas the p80 form is expressed by myeloid cells [18]. To determine the type of receptor induced by IFN-γ, we examined its effects on a wide variety of epithelial and myeloid cell lines. The TNF receptor could be induced by IFN on most myeloid and epithelial cell lines but to a variable degree (Table I). Under identical conditions, among myeloid cell lines, the highest induction was observed in HL-60, whereas HeLa cells showed maximum increase among epithelial cell lines. Interestingly, some epithelial cell lines such as ZR-75-1 and Hep G2 showed a significant reduction in the expression of cell surface receptors after IFN treatment.

3.3. *Both the p60 and the p80 forms of the TNF receptors are induced by IFN-γ*

The expression of p60 and p80 receptors by epithelial and myeloid cells is not exclusive; most cell types express both types of receptor in different proportions. Thus the induction of TNF receptors observed above could be because of induction of either the p60 or p80 form of the receptor. In order to determine the type of receptor induced, we employed receptor-specific antibodies to confirm the induction of each type of receptor by IFN-γ on myeloid cells (e.g. HL-60 and K-562) and epithelial cells (e.g. HeLa). The results of these experiments are shown in Table II. Both HL-60 and K-562 cells displayed both p60 and p80 forms of the receptor and both of these receptors were induced by treatment of cells with IFN. The induction of receptors in HeLa cells was primarily due to the p60 form.

3.4. *IFN-α and IFN-β can also induce both types of TNF receptors*

TNF receptors were also induced by other interferons. The results of these experiments shown in Table III indicate that in HeLa cells both IFN-α and IFN-β increased the cell surface expression of TNF receptors by

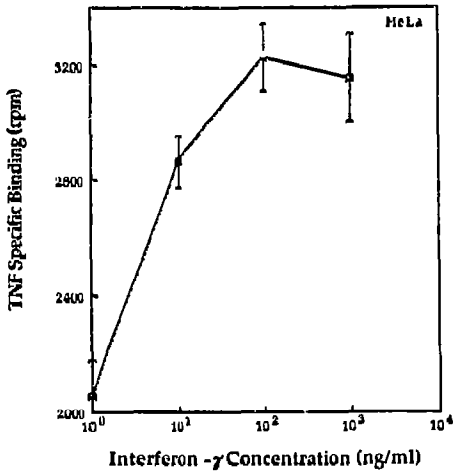

Fig. 1. Induction of cell surface expression of TNF receptors by different concentrations of IFN-γ in HeLa cells. Cells (2×10^5) were incubated with IFN-γ for 6 h, washed, and tested for TNF receptors as indicated in section 2. All determinations were made in triplicate.

35% and 153% respectively, compared with 72% by IFN-γ. However, the induction of receptors in K-562 and HL-60 by IFN-α and IFN-β was less pronounced than that by IFN-γ.

Table I

Induction of p60 and p80 forms of the TNF receptors on different epithelial and myeloid cell lines by interferon-γ

Cell lines	Specific binding (cpm)		Percent induction
	None	IFN-γ	
Myeloid Cells			
Promyelocytic (HL-60)	5,306 ± 534	9,559 ± 1,329	180
Promonocytic leukemias (THP-1)	1,354 ± 234	1,872 ± 95	138
Acute myelogenous leukemia (KG-1)	1,475 ± 342	1,914 ± 2	130
Erythroblastoid (K-562)	8,165 ± 777	10,540 ± 1,054	129
Histocytic lymphoma (U-937)	13,910 ± 904	17,160 ± 871	123
Leukemia (KG-1a)	1,439 ± 345	1,501 ± 204	104
Epithelial Cells			
Epitheloid carcinoma (HeLa)	3,309 ± 230	5,644 ± 413	170
Fibroblasts (FS)	695 ± 4	1,143 ± 47	164
Breast carcinoma (MCF-7)	702 ± 15	996 ± 7	142
Epidermoid carcinoma (A-431)	1,013 ± 74	1,242 ± 32	123
Adenocarcinoma (SK-BR3)	546 ± 7	700 ± 48	128
Cervical carcinoma (ME-180)	808 ± 17	929 ± 67	115
Breast carcinoma (BT-20R)	447 ± 40	520 ± 12	116
Adenocarcinoma (OVCAR)	570 ± 379	612 ± 8	107
Melanoma (RPMI-7951)	540 ± 78	471 ± 42	87
Melanoma (A-375)	1,017 ± 111	970 ± 74	95
Heptoma (Hep G2)	1,646 ± 80	1,056 ± 20	64
Breast carcinoma (ZR-75-1)	736 ± 204	359 ± 35	49

$0.2–1 \times 10^6$ cells were treated overnight with IFN-γ (100 ng/ml) at 37°C and then washed and examined for TNF receptors as indicated in section 2. All determinations were made in triplicate.

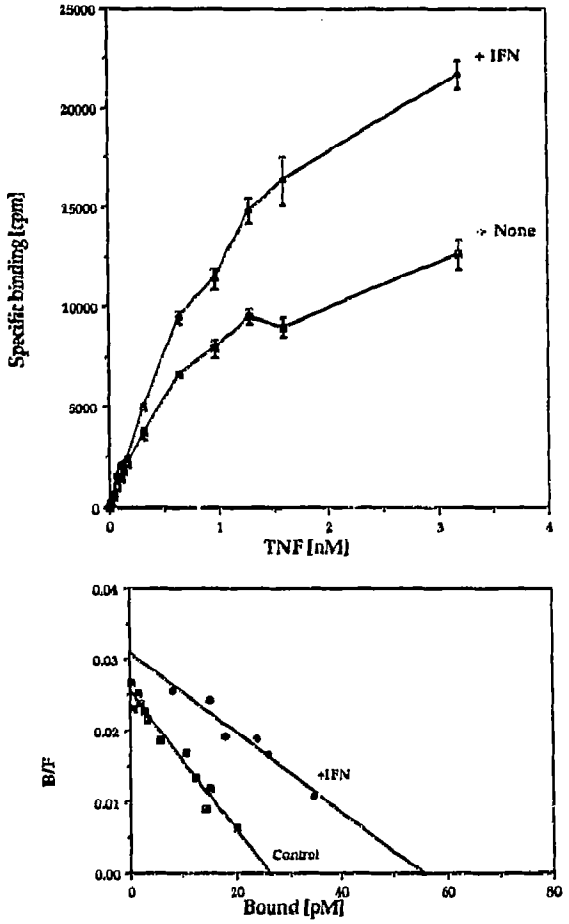

Fig. 2. The TNF binding curve (upper panel) and Scatchard analysis (lower panel) of the cell surface receptors on HeLa cells before (square) and after (diamond) IFN-γ treatment. Cells (2×10^5/well) were treated with IFN (100 ng/ml) for 6 h at 37°C and then binding was determined at 4°C as described in section 2. All determinations were made in triplicate.

Table II

Percent induction of the p80 and p60 forms of TNF receptors on human epithelial and myeloid cell lines by human interferon-γ

Cell lines	Percent induction		
	Total	p60	p80
Myeloid cells			
HL-60	155	146	135
K-562	192	190	229
Epithelial cells			
HeLa	154	150	0

0.2–1 $\times 10^6$ cells were incubated with IFN-γ (100 ng/ml) overnight and then washed. Cells were then preincubated with either pre-immune or anti-p60 (for p60 receptor) or anti-p80 antibodies for 1 h at 37°C and then examined for remaining TNF receptors. TNF binding observed in the presence of pre-immune serum was set at 100%.

4. DISCUSSION

Previously our laboratory and several others have demonstrated that IFN-γ can induce TNF receptors [8–13]. These studies were carried out when the existence of two type of TNF receptors was not known. Recently, two distinct types of TNF receptor, with molecular sizes of 60 kDa (p60) and 80 kDa (p80), have been identified [14–18]. It has been shown that these two receptors are independently regulated and also they transduce distinct signals [19]. The antiproliferative signals of TNF are thought to be mediated through the p60 form of the receptor, whereas the p80 form, which is considered species-specific, mediates the proliferative signal [23]. How these two type of receptors are regulated by IFN-γ is not known. In the present report, we demonstrate that TNF receptors are induced by IFN-γ on epithelial cells (which express primarily the p60 form of the receptor) and on myeloid cells (which mainly express the p80 form of the receptor). Antibodies specific to each type of receptor confirmed that both types of TNF receptor are induced by IFN-γ. The induction of cell surface expression of the TNF receptor was also observed with IFN-α and IFN-β.

In spite of induction of cell-surface expression of both TNF receptors, previous reports showed a lack of effect of IFN-γ on the mRNA transcript of the p60 receptor [15,27]. We confirmed these reports during our study

Table III

Induction of cell-surface expression of TNF receptors by various interferons on different cells

Cell lines	IFNs	Specific binding (cpm)		
		Receptor neutralization		
		Pre-immune	Anti-p60	Anti-p80
HeLa	None	411 ± 108 (100)	79 ± 7	429 ± 110
	IFN-α	556 ± 85 (135)	121 ± 2	1,227 ± 107
	IFN-β	1,040 ± 5 (253)	84 ± 74	762 ± 84
	IFN-γ	709 ± 78 (172)	45 ± 10	600 ± 20
K-562	None	6,790 ± 638 (100)	3,793 ± 274	2,243 ± 17
	IFN-α	7,621 ± 45 (112)	4,327 ± 36	1,708 ± 135
	IFN-β	8,050 ± 23 (118)	5,335 ± 224	1,970 ± 28
	IFN-γ	9,955 ± 238 (147)	6,514 ± 63	2,845 ± 133
HL-60	None	8,897 ± 77 (100)	4,575 ± 503	2,500 ± 10
	IFN-α	9,670 ± 39 (108)	5,375 ± 188	2,170 ± 25
	IFN-β	9,575 ± 15 (107)	6,602 ± 91	2,104 ± 80
	IFN-γ	11,967 ± 220 (134)	8,513 ± 77	2,511 ± 11

Cells (1×10^6 for K-562 and HL-60, and 0.2×10^6 for HeLa) were incubated with either IFN-α or IFN-β or IFN-γ (100 ng/ml) overnight at 37°C. Thereafter, cells were washed and examined for p60 and p80 form of the receptors by preincubation with either pre-immune, or anti-p60 or anti-p80 antibodies for 1 h at 37°C, and then specific TNF binding determined, as described in section 2. Numbers in parentheses indicate % induction by a given IFN when cells treated with pre-immune serum are expressed as 100%.

also (data not shown), These results suggest a difference in the regulation of the two receptors between the transcriptional and translational level.

Since the p60 form of the TNF receptor has been shown to be involved in the antiproliferative effects of the cytokines, it is possible that the synergistic increase in the antiproliferative effect by IFN-γ is due to the induction of the p60 form of the receptor. However, some of the earlier studies suggest that the induction of receptors alone is not sufficient for a synergistic response [20,24]. Since the p80 form of the TNF receptor has been implicated in the proliferation of T cells and thymocytes, whther these effects of TNF are also enhanced by IFN-γ is not known. It has been shown that for proliferation of normal human fibroblasts, however, the signal is mediated through the p60 form of the receptor [25]. Fibroblast proliferation induced by TNF has been shown to be inhibited by IFN-γ [26]. These results also suggest that the induction of receptor alone is not sufficient for the proliferative response. Overall, our results shown here demonstrate that all three IFNs can induce the cell surface expression of both types of TNF receptor.

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