Interferon- α down-regulates the interleukin-6 receptor in a human multiple myeloma cell line, U266

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The effects of interferon- α (IFN- α) on the interleukin-6 (IL-6) receptor in a multiple myeloma cell line, U266, have been examined. IFN- α inhibits [3 H]thymidine incorporation in U266 cells in a time- and dose-dependent manner. Furthermore, IFN- α inhibits the ability of IL-6 to induce increases in [3 H]thymidine incorporation. While IFN- α suppresses the ability of 125 I-IL-6 to bind to the IL-6 receptor on U266 cells, this effect is not due to competition of IFN- α with IL-6 for the IL-6 receptor. Although IFN- α induces IL-6 synthesis in the U266 cell, inhibition of IL-6 binding occurs when IL-6 synthesis is minimal. Furthermore, after pretreatment of U266 cells with neutralizing anti-IL-6 antibodies, IFN- α still inhibits 125 I-IL-6 binding. These data

suggest that IFN- α inhibition of 125 I-IL-6 binding does not involve IL-6 synthesis. IFN- α reduces 125 I-IL-6 binding without affecting its affinity, suggesting that IFN- α inhibits IL-6 receptor expression. Although pretreatment with cycloheximide inhibits 125 I-IL-6 binding, IFN- α does not cause a selective decrease in the levels of gp130 or IL-6 receptor mRNA at times when 125 I-IL-6 binding is inhibited. These observations indicate that IFN- α lowers IL-6 receptor density on U266 cells by mechanisms other than competitive binding or lowering IL-6 receptor mRNA production. Receptor down-regulation may be a mechanism of IFN- α -induced inhibition of growth in U266 cells.

INTRODUCTION

Interleukin 6 (IL-6) is a glycoprotein consisting of 212 amino acids, with a molecular mass of 21–28 kDa. It is produced by a variety of cells such as T cells, monocytes and fibroblasts in response to a variety of factors including antigens, lipopolysaccharide (LPS), IL-1 and tumour necrosis factor. IL-6 has diverse biological properties including induction of B cell differentiation, haematopoiesis and acute-phase proteins [1–4]. These effects are transduced through the IL-6 receptor, which comprises an 80 kDa (gp80) IL-6 binding protein and a 130 kDa glycoprotein (gp130) transducer protein [5].

In addition to the important role of IL-6 in homeostasis, a role for dysregulated IL-6 production in various disease states has been documented. These include a variety of B-cell abnormalities, proliferative diseases, viral infections, myelomas and leukaemias [6–8]. Multiple myeloma is a disease in which IL-6 is an established growth factor. Myeloma cells respond to IL-6 with increased growth, although it is not clear whether myeloma cells produce IL-6 in an autocrine fashion *in vivo* [9]. One agent that has profound anti-proliferative effects and has been investigated in the clinic as a possible maintenance therapy in multiple myeloma is interferon- α (IFN- α) [10–12].

We have examined the growth-inhibitory effects of IFN- α in a cell model of multiple myeloma, the U266 cell line. In addition to studying growth, we examined 125 I-IL-6 receptor binding, IL-6 release and IL-6 receptor synthesis in these cells. Our results suggest that IFN- α may inhibit growth of myeloma cells *in vivo* by down-regulating the IL-6 receptor, thereby blocking the growth-promoting effects of IL-6 in these cells.

MATERIALS AND METHODS

Cell cultures and treatments

U266 cells were obtained from A.T.C.C. (Bethesda, MD, U.S.A.). Cells were grown at 37 °C in suspension culture in RPMI 1640

supplemented with 1% (v/v) each of penicillin, streptomycin, non-essential amino acids, sodium pyruvate, glutamine (Gibco, Grand Island, NY, U.S.A.) and 10% (v/v) fetal bovine serum (Hyclone, Logan, UT, U.S.A.) in a humidified atmosphere of 5% CO₂. Cell densities were maintained between 2.25×10^5 and 0.75×10^6 cells/ml by passaging cells every 2–3 days. U266 cells were harvested at 4 °C by centrifugation and washed in medium containing 1% (v/v) fetal calf serum (FCS). Cells were resuspended at the indicated cell densities and treated by simultaneous addition of various cytokines that were resuspended in RPMI medium containing 1% (v/v) FCS.

Cell proliferation assays and [3 H]thymidine incorporation

To determine the effects of various agents on cell growth, aliquots were removed from cultures and cells were counted by light microscopy in a Neubaur haemocytometer. Viability was assayed by Trypan Blue staining. DNA was labelled by incubating cells in 150 μ l of medium containing 1% (v/v) FCS and various agents in the presence of [3 H]thymidine at 1 μ Ci/well for 4 h. Incorporation of radioactivity into precipitable material was measured using a Skatron cell harvester. Incorporated radioactivity was quantified in a Wallac LKB model 1205 Beta plate reader.

125 I-IL-6 binding assay

U266 cells (5×10^5 /ml) were incubated in medium containing 1% (v/v) FCS at 37 °C with or without IFN- α . At appropriate times after treatment, cells were harvested by centrifugation at 4 °C and washed twice in 1% (v/v) FCS. Washed cells were resuspended in medium containing 1% (v/v) FCS, and 4×10^5 cells in 100 μ l were transferred to multiscreen microtitre plates (0.45 μ m filter; Millipore) containing appropriate amounts of 125 I-IL-6 and a 100-fold excess of unlabelled IL-6 to determine

Abbreviations used: IL-6, interleukin-6; LPS, lipopolysaccharide; IFN, interferon; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FCS, fetal calf serum.

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non-specific binding. After 2 h at 4 °C with shaking, the plates were filtered and washed twice with medium containing 1 % (v/v) FCS. Filters were collected and radioactivity was quantified on a Wallac Gamma counter. K_d and B_{max} values were determined using the non-linear regression program INPLOT (GraphPad Software, San Diego, CA, U.S.A.).

IL-6 ELISA

U266 cells (5×10^5 /ml) were incubated in medium containing 1 % (v/v) FCS at 37 °C in the presence or absence of 500 pM IFN- α . At appropriate times, cells were harvested by centrifugation and the supernatant fractions were assayed for IL-6 using an IL-6 ELISA (Amersham, Arlington Heights, IL, U.S.A.) according to the manufacturer's instructions. The detection limit of this ELISA was 3.13 pg/ml.

RNA extraction and Northern blotting

U266 cells (5×10^5 /ml) were incubated in medium containing 1 % (v/v) FCS at 37 °C. At appropriate times after treatment with or without IFN- α , total RNA was extracted from 20×10^6 cells using RNazol solution (Biotech Labs, Houston, TX, U.S.A.). Samples of 15 μ g of RNA were fractionated on a 0.8 % agarose gel containing 2 % formaldehyde. Gels were run for 3 h at 50 V and then transferred to nylon membranes (USB, Cleveland, OH, U.S.A.). Membranes were blocked by incubation in a prehybridization buffer containing $4 \times$ SSC, 40 % formamide, 20 mM Tris/HCl, pH 7.4, $5 \times$ Denhardt's salt solution, 2.5 % SDS, 5 % dextran sulphate and $1 \times$ blocking reagent (Biotech) for 2 h at 42 °C. Hybridization was accomplished by adding DNA probes that had been 32 P-labelled by random priming (Boehringer, Indianapolis, IN, U.S.A.) according to the manufacturer's instructions. After 16 h of hybridization, the membranes were washed and exposed on Kodak X-Omat film at -70 °C.

In some experiments, poly(A)⁺ RNA was extracted and RNA levels of gp130 and β -actin were analysed with probes that had been biotinylated by 3' end-labelling (Clontech, Palo Alto, CA, U.S.A.).

Probes

The DNA probe for β -actin was purchased from Clontech. The probe for gp130 was synthesized by National Biosciences and corresponded to bp 138–183. Probes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the IL-6 receptor were generated by reverse transcriptase PCR. Primers and templates for GAPDH and the IL-6 receptor were purchased from Clontech. The PCR products corresponding to GAPDH (983 bp) and IL-6 receptor (351 bp) were labelled with 32 P by random priming.

Materials

[3 H]Thymidine (specific radioactivity 70–90 Ci/mmol) and 125 I-IL-6 (specific radioactivity 1400–3200 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). IFN- α and IntronA were from Biosource (Camarillo, CA, U.S.A.) and Schering-Plough Research Institute (Kenilworth, NJ, U.S.A.) respectively. IL-6 and a neutralizing murine anti-(human IL-6) monoclonal antibody (K_d 0.4×10^{-9} M) were purchased from Biosource. Control murine IgG1 antibody was purchased from Chemicon (Temecula, CA, U.S.A.).

Data presentation

Assays were carried out in triplicate. Each set of experiments was performed at least three times. The data presented are from representative experiments. The averages of triplicates in an experiment varied by less than 10 % from the mean.

RESULTS

IFN- α inhibits [3 H]thymidine incorporation in U266 cells

Previous studies have shown that the U266 cell line cultured in FCS without any additional supplements incorporates [3 H]thymidine into DNA and that this [3 H]thymidine incorporation is further increased by exogenous IL-6 [13,14]. Our initial experiments confirmed these findings as exemplified by the data in Table 1. A neutralizing murine monoclonal antibody to human IL-6 inhibited thymidine incorporation by U266 cells. Additional increases in thymidine incorporation stimulated by exogenous IL-6 were also inhibited by the anti-IL-6 monoclonal antibody (results not shown). These data support the previous conclusion that U266 cell growth is regulated by IL-6 via an autocrine mechanism [13].

Table 1 Effects of IL-6 and anti-IL-6 antibody on [3 H]thymidine incorporation in U266 cells

U266 cells (66 000 cells/ml) were incubated in culture medium containing 1 % FCS for various times with or without 500 pM IL-6, 10 μ g/ml of a neutralizing murine anti-(human IL-6) monoclonal antibody or a mouse IgG1 isotype control. [3 H]Thymidine incorporation was quantified as described in the Materials and methods section.

Addition	$10^{-3} \times$ [3 H]Thymidine incorporation (c.p.m.)		
	24 h	48 h	72 h
None	9.4 ± 0.1	12.4 ± 4.0	17.1 ± 0.1
IL-6	15.3 ± 0.5	19.7 ± 2.2	29.5 ± 0.7
Anti-IL-6 antibody	7.5 ± 0.4	8.0 ± 0.3	9.6 ± 0.4
Antibody isotype control	8.9 ± 0.4	14.7 ± 0.9	16.9 ± 0.7

Figure 1 Effect of IFN- α on [3 H]thymidine incorporation in the U266 cell line

Cells (33 000 cells/ml) were incubated in culture medium containing 1 % FCS in the absence (○) or presence of 0.05 pM (●), 5 pM (□) or 500 pM (■) IFN- α . At the indicated times, cells were pulsed with [3 H]thymidine (1 μ Ci/well) for 4 h, and incorporated radioactivity was measured as described in the Materials and methods section.

Figure 2 Effect of IL-6 on IFN- α inhibition of [3 H]thymidine incorporation in the U266 cell line

Cells (66000/ml) were incubated in culture medium containing 1% FCS and various doses of IL-6 in the absence (○) or presence of 5 pM (●), 50 pM (□) or 500 pM (■) IFN- α . At 48 h, cells were pulsed with [3 H]thymidine (1 μ Ci/well) for 4 h, and incorporated radioactivity was measured as described in the Materials and methods section.

Figure 3 IFN- α induces IL-6 synthesis in U266 cells

Cells (500000 cells/ml) were incubated in culture medium containing 1% FCS in the absence (○) or presence (●) of 500 pM IFN- α . At indicated times, aliquots were removed and supernatant fractions were analysed for human IL-6 by ELISA.

IFN- α exerts anti-proliferative effects on various human tumour cell lines [10]. In the present experiments, addition of recombinant human IFN- α to U266 cells produced a time- and dose-dependent inhibition of [3 H]thymidine incorporation (Figure 1). Although exogenous IL-6 resulted in increased [3 H]thymidine incorporation, the inhibition due to IFN- α in U266 cells was evident even in the presence of IL-6 (Figure 2). This inability to overcome the inhibitory effects of IFN- α suggests that IFN- α did not inhibit IL-6 synthesis. Furthermore, direct measurement of endogenous IL-6 by ELISA showed that IFN- α did not inhibit IL-6 synthesis in U266 cells; it actually enhanced it (Figure 3). These data suggest that IFN- α inhibition of U266 growth might be the result of interference with either the IL-6 receptor or some components in the signal transduction pathway.

IFN- α inhibits 125 I-IL-6 binding to U266 cells

The IL-6 receptor in U266 cells was characterized by an 125 I-IL-6 receptor binding filtration assay. U266 cells exhibited specific binding for 125 I-IL-6 with a K_d of 0.153 nM and had approxi-

Figure 4 Equilibrium saturation binding of 125 I-IL-6 to U266 cells

Binding was determined by adding 400000 cells in culture media containing 1% FCS to microtitre wells containing various amounts of 125 I-IL-6 in the presence or absence of a 100-fold excess of unlabelled IL-6. After a 2 h incubation at 4 °C, cells were washed twice with ice-cold medium containing 1% FCS. Specific binding (○) was calculated as the difference between total (●) and non-specific (□) binding.

mately 4700 binding sites per cell (Figure 4). When IFN- α was added to U266 cells there was a decrease in the amount of specifically bound 125 I-IL-6. At 24 h after treatment with 500 pM IFN- α , the receptor number had decreased by 50% (Figure 5a), and this was further decreased to less than 200 receptors (90% decrease) by 48 h (Figure 5b). This effect was seen at lower doses of IFN- α also. At 5 pM and 50 pM IFN- α , the receptor number decreased in 48 h from control levels of 4400/cell to 2000/cell and to 1100/cell respectively (Figure 6). However, despite the 55% and 74% decreases in specifically bound 125 I-IL-6 at 5 pM and 50 pM respectively, the K_d values were practically the same. The K_d for control cells was 0.195 nM, while the values at 5 pM and 50 pM were 0.170 nM and 0.202 nM respectively. These data indicate that IFN- α reduces the number of 125 I-IL-6 binding sites and not the binding affinity, and that this effect of IFN- α is dose-dependent.

IFN- α does not compete with IL-6 for IL-6 binding sites

One mechanism whereby IFN- α might inhibit 125 I-IL-6 binding is through direct competition with IL-6 for the IL-6 receptor. Under the standard conditions of the binding assay, IL-6 inhibited specific binding of 125 I-IL-6 in a dose-dependent manner (Figure 7). When IFN- α was present under identical conditions (2 h, 4 °C), no inhibition of 125 I-IL-6 binding was observed. Leukaemia inhibitory factor, whose receptor shares with IL-6 the gp130 as a signalling component, also did not affect 125 I-IL-6 binding in U266 cells. These data suggest that IFN- α -induced inhibition of 125 I-IL-6 binding is not through direct competition with IL-6 for the IL-6 receptor [15].

IFN- α inhibition of 125 I-IL-6 binding does not involve IL-6 synthesis

In order to address the possibility that endogenously synthesized IL-6 might have resulted in an apparent inhibition of 125 I-IL-6 binding, the effects of pretreatment with a neutralizing anti-(human IL-6) monoclonal antibody on 125 I-IL-6 binding were examined. When U266 cells were treated for 3 h at 37 °C with IFN- α , washed and specific binding determined, there was a

Figure 5 Effect of IFN- α treatment on the specific binding of ¹²⁵I-IL-6 to U266 cells

U266 cells were incubated in culture medium containing 1% FCS in the absence (○) or the presence of various concentrations of IFN- α (●, 5 pM; □, 50 pM; ■, 500 pM) for 24 h (a) or 48 h (b). At appropriate times, cells were counted, washed twice and 4×10^5 cells were suspended in medium containing 1% FCS and taken to microtitre wells containing various amounts of ¹²⁵I-IL-6 in the presence or the absence of a 100-fold excess of unlabelled IL-6. After 2 h at 4 °C, cells were washed twice and specific binding was quantified as described in the Materials and methods section.

Figure 6. Scatchard analysis of the effect of IFN- α on IL-6 binding to U266 cells

Data are from Figure 5(b). Concentrations of IFN- α : ○, 0 pM; ■, 5 pM; □, 50 pM; ●, 500 pM.

Figure 7 IFN- α does not compete with the IL-6 receptor

U266 cells were incubated in culture medium containing 1% FCS and 0.15 nM ¹²⁵I-IL-6 with various concentrations of IL-6 (△), leukaemia inhibitory factor (□) or IFN- α (●) for 2 h at 4 °C. Specific binding was determined as described in the Materials and methods section.

Table 2 Effect of anti-IL-6 antibody on ¹²⁵I-IL-6 binding in U266 cells

U266 cells (5×10^5 /ml) were incubated in culture medium containing 1% FCS with or without anti-(human IL-6) antibody in the presence or absence of 500 pM IFN- α . After 3 h of treatment at 37 °C, the cells were washed twice and resuspended in 1% FCS containing 0.37 nM ¹²⁵I-IL-6 in the presence or absence of excess IL-6. Binding was performed for 2 h at 4 °C as described in the Materials and methods section. Non-specific binding was not different between treatment groups.

Addition	IL-6 bound (specific) (fmol)	Inhibition (%)
None	1.5 ± 0.2	—
Anti-IL-6 antibody	3.4 ± 0.2	—
IFN- α	1.0 ± 0.1	34
IFN- α + anti-IL-6 antibody	1.9 ± 0.1	44

34% decrease in specifically bound ¹²⁵I-IL-6 (Table 2). Under the same pretreatment conditions (3 h, 37 °C), the neutralizing anti-IL-6 monoclonal antibody increased (2.2-fold) the amount of ¹²⁵I-IL-6 specifically bound. Furthermore, when IFN- α was included in the pretreatment with the anti-IL-6 antibody, there was still a 44% decrease in ¹²⁵I-IL-6 binding. Therefore IFN- α exhibited an inhibitory effect after treatment with the antibody which, because it has a similar affinity for IL-6 as the receptor (K_d 0.4×10^{-9} M), would have neutralized any endogenous IL-6 released during this time, thus making endogenous IL-6 unavailable to compete with ¹²⁵I-IL-6. This observation, coupled with the fact that there was barely detectable synthesis of IL-6 at 3 h after IFN- α treatment (Figure 3), suggests that IFN- α inhibition of ¹²⁵I-IL-6 binding does not involve endogenous IL-6 at early time points. Furthermore, overnight incubation of U266 cells with the amount of IL-6 (30 pg/ml) generated by overnight treatment of U266 cells with IFN- α had no effect on ¹²⁵I-IL-6 binding (results not shown).

IFN- α does not decrease IL-6 receptor mRNA levels in U266 cells

To determine whether the effects of IFN- α were dependent on protein synthesis, we investigated the effects of cycloheximide pretreatment on IL-6 receptor binding. A 3 h incubation with

Figure 8 Effect of cycloheximide and IFN- α pretreatment on ^{125}I -IL-6 binding in U266 cells

U266 cells (500 000 cells/ml) were incubated in culture medium containing 1% FCS with no other additions ($\circ$), 10 $\mu\text{g}/\text{ml}$ (36 μM) cycloheximide ($\bullet$), 500 pM IFN- α ($\triangle$) or a combination of IFN- α and cycloheximide ($\blacktriangle$). After 3 h at 37 $^{\circ}\text{C}$, the cells were washed twice and specific binding of ^{125}I -IL-6 was determined as described in the Materials and methods section.

Figure 9 Effect of IFN- α on IL-6 receptor (gp80) and gp130 mRNAs in U266 cells

U266 cells (500 000 cells/ml) were incubated without (—) or with (+) 500 pM IFN- α (a). At the indicated times, total cellular RNA was extracted and analysed by Northern blot analysis as described in the Materials and methods section. (b) Poly (A) $^{+}$ RNA was extracted from U266 cells that had been treated or not with various doses of IFN- α for 24 h and was analysed by Northern blotting as described in the Materials and methods section.

500 pM IFN- α resulted in a 35% inhibition of specifically bound ^{125}I -IL-6 (Figure 8). Incubation with 10 $\mu\text{g}/\text{ml}$ (36 μM) cycloheximide for 3 h resulted in a 65% inhibition of IL-6 binding. Furthermore, IFN- α was not additive with cycloheximide at inhibiting IL-6 binding. These data suggest that the IL-6 receptor undergoes rapid turnover and that protein synthesis is required

for the maintenance of the IL-6 receptor in U266 cells. Therefore the effects of IFN- α on the level of mRNAs for gp80 and gp130 were determined. Both IL-6 receptor (gp80) and gp130 transcripts were detected in untreated U266 cells (Figure 9). When cells were treated with IFN- α for 24 h, there was no inhibition of IL-6 receptor mRNA accumulation at times when ^{125}I -IL-6 binding was inhibited. However at 24 h there was a slight increase (2.8-fold) in gp130 (Figure 9b). These data suggest that IFN- α does not act by decreasing the availability of IL-6 receptor mRNA or gp130 mRNA.

DISCUSSION

IFN- α has profound anti-viral and anti-proliferative effects [10]. The anti-tumour effects have led to the clinical use of IFN- α in a variety of diseases, and IFN- α is currently under investigation as a therapy for multiple myelomas. However, only a limited population of myeloma patients respond to IFN- α and the mechanisms of this therapeutic action of IFN- α are unknown [10–12]. In the present study we have employed a cell line, U266, derived from a myeloma patient [16], to investigate the mechanisms by which IFN- α exerts its anti-tumour effects. Although IL-6 causes differentiation of normal B cells, IL-6 acts as an autocrine growth promoter in U266 cells (Table 1; see also [13]). Our present results clearly demonstrate that treatment of U266 cells with IFN- α inhibits DNA synthesis (Figure 1; see also [17]). Multiple mechanisms have been proposed for the anti-proliferative effects of IFN- α . These include the modulation of mRNA by activation of 2',5'-oligoadenylate synthetase [18], transcriptional regulation of eIF-2A, which affects protein translation [19], and suppression of phosphorylation [20]. In our studies inhibition of DNA synthesis by IFN- α appears to correlate with inhibition of specific binding of ^{125}I -IL-6 to its receptor. Since this inhibitory effect of IFN- α on ^{125}I -IL-6 binding is due to neither direct competition of IFN- α with IL-6 for the receptor (Figure 7; see also [15]) nor increased synthesis of IL-6, and also since IL-6 is an autocrine growth factor for U266 cells, the inhibitory action of IFN- α on IL-6-induced growth promotion in U266 cells must be mediated through a reduction in the cell surface expression of the IL-6 receptor.

Based on the fact that IFN- α reduces IL-6 binding without affecting its K_d value, inhibition of ^{125}I -IL-6 binding could reflect expression of receptor on the cell surface. Such decreases in receptor number could occur by increased receptor internalization [21], increased receptor shedding [22] or inhibition of receptor synthesis. In AF-10 cells, retinoic acid inhibits growth and this growth inhibition is due to down-regulation of the IL-6 receptor brought about by reduced accumulation of IL-6 receptor mRNA [23]. However, in the U266 cells there was no effect of IFN- α on mRNAs for GAPDH or the IL-6 receptor at times when receptor binding was inhibited. It is possible that IFN- α may affect the expression of eIF-2A in U266 cells and thereby modulate translation of the IL-6 receptor. Interestingly, in U266 cells IFN- α seems to cause an increase in gp130 mRNA. These data are consistent with the observation that IL-6 can actually up-regulate gp130 in epithelial cells [24]. Therefore, in contrast to the effects of retinoic acid in the AF-10 cells, the effects of IFN- α in the U266 cell may be at the translational or post-translational level. Consistent with the hypothesis of inhibition of receptor synthesis is the observation that cycloheximide inhibits ^{125}I -IL-6 binding and that this cycloheximide inhibition is not further affected by the presence of IFN- α . The cycloheximide data further suggest that the IL-6 receptor in U266 cells undergoes very rapid turnover. Similar rapid turnover of the IL-6 receptor has been noted in various other systems

including HepG2 cells and M1 cells [25,26]. However, it is not known if IFN- α can enhance receptor internalization or shedding in U266 cells.

A variety of agents, including dexamethasone, histamine, IFN- γ , IL-6, leukaemia inhibitory factor, LPS, phorbol esters, retinoic acid and tumour necrosis factor, affect IL-6 receptor or IL-6 receptor mRNA levels [23,24,27–36] and these effects appear to be cell-specific. For example, histamine treatment down-regulates the IL-6 receptor in HepG2 and U937 cells and up-regulates it in CESS cells [30]. Phorbol esters can also up-regulate the IL-6 receptor in HepG2, HL-60 and K562 cells and down-regulate it in U266 cells [32,35,36]. Dexamethasone can also modulate both the IL-6 receptor and gp130 [33–36]. Furthermore, in a renal cell carcinoma cell line that is autocrine for IL-6, IFN- α inhibits neither cell growth nor IL-6 receptor mRNA accumulation [37]. Our observation that IFN- α inhibits U266 cell IL-6 receptor expression further suggests that IFN- α effects are cell-specific. It is not known whether these cell-specific effects are due to the differential regulation by IFN- α of the JAK-STAT pathway [38].

Enhanced production of IL-6 has been implicated in the genesis of multiple myelomas. Our present data indicate dysregulated IL-6 receptor synthesis and expression as an additional critical component in the initiation, progression and maintenance of multiple myeloma. The ability of IFN- α to be an effective therapy in human myeloma may depend on its ability to down-regulate the IL-6 receptor. The identification of stages in myeloma that are susceptible to IL-6 receptor down-regulation by IFN- α may be a valuable prognostic clinical indicator. Our data suggest that the blockade of IL-6 receptor synthesis may be a mechanism for the growth inhibitory action of IFN- α on U266 cells, a cell model for multiple myeloma.

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