Differential Regulation of Interleukin-6 Receptor and gp130 Gene Expression in Rat Hepatocytes

James E. Nesbitt and Gerald M. Fuller

Department of Cell Biology, University of Alabama at Birmingham,
Birmingham, Alabama 35294-0005

Submitted to Cell Regulation July 3, 1991; Accepted October 8, 1991

Interleukin-6 (IL-6) relays an important signal to hepatocytes during the early stages of an acute inflammatory response, causing an alteration in the expression of several major defense proteins. Additional regulation of this signal could occur either by altering the number of IL-6 receptors (IL-6-R) or of the signal transducing protein, gp130. We employed ribonuclease protection assays to measure the expression of IL-6-R and gp130 mRNA in primary rat hepatocytes in response to IL-6, interleukin-1, dexamethasone, and combinations thereof. Dexamethasone increases receptor mRNA levels 2.7-fold above controls but has no detectable effect on that of gp130. Such treatment increased surface expression of IL-6-R from 600 receptors per cell to >6000, without a change in K_d ($2.5\text{--}4.6 \times 10^{-10}$ M). In contrast to the stimulatory effect of the steroid signal, the inflammatory cytokines, individually and together, down-modulated both the mRNA and the cell surface expression of IL-6-R. These findings demonstrate for the first time that a sensitive control system exists between inflammatory mediators and IL-6-R.

INTRODUCTION

Interleukin-6 (IL-6) is one of the principle cytokines involved in an inflammatory response arising from tissue injury or bacterial infection. IL-6 was originally described as a B-cell differentiation factor because of its ability to induce terminal differentiation of B cells into Ig-secreting plasma cells (Hirano *et al.*, 1986). Subsequent studies revealed that this cytokine also had several other important biological activities associated with inflammatory and immune-related responses (for review, see Kishimoto, 1989; Heinrich *et al.*, 1990; Van Snick, 1990). In addition to its effects on the immune system (Wong and Clark, 1988), IL-6 is a potent inducer of a specific subset of liver-derived plasma proteins known as acute phase respondents (Gauldie *et al.*, 1987).

A cell surface receptor for IL-6 (IL-6-R) has been identified and partially characterized in a number of cells of hematopoietic, fibroblastic, epithelial, and neural origin (Snyers *et al.*, 1989). The number of IL-6-Rs in these cells is very low (typically under 2000 receptors per cell), with binding affinities for IL-6 in the range of 10^{-10} – 10^{-11} M (Taga *et al.*, 1987). The IL-6-R has been cloned from both human lymphocytes (Yamasaki *et al.*, 1988) and rat hepatocytes (Baumann *et al.*, 1990). It is now recognized that the IL-6 signaling pathway is

unique from other second-messenger systems (Nakajima and Wall, 1991). Binding of this ligand to its receptor initiates the association of the receptor to another membrane glycoprotein, gp130 (Taga *et al.*, 1989). Cloning of human gp130 and subsequent analysis have suggested that signal transduction is mediated by gp130-receptor association by a currently unknown mechanism. Increased numbers of high-affinity binding sites were observed when IL-6-R positive cells were transfected with the cDNA for gp130, suggesting that when the IL-6-R interacted with gp130, the association led to a more stable complex (Hibi *et al.*, 1990).

The capability of hepatocytes to respond to IL-6 at the onset of an acute inflammatory reaction can involve regulation of expression of either of these two molecules: the ligand binding protein (IL-6-R) or the signal transducing protein (gp130). The importance of glucocorticoid in the up-regulation of IL-6-R mRNA and the subsequent expression of the receptor has been well documented (Rose-John *et al.*, 1990; Snyers *et al.*, 1990). To gain more information on the mechanisms by which the hepatocyte responds to IL-6, we have examined the expression of IL-6-R and gp130 genes during *in vivo* and *in vitro* stimulation of inflammation. Results from these studies provide information that the regulation of IL-6-R is substantially different from that of gp130 and

that up-regulation of the receptor likely plays a critical role in increasing the sensitivity of hepatocytes to the incoming IL-6 signals by increasing the number of available receptors. The striking diminution of the surface expression of the receptor by the principle inflammatory cytokines suggests that a sensitive up- and down-regulation of the receptor is in operation at the hepatocyte cell surface during the inflammatory response.

METHODS AND MATERIALS

Materials

William's media minus arginine was from Hazelton Research Products, Inc. (Denver, PA). Nu-Serum was from Collaborative Research (Lexington, MA). Fetal Bovine Serum was from HyClone (Logan, UT). Tissue culture antibiotics as well as actinomycin D and cycloheximide were from Sigma Chemical Co. (St. Louis, MO). Radionucleotides were from Amersham Corporation (Arlington Heights, IL). Nitrocellulose paper was from Microseparations, Inc. (Westboro, MA). Guanidine thiocyanate was from U.S. Biochemical Corp. (Cleveland, OH). Molecular biology products and enzymes were purchased from Promega (Madison, WI). All other chemicals were supplied by Fisher Biotech, Fisher Scientific (Pittsburgh, PA), or Boehringer Mannheim Biochemicals (Indianapolis, IN).

Cell Culture

Primary rat hepatocytes were isolated as previously described (Nesbitt and Fuller, 1991). Rat hepatoma cells (FAZA) were cultured as described (Malawista and Weiss, 1974). All experiments were performed using monolayers of cells at 90–95% confluency. In experiments where either transcription or translation were blocked, the following concentrations of inhibitors were used: actinomycin D, 2.5 $\mu\text{g}/\text{ml}$, which inhibits >94% incorporation of [^3H]orotic acid into total cellular RNA; cycloheximide, 2.5 $\mu\text{g}/\text{ml}$, which inhibits >90% incorporation of [^{35}S]methionine into protein, as measured by trichloroacetic acid precipitable counts.

Experimental Inflammation

Experimental inflammation was induced in 200–250 g female Sprague-Dawley rats by one of three methods: subcutaneous injection of 0.5 ml turpentine; subcutaneous injection of 0.4 ml bacterial lipopolysaccharide (1 mg/ml); or intraperitoneal injection of 0.4 ml Freund's adjuvant (complete). At the indicated times, livers were quickly removed from the animals, immediately immersed in liquid nitrogen, and stored at -70°C until RNA isolation.

RNA Isolation

Total cellular RNA was isolated from cultured rat hepatocytes by an acidic-phenol extraction protocol (Scherrer, 1969; Scherrer and Darnell, 1962) exactly as described previously (Nesbitt and Fuller, 1991). After ethanol precipitation, the dried RNA pellets were resuspended in 1 mM EDTA, heated to 57°C for 10 min, and quantitated spectrophotometrically by absorbance at 260 nm. RNA integrity as well as the cells response to the added cytokines were checked by Northern blot hybridizations that were probed with fibrinogen B_β -chain cDNA.

Total rat liver RNA was isolated by the single-step guanidine thiocyanate method (Chomczynski and Sacchi, 1987). Briefly, ~ 10 g of rat liver was placed in 22 ml of ice-cold solution D (4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol, and 0.1% antifoam A emulsion [Sigma Chemical Co.]). The liver was quickly homogenized using a tissuemizer, and the resulting homogenate divided equally into two 50-ml tubes (11

ml each). To each tube was added sequentially 1.1 ml of 2 M sodium acetate, pH 4.0, 11.0 ml water-saturated phenol, and 2.2 ml chloroform-isoamyl alcohol (49:1). This mixture was vortexed vigorously for 10 s, incubated on ice for 15 min, and centrifuged at $11\,500 \times g$ for 30 min at 4°C . The aqueous phase was carefully removed and precipitated with an equal volume of isopropanol (previously chilled to -20°C) for 1 h at -20°C . The RNA was then pelleted by centrifugation as above. The resulting RNA pellets were then resuspended in a total volume of 20 ml solution D, precipitated and pelleted as above, and resuspended in 3 ml RNase-free H_2O . The RNA was then heated to 57°C for 10 min, quick chilled, and quantitated by absorbance at 260 nm. Poly(A) $^+$ mRNA was isolated by oligo(dT)-cellulose chromatography using standard methods (Aviv and Leder, 1972).

Isolation of a Partial Rat Liver gp130 cDNA

Two oligonucleotides were synthesized on the basis of the published sequence of human gp130 (Hibi *et al.*, 1990). The 5' oligonucleotide corresponded to nucleotide 1767 through 1791 of the coding strand. The 3' oligonucleotide corresponded to nucleotide 2480 through 2456 of the noncoding strand. RNA-polymerase chain reaction (PCR) was performed using poly(A) $^+$ RNA from rat liver (Wang *et al.*, 1989).

First-strand synthesis was performed in a 20- μl reaction volume containing 50 mM KCl, 10 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 8.3, 1.5 mM MgCl_2 , 20 units RNasin ribonuclease inhibitor (Promega), 1 μM each dATP, dCTP, dGTP, dTTP, 1 μM 3' oligonucleotide primer, and 1 μg rat liver poly(A) $^+$ RNA. Before reverse transcriptase addition, the sample was heated to 95°C for 2 min and allowed to cool to room temperature. Two hundred units of avian myeloblastosis virus reverse transcriptase (BRL Life Technologies, Inc., Gaithersburg, MD) was then added and the mixture incubated for 55 min at 42°C .

PCR was then performed on this reverse transcriptase reaction using GeneAmp DNA amplification reagent kit as described by the manufacturer (Perkin Elmer-Cetus, Norwalk, CT). The concentration of each primer was 1 μM . Thirty-five PCR cycles were performed in a temperature cycler (Ericomp, San Diego, CA) using 1 min at 94°C (denaturation), 3 min at 37°C (annealing), and 5 min at 72°C (extension). One major amplification product of ~ 680 bp resulted. Digestion of this amplification product with *Pst* I/*Eco*RI resulted in a 579-bp fragment, which was subsequently subcloned into the *Pst* I/*Eco*RI polylinker sites of m13mp18 and m13mp19 phage vectors for sequencing. Single-strand DNA sequencing with the dideoxy-nucleotide technique and Sequenase enzyme (U.S. Biochemical Corp.) was performed by following standard procedures (Sanger *et al.*, 1977). Sequencing data was analyzed using the Genetics Computer Group (University of Wisconsin) sequence analysis software package. Comparison of this rat liver gp130 cDNA with the published sequence of human gp130 revealed 87% identity between nucleotide #1813 and #2391.

In vitro Transcription

A 326-bp *Bam*HI fragment of the rat liver IL-6-R clone pRIL-6-RC.21 (Baumann *et al.*, 1990) was subcloned into the *Bam*HI polylinker site of the pBS vector (Stratagene, La Jolla, CA). This construct was linearized with *Pst* I and used to generate a radiolabeled antisense cRNA probe to the IL-6-R mRNA with T7 RNA polymerase (Promega). A 700-bp *Pst* I/*Bam*HI fragment of a rat cyclophilin clone (Danielson *et al.*, 1988) was subcloned into the *Pst* I/*Bam*HI polylinker site of pBS vector. This construct was linearized with *Nco* I, which digests within the cyclophilin cDNA insert. In vitro transcription of this linearized vector with T7 RNA polymerase results in a 221-base radiolabeled antisense cRNA probe to the rat cyclophilin mRNA. A 579-bp *Pst* I/*Eco*RI cDNA clone to rat gp130 was subcloned into pBS, which was subsequently linearized with *Eco*RI and in vitro transcribed with T3 RNA polymerase to produce an antisense cRNA probe.

In vitro transcriptions were performed in a final volume of 20 μl containing 40 mM Tris-HCl, pH 7.5, 6 mM MgCl_2 , 2 mM spermidine,

10 mM NaCl, 10 mM dithiothreitol, 20 units RNasin ribonuclease inhibitor (Promega), 500 μ M ATP, GTP, and UTP, 50 μ Ci [α - 32 P]CTP (Amersham, 20 mCi/ml, 800 Ci/mmol), 1 μ g linearized DNA template, and 20 units T7 or T3 RNA polymerase (Promega). Transcription reactions were performed for 60 min at 37°C, followed by the addition of 10 units DNase I (Ambion, Austin, TX), and further incubated for 20 min at 37°C. Transcription of the IL-6-R antisense cRNA was carried out in the absence of unlabeled CTP to obtain a probe with high specific activity (2.8×10^9 cpm/ μ g). Transcription of cyclophilin and gp130 antisense cRNA were performed as above, but 50 μ M unlabeled CTP was included to generate riboprobes that had lower specific activities (cyclophilin, 8.4×10^8 cpm/ μ g; gp130, 1.4×10^9 cpm/ μ g). Specific activity for each probe was determined from calculation of percent incorporation by trichloroacetic acid (TCA) precipitation of an aliquot of the transcription reaction.

The resulting radiolabeled riboprobes were purified by electrophoresis in 4% polyacrylamide/8 M urea gels, excised from the gel following a brief X-ray film exposure for localization, and eluted overnight at 37°C in 350 μ l elution buffer containing 0.5 M NH_4OAc , 1 mM EDTA, and 0.1% sodium dodecyl sulfate (SDS).

Ribonuclease Protection Assays

For each experimental sample, 1.25×10^5 cpm of each riboprobe was ethanol precipitated with 15 μ g rat liver RNA for 15 min at -70°C. After centrifugation, the resulting RNA pellets were dried and resuspended in 20 μ l hybridization buffer (80% formamide, 40 mM piperazine- N,N' -bis(2-ethanesulfonic acid), pH 6.4, 400 mM NaOAc, and 1 mM EDTA) and heated to 90°C for 4 min. Hybridizations were carried out at 42°C for 16 h.

RNase digestion was then performed by adding 200 μ l of a 1:100 dilution of ribonuclease cocktail (Ambion, RNase A, 1 mg/ml; RNase T1, 20 000 units/ml) in 10 mM Tris-HCl, pH 7.5, 300 mM NaCl, and 5 mM EDTA for 60 min at 37°C. RNase digestion was terminated by the addition of 20 μ l proteinase K/SDS solution containing 2.5 mg/ml proteinase K, 10% SDS, and 0.5 mg/ml yeast tRNA and incubated for 20 min at 37°C. Samples were then phenol-chloroform-isoamyl extracted, ethanol precipitated, vacuum dried, and resuspended in 10 μ l of RNA loading dye containing 80% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, and 2 mM EDTA.

RNase-resistant RNA products were analyzed by electrophoresis in a 4% polyacrylamide/8 M urea gel followed by autoradiography. Quantitation of protected RNA fragments was performed by scintillation counting of excised bands. Counts for IL-6-R and gp130 were normalized to cyclophilin mRNA levels for each experimental time point and condition. Data that is represented graphically is done so as the ratio of IL-6-R cpm to cyclophilin cpm or gp130 cpm to cyclophilin cpm.

Iodination of Recombinant Murine IL-6

Recombinant murine IL-6 was radiolabeled using Iodo-gen (Pierce Chemical Co., Rockford, IL) as follows. Eighty microliters of Iodo-gen (2 mg/ml in chloroform) was placed in a 12 $\times$ 75-mm glass tube and slowly dried under a stream of nitrogen gas. To this was added 5 μ g recombinant murine IL-6 and 750 μ Ci ^{125}I (Amersham) in a final volume of 100 μ l in 0.25 M phosphate buffer, pH 7.4. The reaction was placed on ice and incubated for 25 min with occasional mixing. Iodinated IL-6 was separated from unincorporated ^{125}I by passing the reaction mixture over a 5-ml G-25 column equilibrated with 0.05 M phosphate buffer containing 1% gelatin and 0.02% Tween-20. Fractions containing the radioactive peak were pooled. Purity and quantitation of the ^{125}I -IL-6 were performed by SDS-polyacrylamide gel electrophoresis/autoradiography and Western blot analysis using a known quantity of unlabeled IL-6 detected with a rabbit polyclonal antibody against recombinant murine IL-6. Specific activity of the ^{125}I -IL-6 was typically 80 000 cpm/ng. The biological activity of ^{125}I -IL-6 was essentially unchanged.

Equilibrium Binding Experiments

Binding assays were performed on confluent monolayers of primary rat hepatocytes plated in 24-well tissue culture dishes (500 000 cells/well). At the start of each binding assay, cells were washed two times with 500 μ l of ice-cold binding media (Dulbecco's modified Eagle's medium containing 20 mM N -2-hydroxyethylpiperazine- N' -2-ethanesulfonic acid, pH 7.4, 2% bovine serum albumin, and 0.02% sodium azide). Binding was performed in 250 μ l binding media using varying concentrations of ^{125}I -IL-6. Nonspecific binding was determined by including a 70-fold excess of unlabeled IL-6. Cells were then incubated on ice for 3 h, after which the media was removed and counted in a γ -counter, and the cells quickly washed three times with 500 μ l ice-cold binding media. Binding was then determined by counting the solubilized cells (250 μ l of 1 N NaOH containing 1% Triton-X 100). Receptor number and binding affinities were determined by Scatchard analysis (Scatchard, 1949).

In experiments determining the effect of cytokines on surface expression of IL-6-R, the following modifications were performed. After the incubation of the cells with the cytokine(s), the cells were washed once with ice-cold binding media and then incubated with 500 μ l of 0.15 M NaCl/0.1 M glycine, pH 3.0, for 3 min. Such treatment removes >95% of bound IL-6 from the cells, without affecting subsequent binding of additional ^{125}I -IL-6. After this treatment, the cells were quickly washed three times with 500 μ l of ice-cold binding media and equilibrium binding then performed as described above.

Cytokines

Recombinant murine IL-6 was prepared for us by Pfizer Research (Groton, CT) as previously described (Grenett *et al.*, 1991) from a full-length cDNA isolated in this laboratory (Grenett *et al.*, 1990). Recombinant murine interleukin-1 β (IL-1 β) was also provided by Pfizer Research. Activity of IL-6 was assayed by measurement of its hepatocyte stimulating activity as measured by fibrinogen mRNA induction and fibrinogen secretion in primary cultures of rat hepatocytes as described (Grenett *et al.*, 1991). IL-1 β activity was measured by the induction of C3 mRNA in hepatocytes exposed to the cytokine (Darlington *et al.*, 1986).

Northern Blot Analyses

Seven micrograms of total RNA (as determined by absorbance at 260 nm) from control and inflamed rat liver were fractionated by electrophoresis through 1.5% agarose-0.6 M formaldehyde gels (Rave *et al.*, 1979; Thomas, 1980), transferred to filters, and cross-linked by irradiation using a Stratilinker 1800 (Stratagene), as previously described (Nesbitt and Fuller, 1991). The membranes were hybridized overnight at 42°C with 1.5×10^6 cpm/ml of [^{32}P]dCTP-labeled fibrinogen B β -chain cDNA. Afterward, the filters were washed three times for 30 min each at room temperature in 2 $\times$ SSC/0.5% SDS, dried, and autoradiographed at -70°C.

RESULTS

IL-6-R and gp130 mRNA Levels in Inflamed Rat

Initial experiments were performed using livers taken from acutely inflamed rats to gain information about IL-6-R and gp130 gene expression in rat liver. We employed ribonuclease protection assays to compare and contrast the expression of both of these mRNAs because this technique is ~10-fold more sensitive than previously reported Northern blot hybridizations. Figure 1A is a representative ribonuclease protection assay illustrating IL-6-R and gp130 mRNA levels in control versus an acutely inflamed rat. IL-6-R mRNA levels increased

Figure 1. Changes in rat liver IL-6-R and gp130 mRNA levels during an acute inflammatory response. Acute inflammation was induced in rats for the indicated times by subcutaneous injection of 0.5 ml turpentine, as described in MATERIALS AND METHODS. (A) Total RNA from these livers was subjected to ribonuclease protection analysis, the autoradiograph of which is shown. The first four lanes show the protected fragments from RNA hybridized with IL-6-R and cyclophilin probes. The second four lanes show the results from RNA hybridized with gp130 and cyclophilin probes. Migration of protected fragments corresponding to gp130, IL-6-R, and cyclophilin mRNAs are indicated on the left. Numbers in parentheses correspond to the sizes of the protected fragments. As controls, the probes were hybridized to 15 μ g of yeast tRNA and subsequently treated (+) or not treated (-) with ribonuclease. The sizes of the nondigested probes are indicated on the right. (B) Seven micrograms of total RNA from these inflamed rat was subjected to Northern blot analysis and probed with 32 P-labeled fibrinogen B β -chain cDNA.

rapidly after the initiation of an acute inflammatory reaction, reaching a level 2.4-fold higher than control within 3 h. By 6 h, IL-6-R mRNA expression returned to essentially that of control levels. Conversely, gp130 mRNA levels increased only slightly during these early times of acute inflammation to a level $\sim$ 1.4-fold above that of control. Separate experiments using different inducers of acute inflammation were performed. The results shown in Figure 1 are from turpentine-injected animals; however, similar results were obtained irrespective of the inducer of inflammation used. Elevated levels of fibrinogen mRNA, as measured by Northern blot hybridization, show that the animal was undergoing an acute inflammatory reaction (Figure 1B). Although two ribonuclease-resistant fragments for the IL-6-R were consistently observed (Figure 1A), both could be specifically competed away with unlabeled IL-6-R antisense RNA.

Kinetics of the Effect of Dexamethasone on IL-6-R and gp130 mRNA Levels in Rat Hepatocytes

To explore differential changes in IL-6-R and gp130 gene expression in a nontransformed cell, we employed primary cultures of rat hepatocytes. Because the pituitary-adrenal axis is activated during the acute phase response (Kushner, 1982) leading to increased concentrations of corticosterone (Besedovsky *et al.*, 1975, 1986), we measured cellular mRNA levels of IL-6-R and gp130 in response to the synthetic glucocorticoid, dexamethasone. Primary rat hepatocytes were plated in dexamethasone-free media for 16 h. At time 0 h, the media was replaced with fresh media containing 1×10^{-6} M dexamethasone. IL-6-R mRNA accumulated rapidly, reaching a maximum level 2.7-fold above that of control within 3 h after addition of the glucocorticoid (Figure 2A). In contrast to the rapid and transient increase in IL-6-R mRNA observed in liver during induced inflammation *in vivo*, continued dexamethasone exposure to hepatocytes resulted in a prolonged high level of IL-6-R mRNA, which gradually declined with time. Unlike IL-6-R mRNA, gp130 mRNA levels remained constant after dexamethasone addition (Figure 2A). A graphical representation of these results is shown in Figure 2B.

Dexamethasone-Induced Increase in IL-6-R mRNA Requires Transcription

To determine if the dexamethasone-induced increase in IL-6-R mRNA was due to transcription, we exposed the cells to actinomycin D at the time of dexamethasone addition. Furthermore, we used an IL-6 responsive rat hepatoma cell line FAZA to examine IL-6-R mRNA responsiveness to dexamethasone in a transformed cell. Confluent monolayers of FAZA cells were incubated with 1×10^{-6} M dexamethasone in the presence or absence of actinomycin D (2.5 μ g/ml) for 3 and 6 h. As observed in primary cultures of rat hepatocytes, addition of dexamethasone increased IL-6-R mRNA levels in these rat hepatoma cells (Figure 3). Quantitation of this induction revealed a 4.5-fold increase above control in IL-6-R mRNA levels after a 3-h treatment with dexamethasone. The addition of actinomycin D, however, completely blocked this increase, indicating that the glucocorticoid induced increase in IL-6-R mRNA is due, in part, to increased transcriptional activity of the IL-6-R gene. Identical results were obtained using primary cultures of rat hepatocytes.

Dexamethasone Increases Cell Surface Expression of IL-6-R in Rat Hepatocytes Without Affecting Binding Affinity

Having demonstrated a significant increase in IL-6-R mRNA levels by glucocorticoid, we determined the changes in cell surface expression of IL-6-R in response to dexamethasone. Primary rat hepatocytes were plated

Figure 2. Effect of dexamethasone on IL-6-R and gp130 mRNA levels in rat hepatocytes. Confluent monolayers of rat hepatocytes were incubated with 1×10^{-6} M dexamethasone for the indicated times. (A) Total RNA from these cells was subjected to ribonuclease protection analysis and the ribonuclease-resistant products analyzed by electrophoresis as described in MATERIALS AND METHODS. Migration of protected fragments corresponding to gp130, IL-6-R, and cyclophilin mRNAs are indicated on the left. Numbers in parentheses correspond to the sizes of the protected fragments. As controls, the probes were hybridized to 15 μ g of yeast tRNA and subsequently treated (+) or not treated (–) with ribonuclease. The sizes of the nondigested probes are indicated on the right. (B) Graphical representation of the effect of dexamethasone on IL-6-R and gp130 mRNA levels in rat hepatocytes. Ribonuclease-resistant fragments were quantitated and normalized to cyclophilin levels as described in MATERIALS AND METHODS. (●) IL-6-R. (▲) gp130. Each point represents the means $\pm$ SEM from three separate experiments.

in 24-well tissue culture dishes in dexamethasone-free media for 16 h. The cells were then exposed to 1×10^{-6} M dexamethasone for 0–12 h. At the indicated times, equilibrium binding analyses were performed as described in MATERIALS AND METHODS. Data presented in Figure 4A shows that under basal conditions (absence of dexamethasone) primary rat hepatocytes express an average of ~ 600 IL-6-R per cell. After a 12-h incubation with dexamethasone, however, surface expression of IL-6-R increased to >6500 receptors per cell (Figure 4B). Calculation of K_d values from these

Scatchard plots reveals no significant change in the binding affinity for the IL-6-R in minus- or plus-dexamethasone-treated cells ($K_d = 245$ and 466 pM, respectively). A time course showing this dexamethasone-induced increase in surface expression of IL-6-R is shown in Figure 4C. A rapid increase in the number of receptors is observed between 3 and 6 h after dexamethasone addition to the cells.

Effect of Inflammatory Cytokines on IL-6-R and gp130 mRNA Levels in Rat Hepatocytes

The liver is a major target for the actions of both IL-6 and IL-1. The hepatocyte responds to these cytokines by altering the expression of a number of different genes as part of an acute inflammatory response (Koj and Gordon, 1985; Heinrich *et al.*, 1990). The possible agonistic and antagonistic effects that these cytokines exert on IL-6-R and gp130 mRNA expression in rat hepatocytes was therefore explored. After an overnight incubation of hepatocytes in dexamethasone-free media, the cells were incubated for 3 h in the presence (1×10^{-6} M) or absence of dexamethasone together with 5 ng/ml of IL-6, IL-1 β , or both. In the absence of dexamethasone, IL-6 and IL-1 β decreased IL-6-R mRNA levels by $19.7 \pm 1.0\%$ and $27.8 \pm 5.7\%$, respectively (Figure 5). A larger decrease was observed when both cytokines were included ($41.2 \pm 11.1\%$ decrease from control). As expected, in the presence of dexamethasone, IL-6-R mRNA levels are higher than that in nondexamethasone treated control cells (Figure 5, lane 5 vs. lane 1). The cytokines had an inhibitory effect on this dexamethasone-induced increase in IL-6-R mRNA. The re-

Figure 3. Effect of actinomycin D on the dexamethasone-induced increase in IL-6-R mRNA levels in a rat hepatoma cell line FAZA. Confluent monolayers of FAZA cells were incubated with 1×10^{-6} M dexamethasone in the presence or absence of 2.5 μ g/ml actinomycin D for the indicated times. Total RNA from these cells was then subjected to ribonuclease protection analysis and the ribonuclease-resistant fragments analyzed by electrophoresis, the autoradiograph of which is shown. Migration of protected fragments corresponding to IL-6-R and cyclophilin mRNA are indicated on the left. Numbers in parentheses correspond to the sizes of the protected fragments.

Figure 4. Effect of dexamethasone on surface expression of IL-6-R in primary rat hepatocytes. Primary rat hepatocytes were incubated with 1×10^{-6} M dexamethasone from 0 to 12 h. At the indicated times, equilibrium bind assays were performed to determine the number of IL-6-R expressed. Scatchard plots of equilibrium binding experiments performed on cells after a 0 h (A) and 12 h (B) exposure to dexamethasone. (C) A graphical representation of the time course of dexamethasone-induced surface expression of IL-6-R. Data presented shows the average number of IL-6-R for each time determined from two separate experiments. Error bars indicate the differences in the number of IL-6-R between each experiment.

sulting IL-6-R mRNA levels were $6.8 \pm 1.7\%$, $12.3 \pm 2.5\%$, and $29.1 \pm 9.5\%$ lower in IL-6-, IL- 1_β -, or IL-6- plus IL- 1_β -treated cells (respectively) when compared with cells treated with the glucocorticoid alone. These percentages are the means $\pm$ SEM derived from three separate experiments.

These inhibitory effects were also observed in cell surface expression of IL-6-R, as determined by equilibrium binding analysis. Primary rat hepatocytes were plated in 24-well tissue culture dishes in the absence of dexamethasone for 16 h before the start of the experiment. The cells were then incubated for 6 h in the presence of 1×10^{-6} M dexamethasone together with 5 ng/ml IL-6, IL- 1_β , or both. The number of IL-6-R was calculated from Scatchard plots of equilibrium binding experiments carried out for each condition. Consistent with the findings reported in Figure 5, IL-6 and IL- 1_β prevent the dexamethasone-induced increase in surface expression of IL-6-R in rat hepatocytes (Table 1).

To examine if these inflammatory cytokines could decrease the number of already expressed IL-6-Rs on the hepatocyte surface, we performed the following experiments. Cells were plated in the presence of 1×10^{-6} M dexamethasone for 16 h before cytokine addition. The number of IL-6 receptors expressed in cells 16 h after their initial plating in dexamethasone-containing media is typically $>10\,000$ receptors per cell (Table 2, controls in conditions A and B). After this 16-h incubation, all cells were incubated for an additional 6 h in the presence of dexamethasone (1×10^{-6} M) together with either 0.5 ng/ml (Table 2, condition A) or 5 ng/ml (Table 2, conditions B) of IL-6, IL- 1_β , or both. The number of IL-6 receptors was calculated from Scatchard plots of equilibrium binding experiments carried out for each condition. Although each individual cytokine decreased the number of IL-6 receptors expressed on the hepatocyte surface, a greater decrease in receptor number was observed when both IL-6 and IL- 1_β were added together. The effect was dose-dependent, as indicated

Figure 5. Effect of IL-6 and IL-1 β on IL-6-R and gp130 mRNA levels in rat hepatocytes. Primary cultures of rat hepatocytes were incubated for 3 h in the presence (right) or absence (left) of 1×10^{-6} M dexamethasone for 3 h. Cytokines were added at the time of dexamethasone addition as follows: lane 1 and 5, control; lane 2 and 6, IL-6 (5 ng/ml); lane 3 and 7, IL-1 β (5 ng/ml); lane 4 and 8, IL-6 + IL-1 β (5 ng/ml each). Total RNA from these cells was then subjected to ribonuclease protection analysis and the ribonuclease-resistant fragments analyzed by electrophoresis, the autoradiograph of which is shown. Migration of protected fragments corresponding to gp130, IL-6-R, and cyclophilin mRNA are indicated on the left. Numbers in parentheses correspond to the sizes of the protected fragments.

in Table 2, condition A (0.5 ng/ml) versus condition B (5 ng/ml).

Determination of IL-6-R and gp130 mRNA Half-Life in Rat Hepatocytes

One mechanism a cell employs to regulate the expression of a gene is to alter the half-life of that gene's mRNA. To explore the endogenous control of IL-6-R and gp130 mRNA in rat hepatocytes, we determined the half-life for each of these two mRNA species. Primary cultures of rat hepatocytes were incubated in dexamethasone-containing media (1×10^{-6} M). At time

Table 1. Effect of cytokines on dexamethasone-induced increase in IL-6-R levels in primary rat hepatocytes

Condition ^a	Receptors/cell	Percent decrease (from control)
Control	5720	0
IL-6	2953	48.4
IL-1	2318	59.4
IL-6 + IL1	1731	69.7

Primary rat hepatocytes were incubated for 6 h in the presence of dexamethasone ($1 \mu\text{M}$) together with 5 ng/ml of the cytokines as indicated. Equilibrium binding assays were then performed as described in MATERIALS AND METHODS.

^a Cytokines = 5.0 ng/ml; DEX = $1 \mu\text{M}$.

Table 2. Effect of cytokines on surface expression of IL-6-R levels in primary rat hepatocytes

Condition	Receptors/cell	Percent decrease (from control)
A. [cytokines] = 0.5 ng/ml		
Control	11 066	0
IL-6	8 973	18.9
IL-1	6 819	38.4
IL-6 + IL-1	6 281	43.2
B. [cytokines] = 5.0 ng/ml		
Control	11 066	0
IL-6	7 450	32.6
IL-1	6 875	37.8
IL-6 + IL-1	4 125	62.7

Primary rat hepatocytes were incubated in the presence of dexamethasone ($1 \mu\text{M}$) for 16 h before the addition of cytokines. Cells were then incubated for an additional 6 h in the presence of dexamethasone and in the presence or absence (control) of cytokines. Equilibrium binding assays were then performed as described in MATERIALS AND METHODS.

0 h, the transcription inhibitor actinomycin D ($2.5 \mu\text{g}/\text{ml}$) was added and IL-6-R and gp130 mRNA levels followed with time. Information reported in Figure 6 shows that the stabilities of these two mRNAs are quite different. The gp130 mRNA exhibited a half-life of over 12 h, whereas that for IL-6-R mRNA was 3 h. Although

Figure 6. Half-life of IL-6-R and gp130 mRNA in rat hepatocytes. Primary rat hepatocytes were incubated in the presence of $2.5 \mu\text{g}/\text{ml}$ actinomycin D for the indicated times. Total RNA from these cells was subjected to ribonuclease protection analysis and the ribonuclease-resistant products analyzed by electrophoresis and quantitated as described in MATERIALS AND METHODS. IL-6-R (●) and gp130 (▲) mRNA levels were normalized to that of cyclophilin and are represented as percent of the level observed at time 0 h for IL-6-R and gp130, respectively. Each point is the means $\pm$ SEM from three separate experiments.

dexamethasone significantly increases the cytoplasmic levels of IL-6-R mRNA, it had no effect on the half-life of this mRNA or on that of gp130.

Effect of Cycloheximide on IL-6-R and gp130 mRNA Accumulation in Rat Hepatocytes

The 3'-untranslated region (UTR) of the hepatocyte IL-6-R mRNA contains a number of sequence motifs thought to be involved in mRNA stability (Caput *et al.*, 1986; Shaw and Kamen, 1986). Messenger RNA molecules possessing these motifs have been shown to display sensitivity to cycloheximide in that their levels can be superinduced in the presence of this translation inhibitor (Mitchell *et al.*, 1985; Rahmsdorf *et al.*, 1987). Figure 7 shows the results of the effect of cycloheximide (2.5 $\mu\text{g}/\text{ml}$) on induction of IL-6-R mRNA and gp130 mRNA in rat hepatocytes. Cycloheximide added to cells incubated in dexamethasone-free media resulted in an approximately threefold increase in IL-6-R mRNA levels after 12 h. When cycloheximide was added to cells in the presence of 1×10^{-6} M dexamethasone, IL-6-R mRNA levels increased nearly sevenfold above control by 12 h, displaying a very linear increase during the first 6 h of this treatment. gp130 mRNA levels were not

Figure 7. Effect of cycloheximide on IL-6-R and gp130 mRNA levels in rat hepatocytes. Primary rat hepatocytes were incubated in the presence or absence of 1×10^{-6} M dexamethasone with 2.5 $\mu\text{g}/\text{ml}$ cycloheximide for the indicated times. Total RNA isolated from these cells was subjected to ribonuclease protection analysis, analyzed by electrophoresis, and quantitated as described in MATERIALS AND METHODS. mRNA levels for IL-6-R and gp130 were normalized to that of cyclophilin and are represented as fold increase above the 0 h control, which was set as 1.0. (□) IL-6-R mRNA levels in cells incubated with cycloheximide in the absence of dexamethasone; (●) IL-6-R mRNA levels in cells incubated with cycloheximide in the presence of dexamethasone (1×10^{-6} M); (▲) IL-6-R mRNA levels in cells incubated with cycloheximide, dexamethasone, and actinomycin D (2.5 $\mu\text{g}/\text{ml}$); (○) gp130 mRNA levels in cells incubated with cycloheximide in the presence of dexamethasone.

affected by cycloheximide treatment, either in the presence or absence of dexamethasone. gp130 data presented in Figure 7 shows the gp130 response to cycloheximide in the presence of 1×10^{-6} M dexamethasone. Addition of actinomycin D at the time of cycloheximide addition did not lead to this increase in IL-6-R mRNA, as indicated in Figure 7.

DISCUSSION

IL-6 relays important information to the liver during the early stages of an acute inflammatory response, causing an alteration in the expression of several major defense proteins. An amplification or modulation of the IL-6 signal could occur if there was a concomitant increase or decrease in the number of IL-6 receptors or signal transducing proteins, gp130, at the surface of the hepatocyte. Changes in the expression of either of these molecules could therefore alter the responsiveness of the hepatocyte to this inflammatory cytokine. To understand more fully the hepatocyte response to IL-6, we examined at the molecular level the regulation of both IL-6-R and gp130 mRNA expression.

We examined the subtle changes in mRNA levels for two important components of the cytokine signaling pathway by using highly sensitive ribonuclease protection assays. Induction of an acute inflammatory reaction in vivo lead to a rapid and transient increase in IL-6-R mRNA levels in rat liver. Although Baumann *et al.* (1990) showed a maximum 4.2-fold increase in IL-6-R mRNA 12 h after the onset of acute inflammation, we saw no change in IL-6-R mRNA levels at this time (compared with noninflamed rat) irrespective of the inducer of inflammation we employed.

Circulating levels of glucocorticoid increase during an inflammatory reaction (Beisel, 1977). These increased glucocorticoid concentrations subsequently inhibit IL-6 production in macrophages (Woloski *et al.*, 1985), thus allowing a mechanism for down-regulation of the expression of these inflammatory mediators. On the other hand, glucocorticoids act in synergy with IL-6 in the induction of a number of hepatically derived acute phase proteins, including fibrinogen (Fuller *et al.*, 1985). Our current findings show that glucocorticoids also increase IL-6-R mRNA levels and cell surface expression in rat hepatocytes in a time- and dose-dependent manner. Similar results have been reported in a human hepatoma cell line, HepG2 (Rose-John *et al.*, 1990; Snyers *et al.*, 1990). Two lines of evidence presented here suggest that the increase in IL-6-R mRNA levels in response to dexamethasone is most likely due to increased transcriptional activity of the IL-6-R gene rather than increased mRNA stability. First, the transcription inhibitor actinomycin D blocked the dexamethasone-induced increase in receptor mRNA. Second, dexamethasone did not alter the half-life of the IL-6-R mRNA.

The rapid increase in IL-6-R mRNA during the initiation of an acute inflammatory response is consistent with the response observed by the addition of dexamethasone to hepatocytes in culture. The rapid decline seen in vivo, however, did not occur in vitro; rather, IL-6-R mRNA levels declined gradually with time. A possible explanation for this may be that the cells in vitro were continually exposed to the glucocorticoid, and hence, subject to its inducing activity. In vivo, however, corticosterone is rapidly removed from circulation. Additionally, we have shown that IL-6 and IL-1 β down-regulate IL-6-R expression in hepatocytes. The rapid removal of glucocorticoid and subsequent increase in IL-6 and IL-1 β as part of the inflammatory response could explain the transient induction of the IL-6-R mRNA observed in vivo.

The dexamethasone-induced increase in IL-6-R mRNA is not inhibited by the translation inhibitor cycloheximide and therefore appears to be mediated through the post-translational activation of existing molecules. Of interest is the finding that addition of cycloheximide lead to a superinduction of IL-6-R mRNA to a level significantly above that in cells treated with the glucocorticoid analogue alone. This superinduction phenomenon occurs in a number of rapidly induced cytokines and proto-oncogenes (Mitchell *et al.*, 1985; Cosman, 1987; Wisdom and Lee, 1991), presumably by preventing the expression of a labile protein(s) involved in mRNA degradation of these genes. A common AUUUA motif located in the 3' UTR of a number of these mRNAs has been suggested as one of the possible regulatory elements involved in this posttranscriptional regulation (Malter, 1989).

Our finding that both IL-6 and IL-1 decrease IL-6-R mRNA levels disagrees with a recent report (Bauer *et al.*, 1989). In that study, IL-6 and IL-1 increased IL-6-R mRNA expression in cultured human primary hepatocytes while decreasing its expression in normal human monocytes. These discrepancies may be due to differences between fetal human hepatocytes and adult rat hepatocytes (as used in the current study). The reproducibility, consistency, quantitation, and sensitivity of our ribonuclease protection assays, as well as the diminution of the number of IL-6-Rs, however, is convincing evidence that the observed findings in rat hepatocytes are reflective of the in vivo response.

In summary, these findings provide evidence that IL-6-R and gp130 are regulated by different mechanisms in rat hepatocytes. The rapid and transient induction and the relatively short half-life of IL-6-R mRNA was complemented by the constant levels and relatively long half-life of gp130 mRNA. None of the inflammatory mediators tested had any effect at altering gp130 mRNA levels in these cells. It is apparent, therefore, that the hepatocyte alters its capability to respond to IL-6 by regulating the expression of the ligand binding protein,

IL-6-R, and not the signal transducing protein, gp130. When these findings are taken in the context of the intact animal, one sees that elegant homeostatic controls exist at several sites in the inflammatory pathway, in which glucocorticoids and cytokines operate in a balanced way to control the defense system of the animal.

ACKNOWLEDGMENTS

We are very grateful to Dr. Georg Fey for the rat liver interleukin-6 receptor cDNA. We thank Dr. Etty Benveniste and Mr. Michael A. Sheffield for critical review of the manuscript and Mr. Nelson Fuentes for his help with photography. This work was supported in part by NIH grant HL-43155 to G.M.F.

REFERENCES

- Aviv, H., and Leder, P. (1972). Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid cellulose. *Proc. Natl. Acad. Sci. USA* 69, 1408-1412.
- Bauer, J., Bauer, T.M., Kalb, T., Taga, T., Lengyel, G., Hirano, T., Kishimoto, T., Acs, G., Mayer, L., and Gerok, W. (1989). Regulation of interleukin-6 receptor expression in human monocytes and monocyte-derived macrophages. *J. Exp. Med.* 170, 1537-1549.
- Baumann, M., Baumann, H., and Fey, G.H. (1990). Molecular cloning, characterization and functional expression of the rat liver interleukin-6 receptor. *J. Biol. Chem.* 265, 19853-19862.
- Beisel, W.R. (1977). Magnitude of the host nutritional responses to infection. *Am. J. Clin. Nutr.* 30, 1236-1247.
- Besedovsky, H., Del Ray, A., Sorkin, E., and Dinarello, C.A. (1986). Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* 233, 652-654.
- Besedovsky, H., Sorkin, E., Keller, M., and Muller, J. (1975). Changes in blood hormone levels during the immune response. *Proc. Soc. Exp. Biol. Med.* 150, 466-470.
- Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S., and Cera, A. (1986). Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA* 83, 1670-1674.
- Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162, 156-159.
- Cosman, D. (1987). Control of messenger RNA stability. *Immunol. Today* 8, 16-17.
- Danielson, P.E., Forss-Petter, S., Brow, M., Calavetta, L., Douglass, J., Milner, R.J., and Sutcliffe, J.G. (1988). p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA* 7, 261-267.
- Darlington, G.J., Wilson, D.R., and Lachman, L.B. (1986). Monocyte-conditioned media, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. *J. Cell Biol.* 103, 787-793.
- Fuller, G.M., Otto, J.M., Woloski, B.M., McGary, C.T., and Adams, M.A. (1985). The effect of hepatocyte stimulating factor on fibrinogen biosynthesis in hepatocyte monolayers. *J. Cell Biol.* 101, 1481-1486.
- Gauldie, J., Richards, C., Harnish, D., Lansdorp, P., and Baumann, H. (1987). Interferon β_2 /B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA* 84, 7251-7255.

- Grenett, H.E., Danley, D.E., Strick, C.A., Otterness, I.G., Fuentes, N., Nesbitt, J.E., and Fuller, G.M. (1991). Isolation and functional characterization of biologically active murine interleukin-6 produced in *Escherichia coli*. *Gene* 101, 267-271.
- Grenett, H.E., Fuentes, N., and Fuller, G.M. (1990). Cloning and sequence analysis of the cDNA for murine interleukin-6. *Nucleic Acids Res.* 18, 6455.
- Heinrich, P.C., Castell, J.V., and Andus, T. (1990). Interleukin-6 and the acute phase response. *Biochem. J.* 265, 621-636.
- Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T., and Kishimoto, T. (1990). Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell* 63, 1149-1157.
- Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T., and Kishimoto, T. (1986). Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* 324, 73-76.
- Kishimoto, T. (1989). The biology of interleukin-6. *Blood* 74, 1-10.
- Koj, A. (1985). In: *The Acute-Phase Response to Injury and Infection: The Roles of Interleukin-1 and Other Mediators. Part III: Liver response to inflammation and synthesis of acute-phase plasma proteins*, eds. A.H. Gordon and A. Koz. Amsterdam: Elsevier, 139-227.
- Kushner, I. (1982). The phenomenon of the acute phase response. In: *Annals of the New York Academy of Sciences; C-Reactive Protein and the Plasma Protein Response to Tissue Injury*, ed. I. Kushner, J.E. Volanakis, and H. Gerwurz, New York: New York Academy of Sciences, 39-48.
- Malawista, S.E., and Weiss, M.C. (1974). Expression of differentiated functions in hepatoma cell hybrids: high frequency of induction of mouse albumin production in rat hepatoma-mouse lymphblast hybrids. *Proc. Natl. Acad. Sci. USA* 71, 927-931.
- Malter, J.S. (1989). Identification of an AUUUA-specific messenger RNA binding protein. *Science* 246, 664-666.
- Mitchell, R.L., Zokas, L., Schreiber, R.D., and Verma, I.M. (1985). Rapid induction of the expression of proto-oncogene *fos* during human monocyte differentiation. *Cell* 40, 209-217.
- Nakajima, K., and Wall, R. (1991). Interleukin-6 signals activating *junB* and *TIS11* gene transcription in a B-cell hybridoma. *Mol. Cell. Biol.* 11, 1409-1418.
- Nesbitt, J.E., and Fuller, G.M. (1991). Transcription and translation are required for fibrinogen mRNA degradation in rat hepatocytes. *Biochim. Biophys. Acta* 1089, 88-94.
- Rahmsdorf, H.J., Schonthal, A., Angel, P., Liffin, M., Ruther, U., and Herrlich, P. (1987). Post-transcriptional regulation of *c-fos* mRNA expression. *Nucleic Acids Res.* 15, 1643-1659.
- Rave, N., Crkvenjakov, R., and Boedtker, H. (1979). Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. *Nucleic Acids Res.* 6, 3559-3567.
- Rose-John, S., Schooltink, H., Lenz, D., Hipp, E., Dufhues, G., Schmitz, H., Schiel, X., Hirano, T., Kishimoto, T., and Heinrich, P.C. (1990). Studies on the structure and regulation of the human hepatic interleukin-6 receptor. *Eur. J. Biochem.* 190, 79-83.
- Sanger, F., Nicklen, S., and Coulson, A.P. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
- Scatchard, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* 51, 660-672.
- Scherrer, K. (1969). In: *Fundamental Techniques in Virology*. Eds. K. Habel and N.P. Salzman. Chapter 38, Isolation and sucrose gradient analysis of RNA. New York: Academic Press, 413-432.
- Scherrer, K., and Darnell, J.E. (1962). Sedimentation characteristics of rapidly labelled RNA from HeLa cells. *Biochem. Biophys. Res. Commun.* 7, 486-490.
- Shaw, G., and Kamen, R. (1986). A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* 46, 659-667.
- Snyers, L., De Wit, L., and Content, J. (1990). Glucocorticoid up-regulation of high-affinity interleukin-6 receptors on human epithelial cells. *Proc. Natl. Acad. Sci. USA* 87, 2838-2842.
- Snyers, L., Fontaine, V., and Content, J. (1989). Modulation of interleukin-6 receptors in human cells. *Ann. N. Y. Acad. Sci.* 557, 388-393.
- Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T., and Kishimoto, T. (1989). Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* 58, 573-581.
- Taga, T., Kawanishi, Y., Hardy, R.R., Hirano, T., and Kishimoto, T. (1987). Receptors for B cell stimulatory factor 2 (BSF-2): quantitation, specificity, distribution and regulation of their expression. *J. Exp. Med.* 166, 967-981.
- Thomas, P.S. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* 77, 5201-5205.
- Van Snick, J. (1990). Interleukin-6: an overview. *Annu. Rev. Immunol.* 8, 253-278.
- Wang, A.M., Doyle, M.V., and Mark, D.F. (1989). Quantitation of mRNA by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* 86, 9717-9721.
- Wisdom, R., and Lee, W. (1991). The protein-coding region of *c-myc* mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. *Genes and Dev.* 5, 232-243.
- Woloski, B.M.R.N.J., Smith, E.M., Meyer, W.J., III, Fuller, G.M., and Blalock, J.E. (1985). Corticotropin-releasing activity of monokines. *Science* 230, 1035-1037.
- Wong, G.G., and Clark, S.C. (1988). Multiple actions of interleukin-6 within a cytokine network. *Immunol. Today* 9, 137-139.
- Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., and Kishimoto, T. (1988). Cloning and expression of the human interleukin-6 (BSF-2/INF β 2) receptor. *Science* 241, 825-828.