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Research Article

Interferons (IFN) elicit antiviral and antineoplastic activities by binding to specific receptors on the cell surface. The binding characteristics of IFN to human lymphocytes were studied using IFN alpha 2 labeled with ^{125}I to high specific activity. The specific binding curves generated were analyzed by the LIGAND program of Munson and Rodbard to determine receptor numbers. The number of receptors in peripheral blood lymphocytes (PBL) and tonsillar B-lymphocytes (TBL) from normal individuals were 505 ± 293 ($n = 10$) and 393 ± 147 ($n = 3$) respectively. When these cells were preincubated in vitro with unlabeled IFN alpha 2, the receptor number decreased to 82 ± 45 and 61 ± 16 respectively. Receptor binding activities recovered gradually over a period of 72 h when the cells were incubated in IFN-free medium. This recovery of receptors could be blocked by the addition of actinomycin D to the incubation medium. A similar decrease in receptor expression was observed in vivo in PBL from patients being treated daily with 5×10^6 units/m² per d of IFN alpha 2 by subcutaneous injection, for acute lymphoblastic leukemia or papilloma virus infections. Receptor numbers in PBL in vivo were further reduced concurrent with the progression of IFN therapy. Thus the reduction in IFN receptor expression observed in vitro can be demonstrated in vivo. These studies [...]

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Regulation of Interferon Receptor Expression in Human Blood Lymphocytes In Vitro and during Interferon Therapy

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Abstract

Interferons (IFN) elicit antiviral and antineoplastic activities by binding to specific receptors on the cell surface. The binding characteristics of IFN to human lymphocytes were studied using IFN α_2 labeled with ^{125}I to high specific activity. The specific binding curves generated were analyzed by the LIGAND program of Munson and Rodbard to determine receptor numbers. The number of receptors in peripheral blood lymphocytes (PBL) and tonsillar B-lymphocytes (TBL) from normal individuals were 505 ± 293 ($n = 10$) and 393 ± 147 ($n = 3$) respectively. When these cells were preincubated in vitro with unlabeled IFN α_2 , the receptor number decreased to 82 ± 45 and 61 ± 16 respectively. Receptor binding activities recovered gradually over a period of 72 h when the cells were incubated in IFN-free medium. This recovery of receptors could be blocked by the addition of actinomycin D to the incubation medium. A similar decrease in receptor expression was observed in vivo in PBL from patients being treated daily with 5×10^6 units/m² per d of IFN α_2 by subcutaneous injection, for acute lymphoblastic leukemia or papilloma virus infections. Receptor numbers in PBL in vivo were further reduced concurrent with the progression of IFN therapy. Thus the reduction in IFN receptor expression observed in vitro can be demonstrated in vivo. These studies indicate that monitoring IFN receptor expression in vivo can provide information regarding the availability of IFN receptors at the cell surface for the mediation of IFN actions during the course of IFN therapy.

Introduction

The interferons (IFN)¹ are a group of proteins which regulate a wide spectrum of cell functions and modulate responses to infectious and malignant diseases (1, 2). Since the discovery of IFN, there has been intensive interest in the application of the modulators for clinical use as both antiviral and antineoplastic agents (3). Recent advances in recombinant DNA technology

in addition to supplying IFN for clinical trials, have also provided IFN preparations of sufficiently high purity to allow radioreceptor assays.

The interaction of IFN with susceptible cells is mediated by binding to specific high affinity cell surface receptors, a step which is necessary for the subsequent biological effects of IFN (4). Cellular processes subsequent to the binding of IFN show general similarities with many other polypeptide hormone-receptor systems such as insulin or epidermal growth factor (5, 6). Interferon-receptor complexes are internalized and degraded intracellularly subsequent to ligand binding (7, 8). Therefore IFN can modulate the expression of its own receptor in target cells. This phenomenon of ligand-induced down-regulation of IFN receptors has been observed in Daudi (lymphoblastoid), WISH (human amnion), and other cultured cell lines (9–12). In addition, we have described the desensitization of IFN α receptors by treatment of a human cell line with IFN γ (13). This clearly results from an indirect mechanism since IFN α and γ do not share the same receptor sites (14).

The regulation of IFN receptors by IFN itself has important implications for the clinical use of IFN. The current use of IFN in patients often involves empiric choice of dosage and dosing interval. The optimal dose of IFN necessary to elicit in vivo cellular responses to treatment is not known. In order to further evaluate the therapeutic potential of IFN, it is important to study cellular responses, including the regulation of IFN receptors during IFN therapy.

In the present study, we have investigated the expression of IFN receptors on freshly isolated blood lymphocytes from normal individuals and from patients on IFN therapeutic trials for acute lymphoblastic leukemia and papilloma virus infection. The phenomenon of down-regulation of IFN α receptors by IFN α_2 itself was demonstrated both in vitro and in vivo.

Methods

Isolation of cells. Peripheral blood lymphocytes (PBL) were isolated from healthy volunteers using Ficoll-Hypaque density gradient centrifugation as described (15). Washed PBL were resuspended in RPMI 1640 medium supplemented with 2% fetal calf serum (Gibco, Grand Island, NY), and containing, per ml, 100 U of penicillin and 100 μg of streptomycin. Tonsillar B-lymphocytes (TBL) were isolated from tonsils (obtained at elective tonsillectomies), and finely minced with a scalpel. The resulting cell suspensions were centrifuged over Ficoll-Hypaque, and the tonsillar lymphocytes obtained were depleted of T lymphocytes by rosetting with neuraminidase-treated sheep erythrocytes as described (16). The cells were suspended in RPMI medium and incubated in a tissue culture dish (Falcon) at 37°C for 30 min for monocyte depletion. The lymphocytes isolated were incubated in RPMI medium with the addition of indicated concentrations of IFN α_2 (Schering Corp., Bloomfield, NJ) for 18 h or as denoted in the figure legends. Cells left untreated with IFN were incubated for the same period of time and used as controls. Subsequent

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1. *Abbreviations used in this paper:* IFN, human interferon; PBL, peripheral blood lymphocytes; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBL, tonsillar B lymphocytes.

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to the incubation period, the cells were washed with fresh medium and used for binding and affinity-labeling experiments, as described below.

To account for the possibility that prior occupancy of cellular IFN α receptors (by unlabeled IFN α_2 in the pretreatment procedure) might be inhibiting the subsequent receptor assay with ^{125}I -IFN α_2 , the following experiments were performed. After incubation at 37°C for 18 h with 1,000 U/ml of IFN α_2 , the cells were washed with acidic phosphate-buffered saline (PBS) (pH 5.5) for 8 min to release receptor-bound unlabeled IFN α_2 (10). Cells not treated with unlabeled IFN α_2 were used as controls. All cells were assayed for ^{125}I -IFN α_2 receptor binding as described.

In vivo studies were done on PBL isolated from patients being treated with IFN α_2 (Schering Corp.), 5×10^6 U/m² per d daily by subcutaneous injection for acute lymphoblastic leukemia or papilloma virus infections. The binding of ^{125}I -IFN α_2 to the patients' PBL were monitored prior to and during the course of IFN therapy. Heparinized blood samples were taken from these patients 20 h after IFN injection on designated days during therapy.

Iodination of IFN. The binding of IFN to lymphocytes was studied using IFN α_2 (kindly provided by Drs. P. Trotta and T. Nagabhushan, Schering Corp, sp act 2×10^8 IU/mg protein) labeled to high specific activity (90–120 Ci/g) with ^{125}I by a solid-phase lactoperoxidase procedure (17). The specific activity of monoiodinated IFN (indicative of 100% mean incorporation) is determined to be 110 Ci/g. The iodinated IFN was separated from unincorporated ^{125}I by chromatography in a Sephadex G-50 column, 0.7×18 cm, as described previously (17). The ^{125}I -IFN α_2 eluted was titrated for antiviral activity against National Institute of Allergy and Infectious Diseases reference standard G-023-901-527 (10). All units are expressed as international units.

Cellular binding assay. Cultured or freshly isolated lymphocytes were washed and suspended in RPMI-1640 medium containing 2% fetal calf serum. Binding reactions were carried out in 200 μl volumes containing 4×10^5 cells and the indicated concentrations of ^{125}I -IFN α_2 , in triplicates (17). After incubation for 4 h at 4°C the reactions were quickly layered over 150 μl of a phthalate oil mixture in a 1.5-ml conical microfuge tube, and centrifuged for 30 s at 12,500 g in an Eppendorf model 5414 microfuge. The culture medium and phthalate oil were then aspirated and cell pellet analysed for cell-bound radioactivity on a Beckman 5500 Gamma Counter. Binding specificity was determined in parallel incubations including a 100-fold excess of unlabeled IFN α_2 at each ^{125}I -IFN α_2 concentration. The nonspecific counts obtained were subtracted from total counts bound to obtain the specific binding activities reported. All bindings are reported as specific counts bound.

Recovery of IFN receptor expression. Freshly isolated TBL in RPMI medium were treated with 1,000 U/ml of IFN α_2 for 3 h at 37°C, or left untreated. The cells were washed and allowed to recover in IFN-free RPMI medium. At indicated time intervals, aliquots of 5×10^6 cells were taken for binding reactions and crosslinking experiments with ^{125}I -IFN α_2 described below. In addition, 5×10^6 cells were taken for re-treatment with 1,000 U/ml of IFN α_2 , at 37°C for 3 h, at the end of the 22-h recovery incubation. The controls were cells incubated and washed in the same manner but without any IFN α_2 treatment.

Effect of actinomycin D. In another experiment, freshly isolated PBL were similarly pretreated with 1,000 U/ml of IFN α_2 at 37°C for 3 h. The cells were washed with acidic PBS (pH 5.5) to dissociate any prebound unlabeled IFN α_2 . The cells were allowed to recover in IFN-free RPMI medium for 22 h at 37°C with or without the presence of actinomycin D at 1 $\mu\text{g}/\text{ml}$. Cellular binding assays and affinity-labeling experiments as described were performed on these cells. Cells not treated with IFN α_2 or actinomycin D, but washed with acidic PBS (pH 5.5), were used as controls.

Affinity labeling of IFN receptors. Subsequent to the incubations outlined above, binding reactions were carried out at 4°C with 5×10^6 cells in 500 μl volumes containing 50 ng/ml of ^{125}I -IFN α_2 , under conditions as described in the cell binding section. Cells were washed three times in cold PBS, resuspended in 1 ml of PBS containing 1 mM disuccinimidyl suberate, and incubated for 30 min at 4°C. Cells were subsequently washed in PBS. Lysis of the cells was performed in a Tris buffer (pH 7.5,

50 mM) containing 0.5% NP-40, 7 mM magnesium acetate, 90 mM potassium chloride, and 150 mM glucose. 1 mM 1,10-phenanthroline, 1 mM benzamidine, 2 mM sodium metabisulphite, 50 μM phenylmethylsulfonyl fluoride, 50 $\mu\text{g}/\text{ml}$ crude soybean trypsin inhibitor and 50 $\mu\text{g}/\text{ml}$ aprotinin were added to the lysis buffer as protease inhibitors (17a). After 1 h of incubation on ice with frequent vortexing, the extract was centrifuged at 12,500 g for 10 min, in an Eppendorf microfuge. The supernatants (20–25 μl) were mixed with an equal volume of electrophoresis buffer (containing 1 mM dithiothreitol and 2-mercaptoethanol), boiled for 5 min, and subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 7.5% gels (18). Gels were dried under vacuum, and exposed to XAR film (Eastman Kodak Co., Rochester, NY) with lightening-plus intensifying screens (DuPont Co., Wilmington, DE) for 8 d at –70°C.

Analysis of binding data. Specific ^{125}I -IFN α_2 binding data were used to determine receptor numbers and dissociation constant, K_D . With the increasing concentrations of ^{125}I -IFN α_2 in the cellular binding reaction, respective specific binding activities corresponding to each ^{125}I -IFN α_2 concentration were calculated. These specific binding data were plotted according to the method of Scatchard (18, 19), and in addition, analyzed by the LIGAND program of P. Munson and D. Rodbard (20). An applesoft version of the LIGAND program adapted by T. Jackson and P. Edwards of Middlesex Hospital, London, England, was employed. The program resolves the equations describing the laws of mass-action binding, and objectively allows the determination of both the number of receptors and the K_D , characterizing the binding reaction.

Results

Expression of IFN receptors on lymphocytes. Specific binding curves were generated when PBL and TBL from normal individuals were incubated at 4°C for 4 h in the presence of increasing concentrations of ^{125}I -IFN α_2 (Figs. 1 and 2). A Scatchard plot of these data indicates a linear interaction of ^{125}I -IFN α_2 with its receptors (insets of Figs. 1 and 2). The LIGAND program of P. Munson was used to determine the number of receptors expressed on the lymphocytes. Normal PBL (Fig. 1) exhibit receptors of $K_D = 1 \times 10^{-9}$ M, 700 sites/cell, and normal TBL possess receptors of $K_D = 9.3 \times 10^{-10}$ M, 504 sites/cell. Data from PBL freshly isolated from 10 individuals and TBL from three individuals are compiled in Table I. Normal PBL exhibit an average of 505 ± 293 sites (range 204–900, $n = 10$) per cell with $K_D = 7.6 \pm 5.0 \times 10^{-10}$ M, whereas normal TBL possess 393 ± 147 sites (range 227–504, $n = 3$) per cell with $K_D = 5.7 \pm 3.5 \times 10^{-10}$ M. The K_D is practically defined as the concentration of ligand (IFN) that is required to saturate 50% of the receptor sites on the cell surface.

Down-regulation of IFN receptor expression. To evaluate the effect of in vitro incubation on receptor binding of PBL, cells were incubated for 18 h at 37°C in the absence of IFN. Subsequent receptor binding assays did not show any significant loss of receptor sites/cell, i.e., 488 ± 181 (range 225–800, $n = 7$). However, on pretreatment of PBL or TBL with unlabeled IFN α_2 (1,000 U/ml, at 37°C for 18 h) there was a marked and reproducible reduction in subsequent receptor expression as measured by ^{125}I -IFN α_2 binding. This phenomenon of down-regulation is observed in both cell types with receptor numbers decreased to 90 sites per cell from a level of 700 sites per cell in PBL (Fig. 1 A), and to 78 sites per cell from a level of 504 sites per cell in TBL (Fig. 2). Data from IFN pretreatment of PBL isolated from six individuals and TBL isolated from three individuals are shown (Table I). The percent in reduction ranged between 60% and 90%. A similar level of reduction in binding

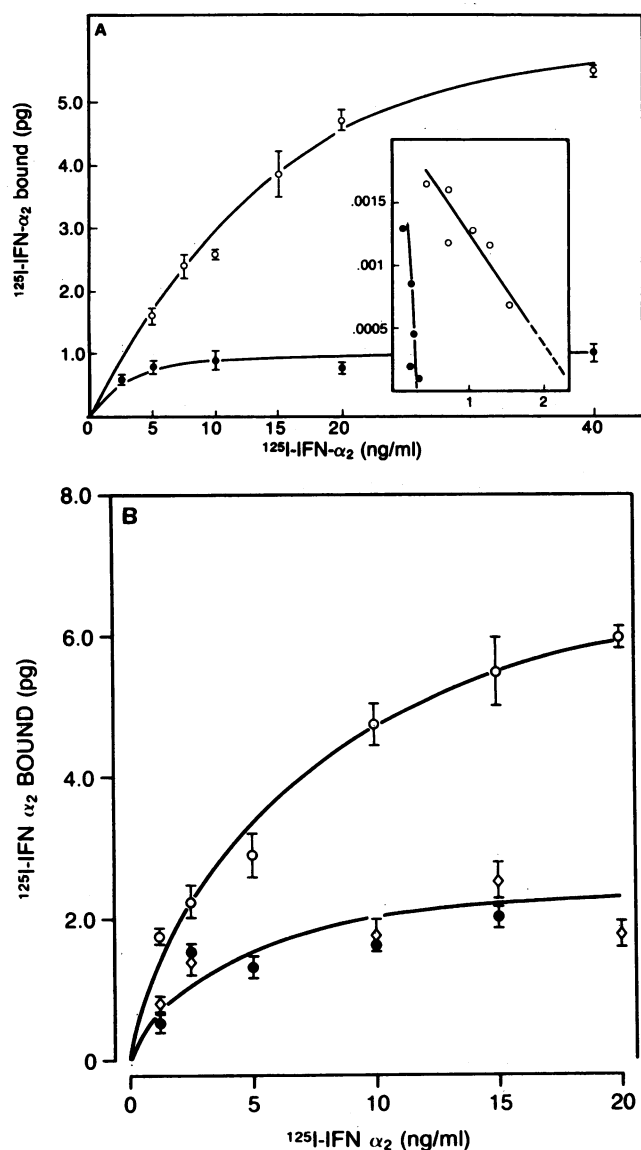


Figure 1. In vitro reduction in IFN α receptor binding in PBL with IFN α_2 pretreatment. (A) PBL were isolated as described in Methods. Cells were treated with IFN α_2 , 1,000 U/ml, at 37°C for 18 h. Binding assays were performed with 4×10^5 cells in a volume of 200 μ l, 4 h at 4°C, with the indicated concentrations of 125 I-IFN α_2 . As controls, parallel determinations were obtained on cells without IFN pretreatment. Linear and nonsaturable nonspecific binding represented, typically, 50% of the total binding for both PBL and TBL. Each point represents the mean of triplicate incubations for each 125 I-IFN α_2 concentration, with error bars indicating one standard deviation about the mean. The Scatchard plot (inset with ordinate as ratio of bound to free ligand, and abscissa as pM bound) is illustrative of the curves resolved by LIGAND. These curves are representative of the data compiled in Table I. \circ , Control; \bullet , IFN pretreated. (B) PBL were pretreated with IFN α_2 (1,000 U/ml) as in (A). Cells were subsequently washed with PBS (pH 7.4), or PBS titrated to acidic pH (5.5) to dissociate prebound unlabeled IFN α_2 . \circ , Control; \bullet , IFN pretreated; \diamond , IFN pretreated plus acidic pH wash.

of 125 I-IFN α_2 was also observed when cells were pretreated with unlabeled IFN α_2 for shorter periods of time (see below Figs. 4 and 6). With the use of lower concentrations of unlabeled IFN

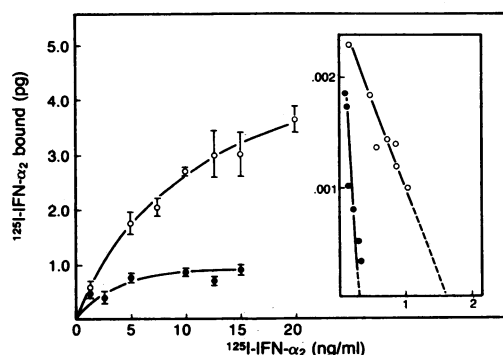


Figure 2. In vitro reduction in IFN α receptor binding in TBL with IFN α_2 pretreatment. TBL were isolated as described in Methods. Cells were treated with IFN α_2 , and subsequent 125 I-IFN α_2 binding assays were performed as described in Fig. 1. Inset axis as in Fig. 1. \circ , Control; \bullet , IFN pretreated.

α_2 in pretreatment, i.e., 50 U/ml and 200 U/ml, the reduction in binding is less pronounced than that observed with 1,000 U/ml pretreatment (Fig. 3). At these lower pretreatment concentrations (Fig. 3), the receptor number decreased to 177 ± 40 and 195 ± 60 sites per cell, respectively. Thus the extent of reduction in subsequent 125 I-IFN α_2 binding was clearly dependent on the concentration of IFN α_2 used in pretreatment.

We next considered the possibility that receptors at the cell surface might be significantly occupied by IFN α_2 during the pretreatment procedure, thereby blocking subsequent assays for IFN α_2 receptor expression. After pretreatment with unlabeled IFN α_2 , PBL were washed with acidic (pH 5.5) PBS prior to 125 I-IFN α_2 receptor binding assays. This procedure ensured that prebound IFN was released from cell surface receptors, without affecting subsequent receptor assays (10). The results ($n = 4$) essentially showed identical specific binding curves and receptor sites as resolved by the LIGAND program, obviating the possibility of prior receptor occupancy in the observed downregulation. In a representative experiment (Fig. 1 B), normal PBL showed 450 ± 50 sites/cells. PBL, pretreated with IFN α_2 , exhibited 177 ± 22 sites/cell, whereas pretreated PBL exposed to the acidic wash protocol exhibited 150 ± 25 sites/cell. Also, the acidic wash did not affect subsequent receptor binding assay on control cells (data not shown).

Table I. Receptor Numbers in Human Lymphocytes Untreated or Treated with IFN

	Receptor sites per cell*	
	PBL	TBL
Normal	505 ± 293 ($n = 10$)	393 ± 147 ($n = 3$)
IFN treatment	85 ± 45 ($n = 7$)	61 ± 16 ($n = 3$)
	$P < .001$	$P < .02$

All binding experiments involved generation of complete isotherms at 4°C. The binding data obtained were then analysed by LIGAND to determine receptor numbers.

* Mean of n replicates ± 1 SD.

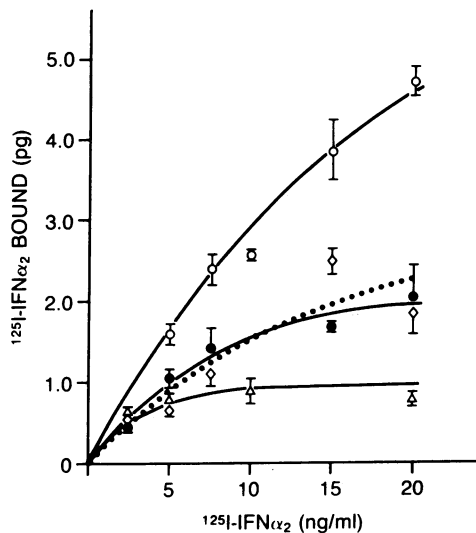


Figure 3. Dose response of IFN receptor binding in PBL pretreated with IFN α_2 . PBL were isolated as described in Methods. Cells were pretreated with different concentrations of unlabeled IFN α_2 , i.e., 50 U/ml, 200 U/ml, and 1,000 U/ml, at 37°C for 18 h. Subsequent to washings, 125 I-IFN α_2 binding assays were performed at 4°C as outlined in Methods. \circ , Control; \diamond , IFN 50 U/ml; \bullet , IFN 200 U/ml; Δ , 1,000 U/ml.

Recovery of IFN receptor expression. The recovery of IFN binding activity can be best illustrated using a bifunctional crosslinking agent, disuccinimidyl suberate, which has been used previously to identify IFN binding cell-surface components (Fig. 4) (21, 22). When the crosslinking agent is added to cells pre-

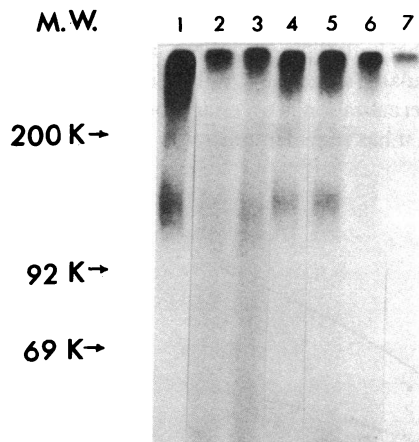


Figure 4. Affinity labeling of IFN receptors. Freshly isolated TBL were pretreated with 1,000 U/ml of IFN α_2 for 3 h at 37°C. These cells were washed and allowed to recover in IFN-free RPMI medium. At indicated time intervals, aliquots of 5×10^6 cells were taken for binding with 50 ng/ml of 125 I-IFN α_2 for 4 h at 4°C. Bound IFN was then crosslinked to receptors with 1 mM disuccinimidyl suberate, and cell extracts subjected to 7.5% SDS-PAGE. The film was exposed to XAR (Eastman Kodak Co.) film for 8 d. Lane 1, controls without IFN pretreatment; lanes 2–5 represent cross-linking at 0 h, 6 h, 12 h, and 22 h after IFN pretreatment; lane 6, after 22 h of recovery incubation, cells were re-treated with 1,000 U/ml of IFN α_2 at 37°C for 3 h; lane 7, control cells with binding incubation including a 100-fold excess of unlabeled IFN α_2 .

bound with 125 I-IFN α_2 , the prebound ligand is covalently linked to the moieties of the receptor which are responsible for the binding of ligand. Subsequent disruption of the plasma membrane by cell lysis buffer releases the 125 I-IFN α_2 -receptor complexes, which can be resolved and visualized by SDS-PAGE and autoradiography. This analysis yields sensitive qualitative information on the expression of cell surface IFN receptors. TBL, when preincubated for a period of 3 h with IFN α_2 at a concentration of 1,000 U/ml, showed ligand-induced downregulation of IFN α receptors (Fig. 4, lane 2). When incubated in IFN-free RPMI medium, following receptor downregulation, cells showed a gradual recovery of 125 I-IFN α_2 binding activity over a 22-h period (Fig. 4, lanes 3–5).

The complete recovery of receptors in PBL took 72 h, as demonstrated by cellular binding assays (Fig. 5). In this experiment PBL were pretreated with 1,000 U/ml of IFN α_2 , and receptor binding activities were measured during the recovery period of 3 d. The pretreated cells showed gradual recovery of binding activity compared to that of the control level over a period of 3 d.

Effect of actinomycin D on recovery of IFN receptor expression. The reappearance of IFN binding during recovery in IFN-free medium can be blocked by the presence of actinomycin D (Fig. 6). Cellular binding assays were performed on these cells in order to generate specific binding curves for the quantitative determination of receptor sites. In this experiment, the control PBL exhibited 480 ± 70 receptor sites/cell (Fig. 6, lane 2). With IFN α_2 pretreatment (followed by acidic PBS wash), both affinity-labeling and cellular binding assays did not detect any receptor expression (Fig. 6, lane 3). These pretreated cells, when allowed to recover in IFN-free medium, exhibited 227 ± 29 receptor sites per cell after 22 h of incubation (Fig. 6, lane 4). However, in the presence of actinomycin D (final concentration at 1 μ g/ml), the recovery of IFN receptor binding was minimal, i.e., 70 ± 10 receptor sites/cell. This result (Fig. 6, lane 5) suggests that the recovery of IFN binding activity, following down-regulation, requires messenger RNA (mRNA) synthesis.

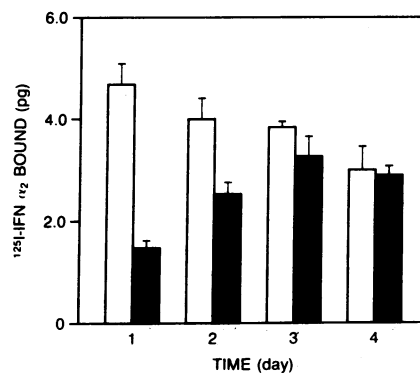


Figure 5. Recovery of IFN receptor binding. PBL were pretreated with 1,000 U/ml of IFN α_2 at 37°C for 18 h. Cells were washed with acidic PBS (pH 5.5) and allowed to recover in IFN-free RPMI medium at 37°C over a period of 3 d. Aliquots of 4×10^5 cells were taken for IFN receptor binding assay with 15 ng/ml of 125 I-IFN α_2 at indicated time intervals. Cells, not treated with IFN α_2 and incubated for the same period of time, were used as controls. Each bar represents the mean of triplicate incubations, with error bars indicating one standard deviation about the mean. Open bar, controls; shaded bar, IFN treated.

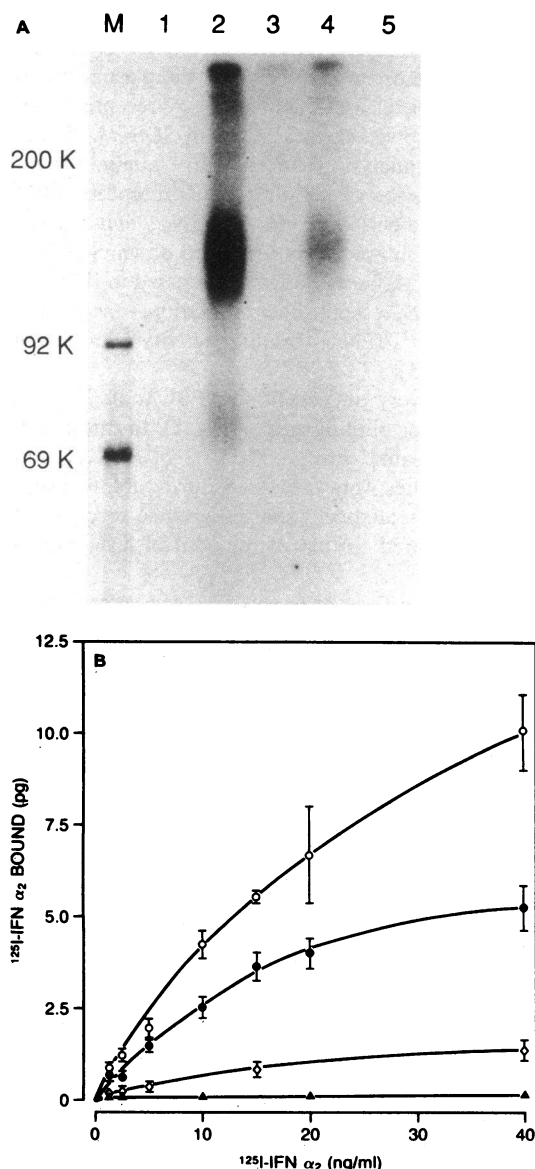


Figure 6. Effect of actinomycin D on the recovery of IFN receptor binding. Freshly isolated PBL were pretreated with 1,000 U/ml of IFN α_2 for 3 h at 37°C. The cells were washed with acidic PBS (pH 5.5) to dissociate any prebound unlabeled IFN α_2 . The cells were allowed to recover in IFN-free RPMI medium for 22 h at 37°C, with or without the presence of actinomycin D (1 μ g/ml). (A) 5×10^6 cells were taken for binding with 125 I-IFN α_2 , crosslinking with disuccinimidyl suberate and SDS-PAGE as described in Fig. 5. Group 1, control cells with binding incubation containing a 100-fold excess of unlabeled IFN. Group 2, control cells without IFN or actinomycin D treatment. Group 3, cells pretreated with IFN without recovery incubation. Group 4, cells pretreated with IFN, recovered for 22 h. Group 5, cells pretreated with IFN, recovered for 22 h in the presence of actinomycin D. (B) Cellular binding assays were performed on each group of cells described in (A). \circ , Group 2; Δ , Group 3; \bullet , Group 4; \diamond , Group 5.

In vivo studies on IFN receptor expression. IFN α_2 receptor binding was monitored in patients on IFN α_2 therapy for either papilloma virus infections or acute lymphoblastic leukemia. The age of the patients ranged from 7 to 34 yr. They were treated daily with IFN subcutaneously, as described in Methods. The

number of IFN α receptors on the PBL of a patient with condyloma acuminata (due to papilloma virus infection) was 834 ± 160 per cell prior to IFN therapy (Fig. 7). With the progression of therapy, the receptor number decreased to 262 ± 42 , 72 ± 8 , and 220 ± 40 on day 5, day 19, and day 25 of IFN therapy respectively (Fig. 7). A similar reduction in IFN receptor binding to PBL during IFN therapy was also observed in the other two patients with either papilloma virus infection or acute lymphoblastic leukemia (Table II). Generally, following 2–5 d of IFN therapy, the receptor numbers decreased by 40–70%, i.e., from 834 ± 160 to 262 ± 42 , 276 ± 50 to 152 ± 19 , and 705 ± 100 to 413 ± 70 sites per cell, respectively (Table II).

Discussion

IFNs are a unique group of glycoproteins that possess potent antiviral, antiproliferative, and immunoregulatory functions (1, 2). As a consequence, they have been used therapeutically in various preliminary clinical trials. Clinical efficacy of IFN α against several infectious diseases (influenza virus, papilloma virus, herpes zoster, and hepatitis B) has been reported (3, 23–26). More recently, antineoplastic activity of IFN has been demonstrated in patients with non-Hodgkin's lymphoma, myeloma, and, most dramatically, hairy cell leukemia (27–29). Most of these studies have focused on the pharmacokinetics, side effects and toxicities of IFN, or clinical responses of the patients. Many of the basic mechanisms involved in the induction by IFN of antiviral and antineoplastic activities in vivo remain to be elucidated.

In vitro studies on cultured cell lines have shown that IFNs elicit antiviral and antineoplastic activities by binding to specific high affinity cell surface receptors (4). Events following the binding of IFN have been the subject of intensive investigation. These events culminate, in part, in the induction of 2,5A synthetase, protein kinase and possibly endonuclease enzymes (1, 2, 30). It has also been reported that both IFN α and IFN γ are internalized and degraded intracellularly subsequent to binding (7, 8). It is not known whether internalization is necessary for the biological activity of IFN. In fact, it has been demonstrated that inhibition

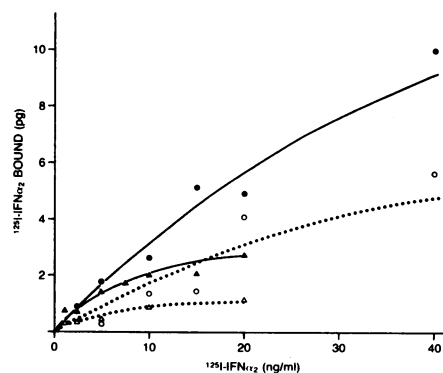


Figure 7. IFN receptor binding in vivo in PBL during IFN therapy. The patient is a 34-yr-old white female with severe condyloma acuminata with cutaneous disseminations. She was treated with IFN α_2 , and IFN receptor binding studies were performed as described in Methods. Nonspecific binding represented 50% of total binding. Points are the means of duplicate or triplicate incubations for each 125 I-IFN α_2 concentration, with maximum deviations of $\pm 10\%$ about the mean. Binding studies were done before (\bullet), at day 5 (\circ), day 19 (Δ), and day 25 (\blacktriangle) of IFN therapy.

Table II. Down-regulation of IFN Receptors in PBL in Response to IFN Therapy

Patient	Age	Sex	Diagnosis	Doses given	Receptor number		
					Basal	IFN	Reduction
A	34 yr	F	Condyloma acuminata	5	834±160	262±42	↓ 69%
B	16 yr	M	A.L.L. Papillomavirus	5	276±50	152±19	↓ 45%
C	7 yr	F	A.L.L.	2	705±100	413±70	↓ 42%

Patient A is a 34-yr-old white female with condyloma acuminata with severe cutaneous dissemination. She had no underlying immune disorder. Patient B is a 16-yr-old white male with acute lymphoblastic leukemia (A.L.L.), diagnosed 1 yr before IFN therapy. He received standard chemotherapy and had been in remission since. He developed persistent flat warts (papilloma virus infection) which were resistant to conventional therapy. Patient C is a 7-yr-old white female with A.L.L. She received standard chemotherapy, and was initially in remission but subsequently developed relapse of the leukemic disease. At the time of IFN therapy, she had leukemic cells in her bone marrow but not in the blood circulation. The IFN binding studies were done before the initiation of intensive chemotherapy. IFN α receptor bindings in PBL (in triplicates) were studied in patients before and during the course of IFN therapy. The binding data were analysed by LIGAND to determine the numbers of receptors. F, female; M, male.

of internalization of IFN-receptor complexes with cytochalasin does not affect 2,5A synthetase induction in Daudi (lymphoblastoid) cells (7), and an IFN-resistant variant of Daudi cells has been shown to internalize 125 I-IFN α_2 similar to the IFN-sensitive Daudi (10).

In this paper, we have demonstrated that human PBL and TBL, when pretreated in vitro with IFN α_2 , show a marked reduction in receptor binding of 125 I-IFN α_2 . The reduced binding represents downregulation of IFN receptor expression at the cell surface in these freshly isolated lymphocytes. This observation is in accord with findings in Daudi, T98G, and other cultured cell lines (10–12). Interestingly, when resting lymphocytes were incubated in IFN-free medium subsequent to downregulation, IFN binding activities gradually recovered over a period of 72 h (Figs. 4, 5). The recovery of receptor binding was blocked by actinomycin D (Fig. 6) and thus synthesis of new receptor mRNA might be required for this recovery.

The in vivo studies reported here on patients receiving IFN therapy revealed a similar phenomenon of reduction in IFN binding (Fig. 7). Concurrent with the progression of therapy, the IFN binding activities in the patients' PBL were further reduced. This reduction in binding most likely results from IFN-induced downregulation of IFN receptor expression in vivo during IFN therapy. However, we cannot exclude the possibility of a loss of an IFN-responsive cellular population in the peripheral circulation during therapy. This latter explanation seems less likely, as our in vivo findings were entirely similar to those of the in vitro observations on downregulation.

The events occurring at the cell surface receptor level are undoubtedly important in the regulation of IFN actions. Two recent studies have demonstrated that natural killer cell activity from patients under continuous IFN therapy are reversibly refractory to in vitro stimulations by IFN (31, 32). Similarly, a reversible resistance to IFN action has been observed in vitro in transformed mouse fibroblasts during continuous IFN exposure (33). Gupta et al. demonstrated that the IFN-induced synthesis of specific polypeptides in human cells reaches a maximum within the first few hours of prolonged IFN treatment. This is followed by a refractory period during which there is a decline in IFN-induced protein synthesis (34). These observations of hyporesponsive states during prolonged, continuous exposure to IFN could be explained by downregulation of IFN receptor expression at the cell surface. On the basis of our in vivo and in vitro findings on the IFN-induced regulation of IFN receptor

expression, together with that of the aforementioned hyporesponsiveness of cells during continuous IFN treatment, it appears that an interval treatment schedule for IFN therapy might be more appropriate than a continuous one, as it allows recovery of cell surface IFN receptors necessary for further binding interaction with exogenously administered interferon.

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References

1. Stiehm, E. R., L. H. Kronenberg, H. M. Rosenblatt, Y. Bryson, and T. C. Merigan. 1982. Interferon: immunobiology and clinical significance. *Ann. of Int. Med.* 96:80–93.
2. DeMayer, E., and H. Schellekens, editors. 1983. The Biology of the Interferon System. Elsevier/North Holland, New York. 549 pp.
3. Cesario, T. C. 1983. The clinical implications of human interferons. *Med. Clin. N. Am.* 67:1147–1162.
4. Zoon, K. C., and H. Arnheiter. 1984. Studies of the interferon receptors. *Pharmac. Ther.* 24:259–278.
5. Standaert, M. L., and R. J. Pollet. 1984. Equilibrium model for insulin-induced receptor down-regulation. *J. Biol. Chem.* 259:2346–2354.
6. Carpenter, J., and S. Cohen. 1979. Epidermal growth factor. *Annu. Rev. Biochem.* 48:193–216.
7. Branca, A. A., C. R. Faltynek, S. B. D'Alessandro, and C. Baglioni. 1982. Interaction of interferon with cellular receptors: internalization and degradation of cell-bound interferon. *J. Biol. Chem.* 257:13291–13296.
8. Anderson, P., Y. K. Yip, and J. Vilcek. 1983. Human interferon gamma is internalized and degraded by cultured fibroblasts. *J. Biol. Chem.* 258:6497–6502.
9. Branca, A. A., and C. Baglioni. 1982. Down-regulation of the interferon receptor. *J. Biol. Chem.* 257:13197–13200.
10. Hannigan, G. E., D. R. Gewert, and B. R. G. Williams. 1984. Characterization and regulation of α -interferon receptor expression in interferon-sensitive and -resistant human lymphoblastoid cells. *J. Biol. Chem.* 259:9456–9460.
11. Sarker, F. H., and S. L. Gupta. 1984. Interferon receptor interaction: internalization of interferon α_2 and modulation of its receptor on human cells. *Eur. J. Biochem.* 140:461–467.

12. Zoon, K. C., H. Arnheiter, D. Z. Nedden, D. J. P. Fitzgerald, and M. C. Willingham. 1983. Human interferon alpha enters cells by receptor-mediated endocytosis. *Virology*. 130:195-203.
13. Hannigan, G. E., E. N. Fish, and B. R. G. Williams. 1984. Modulation of human interferon- α receptor expression by human interferon- γ . *J. Biol. Chem.* 259:8084-8086.
14. Branca, A. A., and C. Baglioni. 1981. Evidence that types I and II interferons have different receptors. *Nature (Lond.)*. 294:768-770.
15. Williams, B. R. G., and S. E. Read. 1981. Detection of elevated levels of the interferon-induced enzyme 2,5-A synthetase, in infectious diseases and on parturition. In *The Biology of the Interferon System*. E. DeMaeyer, G. Galasso, and H. Schellekens, editors. Elsevier/North Holland, New York, 111-114.
16. Gordon, D. S., B. M. Jones, S. E. Browning, T. J. Spira, and D. N. Lawrence. 1982. Persistent polyclonal lymphocytosis of B lymphocytes. *N. Engl. J. Med.* 307:232-236.
17. Hannigan, G. E., G. R. Gewert, E. N. Fish, S. E. Read, and B. R. G. Williams. 1983. Differential binding of human interferon- α subtypes to receptors on lymphoblastoid cells. *Biochem. Biophys. Res. Commun.* 110:537-544.
- 17a. Hannigan, G. E., A. S. Lau, and B. R. G. Williams. 1986. Differential human interferon-alpha receptor expression on proliferating and non-proliferating cells. *Eur. J. Biochem.* In press.
18. Dahlquist, F. W. 1978. The meaning of Scatchard and Hill plots. *Methods Enzymol.* 48:270-299.
19. Scatchard, G. 1949. The attraction of protein for small molecules and ions. *Ann. N.Y. Acad. Sci.* 51:660-672.
20. Munson, P. J., and D. Rodbard. 1980. LIGAND: A versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* 107:220-239.
21. Faltynek, C. R., A. A. Branca, S. McCandless, and C. Baglioni. 1983. Characterization of an interferon receptor on human lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA*. 80:3269-3273.
22. Sarker, F. H., and S. L. Gupta. 1984. Receptors for human γ interferon: Binding and crosslinking of ^{125}I -labeled recombinant human γ interferon to receptors on WISH cells. *Proc. Natl. Acad. Sci. USA*. 81: 5160-5164.
23. Merigan, T., K. Rand, R. Pollard, P. Abdallah, G. Jordan, and R. Fried. 1978. Human leukocyte interferon for the treatment of herpes zoster in patients with cancer. *N. Engl. J. Med.* 298:981-987.
24. Greenberg, H., R. Pollard, L. Lutwick, P. Gregory, W. Robinson, and T. Merigan. 1976. Effect of human leukocyte interferon on hepatitis B virus infections in patients with chronic active hepatitis. *N. Engl. J. Med.* 295:517-522.
25. Desmyter, J., M. Ray, J. DeGrouete, and V. Desmet. 1976. Administration of human fibroblast interferon in chronic hepatitis B virus infection. *Lancet*. 2:645-647.
26. Haglund, S., P. G. Lundquist, K. Cantell, and H. Strander. 1981. Interferon therapy in juvenile laryngeal papillomatosis. *Arch. Oto-rhino-laryngol.* 107:327-332.
27. Gutterman, J., G. Blumenschein, R. Alexanian, H. Yap, A. Buzdar, F. Cabanillas, G. Hortobagyi, E. Hersh, S. Rasmussen, M. Harmon, M. Kramer, and J. Pestka. Leukocyte interferon induced tumor regression in human metastatic breast cancer, multiple myeloma and malignant lymphoma. *Ann. Int. Med.* 93:399-406.
28. Quesada, J. R., J. Reuben, J. T. Manning, E. M. Hersh, and J. U. Gutterman. 1984. Induction of remission in hairy cell leukemia with alpha interferon. *N. Engl. J. Med.* 310:15-18.
29. Quesada, J. R., E. M. Hersh, and J. U. Gutterman. 1984. Biologic therapy of hairy cell leukemia. *Semin. Oncol.* 11(Suppl. 2):507-510.
30. Williams, B. R. G., and E. N. Fish. 1985. Interferon and viruses: in vitro studies. In *Interferons: Their Impact in Biology and Medicine*. J. Taylor-Papadimitriou, editor. Oxford University Press. 40-60.
31. Einhorn, S., H. Blomgren, and H. Strander. 1980. Interferon and spontaneous cytotoxicity in man: enhancement of spontaneous cytotoxicity in patients receiving human leukocyte interferon. *Int. J. Cancer*. 26:419-428.
32. Golub, S. H., P. D'Amore, and M. Rainey. 1982. Systemic administration of human leukocyte interferon to melanoma patients. II. Cellular events associated with changes in natural killer cytotoxicity. *J. Nat. Cancer Inst.* 68:711-717.
33. Chany, C., and M. Vignal. 1970. Effect of prolonged interferon treatment on mouse embryonic fibroblasts transformed by murine sarcoma virus. *J. Gen. Virol.* 7:203-210.
34. Gupta, S. L., B. Y. Rubin, and S. L. Holmes. 1981. Regulation of interferon action in human fibroblasts: transient induction of specific proteins and amplification of antiviral response by actinomycin D. *Virology*. 111:331-340.