Regulation of cell-surface receptors for human interferon- γ on the human histiocytic lymphoma cell line U937

David S. FINBLOOM

Food and Drug Administration, Division of Cytokine Biology, 8800 Rockville Pike, Bethesda, MD 20892, and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, U.S.A.

Interferon- γ (IFN γ) binds to high-affinity receptors on monocytes and is rapidly internalized. This study investigates the ability of the human monocyte-like cell line, U937, to regulate the cell-surface expression of the IFN γ receptor (IFN γ R) during endocytosis of ligand. Recombinant IFN γ was radiolabelled to high specific radioactivity with Bolton–Hunter reagent and used to enumerate IFN γ R on treated U937 cells. Cells which had internalized IFN γ for up to 3 h displayed maximal levels of IFN γ R at all time points tested after all unlabelled IFN γ had been acid-stripped from the cell at pH 2.78. Therefore there was no evidence of down-modulation of the receptor. After trypsin treatment of the IFN γ R, the cells were able to synthesize and insert into the cell membrane up to 1000 IFN γ R molecules/h after a 60 min lag. Since biosynthesis played a minor role during the first 30 min of endocytosis, I examined other possibilities to explain the lack of down-modulation of the receptor. A solubilized-receptor assay revealed the presence of an intracellular pool of receptors equal to about 25 % of the number of cell surface receptors. Using trypsin to differentiate between intracellular and surface receptors, I observed that 43 % of those receptors that were internalized after a 30 min exposure to IFN γ (580 molecules) could be recycled back to the plasma membrane. In addition, equal rates of receptor decay ($t_{1/2} = 5$ h) were observed in the presence of cycloheximide with or without IFN γ . All the data taken together suggest that during the first 30 min of endocytosis both the expression of an intracellular source of receptor and recycling of internalized receptors contribute to maintain optimal receptor expression.

INTRODUCTION

In response to antigen, T lymphocytes secrete interferon- γ (IFN γ), a cytokine that interacts with and influences most cells of the immune system. In particular, IFN γ is necessary for the activation of macrophages [1], which play major roles in the development of delayed-type hypersensitivity [2], chronic inflammation [3] and host defence [4]. Upon exposure to IFN γ , macrophages express enhanced class II major histocompatibility antigens [5], Fc receptors [6], and anti-microbicidal [4] and anti-tumoricidal [7] activities. Induction of such activities requires binding of IFN γ to its receptor and subsequent signal transduction.

It is known from our own recent studies that induction of biological responsiveness requires receptor occupancies of varying durations and degrees [8]. Whereas Fc receptor enhancement results after receptor occupancy of short duration (15–30 min) and minimal occupancy (10–20 %), anti-proliferation results only after multiple exposures for 1 h or more at an occupancy of 50 % or greater [8]. Data are also available which suggest that durations of exposure of 4 h or more to IFN γ are necessary for anti-tumoricidal activity [9]. Although IFN γ is rapidly internalized on binding to the cell [8], the mechanisms underlying the regulation of the receptor itself during endocytosis are much less well understood. Previous studies [10–12] present conflicting data regarding the modulation of IFN γ receptors (IFN γ Rs), and few provide adequate detail as to the possible mechanisms involved in maintaining cell-surface receptor capacity on human monocytes, especially during ongoing endocytosis of ligand. In this study I characterize the ability of the human histiocytic lymphoma monocyte-like cell line U937 to maintain maximal receptor expression in spite of ongoing internalization of ligand and receptor. I observe that during

active endocytosis of rIFN γ there is not down-modulation of the cell-surface expression of receptors. The proficiency of the cell to maintain optimal IFN γ R expression early in endocytosis derives from the cell's ability to mobilize intracellular receptors and to recycle a portion of those receptors that have been internalized with ligand.

MATERIALS AND METHODS

Cells

U937 cells were cultured in spinner flasks (Bellco, Vineland, NJ, U.S.A.) at an initial inoculum of $(1-2) \times 10^5$ /ml in RPMI 1640 medium (Gibco, Grand Island, NY, U.S.A.) with < 25 pg of endotoxin/ml, 10 % (v/v) fetal bovine serum (Hyclone, Logan, UT, U.S.A.) and 50 μ g of gentamicin/ml (Whittaker Bioproducts, Walkersville, MD, U.S.A.). Cells were used when they had reached concentrations of $(0.8-1.2) \times 10^6$ /ml. Viability was > 95 % for all cultures, as determined by Trypan Blue exclusion.

IFN γ

Recombinant human IFN γ , prepared in *Escherichia coli*, was generously provided by Genentech Inc. (San Francisco, CA, U.S.A.) and Hoffmann–La Roche, (Nutley, NJ, U.S.A.). The preparation was greater than 99 % pure, as determined by SDS/PAGE analysis. IFN γ was radioiodinated with Bolton–Hunter reagent (Amersham, Arlington Heights, IL, U.S.A.) as previously described [13,14]. For double-label experiments, rIFN γ was radiolabelled with carrier-free 131 I (New England Nuclear, Boston, MA, U.S.A.) using a modification of the iodine monochloride methodology [15]. A 10 μ g portion (0.5 pmol) of rIFN γ was radiolabelled with 1 mCi of 125 I using

1.7 pmol of ICl in a final volume of 30 μ l. Specific radioactivities ranged from 200 to 400 mCi/mmol. Generally, about 2000–4000 c.p.m. were specifically bound to 10^6 cells. Bindability was > 70 % and bioactivity remained intact, as determined using an anti-viral assay. For each iodination (11 in total) an equilibrium binding curve was generated from data using U937 cells. This was used to calculate the number of receptors per cell and the K_d . The U937 cells expressed 3000–4000 receptors per cell for the duration of these studies, with specificity of binding being 90 % or greater. For calculations in which molecules/cell are used, an average number of 3400 molecules/cell was chosen.

Soluble-receptor assay

The following assay was performed as a modification of a previously described method [16]. U937 cells (8×10^7) were solubilized in 1 ml of either 40 mM-octyl glucoside (Pierce, Rockville, IL, U.S.A.) or 10 mM-CHAPS (Boehringer Mannheim, Indianapolis, IN, U.S.A.) in borate-buffered saline (0.15 M-NaCl/0.2 M- $\text{Na}_2\text{B}_4\text{O}_7$, pH 8.0), containing 1 mg of human serum albumin/ml (American Red Cross), 2 μ M-phenyl-methanesulphonyl fluoride (Boehringer Mannheim) and 5 μ g of each of pepstatin (Sigma, St. Louis, MO, U.S.A.), leupeptin (Sigma) and aprotinin (Calbiochem, La Jolla, CA, U.S.A.)/ml at 4 °C for 15 min. After centrifugation for 15 min at maximal speed in a Beckman Microfuge (Beckman, Palo Alto, CA, U.S.A.), the post-nuclear extract was removed and then used in the binding assay. To 150 μ l of extract (12×10^6 cell equivalents) was added an amount of ^{125}I -rIFN γ that was 1.5 times greater than the estimated number of receptor molecules present in the extract (4×10^{10} molecules). For non-specific controls, a 1000-fold excess of unlabelled IFN γ was added before addition of the radiolabelled ligand. After 1 h on ice, 50 μ l portions were added to 1 ml of 9 % poly(ethylene glycol) (PEG) (Sigma) in Tris-buffered saline (0.15 M-NaCl/0.025 M-Tris, pH 7.4) to which was added carrier proteins [25 μ l of 40 mg/ml solutions of each of human serum albumin and human γ -globulin (Miles Labs., Naperville, IL, U.S.A.)] and incubated for 15 min on ice. The PEG mixture was then centrifuged in the cold for 15 min at maximal speed. The supernatant was aspirated, the tip of the tube containing the pellet was cut off and the radioactivity was assessed in an LKB Clinigamma counter (LKB, Piscataway, NJ, U.S.A.). At this concentration of PEG, 10 % or less of free ^{125}I -rIFN γ was precipitable.

Binding of ^{125}I -rIFN γ to cells

The binding of radiolabelled rIFN γ to U937 cells was performed as previously described [14]. For all binding experiments saturating concentrations of ^{125}I -rIFN γ were utilized (25–50 ng/ml), as determined by the equilibrium binding studies. For all studies, non-specifically bound radioactivity was determined in the presence of a 200-fold molar excess of unlabelled rIFN γ . Internalized rIFN γ was determined by incubating cells, which had been exposed to ^{125}I -rIFN γ at 37 °C, in acid buffer (0.125 M-NaCl/0.025 M-glycine, pH 2.78) for 2 min at 4 °C [8]. This procedure removed up to 95 % of the cell-surface ligand [14].

Trypsin treatment of U937 cells

Washed U937 cells were resuspended in RPMI 1640 medium at $(1-5) \times 10^6$ cells/ml. Cell-surface receptors for IFN γ were degraded by either 0.1 mg of Tos-Phe- CH_2Cl ('TPCK')-treated trypsin/ml (Sigma) for 45 min, or 0.4 mg of trypsin/ml for 15 min at 37 °C. The reaction was quenched with RPMI 1640 containing 5–10 % fetal bovine serum. For re-expression of receptors, cells were then further cultured at 37 °C in RPMI 1640

with 1 mg of human serum albumin/ml for up to 3 h with or without 1–5 μ g of cycloheximide (CHX)/ml (Sigma) or 50 μ M-monomensin (Calbiochem). At various times thereafter cell-surface receptor expression was measured as described. Concentrations of CHX of 1–5 μ g/ml resulted in 90 % inhibition of [^{35}S]methionine (Amersham) incorporation into acid-precipitable proteins. This inhibition was induced within 15 min of incubation with CHX (results not shown).

Assessment of receptor recycling

Two types of experiments were employed to assess the ability of internalized receptors to recycle. In one, cells were cultured in the presence of 1–5 μ g of CHX/ml with or without saturating concentrations of unlabelled rIFN γ at 37 °C. At various times cells were removed, acid-stripped of cell-surface rIFN γ , and residual receptors were assessed by radioligand binding. In the other experiment, cells were precultured with CHX for 15 min at 37 °C, and then one group was exposed to saturating concentrations of unlabelled rIFN γ for an additional 30 min at 37 °C. After a quick wash with warmed media, the cells were resuspended in 0.4 mg of trypsin/ml for 15 min at 37 °C in order to remove all receptors and any IFN γ remaining on the surface. The reaction was then quenched with RPMI 1640 supplemented with 10 % fetal bovine serum, then the cells were washed and continued in culture for an additional 2 h at 37 °C. During this time portions of cells were removed and receptors were enumerated by radioligand binding. The amount of ^{125}I -rIFN γ bound was expressed as a percentage of the total amount of radiolabelled ligand added.

RESULTS

IFN γ R expression during endocytosis of IFN γ

U937 cells internalize 40 % of ^{125}I -rIFN γ molecules bound to the cell during 30 min at 37 °C [8]. Whether or not this results in down-modulation of the IFN γ R was investigated by incubating U937 cells with 25–50 ng of unlabelled rIFN γ /ml (> 90 % receptor occupancy) for 15–180 min at 37 °C. The cells were immediately cooled to 4 °C, and any ligand remaining extracellular was acid-stripped. The cells were then exposed to 25 ng of ^{125}I -rIFN γ /ml for 1–2 h to enumerate the number of IFN γ -binding sites remaining on the cell surface. Data from five separate experiments revealed that at no time during the 3 h of endocytosis was there demonstrable down-modulation of the receptor. At all times there was essentially maximal receptor expression. The mean of the values for each of the various time points was in the range 93–100 % of control (control radioactivity/ 10^6 cells was 2600 c.p.m.).

Since the unlabelled rIFN γ could not be directly assayed, it had to be presumed that the IFN γ was being internalized. This problem was circumvented by carrying out the same experiment using double-labelled IFN γ . ^{125}I -rIFN γ was initially incubated with the cells at 37 °C for 30–45 min. Then the cells were acid-stripped of any remaining extracellular ^{125}I -rIFN γ and exposed to ^{131}I -rIFN γ for 1 h at 4 °C in order to enumerate binding sites still available on the cell surface. After the initial incubation 40 ± 7 % mean $\pm$ S.D., $n = 4$) of the ^{125}I -rIFN γ initially bound to the cell was intracellular. At the same time as 40 % of the ^{125}I -rIFN γ was intracellular, there was maximal binding of the ^{131}I -rIFN γ (108 ± 21 %) following an acid strip to remove cell-surface-bound ^{125}I -IFN γ . The mean radioactivity for ^{131}I -rIFN γ bound to 10^6 cells was 4781 c.p.m. These results confirmed the ability of the cell to maintain full receptor capacity during ongoing endocytosis of ligand.

That the internalized rIFN γ was actually still associated with

Table 1. Soluble receptor assay for IFN γ Rs on cells that have internalized IFN γ

U937 cells were allowed to internalize ^{125}I -rIFN γ as described in the Materials and methods section. The cells were then divided into four groups. One group of cells was immediately assayed for cell-associated rIFN γ and acid-resistant (intracellular) ligand. The other group of cells was solubilized, half of them after an acid treatment to remove extracellular ligand. In the solubilized cells the receptor-IFN γ complex was precipitated in 9% PEG. The values in parentheses refer to the percentages of total radioactivity that are acid-resistant. The mean ($\pm$ S.D.) intracellular ^{125}I was $51 \pm 7\%$ in solubilized cells and $48 \pm 4\%$ in intact cells.

Expt. no.	^{125}I radioactivity (c.p.m.)			
	Solubilized cells		Intact cells	
	Control	Acid	Control	Acid
1	2108	908 (43)	3441	1600 (46)
2	832	478 (57)	1826	924 (50)
3	825	397 (48)	1969	1010 (51)
4	1040	582 (56)	2101	911 (43)

Fig. 1. Effects of CHX and monensin on the re-expression of IFN γ Rs after trypsin treatment

U937 cells were exposed to 0.1 mg of trypsin/ml for 45 min in spinner flasks at 37 °C, washed well with medium containing fetal bovine serum and then cultured for the times shown at 37 °C. At the designated time points the cells were cooled and receptors for IFN γ were determined by ^{125}I -rIFN γ binding. The radioactivity bound to 10^6 control cells was 3615 c.p.m. The Figure is representative of four experiments. ○, Monensin; ●, cycloheximide; Δ, control.

the receptor was suggested by the results of the following experiment. U937 cells were allowed to internalize radiolabelled rIFN γ for 30 min at 37 °C. Some of the cells were then assayed immediately (intact cells, Table 1) for both total cell-associated rIFN γ and intracellular rIFN γ (acid-resistant). The other cells (one set untreated and the other set acid-stripped) were solubilized (solubilized cells, Table 1) in non-ionic detergent and the post-nuclear extract was analysed for the receptor-IFN γ complex by

precipitation with 9% PEG. Only those ^{125}I -rIFN γ molecules complexed to the receptor are precipitated at this concentration of PEG. Since there was not 100% recovery of receptor in the solubilized cells, the results were expressed as the acid-resistant radioactivity as a % of total cell-associated, and the values compared between solubilized and intact cells. As can be seen in Table 1, both groups of cells had a similar percentage of acid-resistant radioactivity (intact cells 48%; solubilized cells 51%). This suggested that the internalized rIFN γ was still bound to its receptor at the time studied.

Re-expression of receptor after trypsin treatment

In order to obtain information on the capacity of the cell to synthesize IFN γ receptors, I treated U937 cells with trypsin to remove 80–90% of cell-surface receptors and then monitored the re-expression of receptors by radioligand binding in the absence or presence of various inhibitors. The data presented in Fig. 1 are representative of four such experiments. Exposure of the cells to 0.1 mg of trypsin/ml for 45 min at 37 °C typically resulted in the removal of at least 80% of the cell-surface receptors. When allowed to recover at 37 °C, there was usually a lag of 30–60 min followed by a rapid re-expression of receptors to near-original levels during the next 2 h of culture. Since these cells generally expressed about 3400 receptors/cell [8,14], during maximal recovery the cells could place about 1000 receptors/h in the plasma membrane. When CHX was added to cells after trypsin treatment there was essentially complete inhibition of receptor re-expression. In addition to abrogating any recovery of receptor expression, the cells treated with monensin continued to show a fall in receptor number over the 3 h period studied. If U937 cells were exposed to CHX for only 1.5 h after the trypsin treatment, the cells began to re-express surface receptors for IFN γ soon after the CHX was washed out. Recovery comparable with that in cells which had no prior exposure to CHX occurred within 2 h (results not shown).

Assessment of recycling of internalized receptors

I initially studied the ability of the cells to maintain receptor levels during treatment with monensin. However, since monensin not only inhibits receptor recycling but also protein glycosylation and transport, several mechanisms could explain the results obtained. U937 cells were exposed to 50 μM -monensin in the presence or absence of IFN γ (25 ng/ml) for 30 min at 37 °C. There was a loss of about 50–60% of the receptors exposed on the cell surface. Since the effect of monensin occurred in both the presence and the absence of IFN γ , this suggested that the IFN γ R might be internalized irrespective of receptor occupancy, and that monensin prevented presynthesized or recycled receptors from inserting in the plasma membrane. I next carried out some experiments to assess more directly receptor recycling as a possibility.

Cells were allowed to internalize IFN γ for 30 min at 37 °C. These cells had been pretreated with CHX at 5 $\mu\text{g}/\text{ml}$ for 15 min and were continued in culture with CHX so that protein synthesis was inhibited throughout the experiment. After internalization of IFN γ had occurred, the cells were trypsin-treated for 15 min at 37 °C so that all surface receptors and any remaining IFN γ was removed. The cells were washed and then allowed to recover over the next 2 h at 37 °C. In those cells exposed to IFN γ , intracellular receptor-ligand complexes would now be resistant to trypsin. If these receptors were recycled, then during the post-trypsin recovery period these cells should express more ligand-binding sites sooner than control cells. This result was observed in four experiments, the means of which are presented in Fig. 2. The data are presented as percentages of total ^{125}I -rIFN γ added

Fig. 2. Assessment of recycling of IFN γ R

U937 cells were precultured for 15 min in either medium alone (control; not shown) or with CHX (5 μ g/ml). The CHX-treated cells were divided into two groups; unlabelled IFN γ was added for 30 min at 37 °C to one group but not to the other. Both groups of cells were washed after the 30 min incubation and then trypsin-treated for 15 min to remove all IFN γ and receptors. The cells were allowed to recover at 37 °C, and at various times the numbers of receptors were determined by 125 I-rIFN γ binding. The data from four experiments are presented as means $\pm$ S.E.M. of the amount of IFN γ bound per 10^6 cells, expressed as a percentage of the total 125 I-rIFN γ added to each culture. Maximal binding for control cells (3400 molecules/cell) was 35% at 2 h post-trypsin. The mean radioactivity specifically bound to 10^6 cells not exposed to trypsin was 2366 c.p.m. ●, CHX alone; Δ , IFN γ plus CHX.

to the assay. In cells not exposed to CHX or IFN γ , a value of 35% for maximal binding occurred at 2 h after trypsin treatment. This was the equivalent of 3400 molecules bound/cell. At 15 min post-trypsin, both groups demonstrated an abrupt increase in receptor number, which could be explained by the appearance of presynthesized receptors. In the CHX alone group, the difference (5.6%) from time zero ($3.9 \pm 1.4\%$) to 15 min ($9.5 \pm 0.7\%$) represented the equivalent of 16% of the total cell surface number, or about 550 receptors, that could be mobilized from an intracellular source. In those cells pre-exposed to IFN γ , however, the difference (11.3%) from zero time ($4.2 \pm 1.8\%$) to 15 min ($15.5 \pm 1.7\%$) was 2-fold greater than that seen with cells exposed to CHX alone and was consistent with the recycling of protected internalized receptors. The difference (6% of total 125 I-rIFN γ added) between the two values at 15 min ($15.5 \pm 1.7\%$ versus $9.5 \pm 0.7\%$; means $\pm$ S.E.M.; $P < 0.02$) represents 580 molecules recycled. Therefore, if 1360 molecules (40% of 3400) are intracellular at 30 min, 43% of those molecules were recycled during the first 15 min of recovery after trypsin treatment.

Further evidence for recycling of receptors was obtained from the following experiment. U937 cells were incubated with CHX in the presence or absence of IFN γ for up to 120 min at 37 °C. The cells were subjected to an acid strip to remove any remaining cell-bound unlabelled IFN γ , and then the number of IFN γ receptors was assayed by radioligand binding. If receptors were internalized and not recycled, the decay of receptor number over time could be greater in the cells incubated with IFN γ plus CHX. As seen in Fig. 3, the decay of the IFN γ R was similar in both groups of cells. This suggested that the internalization of receptors in the IFN γ group during the incubation period was matched by

Fig. 3. Determination of decay of IFN γ R in the absence or presence of IFN γ

U937 cells were cultured for 2 h at 37 °C in the absence or presence of 50 ng of IFN γ /ml with 1–5 μ g of CHX/ml. The cells were then washed, acid-stripped to remove all surface-bound IFN γ and exposed to 125 I-rIFN γ to determine receptor number. The data are means $\pm$ S.E.M. of 5–7 experiments and are presented as percentages of control values at time zero. ○—○, CHX alone; ●—●, IFN γ plus CHX. Radioactivity specifically bound in one of the experiments for 10^6 cells was 2427 c.p.m.

Table 2. Determination of the amount of intracellular IFN γ R using a soluble-receptor assay

U937 cells were solubilized as described in the Materials and methods section either without treatment or after trypsin treatment. 'Receptor remaining' refers to the amount (% of control) of receptor remaining after treatment of the cells with trypsin. 'Intracellular receptor' was calculated by subtracting the percentage of receptors remaining after trypsin treatment in intact cells from that in solubilized cells. The mean ($\pm$ S.D.) intracellular receptor content was $24 \pm 16\%$.

Expt.	Solubilized cells			Intact cells: receptor remaining (%)	Intracellular receptor (%)
	Control (c.p.m.)	Trypsin- treated (c.p.m.)	Receptor remaining (%)		
1	940	325	34	19	15
2	803	354	44	20	24
3	758	361	47	8	39
4	948	191	20	20	0

a re-expression of receptors by mechanisms not requiring protein synthesis. In addition, these studies were consistent with a half-life for the IFN γ receptor of 5 h.

Intracellular pool of receptors

One final mechanism for the regulation of cell-surface receptor expression involves the transport to the cell surface of receptors presynthesized within the cytoplasm. In order to obtain some additional information regarding an intracellular pool of receptors, I directly measured soluble receptors in intact cells and trypsin-treated cells (Table 2). Since the assay did not allow the recovery of all available receptors, the data are presented as intracellular receptors as a percentage of the total 125 I-rIFN γ

bound to the iFN γ R for both intact and solubilized cells. Although there was wide variation from experiment to experiment, an amount of intracellular receptors equivalent to about one-quarter of that exposed on the cell surface was demonstrated.

DISCUSSION

IFN γ is the predominant factor leading to activation of monocytes, resulting in enhanced expression of a variety of surface antigens and receptors that play a vital role in the cell-to-cell communication needed for effective immune responsiveness. In this report I have characterized how the monocyte-like cell line U937 maintains optimal expression of cell-surface IFN γ receptors during endocytosis of both ligand and receptor. There is no evidence from the experiments presented herein that in U937 cells the receptor for IFN γ is down-modulated by ligand during endocytosis. The cells optimize receptor expression both by recycling internalized receptors and by mobilizing a presynthesized source of intracellular receptors into the plasma membrane. If necessary, the cell has the additional capability to synthesize and insert into the membrane up to about 1000 molecules of IFN γ R/h. However, biosynthesis of receptors appears to play a minor role in maintaining optimal levels of surface receptor during the first 30–60 min of endocytosis.

Although no down-modulation of the IFN γ R is observed in U937 cells, ligand-induced changes in receptor expression may be dependent upon the cell type studied. In the leukaemic murine cell line L1210, low concentrations of IFN γ (100 units/ml) are able to lower the receptor number within 3 h [11]. However, acid-stripping of cells was not done in this study, and the possibility exists that IFN γ remained bound to a portion of the receptors on the surface. In contrast, no change in receptor number is demonstrable in murine macrophages [10]. HeLa cells also show no alteration in receptor expression in response to ligand [12]. In the same study, however, human monocytes down-modulated their receptor in the presence of 500 units of IFN γ /ml. In these experiments monocytes were exposed to 125 I-IFN γ for 3 h at 37 °C and then the receptor number was evaluated by measuring acid-dissociable radioactivity following a further incubation at 4 °C with 125 I-IFN γ . Since an acid-strip of radioactivity after the initial incubation was not performed, and since both the initial and subsequent incubation used 125 I-rIFN γ , an accurate assessment of the number of molecules remaining bound to the receptors after the 37 °C incubation cannot be made in this study.

The ability of U937 cells to maintain optimal IFN γ R expression during endocytosis results from both the recycling of a portion of the internalized receptors and the expression of a presynthesized intracellular source of receptor. The evidence for the recycling comes from two types of experiments (Figs. 2 and 3) similar to those used to study the tumour-necrosis-factor receptor [17]. From the experiments described in Fig. 2, the data are consistent with about 600 internalized IFN γ R molecules being recycled back to the surface of the cell and about 550 receptors being mobilized into the membrane from a presynthesized intracellular source. In the other experiment (Fig. 3), the rate of decay of IFN γ R was similar in cells exposed to CHX in both the presence and the absence of IFN γ . The lack of an increase in the decay rate in IFN γ -treated cells implies that these cells are able to maintain receptor expression in spite of active internalization of IFN γ -receptor complexes. This can occur by recycling of internalized receptors or possibly by the insertion into the membrane of presynthesized intracellular receptors. Although not directly addressed in this report, the finding that monensin alone (without IFN γ) induces a decrease in IFN γ R expression can be consistent with constitutive

internalization of the receptor. Whether or not this potential pathway is similar to a ligand-driven route remains to be determined.

The presence of intracellular receptors has been suggested by some studies employing permeabilization agents such as saponin and digitonin [18]. Since I am unable to demonstrate specific binding of 125 I-rIFN γ to permeabilized U937 cells, a soluble-receptor assay which measures specifically bound IFN γ has been employed. With this assay intracellular receptors exist at an amount equal to about 25 % of the surface receptor population. This is not that dissimilar to the 50 % value reported elsewhere [18]. Whether or not these receptors represent an actual receptor pool or merely represent newly synthesized receptors is as yet unknown.

As measured in the present report, the IFN γ R has a half-life of 5 h. This half-life is in between those of receptors which are known to be internalized and degraded (tumour necrosis factor, 2 h [17]; interleukin-2, 1 h [19]) and those known to be recycled (transferrin [20] and asialoglycoproteins, 20 h [21]). Therefore exclusively one mechanism or the other probably does not occur in U937 cells. As the IFN γ receptors enter the cell, a portion may become available for recycling back to the plasma membrane, whereas some will remain with IFN γ and become degraded. With 3400 receptors/cell, 1360 (40 %) are internalized at 30 min, and therefore 1360 receptors have entered the plasma membrane in order to maintain optimal receptor expression; 550 of these 1360 receptors can be derived from presynthesized intracellular sources. Another 580 receptors (43 % of those internalized) can be recycled back to the plasma membrane. Biosynthesis may contribute up to 200 receptors during the first 30 min of internalization (see Fig. 1). After the first 1 h of endocytosis, rates of biosynthesis can almost approach internalization rates and therefore will more than likely play an increasingly important role.

Thus both recycling and re-expression of presynthesized receptors help equally to maintain optimal receptor density on the surface of U937 cells during the first 30 min of endocytosis. In addition, the ability of these cells to rapidly synthesize the receptor clearly enables the cells to insert newly synthesized receptors over a longer time period (1–3 h). Since the precise role of the internalized IFN γ -receptor complex is still being currently defined with respect to biological effect [22], studies which clarify its behaviour during endocytosis will be of value in determining its importance in the activation of cells.

I acknowledge Paula Smith and Karen Winestock for excellent technical support and I thank Dr. Kathryn Zoon, Dr. Robert Kozak and Dr. Gerald Feldman for critical review of the manuscript.

REFERENCES

- Schreiber, R. D. (1984) *Contemp. Top. Immunobiol.* **13**, 171–198
- Issekutz, T. B., Stoltz, J. M. & van der Meide, P. (1988) *Clin. Exp. Immunol.* **73**, 70–75
- Cooper, S. M., Sriram, S. & Ranges, G. E. (1988) *J. Immunol.* **141**, 1958–1962
- Murray, H. W. (1988) *Ann. Intern. Med.* **108**, 595–608
- Becker, S. (1984) *J. Immunol.* **132**, 1249–1254
- Guyre, P. M., Morganelli, P. M. & Miller, R. (1983) *J. Clin. Invest.* **72**, 393–397
- Meltzer, M. S., Crawford, R. M., Finbloom, D. S. & Nacy, C. A. (1988) in *Biological Response Modifiers and Cancer Research* (Chiao, J. W., ed.), pp. 335–362, Marcel Dekker, New York
- Finbloom, D. S. (1988) *Clin. Immunol. Immunopathol.* **47**, 93–105
- Meltzer, M. S., Occhionero, M. & Ruco, L. P. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2198–2205
- Celada, A. & Schreiber, R. D. (1987) *J. Immunol.* **139**, 147–153
- Wietzerbin, J., Gaudelet, C., Aguet, M. & Falcoff, E. (1986) *J. Immunol.* **136**, 2451–2455

12. Fischer, D. G., Novick, D., Orchansky, P. & Rubinstein, M. (1988) *J. Biol. Chem.* **263**, 2632–2637
13. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–538
14. Finbloom, D. S., Hoover, D. L. & Wahl, L. M. (1985) *J. Immunol.* **135**, 300–305
15. Helmkamp, R. W., Goodland, R. L., Bale, W. G., Spar, I. L. & Mutschler, L. E. (1960) *Cancer Res.* **20**, 1495–1500
16. Calderon, J., Sheehan, K. C. F., Chance, C., Thomas, M. L. & Schreiber, R. D. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4837–4841
17. Watanabe, N., Kuriyama, H., Sone, H., Neda, H., Yamauchi, N., Maeda, M. & Niitsu, Y. (1988) *J. Biol. Chem.* **263**, 10262–10266
18. Celada, A., Allen, R., Esparza, I., Gray, P. & Schreiber, R. D. (1985) *J. Clin. Invest.* **76**, 2196–2205
19. Duprez, V. & Dautry-Varsat, A. (1986) *J. Biol. Chem.* **261**, 15450–15454
20. Klausner, R. D., van Renswoude, J., Ashwell, G., Kempf, C., Schechter, A. N., Dean, A. & Bridges, K. R. (1983) *J. Biol. Chem.* **258**, 4715–4724
21. Schwartz, A. L., Fridovich, S. E. & Lodish, H. F. (1982) *J. Biol. Chem.* **257**, 4230–4237
22. Smith, M. R., Muegge, K., Keller, J. R., Kung, H., Young, H. A. & Durum, S. K. (1990) *J. Immunol.* **144**, 1777–1782

Received 13 August 1990/24 October 1990; accepted 1 November 1990