Two Molecular Forms of the Human Interferon-γ Receptor

LIGAND BINDING, INTERNALIZATION, AND DOWN-REGULATION*

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Dina G. Fischer, Daniela Novick, Patricia Orchansky, and Menachem Rubinstein‡

From the Department of Virology, Weizmann Institute of Science, Rehovot 76100, Israel

The receptors for human interferon-γ (IFN-γ) on peripheral blood monocytes and various cells of nonhematopoietic origin were thoroughly characterized and compared. The receptors of all cell types exhibited a similar affinity for IFN-γ (K<sub>d</sub> ~ 1 x 10<sup>-10</sup> M), and in all cases receptor-mediated endocytosis and ligand degradation were demonstrated. However, the receptors differed in their molecular weights (95,000 in HeLa cells and 140,000 in monocytes, assuming a 1:1 ligand to receptor ratio) as concluded from experiments of cross-linking to <sup>125</sup>I-IFN-γ. Lower molecular weight species were obtained as well, particularly in monocytes. Such species could represent either degradation products or subunit structures. The monocyte and HeLa receptor responded differently to an excess of ligand. A significant receptor down-regulation was observed when monocytes were incubated with an excess of <sup>125</sup>I-IFN-γ, whereas no such down-regulation was observed in HeLa cells or in normal fibroblasts. This differential response was observed both in the presence or in the absence of a protein synthesis inhibitor. The receptor on monocytes was found to be acid-labile whereas that on HeLa cells was resistant to acid treatment. These and additional experiments indicate that the monocyte receptor is inactivated following treatment. These and additional experiments indicate that the monocyte receptor is inactivated following internalization, whereas the HeLa receptor retains its structure and recycles back to the cell surface. The difference in the properties and fate of these two receptor subtypes is probably related to the differential functions of IFN-γ in various cell types.

Interferon-γ (IFN-γ) is a lymphokine produced by activated T-lymphocytes. It exerts antiviral activity, growth inhibitory effect, and several immunoregulatory activities on a variety of cell types (1). IFN-γ elicits its various activities via a specific cell-surface receptor. The existence of a specific receptor for IFN-γ on various human and mouse cells was demonstrated in several studies (2–19). In all cases, specific receptors were demonstrated by high affinity binding of <sup>125</sup>I-labeled IFN-γ (K<sub>d</sub> < 10<sup>-10</sup> M) and by specific competition with unlabeled IFN-γ. The exclusivity of the receptor for IFN-γ was concluded from the finding that other types of IFN did not compete for binding to the receptor (4, 6, 7, 10, 19). Cross-linking experiments of labeled IFN-γ to intact cells yielded in most studies complexes of apparent molecular weights of 90,000–125,000 (6, 7, 9, 12). In some other studies complexes of an apparent molecular weight of 165,000 were observed (18, 20).

We have recently presented evidence that human peripheral blood monocytes have an IFN-γ receptor (IFN-γR) that is structurally and functionally different from its counterpart in cells of nonhematopoietic origin (11). In the present study, we have further characterized and compared the IFN-γR of human monocytes to the receptor from cells of nonhematopoietic origin. We demonstrate here that both forms of the IFN-γR have a similar affinity for IFN-γ and in both cases the ligand is internalized and degraded. However, these receptors differ significantly with respect to their acid lability and their fate following internalization.

EXPERIMENTAL PROCEDURES*

RESULTS

Surface Binding of IFN-γ—Various cell types of both hematopoietic and nonhematopoietic origin were found to exhibit high affinity for <sup>125</sup>I-IFN-γ. In all cases, one class of binding sites was deduced from the linear Scatchard plots of <sup>125</sup>I-IFN-γ binding (Figs. 1 and 2, insets). Dissociation constants of 1.3 x 10<sup>-10</sup> M ± 7% and 1 x 10<sup>-10</sup> M ± 13% were determined by competition studies with unlabeled ligands for HeLa cells and monocytes, respectively (Figs. 1 and 2). Similar values were obtained for binding to many other cell types such as foreskin fibroblasts (FS11), epithelial cell lines (WISH), and cell lines of hematopoietic origin (e.g. U-937, KG-1, and Daudi; data not shown). The number of receptors per cell was found to be 8400 ± 200 and 1400 ± 100 for HeLa cells and monocytes respectively (Figs. 1 and 2). In all cases, nonspecific binding was less than 1% as found by the LIGAND program and less than 5% by using an excess of unlabeled IFN-γ.

Cross-linking of <sup>125</sup>I-IFN-γ to Cells—Cross-linking of <sup>125</sup>I-IFN-γ to various cells revealed the presence of different molecular forms of IFN-γR. In HeLa cells, a complex of molecular weight 120,000 was seen in autoradiographs of SDS-polyacrylamide gel electrophoresis. Additional minor bands corresponding to molecular weights of 90,000 and 70,000 were also seen (Fig. 3). A similar pattern of bands was observed in other cell lines of nonhematopoietic origin such as FS11 and WISH. Cross-linking of <sup>125</sup>I-IFN-γ to monocytes gave a faint band corresponding to a molecular weight of 165,000 as well as intense broad bands of molecular weights 60,000–80,000 and 90,000 (Fig. 3). The 120,000 and the 165,000 bands were mutually exclusive in the various cell

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‡Has the Edna and Maurice Weiss Chair in Interferon Research.

§The abbreviations used are: IFN-γ, interferon-γ; IFN-γR, interferon-γ receptor; SDS, sodium dodecyl sulfate.

2 Portions of this paper including "Experimental Procedures" and Figs. 1, 2, and 4–6 are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
The level of surface-bound IFN-γ decreased by 50% within 45 min in HeLa cells and within 1 h in monocytes. The level of internalized 125I-IFN-γ rose rapidly during the first 30 min. However, in the absence of free 125I-IFN-γ, the rate of internalization was reduced on further incubation. This reduction coincided with the disappearance of surface-bound 125I-IFN-γ. The level of internally accumulated IFN-γ started to decline after 2 h due to a process of degradation followed by excretion of the degradation products which was apparent after a lag of 30 min. The efficiency of the degradation process was demonstrated by an additional experiment in which the cells were preincubated at 37 °C with an excess of 125I-IFN-γ, washed, and further incubated at 37 °C. Under these conditions, extensive internalization was allowed before excess free IFN-γ was washed away. Thereafter, most of the cell-associated 125I-IFN-γ was released within 3 h as degradation products, and its level dropped to 5% and 20% of the initial values in HeLa cells and in monocytes, respectively (Fig. 6). Some of the cell-associated 125I-IFN-γ was released intact as revealed by trichloroacetic acid precipitation. This release was due to the dilution effect, it corresponded to less than 15% of the total radioactivity, and it did not increase after the first 15 min of incubation (not shown). In conclusion, both cell types were capable of internalizing and degrading IFN-γ with a similar efficiency.

**Down-regulation of the Receptors**—The level of surface-bound 125I-IFN-γ was determined after incubation of cells with an excess of 125I-IFN-γ for different time periods at 37 °C. The level of surface-bound 125I-IFN-γ in HeLa cells was not reduced during the entire incubation period (3 h, Fig. 7). On longer incubations (more than 6 h, data not shown), a reduction in the level of surface binding was noticed, but it was attributed to depletion of 125I-IFN-γ from the medium. Indeed, when the medium was replaced by a fresh medium containing 125I-IFN-γ at the initial concentration, the original level of surface binding was restored. Similar results were observed in experiments with WISH cells and with fibroblasts (not shown). In contrast, the level of surface-bound 125I-IFN-γ on monocytes was gradually reduced and reached 50% of

![Fig. 3. SDS-polyacrylamide gel electrophoresis of cross-linked complexes of 125I-IFN-γ and cells.](image-url)

Monocytes (1.5 × 10^6, lanes b and c) or HeLa cells (1 × 10^6, from EDTA-treated monolayers, lanes d and e) were incubated with 125I-IFN-γ (20,000 units) at 4 °C for 90 min in the presence (lanes b and c) or absence (lanes d and e) of an excess of unlabeled IFN-γ (2 × 10^6 units). Following cross-linking with disuccinimidyl suberate, the cells were washed and solubilized. 125I-IFN-γ and its cross-linked products were immunoprecipitated by rabbit anti-IFN-γ and protein-A-agarose. The agarose beads were suspended in a sample buffer, and the supernatant was analyzed by SDS-polyacrylamide gel electrophoresis (7.5%) followed by autoradiography. Lane a, "C-labeled molecular weight markers (indicated on the left in thousands); lane f, 125I-IFN-γ cross-linked to itself in the absence of cells.

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The fate of cell-associated IFN-γ was then studied both in HeLa cells and in monocytes. Both cell types were preincubated at 4 °C for 2 h with an excess of 125I-IFN-γ, washed, and further incubated in the absence of IFN-γ for different time periods at 37 °C. In both cell types, a rapid internalization of the surface-bound 125I-IFN-γ was observed (Fig. 5).
Variability may be the basis for the different activities of IFN-γ binding, internalization, and degradation as well as receptor nonspecific binding. In this study, glycosylated IFN-γ which was studied. These studies led us to conclude that there are two types of IFN-γ receptors which share several properties such as binding affinity, internalization, and degradation of IFN-γ. However, the two receptor types differ in their molecular weight, stability in acid, and in their regulation. Such variability may be the basis for the different activities of IFN-γ on different cell types.

The two molecular forms of the IFN-γ-R reside in different cells and are mutually exclusive. However, both receptors exhibit a similar affinity for IFN-γ. We had reported that the binding of IFN-γ to nonhematopoietic cells (WISH) yielded a linear scatchard plot, whereas a biphasic plot was obtained with monocytes (11). A later analysis of the same binding data by the LIGAND program indicated that there was only one class of high affinity binding sites for IFN-γ in monocytes. The low affinity phase in the plot was therefore due to nonspecific binding. In this study, glycosylated IFN-γ which exhibited a high specific activity (5 × 10⁻⁸ units/mg) was used for the measurement of $K_a$. Other studies were performed with recombinant IFN-γ produced in Escherichia coli and this IFN exhibited a somewhat lower specific activity as compared to that of natural IFN-γ ($10^{-7}-3 \times 10^{-8}$ units/mg, e.g. Ref. 10).

Calculation of the binding data employing glycosylated IFN-γ revealed that 50% saturation of the receptors occurred at 200–400 units/ml, whereas half-maximal biological effects were observed at 1 unit/ml. Thus, there is a considerable amount of spare receptors on the cell surface. Based on the specific activity and the $K_a$ we calculated that at 1 unit/ml of IFN-γ, one cell will bind 10–15 molecules of IFN-γ and this quantity is sufficient for exerting half-maximal biological effect. It is therefore conceivable that signal transduction into the cell is accomplished by signal amplification.

Cross-linking experiments provided evidence for the existence of two receptor subtypes. The observed molecular weight of 120,000 for the $^{125}$I-IFN-γ-receptor complex in cells of nonhematopoietic origin was in accordance with previously reported values (6, 7, 12). The molecular weight of the receptor was calculated as 95,000 assuming a 1:1 ligand to receptor ratio in the complex. A major protein band of this molecular weight was obtained recently by ligand affinity chromatography of the IFN-γ-R from fibroblasts (21). Similarly, the 165,000 complex from monocytes corresponded to a molecular weight of 140,000 for the monocyte receptor. However the majority of cross-linked complex in monocyte had a molecular weight of 60,000–80,000 and 90,000. Such bands were also seen in preparations from HeLa cells but in a much lower intensity. These lower molecular weight complexes could represent degradation products or, as recently suggested, subunit structures (18). Interestingly, Ucer et al. (18) did not report the presence of the 120,000 complex in any cell type. In contrast, a complex of molecular weight 120,000, similar to that of nonhematopoietic cells was obtained by cross-linking of $^{125}$I-IFN-γ to U937 cells (9), which are a monocyte-like cell line. Such a discrepancy between U937 cells and peripheral blood monocytes may be related to differences in their state of differentiation. Since the 120,000 and the 165,000 complexes were mutually exclusive in different cell types, it is not likely that they represent different levels of degradation of the same receptor molecule. However, preliminary studies indicate that the receptors on these two cells are immunologically cross-reactive. The extent of the structural homology between the two subtypes will be determined by sequencing the receptors or their gene(s).

In terms of post-binding events, both receptors behaved as typical polypeptide hormone receptors (22). Receptor-mediated endocytosis followed by ligand degradation and release of degradation products were demonstrated in both cell types in accordance with previous studies (3, 19). An unusual effect of chloroquine was, however, observed. Chloroquine was found to prevent the degradation of various ligands by increasing lysosomal pH, thereby causing an accumulation of intact ligands within the cell. However, the elevation of pH by

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**Table 1**

| Monocytes | HeLa | Receptors/cell |
|-----------|------|----------------|
| $K_a$ M | 2.3 x 10⁻¹⁰ ± 45% | 2.4 x 10⁻¹² ± 23% |
| 1500 ± 36% | 8600 ± 19% |
| $K_a$ M | 1.5 x 10⁻¹⁰ ± 25% | 2.4 x 10⁻¹⁵ ± 15% |
| 680 ± 25% | 8600 ± 14% |

*a Cells treated at pH 4.5 for 10 min at room temperature, prior to the binding assay.
TABLE II

Comparison of the two forms of the IFN-γ receptor

| Property                  | Monocytes | HeLa |
|---------------------------|-----------|------|
| Molecular weights of complexes* | 165,000 (minor), 120,000 (major). | 70,000 and 70,000 and 90,000 (major), 90,000 (minor) |
| Affinity for ligand (Kd,M) | 1 x 10^-38 | 1 x 10^-37 |
| Ligand internalization     | +         | +    |
| Ligand degradation         | +         | +    |
| Down-regulation by excess ligand | + | -    |
| Lability to acid treatment | +         | +    |

* Cross-linked complexes of 125I-IFN-γ and the cell surface receptor.

IFN-γ exhibits antiviral activity in nonhematopoietic cells such as fibroblasts, WISH, and HeLa, but not in monocytes (11). Similarly, IFN-γ induces (2'-5') digo A synthetase in the nonhematopoietic cells, but not in monocytes. In this study, we demonstrated that the receptors of IFN-γ in monocytes differ significantly from those in HeLa cells, the lysis of which is not resistant to acid pH even before digestion by lysosomal proteases. In contrast, the receptor in HeLa cells is resistant to the low endosomal pH. The properties of the two receptors are summarized in Table II.
Characterization of Two IFN-γ Receptor Subtypes

Supplemental Material to
Two Molecular Forms of the Human Interferon-γ Receptor: Ligand Binding, Internalization and Down Regulation
Dina G. Fischer, Daniela Nowick, Patricia Oorschansky and Melchior Rizzuto

Experimental Procedures
Materials: IFN-γ was produced in recombinant Chinese hamster ovary cells (2G) and purified as described in a previous report (29). Purified IFN-γ (IFN-γ: N terminal 28,000; C-terminal 22,000 dalton) was labeled with the Bolton and Hunter reagent (27) to a specific activity of 3,000,000 cpm/μg (Bolton & Hunter label). The labeled IFN-γ retained most of its original specific activity. Fluorescein- and pyrene-labeled IFN-γ was prepared from Protease-4-chromatographed IFN-γ. In order to minimize nonspecific labeling, IFN-γ was purified by chromatography on DEAE-cellulose (28).

Tuberculosis hypersusceptible mice were used in Fishel cells (28). A reference standard of interferon-γ (G202) was obtained from NIH and used for the calibration in all assays. Cells: Fishel cells (ATCC CCL-28), an epidermal cell derived from human epidermis reacting to vitamin D, was grown in RPMI-1640 medium; WI-38 (ATCC CCL-70), an embryonic cell derived from human amnion was grown in minimal essential medium F12 + 5% fetal bovine (29). Cells were suspended in 0.2 M acetic acid (0.2 M in saline) and incubated at 37°C for 30 min. Suspended cells were then washed with cold PBS and with 2% diisothiocyanate as described, detached and counted. These cells were then taken with trypsin and the cell-associated radioactivity was counted. Counting was taken at the indicated times. Non-specific binding and internalization were removed by the presence of a 1,000-fold excess of IFN-γ (30).

The effect of chloramphenicol on the internalization of IFN-γ was measured at 4°C in four different filters with different periods with IFN-γ (100 units/m). The supernatant was removed, the wells were washed with cold PBS and with 2% diisothiocyanate as described, detached and counted. These cells were then taken with trypsin and the cell-associated radioactivity was counted. Counting was taken at the indicated times. Non-specific binding and internalization were removed by the presence of a 1,000-fold excess of IFN-γ (30).

The effect of chloramphenicol on the internalization of IFN-γ was measured in the following: murine macrophage cell lines were incubated at 37°C for 2 h. IFN-γ (100 units/m) was added and the amount of IFN-γ bound was determined. In a parallel set of tubes, chloramphenicol (200 μM) was added to the supernatant at 30 min prior to the addition of IFN-γ. At the end of the incubation all tubes were washed and processed as described above for determining the level of IFN-γ internalization. Number and number of the monocytes after the various treatments was determined to ensure that an equal number of cells was present in all samples.

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Characterization of Two IFN-γ Receptor Subtypes

Fig. 2. Binding of IFN-γ to monocytes. Monocytes were incubated at 4°C for 2.5 h with 125I-IFN-γ (40 μCi/ml) together with increasing concentrations of unlabeled IFN-γ. Cell-associated radioactivity was measured and the data analyzed by the LIGAND program. Inset: Scatchard analysis of a saturation binding experiment with increasing concentrations of 125I-IFN-γ at 4°C. The data was analyzed by the LIGAND program. (Bound ligand), Total ligand. Further legend.

Fig. 3. Internalization and degradation of surface-bound 125I-IFN-γ. Human cells (A) or monocytes (B) were incubated at 37°C for 2.5 h with 125I-IFN-γ [100 μCi/ml]. Free 125I-IFN-γ was removed by washing and the cells were then further incubated at 37°C for different time periods. Surface-bound (○), internalized (△) and degraded (■) 125I-IFN-γ were then determined. (See experimental procedures).

Fig. 4. Degradation of cell-associated 125I-IFN-γ. Human cells (A) or monocytes (B) were incubated at 37°C for 2.5 h with 125I-IFN-γ [100 μCi/ml]. Free 125I-IFN-γ was removed by washing and the cells were then further incubated at 37°C for different time periods. Surface-bound (○), internalized (△) and degraded (■) 125I-IFN-γ were then determined.