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Immunology

Purification and characterization of the human interferon-γ receptor from placenta

(monoclonal antibody/lymphokines)

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ABSTRACT **Purification of the human interferon-γ (IFN-γ) receptor was facilitated by identification of human placenta as a large-scale receptor source. When analyzed in radioligand binding experiments, intact placental membranes and detergent-solubilized membrane proteins expressed 1.3 and 5.9 × 10^{12} receptors per mg of protein, respectively, values that were 13–163 times greater than that observed for U937 membranes. Two protocols were followed to purify the IFN-γ receptor from octyl glucoside-solubilized membranes: (*i*) sequential affinity chromatography over wheat germ agglutinin- and IFN-γ-Sepharose and (*ii*) affinity chromatography over columns containing receptor-specific monoclonal antibody and wheat germ agglutinin. Both procedures resulted in fully active preparations that were 70–90% pure. Purified receptor migrated as a single molecular species of 90 kDa either when analyzed on silver-stained $NaDodSO_4$/polyacrylamide gels or when subjected to electrophoretic transfer blot analysis using a labeled IFN-γ receptor-specific monoclonal antibody. The identity of the 90-kDa component as the receptor was confirmed by demonstrating its ability to specifically bind ^{125}I-labeled IFN-γ following $NaDodSO_4$/PAGE and transfer to nitrocellulose. Certain receptor preparations converted into a 55-kDa fragment either during purification or upon storage at 4°C. On the basis of N-Glycanase digestion studies, the IFN-γ receptor appeared to contain 17 kDa of N-linked carbohydrate. The ligand binding site, the epitope for the receptor-specific monoclonal antibody, and all of the N-linked carbohydrate could be localized to the 55-kDa domain of the molecule.**

Interferon-γ (IFN-γ) is a lymphokine produced by T cells (1, 2) and possibly natural killer cells (3, 4) that has important immunomodulatory activities. In addition to its antiviral and antiproliferative activities, IFN-γ participates in the afferent limb of the immune response by inducing expression of major histocompatibility complex class II antigens on numerous cell types (5, 6) and by regulating production of other immunomodulatory cytokines, such as interleukin 2 (7), interleukin 1 (8, 9), and tumor necrosis factor (10–12). It also functions in the efferent limb of the immune response by enhancing cytocidal activities of mononuclear phagocytes (13–16) and by regulating production and class selection of immunoglobulins (17, 18). IFN-γ manifests these effects by binding to specific IFN-γ receptors on cell surfaces. During the past 4 years, several laboratories have defined and partially characterized IFN-γ receptors on a variety of different cell types, including mononuclear phagocytes (19–21), fibroblasts (22, 23), lymphocytes (24–26), platelets (27), and numerous primary and cultured tumor cell lines (28–30). On the basis of radioligand binding and crosslinking techniques, the IFN-γ receptors on these cells were shown to have relatively high affinity for ligand (K_a = 10^9–10^{10} M^{-1}) and were estimated to display molecular masses of 87–120 kDa.

Recently, IFN-γ receptors were isolated on an analytical scale from Raji cells (31) and human fibroblasts (32). The isolated receptor preparations in both studies displayed multiple components with molecular masses of 90–95, 80, and 50–60 kDa when analyzed by $NaDodSO_4$/PAGE and electrophoretic transfer blotting. However, these studies were unable to establish whether the different components were related. These earlier studies were hampered by the limited availability of purified IFN-γ receptor. In the current report we demonstrate the high expression of IFN-γ receptor in human placenta and document its preparative-scale purification. Moreover, we show that the IFN-γ receptor is a single-chained glycoprotein of molecular mass 90 kDa that can be cleaved by limited proteolysis to a 55-kDa fragment that contains the ligand binding site.

MATERIALS AND METHODS

Reagents. Recombinant human IFN-γ was generously provided by H. Michael Shepard of Genentech (South San Francisco, CA). Preparations displayed a specific antiviral activity of 3.0 × 10^7 international reference units/mg. Monoclonal antibodies to the human IFN-γ receptor were produced as described (33). The particular antibody used in this study (GIR-301) is an IgA κ chain that inhibits binding of ^{125}I-labeled IFN-γ (^{125}I-IFN-γ) to soluble, purified receptor, isolated human placental membranes, intact mononuclear phagocytes (U937), fibroblasts (WISH), or epithelial tumor cells (Colo 205). GIR-301 was purified from culture supernatants by affinity chromatography on columns containing 30 mg of goat anti-mouse IgA (Nordic Immunological Laboratories, El Toro, CA) covalently coupled to Sepharose. Proteins were radioiodinated to specific activities of 2–10 μCi/μg (1 Ci = 37 GBq) by using the Bolton–Hunter reagent (ICN Chemicals, Radioisotope Division) as described (20).

Preparation and Solubilization of Human Placental Membranes. Human term placenta, minus cord and membranes (392 g), was minced in 300 ml of 50 mM Tris buffer (pH 7.4) containing 0.25 M sucrose, 10 μg of leupeptin per ml, 2100 kallikrein international units of aprotinin per ml, 5 mM iodoacetamide, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$), and 0.1% (wt/vol) bacitracin and homogenized with a Tekmar Tissumizer (Tekmar, Cincinnati, OH) for five 1-min intervals at 4°C. The homogenate was cleared of debris by centrifugation at 10,000 × *g* for 30 min at 4°C and then the membranes were sedimented by ultracentrifugation at 100,000 × *g* for 2 hr at 4°C. Membrane

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Abbreviations: IFN-γ, interferon-γ; $PhMeSO_2F$, phenylmethylsulfonyl fluoride; WGA, wheat germ agglutinin.

pellets were washed three times by resuspension in sucrose free-homogenization buffer and ultracentrifugation.

Placental membranes were solubilized by treatment for 2 hr at 4°C with 80 mM octyl glucoside at a detergent:protein ratio of 2.5:1 in phosphate-buffered physiologic saline (PBS) (pH 7.4) containing all of the above-mentioned protease inhibitors. After ultracentrifugation (1 hr, 100,000 × g, 4°C) the soluble fraction was dialyzed overnight against 10 vol of PBS containing 30 mM octyl glucoside, iodoacetamide, EDTA, and $PhMeSO_2F$ and cleared again by ultracentrifugation. Under these conditions, all of the receptor but only 30% of the total protein is solubilized.

Binding Assays. The binding of radioiodinated IFN-γ to placental membranes was analyzed by using a modification of the cell binding assay described elsewhere (20). The modified assay utilized 25–50 μg of membrane protein. Membrane-associated and free ^{125}I-IFN-γ were separated by centrifugation over a 300-μl cushion of 50% fetal calf serum for 10 min at 10,000 × g in a Beckman Microfuge 12.

The assay used to quantitate the soluble IFN-γ receptor was adapted from the soluble insulin receptor binding assay devised by Cuatrecasas et al. (34). Reaction mixtures contained 5–200 ng of human ^{125}I-IFN-γ in 25 μl, 25 μl of either medium or unlabeled IFN-γ in 500-fold excess (to determine nonspecific binding), and 175 μl of receptor solubilized from isolated placental membranes (0–50 μg of protein containing 0–10 ng of receptor) in PBS/30 mM octyl glucoside. After incubation for 2 hr at 4°C, 25 μl of a solution containing bovine gamma globulin (40 mg/ml) and bovine serum albumin (40 mg/ml) was added as carrier protein, and the receptor–ligand complex was differentially precipitated away from free ligand by addition of 1 ml of 11.25% (wt/vol) PEG 8000 in PBS. After incubation for 15 min at 4°C, precipitates were pelleted by centrifugation at 10,000 × g for 15 min at 4°C. Pellets were washed one time with 1 ml of 9% PEG, and the radioactivity was quantitated in the pellet and the combined supernatants.

Purification of the Human IFN-γ Receptor. Two different protocols were used to purify the IFN-γ receptor from solubilized human placental membranes. In the first method, 1.18 g of solubilized membrane proteins in 145 ml from two placentas (total wet weight = 850 g) was applied at 4°C to a 3.5 × 20 cm column containing 144 ml of wheat germ agglutinin (WGA)/agarose (7 mg of WGA per ml of gel) (Vector Laboratories, Burlingame, CA) equilibrated in PBS containing 30 mM octyl glucoside, 5 mM iodoacetamide, 2 mM EDTA, and 1 mM $PhMeSO_2F$. The column was washed with 10 column vol of starting buffer and then with 10 column vol of buffer containing 0.5 M NaCl. The WGA-associated receptor was eluted by using buffer that contained 0.5 M NaCl and 0.5 M *N*-acetylglucosamine (GlcNAc). Receptor-containing fractions, identified by the PEG-soluble receptor binding assay, were pooled, dialyzed against 10 vol of PBS/30 mM octyl glucoside, and then applied at 4°C to a 1.0 × 13 cm column containing 30 mg of human recombinant IFN-γ covalently attached to 10 ml of cyanogen bromide-activated Sepharose prepared by the method of March et al. (35). After washing, the column was stripped with 0.2 M carbonate buffer (pH 10.3) containing 30 mM octyl glucoside and 0.5 M NaCl. Fractions were neutralized and screened for receptor activity. Pooled fractions were concentrated and dialyzed against 10 vol of PBS/30 mM octyl glucoside containing iodoacetamide, EDTA, and $PhMeSO_2F$. Protein content of the purified preparation was determined by the bicinchoninic acid method (Pierce).

The second protocol used a 1.0 × 15 cm column of Sepharose containing 30 mg of covalently coupled purified GIR-301, the IFN-γ receptor-specific monoclonal antibody. The chromatographic procedure was essentially the same as that described above with two exceptions: (*i*) column-associated receptor was eluted with 50 mM diethylamine buffer (pH 11.5) containing 0.15 M NaCl, 30 mM octyl glucoside, 5 mM iodoacetamide, 2 mM EDTA, and 1 mM $PhMeSO_2F$ and (*ii*) the material eluted from the first antibody column was rechromatographed over the same column a second time. Following immunoaffinity chromatography the receptor preparation was further purified by using a 2-ml column of WGA-Sepharose that was washed and eluted with GlcNAc as described above.

N-Glycanase Treatment of Purified IFN-γ Receptor. Five micrograms of receptor in 104 μl of 0.2 M phosphate buffer (pH 8.3) containing 0.1% $NaDodSO_4$, 1.7 mM 2-mercaptoethanol, 0.5% Nonidet P-40, and 5 mM EDTA was mixed with 10 μl of N-Glycanase (13.6 units/ml) purified as described (36) and obtained from J. Baenziger at this institution. After a 16-hr incubation at 37°C, the digestion was terminated by addition of Laemmli sample buffer and boiling.

$NaDodSO_4$/PAGE and Immunoblotting. $NaDodSO_4$/PAGE was performed according to the method of Laemmli (37). Bands were visualized by silver staining. Electrophoretic transfer blot analysis was performed following electrotransfer of the proteins from $NaDodSO_4$ gels to nitrocellulose membranes at 4°C at constant current (400 mA for 2 hr or 150 mA overnight) (38). Membranes were blocked with 3% milk in 20 mM Tris/0.15 M NaCl buffer, pH 7.4, for 1 hr at room temperature, washed twice with PBS containing 0.05% Tween-20, and then incubated with 3-ml volumes of biotinylated GIR-301 (1 μg/ml), ^{125}I-labeled GIR-301 (0.12 μg/ml), or ^{125}I-IFN-γ (30 ng/ml) for 2 hr at room temperature. Autoradiography was performed with Kodak X-Omat AR5 film using an intensifying screen. Strips treated with biotinylated antibody were developed by incubation with streptavidin–peroxidase conjugate (1:600) and diaminobenzidine/H_2O_2 substrate solution.

RESULTS

Quantitation of the IFN-γ Receptor on Human Placental Membranes. One of the major problems hampering past efforts to purify the IFN-γ receptor has been the lack of a large-scale cell source expressing high levels of the protein. Fig. 1 demonstrates that human placental membranes are a particularly rich receptor source. Binding of ^{125}I-IFN-γ to either isolated placental membranes or detergent-solubilized membrane proteins at 4°C was saturable (as confirmed in additional experiments using higher doses of radiolabeled ligand), and Scatchard analysis of the binding data indicated the presence of only a single class of binding sites. Isolated placental membranes expressed 0.5–1.3 × 10^{12} receptors per mg of membrane protein, whereas solubilized membranes showed 2–5.9 × 10^{12} receptors per mg of protein. These values are 13–163 times higher than the amount of receptor expressed on the membranes of the U937 cell line (3.6 × 10^{10} receptors per mg). The parallel nature of the Scatchard plots demonstrated that IFN-γ bound to membrane-associated and soluble receptors with identical affinities (1 × 10^8 M^{-1}). This result thus confirmed that the IFN-γ receptor could be solubilized in fully active form.

Purification of the IFN-γ Receptor. Two different strategies were used to purify the IFN-γ receptor. Initially, isolated placental membranes were solubilized and subjected to sequential affinity chromatography over Sepharose columns containing WGA and recombinant human IFN-γ. This protocol led to the purification of 23 μg of receptor in 12% yield. Based on the soluble receptor binding assay and assuming a 1:1 receptor:ligand stoichiometry, this preparation was at least 70% pure. Silver-stained $NaDodSO_4$/polyacrylamide gels of this preparation showed only a single component of 80–90 kDa. This material was used to prepare the monoclonal antibodies described elsewhere (33). This method presented

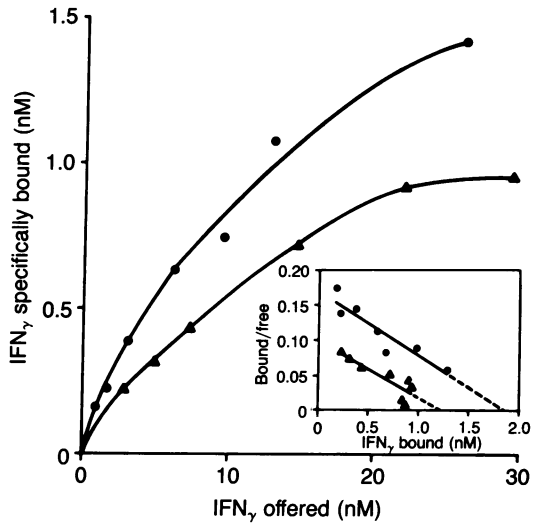

FIG. 1. Comparable binding of ^{125}I-IFN-γ to membrane-associated and soluble forms of the IFN-γ receptor from human placenta. Reaction mixtures contained either isolated placental membranes (50 μg of membrane protein) (▲) or 50 μg of protein solubilized by treatment of membranes with octyl glucoside (●). Binding assays for each receptor source were performed as outlined in the text. Specific binding was defined as that component of the total binding inhibitable by a 500-fold excess of unlabeled ligand. Nonspecific binding: intact membrane assay, 1.7%; soluble receptor assay, 24%.

two difficulties: (*i*) the yields were low and (*ii*) the amount of material that could be purified was limited because (*a*) limited amount of recombinant IFN-γ was available and (*b*) the ligand column was destroyed by the extremes of pH needed to dissociate the column-bound receptor. When receptor-specific monoclonal antibodies became available, an improved purification protocol was devised based on immunoaffinity and lectin chromatography using Sepharose columns containing covalently attached GIR-301 and WGA. This protocol permitted the 3700-fold purification (theoretical = 3800) of 284 μg of IFN-γ receptor from the membrane fraction of four placentas (containing 804 μg of receptor and 3.05 g of protein as starting material) with an overall yield of 35%. Purified receptor and crude solubilized receptor preparations showed identical ligand binding affinities when examined by Scatchard analysis (data not shown). Fig. 2*a* (lane 2) represents the silver-stained NaDodSO$_4$/PAGE pattern of an immunoaffinity-purified preparation of IFN-γ receptor derived from human placental membranes. This gel reveals a single predominant band of 90 kDa. Identical patterns were obtained when the sample was run under either reducing or nonreducing conditions.

Electrophoretic Transfer Blot Analysis of the Purified IFN-γ Receptor. Purified receptor was also analyzed by electrophoretic transfer blot analysis. Fig. 2*b* (lane 2) documents the presence of a single molecular species detected by the biotinylated receptor-specific GIR-301 monoclonal antibody. When compared to biotinylated protein standards (lane 1), the component displayed an apparent molecular mass of 90 kDa, which is in agreement with the silver-stain pattern. Identical patterns were obtained by using two other receptor-specific monoclonal antibodies (GIR-208 and GIR-94). No bands were observed when electrophoretic transfer blots were developed with irrelevant biotinylated monoclonal antibodies. Moreover, no additional bands were detected by GIR-301 when blotting was performed on either unfractionated, solubilized placental membranes or membrane proteins that bound to WGA-Sepharose (data not shown).

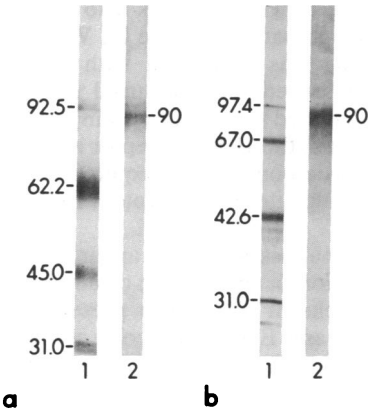

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoretic analysis of the purified human IFN-γ receptor. An IFN-γ receptor preparation purified from human placental membranes by immunoaffinity chromatography over GIR-301-Sepharose and lectin chromatography was analyzed by NaDodSO$_4$/PAGE under nonreducing conditions. (*a*) Silver-stained 9% NaDodSO$_4$ gels of molecular mass standards (lane 1) and 2.5 μg of purified receptor (lane 2). (*b*) Electrophoretic transfer blot analysis performed on biotinylated molecular mass standards (lane 1) and 0.25 μg of purified receptor (lane 2) following electrophoresis in 7.5–20% gradient polyacrylamide gels and electrotransfer to nitrocellulose. Filters were developed with biotinylated GIR-301 and/or streptavidin–peroxidase. Molecular masses are given in kDa.

Confirmation of the Identity of 90-kDa Polypeptide as the IFN-γ Receptor. To conclusively show that the 90-kDa band seen on silver-stained gels and electrophoretic transfer blots was the IFN-γ receptor, the electrotransferred protein was examined for its ability to interact with ligand. In Fig. 3, lane c, ligand blotting using iodinated IFN-γ documented the ligand binding capacity of the 90-kDa component. The band developed with ^{125}I-IFN-γ was indistinguishable from the band developed with labeled monoclonal anti-receptor antibody (lane a). Ligand blotting appeared to be specific because no bands were observed when the nitrocellulose strips were pretreated with a 250-fold excess of unlabeled IFN-γ (lane d) and because blotting was eliminated when the receptor was reduced prior to NaDodSO$_4$/PAGE (lane e). Electrophoretic

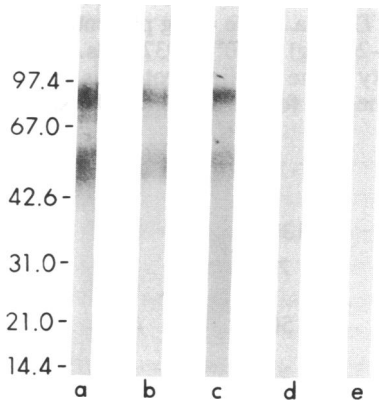

FIG. 3. Electrophoretic transfer blot analysis performed on the purified human IFN-γ receptor using radiolabeled monoclonal antibody or ligand. The same receptor preparation as depicted in Fig. 2 was run on a 7.5–20% NaDodSO$_4$ gradient polyacrylamide gel and then electrotransferred to nitrocellulose. Lane a, nonreduced sample exposed to GIR-301 (100 ng/ml); lane b, reduced sample exposed to the same antibody; lane c, nonreduced sample developed with IFN-γ (51 ng/ml); lane d, transferred nonreduced sample preincubated with 12.75 μg (250-fold excess) of unlabeled IFN-γ before exposure to 51 ng of ^{125}I-IFN-γ; lane e, reduced sample exposed to IFN-γ. Molecular masses are given in kDa.

transfer blotting by GIR-301 was also diminished with reduced receptor preparations (lane b). When electrophoretic transfer blotting was performed with GIR-208, the same bands were observed for unreduced preparations but no staining was detected with reduced samples of receptor (data not shown). Thus, the IFN-γ receptor appears to contain at least one disulfide bond that is required to renature at least the ligand binding site on the protein following removal of $NaDodSO_4$.

During this analysis it became apparent that occasionally during purification or upon aging of purified receptor preparations (2 weeks at 4°C in the presence of $PhMeSO_2F$, iodoacetomide, and EDTA) a second component of 55 kDa could be visualized (Fig. 3). This second component reacted with ligand and antibody. Preparations showing only the 90-kDa form occasionally converted at 4°C into 90- and 55-kDa components and eventually converted entirely into the 55-kDa form. This conversion could be prevented by storage at −70°C. These results indicated that the smaller component arose from fragmentation of the 90-kDa polypeptide.

Demonstration That the IFN-γ Receptor Is a Glycoprotein. The broad $NaDodSO_4$/PAGE patterns of purified preparations of IFN-γ receptor suggested that it might be a glycoprotein. This possibility was explored by determining whether the receptor carried N-linked carbohydrate. The particular receptor preparation analyzed showed the broad 90- and 55-kDa components (Fig. 4, lane a). However, after treatment with the endoglycosidase N-Glycanase (lane b), both bands were converted to sharper components with reduced mass of 73 and 38 kDa. Thus, the intact receptor and its breakdown product carried the same amount (17 kDa) of N-linked carbohydrate.

DISCUSSION

The data presented in this paper document the preparative-scale isolation of the human IFN-γ receptor. The key to this study was the identification of human placenta as a crude receptor source. On the basis of Scatchard analysis, membranes derived from this easily obtained tissue were found to express >30 times the amount of receptor per weight of membrane protein than the U937 cell line, and placental membrane-associated receptor could be solubilized in fully active form. Thus, a single 400-g placenta supplied as much receptor (60–200 μg) as 10^{12} U937 cells.

The identity of the purified protein as the IFN-γ receptor was established by four criteria. (*i*) The specific activity of purified receptor preparations, as determined by Scatchard analysis of data obtained by using the soluble receptor binding assay, indicated a level of purity that approached 90% (assuming a receptor:ligand stoichiometry of 1:1). (*ii*) Only a single molecular mass species of 90 kDa was observed when the purified receptor was analyzed on silver-stained $NaDodSO_4$/polyacrylamide gels. (*iii*) When examined by electrophoretic transfer blot analysis, the 90-kDa component reacted with IFN-γ receptor-specific monoclonal antibodies. The specificity of these antibodies has been confirmed elsewhere by demonstrating their ability to inhibit the binding of IFN-γ to placental membranes, U937, fibroblasts, and epithelial cells as well as inhibiting induction of IFN-γ-dependent antiviral activity in human WISH fibroblasts (33). (*iv*) The same 90-kDa component recognized by the antibody displayed ligand binding activity following $NaDodSO_4$/PAGE under nonreducing conditions and transfer to nitrocellulose.

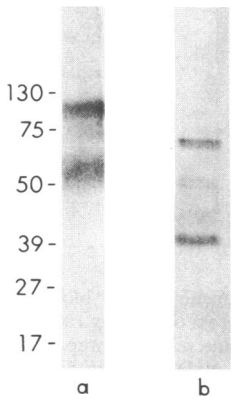

FIG. 4. Demonstration of the presence of N-linked carbohydrate on the intact IFN-γ receptor and its 55-kDa fragment. Purified IFN-γ receptor was incubated either with buffer (lane a) or N-Glycanase (lane b) prior to $NaDodSO_4$/PAGE on 7.5–20% gradient gels under nonreducing conditions. Molecular masses are given in kDa.

It was somewhat surprising to find that IFN-γ bound to either the membrane-associated or soluble placental receptor with an affinity of 10^8 M^{-1}. This affinity is ≈10–20 times lower than that observed for IFN-γ receptors on a variety of other cell types (19–30). However, we have recently observed that the ligand binding affinity of IFN-γ receptors on whole U937 cells or isolated U937 membranes dropped 1 order of magnitude following solubilization of the receptor. Thus, the affinity of the IFN-γ receptor for its ligand appears to be highly dependent on the local environment. Further work is necessary to establish the molecular basis for this observation.

The results presented here significantly clarify earlier attempts to define the structure of the human IFN-γ receptor. Based on radioligand crosslinking experiments, several groups proposed that the molecular mass of the IFN-γ receptor was 80–120 kDa (20, 23, 28–30). However, these studies were indirect and needed to be confirmed by an analysis of the purified receptor in ligand-free form. Recently, Aguet and Merlin (31) and Novick *et al.* (32) achieved the partial purification of small amounts of receptor from Raji cells and fibroblasts, respectively. However, both receptor preparations contained multiple molecular species [90 and 50 kDa for Aguet and Merlin (31) and 95, 80, and 60 kDa for Novick *et al.* (32)] and, due to the limited amounts of receptor obtained, no efforts were made to establish whether the different components were related. The purification protocol employed in the present study enabled us to prepare microgram quantities of receptor that, on $NaDodSO_4$/PAGE, behaved only as a single component with a molecular mass of 90 kDa. However, occasionally upon aging or during purification, an additional 55-kDa component was detected. We have established that the two protein forms are interrelated (*i*) by demonstrating that the 90- and the 55-kDa forms contain the ligand binding site and the epitopes for the receptor-specific monoclonal antibodies and (*ii*) by observing the time-dependent conversion of the 90-kDa form into the 55-kDa form. These results suggest that the intact IFN-γ receptor is indeed a 90-kDa protein that is extremely sensitive to limited fragmentation by proteases that may be trace contaminants in the preparation. The resulting 55-kDa fragment appears to be relatively protease-resistant and contains the binding site for ligand. It is likely that the additional molecular components observed in the previous studies represented these receptor breakdown products.

The data obtained in this laboratory indicate that the IFN-γ receptors on different cells are similar. As documented here, only a single molecule was purified from human placenta, which is a tissue comprised of a variety of different cell types. As shown elsewhere, binding of IFN-γ to placental membranes, mononuclear phagocytes, fibroblasts, and epithelial-like tumor cells was completely inhibitable by GIR-301 and

another receptor-specific monoclonal antibody, GIR-208 (33). Finally, preliminary electrophoretic transfer blot experiments have suggested that the IFN-γ receptor on U937 is antigenically similar to the placental protein. However, these results do not preclude the possibility that IFN-γ receptors on different cells may show distinct glycosylation patterns.

Thus, based on the above data, we propose the following model for the human IFN-γ receptor. The receptor is minimally a single-chained 90-kDa glycoprotein. The intact protein carries 19% N-linked carbohydrate and contains a critical disulfide bond necessary for maintaining ligand binding activity. The receptor is labile and is easily cleaved to a fragment that displays a molecular mass of 55 kDa. The 55-kDa receptor fragment carries the ligand binding site, the epitopes for the GIR-301, GIR-208, and GIR-94 monoclonal antibodies, and all of the N-linked carbohydrate. It thus comprises at least part of the extracellular portion of the receptor. The IFN-γ receptors on different cell types appear to be similar. The purification of the IFN-γ receptor in relatively large quantities is an important step that should facilitate the analysis of this protein at the molecular level and provide insights into its role in regulating immune cell function.

Note Added in Proof. Recently a murine IFN-γ receptor has been isolated and partially characterized as a 95-kDa protein (39).

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