Configuration of the Interferon-α/β Receptor Complex Determines the Context of the Biological Response

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Constituents of the Type 1 interferon (IFN) receptor (IFNABR) identified to date include the α and β transmembrane subunits and the associated intracellular kinases, Jak1 and Tyk2. In this report, we demonstrate that a human cell type that expresses both subunits of IFNABR, together with Jak1 and Tyk2, exhibits a limited binding capacity for and is only partially sensitive to the effects of IFN-α/β, despite adequate levels of the cytoplasmic transcription factors Stat1, Stat2, and Stat3. Specifically, a low affinity interaction between IFN-α/β and cell surface receptors results in ISGF3 (Stat1:2) activation and an antiviral response, yet no IFN-inducible growth inhibition. Using a panel of murine cells that are variably configured with respect to the human IFNABR-α/β subunits, we provide evidence that an additional component(s) encoded on human chromosome 21 is required to confer high affinity binding and IFN-inducible growth inhibition to cells that express the α and β subunits of the IFNABR. The data indicate that transcriptional activation that leads to an antiviral response is mediated by IFN-α/β activation of IFNABR-α and IFNABR-β in the context of a low affinity interaction, yet a high affinity interaction is necessary for signal transducing events that mediate growth inhibition. We provide evidence that the extent of ISGF3 activation correlates directly with the magnitude of an antiviral but not a growth inhibitory response.

Type 1 interferons (IFN), comprising α and β subtypes, are a family of biologically active related proteins that exhibit target cell specificity (1, 2). This specificity is mediated by a high affinity interaction between the IFN and its cell surface receptor (3–5). Accumulating evidence indicates that the Type 1 IFN receptor is a multicomponent. A cDNA coding for a human IFN-α receptor peptide (IFNABR-α) was reported to code for a functional human IFN-α receptor when transfected into murine cells (6). However, sensitivity to the biological effects of human IFN was restricted to a specific IFN-α species, IFN-α8. More recently, a distinct cell surface receptor protein has been identified (7). This receptor, IFNABR-β, may represent the primary ligand recognition chain of the IFN receptor complex. Studies from our laboratory demonstrate that specific species of membrane glycosphingolipids containing a terminal Galα1–4Gal associate with IFNABR-α to create a functional receptor (8).

The initial interaction between a Type 1 IFN and its specific cell surface receptor apparently leads to ligand-induced tyrosine phosphorylation of IFNABR-α and a rapid phosphorylation activation, in the absence of protein synthesis, of a latent cytosolic transcription factor, ISGF3 (reviewed in Ref. 10). Complementation of IFN-resistant mutant cell lines with two members of the Janus family of nonreceptor protein tyrosine kinases (Jak) Tyk2 (11, 12) and Jak1 (13), that are required for IFN-induced signal transduction further defines a potential role for receptor-associated phosphorylation events in the signaling cascade initiated by ligand binding. Indeed, the data imply that Jak1 associates with IFNABR-β (7) and Tyk2 with IFNABR-α (9, 11, 12, 14). Current models for IFN-induced signaling invoke kinase-mediated phosphorylation of the STAT proteins Stat1, Stat2, and Stat3 (15–19). These phosphorylated STATs dimerize via SH2-phosphotyrosyl interactions (20, 21) and translocate to the nucleus where they bind to specific promoter sequences, thereby regulating gene expression. Homodimers and heterodimers of Stat1 and Stat3 bind the palindromic IFN response element, pIRE, (22, 23). A heterodimer of Stat1 and Stat2 associates with a DNA-binding adapter protein, p48, to form the Stat complex designated ISGF3 (24, 25). ISGF3 transcriptionally activates a subset of genes that contain an IFN-stimulated response element (ISRE) (26).

The existence of multiple, distinct signaling pathways that effect different biological outcomes in response to a single IFN suggests that component configuration is critical within a multimolecular transmembrane receptor complex. Members of the hematopoietic growth factor family of cytokines, which include interleukin-γ, interleukins, erythropoietin, growth hormone, granulocyte colony-stimulating factor, and granulocyte-macrophage colony stimulating factor, mediate their pleiotrophic effects through interactions with multicomponent receptors (27–35). Generally, specific ligands exhibit low affinity binding to individual receptor components, yet high affinity binding occurs when the intact receptor complex is assembled. For certain cytokine receptors, such as the interleukin-2 receptor complex, an intermediate affinity interaction between the ligand and a single receptor component, the interleukin-2β chain, may lead to a limited signal transduction (36). Thus, there may be some redundancy associated with multimeric receptor complexes that allows for partial responses when specific receptor components are not expressed at the cell surface.

In agreement with affinity studies with 125I-IFN-α (37–44), here we report that IFNABR-α by itself is not sufficient for the appropriate high affinity binding and complete biological responses effected by IFN-α and -β. We provide evidence that a low affinity interaction between IFN-α/β and the two receptor chains IFNABR-α and IFNABR-β is associated with ISGF3 activation and antiviral responses. Cell surface expression...
of human chromosome 21-encoded factors, which include IFNABR-α and IFNABR-β forms, permits a high affinity interaction between the IFN and the receptor complex, such that target cells exhibit both antiviral and growth inhibitory activities.

**EXPERIMENTAL PROCEDURES**

Cell Cultures and Virus—Human Daudi (lymphoblastoid derived from B cells) cells were grown in 10% RPMI 1640 medium as suspension cultures. T98G (human glioblastoma), MRC-5 (human fetal fibroblast), and Chinese hamster ovary cells were grown as monolayer cultures in 10% modified Eagle’s medium. Murine A9 cells are a mouse-hamster hybrid cell type composed of murine A9 cells containing 1-5 copies of intact chromosome 21 from WI-38 human fibroblast cells were grown as monolayer cultures in 20% modified Eagle’s medium. Encephalomyocarditis virus, passaged on murine L929 cells, was used for this study.

Interferons—IFN-Con1, as a consensus IFN-α, was a gift from Amgen, Inc. (Thousand Oaks, CA) and had a specific activity of 3.0 × 10^6 units/mg protein. IFN-Con1, was designed to represent an average IFN-α with an amino acid chosen at each site that was found most frequently in the known family of IFN-αs. IFN-β was provided by Biogen Inc. (Cambridge, MA) and had a specific activity of 2.0 × 10^6 units/mg protein. IFN-α2b was a gift from Schering Corp. and had a specific activity of 2.0 × 10^6 units/mg protein. Murine IFN-α was purchased from Biogen Inc. (Cambridge, MA) and had a specific activity of 2.0 × 10^6 units/mg protein.

Antiproliferative Assay—This assay has been previously described (45).

**RESULTS**

ISGF3 Activation in the Context of IFNABR-α and IFNABR-β Expression Confers an Antiviral Response—Human MRC-5 cells, although responsive to the antiviral effects of human IFN-Con1 and IFN-β (Fig. 1B), are about 10-fold less sensitive than T98G cells (Fig. 1A) or other IFN-sensitive cells, eg, A549 and HeLa cells (data not shown). In contrast to T98G cells that are growth inhibited by IFN-Con1 and IFN-β, with ID_{50} of ~100 pg/ml (Fig. 1C), MRC-5 cells are insensitive to the antiproliferative effects of these IFN (Fig. 1D). Fig. 2 describes the steady-state receptor binding characteristics of IFN-Con1 on proliferating MRC-5 (A) and T98G (B) cells, at 4°C. Specific binding was resolved into the characteristic high and low affinity biphasic Scatchard plot for T98G cells, yet the low affinity, monophase plot was obtained for the MRC-5 cells (Fig. 2C). Indeed, the binding capacity of MRC-5 cells for IFN-α is significantly reduced compared with T98G cells (Fig. 2, A and B).

Total RNA from MRC-5 cells, when reverse transcribed, contained cDNA that could be amplified by PCR using IFNABR-β-specific primers (7). Analysis of the PCR products revealed an expected 397-bp band (data not shown). To further examine IFNABR-β expressed in MRC-5 cells, a 992-bp fragment was subcloned into the vector PCR II and sequenced. Our results indicate that an IFNABR-β form expressed in MRC-5 cells is encoded by a gene whose sequence is essentially identical to the previously reported sequence, with one codon difference that would result in a conservative change to the amino acid residue at position 216: Asp to Asn. In Northern blots, however, two transcripts of 1.55 and 4.5 kilobase pairs were revealed that may represent differentially spliced products of the same gene (Fig. 2D). Whole cell extracts from MRC-5 and T98G cells exhibit comparable levels of IFNABR-β when Western blots are probed with a polyclonal Ab to IFNABR-β (Fig. 2E). Flow cytometric analysis of IFNABR-β mAb (Fig. 2F) binding to native IFNABR-α on MRC-5 and T98G cells identifies similar
levels of IFNABR-α cell surface expression on these cell types. When viewed together, the data suggest that IFNABR-α and IFNABR-β expression are not limiting factors that affect the binding capacity of MRC-5 cells.

There is emerging evidence to suggest that the Jaks, Tyk 2 and Jak 1, constitutively associate with the intracellular regions of IFNABR-α and IFNABR-β, respectively, and that this association creates a productive receptor, both in terms of ligand recognition and signal transduction. IFN-α/β-induced receptor-mediated ISGF3 activation requires both Tyk 2 and Jak 1. Because there is good evidence to suggest that transcriptional activation that precedes IFN-induced responses is mediated by ISGF3 activation, we examined the extent of IFN-induced ISGF3 activation in both T98G and MRC-5 cells. The results in Fig. 3A demonstrate that IFN-Con1/IFN-β treatment of both T98G and MRC-5 cells results in specific activation of ISGF3. Antisera to Jak 1 and Tyk 2, as well as monoclonal antibodies raised against Stat1, Stat2 and Stat3, detected these factors in extracts from unstimulated T98G and MRC-5 cells (Fig. 3B), indicating that these are not limiting in the MRC-5 cells.

To further examine the specific interaction between Type 1 IFN and IFNABR-α, we investigated the biologic effects of a number of different human Type 1 IFN in murine A9 cells that were stably transfected with the human IFNABR-α. Northern blots confirmed that human IFNABR-α mRNA is expressed in the transfectants (data not shown), and flow cytometric analysis of IFNABR-α mAb binding to human IFNABR-α on A9 transfectants containing plasmid only and the A9+ transfectants containing IFNABR-α identified IFNABR-α cell surface expression on the A9+ transfectants alone (Fig. 4). The results in Fig. 5 demonstrate that murine A9+ cells exhibiting cell surface expression of the human IFNABR-α are restricted in their antiviral responsiveness to selected human Type 1 IFN. Whereas the parental A9 cells are relatively insensitive to the antiviral effects of human IFN-α2b (ID50 = 2.5 μg/ml/5 × 10^5 units/ml), IFN-Con1 (ID50 = 50 ng/ml/1.5 × 10^3 units/ml), and IFN-β (ID50 = 100 ng/ml/2 × 10^4 units/ml) compared with murine IFN-α (60 ng/ml/90U/ml), when challenged with the same infecting dose of encephalomyocarditis virus (Fig. 5A), the A9+ cells exhibit a 200-fold increase in sensitivity to the antiviral effects of IFN-Con1 (ID50 = 0.5 ng/ml/1.5 × 10^3 units/ml) (Fig. 5B). The A9+ transfectants remain relatively unresponsive to the antiviral effects of human IFN-α2b and IFN-β, with dose-response curves similar to those depicted in Fig. 5A. Examination of the sensitivity of the transfectant A9+ cells to the growth inhibitory effects of IFN-α2b, IFN-Con1, and IFN-β revealed that in contrast to the partial antiviral responsiveness, the A9+ cells remained refractory to the antiproliferative effects of each of these IFN (Fig. 6). Gel retardation assays to determine IFN-induced ISGF3 activation in the transfectant versus the A9− cells showed that there was a direct correlation between IFN-induced ISGF3 activation in the transfectants and IFN-induced antiviral activity; whereas both IFN-α2b and IFN-β failed to induce ISGF3 activation in the A9+ cells and failed to elicit an antiviral response in these cells, IFN-Con1 induced ISGF3 activation and an antiviral response in the A9+ transfectants (Fig. 7).

In an attempt to restore complete sensitivity to the full range of biologic responses inducible by Type 1 IFN, we examined the influence of chromosome 21-encoded factors, in addition to IFNABR-α and IFNABR-β expression, on biological outcome. We examined the biological effects of different human Type 1 IFN, both IFN-α and IFN-β, in murine A9 cells that contain 1–5 copies of intact human chromosome 21, designated A9−21. At the outset we determined that the levels of cell surface
expression for IFNABR-α were comparable in A9+ and A9+21 cells (Fig. 4). These data confirmed that the extent of cell surface expression for IFNABR-α was not a limiting factor in the A9+ transfectants. Next, we examined cells for IFNABR-β expression, and our results demonstrate that in contrast to the IFNABR-α-containing A9+ cells, A9+21 cells express IFNABR-β RNA (Fig. 8).

In subsequent studies we demonstrated that those additional factors encoded on chromosome 21 that are expressed in the A9+21 cells are sufficient to enhance the antiviral effectiveness of IFN-Con1 by 150-fold over that detected in the A9+ cells and to restore sensitivity to the antiviral effects of IFN-α2b and IFN-β (Fig. 5). Moreover, the A9+21 cells were fully responsive to the growth inhibitory effects of the different human IFN-αs and IFN-β (Fig. 6). Whereas the growth inhibitory effects of murine IFN-α remained unchanged in the parental A9 and A9+ cells (ID$_{50}$ = $10^3$ units/ml) due to the rapid doubling time of the A9+21 cells ($\sim$12 h), $10^4$ units/ml murine IFN-α are required to achieve an ID$_{50}$ in these cells (data not shown). Similarly, the effectiveness of the human IFN in the A9+21 cells is apparently reduced by approximately 1 order of magnitude compared with T98G cells. Of note, our data indicate a correlation again between antiviral activity and ISGF3 activation, because we were able to restore ISGF3 activation in response to IFN-α2b and IFN-β in the A9+21 cells (Fig. 7).

The Interaction between IFN-Con1 and IFNABR-α Is a Low Affinity Event—Because the level of cell surface expression of IFNABR-α on the murine A9+ cells does not appear to be a limiting factor, we examined the binding characteristics of 125I-IFN-Con1 on A9+ cells. Whether binding capacity was assessed at 4 or 37°C, we were unable to detect any specific high affinity binding, as determined by competition with 100-fold excess of unlabeled ligand. Apparently, any interaction between IFN and the IFNABR-α on the surface of the A9+ cells must be a low affinity event that is undetectable in our standard binding reaction. We modified the experimental conditions of our binding studies by altering the incubation time with 125I-IFN-α and increasing the ligand:cell ratio, but with identical outcome: no significant specific high affinity binding was
Type 1 Interferon Receptor

Fig. 3. STAT and Jak levels in MRC-5 cells. MRC-5 cells were either left untreated or treated with 10 ng/ml of IFN-Con1 (con1) or IFN-β (b). T98G cells were similarly either left untreated or treated with 1 ng/ml of IFN-Con1, or IFN-β. A, 5 μg of whole cell extracts prepared as described under “Experimental Procedures” were reacted with 30,000 cpm of a 32P-end-labeled ISRE, representing nucleotides −107 to −87 of the human 2-5A synthetase gene, which contains a functional ISRE. Complexes were resolved by using native gel electrophoresis and visualized by autoradiography. Mobility of ISGF3 is indicated. Specific complexes were identified by the ability of 2 distinct mAbs, which recognize native cell surface IFNABR-α, to immunoprecipitate the fusion protein (data not shown). Differential recognition of the IFNABR-α fusion protein by these antibodies, together with the ability of the fusion protein to associate with a specific glycosphingolipid species normally associated with cell surface IFNABR-α (8), suggests that this fusion protein is conformationally similar to native cell surface IFNABR-α.

Using 125I-IFN-Con1, we were unable to identify specific, competent binding to the fusion protein to any significant extent, nor were we able to demonstrate that IFN-α2b or IFN-β exhibited any appreciable affinity for this fusion protein (data not shown). Our approach was to incubate the fusion protein for 2 h with varying doses of 125I-IFN-Con1, 4 °C, as per our standard binding reaction, in the presence or the absence of a 10-fold excess of unlabeled IFN-Con1 or IFN-β. The amount of 125I-IFN-Con1, specifically bound to the fusion protein was then determined by either (i) conjugating the fusion protein to protein A-Sepharose and measuring bound cpm in the pellet fraction separated by centrifugation or by (ii) covalently cross-linking bound 125I-IFN to the fusion protein with disuccinimidyl suberate, affinity purifying the fusion protein on protein A-Sepharose, and then visualizing the salt-eluted fraction by SDS-polyacrylamide gel electrophoresis. Failure to detect 125I-IFN-Con1 bound to the fusion protein by either method supports our findings that IFNABR-α alone does not bind the IFN-α2b or IFN-β.

Our data would suggest that different Type 1 IFN, both α and β, exhibit poor affinity for IFNABR-β that is not associated with an intact, functionally competent receptor complex; A9+ murine cells that lack human IFNABR-β do not bind these IFN with high affinity. Accordingly, we examined the ability of different IFN to bind to a fusion protein that comprised the extracellular portion of IFNABR-α linked to the Fc region of IgG1. The conformational integrity of the IFNABR-α/Fc fusion protein is suggested by the ability of 2 distinct mAbs, which recognize native cell surface IFNABR-α in flow cytometry (51), to immunoprecipitate the fusion protein (data not shown). Differential recognition of the IFNABR-α fusion protein by these antibodies, together with the ability of the fusion protein to associate with a specific glycosphingolipid species normally associated with cell surface IFNABR-α (8), suggests that this fusion protein is conformationally similar to native cell surface IFNABR-α.

Fig. 4. IFNABR-α cell surface expression on A9+, A9+, and A9+21 cells. Flow cytometric analysis of IFNABR-α mAb binding to native IFNABR-α on A9+-transfected containing plasmid only, A9+ transfectants expressing IFNABR-α mRNA, and A9+21 cells that contain 1–5 copies of human chromosome 21 on the murine A9 background. For details refer to Fig. 2. Incubation with either medium alone or secondary and tertiary reagents alone resulted in superimposable, negative cytograms, which are represented as open cytofluorograph profiles; positive cytograms (+IFNABR-α mAb), represented as filled cytofluorograph profiles, gave mean fluorescent intensities of 148 for A9+ and 139 for A9+21 cells.
not constitute a binding receptor. Apparently contradictory reports that suggest that IFNABR-α is a binding receptor (52, 53) did not evaluate the binding capacity of the receptor chain alone.

DISCUSSION

Human IFNABR-α stably expressed in murine cells apparently confers sensitivity only to select human IFN-αs, IFN-α8 (6, 44, 54, 55), and IFN-Con1 (our data, 9, 55). In contrast, a monoclonal antibody to human IFNABR-α inhibits the biologic activity of several species of human IFN-α, IFN-β, and IFN-ω in human cells (56), suggesting a structural heterogeneity of the IFNABR-α that would allow for differential affinities amongst the different Type 1 IFN, dependent on the presentation/accessibility of IFNABR-α on the cell type in question. The implications are that additional components influence the in-
It is likely that the different Type 1 IFN share a similar structural conformation and that the IFNABR-α peptide, when optimally configured at the cell surface in association with IFNABR-β, is able to accommodate for the minimal structural variations. The preceding data would suggest that the origin of the cell type dictates the accessibility of IFNABR-α to the different Type 1 IFN. Interestingly, the IFNABR-α mAb that we employed for flow cytometry is able to distinguish human IFNABR-α, regardless of the host cell, implying that the epitope(s) to which it is directed on the extracellular portion of IFNABR-α is unaffected by cell type yet is specific for human IFNABR-α alone. We infer that the IFN recognition epitope(s) on IFNABR-α are influenced by cell factors.

IFNABR-α expressed on human MRC-5 cells that also express IFNABR-β is able to interact with IFN-α/β to invoke ISGF3 activation and an antiviral response, yet the limited binding capacity of MRC-5 cells and the low affinity ligand-receptor interaction apparently precludes their sensitivity to the growth inhibitory effects of the same IFN. The implications are that MRC-5 cells express functionally deficient Type 1 IFN receptors. We observed that a 10-fold increase in IFN-α/β dose is required in MRC-5 cells to achieve a similar level of ISGF3 activation to that obtained in T98G cells and that there is a direct correlation between the extent of IFN-induced ISGF3 activation and the magnitude of an antiviral response. Using monoclonal antibodies directed against individual Jak and STATs, we have shown that cytoplasmic extracts from MRC-5 cells express Jak1, Tyk2, Stat1, Stat2, and Stat3, thus we infer that none of these factors are limiting in the MRC-5 cells. Apparently, the receptor configuration in the MRC-5 cells precludes the appropriate IFN-induced activation of at least Stat1a and Stat2. Furthermore, our data indicate that ISGF3 activation mediated by IFN-α/β-induced activation of IFNABR-α and IFNABR-β does not necessarily invoke an antiproliferative response. When viewed solely in the context of the affinity characteristics of the ligand-receptor interaction, the implications are that the threshold of sensitivity to the antiviral effects of IFN is lower than that required to induce a growth inhibitory response. We have demonstrated that MRC-5 cells express comparable levels of cell surface IFNABR-α and IFNABR-β with Daudi (8) and T98G cells that are responsive to the growth inhibitory effects of Type 1 IFN. These observations suggest that accessory factors directly influence the binding capacity of cells for IFN-α and β, perhaps through an interaction with IFNABR-α and/or IFNABR-β, thereby affecting signal transduction events that mediate an antiproliferative response. In a similar manner to the Jak, the accessory component may contribute to the overall affinity of the IFN receptor complex through an association with an intracellular domain in either IFNABR-α and/or IFNABR-β. The implications are that this accessory component mediates signal transduction that leads to a growth inhibitory response. Our results with murine cells transfected with the human IFNABR-α indicate that the subtle structural variations among the different human IFN-α/β and IFN-β cannot be accommodated readily when human IFNABR-α is expressed on murine cells and, indeed, receptor recognition is dramatically restricted. Not surprisingly, only the low affinity signaling pathway associated with an antiviral response is affected in the murine transfectants. Human IFNABR-α, perhaps associated with murine specific components, will accommodate IFN-Con1. This may be a consequence of the inherent higher specific affinity of IFN-Con1 for the Type 1 IFN receptor, per se, that allows recognition of IFNABR-α on the murine background. By contrast, IFN-α/β, which has been shown to be active on murine cells transfected with human IFNABR-α (6, 52, 55), is distinct from the other human IFN-α species in that region associated with species-specific receptor recognition (5, 57) and may indeed resemble a murine IFN in this region of the molecule. Full
biological sensitivity was restored to IFNABR-α by complementation with chromosome 21-encoded factors that include IFNABR-β. From our experimental results we infer that distinct signaling pathways invoke antiviral and growth inhibitory responses.

The challenge is to elaborate those regions on IFNABR-α and IFNABR-β that interact with the ligand IFN, that are possibly involved in receptor-receptor interactions, and that associate with accessory factors (e.g. Jak s) that are necessary for full ligand-receptor activation. Our data indicate that co-expression of IFNABR-α and IFNABR-β is not sufficient to invoke a complete biological response to IFN-α/β. Apparently, ISGF3 activation that leads to an antiviral response is mediated by co-expression with chromosome 21-encoded factors that include accessory factors.

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