The Short Form of the Interferon α/β Receptor Chain 2 Acts as a Dominant Negative for Type I Interferon Action*

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We have characterized the functional properties of the short form of the human interferon α/β receptor chain 2 (IFNAR2), denoted IFNAR2.1. IFNAR2.1 contains a shortened cytoplasmic domain when compared with the recently cloned full-length IFNAR2 chain (IFNAR2.2). We show that IFNo8 and IFNβb induce antiviral and antiproliferative activity in mouse cell transfectants expressing the human IFNAR1 chain of the receptor and induce the formation of STAT1/STAT2 dimers in IFN-stimulated response element (ISRE)-dependent gel shift assays. In contrast, coexpression of IFNAR2.1 with IFNAR1 reduces the IFN-induced antiviral, antiproliferative and ISRE-dependent gel shift binding activity conferred by IFNAR1 alone. No antiviral or antiproliferative response to IFN, nor IFN-induced ISRE-dependent gel shift binding activity, was observed when IFNAR2.1 was expressed alone in murine cells. Therefore, IFNAR2.1 acts as a dominant negative for these IFN-induced activities. Our results suggest that IFNAR2.1 represents a nonfunctional version of the full-length chain (IFNAR2.2).

Type I interferons (IFNs), consisting of α and β subtypes, are a multifunctional cytokine family capable of inhibiting cell proliferation and viral replication, in addition to modulating cellular immune functions (1). Type I IFNs compete with one another for binding to a common multisubunit receptor present on the surface of target cells. The type I IFN receptor consists of at least two distinct subunits IFNAR1 and IFNAR2 (2–5). The IFNAR1 chain appears to be involved primarily in signal transduction (2, 6, 7), while the IFNAR2 chain plays a role both in ligand binding and signal transduction (3–5). The IFNAR2 chain was originally identified as a ~50-kDa protein (IFNAR2.1) (3), having a truncated cytoplasmic domain due to alternative mRNA splicing (8). A 100-kDa form of the IFNAR2 chain (IFNAR2.2) has been cloned which reconstitutes biological activity in both murine (4) and human cells (5). In most mammalian cells IFNAR2.1 is expressed at low levels relative to the full-length IFNAR2 chain (9). These findings call into question the biological function of IFNAR2.1.

We have examined the biological consequences of expression of the human IFNAR1 and IFNAR2.1 chains on the sensitivity of murine cells to several type I IFNs (IFNo2, IFNo8, and IFNβb). In murine L929 cells this form is completely unresponsive to human IFN (6, 7). We previously established that the IFNAR1 subunit acts as a species-specific signal transduction component of the human type I IFN receptor complex, but it does not directly bind IFN (6, 7).

In the present study, we demonstrate that IFNAR1 expression in L929 cells confers sensitivity to the antiviral, antiproliferative, and ISRE-dependent gel shift binding activities of IFNo8 and to a lesser extent IFNβb. However, expression of IFNAR1 in L929 cells does not confer sensitivity to the biological activities of IFNo2. IFNAR2.1 expression in murine cells does not confer sensitivity to any IFN subtype examined. Interestingly, cells expressing both receptor chains exhibited a markedly reduced sensitivity to the antiviral, antiproliferative, and ISRE-dependent gel shift binding activities of IFNo8 and IFNβb, when compared with cells expressing only IFNAR1. These results suggest that IFNAR2.1 acts in a dominant negative manner for the induction of biological activity by several type I IFNs.

MATERIALS AND METHODS

Cells and IFNs—Isolation of mouse L929 cells expressing IFNAR1, IFNAR2.1, or both IFNAR1 and IFNAR2.1 together has been described previously (10). IFNAR1 and IFNAR2.1 transfectants were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine calf serum, geneticin (1.5 mg/ml), and hygromycin (0.3 mg/ml). Recombinant IFNo2 (4 × 107 IU/mg), IFNo8 (2 × 107 IU/mg), and IFNβb (4 × 107 IU/mg) was kindly provided by P. Trotta, Schering-Plough (Kenilworth, NJ), H. Hochkoppel, Ciba-Geigy AG (Basel, Switzerland), and Berlex Biosciences (Richmond, CA), respectively. IFN activities are expressed in international reference units/ml as assayed by protection against the cytopathic effect of vesicular stomatitis virus (VSV) on human fibroblasts, using the NIH human IFNo standard for reference.

Antiviral and Antiproliferative Assays—For determining antiviral activity, cell cultures (5 × 105 cells/25 cm2 flask) were preincubated overnight with IFN, followed by infection with VSV (Indiana strain) for 1.5 h at 0.1 plaque-forming units (pfu) per cell. At 24 h post-infection, the virus yield in the medium was assayed by plaque formation on Vero cells (11). Assay of the antiproliferative action of IFN was performed by treating cells (1 × 105 cells/25 cm2 flask) with IFN. After 4 days, the cells were trypsinized and enumerated in a Coulter Counter (12, 13).

Nuclear Extracts and Gel Shift Assays—Nuclei were extracted from control and IFN-treated cells (3000 IU/ml, 15 min) with buffer containing 20 mM Tris-HCl, pH 7.85, 250 mM sucrose, 0.4 mM KCl, 1.1 mM MgCl2, 5 mM β-mercaptoethanol, and 0.4 mM phenylmethylsulfonyl fluoride, and proteins were frozen in dry ice and stored at −80°C (11, 14, 15). Protein gel shift analysis, the nuclear extracts were incubated with a 32P-labeled ISG15 ISRE promoter probe (5′-GATCCATGCCTGGAAGGAAAACCGGAAATGAGG-3′) (16, 17) at 25°C for 30 min, and the unbound radiolabeled probe was separated from protein-DNA.

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complexes on 5% polyacrylamide gels. Gels were quantitated by phosphorimage autoradiography (Molecular Dynamics).

RESULTS AND DISCUSSION

Antiviral Activity of Human IFNs in Murine Transfectants—We have previously described the isolation of murine L929 cells stably expressing the cloned human IFNAR1 chain (L929R1 cells), the 51-kDa IFNAR2.1 chain (L929R2 cells), or both IFNAR1 and IFNAR2.1 (L929R12 cells) (10). IFNs are defined by their ability to inhibit the replication of a wide variety of RNA and DNA viruses. Mouse L929 cells are resistant to the antiviral action of huIFN, but are highly responsive to mouse IFN (6). Expression of the human IFNAR1 (huIFNAR1) chain in L929 cells has been found to result in antiviral protection by some human type I IFNs, without affecting sensitivity to mouse IFNα/β (6, 7). In the present study, we determined the effect of huIFNAR2.1 expression on IFN’s antiviral activity in cells expressing IFNAR1. Cells were treated overnight with human IFN, then infected with VSV (at a multiplicity of infection of 0.1 pfu/cell) and the virus released into the culture medium at one day post-infection was titrated by plaque assays on the indicator Vero cell line.

Expression of huIFNAR1 or huIFNAR2.1 had no effect on the ability of L929 cells to support VSV replication, since virus replication was similar in all cells lines examined (L929, L929R1, L929R2, or L929R12) varying between 0.8 and 1.3 × 10⁸ pfu/ml. As shown in Fig. 1 even at a concentration as high as 3,000 IU/ml, IFNα8, or IFNβ1 did not markedly inhibit VSV replication in L929 cells or in L929R2 cells. Consistent with our previous studies (6), L929R1 cells, which only express huIFNAR1, were sensitive to the antiviral action of human IFNα8 and to a lesser extent IFNβ1b. In contrast, coexpression of huIFNAR2.1 with huIFNAR1 in L929R12 cells markedly reduced sensitivity to the antiviral action of human IFNα8 or IFNβ1b. For example, IFNα8 treatment (3,000 IU/ml) induced only a 225-fold reduction in viral titer in L929R12 cells, as compared with a >6,000-fold reduction in L929R1 cells (>100-fold reduction in antiviral activity). A dose-dependent reduction of viral titer in L929R1 and L929R12 cells was observed with IFNα8 concentrations varying from 100 to 10,000 IU/ml (Fig. 1B). However, at all concentrations the antiviral effect of IFNα8 was markedly reduced in cells coexpressing IFNAR2.1. These results show that the expression of IFNAR2.1 reduces the sensitivity of IFNAR1-expressing cells to the antiviral action of human IFNα8 and IFNβ1b.

Antiproliferative Activity of Human IFNs in Murine Transfectants—Besides their broad antiviral action, IFNs also inhibit cell proliferation. Since L929R1 transfectants were highly sensitive to the antiviral action of human IFNα8, we extended our studies to determine if they were also sensitive to IFN’s antiproliferative action. Although cells sensitive to the antiviral action of IFN are usually also sensitive to the antiproliferative action, there are instances where there is a divergence in sensitivity to these actions of IFNs (18). Thus, the various L929 transfectants were treated with human type I IFNs and cell numbers quantitated after 4 days. As shown in Fig. 2, treatment with human IFNα8 or IFNβ1b inhibited the proliferation of L929R1 cells which express IFNAR1 alone, or of L929R12 cells, which coexpress IFNAR1 and IFNAR2.1. However, the
antiproliferative effect in L929R12 cells was markedly less when compared with the effect with L929R1 cells. IFNα2, IFNα8, or IFNβ1b did not affect the proliferation of L929 cells or L929R2 cells that express the IFNAR2.1 chain. IFNα2 had no significant antiproliferative activity in any of the transfectants (L929R1, L929R2, or L929R12) cell lines. Although an antiproliferative effect could be detected in L929R2 cells at 100 IU/ml IFNα8 (Fig. 2B) or IFNβ1b (data not shown), the maximal antiproliferative effect was observed at 10,000 IU/ml, and no further inhibition of proliferation was observed at higher IFN concentrations. Most importantly, IFNα’s antiproliferative effect in L929R12 cells was reduced at all IFN concentrations when compared with the effect in L929R1 cells. For example, the IC50 of the antiproliferative effect of IFNα8 was 125 IU/ml in L929R1 transfectants as compared with an IC50 of ~30,000 IU/ml for IFNα8 in L929R12 cells. Thus, expression of huIFNAR1 in murine cells reduced the sensitivity of cells expressing huIFNAR1 to the antiviral and antiproliferative effects of IFNα8 and IFNβ1b.

**Induction of ISGF3 Activity by Human IFNs in Murine Transfectants**—An early event induced by the binding of IFNs to their receptor is specific gene induction through the activation of the STAT-containing ISGF3, which binds to the conserved ISRE present in the promoter of many IFN-responsive genes. Because of the importance of ISGF3 in IFN signaling, we determined the IFN-induced formation of the ISGF3 transcription factor in the various murine transfectants. Nuclear extracts prepared from control and IFN-treated (3,000 IU/ml, 15 min) transfectants were incubated with a labeled ISRE probe, and the resultant protein-DNA complexes were analyzed by the gel shift assay. The data presented here indicate that IFNAR1 is able to transduce signals not only for the antiviral action of some type I IFNs as shown previously (6, 7), but also for the antiproliferative action of human IFN. This presumably occurs through the interaction of the human IFNAR1 chain with components of the mouse type I IFN receptor (19), since IFNAR1 expression does not increase human IFN binding to murine cells. It is unclear why particular human type I IFNs such as IFNα2 are unable to transduce signals through the available murine IFN receptor subunits, while IFNα8, and to a lesser extent IFNβ1b, are able to elicit biological effects through the endogenous receptor. These results suggest that type I IFN subtypes may differentially interact with their common multisubunit receptor. For example, while IFNα8 productively interacts with human type I IFN receptor components in a murine context, IFNα2 does not.

In addition coexpression of IFNAR1 and IFNAR2.1 in L929 cells markedly reduced sensitivity to the biological activities of human IFNα8 and IFNβ1b relative to cells expressing only IFNAR1, although the sensitivity to murine IFNβ/β was similar in all transfectants (data not shown). Therefore, IFNAR2.1 acts in a dominant negative manner for the antiviral and antiproliferative actions of human IFNα8 and IFNβ1b, as well as for the induction of ISRE-dependent gel shift binding activity. The level of human IFNAR2.1 chain expression relative to that of the endogenous murine IFNAR2 chain in the various transfectants is unknown. Presumably murine cells do not express a short form of IFNAR2, because human IFNAR2.1 is encoded by an Alu cassette, which is a sequence present solely in full-length IFNAR2 for interaction with type I IFNs to bind ligand and transduce biological effects.

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