Differential Responsiveness of a Splice Variant of the Human Type I Interferon Receptor to Interferons*

Jeffry R. Cook, Cathleen M. Cleary, Thomas M. Mariano, Lara Izotova, and Sidney Pestka‡

From the Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854-5635

Chinese hamster ovary cells containing the yeast artificial chromosome F136C5 (αYAC) respond to all type I human interferons including IFN-αA, IFN-β, and IFN-ω. The αYAC contains at least two genes encoding interferon-α receptor (IFN-αR) chains that are required for response to type I human interferons: Hu-IFN-αR1 and Hu-IFN-αR2. We previously isolated a splice variant of the Hu-IFN-αR1 chain designated Hu-IFN-αR1s. Chinese hamster ovary cells containing a disrupted αYAC, which contains a deletion in the human IFNAR1 gene, were transfected with expression vectors for the Hu-IFN-αR1 and Hu-IFN-αR1s chains. With these cells, two type I interferons have been identified which can interact with the splice variant (Hu-IFN-αR1s) and with the Hu-IFN-αR1 chains: Hu-IFN-αA and IFN-ω. Two other type I interferons, Hu-IFN-αB2 and Hu-IFN-αF, are capable of signaling through the Hu-IFN-αR1 chain only and cannot utilize the splice variant Hu-IFN-αR1s. Hu-IFN-αR1 and Hu-IFN-αR1s differ in that the latter is missing a single subdomain of the receptor extracellular domain encoded by exons 4 and 5 of the IFNAR1 gene. These results therefore indicate that different type I interferons require different subdomains of the Hu-IFN-αR1 receptor chain, and that the splice variant chain (Hu-IFN-αR1s) is functional.

Functional screening of yeast artificial chromosomes was previously used to isolate and clone the accessory factor, or second chain, of the human IFN-α previously used to isolate and clone the accessory factor, or second chain, of the human IFN-α. The αYAC contains at least two genes encoding interferon-α receptor (IFN-αR) chains that are required for response to type I human interferons: Hu-IFN-αR1 and Hu-IFN-αR2. We previously isolated a splice variant of the Hu-IFN-αR1 chain designated Hu-IFN-αR1s. Chinese hamster ovary cells containing a disrupted αYAC, which contains a deletion in the human IFNAR1 gene, were transfected with expression vectors for the Hu-IFN-αR1 and Hu-IFN-αR1s chains. With these cells, two type I interferons have been identified which can interact with the splice variant (Hu-IFN-αR1s) and with the Hu-IFN-αR1 chains: Hu-IFN-αA and IFN-ω. Two other type I interferons, Hu-IFN-αB2 and Hu-IFN-αF, are capable of signaling through the Hu-IFN-αR1 chain only and cannot utilize the splice variant Hu-IFN-αR1s. Hu-IFN-αR1 and Hu-IFN-αR1s differ in that the latter is missing a single subdomain of the receptor extracellular domain encoded by exons 4 and 5 of the IFNAR1 gene. These results therefore indicate that different type I interferons require different subdomains of the Hu-IFN-αR1 receptor chain, and that the splice variant chain (Hu-IFN-αR1s) is functional.

Experimental Procedures

Cells—The 16-9 cell line is a human × hamster hybrid containing the long arm of human chromosome 6 and a transfected HA-B7 gene (Cook et al., 1994). The 16-9/ΔYAC cells were obtained by transforming 16-9 cells with the αYAC (F136C5) as described (Soh et al., 1994b). 16-9/ΔYAC cells contain the deleted ΔYAC whose IFNAR1 gene was disrupted by homologous recombination (Cleary et al., 1994). 16-9/ΔYAC cells are the 16-9/ΔYAC cell line transfected with the cDNA for Hu-IFN-αR1 and the 16-9/ΔYAC cell line is the 16-9/ΔYAC cell line transfected with the cDNA for Hu-IFN-αR1s. The 16-9 cell line was maintained in F12 medium plus 10% fetal bovine serum. The 16-9/ΔYAC and 16-9/ΔYAC cell lines were grown in F12 plus 10% fetal bovine serum and 350 μg/ml antibiotic G418. The 16-9/ΔYAC and 16-9/ΔYAC cell lines were grown in F12 plus 10% fetal bovine serum and 350 μg/ml each of antibiotic G418 and hygromycin.

Interferons—The following interferons were prepared according to the methods described by Wang et al. (1994) and provided by PBL Biomedical Laboratories (New Brunswick, NJ): human IFN-β2, -ω, -αD, -αF, -αG, -αH, -αI, and -αK. Hu-IFN-αA was prepared as described by Staehelin et al. (1981). Human IFN-β was purified as described by Moschera et al. (1986). Human IFN-ω was a gift from Dr. G. Adelf (Ernst-Boehringer Institut fur Arzneimittelforschung, Austria). Specific activity was based on anti-VSV activity with human WIHS cells in a cytopathic effect inhibition assay (Famletti et al., 1981). Specific activities ranged from 1.5 × 10⁴ to 5.0 × 10⁴ units/mg of protein.

Assay of Class I MHC Antigen Expression—The assay of class I MHC antigen expression was conducted as described previously (Cook et al., 1992) except that the cells were exposed to interferons for 72 h before
assay. Class I MHC antigen expression was quantified as the mean logit of relative fluorescence for 10,000 cells as recorded by a Coulter Epics Profile cytofluorograph.

IFN Binding Assay—The phosphorylatable interferons Hu-IFN-αR1 and Hu-IFN-αR2 were supplied by PBL Biomedical Laboratories. These interferons were phosphorylated as described (Li et al., 1989; Soh et al., 1994b; Wang et al., 1994). Labeled interferons were incubated with 7.5 × 10⁶ cells in a 0.15-ml reaction. The ³²P-labeled interferons were present at a concentration of 2.5 × 10⁶ cpm per reaction. Unlabeled competitor interferons were present at a 100-fold excess relative to the ³²P-labeled interferons. Cells were incubated with ligand at 14°C for 2 h and bound IFN was separated from unbound IFN by centrifuging an aliquot (0.1 ml) of the reaction through 10% (w/v) sucrose (Langer and Pestka, 1986).

Screening of Libraries—Host cells and phage were grown according to standard procedures (Maniatis et al., 1982). Plaques were lifted onto nylon membrane circles (Micron Separations, Inc.) and lifts were processed for 5 min each in denaturing buffer (0.5 M NaOH, 0.5 M NaCl), neutralizing buffer (0.5 M Tris.Cl, pH 8.0, 0.5 M NaCl), and 2 × SSPE (1 × SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, 1 mM EDTA)). Lifts were dried in air and then baked at 80°C for 2 h in a vacuum oven. Hybridizations were performed in 50% formamide, 1.0% SDS, 5 × SSPE and 5 × Denhardt’s solution (1 × Denhardt’s solution is 0.002% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin) at 42°C.

Blots were washed in 2 × SSPE, 0.1% SDS at 60°C. Positive plaques were purified through three rounds of screening before phage were grown for λDNA isolation.

RESULTS

The 3 × 15 cell line is a hamster-human somatic cell hybrid that contains approximately 1 to 3 kilobases of human chromosome 21 to which the genes for the type I interferon receptor complex have been localized (Langer et al., 1990; Jung, 1991). These cells showed increased expression of hamster MHC class I cell surface antigens in response to treatment with Hu-IFN-αB2 at 1000 units/ml for 72 h (Langer et al., 1990). A cDNA library was prepared from 3 × 15 cell mRNA by the automatic directional cloning (ADC) system with the λ-pCEV27 phagemid vector (Miki et al., 1991). The 3 × 15 λ-pCEV27 cDNA library was screened with an 878-base pair fragment of the Hu-IFN-αR1 cDNA (Uzé et al., 1990), covering in part the first seven exons of the gene (Fig. 1). Approximately 3.2 × 10⁶ plaques were screened; 9 positive clones were identified. After a second and third round of screening with both the aforementioned probe and a 478-base pair probe corresponding to portions of exons IX to XI, one clone, λ-pCEV27-17-1a, was purified which hybridized to both probes (Cleary et al., 1992).

Sall restriction endonuclease released an insert of 1.6–1.7 kilobases. The plasmid was rescued from clone λ-pCEV27-17-1a by digestion with restriction enzyme PvuI at the multiple excision site followed by ligation and transformation, as described (Miki et al., 1991). The nucleotide sequence of this cDNA was determined by the dideoxynucleotide chain termination method (Sanger et al., 1977). Sequence alignment of clone 17-1a with the Hu-IFN-αR1 cDNA revealed that they shared complete homology at the nucleotide level except for gaps in the sequence at three sites in clone 17-1a. Mapping of the human IFNAR1 gene (Mariano et al., 1992; Lutfalla et al., 1992) permitted localization of the gaps with respect to the intron/exon structure of the gene as depicted in Fig. 1. First, the 5' nucleotide of clone 17-1a aligns with nucleotide 306 of the Hu-IFN-αR1 cDNA, which lies within exon III of the gene. Thus, clone 17-1a lacked a 5' untranslated region, a leader sequence and the amino-terminal coding region of the cDNA. Second, a gap was observed in the alignment of the extracellular regions of these cDNAs. This gap corresponds precisely to the sequence encoding exons IV and V of the Hu-IFN-αR1 cDNA, and thus an alternatively spliced extracellular domain is encoded by clone 17-1a. Alignment then continues through the remaining extracellular, transmembrane, and intracellular coding regions. The third gap appears within the 3' untranslated region where the 17-1a cDNA utilizes the first of 2 polyadenylation signals present at nucleotide 2227 in the Hu-IFN-αR1 cDNA, and therefore lacks the remaining 500 base pairs of 3' untranslated sequence between the two polyadenylation sites. Thus, the 17-1a cDNA clone represents an alternatively spliced form of the Hu-IFN-αR1 cDNA in which exons IV and V of the sequence are precisely spliced out. This splicing event does not interrupt the open reading frame, but deletes the segment encoding the 99 amino acids of these two exons, and therefore, this alternatively spliced cDNA may code for a functional protein. The exons spliced out correspond roughly to the SD100B domain in the subdomain structure of the Hu-IFN-αR1 component (Bazan, 1990a, 1990b).

Clone 17-1a was digested with Sall and SpeI and subcloned into pYH12, which contains the full-length Hu-IFN-αR1 cDNA. (This clone is designated "Hu-IFN-αR-CC17.") Hu-IFN-αR-CC17 was digested with NheI and Smal and the released cDNA insert was subcloned into pB942, an expression vector with hygromycin resistance. The resulting plasmid was transfected...
into the 16-9/ΔαYAC cell line to produce the 16-9/ΔαYAC R1s cell line. We then examined the activity of this cell line, in addition to the 16-9, 16-9/YAC, 16-9/ΔαYAC, and 16-9/ΔαYAC R1 R1s cell lines in response to Hu-IFN-αA and Hu-IFN-αB2. As shown in Fig. 2, induction of class I MHC antigen by Hu-IFN-αA (left panels) and Hu-IFN-αB2 (right panels) was not seen in the parental 16-9 hamster cell line (panels A and B), but was seen in 16-9/YAC cells (panels C and D). When the IFNAR1 gene on the YAC was disrupted (Cleary et al., 1994), no response to either IFN-αA or IFN-αB2 was seen in the resulting 16-9/ΔαYAC cell line (panels E and F). To examine the function of the splice variant Hu-IFN-αR1s, 16-9/ΔαYAC cells were transfected with either the expression vectors for Hu-IFN-αR1 or Hu-IFN-αR1s to produce the 16-9/ΔαYAC/αR1 and the 16-9/ΔαYAC/αR1s cell lines, respectively, as described by Cleary et al. (1994). As shown in Fig. 2, 16-9/ΔαYAC R1 R1s cells responded to both Hu-IFN-αA and Hu-IFN-αB2 (panels G and H). However, 16-9/ΔαYAC R1 R1s cells, expressing the Hu-IFN-αR1s splice variant, failed to respond to Hu-IFN-αB2 at 500 units/ml, but responded to Hu-IFN-αA at the same concentration (panels I and J). These results indicate that the receptor complex containing the splice variant responds to Hu-IFN-αA, but not to Hu-IFN-αB2.

Fig. 3 shows class I MHC antigen expression of the above cell lines as a function of concentration of Hu-IFN-αA and Hu-IFN-αB2. The parental 16-9 cell line exhibited no response to either Hu-IFN-αA or Hu-IFN-αB2 at any of the concentrations tested (panel A). Similar results were obtained for the cell line containing the ΔαYAC, 16-9/ΔαYAC (panel C) at concentrations of 500 units/ml or less of Hu-IFN-α or Hu-IFN-αB2. However, a significant response was observed at high concentrations (1000 units/ml) of Hu-IFN-αA. The 16-9/YAC (panel B) and 16-9/ΔαYAC/αR1 (panel D) cell lines responded to both Hu-IFN-αA and Hu-IFN-αB2, essentially as described previously (Cleary et al., 1994). However, the cell line expressing the splice variant (16-9/ΔαYAC R1 R1s; panel E) responded to Hu-IFN-αA at concentrations above 100 units/ml, but failed to respond to Hu-IFN-αB2 at any concentration assayed. These results indicated that the inability of cells containing the Hu-IFN-αR1s chain to respond to Hu-IFN-αB2 may be due to the absence of an IFN-αB2-specific ligand docking site in the splice variant receptor. The fact that the response to IFN-αA (panel E) was not observed at or below 100 units/ml probably also indicates that the splice variant is less efficient in responding to this interferon than to the Hu-IFN-αR1 chain, possibly because of lower affinity of the receptor complex with the splice variant for Hu-IFN-αA.

Because Hu-IFN-αA and Hu-IFN-αB2 displayed different interactions with the Hu-IFN-αR1s chain, it was of interest to determine whether other type I interferons behave similarly. Therefore, we examined the effect of Hu-IFN-αC, -αD, -αF, -αG, -αH, -αI, -αK, and -ω on class I MHC antigen induction and found that most of these interferons stimulated class I MHC antigen expression in the parental hamster cells (Table I), thereby preventing evaluation of their effect in hamster cells containing the αYAC and other constructs. Hu-IFN-β was not tested as previous studies had shown that it is functional with the parental hamster cells (data not shown). Only Hu-IFN-αF...
Type I Interferon Receptor

Table I

| IFN group | Cell linea | 16-9 | 16-9ΔαYAC | 16-9ΔαYAC/αR1s |
|-----------|------------|------|----------|-----------------|
| 1. Hu-IFN-αβ2 | - | - | + | + |
| 2. Hu-IFN-αf | - | - | + | + |
| 3. Hu-IFN-αA, Hu-IFN-ω | - | - | + | + |
| 4. Hu-IFN-αC, -αD, -αG, -αH, -αI, -αJ, -αK | ++ | NDb | ND | ND |

* a - indicates no activity in MHC class I antigen assay, ± indicates some activity in MHC class I antigen assay, and ++ indicates full activity in the MHC assay.

b ND, not done.

Fig. 4. Class I MHC antigen induction as a function of Hu-IFN-αf concentration. Results for 16-9, 16-9ΔαYAC, and 16-9ΔαYAC/αR1s are shown in A, and results for 16-9ΔαYAC/αR1s are shown in B. Class I MHC antigen expression was determined as described in the legend to Fig. 2. Relative fluorescence values were normalized so that untreated controls have a relative fluorescence of 1.0.

Fig. 5. Class I MHC antigen induction as a function of Hu-IFN-α concentration. Results for 16-9, 16-9ΔαYAC, and 16-9ΔαYAC/αR1s are shown in A, and results for 16-9ΔαYAC/αR1s are shown in B. Class I MHC antigen expression was examined competition by interferons for binding of 32P-labeled Hu-IFN-αA-P1 and 32P-labeled Hu-IFN-β2-P to 16-9ΔαYAC/αR1s cells. As is shown in Table II, 16-9ΔαYAC/αR1s cells could bind labeled Hu-IFN-αA-P1, and this binding was reduced by unlabeled Hu-IFN-αA. In addition, Hu-IFN-αB, Hu-IFN-ω, and Hu-IFN-αF all were able to compete with labeled Hu-IFN-αA-P1 for binding to 16-9ΔαYAC/αR1s cells. This indicates that Hu-IFN-αB and Hu-IFN-αF both bind to the 16-9ΔαYAC/αR1s cells. In the similar experiment performed with labeled Hu-IFN-β2-P it is also clear that 32P-labeled Hu-IFN-αA-P1 binds to the receptor containing the splice variant and that the other type I interferons (Hu-IFN-αB, Hu-IFN-αA, Hu-IFN-ω, and Hu-IFN-αF) compete with binding of 32P-labeled Hu-IFN-αA-P1 to this receptor (Table II).

Discussion

Our results enable us to identify four groups of human type I interferons (Table I). The first group consists of at least Hu-IFN-αB2. This interferon interacts with the subdomain of the Hu-IFN-αR1 chain which is encoded by exons 4 and 5 of the IFNAR1 gene. Elimination of these exons by alternative splicing produces an mRNA which is translated into a receptor which can bind ligand, but which cannot transduce the appropriate signal for class I MHC antigen induction in response to Hu-IFN-αB2. A second group represented by Hu-IFN-αF requires the subdomain which is absent in the splice variant Hu-IFN-αR1s for maximum activity, but still exhibits some activity in cells lacking a functional IFNAR1 gene (16-9ΔαYAC cells). Like Hu-IFN-αB2, Hu-IFN-αF is inactive in cells expressing the splice variant (16-9ΔαYAC/αR1s). A third group of type I interferons includes at least Hu-IFN-αA and Hu-IFN-ω. These interferons interact primarily or exclusively with

and Hu-IFN-ω did not react with the parental hamster 16-9 cells as judged by class I MHC antigen induction; therefore, these two interferons were studied further. As is shown in Fig. 4, Hu-IFN-αf behaves somewhat like Hu-IFN-αβ2. At 1000 units/ml and below, there was no apparent MHC class I antigen induction with parental hamster cells. Cells containing the αYAC (16-9ΔαYAC) responded to Hu-IFN-αf even at 10 units/ml. The cells with the disrupted IFNAR1 gene (16-9ΔαYAC) exhibited a significantly lower response to Hu-IFN-αf than the 16-9/αYAC cells; nevertheless, there was a residual response of these cells to Hu-IFN-αf. In 16-9ΔαYAC/αR1s, however, Hu-IFN-αf stimulated class I MHC antigen induction somewhat more than the cells containing the disrupted IFNAR1 gene. Significantly, the 16-9ΔαYAC/αR1s cells expressing the splice variant did not respond to Hu-IFN-αF. Similar experiments were performed with Hu-IFN-ω. This interferon had previously been shown to act through the Hu-IFN-α receptor complex (Flores et al., 1991). As shown in Fig. 5, Hu-IFN-ω resembles Hu-IFN-α in that it stimulated class I MHC induction when either Hu-IFN-αR1 or Hu-IFN-αR1s was included in the type I interferon receptor complex. It should be noted that, at 10 and 100 units/ml of Hu-IFN-ω, there was a significant induction of class I antigens in 16-9ΔαYAC cells, 16-9/ΔαYAC/αR1, and 16-9ΔαYAC/αR1s cells, but no significant induction in parental 16-9 cells or 16-9ΔαYAC cells.

In order to determine whether the absence of the subdomain encoded by exons 4 and 5 of the IFNAR1 gene produces a receptor without affinity for Hu-IFN-αB2 and Hu-IFN-αF, we...
regions of the receptor which are included in the Hu-IFN-aR1s chain and are therefore active when this receptor replaces the Hu-IFN-aR1 chain in the type I interferon receptor complex. It is likely that other type I interferons will be found to belong to one or the other of these receptor groups. A fourth group of type I interferons consists of those species which interact with the hamster receptor, even at relatively low concentrations (Table I).

The YAC is known to encode two receptor chains involved in IFN-α action: Hu-IFN-αR1 and Hu-IFN-αR2 (Emmanuel, 1995). A third component (CRF84), also on the YAC, may or may not be a functional component of the IFN-α pathway. Disruption of the IFNAR1 gene (Cleary et al., 1994) effectively eliminated Hu-IFN-αR1 expression and should block IFN-α signal transduction in so far as Hu-IFN-αR1 is a required component. For Hu-IFN-αA and Hu-IFN-αB there was no residual activity in the 16-9ΔYAC cell line at ≤ 500 units/ml. However, at 1000 units/ml, some class I MHC antigen induction was noted with Hu-IFN-αA (Fig. 3). Similarly, a small but significant level of activity was observed with Hu-IFN-αF (Fig. 4) in 16-9ΔYAC cells treated with ≥ 500 units/ml of Hu-IFN-αF. Hu-IFN-ω also elicited some class I MHC antigen induction in the 16-9ΔYAC line at ≥ 500 units/ml (Fig. 5). The experiments described in this study do not permit us to define the cause of the residual activity in 16-9ΔYAC cells. Two hypotheses can be suggested, however. First, the hamster IFN-αR1 or IFN-αR1s chain could be substituting for the comparable human chain to one extent or another. Second, the remaining components of the human type I interferon receptor complex could be capable of signal transduction to a certain extent without the Hu-IFN-αR1 chains.

Transfecting the Hu-IFN-αR1s receptor into the 16-9ΔYAC cell line reconstituted responsiveness to Hu-IFN-αA. As is shown in Fig. 3, a significant response to Hu-IFN-αA was observed at 500 units/ml in 16-9ΔYAC/R1s cells, whereas no response was observed at this concentration in the 16-9ΔYAC cells (Fig. 2). These results were also confirmed by antiviral studies (Cleary, 1995) in which the 16-9ΔYAC/R1s cells responded to Hu-IFN-αA (ED50 = 3536 units/ml) but not to Hu-IFN-αB2 (ED50 > 40,000 units/ml). The responsiveness of the 16-9ΔYAC/R1s cell line to Hu-IFN-αω was pronounced (Fig. 5). In fact, the induction of class I MHC antigen in 16-9ΔYAC/R1s cells by Hu-IFN-ω exceeded that observed in the 16-9ΔYAC/R1 cell line at every concentration tested, possibly indicating that Hu-IFN-ω interacts preferentially with a type I receptor complex containing the splice variant receptor. As is shown in Table II, these results also confirmed those with the ED50 of the 16-9ΔYAC/R1 cell line being 312 units/ml with Hu-IFN-ω and the ED50 of the 16-9ΔYAC/R1s cell line being only 56 units/ml (Cleary, 1995).

The examination of the primary amino acid sequences of the human type I interferons reveals several homologies among the groups of interferons distinguished in the above studies. Hu-IFN-αA and Hu-IFN-ω (group 3) both contain a serine at position 11 and a threonine at position 14 in place of the consensus asparagine and alanine (Fig. 6). The serine substitution is unique to Hu-IFN-αA and -ω, Hu-IFN-κ, -ωB2, -ωD, -ωH1, and -ωG1 and also contains the threonine substitution. At position 64, Hu-IFN-αA and Hu-IFN-ω contain an isoleucine substituted for the consensus threonine, which is present in all other type I interferons. At position 132 Hu-IFN-ω and Hu-IFN-αA contain a threonine to isoleucine substitution which is unique to these two interferons. Also, at position 156 and 157, respectively, Hu-IFN-αB2 and Hu-IFN-ωF have an isoleucine, whereas Hu-IFN-αA and Hu-IFN-ω have threonine or asparagine. Some or all of these amino acid substitutions may be involved in the interaction of Hu-IFN-ωR1s with type I interferons. Site-directed mutagenesis of either Hu-IFN-αA or Hu-IFN-ω could be utilized to demonstrate which of the candidate residues, if any, are in fact required for signal transduction through Hu-IFN-ωR1s. The search for residues responsible for interaction of the human type I interferon with the hamster receptor is not likely to be as straightforward as analysis of primary sequences (Fig. 6) does not reveal any homologies unique to group 4 interferons.

The differential responsiveness of the splice variant receptor should be viewed in terms of earlier studies showing that different species of type I interferons have different activity profiles. Evinger et al. (1981) found that individual purified Hu-IFN-α species produced by leukocytes had varied ratios of growth inhibitory activity to antiviral activity. Rehberg et al.

| Unlabeled competitor | Percent of control binding to 16-9ΔYAC/R1s cells | 3P-labeled Hu-IFN-αA | 3P-labeled Hu-IFN-αB |
|----------------------|-----------------------------------------------|----------------------|----------------------|
| None                 | 100                                          | 100                  | 100                  |
| Hu-IFN-αA            | 25                                           | 48                   |                      |
| Hu-IFN-αB2           | 35                                           | 34                   |                      |
| Hu-IFN-ω             | 43                                           | 34                   |                      |
| Hu-IFN-αF            | 36                                           | 29                   |                      |

Table II

Fig. 6. Comparative sequence analysis of type I interferons used in this study. Top row shows the consensus sequence of type I interferons. The other rows show residues at which Hu-IFN-αA, -ω, -ωB2, and -ωF differ from the consensus sequence. Underlined amino acids indicate substitutions which may account for the differential activity of Hu-IFN-αA and -ω versus Hu-IFN-ωB2 and -ωF on cells expressing Hu-IFN-ωR1s. Sequence of Hu-IFN-αA, -ωB2, and -ωF are from Pestka (1986).
The ratio of the Hu-IFN-αA and Hu-IFN-αD had different antiviral activities depending on the type of target cell, with Hu-IFN-αA having high antiviral activity on human cells and Hu-IFN-αD having much less. Similarly, Hu-IFN-αA exhibited high antiproliferative activity whereas Hu-IFN-αD exhibited less. Ortaldo et al. (1984) found that Hu-IFN-αA was virtually incapable of augmenting NK activity, while other type I interferons exhibited various levels of NK stimulating activity. It was suggested that the lack of NK activity in response to Hu-IFN-αA could be due to the structure of Hu-IFN-αA or to the existence of different type I receptors. Finally, Fish et al. (1983) also examined the antiproliferative and antiviral activities of several type I interferons and found that Hu-IFN-αA, -αC, and -αF had higher antiproliferative activities on human cells than did Hu-IFN-αB, -αD, and -αA. Nevertheless, Hu-IFN-αA, -αD, and -αA/D stimulated greater antiviral protection than did Hu-IFN-αB, -αC, and -αF. These results are consistent with the class I MHC antigen induction data presented here. Of especial interest is the fact that both Hu-IFN-αA and -αB can be distinguished from Hu-IFN-αA in terms of their antiviral and antiproliferative activities. When Hu-IFN-αD was tested for the present report, its interaction with the parental hamster cell lines made further study of its interaction with the splice variant receptor possible. The previous studies clearly established that type I interferons do have differential activities and our results suggest that the various Hu-IFN-α species and other type I interferons interact with the receptor differentially, thereby accounting for their differential activities. The tissue-specific expression of the Hu-IFN-αR1s has not yet been examined. However, it is conceivable that the ratio of the Hu-IFN-αR1s to Hu-IFN-αR1 may vary from tissue to tissue thereby altering the responsiveness to the various type I interferons. The ratio of the Hu-IFN-αR2 chain to the other type I receptor components may also be important. The conservation of the multitude of type I interferons throughout evolution of the mammals is consistent with unique functional roles for each of the type I interferons.

The phenomenon of differential regulation by expression of alternate forms of a receptor component has been reported in other systems. The human fibroblast growth factor receptor-2 (FGFR-2) and keratinocyte growth factor receptor (KGF FR) are encoded by alternatively spliced forms of the same cDNA (Miki et al., 1992). The FGFR-2 cDNA encodes a receptor lacking 2 exons present in the extracellular domain of the KGF FR (Miki et al., 1992). The FGFR-2 binds acidic and basic FGF with high affinities but does not bind KGF, while the KGF FR binds both KGF and acidic FGF with high affinity. Also, the murine FGFR-1 was found to exist in three forms, one of which is secreted (Werner et al., 1992). Three exons encoding extracellular immunoglobulin-like domains are encoded by the FGFR-1 gene and the three forms of this receptor bind acidic FGF with comparable affinities, but display a 50-fold difference in binding affinity for basic FGF. Heterogeneity of glutamate receptors with different ion conductance properties has been determined to be the result of an arginine/glutamine substitution in the transmembrane region. In this case, the alternate receptor forms are produced by RNA editing rather than alternative splicing (Sommer et al., 1991). Our current results begin to dissect the complexity of the type I interferon receptor and to provide a basis for understanding the interactions of the 14 or more different type I interferons with this receptor complex.

Acknowledgments—We thank Ed Yurchow for cytofluorography and Eleanor Kells for assistance in preparation of this manuscript.

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