A Gene on Human Chromosome 21 Located in the Region 21q22.2 to 21q22.3 Encodes a Factor Necessary for Signal Transduction and Antiviral Response to Type I Interferons*

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The type I interferons (IFNs) are a family of multifunctional cytokines which includes the 15 IFNα subtypes and IFNβ. These IFNs compete for binding to cell surface receptors. However, murine cells transfected with a cDNA for a human IFNα receptor (IFNAR) developed an antiviral response only to human IFNαB, but not to human IFNα2 nor -β(1). In this study we show, using a panel of CHO-human chromosome 21 hybrid cell lines which all express IFNAR, that only those containing the region 21q22.2 to 21q22.3 transduce signals for IFN responses. Two such hybrid cell lines responded to IFNα2, -αB and -β by induction of 2′-5′ oligoadenylate synthetase and resistance to viral infection. Other hybrid cell lines, that lacked the region 21q22.2-3, failed to transduce signals as above; even though they expressed IFNAR and bound human IFNα2, -αB, and -β. These data demonstrate that a gene(s) located in the region 21q22.2-3 encodes a factor(s) which is necessary for signaling but does not influence ligand binding. This factor is not the cofactor required for IFNγ signaling which is located in the region 21p to 21q22.2(12).

The interferons (IFNs) are a large family of cytokines whose functions include the protection of cells against viral infection, the regulation of cell proliferation and differentiation, and activation of cells (such as NK cells and macrophages) (3). The type I IFNs include the 15 subtypes of IFNα, one IFNβ, two IFNα0 (and several trophoblast proteins in some species) (4, 5). This classification is based on similarities in structure (4) and biological activities (3), a shared receptor or receptor component (5), and induction in response to virus and location of the genes on chromosome 9q (3). Type II IFN, or IFNγ, differs from Type I IFNs in all of the above respects, except that some overlap in biological function occurs. The structures of the type I IFNs are conserved with 75–95% amino acid identity between IFNα subtypes, 35% identity between IFNα and -β, and 50–65% identity between IFNα and the remaining type I IFNs (4). The structural differences among the type I IFNs are reflected in functional differences, for example in antigenicity (7) and in biological specific activities which can vary by up to 1000-fold between subtypes (8). Receptor binding has been shown to be necessary, but not sufficient, for the biological activities of type I IFNs (3, 6). Post-receptor binding events such as signal transduction and induction of IFN response genes are also necessary for biological responses.

Many type I IFN-induced genes have been identified, and some, in particular those encoding the double-stranded RNA-dependent 2′-5′ oligoadenylate synthetase, 68 kinase, and Mx proteins, have been assigned a role in antiviral and/or antiproliferative effects of IFNs (9). Recently, early events in the induction of certain IFN-sensitive genes have been characterized. The protein kinases tyk-2 (10) and JAK-1 (11) are involved in signal transduction by type I IFNs and a transcription factor complex, termed ISGF3α, is activated by phosphorylation as early as 2 min after receptor binding of IFNα (12, 13). However the precise mechanisms by which IFN ligand-receptor interaction transduces signals to activate the intracellular pathways for antiviral or antiproliferative effects remain to be elucidated.

Most of the type I IFNs have been presumed to bind to a common cell surface receptor component based on their ability to compete with each other for receptor binding (6). Furthermore the gene encoding the receptor (or a component of the receptor) for the type I IFN has been shown to be localized on human chromosome 21 in the region 21q22.1 (1, 6, 14). The cDNA encoding IFNAR has been isolated (1). Unexpectedly, murine cells transfected with IFNAR developed an antiviral response only to human IFNαB, but not to IFNα2 or IFNβ (1). Since receptor binding data were not presented for IFNα2 or -β it, remains unclear whether the failure to elicit an antiviral response in these transfected cells by these two ligands was due to an inability to bind the cloned receptor component or whether another factor(s) is required to transduce signals.

In the present study we have examined the receptor binding and biological responses of several human type I IFNs. We have used a panel of CHO-human chromosome 21 hybrid cell lines which contain different portions of human chromosome 21 to examine (i) the binding of IFNs and (ii) the biological consequence of such binding as manifest by the induction of 2′-5′ oligoadenylate synthetase activity and the development of antiviral responses. Our studies show that a gene located on the distal portion of human chromosome 21 in the region q22.2 to q22.3 encodes a factor which does not affect ligand-receptor binding, but is necessary for signal transduction by type I IFNs. Furthermore this factor is distinct from a signaling factor required for responses to type II IFNγ, the gene for which maps to a different part of chromosome 21.

MATERIALS AND METHODS

Cell Lines and Interferons—CHO-K1 cell line (American Type Culture Collection) and CHO-human chromosome 21 containing hybrid cell lines (D. Patterson, Eleanor Roosevelt Institute Denver, CO) were...
grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin, 2.3 mg/ml proline, and 0.1 mM uridine. The 72532x6 cell line contains an entire human chromosome 21, 643C-13 contains 21p-21q22, 21q" contains 21p to 21q22.1, and MRC 2G contains a human ring chromosome 21, 21p to 21q22.1, with a deletion in the 21q22 region (15) (Fig. 1a). Recombinant human IFNα2a, antiviral specific activity 2.0 ± 30 pg of RNA in 50% formamide were run on 1% agarose gels, transcribed with and without excess unlabeled IFN (100 IU/mg of protein). IFNα2a was iodinated also using chloramine T (19) to a radioactive specific activity of approximately 50 pCi/pg of protein. IFNβα was iodinated also using chloramine T (19) to a radioactive specific activity of approximately 100 pCi/pg of protein. Integrity of the iodinated IFNs was monitored by comparative antiviral and receptor binding activities relative to unlabeled IFNs and was shown to be unaffected for the period of use.

Binding assays were performed using monolayers of CHO cells or CHO hybrid cells in six-well cell dishes containing 1-3 × 10^5 cells/well. Duplicate wells were incubated with each of a range of concentrations of 125I-labeled IFN in 0.5 ml of binding medium (Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 50 mM Hepes, 1.0 mM CaCl₂, pH 7.4) with and without excess unlabeled IFN (100 x for IFNα2a and -αβ or 1000 x for IFN βα, as appropriate) to determine nonspecific binding. The plates were incubated at 20 °C for 2 h with moderate shaking. The incubation mixture was then removed and the monolayers washed four times with cold binding medium. The cells were harvested and counted in a γ-counter. Scatchard analyses of the binding curves were performed using the LIGAND program. In all cases where significant binding was detected, a "one-site" fit was statistically significant (p < 0.05) and a "two-site" fit was not (20). The binding of 125I-labeled IFNα2a and IFNβα to the CHO cell line was so low that it could not be resolved by a Scatchard plot. Binding curves and Scatchard plots were produced using the INPLOT program.

Receptor Binding Studies—IFNα2a and IFNβα were iodinated using chloramine T as described previously (18) to a radioactivity specific activity of approximately 50 μCi/μg of protein. IFNβα was also iodinated using chloramine T (19) to a radioactive specific activity of approximately 100 μCi/μg of protein. Northern Blots—Cells were grown to mid-log phase, harvested, and RNA extracted using guanidinium isothiocyanate (16). Approximately 30 μg of RNA in 50% formamide were run on 1% agarose gels, transferred to Hybond-N, hybridized with a 32P-labeled 1.2 kilobase pair NcoI fragment of the human IFNAR cDNA at 55 °C overnight and washed at 65 °C, 0.1 x SSPE, 0.1% SDS. As a control for RNA loading, filters were stripped and rehybridized with a 32P-labeled 1.1-kilobase pair glyceraldehyde phosphate dehydrogenase cDNA probe (17).

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Results

Expression of IFNAR and Ligand Binding—In initial experiments we examined the four different CHO-human chromo-some 21 hybrid cell lines (Fig. 1a) for expression of IFNAR (Fig. 1b). Northern blot analysis of these four cell lines as well as the CHO cell line demonstrated that all the hybrid cell lines expressed a transcript of approximately 2.7 kilobase pairs for human IFNAR, whereas the CHO cell line did not express the human transcript (Fig. 1b). The transcript size was as expected on the basis of sequence and published data, including Northern blots of RNA from Daudi cells (from which the IFNAR cDNA was cloned) (1). The apparent difference in mobility of the transcript from the 21q cell line is an artifact of the running conditions used for this gel, thus the GAPDH transcript also has a similarly affected mobility (Fig. 1b). Furthermore in other Northern blot experiments where RNA from 21q' and 72532x6 only were tested, the IFNAR transcript had the same mobility (data not shown).

Analyses of the binding of human type I IFNs to receptors on the hybrid cell lines are presented as binding curves and Scatchard plots (insets) (Fig. 2). Scatchard analysis of the binding of IFNs α2, αβ, and β to the hybrid cell lines was resolved into a one-site fit in all cases (p < 0.05) (Fig. 2). The very low level of binding of 125I-IFNs α2 or αβ to the CHO cell line could not be resolved by Scatchard analysis (Fig. 2a). The binding of 125I-IFNβα to the CHO cell line was also lower than to the hybrid cell lines. Although there may appear to be some high affinity binding component evident from the Scatchard plots, this could not be significantly resolved at these very low levels of binding (and even if it was, the numbers would have been very small and similar for the four hybrid cell lines). Such a low level of high affinity binding may be a consequence of some low level inter-
action of the human IFN ligand-human IFNAR complex with other components of the endogenous hamster type I IFN receptor(s).

All four hybrid lines bound $^{125}$I-IFN$\alpha$2 with similar dissociation constants of about 200 pM and had similar number of receptors per cell, about 800 (Table I and Fig. 2a). Similarly, the binding of $^{125}$I-IFN$\alpha$B and $^{125}$I-IFN$\beta$ was comparable in the various hybrid cell lines. For instance, the binding of IFN$\alpha$B to 72532x6 and 21q$^q$ had dissociation constants of 170 and 150 pM, respectively, and the number of receptors per cell were 300 and 400, respectively (Fig. 2b). For IFN$\beta$, binding to 72532x6 and 21q$^q$ dissociation constants were 54 and 68 pM, respectively, and the number of receptors per cell were 1300 and 720, respectively.

**FIG. 2.** Binding of $^{125}$I-labeled type I IFNs to CHO-human chromosome 21-containing hybrid cell lines and the parental CHO cell line. Data are plotted as specific binding versus concentration of added IFNs and as Scatchard plots (insets) obtained using the LIGAND program. The binding of $^{125}$I-IFN$\alpha$2 is shown in Fig. 2a, $^{125}$I-IFN$\alpha$B in Fig. 2b, and $^{125}$I-IFN$\beta$ in Fig. 2c.
**Type I Interferon Signaling Factor**

Induction of 2'-5' Oligoadenylate Synthetase—The ability of the receptor-bound IFNs α2, α8, and β to transduce signals is in the hybrid cell lines was measured first by the induction of 2'-5' oligoadenylate synthetase, a double-stranded RNA-dependent IFN-induced enzyme which is induced early and plays an important role in the antiviral response (24). IFNα2 significantly induced 2'-5' oligoadenylate synthetase activity in 72532x6 and 643C-13, but surprisingly not in 21q+ nor MRC 2G nor the CHO cell lines (Fig. 3a). The enzyme activity of the untreated cells in nanomoles of P incorporated per mg of protein was: 108 ± 49 (mean ± S.E., n = 5) for CHO, 56 ± 37 (n = 4) for 21q+, 62 ± 46 (n = 3) for 72532x6, 73 ± 35 (n = 4) for MRC 2G and 62 ± 27 (n = 4) for 643C-13. The induction of 2'-5' oligoadenylate synthetase occurred in an apparent dose-related fashion and was already significantly increased in 72532x6 by 100 IU/ml of IFNα2 (Fig. 3a). On the contrary, the treatment of 21q+, MRC 2G, and CHO cell lines, which all lack the region of human chromosome 21q22.2-3, showed no (or very little) induction of 2'-5' oligoadenylate synthetase activity, even at concentrations of IFNα2 up to 1000 IU/ml (Fig. 3a).

Induction of 2'-5' oligoadenylate synthetase by IFNβ and IFNγ was also only observed in 72532x6, which contains the entire human chromosome 21, but not in the 21q+ cell lines Fig. 3, b and c). Furthermore, we also found in one experiment that IFNβ (1000 IU/ml) increased the activity of 2'-5' oligoadenylate synthetase in 643C-13 cells (increase of 360 nmol of P/mg of protein), but not in MRC 2G cells. These data demonstrate that a factor(s) encoded by a gene(s) located at 21q22.2-3 is necessary for eliciting a response resulting from IFN ligand-receptor interaction and show that the factor is required prior to induction of IFN-induced genes such as 2'-5' oligoadenylate synthetase which occurs early in the response.

**Antiviral Responses**—Even though all the hybrid cell lines bound type 1 IFNs with similar affinities, we show that only 72532x6, which contains the human chromosome 21q region 22.2-q22.3, elicited antiviral responses to α2. The 72532x6 cell line showed antiviral activity to SFV in response to as little as 20 IU/ml of IFNα2, whereas the CHO cells were relatively insensitive, responding to IFNα2 only at concentrations between 750 and 1500 IU/ml (Fig. 4). Interestingly, the 21q+ cell line, which lacks the human chromosome 21 region 21q22.2-q22.3, was as insensitive to IFNα2 as the CHO cell line (Fig. 4). From several such experiments, the ratios (relative to 72532x6 = 1) of IFNα2 required for antiviral response to SFV were 164 ± 80 for 21q+ (mean ± S.E., n = 5) and 78 ± 38 for CHO-K1 (n = 5).

Additional experiments demonstrated that only 72532x6, which contains the entire human chromosome 21, demonstrated efficient antiviral activity regardless of type 1 IFN ligand used or the cytopathic virus used. Thus, about 50-fold less IFNβ (47 ± 27, n = 3) was required to protect 72532x6 (relative to 21q+ and CHO) from the cytopathic effect of SFV. Also, when encephalomyocarditis virus was used as the challenge virus, 21q+ was as insensitive as CHO to the antiviral effects of IFNα2 (10-fold more IFN required than 72532x6). Together, these data demonstrate that a gene(s) encoded in the region 21q22.2-3 is necessary for antiviral responses triggered by the binding of these type 1 IFNs to the receptor. These results confirm those on the induction of 2'-5' oligoadenylate synthetase.

**DISCUSSION**

These studies demonstrate that a factor encoded on human chromosome 21 q22.2 to q22.3 is required for transducing signals necessary for antiviral response induced by several type 1 IFNs, namely α2, α8, and β. The hybrid cell lines that contained the entire human chromosome 21 showed an up-regulation of 2'-5' oligoadenylate synthetase activity and an antiviral response. Hybrid cell lines that contained human chromosome 21 but lacked the region q22.2 to q22.3 failed to show evidence of a biological response, even though they bound the three type 1 IFN ligands. The localization of the gene encoding this factor to human chromosome 21q22.2-22.3 and not to other random human genomic fragments that may be present in such interspecies hybrids (25) is confirmed by its presence in two hybrid cell lines containing

**Table I**

Receptor binding of IFNα2 to hybrid cell lines

Data are presented as mean ± S.E. of three experiments or mean and range of two experiments.

| Cell line | No. Expts. | Dissociation constant (Kd) | Number of sites/cell |
|-----------|------------|---------------------------|----------------------|
| 72532x6   | 3          | 270 ± 44                  | 739 ± 42             |
| 643C-13   | 2          | 175 (105, 244)            | 995 (762, 1228)      |
| 21q+      | 3          | 277 ± 28                  | 814 ± 119            |
| MRC 2G    | 2          | 208 (192, 233)            | 880 (690, 1070)      |

**Fig. 3.** Induction of 2'-5' oligoadenylate synthetase activity by IFNα2 (a) and IFNβ (b), and IFNγ (c). 2'-5' oligoadenylate synthetase activity of cells treated with 100 or 1000 IU/ml of IFN is expressed as the increase in nanomoles of P incorporated per mg of protein in activity over that of untreated cells. Data are expressed as mean ± S.E. of at least three experiments for IFNα2 induction and as the mean and range of two experiments for IFNβ and IFNγ inductions.
CHO-K1    72532X-6    21q+ IFN-α2 (IU/ml)

3000
1500
750
375
187
94
47
23

Fig. 4. Antiviral activity of type I IFNs in CHO-human chromosome 21 hybrid cell lines. A typical antiviral assay of IFN-α2a on CHO-K1, 72532X-6, and 21q+. Quadruplicate wells of each cell line were exposed to serial dilutions of IFN-α2a ranging from approximately 20 to 3000 IU/ml, then SFV for 3 days. Thereafter, viable cells were stained (red) using 3-(4,5-di methylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide as described in the text.

this region as well as its absence from two hybrid cell lines which lack this region. These findings are also significant because they show that the factor(s) necessary for signal transduction of an antiviral response is apparently not involved in receptor binding. The cloning of this molecule is currently in progress and will be aided by the recent mapping of the entire human chromosome 21 and the availability of yeast artificial chromosomes spanning this chromosome (26).

The data in this study also give insight into the failure of murine cells transfected with IFNαR to respond to human IFNs α2 and β as reported by Uzé et al. (1). The absence of the human chromosome 21-encoded factor identified herein may, in part at least, explain why the murine IFNαR transfectants did not develop an antiviral response to IFNs α2 and β (1). The development of a response only to IFNβR reported in those studies could be explained if IFNβR, but not IFNα2 and β, interacted with the murine homologue(s) of the “signaling” factor(s), located at human chromosome 21q22.2 to 22.3. For this to occur, IFNαβ should have structural differences from both IFNα2 and β and a similarity with murIFNα. Indeed IFNβR has 26 amino acid residues different from IFNα2 and many more differences from IFNβ; 6 of these residues are conserved between human IFNαβ and the majority of murine IFNαβ, namely Asn11, Ile14, Pro26, Gln28, Ala39, and Met100 (4). Perhaps one or more of these residues is important for interactions with the murine signaling factor(s).

In the present study the hamster homologue of the human chromosome 21-encoded signaling factor did not reconstitute signal transduction by the human IFN ligand α2, β, or αβ-human receptor complex, indicating the species specificity of this interaction. It is unlikely that this factor is one of the JAK family of protein tyrosine kinases known to be involved in type I IFN signaling (10, 11). The activity of JAK-1 is not species-specific, since human cells defective in IFN signal transduction exhibit reconstituted signaling when transfected with murine JAK-1 cDNA (11). Another JAK family member, tyk-2, is known to be encoded by a gene located on human chromosome 19 (27). Furthermore, none of the known JAK family members have been localized to human chromosome 21 (37).

The finding that a type I IFN signaling molecule in addition to IFNAR (and an IFNγ signaling molecule (28) and IFN-inducible Mx genes (15)) are located on chromosome 21 may also have relevance to Down's syndrome (trisomy 21). This may explain why cells from individuals with Down's syndrome (trisomy 21) have shown approximately 8-fold greater antiviral response to IFN (29), which is greater than a 1.5-fold gene dosage effect, which would be expected from the overexpression of IFNAR by itself. Some of the features of Down's syndrome, such as inhibited growth and immunological abnormalities, may be due (in part) to dysregulation of the antiproliferative
and immunoregulatory actions of IFNs.

The chromosome 21-encoded signaling factor identified in this study does not apparently modify ligand-receptor binding. All hybrid cell lines showed virtually identical ligand binding of the IFNα subtypes as analyzed by Scatchard plots, which showed similar numbers of receptors per cell and a Kd value of approximately 200 pM. This Kd is virtually the same as we have observed for some human cell lines (data not shown). Thus the 7253kx6, 21q, 643C-13, and even the MRC 2G cell lines contain all components required to give the described receptor binding characteristics. Since cells transfected with IFNAR alone failed to bind α2 or β1, then other binding components of the receptor (distinct from the signaling factor described herein) may be encoded on chromosome 21. Recently receptor-ligand cross-linking experiments (30) and immunoprecipitation (31) using antibodies to Daudi cell surface molecules putatively designated as components of the type I IFN receptor complex suggest that there may be membrane components other than IFNAR associated with the type I IFN receptor complex. Furthermore, one such component may be encoded by another gene located on human chromosome 21, in the region 21p to 22.2. Taken together with our data showing the binding of type 1 IFNs α2, α5, and β by MRC 2G (the hybrid cell line in this study with the smallest amount of chromosome 21), an additional factor which is necessary for binding α2 and β must be located in the region 21p-21q 21 (proximal of MRC2, Fig. 1, upper panel) or in 21q22.1 (between MRC2 and 8;21 markers).

Interferon γ has also been shown to require a factor for signal transduction subsequent to receptor-ligand interaction (28). Curiously, this factor is also located on human chromosome 21, but proximal to 21q22.1 (6). Thus there are two distinct factors for transducing signals resulting from type I and II IFN binding to their cognate receptors. These factors (for both type I and II IFN) do not appear to modify the affinity of the ligands for their respective receptors and are therefore distinct from the “affinity converters” (or β subunits of receptors) which function in signaling for other cytokines, e.g. gp130 for interleukin 6, leukemia inhibitory factor, ciliary neurotrophic factor (32) and KHR7 for interleukin 3, interleukin 5, granulocyte/macrophage colony-stimulating factor (33). Thus the data in this study provide further evidence of similarities between the IFNα and IFNγ receptors, i.e. in addition to sequence motif and predicted structural similarities (34), both require additional signaling molecules located on human chromosome 21 (Fig. 5).

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