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Identification, Cloning, and Characterization of a Novel Soluble Receptor That Binds IL-22 and Neutralizes Its Activity$^{1,2}$

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With the use of a partial sequence of the human genome, we identified a gene encoding a novel soluble receptor belonging to the class II cytokine receptor family. This gene is positioned on chromosome 6 in the vicinity of the IFNGRI gene in a head-to-tail orientation. The gene consists of six exons and encodes a 231-aa protein with a 21-aa leader sequence. The secreted mature protein demonstrates 34% amino acid identity to the extracellular domain of the IL-22R1 chain. Cross-linking experiments demonstrate that the protein binds IL-22 and prevents binding of IL-22 to the functional cell surface IL-22R complex, which consists of two subunits, the IL-22R1 and the IL-10R2c, chains. Moreover, this soluble receptor, designated IL-22-binding protein (BP), is capable of neutralizing IL-22 activity. In the presence of the IL-22BP, IL-22 is unable to induce Stat activation in IL-22-responsive human lung carcinoma A549 cells. IL-22BP also blocked induction of the suppressors of cytokine signaling-3 (SOCS-3) gene expression by IL-22 in HepG2 cells. To further evaluate IL-22BP action, we used hamster cells expressing a modified IL-22R complex consisting of the intact IL-10RZ$^2$, and the chimeric IL-22R1/γR1 receptor in which the IL-22R1 intracellular domain was replaced with the IFN-γR1 intracellular domain. In these cells, IL-22 activates biological activities specific for IFN-γ, such as up-regulation of MHC class I Ag expression. The addition of IL-22BP neutralizes the ability of IL-22 to induce Stat activation and MHC class I Ag expression in these cells. Thus, the soluble receptor designated IL-22BP inhibits IL-22 activity by binding IL-22 and blocking its interaction with the cell surface IL-22R complex. The Journal of Immunology, 2001, 166: 7096–7103.

Cytokines elicit biological activities via binding to specific membrane-bound receptors. Ligand-induced oligomerization of the receptor extracellular domains causes the interaction of the receptor intracellular domains and receptor-associated cytoplasmic proteins, participants of the signal transduction cascade. In this way, the extracellular signal is transmitted through the cellular membrane to the cytoplasm and subsequently to the nucleus (1–4). However, functions of several cytokines are also negatively regulated by membrane-bound decoy receptors or by soluble receptors. For example, the role of such cytokine inhibitors is well characterized for IL-1 and IL-18 signaling (5–10). These cytokine signal through receptors belonging to the class II cytokine receptor family (CRF$^2$) using structurally homologous but distinct receptor complexes. Functional receptor complexes for both IL-1 and IL-18 consist of the unique ligand-binding chains (IL-1RI and IL-18R, respectively) and accessory chains (IL-1R accessory protein (AcP) and IL-18 AcP-like, respectively), which are required to initiate signaling and increase cytokine binding affinity of the receptors. The type II IL-1R (IL-1RII) plays the role of decoy receptor for IL-1. IL-1RII has a short intracellular domain and, although it is able to bind the ligand and interact with IL-1 AcP, it is unable to transduce signaling. The IL-1RII binds IL-1, particularly IL-1β, preventing ligand binding to the signal-transducing IL-1RI. It may also act by sequestering the IL-1 AcP and, thus, reducing its availability for the functional IL-1R complex. In contrast, the IL-18-binding protein (BP) does not possess a transmembrane domain and functions as a soluble inhibitor of IL-18 actions. The use of decoy or soluble receptors for cytokines using members of the class II CRF for signaling (3) has been demonstrated only for IFN-α. The human IFN-αR2c, one of the functional chains of the IFN-αR complex, has two splice variants, the membrane-bound IFN-αR2c and a short intracellular domain, which appears unable to transduce the signal, and the soluble IFN-αR2a form (6, 11–16). However, the functions of these receptors have not been well characterized.

IL-22 (or IL-10-related T cell-derived inducible factor) is a member of the family of IL-10 homologues and requires two receptor chains to assemble the functional IL-22R complex, the unique IL-22R1 chain and the IL-10R2c chain (17, 18), which also

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$^2$ The cDNA and deduced amino acid sequences of CRF2-10 and its splice variants were submitted to the GenBank.

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$^5$ Abbreviations used in this paper: CRF, cytokine receptor family; AcP, accessory protein; IL-22BP, IL-22-binding protein; F, forward; R, reverse; FL, tagged at N terminus with the FLAG epitope; AP, adapter primer; BAC, bacterial artificial chromosome; CRF, cytokine receptor family; ESF, expressed sequence tag; UTR, untranslated region; SOCS, suppressors of cytokine signaling; P, phosphorylatable.

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serves as a second chain of the IL-10R complex (19). Both chains belong to the class II CRF (3). The other family members are two receptor chains for type I IFNs, another chain of the IL-10R complex, and tissue factor. Several orphan receptors from this family have also been identified (3). The IL-22R complex demonstrates unique features compared with other receptor complexes in that both chains are able to bind IL-22 independently, whereas other ligands (type I and II IFNs and IL-10) bind with high affinity to only one chain of the corresponding receptor complexes, with the second chain only modulating affinity of the entire receptor complex (reviewed in Ref. 3).

In a search of novel receptors from the class II CRF, we identified and cloned a soluble receptor designated CRF class II member 10 (CRF2-10) or IL-22BP, which is the topic of this report. We show that CRF2-10 binds to IL-22, prevents its interaction with the functional IL-22R complex, and, thus, blocks the activity of IL-22.

### Materials and Methods

#### PCR, primers, cDNAs, and expression vectors

Primers 5'-CAATCGGAAAATAGAAGACTTGGTGG-3' (R10-1 forward (F) primer, R10-1p), 5'-GATGATCAAGACAACTCTCCTTGGAC-3' (R10-2p), 5'-TACCTCTAACATGGCCTCTCACCA-3' (R10-5 reverse (R) primer, R10-5p), 5'-CTCAGAAGACTGAGATTGTCATC-3' (R10-6), 5'-CTCAGAAGACTGAGATTGTCATC-3' (R10-6p), containing cDNA isolated from human placenta (catalog number HL4252AH, Clontech Laboratories, Palo Alto, CA) were used for nested PCR. The first round was performed with R10-1p and R10-6p primers followed by the second round with R10-2p and R10-5p primers and the PCR product of the first round as a template. The resultant PCR product was cloned and sequenced. Three additional primers, 5'-GGTCCTACAGAGCTCTGCTC-3' (R10-9p), 5'-TCTCACTAG-AGCTCTGCTC-3' (R10-9R), 5'-GATTAATACGCTACT-3' (R10-10R), and 5'-CTCCCACCGGAGGCGG-3' (R10-10p), were designed to clone the 5' end of the CRF2-10 cDNA. Nested PCR was performed with these three primers and adapter primers (AP) and 2 and placental cDNA provided with the Marathon cDNA amplification kit (catalog number K1802-1, Clontech Laboratories) following the manufacturer's protocol. The first round was performed with the R10-10p primer and AP1 and was followed by the second round with either the R10-11 or R10-9 primer and AP2 and the PCR product of the first round as a template. Several PCR products were obtained and sequenced.

CRF2-10-specific primers 5'-AGGAAACAGTTCTGCTCAGGGAATGG-3' (R10-1p), 5'-TACAGATCGCAGATCTCGCTCTTGGACGCTC-3' (R10-2p), 5'-ACGATCTGACCTCTCCTCAGGAGGACGCCAGC-3' (R10-10p), and 5'-AGATCTAGAATTCCTCCTCCAGGACACC-3' (R10-9p) were used to clone a fragment of the CRF2-10 cDNA encoding the mature protein into the pEF-SPFL vector (20). These primers and either the library containing cDNA isolated from human placenta or cDNAs synthesized with total RNA from human placenta were ligated into the pEF-SPFL vector (GenBank accession number XM004438) (20), resulting in plasmid pEF-SPFL-FLAG (FL-CRF2-10).

To clone the extracellular domain of the CRF2-8 protein (or ZCYTOR7, homology search is a part of the Hs.126891 gene), primers 5'-CAATGGAAAAATAAAGAAGACTGTTGG-3' (R10-1 forward (F) primer, R10-1p), 5'-GCCGGATCCCTGTGTCTCTGGTACGAGATTGACTCCTCCTTGGAC-3' (R10-2p), and 5'-TTACTCTTAATTCATCGCCCTCTCCAC-3' (R10-5 reverse (R) primer, R10-5p) were used. The first round was performed with R10-1p and R10-5p primers followed by the second round with R10-2p and R10-5p primers and the PCR product of the first round as a template. Several PCR products were obtained and sequenced. The analysis of the sequence revealed the presence of an additional exon (exon 5; Fig. 1; exons are numbered based on the structure of the CRF2-10 gene) between exon 4 (primers R10-1 and R10-1p) and exon 5 (primers R10-5 and R10-5p) of the CRF2-10 gene. The other family members are two members of related ESTs. We speculated that the exon identified in the homology search is a part of the Hs.126891 gene. This hypothetical exon. These ESTs represented the 3' untranslated UTR sequence (see Materials and Methods).

#### EMSAs and Western and Northern blotting

Cells were starved overnight in serum-free medium and then treated with IL-10 or IL-22 for 15 min at 37°C and used for EMSA experiments to detect activation of Stat1, Stat3, and Stat5 as previously described (19). EMSAs were performed with a 22-bp sequence containing a Stat-1a binding site corresponding to the IFN-y activation sequence element in the promoter region of the human IFN regulatory factor-1 gene (5'-GATTC GATTCCCGAATCATG-3') as previously described (12, 23).

Three days after transfection, conditioned medium from COS-1 cells transiently transfected with expression plasmids was collected. FLAG-tagged proteins in the conditioned medium were identified by Western blotting with anti-FLAG epitope-specific M2 mAb (Sigma) as previously described (20). FLAG-tagged proteins were purified from conditioned medium by immunoaffinity chromatography with the anti-FLAG M2 gel (Sigma) according to the manufacturer's suggested protocols.

Cells were lysed and analyzed previously (17) with suppressors of cytokine signaling (SOCS)-3 or β-actin probes. The FLAG-tagged P human IL-22 (FL-IL-22-P) was labeled with [32P]ATP and used for cross-linking to cells as previously described (17, 23, 25). [32P]FL-IL-22-P and soluble receptors were mixed in 200 μl PBS and incubated at 22°C for 1 h followed by the addition of bis(sulfosuccinimidyl) suberate to the final concentration 0.5 mM. The reaction mixture was incubated at 22°C for 20 min and 3% Triton X-100 (0.05%) was added to the final concentration at 50 mM for 10 min. The complexes were later analyzed on SDS-PAGE.

#### Results

### Cloning of the CRF2-10 cDNA

A search of the GenBank database with the TBLASTN program for possible IL-22R1 (CRF2-9) homologues revealed a genomic fragment from human chromosome 6 contained within a bacterial artificial chromosome (BAC) (GenBank accession number AL050337) potentially encoding a fragment of a protein with homology to IL-22R1. We hypothesized that this genomic fragment represented an exon of a novel receptor with homology to IL-22R1. We selected two sets of primers for PCR: the sequences of primers R10-1 and R10-2 were derived from the identified hypothetical exon sequence, and the sequences of primers R10-5 and R10-6 were derived from the 3' UTR sequence (see Materials and Methods). The primers and the human placental cDNA library were used for nested PCR. The resultant PCR product was cloned and sequenced. The analysis of the sequence revealed the presence of an additional exon (exon 5; Fig. 1; exons are numbered based on the structure of the CRF2-10 gene) between exons 4 (primers R10-1 and

### Cells, transfection, and cytofluorographic analysis

The 16-9 hamster × human somatic cell hybrid line is the Chinese hamster ovary cell K1 hybrid containing a translocation of the long arm of human chromosome 6 encoding the human IFNGR1 gene and a transfected human HLA-B7 gene (21). The cells were maintained in F12 (Ham) medium (Sigma, St. Louis, MO) containing 5% heat-inactivated FBS (Sigma). On day 1, an SV40-transformed fibroblast-like simian CV-1 cell line, were maintained in DMEM (Life Technologies, Rockville, MD) with 10% heat-inactivated FBS. Cells were transfected as previously described (19, 20). COS-1 cell supernatants were collected at 72 h as a source of the expressed proteins.

To detect cytokine-induced MHC class I Ag (HLA-B7) expression, cells were treated with COS-1 cell supernatants or purified recombinant proteins as indicated in the text for 1 h and analyzed by flow cytometry. Cell surface expression of the HLA-B7 Ag was detected by treatment with mouse anti-HLA (W6/32) (22) mAb followed by FITC-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA). The cells then were analyzed by cytofluorography as previously described (13).
and R10-2) and exon 6 (primers R10-5 and R10-6) containing the entire 3′ UTR.

Three additional primers (R10-9, R10-10, and R10-11) were designed based on the sequence of exon 4 to clone the 5′ end of the CRF2-10 cDNA. Nested PCR was performed with these three primers and placent cDNA provided with the Marathon cDNA amplification kit. Several PCR products were cloned and sequenced. Different sites of the PCR products reflected the variability of the 5′ UTR of the CRF2-10 cDNA. The CRF2-10 cDNA sequence (Fig. 2) represents the sequence with the longest 5′ UTR.

The comparison of the sequences with the genomic sequence (GenBank accession number AL050337) revealed exons 1–3 of the CRF2-10 gene (Figs. 1 and 2). One of the PCR products represented a splice variant of the CRF2-10 cDNA, revealing the existence of an additional exon 4a (Fig. 1, B and C, and 2). This long splice variant was designated CRF2-10a. When the fragment of the CRF2-10 cDNA encoding mature protein was amplified by PCR either from the placent cDNA library or cDNAs synthesized with total RNA isolated from LPS-stimulated PBMCs, the existence of another splice variant lacking exon 5 was revealed (Figs. 1 and 2C). Joining exons 4 and 6 causes a frame-shifting event, resulting in premature termination and, thus, the generation of a short protein, which was designated CRF2-10b (Figs. 1 and 2C). Thus, the entire CRF2-10 gene is composed of 6 exons (Fig. 1). The entire first exon and a part of the second exon encode the 5′ UTR. The rest of the second exon encodes the signal peptide of the protein. Exons 3–5 and a portion of exon 6 encode the secreted CRF2-10. The rest of the exon 6 encodes the long 3′ UTR with the presence of multiple polyadenylation signals. In addition, there are alternative splicing events that either insert the exon 4a between exons 4 and 5, creating a longer CRF2-10 splice variant, or eliminate the exon 5, creating prematurely terminated short protein (Figs. 1 and 2).

This BAC (GeneBank accession number AL050337) was mapped to human chromosome 6 (6q24.1–25.2). In addition to containing the entire CRF2-10 gene, the BAC also contains the entire IFNGR1 gene (Fig. 2). Analysis of the DNA segment demonstrates that the CRF2-10 gene is positioned near the IFNGR1 gene and transcribed in the same direction as the IFNGR1 gene (Fig. 2). All exon/intron and intron/exon splice sites conform well to the consensus motifs (exon/GT-intron-AG/exon).

Comparison of the sequence of the CRF2-10 protein with those of other members of this family of receptors revealed that CRF2-10 is mostly homologous to the extracellular domain of the IL-22R1 chain (3, 17, 18) and to the extracellular domain of CRF2-8 (3) (Fig. 2D). The function of CRF2-8 was recently characterized as a receptor subunit for the IL-20R complex (26). The comparison reveals that the mature CRF2-10 protein demonstrates 34 and 33% amino acid identity to the extracellular domains of IL-22R1 and CRF2-8, respectively.

**Expression and purification of CRF2-10 and cross-linking**

To obtain the CRF2-10 protein, an expression vector was created encoding FL-CRF2-10. COS-1 cells were transiently transfected with the expression vector and, 3 days later, conditioned medium containing FL-CRF2-10 was collected and tested for protein expression. Western blotting with anti-FLAG Ab revealed that FL-CRF2-10 was produced and secreted from COS-1 cells (data not shown). FL-CRF2-10 was purified from conditioned medium by affinity column chromatography and analyzed by Western blotting with anti-FLAG Ab (Fig. 3A, lane 2). The purified protein appeared on SDS-PAGE as a broad band in the region of ~35–45 kDa, suggesting possible glycosylation of the protein. Indeed, there are five potential sites for N-linked glycosylation (Asn-X-Thr/Ser) in CRF2-10. The FL-CRF2-8 protein was also expressed in COS-1 cells and was purified following similar protocols and used in experiments as a control (Fig. 3A, lane 3).

FL-IL-22-P was described previously (17). This protein contains the FLAG epitope at its N terminus and the consensus amino acid sequence Arg-Arg-Ala-Ser-Val-Ala (P site), recognizable by the catalytic subunit of the cAMP-dependent protein kinase (24, 25) fused to its COOH terminus. Purified FL-IL-22-P is a glycosylated protein migrating on SDS-PAGE as several bands in the region of ~25–40 kDa (Fig. 3A, lane 4). The protein can be labeled with [32P]phosphate (Fig. 3A, lane 5) and cross-linked to the IL-22R chains expressed on the cell surface (17).

To determine whether CRF2-10 is capable of binding IL-22, cross-linking experiments were performed in solution. The FL-IL-22-P protein was labeled, and the [32P]-labeled FL-IL-22-P was incubated with the conditioned medium of COS-1 cells expressing FL-CRF2-10 (Fig. 3B, lane 8). After 1 h incubation, bis(sulfosuccinimidyl)suberate was added and cross-linking was performed. As a control, [32P]-FL-IL-22-P was incubated with the conditioned medium of COS-1 cells transfected with the empty vector and either left untreated (Fig. 3B, lane 6) or cross-linked after 1 h of incubation (Fig. 3B, lane 7). In addition, [32P]-FL-IL-22-P was incubated in solution with either purified FL-CRF2-10 (Fig. 3B, 1 μg, lane 9; 0.1 μg, lane 10) or with purified FL-CRF2-8 (Fig. 3B, 1 μg, lane 11). After a 1-h incubation, cross-linking was performed, and cross-linked complexes were resolved on the gel and autoradiographed (Fig. 3B). Radiolabeled IL-22 migrates as a broad band in the region of 25–40 kDa (Fig. 3, A and B). Cross-linking of the IL-22 in solution resulted in the appearance of an additional broad band in the region of 100–140 kDa, likely representing an IL-22 tetramer. In the presence of the CRF2-10 protein, cross-linking produced an additional complex migrating as an intense broad band in the region of 55–80 kDa. Moreover, the intensity of bands corresponding to the IL-22 monomer was greatly reduced, and cross-linking bands corresponding to the possible tetramer of IL-22 were depleted, indicating that IL-22 tightly binds CRF2-10, interfering with the formation of IL-22 oligomers. The pattern of cross-linking in the presence of the CRF2-8 soluble extracellular domain in the mixture looks identical with the pattern of complexes generated by cross-linking of IL-22 to itself (Fig. 3B, lanes 11 and 7, respectively) indicating the lack of interaction between CRF2-8 and IL-22.
FIGURE 2. The cDNA and predicted amino acid sequence of CRF2-10. A, The cDNA and the deduced amino acid sequence of CRF2-10. Amino acid residues of the putative signal peptide of CRF2-10 are boxed. Potential glycosylation sites are underlined. Positions of introns and an alternative exon 4a are indicated by arrows.

B, The nucleotide sequence of the alternative exon 4a and the encoded amino acid sequence.

C, CRF2-10 and its two splice variants, CRF2-10 L and CRF2-10 S, are aligned.

D, Alignment of amino acid sequences of CRF2-10 (present study), IL-22R1 (3, 17, 18), and CRF2-8 or IL-20Rα (3, 26). A consensus sequence is shown on the bottom. Identical amino acids corresponding to the consensus sequence are shown in black outline with white lettering. Similar amino acids are shown in gray outline with white lettering. Amino acid residues are numbered starting from the first Met residue (signal peptide amino acids are included). The program PILEUP of the Wisconsin Package Version 9.1 from the Genetics Computer Group (Madison, WI) was used with the following parameters: the gap creation penalty 1, the gap extension penalty 1. The BOXSHADE 3.21 program was used for shading of the alignment file.
had no effect on IL-22 activity in this assay (Fig. 5, left panel). STAT1 DNA-binding complexes in a dose-dependent manner (Fig. 5). Pretreatment for 1 h of IL-22 with the FL-CRF2-10 protein before addition to the cells eliminated formation of the IL-22-induced Stat1 DNA-binding complexes after IL-22 treatment (Fig. 5, left panel) (17). Pretreatment for 1 h of IL-22 with the FL-CRF2-10 protein before addition to the cells eliminated formation of the IL-22-induced Stat1 DNA-binding complexes in a dose-dependent manner (Fig. 5, left panel). The FL-CRF2-8 protein was used as a control and had no effect on IL-22 activity in this assay (Fig. 5, left panel).

**Biological activity**

The functional IL-22R complex is composed of two subunits, the IL-22R1 chain and the second IL-10R2c chain (17–19). Both chains are required for signaling; however, each chain alone is capable of binding IL-22 (17, 18). The IL-10R2c chain also plays the role of the second chain of the IL-10R complex (19). To determine whether CRF2-10 can neutralize activity of IL-22, we used hamster cells expressing a chimeric IL-22R complex with the IL-22R1 intracellular domain replaced with the IFN-γR1 intracellular domain (17). The native receptor complex was modified to facilitate detection of IL-22-induced biological activities. With this exchange, IL-22 can activate IFN-γ-like biological responses, such as MHC class I Ag induction and Stat1 activation, in hamster cells expressing the chimeric IL-22R1/γR1 chain and the intact second chain, IL-10R2c (17). COS-1 cell-conditioned medium containing IL-22 was left untreated or incubated with purified FL-CRF2-10 for 1 h. The medium was then added to the cells expressing the IL-22R1/γR1 chain and IL-10R2c, and the ability of IL-22 to induce IFN-γ-like biological activities in these cells was tested. IL-22 up-regulated MHC class I Ag expression in these cells (Fig. 4, thin line, open area) (17). Incubation of IL-22 with CRF2-10 before addition to the cells resulted in complete neutralization of the ability of IL-22 to induce MHC class I Ag expression in these cells (Fig. 4, thin line, shaded area).

EMSA was also performed in these cells. IL-22 activates Stat1 in these cells as demonstrated by the formation of the Stat1 DNA-binding complexes after IL-22 treatment (Fig. 5, left panel) (17). Pretreatment for 1 h of IL-22 with the FL-CRF2-10 protein before addition to the cells eliminated formation of the IL-22-induced Stat1 DNA-binding complexes in a dose-dependent manner (Fig. 5, left panel). The FL-CRF2-8 protein was used as a control and had no effect on IL-22 activity in this assay (Fig. 5, left panel).

Human lung carcinoma A549 cells are responsive to IL-22 as measured by the ability of IL-22 to induce Stat activation in these cells (17). In the presence of CRF2-10, the formation of Stat1/Stat3 DNA-binding complexes in response to IL-22 as measured by EMSA was blocked (Fig. 5, right panel).

Many cytokines induce the expression of the SOCS genes. In IL-22-responsive HepG2 human hepatoma cells (27), the expression of the SOCS-3 gene was induced within 4 h after treatment with IL-22 (Fig. 6). IL-22-driven induction of the SOCS-3 gene expression was inhibited by the addition of CRF2-10 (Fig. 6).

**FIGURE 3.** CRF2-10 binds IL-22. **A.** Analysis of soluble proteins used in the study. CRF2-10 was tagged at the N terminus with the FLAG epitope (FL-CRF2-10). FL-CRF2-8 is the soluble extracellular domain of CRF2-8 (3) tagged at the N terminus with the FLAG epitope. IL-22 was tagged at the N terminus with the FLAG epitope and at the C terminus with the Arg-Arg-Ala-Ser-Val-Ala sequence that contains the consensus amino acid motif recognizable by the catalytic subunit of the cAMP-dependent protein kinase (FL-IL-22-P) (17). The proteins were expressed in COS-1 cells, purified from conditioned medium by affinity chromatography, and evaluated by Western blotting with anti-FLAG Ab (lanes 2–4). The 32P-labeled FL-IL-22-P was also loaded as a control (lane 1). The ordinate represents relative cell number, and the abscissa relative fluorescence.

**FIGURE 4.** MHC class I Ag induction. Chinese hamster cells expressing the intact IL-10R2c chain and the chimeric IL-22R1/γR1 chain were used (17). In these cells, IL-22 is able to induce IFN-γ-specific biological activities such as MHC class I Ag expression as demonstrated here by flow cytometry. The cells were left untreated (open area, thick line) or treated with conditioned medium (30 μl) from COS-1 cells containing FL-IL-22 that was untreated (open area, thin line) or incubated with the purified FL-CRF2-10 protein (5 μg) for 1 h before addition to the cells (shaded area, thin line). The ordinate represents relative cell number, and the abscissa relative fluorescence.
cross-linking experiments demonstrated that CRF2-10 binds IL-22 and prevents its binding to the cell surface IL-22R complex.

Discussion

IL-22 (18, 27, 28) is one of six recently identified IL-10 homologues of human and viral origin. In addition to IL-22, the list includes melanoma differentiation-associated-7 gene (29), IL-19 (30), ak155 (31), IL-20 (26), and CMV-encoded IL-10 (20). IL-22 acts through the IL-22R complex consisting of the specific IL-22R1 chain and the IL-10R2c chain, which also functions as the second chain of the IL-10R complex (17–19). Both chains of the IL-22R complex belong to the class II CRF, which, in addition, includes the first chain of the IL-10R complex, both chains of type I and type II IFNs, tissue factor, and several orphan receptors (3, 17). Searching the available sequence of the human genome, we

FIGURE 7. Cross-linking. The hamster cells described in the legend to Fig. 3 were incubated only with [32P]FL-IL-22-P (lane 1) or with [32P]FL-IL-22-P with the addition of either a 100-fold excess of unlabeled IL-22 (competitor, lane 2), purified FL-CRF2-10 (5 µg, lanes 3 and 5), or purified FL-CRF2-8 (5 µg, lanes 4 and 6). [32P]FL-IL-22-P was incubated with each of soluble receptors before the addition to the cells (lanes 3 and 4), or the proteins were added at the same time to the cells without prior incubation (lanes 5 and 6). Cells were incubated with the proteins, washed, harvested, and cross-linked. The extracted cross-linked complexes were analyzed on 7.5% SDS-PAGE. The molecular mass markers are shown on the right.
have identified the gene and subsequently cloned the cDNA of a novel receptor (Figs. 1 and 2) belonging to this family of receptors that was first designated CRF2-10.

Comparison of the sequence of the CRF2-10 protein with those of other members of this family of receptors revealed that CRF2-10 is most homologous to the IL-22R1 chain and to the extracellular domain of CRF2-8. The function of CRF2-8 was recently characterized as a receptor subunit for the IL-20R complex (26). However, unlike IL-22R1 or CRF2-8, CRF2-10 is a soluble secreted protein lacking the transmembrane and cytoplasmic domains. Based on the homology of CRF2-10 to the IL-22R1 extracellular domain, we hypothesized that it would bind IL-22 and act as an IL-22 antagonist. Results of several experiments confirm this hypothesis. By cross-linking, we demonstrated that CRF2-10 binds radiolabeled IL-22 in solution (Fig. 3B). Also, in the presence of the CRF2-10 protein, IL-22 was unable to interact with the membrane-bound IL-22R complex (Fig. 7). In addition, CRF2-10 neutralized IL-22 activity. CRF2-10 inhibited the ability of IL-22 to induce Stat activation in intact IL-22-responsive cells such as A549 human lung carcinoma cells (Fig. 5, right panel). Moreover, to further evaluate the function of the CRF2-10 protein, we used IL-22-responsive hamster cells (17). These cells express the human functional chimeric IL-22R complex composed of the intact second chain the IL-10R2, and the chimeric IL-22R1/γc1 chain with the IL-22R1 intracellular domain replaced by the IFN-γR1 intracellular domain (17). In these cells, IL-22 induced Stat1 activation and up-regulated MHC class I Ag expression (Figs. 4 and 5, left panels), activities characteristic of IFN-γ signaling (17). The addition of CRF2-10 inhibited the ability of IL-22 to induce Stat1 activation and MHC class I Ag expression in these cells (Figs. 4 and 5, left panels). We also demonstrated that CRF2-10 inhibited the ability of IL-22 to induce the expression of SOCS-3 gene in HepG2 human hepatoma cells (Fig. 6). The results demonstrate that the CRF2-10 protein binds IL-22 and, thus, can be designated the IL-22BP. By binding IL-22, CRF2-10 blocks the activities of IL-22 (Figs. 4–6).

Although both chains of the IL-22R complex are required to assemble the functional receptor, each chain alone is able to bind IL-22 (17, 18). Thus, it is likely that the IL-22 activity can be negatively regulated by the expression on the cell surface of the R2, chain unpaired by the IL-22R1 chain. Because IL-22 binding to the R2, chain expressed alone does not lead to signaling, it may prevent shedding of IL-22 into the circulation (local suppression). In addition, the secretion of the soluble IL-22BP into the circulation can provide systemic inhibition of IL-22 action. Because incubation of cells expressing the functional IL-22R complex with IL-22 in the presence of IL-22BP inhibited binding of IL-22 to the cellular receptors (Fig. 7, lane 5), it seems likely that IL-22BP has higher affinity for IL-22 binding than the membrane-bound IL-22R complex. The fact that the complex of IL-22BP and IL-22, present for 3 days in conditioned medium of cells expressing modified functional IL-22R complex, was still unable to induce biological activities (Fig. 4) indicates that the complexes are stable, and little or no dissociation of the complexes occurs.

IL-22 has been demonstrated to induce production of acute-phase proteins in liver (27). The production of IL-22BP may be one of the mechanisms to precisely regulate IL-22 function (Fig. 8). It is interesting to note that all ESTs for the UniGene Hs.126891 (the CRF2-10 or IL-22BP gene) were derived from lung tissue, suggesting that IL-22BP is normally expressed in this tissue and perhaps functions in local inflammation. It would be of interest to test whether the profile of the expression of this protein differs between asthmatics and healthy individuals. The homology between IL-22BP and the IL-22R1 extracellular domain (34% identity) is comparable to that between IL-22BP and the CRF2-8 extracellular domain (33% identity). It was recently demonstrated that CRF2-8 is a receptor subunit for IL-20, which may play a role in psoriasis (26). Thus, it will be of a great interest to determine whether IL-22BP can bind IL-20 (and/or other IL-10 homologues) and whether it plays role in psoriasis. Moreover, although it is unlikely that the short splice variant (CRF2-10S) would bind IL-22, it is possible that the long splice variant (CRF2-10L) may still bind IL-22 and perhaps other IL-10 homologues. It is noteworthy that there is a mouse EST (GeneBank accession number BB222214) and a bovine EST (GeneBank accession number BE809214) that encode a mouse and bovine protein, respectively, homologous to human CRF2-10, indicating that the CRF2-10 gene is conserved between the species.

In conclusion, we have identified, cloned, and characterized a novel soluble receptor belonging to the class II CRF. The gene encoding this receptor maps to human chromosome 6 in the vicinity of the IFNGRI gene. The protein was designated IL-22BP based on its ability to bind IL-22. Moreover, IL-22BP inhibits IL-22 activity by binding IL-22 and preventing its interaction with the functional IL-22R complex. Thus, IL-22BP is a naturally occurring IL-22 antagonist, but its physiological role remains to be determined.

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References


