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CUTTING EDGE

Cutting Edge: IL-26 Signals through a Novel Receptor Complex Composed of IL-20 Receptor 1 and IL-10 Receptor 2[1]

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***The receptor for IL-26 (AK155), a cytokine of the IL-10 family, has not previously been defined. We demonstrate that the active receptor complex for IL-26 is a heterodimer composed of two receptor proteins: IL-20R1 and IL-10R2. Signaling through the IL-26R results in activation of STAT1 and STAT3 which can be blocked by neutralizing Abs against IL-20R1 or IL-10R2. IL-10R2 is broadly expressed on a wide variety of tissues, whereas only a limited number of tissues express IL-20R1. Therefore, the ability to respond to IL-26 is restricted by the expression of IL-20R1. IL-10, IL-19, IL-20, IL-22, and IL-24 fail to signal through the combination of IL-10R2 and IL-20R1 proteins, demonstrating that this receptor combination is unique and specific for IL-26. The Journal of Immunology,* 2004, 172: 2006–2010.**

The biological activities of cytokines are mediated by signaling through specific membrane-spanning receptors. Cytokine receptors can be divided into class I and class II, based on unique conserved sequence motifs present in their extracellular domains. The class II cytokine receptor family (CRF2)[3] is composed of 12 receptors that share a characteristic pattern of cysteine, proline, and tryptophan residues in their extracellular domains. As a result, cytokines that signal through CRF2 receptors also share primary and structural similarity. Cytokines of two ligand families, the IFN and IL-10 families, use CRF2 members to exert their functions (1–5). Type I IFNs (IFN-$\alpha\beta$) comprise the core of the IFN family. The IL-10 family is composed of six cellular cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26) and several viral homologs (1–5). Three cytokines of the recently discovered IFN-λ family (6, 7) demonstrate limited homology to cytokines from both the type I IFN and IL-10 families (6, 7). A single type II IFN, IFN-γ, has diverged from other CRF2 cytokines, although its tertiary and quaternary structure is similar to that of IL-10. Several CRF2 ligands, including IL-26, were only recently identified, and their functions are just beginning to be characterized. Nevertheless, the functional receptor complexes for all CRF2 ligands except IL-26 have been defined.

Originally designated as AK155, IL-26 was cloned as a protein expressed by herpesvirus saimiri-transformed T cells (8). IL-26 mRNA was also detected in several T cell lines, including human T cell leukemia virus-transformed cell lines. IL-26 mRNA is expressed in activated NK cells and T cells with enhanced expression upon type 1 polarization (9). IL-26 is also produced by activated memory but not by naive $CD4^+$ T cells, independently of costimulation (9). Although these data suggest that IL-26 may influence certain aspects of the immune response, the major function(s) of IL-26 remains to be defined. As a first step in defining IL-26 activities, we identified a receptor complex specific for IL-26 and characterized the signal transduction events induced by this cytokine. A combination of two receptor subunits, IL-20R1 (CRF2-8) and IL-10R2 (CRF2-4), is required to form the functional IL-26R complex. Although it was previously shown that IL-20R1 can dimerize with IL-20R2 to form the receptor for IL-19, IL-20, and IL-24, and IL-10R2 can dimerize with either IL-10R1, IL-22R1, or IFN-λR1 to form receptors for IL-10, IL-22, and IFN-λ, respectively, this is the first demonstration that IL-20R1 and IL-10R2 can also dimerize to generate the receptor for IL-26. IL-26-mediated signaling through this receptor complex induced activation of two members of the STAT family, STAT1 and STAT3.

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[3] Abbreviations used in this paper: CRF, cytokine receptor family; GAS, IFN-γ activation sequence; IL-22BP, IL-22 binding protein; s, soluble; Jak, Janus kinase.

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Materials and Methods

Plasmid construction

Expression vectors for IL-10R2 and the IL-22R1/γR1chimeric receptor were described (10, 11). The cDNA fragment encoding the IL-20R1 extracellular domain was amplified from a library containing cDNA isolated from human placenta (catalog no. HL4025AH; Clontech, Palo Alto, CA) with primers 5′-GCCGGATCCATGCGGCCGCTGCCGCTGCCG-3′ and either 5′-ATC GCTAGCCATTTAGCCTTGAACTCTGATG-3′ or 5′-CGACTAGTTT TAGCCTTGAACTCTGATG-3′. PCR products were digested with *Bam*HI restriction endonuclease and cloned into *Bam*HI and *Eco*RV sites of the pcDEF3 vector, generating plasmids pEF2-IL-20R1$_{EC/Nhe}$ and pEF2-IL-20R1$_{EC/Spe}$, respectively. Plasmid pEF2-IL-20R1/γR1 was created by reclonning IFN-γR1 intracellular domain from plasmid pEF3-IL-10R1/γR1 (10) to plasmid pEF2-IL-20R1$_{EC/Nhe}$ with *Nhe*I and *Bss*HII restriction endonucleases. The PCR product encoding the transmembrane and intracellular domains of IL-20R1 was generated with primers 5′-GTGGCTAGCCTGGTATGTTT TGCCCAT-3′ and 5′-GCGAATTCGTCTGGCAAACATTTATTGA-3′ and cloned into *Nhe*I and *Eco*RI sites of plasmids pEF2-IL-20R1$_{EC/Nhe}$ or pEF2-FL-IL-10R1/CRF2-12 (6), resulting in plasmids pEF2-IL-20R1 and pEF2-FL-IL-10R1/IL-20R1. The PCR product encoding the Fc fragment of human IgG1 was generated with primers 5′-CGACTAGTGACAAAACTCA CACAT-3′ and 5′-GCCGAATTCATTTACCCGGAGACAG-3′ and human PBMC cDNA, and cloned into *Spe*I and *Eco*RI sites of plasmid pEF2-IL-20R1$_{EC/Spe}$, resulting in plasmid pEF2-IL-20R1-Fc. Plasmid pEF2-IL-10R2-Fc was created by cloning PCR product encoding the extracellular domain of IL-10R2 into *Kpn*I and *Spe*I sites of plasmid pEF2-IL-20R1-Fc, resulting in plasmid pEF2-IL-10R2-Fc. Plasmids encoding soluble IL-20R1 and IL-10R2 were generated by introducing a stop codon at the end of the extracellular domains of the receptors by PCR.

The nucleotide sequences of the modified regions of all constructs were verified in their entirety by DNA sequencing.

Cells, reagents, EMSA, and Western and Northern blotting

COS-1 cells, an SV40-transformed fibroblast-like simian CV-1 cell line, colorectal adenocarcinoma HT-29 cells, colon carcinoma COLO-205 cells, human embryonic kidney 293 cells, and breast adenocarcinoma MCF7 cells were maintained and propagated as recommended by American Type Culture Collection (Manassas, VA). Cells were transfected as described (6, 10, 12).

To evaluate STAT activation, cells were treated for 15 min at 37°C with various cytokines as indicated in the text and used for either EMSA experiments with IFN-γ activation sequence (GAS) probe or for Western blotting as described (6, 10). IL-26 and neutralizing IL-10R2 and IL-26 Abs were purchased from R&D Systems (Minneapolis, MN). IL-10, IL-19, IL-20, and IL-22 were from PeproTech (Rocky Hill, NJ). IL-24, IL-22 binding protein (IL-22BP), soluble and Fc fusion receptors were produced in COS cells. IL-22R1 Ab has been described (13). To produce IL-20R1 Abs, P815 mastocytoma cells were transfected with the human IL-20R1 cDNA cloned into the pEF-BOS plasmid before injection into DBA/2 mice. After rejection of the tumors, the sera of these mice had high titers of neutralizing IL-20R1 Abs.

Two blots containing RNA derived from various normal tissues (Human Brain MTN Blot II; catalog no. 7755-1; Clontech; and Human Fetal mRNA Blot II; catalog no. D2802-50; Invitrogen, San Diego, CA) were hybridized with radiolabeled cDNA specific for IL-20R1 or IL-10R2 as described (11, 14).

IL-26 binding assay

ELISAs were performed as follows. Greiner (Nurtingen, Germany) Hybind plates were coated overnight at 4°C with 12.5 μg/ml either BSA or IL-26 in sodium carbonate (pH 9.5). The plates were then incubated 3 h at 22°C with 100 μl of COS cell-conditioned medium (mock) or COS cell supernatants containing Fc fusion receptors with or without soluble (s)IL-20R1. Receptor-Fc binding was measured with the use of goat anti-human IgG polyclonal Ab coupled to peroxidase as described (13). Relative binding was determined by dividing values obtained for specific binding to IL-26 by those for unspecific binding to BSA.

Size-exclusion chromatography was performed as described (15) with sIL-20R1 (15) and IL-26 produced in *E. coli* cells with the use of maltose-binding protein fusion protein and purification system (pMAL-p2x; New England Biolabs, Beverly, MA).

Results

IL-20R1 is a part of the IL-26 receptor complex

Functional CRF2 receptor complexes are heterodimers. Each subunit plays its unique role within a given receptor complex. A receptor with longer intracellular domain, denoted the R1 type receptor subunit, orchestrates recruitment of various signaling proteins and, therefore, determines the specificity of cytokine signaling. The R2 type receptor chain has a short intracellular domain that mainly does not influence the specificity of signaling. Nevertheless, both R1 and R2 receptors associate with Janus (Jak) tyrosine kinases, which trigger intracellular signal transduction events following ligand binding (3, 4).

The phylogenetic tree of the family of IL-10-related cytokines demonstrates that the family can be further divided into two groups (Fig. 1). IL-19, IL-20, and IL-24 belong to one group, whereas IL-26 along with IL-10 and IL-22 form a distinct group. The members of each group share stronger homology within the same group than with members of the other group. In addition, IL-19, IL-20, and IL-24 use the common R2 type receptor chain, IL-20R2, for signaling, suggesting that the cytokines of the other group, IL-10, IL-22, and IL-26, may also share a common R2 chain as a part of their specific receptor complexes. In fact, IL-10R2 is a shared receptor chain for the IL-10, IL-22, and IFN-λ receptor complexes. This observation suggested that IL-10R2 may also be the second chain for the IL-26 receptor complex.

We hypothesized that either IL-20R1 or IL-22R1 could serve as the first chain of the IL-26 receptor complex. To distinguish between signaling through intact endogenous receptors and signaling through exogenously expressed receptors, we used chimeric receptors. Both IL-20R1 and IL-22R1 predominantly activate STAT3 upon ligand binding. However, replacement of the intracellular domains of these receptors by the IFN-γR1 intracellular domain enables chimeric IL-22R1/IFN-γR1 (IL-22R1/γR1) and IL-20R1/IFN-γR1 (IL-20R1/γR1) chains to activate STAT1 upon ligand binding and induce various IFN-γ-specific biological activities such as up-regulation of MHC class I Ag expression (11). Thus, to investigate whether IL-20R1 or IL-22R1 plays a functional role in the IL-26 receptor complex, each chimeric receptor was transiently expressed in monkey (COS) cells or human embryonic kidney 293 cells. Stable transfectants were also obtained for the human colorectal adenocarcinoma cell line HT29 and the breast adenocarcinoma cell line MCF7. In all cell lines expressing IL-20R1/γR1, IL-26 activated STAT1 DNA-binding complexes as determined by EMSA, whereas IL-26 failed to induce STAT1 activation in parental cell lines or in cells expressing IL-22R1/γR1 (Fig. 2*A* and data not shown). In addition, in COS cells, IL-26-induced transcription of the luciferase reporter gene driven by a minimal TK promoter containing three copies of a GAS element was

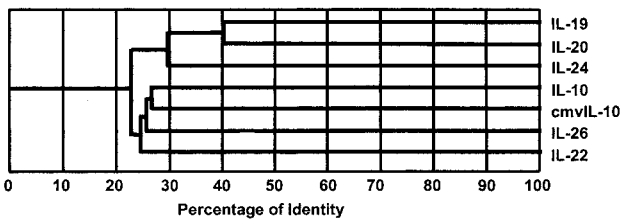

FIGURE 1. IL-10 family. A phylogenetic tree was generated by alignment of amino acid sequences of IL-10-related cytokines, including CMV-encoded IL-10 homolog (cmvIL-10) (4). Alignment was generated using the program PILEUP of the Wisconsin package, version 9.1 (Genetics Computer Group, Madison, WI). This program was used with the following parameters: the gap creation penalty 1, the gap extension penalty 1. The CLUSTAL X program was used to create the phylogenetic tree.

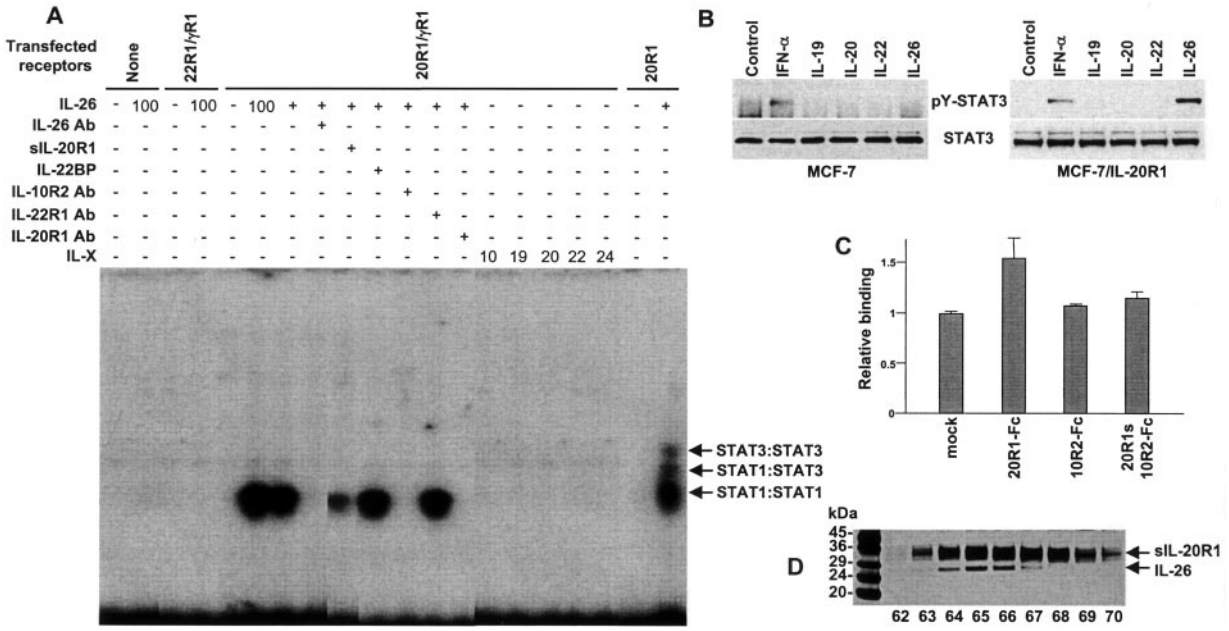

FIGURE 2. Characterization of IL-26R chains performed on transfected human MCF7 cells and in solution. *A* and *B*, STAT activation in parental human MCF7 cells or MCF7 cells expressing IL-22R1/γR1 (22R1/γR1), IL-20R1/γR1 (20R1/γR1), or IL-20R1 (20R1) in response to IL-26 (10 ng (+); 100 ng (100)) was evaluated by EMSA with GAS probe (*A*) or by Western blotting (*B*). The position of STAT DNA-binding complexes in EMSAs is indicated by arrows. IL-26 was incubated for 60 min with either IL-26 Ab (1 μg), IL-22BP (0.1 μg), or sIL-20R1 (0.1 μg), before adding to the cells. IL-10R2, IL-22R1, or IL-20R1 Ab (1 μg or 1/100 dilution) was added to the cells 60 min before IL-26 treatment. Cells were also treated with 100 ng each of IL-10, IL-19, IL-20, IL-22, IL-24, and IFN-α as indicated. *C*, ELISA plate coated with IL-26 was incubated with IL-20R1-Fc (20R1-Fc) and IL-10R2-Fc (10R2-Fc) alone or in combination with sIL-20R1. Specific interaction was detected with peroxidase-conjugated secondary Ab. The plate coated with BSA was used as a control of unspecific binding. Relative binding (specific/unspecific) is shown. *D*, A mixture of IL-26 and sIL-20R1 incubated for 2 h at 22°C was analyzed on gel filtration chromatography. Fractions 62–70 were resolved on SDS-PAGE.

dependent on the expression of IL-20R1. IL-10, IL-19, IL-20, IL-22, and IL-24 did not induce STAT activation in parental MCF7 cells or MCF7 cells transfected with IL-20R1/γR1 (Fig. 2*A*). We also demonstrated that expression of the intact IL-20R1 in MCF7 cells enables IL-26 to induce activation of STAT1 and STAT3 as determined by EMSA (Fig. 2*A*). Western blotting revealed that IL-26 predominantly activated STAT3 in these cells (Fig. 2*B*).

All of the cell lines used in these experiments expressed endogenous IL-10R2 (10, 11, 13). To determine whether this chain is a part of the IL-26 receptor complex, the ability of IL-10R2 Ab to neutralize IL-26-induced STAT1 activation was evaluated. Pretreatment of cells with the Ab before addition of IL-26 completely blocked ligand-induced STAT1 activation (Fig. 2*A*). An Ab against IL-20R1 but not against IL-22R1 was also capable of inhibiting IL-26-induced STAT1 activation. IL-26 activity was also completely neutralized by IL-26 Ab. The ability of soluble receptors to neutralize IL-26 activity was also evaluated. Pretreatment of IL-26 with sIL-20R1 caused reduction of STAT1 activation, whereas pretreatment with sIL-10R2 had no effect. IL-22BP, a soluble receptor that functions as an IL-22 antagonist (12, 16, 17), did not inhibit IL-26 signaling (Fig. 2 and data not shown). Thus, sIL-20R1 appears to bind IL-26 with sufficient affinity to compete with the membrane-associated receptor.

Interaction between IL-26 and its receptors was further tested by modified ELISA and size-exclusion chromatography. IL-20R1-Fc fusion protein was able to bind to the IL-26-coated plate, whereas IL-10R2-Fc was not capable of binding (Fig. 2*C*). However, in the presence of the sIL-20R1, we detected low-level binding of IL-10R2-Fc to the plate, suggesting that IL-10R2-Fc protein interacted with the complex composed of IL-26 and sIL-20R1. Size-exclusion chromatography demonstrated that IL-26 and sIL-20R1 were fractionated together (Fig. 2*D*), further confirming that IL-26 and IL-20R1 do interact in solution.

Expression of the IL-20R1 *and* IL-10R2 *genes in normal tissues*

IL-10R2 is ubiquitously expressed (9, 18). Thus, the expression of IL-20R1 should be a factor limiting responsiveness of cells to IL-26. A high level of IL-20R1 mRNA expression was previously detected in skin, testis, heart, placenta, salivary gland, and prostate by RT-PCR (19). Moderate expression was observed in brain, lung, stomach, pancreas, ovary, uterus, thyroid, and adrenal glands. Barely detectable expression was seen in kidney, liver, colon, muscle, and small intestine. Interestingly, IL-20R1 expression was found to be elevated in psoriatic skin (19). Receptor expression was also detected in keratinocytes, endothelial cells, and immune cells in psoriatic lesions.

We also evaluated several tissues for IL-20R1 expression by Northern blotting. Two distinct IL-20R1 transcripts (2.2 and 4 kb), were detected in skin and lung tissues (Fig. 3*A*). Although RT-PCR detected IL-20R1 expression in adult heart (19), expression of this gene was undetectable by Northern blotting in fetal heart (Fig. 3*A*), which could indicate the different pattern of expression between adult and fetal tissues. Database searches demonstrate that many IL-20R1 expressed sequence tags were derived from various brain cDNA libraries (UniGene no. Hs.21814). Indeed, Northern blotting revealed that cerebellum has a very high level of *IL-20R1* gene expression (Fig. 3*B*).

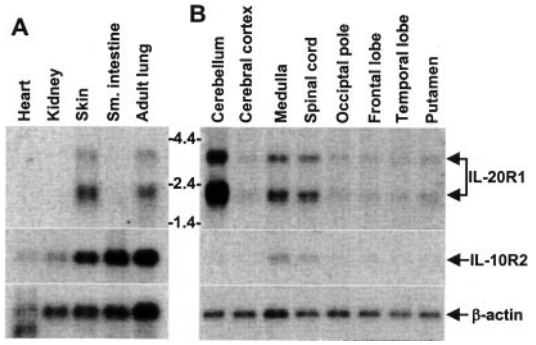

FIGURE 3. Expression pattern of IL-20R1 and IL-10R2 mRNAs. Northern blotting was performed on two blots containing mRNA isolated from normal human fetal tissues (heart, kidney, skin, small intestine) and adult lung (*A*), and normal human brain (cerebellum, cerebral cortex, medulla, spinal cord, occiptal pole, frontal lobe, temporal lobe, and putamen) (*B*). Arrows point to IL-20R1 and IL-10R2 transcripts. RNA loading was assessed by evaluating the expression of the *β-actin* gene.

Lower levels were seen in medullar and spinal cord. In contrast, only a very low level of IL-10R2 mRNA expression was detected in certain brain compartments (Fig. 3*B*). Thus, IL-20R1 may have a unique function in brain tissues.

IL-26 activates STAT1 and STAT3

To define which STATs are activated by signaling through the IL-20R1 chain, we examined the activation of STATs in HT-29 cells that were stably transfected with a chimeric receptor, FL-IL-10R1/IL-20R1, composed of the FLAG epitope-tagged extracellular domain of the IL-10R1 chain linked to the intracellular domain of IL-20R1 (Fig. 4*A*). This chimeric receptor is able to bind IL-10 and dimerize with IL-10R2, the shared second chain for the IL-10, IL-22, IFN-λ, and IL-26 receptor complexes, to transduce a signal. Thus, IL-10-mediated signaling through the FL-IL-10R1/IL-20R1 chimeric receptor mimics signaling through the native IL-26 receptor complex. The use of the chimeric receptor allowed us to dissect signaling

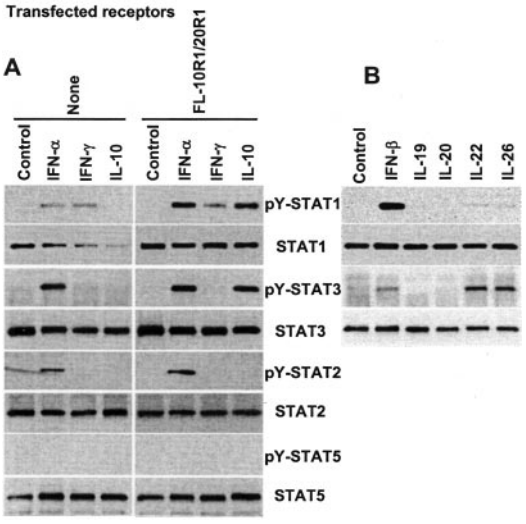

FIGURE 4. STAT activation performed on human cells. STAT activation in parental HT-29 cells or HT-29 cells expressing FL-IL-10R1/IL-20R1 (FL-10R1/20R1) in response to IFN-α, IFN-γ, and IL-10 (as indicated on the figure) (*A*) and in COLO-205 cells in response to IFN-β, IL-19, IL-20, IL-22, and IL-26 (*B*) was evaluated by Western blotting.

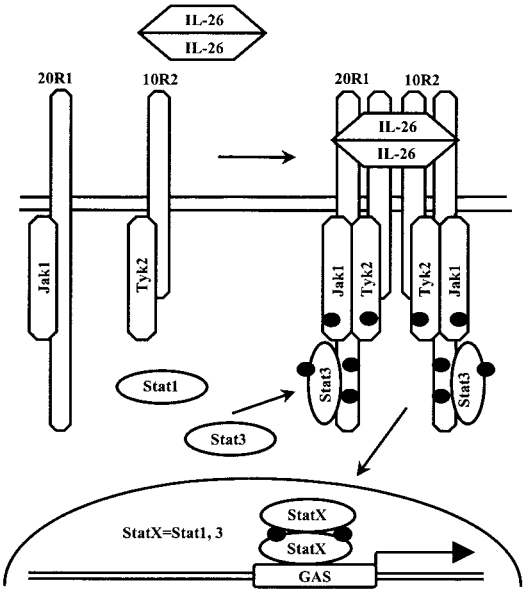

FIGURE 5. The model of IL-26 receptor complex. IL-26 was reported to be a dimer (8) and thus is likely to bind to two IL-20R1 and two IL-10R2 chains. IL-20R1 seems to be major ligand binding chain. Ligand binding leads to the initiation of a signal transduction cascade. IL-10R2 is associated with Tyk2 (10), and IL-20R1 is likely to interact with Jak1. Upon the ligand-induced heterodimerization of IL-26R chains, receptor-associated Jaks crossactivate each other and phosphorylate the IL-20R1 intracellular domain, resulting in the activation of STAT1 and STAT3.

events activated through the IL-20R1 intracellular domain. This also eliminated the possibility that IL-26 may cross-react with receptors other than the IL-20R1 and IL-10R2 complex. Thus, the STAT proteins that are activated by IL-10 in these cells represent those that are normally recruited to the full-length IL-20R1 intracellular domain. STAT activation was measured by immunoprecipitating specific STAT proteins from whole-cell lysates, and then Western blotting with phosphotyrosine-specific Abs.

HT-29 cells do not express endogenous IL-10R1; therefore, treatment with IL-10 did not induce activation of any STATs in these cells (Fig. 4*A*). However, in HT29 cells expressing the FL-IL-10R1/IL-20R1 chimeric chain, treatment with IL-10 induced activation (tyrosine phosphorylation) of STAT1 and STAT3 but failed to activate STAT2 or STAT5. IL-26 also induced activation of STAT1 and STAT3 in a colorectal adenocarcinoma cell line, COLO-205, which expresses the two chains needed to form the endogenous IL-26 receptor complex: IL-20R1 and IL-10R2 (Fig. 4*B*). The activation of STAT3 by IL-26 was consistently stronger than the activation of STAT1, demonstrating that IL-26 signals predominantly through STAT3. These results are in agreement with the observation that the IL-20R1 intracellular domain possesses two classical (YXXQ) STAT3 docking sites (3, 4).

Discussion

IL-20R1 is an essential component of the IL-20 receptor complex (19). Three cytokines, IL-19, IL-20, and IL-24, share the receptor complex composed of IL-20R1 and IL-20R2 (13, 20). It was previously demonstrated that IL-20R2 has a more limited pattern of expression in tissues than IL-20R1 (19). This observation suggested that, in tissues where expression of these

receptors did not match, IL-20R1 could function independently of IL-20R2.

As demonstrated in this study, IL-20R1 can couple with IL-10R2 to form the IL-26 receptor complex (Fig. 5). The complex is specific for IL-26, because other IL-10-related cytokines, IL-10, IL-19, IL-20, IL-22, and IL-24, do not signal through the IL-26 receptor complex. IL-26 activates the Jak/STAT signaling pathway, resulting in rapid tyrosine phosphorylation of STAT1 and STAT3 in cells that express endogenous IL-20R1 and IL-10R2 (Fig. 5). IL-10R2 is broadly expressed on a wide variety of tissues, whereas only a limited number of tissues express IL-20R1. Therefore, the ability to respond to IL-26 is largely determined by expression of IL-20R1. IL-10R2 also functions as a receptor subunit for the IL-10, IL-22, and IFN-λ receptor complexes (6, 7, 10, 11, 21). Whereas IL-20R1 binds IL-26 directly, IL-10R2 does not. Nevertheless, IL-10R2 Ab completely blocked IL-26-induced signal transduction, providing strong direct evidence that IL-10R2 is required to assemble the functional IL-26 receptor complex. Thus, it seems that a common functional role of IL-10R2 is well conserved in all of the receptor complexes that use IL-10R2 as a receptor subunit. IL-10R2 does not interact with cytokines directly; nevertheless, its engagement is required to assemble functional receptor complexes for IL-10, IL-22, IFN-λ, and IL-26, where each of these cytokines specifically interacts with its own unique receptor chain. Our characterization of the functional IL-26 receptor complex provides another interesting example of receptor sharing by CRF2 cytokines. Although unique receptor complexes can be formed through the shared use of a limited number of transmembrane proteins, the full biological significance of class II cytokine receptor sharing remains to be determined.

One likely result is that the functions of several CRF2 cytokines will overlap due to receptor sharing. Each receptor heterodimer is composed of a subunit with a long intracellular domain (R1 type subunit) and another one with a short intracellular domain (R2 type subunit). Specificity of signaling and subsequent biological activities are mainly determined by the R1 type subunit. Because both IL-19 and IL-26 signal through the same R1 subunit, IL-20R1, the biological activities of IL-26 may recapitulate at least some of those induced by IL-19. However, IL-19 and IL-26 use different R2 subunits, IL-20R2 and IL-10R2, to assemble their receptor complex. IL-20R2 has restricted expression pattern, whereas IL-10R2 is broadly expressed. This predicts that IL-26 can activate a larger number of cell types than IL-19.

A second general consequence of receptor sharing may be that CRF2 cytokines can cross-regulate their activities by competing for receptor components. It is interesting to speculate that IL-10, a cytokine with strong anti-inflammatory activity, could sequester IL-10R2 and thereby regulate the functions of other CRF2 cytokines that require the IL-10R2 chain for signaling. Among these cytokines, IL-22 demonstrates proinflammatory activities, and inhibition of its function at the receptor level would be consistent with IL-10's predominant anti-inflammatory function.

It was recently demonstrated that IL-20 stimulates hemopoiesis, with a specificity toward multipotential progenitors (22). Based on partial receptor sharing between IL-20 and IL-26, it is interesting to speculate that IL-26 may have a similar activity. With the IL-10 family of cytokines and their receptors defined, it should now be possible to dissect their ligand-specific, shared, and mutually regulatory functions.

Acknowledgments

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References

1. Dumoutier, L., and J. C. Renauld. 2002. Viral and cellular interleukin-10 (IL-10)-related cytokines: from structures to functions. *Eur. Cytokine Network 13:5.*
2. Fickenscher, H., S. Hor, H. Kupers, A. Knappe, S. Wittmann, and H. Sticht. 2002. The interleukin-10 family of cytokines. *Trends Immunol. 23:89.*
3. Kotenko, S. V., and S. Pestka. 2000. Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene 19:2557.*
4. Kotenko, S. V. 2002. The family of IL-10-related cytokines and their receptors: related, but to what extent? *Cytokine Growth Factor Rev. 13:223.*
5. Renauld, J. C. 2003. Class II cytokine receptors and their ligands: key antiviral and inflammatory modulators. *Nat. Rev. Immunol. 3:667.*
6. Kotenko, S. V., G. Gallagher, V. V. Baurin, A. Lewis-Antes, M. Shen, N. K. Shah, J. A. Langer, F. Sheikh, H. Dickensheets, and R. P. Donnelly. 2003. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol. 4:69.*
7. Sheppard, P., W. Kindsvogel, W. Xu, K. Henderson, S. Schlutsmeyer, T. E. Whitmore, R. Kuestner, U. Garrigues, C. Birks, J. Roraback, et al. 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol. 4:63.*
8. Knappe, A., S. Hor, S. Wittmann, and H. Fickenscher. 2000. Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. *J. Virol. 74:3881.*
9. Wolk, K., S. Kunz, K. Asadullah, and R. Sabat. 2002. Cutting edge: immune cells as sources and targets of the IL-10 family members? *J. Immunol. 168:5397.*
10. Kotenko, S. V., C. D. Krause, L. S. Izotova, B. P. Pollack, W. Wu, and S. Pestka. 1997. Identification and functional characterization of a second chain of the interleukin-10 receptor complex. *EMBO J. 16:5894.*
11. Kotenko, S. V., L. S. Izotova, O. V. Mirochnitchenko, E. Esterova, H. Dickensheets, R. P. Donnelly, and S. Pestka. 2001. Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rβ) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. *J. Biol. Chem. 276:2725.*
12. Kotenko, S. V., L. S. Izotova, O. V. Mirochnitchenko, E. Esterova, H. Dickensheets, R. P. Donnelly, and S. Pestka. 2001. Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. *J. Immunol. 166: 7096.*
13. Dumoutier, L., C. Leemans, D. Lejeune, S. V. Kotenko, and J. C. Renauld. 2001. Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types. *J. Immunol. 167:3545.*
14. Gallagher, G., H. Dickensheets, J. Eskdale, L. S. Izotova, O. V. Mirochnitchenko, J. D. Peat, N. Vazquez, S. Pestka, R. P. Donnelly, and S. V. Kotenko. 2000. Cloning, expression and initial characterization of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). *Genes Immun. 1:442.*
15. Pletnev, S., E. Magracheva, S. Kozlov, G. Tobin, S. V. Kotenko, A. Wlodawer, and A. Zdanov. 2003. Characterization of the recombinant extracellular domains of human interleukin-20 receptors and their complexes with interleukin-19 and interleukin-20. *Biochemistry 42:12617.*
16. Dumoutier, L., D. Lejeune, D. Colau, and J. C. Renauld. 2001. Cloning and characterization of IL-22 binding protein, a natural antagonist of IL-10-related T cell-derived inducible factor/IL-22. *J. Immunol. 166:7090.*
17. Xu, W., S. R. Presnell, J. Parrish-Novak, W. Kindsvogel, S. Jaspers, Z. Chen, S. R. Dillon, Z. Gao, T. Gilbert, K. Madden, et al. 2001. A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. *Proc. Natl. Acad. Sci. USA 98:9511.*
18. Aggarwal, S., M. H. Xie, M. Maruoka, J. Foster, and A. L. Gurney. 2001. Acinar cells of the pancreas are a target of interleukin-22. *J. Interferon Cytokine Res. 21:1047.*
19. Blumberg, H., D. Conklin, W. F. Xu, A. Grossmann, T. Brender, S. Carollo, M. Eagan, D. Foster, B. A. Haldeman, A. Hammond, et al. 2001. Interleukin 20: discovery, receptor identification, and role in epidermal function. *Cell 104:9.*
20. Wang, M., Z. Tan, R. Zhang, S. V. Kotenko, and P. Liang. 2001. Interleukin 24 (MDA-7/MOB-5) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. *J. Biol. Chem. 277:7341.*
21. Xie, M. H., S. Aggarwal, W. H. Ho, J. Foster, Z. Zhang, J. Stinson, W. I. Wood, A. D. Goddard, and A. L. Gurney. 2000. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J. Biol. Chem. 275:31335.*
22. Liu, L., C. Ding, W. Zeng, J. G. Heuer, J. W. Tetreault, T. W. Noblitt, G. Hangoc, S. Cooper, K. A. Brune, G. Sharma, et al. 2003. Selective enhancement of multipotential hematopoietic progenitors in vitro and in vivo by IL-20. *Blood 102:3206.*