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*

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Interleukin-10 (IL-10)-related T cell-derived inducible factor (IL-TIF; provisionally designated IL-22) is a cytokine with limited homology to IL-10. We report here the identification of a functional IL-TIF receptor complex that consists of two receptor chains, the orphan CRF2-9 and IL-10R2, the second chain of the IL-10 receptor complex. Expression of the CRF2-9 chain in monkey COS cells renders them sensitive to IL-TIF. However, in hamster cells both chains, CRF2-9 and IL-10R2, must be expressed to assemble the functional IL-TIF receptor complex. The CRF2-9 chain (or the IL-TIF-R1 chain) is responsible for Stat recruitment. Substitution of the CRF2-9 intracellular domain with the IFN-γR1 intracellular domain changes the pattern of IL-TIF-induced Stat activation. The CRF2-9 gene is expressed in normal liver and kidney, suggesting a possible role for IL-TIF in regulating gene expression in these tissues. Each chain, CRF2-9 and IL-10R2, is capable of binding IL-TIF independently and can be cross-linked to the radiolabeled IL-TIF. However, binding of IL-TIF to the receptor complex is greater than binding to either receptor chain alone. Sharing of the common IL-10R2 chain between the IL-10 and IL-TIF receptor complexes is the first such case for receptor complexes with chains belonging to the class II cytokine receptor family, establishing a novel paradigm for IL-10-related ligands similar to the shared use of the gamma common chain (γc) by several cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-15.

Six new ligands with limited sequence homology (19–27% identity) to IL-10 have been recently identified (1–5). One of these IL-10 homologs is a viral protein, whereas others are encoded in the genome. Cytomegalovirus-encoded IL-10, designated cmvIL-10 (5), demonstrates only 27% identity to human IL-10. Despite this limited homology, cmvIL-10 binds to and signals through the canonical IL-10 receptor complex (5, 6). cmvIL-10 is produced by cytomegalovirus-infected cells and is likely to play a role in immune evasion helping virus to avoid clearance by the host immune system (5, 7). Another IL-10 homolog was cloned as a protein whose expression is elevated in terminally differentiated human melanoma cells and was designated mda-7, for melanoma differentiation-associated gene 7 (3). The expression of the rat mda-7 analog was linked to wound healing (8) (the protein was designated e49a) and to ras transformation (9) (the protein was designated mob-5). The expression of rat mda-7 (e49a) was localized primarily to fibroblast-like cells at the wound edge and base. During wound healing the level of e49a mRNA was transiently elevated 9- to 12-fold above unwounded controls (8). In addition, expression of rat mda-7 (mob-5) was demonstrated to be induced by expression of oncogenic ras. Moreover, mob-5 and its putative receptor are oncogenic ras-specific targets; mob-5 binds to the cell surface of ras-transformed cells but not of parental untransformed cells (9).

Another IL-10 homolog, designated ak155, was cloned as a protein expressed by Herpesvirus saimiri-transformed T lymphocytes (4). Transcription of the gene of a fourth IL-10 homolog, designated IL-19, was demonstrated to be induced in monocytes by LPS treatment. The appearance of IL-19 mRNA in LPS-stimulated monocytes coincided with the expression of IL-10 mRNA (2). An additional protein with homology to IL-10 was designated Zcyto10 (GenBank accession number AF224266), but there is no published information available about its activities or expression.

Finally, an IL-10 homolog, designated IL-TIF (IL-10-related T cell-derived inducible factor), is expressed by IL-9-treated murine T cells (1). Its human analog (human IL-TIF or, provisionally, IL-22) was recently reported (10, 11). Murine IL-TIF expression can be induced by IL-9 in thymic lymphomas, T cells, and mast cells in vitro and by LPS in various organs in vivo. It was also demonstrated that IL-TIF injection induced production of acute-phase reactants in mouse liver, suggesting involvement of IL-TIF in the inflammatory response (10). IL-TIF reaction; MHC, major histocompatibility complex; EMSA, electrophoretic mobility shift assay; PAGE, polyacrylamide gel electrophoresis; PNGase F, peptide:N-glycosidase F; cmvIL-10, cytomegalovirus-encoded IL-10.
TIF can induce activation of Stat proteins (Stat1, Stat3, and Stat5) in several cell lines, including mesangial MES13, neuronal PC12, and hepatoma HepG2 cell lines (1, 10). In addition, there are data linking IL-TIF to allergy and asthma. IL-TIF is induced by IL-9, a Th2 cytokine active on T and B lymphocytes, mast cells, and eosinophils, and potentially involved in allergy and asthma (12–14). The IL-TIF gene (and also the ak155 gene) is located on human chromosome 12q, where several loci potentially linked to asthma and atopy have been identified by genetic studies, particularly in the 12q13.12-q23.3 region (for review see Ref. 15). The strongest evidence for linkage is in a region near the gene encoding IFN-γ (16–19). However, the gene for IFN-γ appears to be highly conserved (no sequence variations were detected in 265 individuals), suggesting that mutations of the IFN-γ gene are unlikely to be a significant cause of inherited asthma (20). The IL-TIF and ak155 genes are positioned next to the IFN-γ gene on the bacterial artificial chromosome BAC RPCI11-444B24 (GenBank® accession number AC007458) and, thus, are possible candidates for linkage to asthma.

Cytokines exert their actions by binding to specific cell surface receptors that leads to the activation of cytokine-specific signal transduction pathways. The functional IL-10 receptor complex consists of two chains (6), the ligand binding IL-10R1 subunit (21) and the second IL-10R2 subunit that supports signaling through the IL-10R1 chain (6). Both chains belong to the class II cytokine receptor family (22, 23), which also includes two receptor chains for type I interferons (IFNs), two receptor chains for type II IFN, and the tissue factor that binds coagulation factor VIIa (for review see Ref. 24). In addition, there are currently at least five orphan receptors CRF2-8, CRF2-9, CRF2-10, CRF2-11, and CRF2-12 (cytokine receptor family class II members) and the extracellular domains of CRF2-8, CRF2-9, and CRF2-10 are mostly homologous to the IL-10R1 extracellular domain (24).  

In this study we demonstrate that the functional IL-TIF receptor complex consists of two receptor chains, the orphan CRF2-9 chain and the IL-10R2 chain, which we demonstrate to be a common shared chain between the IL-TIF- and the IL-10 receptor complexes.

MATERIALS AND METHODS

Plasmid Construction—Primers 5'-CCGGTACCAATGCGGCCTG-CCGAGAATCTG-3' and 5'-GGCTCAAATTGGCGCTTCTC-3' (tifi) and total RNA isolated from PBMCs obtained from a healthy donor were used for reverse transcription-PCR to clone the human IL-TIF cDNA into plasmid pEF3 (25) with the use of KpnI and EcoRI restriction endonucleases, resulting in plasmid pEF-IL-TIF. The PCR product obtained with primers 5'-CCGGATCCAGGAGGAGGACAGTGCAGCCGCC-3' (tifi) and tifi and plasmid pEF-IL-TIF as a template was digested with BamHI and EcoRI restriction endonucleases and cloned into corresponding sites of the pEF-SPFL vector (5), resulting in plasmid pEF-SPFL-IL-TIF. This plasmid encodes IL-TIF tagged at its N terminus with the FLAG epitope (FL-IL-TIF). The PCR product obtained with primers 5'-CCGGATCCAGGAGGAGGACAGTGCAGCCGCC-3' (tifi) and tifi and plasmid pEF-IL-TIF as a template was digested with BamHI and EcoRI restriction endonucleases and cloned into corresponding sites of the pEF-SPFL vector, resulting in plasmid pEF-SPFL-IL-TIF-P. This plasmid encodes FL-IL-TIF tagged at its C terminus with the Arg-Arg-Ala-Ser-Val-Ala sequence (FL-IL-TIF-P), which contains the consensus amino acid sequence recognizable by the catalytic subunit of the cAMP-dependent protein kinase (26–29).

Primers 5'-GGCGGTACGAGGACGCTGCTCGACATC-3' and 5'-GGCGGTACGAGGACGCTGCTCGACATC-3' and a library containing cDNA isolated from human fetal liver (CLONTECH, catalog no. HL4029AH) were used for PCR to clone the extracellular domain of the CRF2-9 protein (24) into plasmid pEFL-IL-10R1/R1 with the use of Kpnl and NheI restriction endonucleases, resulting in plasmid pEF-CRF2-9/R1. Primers 5'-GGCGGTACGAGGACGCTGCTCGACATC-3' and 5'-GGCGGTACGAGGACGCTGCTCGACATC-3' and the same library were used for PCR to clone the CRF2-9 intracellular domain into plasmid pEF-CRF2-9/R1 with the use of NheI and EcoRI restriction endonucleases, resulting in plasmid pEF-CRF2-9.

A “tandem vector” encoding two receptors, the CRF2-9/R1 and the IL-10R2 chains, in which the expression of each receptor is controlled by separate promoters and polyadenylation signals was created as follows. The fragment containing the EF-1α promoter, the IL-10R2 coding sequence, and the bovine growth hormone polyadenylation signal was released from the pEF-CRF (or pEF-IL-10R2) vector (6) by digestion with BsmI and PstHI restriction endonucleases and ligated into the BsaI and MluI sites of the pEF-CRF2-9/R1 plasmid. The digestion plasmid was designated pEF-CRF2-9/R1-IL-10R2.

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

Cells, Transfection, and Cytofluorographic Analysis—The 16-9 hamster x human somatic cell hybrid line is the Chinese hamster ovary cell (CHO-K1) hybrid containing a translocation of the long arm of human chromosome 6 encoding the human IFNGR1 (Hu-IFN-γR1) gene and a transfected human HLA-B7 gene (30). The cells were maintained in F-12 (Ham) medium (Sigma) containing 5% heat-inactivated fetal bovine serum (FBS; Amstream). COS-1 cells, an SV40-transformed simian CV-1 cell line, were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) with 10% heat-inactivated fetal bovine serum. Cells were transfected as described previously (5, 6) except that stable COS cell transfecants were selected with 350 µg/ml G418. COS cell supernatants were collected at 72 h as a source of the expressed proteins.

Leukocytes were obtained from a normal donor by leukapheresis. Peripheral blood mononuclear cells (PBMCs) were then isolated by density centrifugation with polyacrylamide and sodium diatrizoate according to the manufacturer’s suggested protocol (Sigma, HISTOPAUSE-1077).

To detect cytokine-induced MHC class I antigen (HLA-B7) expression, cells were treated with COS cell supernatants or purified recombinant proteins as indicated in the text for 72 h and analyzed by flow cytometry. Cell surface expression of the HLA-B7 antigen was detected by treatment with mouse anti-HLA (W6/32) (31) monoclonal antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology Inc., catalog no. SC-2010). The cells then were analyzed by cytofluorography as described previously (32).

Electrophoretic Mobility Shift Assays and Western and Northern Blotting—Cells were starved overnight in serum-free media and then treated with IL-10 or IL-TIF as indicated in the text for 15 min at 37 °C and used for EMSA experiments to detect activation of Stat1, Stat3, and Stat5 as described (6). EMSAs were performed with a 22-base pair sequence containing a Stat1a-binding site corresponding to the GAS (IFN-γ activation sequence) element in the promoter region of the human IRF-1 gene (5' -GATCGATTTCCCCGAAATCATG-3') as described (33, 34).

Three days after transfection, conditioned media from COS-1 cells transiently transfected with expression plasmids was collected and subjected to Western blotting with anti-FLAG epitope-specific M2 monoclonal antibody (Sigma) as described (5).

Northern blotting was performed with two blots (CLONTECH, catalog nos. 7757-1 and 7780-1) and a CRF2-9 probe corresponding to the coding region of the CRF2-9 cDNA as described (2). The RNA loading was adjusted by the manufacturer with a β-actin signal.

Cross-linking—The FL-IL-TIF-P protein was transiently expressed in COS cells and purified from conditioned media by immunoprecipitation. Cells were treated with the anti-FLAG M2 monoclonal antibody (Sigma) and the blot was developed with 125I-ATP and used for cross-linking as described (28, 29, 33).

RESULTS

Ligands, Receptors, and Their Derivatives—The following ligands and receptors and their derivatives were created and used in this study. Human IL-TIF (IL-21) (GenBank® accession no. AJ277247) is a cytokine with limited homology to IL-10 (10, 11). Three expression vectors were created (Fig. 1A) encoding intact human IL-TIF, N-terminal FLAG-tagged IL-TIF (FL-IL-TIF), or FL-IL-TIF with the consensus amino acid sequence Arg-Arg-Ala-Ser-Val-Ala (phosphorylatable site, P), recognizable by the catalytic subunit of the cAMP-dependent protein kinase.
Functional IL-TIF (IL-22) Receptor Complex

Fig. 1. Ligands and receptors. A, intact human IL-TIF and its two derivatives. IL-TIF was tagged at the N terminus with the FLAG epitope (FL-IL-TIF) and was tagged 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-TIF-P). B, expression of FL-IL-TIF and FL-IL-TIF-P in COS cells. COS cells were transiently transfected with the pEF-SPL (lane 1, mock), the pEF-SPL-IL-TIF (lane 3, FL-IL-TIF), and the pEF-SPL-IL-TIF-P (lane 5, FL-IL-TIF-P) expression vectors. Three days later, 20 µl of the conditioned media was subjected to Western blotting experiments with anti-FLAG antibody. FL-IL-10-P was used as a control (lane 2). Conditioned medium containing FL-IL-TIF was treated with peptide:N-glycosidase F (PNGase F) to demonstrate that the protein is glycosylated (lane 4). FL-IL-TIF-P was purified from conditioned media by affinity chromatography and evaluated by Western blotting with anti-FLAG antibody (lane 5). Lane 6 represents an autoradiograph of the SDS-PAGE gel containing radiolabeled FL-IL-TIF-P. The molecular weight markers are shown on the left. C, expression vectors encoding the intact CRF2-9 chain, an orphan receptor from the class II cytokine receptor family, and the chimeric CRF2-9/γR1 receptor that has the CRF2-9 extracellular domain fused to the transmembrane and intracellular domains of the IFN-γR1 chain were constructed. IL-10R2 is the intact second chain of the human IL-10 receptor complex (6). D, predicted amino acid sequence of CRF2-9. The comparison of sequences of the extracellular domains of CRF2-9 and other receptors from the class II cytokine receptor family demonstrates that the CRF2-9 chain belongs to this receptor family and is most similar to the IL-10R1 chain and the orphan receptor CRF2-8 (24). Amino acid residues of the putative signal peptide and of the putative transmembrane domain of the CRF2-9 are boxed. Tyrosine residues are underlined (Y). Stat3 recruitment or docking sites are also underlined (YXXQ motif). Potential glycosylation sites are noted by lines over these sequences.

protein kinase (26–29) fused to its C terminus (FL-IL-TIF-P). COS cells were transiently transfected with the expression vectors, and 3 days later conditioned media containing FL-IL-TIF or FL-IL-TIF-P were tested by Western blotting with anti-FLAG antibody for protein expression (Fig. 1B, lanes 3 and 4). Western blotting revealed that FL-IL-TIF was secreted from COS cells and migrated on the SDS-PAGE gel as several bands in the region of about 25–40 kDa, suggesting possible glycosylation of the protein. Indeed, there are three potential sites for N-linked glycosylation (Asn-Xaa-Thr/Ser) in human IL-TIF. Treatment of the conditioned media with peptide:N-glycosidase F (PNGase F) resulted in the disappearance of the higher bands and enhancement of a band in the region of 25 kDa (Fig. 1B, lane 4), consistent with glycosylation of the 25–40 kDa proteins. FL-IL-TIF-P, purified by affinity column chromatography, was also analyzed by Western blotting with anti-FLAG antibody (Fig. 1B, lane 5). The 32P-labeled FL-IL-TIF-P ([32P]FL-IL-TIF-P) was also resolved on the gel and autoradiographed (Fig. 1B, lane 6). Human IL-10 tagged with the FLAG epitope at the N terminus and with the phosphorylation site at the C terminus was used as a control (Fig. 1B, lane 2).

CRF2-9 is an orphan human receptor from the class II cytokine receptor family as shown in Fig. 1D (24). We constructed expression vectors encoding intact CRF2-9 and a chimeric CRF2-9/γR1 receptor that has the CRF2-9 extracellular domain fused to the transmembrane and intracellular domains of the human IFN-γR1 chain (Fig. 1C). The previously constructed pEF-CRF (or pEF-IL-10R2) vector (6) was also utilized in this study. In addition, to express both receptor chains in a single transfected cell the tandem vector encoding two receptors, the CRF2-9/γR1 and the IL-10R2 chains, in which expression of each receptor is controlled by separate set of promoter and polyadenylation signal was constructed.

Experiments in COS Cells—COS cells were transfected with the expression plasmid encoding CRF2-9, and transfectants were selected by growth in 350 µg/ml G418 for 3 weeks and pooled. To test for responsiveness to IL-TIF, pooled cells were treated with conditioned media from COS cells expressing FL-IL-TIF or left untreated as control, and the detergent-free total cellular lysates were prepared for electrophoretic mobility shift assays (EMSAs). The formation of Stat DNA-binding complexes was detected in FL-IL-TIF-treated COS cells transfected with the plasmid expressing the CRF2-9 chain and not in untreated cells or in FL-IL-TIF-treated control COS cells transfected with the blank expression vector (Fig. 2). The DNA-binding complexes (Fig. 2) were shown to consist mainly of two Stats with anti-Stat1 and anti-Stat3 antibodies: Stat1α and Stat3. Thus, the pattern of IL-TIF-induced Stat DNA-binding complexes observed in COS cells expressing CRF2-9 correlates with the pattern of Stat activation demonstrated for IL-TIF signaling in PC-12 or MES-13 cells (1). COS cells were also stably transfected with an expression vector encoding the chimeric CRF2-9/γR1 receptor with the CRF2-9 extracellular domain fused to the transmembrane and the intracellular domains of the IFN-γR1 chain (Fig. 1C). This chimeric receptor was made to enable us to detect IFN-γ-like biological activities induced by IL-TIF. Because IL-TIF-specific biological activities are not well characterized and may be restricted to specific cell types, and because we expect that the CRF2-9 receptor complex structurally mimics the IL-10 receptor complex, we followed the same approach that was used to create the chimeric IL-10-IFN-γ receptor complex (6). We predicted that, in cells express-
with the chimeric CRF2-9/IL-10 receptor complex, and to consist of two receptor chains with one common chain shared between these two receptor complexes. It has been demonstrated that, in hamster cells, expressing either the chimeric human CRF2-9/γ1 chain, the intact human IL-10R2 chain, or both, and cells expressing the human IL-10R1/γ1 and the IL-10R2 chains together were incubated with radiolabeled FL-IL-TIF-P. The cells were washed to remove unbound ligand, then bound ligand was cross-linked to the cells. After cross-linking, cells were lysed and cross-linked complexes were resolved on 7.5% SDS-PAGE (Fig. 4). The appearance of several labeled cross-linked complexes was observed in all cell lines except parental hamster cells, and the specificity of binding was shown by competition with an excess of unlabeled IL-TIF (Fig. 4). In cells expressing the CRF2-9/γ1 chain, major cross-linked complexes migrated in the region of 115 kDa, with less intense bands in the region of 60–85 kDa. In cells expressing the IL-10R2 chain, major cross-linked complexes migrated in the region of 105 kDa, with possibly three additional lower molecular mass bands. These additional bands may represent ligand oligomers not cross-linked to receptors. The cross-linking pattern in cells expressing both the IL-10R1/γ1 and the IL-10R2 chains was identical to the pattern obtained with cells expressing the IL-10R2 chain alone. We did not observe any cross-linked complexes in parental hamster cells. Major cross-linked complexes from cells expressing both the CRF2-9/γ1 chain and the IL-10R2 chain migrated on SDS-PAGE in the region of 105 and 115 kDa, corresponding in size to the complexes obtained with cells expressing either chain alone. The amount of each sample loaded on the gel was normalized to a constant number of cells used for cross-linking experiments. Thus, it appears that there is more IL-TIF binding to cells expressing both CRF2-9 and IL-10R2 chains than to cells expressing each chain alone. This is consistent with our preliminary direct binding data that indicate the increased binding of IL-TIF to cells with both chains is due to increased affinity rather than to an increase in the number of binding sites. The faster migrating species were also seen with cells expressing both chains of the IL-TIF receptor complex. Moreover, cross-linked complexes migrating in the region of 200 kDa appeared only in cells expressing both chains of the IL-TIF receptor complex and not in cells expressing either chain alone. These complexes are likely to contain oligomers of IL-TIF and both receptor chains, formed as a result of the association of the IL-TIF receptor chains induced by ligand binding. The cross-linking experiments provide direct strong evidence that IL-TIF can bind to each chain of the IL-TIF receptor complex independently.

Expression of the CRF2-9 mRNA—Northern blots containing RNA from multiple human tissues and human cancer cell lines

| Transfected Receptors | COS Cells | Hamster Cells |
|-----------------------|-----------|---------------|
| IL-TIF                | -         | +             |
| IL-10                 | +         | -             |
| anti-Stat1            | -         | +             |
| anti-Stat3            | -         | +             |
| Stat1:Stat3           | +         | +             |
| Stat1:Stat3           | +         | +             |
| Stat1:Stat1           | +         | +             |

Fig. 2. IL-TIF-induced Stat activation in monkey COS cells and hamster 16-9 cells. Electrophoretic mobility-shift assay (EMSA) was used to determine Stat DNA-binding complexes activated by IL-TIF treatment in COS or hamster cells transfected with different receptors as indicated on the figure. COS or hamster cells were transfected with blank vector or with vectors encoding receptors as indicated on the figure, grown for 3–4 weeks, and G418-resistant clones were pooled and used for EMSA. Cells were incubated with conditioned media from COS cells expressing either FL-IL-TIF or FL-IL-10 (100 μl per 10⁶ cells in 1 ml). Cellular lysates were prepared and assayed for Stat activation in the EMSA as described previously (6). Positions of Stat DNA-binding complexes are indicated by arrows. Antibodies against Stat1 and Stat3 were added as indicated to reduce the mobility of complexes containing these proteins. To detect a comparable amount of Stat DNA-binding complexes in the EMSA the portion of the gel with samples obtained from COS cells was exposed 2 days, whereas the portion of the gel with samples from hamster cells was exposed for 8 h.

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were used to assess expression of the CRF2-9 mRNA (Fig. 5). In normal tissues a transcript in the region of 3.0 kb was detected in kidney and liver (Fig. 5B). The size of the transcript is comparable with the size of the CRF2-9 cDNA 2.8 kb. Among tested cancer cell lines, expression of the CRF2-9 mRNA was detected in three solid tumor cell lines, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361, and not in promyelocytic leukemia HL-60, epitheloid carcinoma HeLa S3, lymphoblastic leukemia MOLT-4, and Burkitt’s lymphoma Raji (Fig. 5A). A549 cells were responsive to IL-TIF as demonstrated by IL-TIF-induced Stat activation determined by EMSA (data not shown). It is noteworthy that the five cell lines expressing the CRF2-9 mRNA (SW480, A549, G-361, HepG2, and Caki) are nonhematopoietic tumor cell lines. In addition, the fact that the CRF2-9 gene is expressed in normal liver and kidney tissue correlates with a recent report demonstrating that IL-TIF functions as a hepatocyte-stimulating factor (10).

FIG. 3. Ligand binding and MHC class I antigen induction. Row I, Schematic of five cell lines (clonal populations) used in these experiments: the parental Chinese hamster 16-9 cells, cells expressing the intact IL-10R2 chain (6), the chimeric CRF2-9/γR1 chain, or both receptors together, and cells expressing the modified IL-10 receptor complex containing the intact IL-10R2 chain and the chimeric IL-10R1/γR1 chain (5, 6). Row II, A–E, the ability of IL-TIF or IL-10 to induce MHC class I antigen expression was demonstrated by flow cytometry as described previously (6). The cells described in row I were left untreated (open areas, thick lines) or treated with conditioned media (100 μl) from COS cells transfected with the pEF-SPL-IL-TIF plasmid (shaded areas, thin lines) or with Hu-IL-10 (100 units/ml; open areas, thin lines). The ordinate represents relative cell number, and the abscissa represents relative fluorescence.

FIG. 4. Cross-linking. The hamster cells described in Fig. 3, row I, were incubated with 32P-labeled FL-IL-TIF-P with or without addition of a 100-fold excess of unlabeled IL-TIF (competitor), washed, harvested, and cross-linked. The extracted cross-linked complexes were analyzed on 7.5% SDS-PAGE. The molecular weight markers are shown on the right.

FIG. 5. The expression of the CRF2-9 mRNA. Northern blotting was performed on two blots containing mRNA isolated from: A, human cancer cell lines (promyelocytic leukemia HL-60, epitheloid carcinoma HeLa S3, lymphoblastic leukemia MOLT-4, Burkitt’s lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361); and B, normal human tissues (brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, peripheral blood leukocytes (PBL)). The arrow points to the CRF2-9 transcript. Equal RNA loading was assessed by evaluating the expression of the β-actin gene.

Hypothesis is that the IL-10R2 chain is shared by receptors for ligands other than IL-10. If such ligands existed, they should demonstrate homology to IL-10. Thus, we investigated whether such ligands existed. Our initial screening of the GeneBank® EST and genomic data bases resulted in identification of the cytomegalovirus-encoded IL-10 homolog (cmvIL-10) (5), which can bind and signal through the canonical IL-10 receptor complex (6) despite the low homology between cmvIL-10 and IL-10 (27% identity). The discovery of cmvIL-10 demonstrated that only 27% identity is sufficient to allow ligands to share both chains of the IL-10 receptor complex and, thus, suggested that ligands of lower homology might share only one receptor chain, particularly the IL-10R2 chain.

Human IL-TIF was cloned and expressed with a FLAG tag at its N terminus (FL-IL-TIF) in COS cells to enable detection of

DISCUSSION

The IL-10R2 chain is ubiquitously expressed, whereas the IL-10 activity is restricted mainly to cells of hematopoietic origin (35, 36). This raised the question of why the second chain of the IL-10 receptor complex is widely expressed when its function was required only in limited cellular subsets. One

human IL-TIF was cloned and expressed with a FLAG tag at its N terminus (FL-IL-TIF) in COS cells to enable detection of
the protein in COS cell-conditioned media. The anti-FLAG antibody recognized several proteins of about 25–40 kDa, suggesting possible glycosylation of FL-IL-TIF (Fig. 1). Treatment of conditioned media with PNGase F resulted in the disappearance of the higher molecular mass bands and appearance of a 21-kDa band comparable in size to FL-IL-10 (Fig. 1) demonstrating glycosylation of the 25–40 kDa protein forms. FL-IL-TIF does not utilize the canonical IL-10 receptor complex for signaling as demonstrated by the inability of human IL-TIF to induce MHC class I antigen expression and Stat1 activation in hamster cells expressing the chimeric IL-10R1γR1 chain and the intact IL-10R2 chain, whereas human IL-10 did (Figs. 2 and 3) (5, 6). IL-TIF also failed to induce Stat DNA-binding complexes in PBMCs (data not shown), whereas IL-10 did (6). Thus, we hypothesized that IL-TIF requires its own specific receptor complex for signaling, but might share the IL-10R2 chain with the IL-10 receptor complex.

Because IL-10 receptor chains belong to the class II cytokine receptor family, new ligands that have homology to IL-10 might signal through receptors from the same family. Several orphan receptors from the class II cytokine receptor family were identified by searching available public EST and genomic data bases with the sequence of the IL-10R1 extracellular domain as the query sequence (24).2 We examined whether one of them was a subunit of the IL-TIF receptor complex.

Our initial experiments demonstrated that, in COS cells expressing the CRF2-9 chain, IL-TIF induced formation of Stat DNA-binding complexes characteristic of IL-TIF signaling in MES-13 and PC-12 cells (1). Thus, the expression of the CRF2-9 chain alone in COS cells is sufficient to render the cells responsive to IL-TIF. We hypothesized that the second IL-TIF receptor chain, likely the IL-10R2 chain, is expressed in COS cells analogous to the results with IL-10 (6, 21). It was previously demonstrated that the IL-10R2 chain has limited species specificity: The IL-10R2 chain of mouse, human, or monkey origin can support signaling through the IL-10R1 chain of these species (6, 21). However, the hamster IL-10R2 chain can support signaling only through mouse IL-10R14 but not through human IL-10R1 (6). In addition, to enable us to detect IL-TIF-induced biological activities, we utilized the chimeric receptor approach that we used to characterize other receptor complexes (5, 6, 32). The human CRF2-9 extracellular domain was fused to the transmembrane and intracellular domains of the human IFN-γR1 chain resulting in the chimeric CRF2-9/γR1 chain. We hypothesized that, in cells expressing the modified IL-TIF receptor complex, in which the intact CRF2-9 is replaced with the chimeric CRF2-9/γR1 chain, IL-TIF would induce IFN-γ-like biological activities such as MHC class I antigen expression and Stat1 activation, which can be readily measured. To test this hypothesis we expressed the chimeric CRF2-9/γR1 chain in COS cells. Indeed, the pattern of IL-TIF-induced Stat activation changed to that characteristic of IFN-γ signaling (Fig. 2); only Stat1 DNA-binding complexes were observed in IL-TIF-treated COS cells expressing the chimeric CRF2-9/γR1 chain. These experiments also demonstrate that the disruption of biological activity of IL-TIF signaling is mediated by the CRF2-9 intracellular domain, because the substitution of the CRF2-9 intracellular domain by the IFN-γR1 intracellular domain caused a change in the pattern of Stat activation (Fig. 2).

We then repeated a similar series of experiments in hamster cells. We did not observe the IL-TIF-induced activation of Stats in hamster cells expressing the chimeric CRF2-9/γR1 chain (Fig. 2). Because we hypothesized that the functional IL-TIF receptor complex might contain both CRF2-9 and the IL-10R2 chain, we expressed both the chimeric CRF2-9/γR1 chain and the intact IL-10R2 chain in hamster cells. In these cells IL-TIF was able to induce Stat1 activation as measured by EMSA (Fig. 2). Moreover, IL-TIF treatment causes up-regulation of class I MHC antigen expression only in cells expressing both receptors (Fig. 3D) and failed to do so in cells expressing each of the receptors alone or in parental cells (Fig. 3, A–C). We also used hamster cells expressing various receptor combinations to demonstrate ligand binding. By cross-linking we demonstrated that IL-TIF did not bind to parental cells but was bound to hamster cells expressing either the CRF2-9/γR1 or the IL-10R2 chain, and to cells expressing both chains (Fig. 4). Low levels of binding of FL-IL-TIF to cells expressing either receptor alone or both together, but not to parental hamster cells, were also detected by flow cytometry with anti-FLAG antibody (data not shown). The ability of the IL-10R2 alone to bind IL-TIF was a surprising result, because, when expressed alone, this chain is unable to bind IL-10 (5, 6). Furthermore, other ligands (IFN-α and IFN-γ) signaling through receptor complexes whose chains belong to the class II cytokine receptor family do not bind to their “second” chains with high affinity (24). Whether this unusual binding is of functional significance remains to be determined. Because IL-10R2 is ubiquitously expressed but unable to transduce a signal without an additional chain (IL-10R1 or CRF2-9), it is possible that secreted IL-TIF will be retained at the site of secretion by being bound to the IL-10R2 chain, providing local action but preventing its action at remote sites.

These experiments demonstrate that IL-TIF specifically binds to and signals through the CRF2-9 chain and that the second chain of the IL-10 receptor complex, the IL-10R2 chain, also functions as the IL-TIFR2 chain. It is possible that the IL-10R2 chain may be shared by receptors for the other IL-10 homologs: ak155, mda-7, IL-19, and Zcyto10. The fact that all IL-10 homologs have most of their identical residues located in the C-terminal half of the protein, with the highest homology in the region of the helix F (37), suggests that this region is involved in the interaction with the same receptor component. New ligands are likely to possess their own unique ligand binding chains (like CRF2-9 for IL-TIF), sharing the second IL-10R2 chain, providing local action but preventing its action at remote sites.

The IL-TIF receptor (Fig. 6) is likely to be structurally homologous to the IL-10 and IFN-γ receptor complexes (24). IL-TIF binding is likely to induce oligomerization of two CRF2-9 (or IL-TIF-R1) chains and two IL-10R2 (or IL-10hR2) chains. A distinct feature of the IL-TIF receptor complex is that both chains can independently bind ligand, whereas in the IL-10 and IFN-γ receptor complexes, only one chain (the R1 chain) can bind ligand in the absence of the other. In all of these receptors, the second (R2) chains are necessary for signaling.

Identification of the receptor for a particular cytokine provides information about possible signal transduction cascades. It has been demonstrated that IL-TIF induces Stat1, Stat3, and Stat5 activation (1). There are several Tyr residues in the CRF2-9 intracellular domain that are potential sites for phosphorylation (Fig. 1D). Analysis of amino acids surrounding Tyr residues within the CRF2-9 intracellular domain reveals the presence of four potential Stat3 recruitment sites, phospho-

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4 S. V. Kotenko, Wen He, and S. Pestka, unpublished data.
FIG. 6. Model of the IL-22 (IL-TIF) receptor complex and signal transduction. The functional IL-22 (IL-TIF) receptor complex consists of two receptor chains, the IL-22R1 (IL-TIF-R1 or CRF2-9) chain (24) and the IL-10R2 chain (6) and is likely to be structurally homologous to the functional IL-10 receptor complex (C). The IL-10R2 chain is a shared common chain for at least two receptor complexes, the IL-10 receptor complex, and the IL-22 receptor complex and is likely to be a shared receptor chain with receptor complexes for other IL-10 homologs. Thus, this chain can be designated receptor two (2) common chain (R2c). Both chains of the IL-22 receptor complex are ligand binding chains, however, none of them are capable of transducing IL-22-signaling alone (A). Both chains are necessary to assemble the functional receptor complex able to induce signaling after binding IL-22 (B). The IL-10R2 chain is associated with Tyk2 (6, 34). Both cytokines, IL-10 and IL-22, activate a similar combination of Stat proteins, Stat1, Stat3, and Stat5 (1, 40, 41).

Tyr-Xaa-Xaa-Gln sequence (Fig. 1, YXXQ motif). It remains to be determined how Stat1 and Stat5 are recruited to the IL-TIF receptor complex and also whether all four Stat3 docking sites are active or only a subset of them.

A recent report (11) is in agreement with our results, although the authors did not demonstrate that the expression of the CRF2-9 receptor (they named IL-22R) in COS cells rendered them sensitive to IL-TIF. In our experiments, COS cells expressing intact or modified CRF2-9 are responsive to IL-TIF treatment; thus, the conclusion about the necessity of the R2 chain to assemble the functional IL-TIF receptor complex required experiments in hamster cells. Our data demonstrate that endogenous IL-10R2 in COS cells can support signaling through the human IL-TIF receptor complex as we previously demonstrated for the IL-10 receptor complex (6, 24). The discrepancy can be explained by the fact that we used stable transfectants of COS cells, whereas Xie et al. (11) used transiently transfected cells. Transient expression results in overexpression of the CRF2-9 chain so that most of the CRF2-9 chains do not interact with the limited level of the endogenous IL-10R2 chain resulting in nonfunctional CRF2-9-IL-TIF complexes (38, 39). Our experiments in hamster cells, in which the hamster IL-10R2 chain cannot support signaling through the human IL-10R1 chain (6, 24), demonstrate the requirement for both CRF2-9 (IL-TIF-R1) and IL-10R2 chains for reconstitution of a functional IL-TIF receptor complex and that IL-10R2 (R2c) serves as a common receptor chain for both IL-10 and IL-TIF.

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Identification of the Functional Interleukin-22 (IL-22) Receptor Complex: THE IL-10R2 CHAIN (IL-10R β) IS A COMMON CHAIN OF BOTH THE IL-10 AND IL-22 (IL-10-RELATED T CELL-DERIVED INDUCIBLE FACTOR, IL-TIF) RECEPTOR COMPLEXES

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