The Fas receptor is a member of a family of cell death receptors, including tumor necrosis factor receptor I (TNFR I), death receptor 3 and 4 (DR3 and DR4), and cytotoxic avian receptor 1 (CAR1). The Fas receptor is composed of several discrete domains, including three cysteine-rich domains (CRDs), a transmembrane domain, and an intracellular domain responsible for transmitting an apoptotic signal. While the mechanism of Fas-mediated cell death has become elucidated, the requirements for Fas ligand binding to the receptor have not been fully defined. Using a series of chimeric Fc receptor fusion proteins between the human Fas receptor and TNFR I, each cysteine-rich domain of Fas was found to be required for interaction with the Fas ligand. Interestingly, TNFR I CRD1 could partially substitute for the Fas CRD1. The importance of this domain was underscored by the analysis of a Fas extracellular mutation (C66R), which resulted in a complete loss of ligand binding. This mutation was cloned from a human patient suffering from Canale-Smith syndrome, which is characterized by autoimmunity resembling that observed in the lpr and lprSK mice. The localization of essential ligand binding domains in the Fas receptor correlated exactly with the ability of the Fas receptor fusion proteins to prevent cell death mediated by the Fas ligand.

The Fas receptor, a member of the tumor necrosis factor (TNF) family of receptors, is synthesized as a 45-kDa type I transmembrane protein that functions to initiate a signal transduction cascade, leading to programmed cell death. Fas receptor mRNA is expressed at particularly high levels in activated lymphocytes, liver, ovary, heart, and thymus and acts as a key mediator in the peripheral deletion of lymphocytes that are removed after activation and expansion in response to antigen (1–3). Mice with loss of function mutations at the fas locus demonstrate marked lymphadenopathy, splenomegaly, autoimmunity, glomerulonephritis, and arthritis (4–8). Recently, roles for Fas and Fas ligand have been defined in the pathogenesis of viral hepatitis, Hashimoto’s thyroiditis, and autoimmune diabetes as well as mediation of the immune privilege status of the eye, testis, and some cancer cells (9–15).

The TNF receptor family is defined by the presence of between two and six cysteine-rich domains (CRDs) in their extracellular regions (16). The CRDs are thought to fold independently and are stabilized by extensive intrachain disulfide bonding. The Fas receptor and TNFR I also share significant homology within their intracellular domains. An 80-amino acid region called the death domain is required for the apoptosis-promoting activities of these receptors (17, 18). The death domain is a novel protein-protein association motif found in many proteins, such as the Drosophila pro-apoptotic protein Reaper and the mammalian pro-apoptotic proteins FADD, TRADD, and RIP (8). These last three death domain proteins are recruited to the Fas receptor or TNFR I and mediate the initiation of an apoptotic program requiring activation of interleukin-1β converting enzyme-like proteases (19–21).

Receptor-ligand interactions are believed to occur predominantly via the CRDs in the extracellular regions of these receptors. However, there is little information regarding sequences of the Fas receptor required for engagement of its ligand. Structure-function and x-ray crystallographic structure analyses of TNFR I have demonstrated that all four CRDs contribute to TNF binding (22–25). However, several family members such as cytotoxic avian receptor 1 (CAR1) and death receptor 4 (DR4) possess as few as two CRDs, yet are still able to bind ligand (26, 27). Thus, these receptors have the capacity to engage their respective ligands in a distinct manner.

To define Fas ligand-receptor interactions, a series of chimeras between the human Fas receptor and TNFR I extracellular domains were constructed and fused to the Fc region of human IgG1. The Fas receptor contains three CRDs, whereas TNFR I has four CRDs. A sequence analysis of the TNF receptor family indicated that the three CRDs of the Fas receptor are most homologous to the first three CRDs of TNFR I (28). The only significant difference exists within CRD1. The human Fas receptor contains two disulfide bonds compared with three for the human TNFR I CRD1. The Fc receptor fusion proteins permitted the production of mammalian cell-derived soluble recombinant forms that could be easily purified and analyzed for ligand binding (22, 29). The results indicated that all three CRDs of the Fas receptor are required for ligand binding, as mutations in each CRD led to a complete loss in ligand binding. Interestingly, CRD1 from TNFR I was able to partially substitute for the homologous domain of Fas, yielding a chimeric receptor that recognized the Fas ligand with full efficacy but reduced potency.
Cysteine-rich Repeats of the Fas Receptor

MATERIALS AND METHODS

Preparation of Fc Receptor Fusion Constructs—The vector pCDSL1g was a kind gift from Brian Seed. The human IgG Fc, domain genomic coding sequence was excised using BamHI and XhoI and ligated into these sites in pCDNA3 (Invitrogen), generating the new plasmid pCDNA3-Fc.

The coding sequence for the extracellular domains of the wild-type human Fas receptor and TNFR I was generated using the polymerase chain reaction (PCR). The following primers were used for the PCRs: A, 5’-CGGATCCGCTATGAGCCGCTAGCTCTCCTC-3’; B, 5’-CCGGT-ACCACCATGGGCTTCCCTCAGGCTG-3’; C, 5’-CCGGATCCACCATGGGCTTCCCTCAGGCTG-3’; and D, 5’-CCGGATCCGGCGGTCC-TGATGTCTCAGG-3’. Each reaction contained 1 ng/ml template (pCDNA3-Fc or pCMV5-hTNFR I), 1 ng/ml of each primer for pairs A + C (F, -FasR) or B + D (F, -TNFR I), 200 µM dNTPs, 1 x Taq polymerase buffer, and 2.5 units of Taq polymerase (Boehringer Mannheim). PCR reactions were performed as follows: cycles 1–10 (denaturation, 94 °C for 1 min; annealing, 45 °C for 1 min; and extension, 72 °C for 1 min); cycles 11–30 (denaturation, 94 °C for 1 min; annealing, 65 °C for 1 min; and extension, 72 °C for 1 min); and incubation at 72 °C for 8 min after the last cycle. The PCR products were digested with Kpn I and BamHI and ligated into pKpnI and BamHI-digested pCDNA3-Fc, to yield the plasmids pCDNA3-Fc-wtFasR and pCDNA3-Fc-wtTNFR I.

Constructs encoding chimeric Fc-FasR mutants were prepared using a hybrid PCR-based approach. The N-term Swap mutant was generated as follows. The first round of PCRs was performed in parallel using primer pair B + E (5’-CAAGTGCATCCTCCTCTCCCTC-3’) with pCDNA3-Fc-wtFasR and primer pair A + F (5’-AGAGATTGCTCGAAGAAGCATGGCTG-3’) with pCDNA3-hFasR as a template and primer pair A + H (5’-ACTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template; as described above for the preparation of the pCDNA3-Fc-wtFasR construct, except that for cycles 11–30 annealing was performed at 60 °C. The products were then isolated, denatured, annealed, and extended using 1 unit of Klenow (Boehringer Mannheim), 1 x Klenow buffer, and 250 µM dNTPs for 30 min at 37 °C. The extended product was then used as a template for a second round of PCR using primer pair A + F as described above. The PCR fragment was isolated and digested with KpnI and BamHI and ligated into KpnI- and BamHI-digested pCDNA3-Fc, to yield the plasmid pCDNA3-Fc-N-term Swap.

The CRD1, CRD2, and CRD3 chimeric mutants were described for pCDNA3-Fc-N-term Swap except that the following primers and templates were used for the first round of PCRs: CRD1 chimeric mutant, primer pair B + G (5’-TGCGACGGGCGCCCTCCTCTCAGG-3’) with pCMV5-hTNFR I as a template and primer pair A + H (5’-GACTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template; CRD2 chimeric mutant, primer pair C + I (5’-CTCTTGATAGATTGGACGCTACTGCAGCTGGG-3’) with pCDNA3-Fc-hFasR as a template; as described above for the preparation of the pCDNA3-Fc-wtFasR construct; and CRD3 chimeric mutant, primer pair C + K (5’-CTCTTCTTCACATCGCGGCTCAGCTGGG-3’) with pCDNA3-Fc-hFasR as a template and primer pair L (5’-AAGTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template.

The Crd1 mutant construct was prepared as described above for the preparation of the pCDNA3-Fc-wtFasR construct, except that the following primer pairs were used: primer pair A + H (5’-GACTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template and primer pair A + H (5’-GACTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template; and primer pair A + H (5’-GACTGAGAGCCTGCAAGAAGGAGAG-3’) with pCDNA3-hFasR as a template. Each PCR reaction was performed as follows: cycles 1–10 (denaturation, 94 °C for 1 min; annealing, 45 °C for 1 min; and extension, 72 °C for 1 min); cycles 11–30 (denaturation, 94 °C for 1 min; annealing, 65 °C for 1 min; and extension, 72 °C for 1 min); and incubation at 72 °C for 8 min after the last cycle. The PCR products were digested with Kpn I and BamHI and ligated into pKpnI and BamHI-digested pCDNA3-Fc, to yield the plasmids pCDNA3-Fc-wtFasR and pCDNA3-Fc-wtTNFR I.

Binding of the Fc-receptor fusion protein was determined by boiling the matrix in 1x SDS-PAGE sample buffer, and the dissociated material was analyzed by Western blot after transfer to a nitrocellulose membrane. The membrane was blocked using 1% bovine serum albumin, incubated with the anti-human Fas ligand monoclonal antibody (Pharmingen; clone G-247) was added to a concentration of 1 µg/ml. To each reaction, 15 µl of protein-A-Sepharose was added and incubated for 2 h at 4 °C. The concentrations were determined empirically by performing a dose-response analysis using the wild-type Fc-FasR fusion protein as a control. As an anti-human Fas ligand monoclonal antibody (Pharmingen; clone G-247) was added to a concentration of 1 µg/ml. To each reaction, 15 µl of protein-A-Sepharose was added and incubated for 2 h at 4 °C. The concentrations were determined empirically by performing a dose-response analysis using the wild-type Fc-FasR fusion protein as a control.

Preparation of Soluble Human Fas Ligand—Soluble human Fas ligand was prepared as described previously. Briefly, CHO-K1 cells were stably transfected with a mammalian expression vector that drives the synthesis of a secreted, biologically active form of the human Fas ligand containing residues 103–281. Supernatant containing the soluble Fas ligand was prepared by culturing the CHO-K1 line in CHO-SFMII serum-free medium (Life Technologies, Inc.) in spinner culture for 72 h at 37 °C in 5% CO2. The cell-free supernatant was filtered through a 0.2-µm filter and used directly for experiments. The ability to induce cell death was assayed using mouse A20B cells. The LDR was achieved using 10-fold diluted crude supernatants.

Fc-Receptor Protein Ligand Binding Assay—Binding of the Fc-receptor fusion proteins to soluble human Fas ligand was assessed as follows. CHO-K1 supernatant containing soluble Fas ligand was diluted with an equal volume of Nonidet-P40 Buffer to a final volume of 500 µl. Fc fusion protein was added to each dilution at a concentration of 0.5 or 2.5 µg/ml and incubated for 2 h at 4 °C. The concentrations were determined empirically by performing a dose-response analysis using the wild-type Fc-FasR fusion protein. A20B cells were grown exponentially in RPMI 1640 supplemented with 10% fetal bovine serum, 2% L-glutamine, and 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were harvested by centrifugation at 1000 x g for 10 min, washed with Tris-buffered saline (TBS), and 2 x 105 cells were added to each well in 100 µl of the indicated medium. The cells were centrifuged at 1000 x g for 10 min, washed with Tris-buffered saline/Tween-20, and the RIPA buffer at the target temperature. Any bound soluble ligand was dissociated from the Fc fusion protein by boiling the matrix in 1 x SDS-PAGE sample buffer, and the dissociated material was analyzed by Western blot after transfer to a nitrocellulose membrane. The membrane was blocked using 1% bovine serum albumin, incubated with the anti-human Fas ligand monoclonal antibody (Pharmingen; clone G-247) was added to a concentration of 1 µg/ml. To each reaction, 15 µl of protein-A-Sepharose was added and incubated for another 30 min at 4 °C. The matrix was isolated by centrifugation and washed with 6 x 1 x Nonidet-P40 buffer at the target temperature. The ability to induce cell death was assayed using mouse A20B cells. The LDR was achieved using 10-fold diluted crude supernatants.

Bioassay of Fc-Receptor Proteins—The ability of the various Fc-receptor fusion proteins to neutralize the cytotoxic activity of soluble human Fas ligand was performed using A20B Fc receptor cells as the target (32). A20 cells were grown exponentially in RPMI 1640 + 10% fetal calf serum (heat-inactivated) with Pen-Strep and Fungizone. The cells were diluted to 5 x 10^5/ml, and 50 µl was added to each well in a 96-well plate. Soluble human Fas ligand (50 µl of CHO-K1 supernatant) with or without Fc-receptor fusion protein at a known concentration was added to the A20 cells. Cultures were incubated for 18 h in the presence of ligand, and viability was determined using the MTT assay. The viability of the A20 cells cultured with ligand and no Fc-fusion protein was <10% of that of untreated cells. The ability of the Fc-fusion proteins to rescue the A20 cells from soluble Fas ligand was 2 Orlinick, J., Elkon, K., and Chao, M. V., J. Biol. Chem., in press.
Cysteine-rich Repeats of the Fas Receptor

To begin to define the requirements of Fas receptor binding to its ligand, a series of extracellular domain chimeras were constructed between the Fas receptor and TNFR I. The choice of TNFR I was prompted by the close homology between CRD1, CRD2, and CRD3 of the Fas receptor and TNFR I (28). To facilitate the binding analysis, the chimeric constructs were prepared by fusing the extracellular domains to the hinge region of the human IgG1 Fc region. This allowed for the isolation of the parental and mutant extracellular receptor sequences in native form from mammalian cells (22, 29).

Each of the coding sequences for the three CRDs or the short sequence N-terminal to CRD1 from the human Fas receptor was exchanged with the corresponding sequence from the human TNFR I by PCR (Fig. 1). This yielded the following mutants: CRD1, CRD2, CRD3, and N-term Swap. The cDNAs encoding the extracellular domains of the wild-type human Fas receptor or TNFR I or the mutant forms of the Fas receptor in a human IgG1 Fc expression vector were transiently transfected into 293T cells. Transfected cells were cultured under serum-free conditions, and Fc-fusion proteins were purified by protein A-Sepharose chromatography. The fusion proteins were analyzed by SDS-PAGE under reducing conditions (Fig. 2). Each recombinant protein was efficiently expressed and could be detected with the predicted molecular mass. Analysis under nonreducing conditions demonstrated that each protein shifted in apparent molecular mass by a factor of two, as expected for Fc-fusion proteins (data not shown).

The ability of each chimeric fusion protein to bind the human Fas ligand was assessed using a soluble version of the ligand. Biologically active soluble ligand containing residues 103–281 was produced from stably transfected CHO-K1 cells.2 Purified Fc-fusion proteins were incubated with supernatant containing soluble Fas ligand and recovered by incubation with protein A-Sepharose. To determine whether the ligand bound to the Fc fusion proteins, the protein A-Sepharose-precipitated material was analyzed by Western blotting with an anti-human Fas ligand specific monoclonal antibody (Fig. 3A). The monomeric, dimeric, and trimeric forms of the ligand were observed. Concentrations of 0.5 and 2.5 $\mu$g/ml were chosen after dose-response analysis using the wild-type Fc-Fas receptor: ~50% binding was observed at 0.5 $\mu$g/ml, whereas full binding was observed at 2.5 $\mu$g/ml. The specificity of the assay was demonstrated by the lack of ligand binding to the wild-type Fc-TNFR I. The results demonstrated that whereas the N-terminal Swap mutant bound the ligand as well as the wild-type Fc-Fas receptor, the CRD2 and CRD3 mutants showed no binding to ligand. Interestingly, although the CRD1 mutant did not bind ligand at 0.5 $\mu$g/ml, at the higher concentration of 2.5 $\mu$g/ml, ligand binding could be detected (Fig. 3A). The CRD1 chimera was the only Fas mutant examined that exhibited this behavior. As a control, an anti-human Fas ligand monoclonal antibody was used to immunoprecipitate ligand from the CHO-K1 supernatant to demonstrate the total amount of ligand used in the assay. The immunoglobulin heavy-chain band can be seen at 55 kDa.

Since the CRD1 mutant was generated by exchanging CRD1 and the short sequence N-terminal to it from the Fas receptor with that from TNFR I, the observed residual binding to ligand could be explained by one of several possibilities. First, the sequence exchanged by the CRD1 mutant may not be absolutely necessary for ligand binding. Alternatively, the sequence exchanged is required for ligand binding, but the homologous region from TNFR I can substitute for the loss of Fas receptor sequence. To address this issue, two additional Fc-receptor mutants were prepared: DCRD1 and C66R (Fig. 1). The DCRD1 deletes CRD1 and the short sequence N-terminal to it, leaving only CRD2 and CRD3 of the Fas receptor extracellular domain. The C66R mutation was identified during analysis of a patient with Canale-Smith syndrome, which is a human counterpart of the lpr-based disorder in mice (30). Cloning and sequencing of the Fas receptor cDNA from this individual's peripheral blood mononuclear cells demonstrated the presence of a single mutation in the extracellular domain, resulting in TGC changed to CGC at codon 66 (Cys to Arg). As this cysteine is involved in one of two disulfide bonds that stabilize the structure of the Fas

![Figure 1](image1.png)

**Fig. 1. Human Fas receptor mutant constructs.** A series of Fc-receptor proteins were prepared as described under “Materials and Methods.” The human Fas receptor extracellular domain is composed of three CRDs, whereas the human TNFR I contains four CRDs. Each CRD from the Fas receptor was exchanged with the homologous CRD from the TNFR I to generate the chimeric receptor constructs designated CRD1, CRD2, and CRD3. The protein sequences N-terminal to CRD1 from the Fas receptor were exchanged with the homologous sequence from TNFR I to generate the N-term Swap mutant. Mutants in which CRD1 was either deleted or disrupted were prepared and designated DCRD1 or C66R, respectively. The wild-type Fas receptor and wild-type TNFR I fusion proteins are also shown.

![Figure 2](image2.png)

**Fig. 2. SDS-PAGE analysis of Fc-receptor fusion proteins.** The fusion proteins depicted in Fig. 1 were expressed in 293T cells after transient transfection and purified from serum-free supernatants using protein A-Sepharose affinity chromatography. Each fusion protein (5 $\mu$g) was analyzed by 8% SDS-PAGE under reducing conditions. The fixed gel was stained using Coomassie Brilliant Blue. wt, wild type.
Cysteine-rich Repeats of the Fas Receptor

Gand-induced cell death bioassay was employed. Each fusion protein was assessed for its ability to protect A20 FasR* cells from apoptosis induced by soluble human Fas ligand. A20 cells undergo rapid Fas-dependent cell death in the presence of agonist (32). After an 18-h incubation with the Fas ligand and increasing concentrations of each Fc-fusion protein, A20 cell viability was determined by MTT assay. The wild-type and N-term Swap Fc-Fas receptor proteins were equipotent in protecting A20 cells from ligand-induced cell death with an IC_{50} = 0.5 μg/ml (Fig. 3B). Consistent with the inability of the TNFR I, CRD2, CRD3, C66R, and ΔCRD1 Fc-fusion proteins to bind to the soluble Fas ligand, A20 cells were not protected from apoptosis (Figs. 3B and 4B). Finally, the CRD1 Fas mutant demonstrated full efficacy in this assay but with a significantly reduced potency (IC_{50} = 7 μg/ml)

These findings were entirely consistent with the results from the ligand binding assays.

**DISCUSSION**

The engagement of the Fas receptor by its ligand initiates an irreversible set of events culminating in apoptosis. In this study we have begun to define the requirements of the Fas receptor for binding to its ligand. We have utilized the homology found within the extracellular domains of all TNF receptor family members to generate a series of chimeric receptor bodies between the human Fas receptor and TNFR I (Fig. 1). One advantage of this approach is that the folding of the resulting mutant fusion proteins should not be significantly altered with respect to the wild-type protein due to the modular structure of the CRD sequence (23). A second advantage is that the specificity of the Fas receptor-ligand interaction can be clearly defined relative to TNFR I, i.e. which domains from the Fas receptor uniquely contribute specificity to Fas ligand binding.

We have also taken advantage of fusion proteins with a deleted (ΔCRD1 mutant) or disrupted (C66R mutant) CRD1 to extend the binding study.

The analysis of the binding and bioactivities of mutant Fc-fusion proteins indicated that all three CRDs of the human Fas receptor are required for ligand binding. Although exchanging the short sequences N-terminal to CRD1 had little effect, exchanges of CRD2 or CRD3 of the Fas receptor for the homologous domain found in TNFR I resulted in a complete loss of binding to the Fas ligand. Interestingly, exchanging the TNFR I CRD1 for the Fas receptor CRD1 led to a 14-fold diminution in ligand binding potency (Figs. 3, A and B). That CRD1 is absolutely necessary for binding to the Fas ligand is supported by the analysis of the ΔCRD1 and C66R mutant fusion proteins (Figs. 4, A and B). The removal of CRD1 or a single amino acid change that disrupts one of two disulfide links in this domain resulted in an inability to bind the Fas ligand. These findings, along with the observation that the CRD1 chimera can interact with the Fas ligand, indicates that much of the observed specificity of the Fas receptor for its ligand relative to TNFR I is dictated by sequences represented by CRD2 and CRD3 and to a lesser extent CRD1.

Another conclusion from this study is that no single CRD or pair of CRDs is sufficient for Fas ligand binding. This is particularly relevant given recent findings of multiple splice variants of the human Fas receptor mRNA (33, 34). Cascino et al. (33) have suggested that some of these splice variants, which encode soluble Fas receptor truncated within the extracellular domain, could play an important physiological role by binding to Fas ligand, thereby competing with the full-length Fas receptor. This represents an unlikely scenario, since Fas ligand binding requires all three Fas receptor CRDs. However, a splice variant encoding a soluble Fas receptor lacking the transmembrane domain may be a very important physiological or patho-

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**FIG. 3.** Binding analysis of the Fc-Fas receptor-TNFR I chimera to soluble human Fas ligand. A, direct binding assay. Different concentrations of the parental or chimeric mutant Fc-fusion proteins (0.5 or 2.5 μg/ml) were incubated with soluble Fas ligand prepared from CHO-K1 cells as described under “Materials and Methods.” The Fc-fusion proteins bound to ligand were harvested by adding 15 μl of protein A-Sepharose. The washed matrix was incubated with SDS-PAGE sample buffer and analyzed by Western blot for the presence of protein A-Sepharose. The results are plotted as “% Rescue,” using E_{50}; f, wild-type FasR; j, wild-type TNFR I; x, N-term Swap; 0, CRD1; ■, CRD2; □, CRD3.

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**FIG. 4.** Binding of the parental and chimeric mutant Fc-fusion proteins to soluble Fas ligand. A20 cells were not protected from apoptosis induced by soluble human Fas ligand. A20 cell viability was determined by MTT assay. The wild-type and N-term Swap Fc-Fas receptor proteins were equipotent in protecting A20 cells from ligand-induced cell death with an IC_{50} = 0.5 μg/ml (Fig. 3B). Consistent with the inability of the TNFR I, CRD2, CRD3, C66R, and ΔCRD1 Fc-fusion proteins to bind to the soluble Fas ligand, A20 cells were not protected from apoptosis (Figs. 3B and 4B). Finally, the CRD1 Fas mutant demonstrated full efficacy in this assay but with a significantly reduced potency (IC_{50} = 7 μg/ml).
Cysteine-rich Repeats of the Fas Receptor

The ability of the CRD1 and CRD6 mutants to bind soluble Fas ligand was assessed by Western blot analysis, as described in Fig. 3A. B, competitive bioassay. The ability of the CRD1 and CRD6 mutants to compete with endogenous Fas receptor for Fas ligand was assessed as described in Fig. 3B. wt, wild type; □, wild-type FasR; ■, CRD6; ●, CRD1.

physiological modulator of the Fas receptor-ligand system, as the encoded protein should contain three intact CRDs.

Notably, some human Canale-Smith syndrome patients are heterozygous for mutations at the Fas locus that result in truncated soluble forms of the Fas extracellular domain (35, 36). The observed autoimmune phenotype could thus result from reduced expression of the normal receptor due to reduced copy number or from a direct effect of the truncated form on the wild-type receptor. The results presented here exclude the possibility that the soluble mutant protein acts to compete with the normal receptor for ligand.

The findings of this study add to previous studies of other TNF receptor family members. For TNFR I, ligand binding involves contacts to all four CRDs. CRD4, however, does not appear to be required for NGF binding, but both CRD3 and CRD4 are necessary and sufficient. Thus, for each TNF receptor family member, a different requirement exists for ligand binding that involves use of alternative cysteine-rich repeats. This is highlighted by the variability in the number of CRDs among receptor family members, ranging from as few as two (e.g. DR4 and CAR1) to as many as six CRDs (e.g. CD30).

Recently, serine-scanning mutagenesis was performed on the extracellular domain of the human Fas receptor that revealed several contact residues for binding Fas ligand (31). Our study indicates that Fas receptor binding to its ligand requires all three cysteine-rich repeats, and this interaction is distinct from a closely related receptor, TNFR I. Furthermore, CRD1 from TNFR I can partially substitute for the homologous region in the Fas receptor. Additional structural analysis may allow for the production of a modified Fas receptor with a higher affinity for ligand, from which more potent Fas receptor-ligand antagonists could be developed.

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