CD30 Contains Two Binding Sites with Different Specificities for Members of the Tumor Necrosis Factor Receptor-associated Factor Family of Signal Transducing Proteins*

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CD30 is a member of the tumor necrosis factor (TNF) receptor family of proteins. CD30 can regulate proliferation of lymphocytes and may also play an important role in human immunodeficiency virus replication. However, little is known about CD30 signal transduction. We performed a yeast two-hybrid library screen with the cytoplasmic domain of CD30 and isolated multiple independent cDNAs encoding human tumor necrosis factor receptor-associated factor 1 (TRAF1), TRAF2, and CRAF1 (TRAF3). The ability of TRAF1, TRAF2, and CRAF1 to associate with CD30 was confirmed using an in vitro coprecipitation assay, further demonstrating that the interaction was specific and direct. The TRAF-binding domain of CD30 was mapped to the COOH-terminal 36 amino acid residues, which contained two independent binding sites. CRAF1 bound only a single site, which contained the sequence PEQET, whereas TRAF1 and TRAF2 were capable of binding to either the PEQET site or an additional downstream domain. These data indicate that the TRAF protein binding pattern of CD30 differs from other TNF receptor family members and suggest that signaling specificity through TNF receptor family proteins may be achieved through differences in their abilities to bind TRAF proteins.

CD30 was originally identified as an antigen expressed on the surface of Hodgkin's lymphoma cells (1, 2). Subsequently, CD30 was shown to be expressed by lymphocytes with an activated phenotype (3-7), cells on the periphery of germinal centers (2, 7), CD45RO activated phenotype (3–7), and Epstein-Barr virus-and human T cell lymphotropic virus type I and II-transformed cells (3, 5, 7, 8). CD30 is expressed as both membrane bound and soluble forms (9). Increases in the amount of soluble CD30 have been detected in patients with human immunodeficiency virus infections, leading to speculation that CD30 may play a role in the development and progression of AIDS (10–12). CD30 may also play a role in the development of T helper 2 type cells (13–15).

CD30 is a member of the TNF receptor superfamily of receptor proteins (16, 17). The membrane bound form of CD30 is a 120-kDa 595-amino acid glycoprotein with a 188-amino acid cytoplasmic domain (7, 16–20). Cross-linking of CD30 with either antibodies or with CD30 ligand produces a variety of effects in cells, including augmenting the proliferation of primary T cells following T cell receptor engagement (5, 21), induction of the NF-kB transcription factor (11, 22), induction of human immunodeficiency virus gene expression in infected T cells (11, 12), increases in intracellular calcium (4), and cell death (5). However, little is known about the mechanisms of CD30 signal transduction.

Most of the homology between TNF receptor family members occurs in the extracellular domain, with little homology in the cytoplasmic domain (17). This suggested that different members of the TNF receptor family might utilize distinct signaling pathways. Consistent with this hypothesis, the TNF receptor type 1 and Fas have been shown to interact with a set of intracellular signaling molecules through a 65-amino acid domain termed a death domain (17, 23–26), whereas the TNF receptor type 2 and CD40 have been found to associate with members of the tumor necrosis factor receptor-associated factor (TRAF) family of signal transducing molecules (27–32). The T cell activation properties of the TNF receptor family member 4–1BB have been shown to involve the specific ability of its cytoplasmic domain to associate with the tyrosine kinase p56lck (33). The sequence of the cytoplasmic domain of CD30 shows little sequence similarity to any of these receptors (17). CD30 lacks an obvious death domain or a p56lck-binding site. Therefore, the yeast two-hybrid system was used to screen a cDNA library in order to identify proteins that interacted with the cytoplasmic domain of CD30. Multiple independent cDNA isolates of human TRAF2 (TRAP; 27, 31), TRAF1 (EB16; 27, 32), and CRAF1 (TRAF3, CAP1, CD40-bp, LAP1; 28–30, 32) were identified. Each of these proteins was found to associate specifically with CD30 in yeast and in an in vitro coprecipitation assay using a glutathione S-transferase (GST)-CD30 fusion protein. This is the first demonstration of a direct interaction between TRAF1 and a receptor protein. The region of CD30 required for the interaction with the TRAF proteins was mapped to the COOH-terminal 36 amino acids. This region of CD30 was found to contain a single binding site for CRAF1. However, both TRAF1 and TRAF2 were capable of binding either to the CRAF1-binding site or a downstream site. These results demonstrate that CD30, in addition to its extracellular similarities to TNF receptor type 2 and CD40, shares with these receptors the ability to bind members of the TRAF family of signal transducing molecules. Nevertheless, there appear to...
be different binding specificities of individual TNF receptor proteins for the TRAF proteins. The differential ability of these receptors to bind distinct members of the TRAF family may, at least partially, account for the differences in their biological activities.

MATERIALS AND METHODS

Plasmids—The yeast expression vectors pAS1 and pACT have been described previously (34). The Matchmaker human B cell library was purchased from Clontech and is derived from Epstein-Barr virus-transformed peripheral blood lymphocytes (B cell population).

Polymerase chain reaction (PCR) was used to construct the wild type and all deleted forms of the CD30 cytoplasmic domain (CD30 cyto). In all cases described below, Phusion polymerase (Stratagene) was used in order to minimize the possibility of PCR introduced errors. pAS1-CD30 cyto contains the coding sequence for the entire cytoplasmic domain of CD30 (amino acid residues 408–595). Arginine 409 was mutated to a methionine in order to create an in-frame with pAS1 upstream of the appropriate codon. The NdeI site to facilitate cloning into NdeI site of Bluescript SK(+). The COOH-terminal deletions of CD30 cyto (deletion at the NdeI site of Bluescript SK(+)) were made by PCR using antisense oligonucleotides that introduced in-frame stop codons in the appropriate positions. NH2-terminal deletions were also constructed by PCR using 5′-ACGTAGCTCATAGGGGCCCT-GCAGGAAAGGAATTCGG-3′ and sense, 5′-ACGTAGCTCATAGGGGCCCTGCAGGAAAGGAATTCGG-3′, and antisense, 5′-ACGTAGCTCATAGGGGCCCTGCAGGAAAGGAATTCGG-3′, using the oligonucleotides: sense, 5′-ACGTAGCTCATAGGGGCCCTGCAGGAAAGGAATTCGG-3′, and antisense, 5′-ACGTAGCTCATAGGGGCCCTGCAGGAAAGGAATTCGG-3′. The PCR products were then ligated into the NdeI and BamHI sites of pAS1. The COOH-terminal deletions of CD30 (Δ124, Δ171, Δ136, Δ119, and Δ19) were made by PCR using antisense oligonucleotides that introduced in-frame stop codons in the appropriate positions. NH2-terminal deletions (ΔN96, ΔN117, and ΔN146) were also constructed by PCR using 5′-primers that inserted an NdeI site in-frame with pAS1 upstream of the appropriate codon. The ΔPEQET internal deletion and point mutations of this sequence were made using the Chameleon mutagenesis system (Stratagene) according to the manufacturer’s protocol. Mutations were verified by sequencing.

Full-length TRAF1, TRAF2, and CRAF1 DNAs that were isolated in the yeast two-hybrid screen were subcloned into Bluescript SK(+) (Stratagene) in order to facilitate in vitro transfection and translation. pACT plasmids containing the full-length TRAF clones were digested with Xhol (in the case of human TRAF2 the digestion was partial), and the fragments were ligated into the Xhol site of Bluescript SK(+).

GST-CD30 cyto was constructed by using PCR to amplify the CD30 cytoplasmic domain coding sequence from pAS1-CD30 cyto. The sense primer, 5′-GTTGAACTCCACCGAGGAGGCGAGGAAATTCGG-3′, added an in-frame BamHI site to the 5′ end that facilitated subcloning into pGEX-2TK (Pharmacia Biotech Inc.). GST-CD28 cyto was constructed similarly by using PCR to amplify the CD28 cytoplasmic domain coding sequence from a plasmid containing a full-length mouse CD28 cDNA. The oligonucleotide primers inserted an in-frame BamHI site at the 5′ end and an EcoRI site at the 3′ end that facilitated subcloning into pGEX-2TK.

Yeast Two-hybrid Screening—Yeast two-hybrid library screening and analysis were performed essentially as described in the Matchmaker protocol provided by Clontech using the yeast strain Y153. Briefly, Y153 was transformed with the appropriate pAS1 and pACT plasmid DNA derivatives (for directed assays, 200 ng of each plasmid; for library screening, 30 μg of each plasmid) and then plated on plates lacking leucine, tryptophan, and histidine and containing 25 mm 3-amino- nitroizazole (3-AT) and plates lacking leucine and tryptophan. After 3 days at 30°C, colonies from plates lacking leucine, tryptophan, and histidine and containing 3-AT were transferred to filters and assayed for β-galactosidase activity. However, in all cases tested, colonies from the plates lacking leucine and tryptophan gave identical results. In general, β-galactosidase activity was apparent within 1 h, but the filters were allowed to incubate for 24 h. In all assays, transformants were tested for both the ability to grow on medium lacking histidine and containing 3-AT and for β-galactosidase expression. Without exception, protein-protein interactions were considered to be positive if both growth in the absence of histidine and β-galactosidase expression were observed.

GST CoPrecipitation Assays—GST co-precipitation assays were performed as described by Lewis et al. (35). GST and GST fusion proteins were expressed in XL1 Escherichia coli. 35S-Labeled full-length TRAF1, TRAF2, and CRAF1 were in vitro transcribed/translated from the Blue- script plasmid templates described above in rabbit reticulocyte lysate using the TNT T7/T5-coupled reticulocyte lysate system (Promega). Precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis. Gels were fluorographed with ECL (DuPont NEN) and visualized by autoradiography.

RESULTS

Yeast Two-hybrid Screening—To identify proteins that associate with the cytoplasmic domain of CD30, we used the yeast two-hybrid system. The DNA sequence encoding the 188-amino acid cytoplasmic domain of CD30 was cloned into the pAS1 expression vector (34) fusing this domain to the DNA-binding domain of GAL4. This vector was used to screen a cDNA library prepared from Epstein-Barr virus-transformed B cells cloned into the GAL4 transcriptional activation domain vector pACT (34). Approximately 700,000 transformants were screened yielding 25 positive clones as defined by growth on medium lacking histidine and containing 3-AT and β-galactosidase expression. These positive clones interacted specifically with the CD30 fusion construct but not with either the GAL4 DNA-binding domain of GAL4 or a GAL4-CD28 fusion (data not shown). Sequence analysis revealed that 13 of the 25 cDNA clones encoded members of the TRAF family of signal transducing proteins. As shown in Fig. 1, seven independent isolates of human TRAF2, four independent isolates of human TRAF1, and two independent isolates of CRAF1 were obtained.

In addition to full-length clones for each member of the TRAF family, several NH2-terminal deletions were obtained, indicating that this domain is not required for interaction with CD30. The CRAF1ΔN125, C6 (Fig. 1), which has a 125-amino acid NH2-terminal and a 6-amino acid COOH-terminal dele-

![Figure 1](http://www.jbc.org)
tion, was the only isolate with a COOH-terminal deletion. Thus, all of the TRAF2, TRAF1, and CRAF1 clones obtained in this screen contain intact TRAF domains at their COOH termi- 

nism, consistent with previously published data indicating that this domain is necessary and sufficient for association with receptor proteins (28, 36).

TRAF2, TRAF1, and CRAF1 Associate with CD30 in Vitro—

The possibility existed that the interaction between the TRAF proteins (TRAF1, TRAF2, and CRAF1) and CD30 in yeast was nonspecific. Furthermore, TRAF1 has not been previously shown to interact directly with receptor proteins. TRAF1 has been shown to interact indirectly with the TNF receptor type 2 by forming heteromers with TRAF2 (27). Thus, it was formally possible that a TRAF-like protein in yeast might be facilitating the interaction between TRAF1 and CD30. To confirm the specificity of the interaction between CD30 and TRAF1, TRAF2, and CRAF1, the CD30 cyto was fused to glutathione S-transferase. Affinity purified GST-CD30 cyto was tested for its ability to precipitate 35S-labeled full-length TRAF2, TRAF1, and CRAF1. As shown in Fig. 2, each of these proteins could be precipitated with GST-CD30 cyto. In contrast, neither GST alone nor a GST fusion protein contain-

ing the cytoplasmic domain of the mouse CD28 protein copre-

cipitated detectable amounts of TRAF2, TRAF1, or CRAF1, indicating that the interactions between CD30 and the TRAF proteins observed in yeast are specific. In addition, the ability of GST-CD30 cyto to coprecipitate TRAF1 in vitro argues that these proteins associate directly.

TRAF Proteins Associate with the Carboxyl-terminal Region of CD30—In order to map the domain(s) of CD30 required for interaction with TRAF1, TRAF2, and CRAF1, COOH-terminal deletion mutants of the CD30 cytoplasmic domain were con- 

structed (Fig. 3A). These mutants were then tested for their ability to interact with the TRAF proteins in yeast (Fig. 3B). In these and subsequent experiments shown in this paper, TRAF1, TRAF2, and CRAF1 clones containing the largest NH2-terminal truncations were used in order to examine the mini- 

mal CD30 interaction domain. However, in those cases tested, identical results were obtained with full-length TRAF clones (data not shown). The yeast were cotransformed with plasmids expressing the various CD30 COOH-terminal deletion mutants and the TRAF expression vectors and then assayed for their ability to grow on medium lacking histidine and containing 3-AT and for β-galactosidase activity. The mutants Δ9 and Δ19, which lack the COOH-terminal 9 and 19 amino acids, respec-


tively, associated with TRAF2, TRAF1, and CRAF1. Interest-

ingly, yeast coexpressing the Δ19 mutant and TRAF1 repro-

ducibly did not grow as well or express as much β-galactosidase as wild type or the Δ9 mutation (Figs. 3B and 4C and data not shown), suggesting that although it can interact with TRAF1, the Δ19 mutant has a lower affinity for TRAF1. Mutants Δ36, Δ71, and Δ124 did not interact with either TRAF2, TRAF1, or CRAF1. These data indicate that the domain of CD30 required for interaction with these proteins lies within the COOH-terminal 36 amino acids.

Deletion mutants lacking the NH2-terminal 96, 117, and 146 amino acids (ΔN96, ΔN117, and ΔN146, respectively) of the CD30 cytoplasmic domain were also constructed and tested in the yeast two-hybrid assay for the ability to interact with the TRAF proteins (Fig. 3C). Yeast were cotransformed with the vectors expressing the individual NH2-terminal mutations and vectors expressing the TRAF proteins and then assayed for growth on medium lacking histidine and containing 3-AT and for β-galactosidase activity. The empty pACT vector, which expresses only the GAL4 transcriptional activation domain, was also tested to rule out the possibility that the CD30 NH2-

terminal mutations could activate transcription in the absence of an interaction with the TRAF proteins. As shown in Fig. 3C, all of the NH2-terminal mutants, including ΔN146, which encodes only the COOH-terminal 42 amino acids of CD30, were capable of interacting with TRAF1, TRAF2, and CRAF1 but not the GAL4 activation domain (pACT) alone. This correlates with the data obtained from the COOH-terminal deletion analysis and indicates that the COOH-terminal 42 amino acids of CD30 are necessary and sufficient for binding to TRAF1, TRAF2, and CRAF1.

The Carboxyl-terminal Region of CD30 Contains a Single CRAF1-binding Site and Two Independent Binding Sites for TRAF1 and TRAF2—Computer alignments of the cytoplasmic domain of CD30 with the cytoplasmic domains of other TNFR family members revealed little overall homology. The cytoplas-

mic domain of CD40 was most similar to the COOH-terminal region of CD30, which contains the putative binding domain for the TRAF proteins (Fig. 4A). The CD40 cytoplasmic domain has been shown to associate with both TRAF2 and CRAF1 (37). There are two regions of particularly high similarity between CD40 and CD30. The first is a 5-amino acid stretch with the sequence PEQET, which is almost identical to the sequence PVQET in CD40. The threonine residue (Thr326) in this se-

quence is known to be required for several functions of CD40 (38, 39), including binding of CRAF1 (30). The PEQET sequence was deleted in the Δ36 mutant (Fig. 4A), which failed to
bind TRAF2, TRAF1, and CRAF1. The second region of homology is a 6-amino acid stretch with the sequence EEEDKE. This sequence is contained in the Δ9 mutant but is deleted in the Δ19 mutant (Fig. 4, A and B). As noted above, both the Δ9 and Δ19 constructs were capable of interacting with all of the TRAF proteins in a yeast two-hybrid assay (Fig. 3B). This suggested that the 10-amino acid sequence between the Δ9 and Δ19 mutations, which contains the EEEDKE sequence, is not required for binding and that the PEQET sequence is most likely the domain involved in binding to the TRAF proteins.

To test this possibility, an internal deletion mutant (ΔPEQET) lacking the amino acid sequence PEQET in the context of the wild type CD30 cytoplasmic domain was constructed and then analyzed in a yeast two-hybrid assay for its ability to interact with the TRAF proteins (Fig. 4C). The ΔPEQET mutant failed to bind CRAF1, indicating that this sequence is necessary for CRAF1 binding. Individual alanine substitutions for each of the residues conserved in CD40 (i.e., Pro, Gln, Glu, and Thr) also abolished the ability of CD30 to bind to CRAF1 in the yeast two-hybrid assay (not shown).

Fig. 3. Effects of carboxy-terminal and amino-terminal deletions on the ability of the CD30 cytoplasmic domain to associate with TRAF proteins. A, schematics depicting the structures of wild type (wt) and the various COOH-terminal CD30 deletions. The deletion mutants were constructed as described under "Materials and Methods." B, β-galactosidase filter assay from the yeast two-hybrid assay using the COOH-terminal deletion mutants. The yeast were cotransformed with plasmids expressing the GAL4 DNA-binding domain fused to wild type (wt) or deleted CD30 cytoplasmic domain and plasmids expressing the GAL4 activation domain fused to TRAF2, TRAF1, or CRAF1 as described under "Materials and Methods." These results are representative of at least three independent experiments. C, schematics depicting the structures of the NH2-terminal deletions of the CD30 cytoplasmic domain (left) and the results of the yeast two-hybrid assays with these constructs (right). These results are representative of at least three independent experiments. WT, wild type.
sites for TRAF1 and TRAF2 in the COOH-terminal 36 amino acids of CD30 and that each site alone was capable of binding either TRAF1 or TRAF2 independent of the other. We tested the second possibility by combining the ΔPEQET mutation with the Δ19 and Δ9 mutants were described in Fig. 3A. The ΔPEQET mutation deletes the homology to the site in CD40 that contains the threonine residue (Thr254) previously shown to be required for binding to CRAF1 (30). Construction of ΔPEQET, ΔPEQET/Δ19, and ΔPEQET/Δ9 is described under “Materials and Methods.” C, β-galactosidase filter assay from the yeast two-hybrid assay. Identical results for β-galactosidase activity were obtained using plates lacking leucine and tryptophan. The results shown are representative of three independent experiments. WT, wild type.

As shown in Fig. 4C, neither the ΔPEQET nor the Δ19 mutations alone abolished TRAF1 or TRAF2 binding. However, the ΔPEQET/Δ19 mutant, which lacks both regions of homology to CD40, did not bind either TRAF1 or TRAF2. In contrast, the ΔPEQET/Δ9 mutation had no effect on either TRAF1 or TRAF2 binding. These data indicate that the region between the Δ9 and Δ19 mutations, which contains the EEEGKE sequence, contains a second binding site for TRAF1 and TRAF2 that can function independently from the PEQET sequence. As seen in Figs. 3B and 4C, TRAF1 does not interact with the Δ19 mutant as well as wild type or Δ9, as judged by the β-galactosidase activities. This suggests that although TRAF1 is capable of interacting with either the PEQET or the EEEGKE sites in yeast, it prefers the EEEGKE site. These results are summarized in Fig. 5.

**DISCUSSION**

The TNF receptor (TNFR) type 2 and CD40 are known to regulate cellular proliferation (40-42) and induce the NF-κB transcription factor (27, 39, 43, 44), and these abilities appear to correlate at least in part with their ability to bind TRAF
proteins (27, 37). The domain of TNFR type 2 required for induction of NF-κB is also required for its ability to coprecipitate TRAF1 and TRAF2 (27). The role of TRAF2 in TNFR type 2 signal transduction is further supported by the observation that the overexpression of TRAF2 can induce NF-κB (37). Threonine 254 in CD40 has been shown to be essential for signaling (38), and a threonine to alanine substitution in this position was found to abolish CRAF binding (26). In addition, the cell survival function of CD40 (45) was found to correlate with its ability to induce Bcl-xL expression (46), and a COOH-terminal deletion of CD40, which removed the region containing threonine 254, abolished the induction of Bcl-xL, suggesting that CRAF binding is required for this effect (47). Thus, the TRAF proteins appear to play a role in regulating cell proliferation or survival. CD30 has also been shown to regulate cellular proliferation and to induce NF-κB (5, 11, 22). Like CD40, CD30 can directly associate with TRAF2 and CRAF1. Unlike TNFR type 2 and CD40, CD30 was also found to directly associate with TRAF1. Previously, TRAF1 was only found to associate indirectly with receptor proteins by forming heteromers with TRAF2 (27). We also found that CD30 contained two independent binding sites for the TRAF proteins and that these proteins had different specificities for each site. CRAF1 binding was the most restricted because it recognized only the domain containing the sequence EEGKE. TRAF1 was capable of binding to the cytoplasmic tail of CD30 through either the EEGKE domain or through the downstream domain, which contains the sequence EEGKE. However, TRAF1 formed a stronger interaction with the EEGKE-containing domain as measured by yeast two-hybrid analysis. TRAF2 was the most promiscuous of the three proteins, binding well to either site. The ability of CD30 to bind directly to TRAF1 suggests a mechanism by which CD30 can mediate novel signaling effects. Furthermore, these data indicate that the TRAF protein binding pattern of CD30 differs from that of other members of the TNF receptor family. This suggests that the signaling specificity of these receptors may be determined by their ability to bind different combinations of TRAF proteins.

The TRAF proteins are most related in the COOH-terminal TRAF domain, which has been further subdivided into a TRAF-N domain and TRAF-C domain (28, 36). The TRAF domain is required for the ability of TRAF proteins to form multimers and, in the case of TRAF1 and TRAF2, heteromers (27, 28, 37). In addition, experiments using CRAF1 and TRAF2 have demonstrated that the TRAF domain encodes the receptor binding function (28, 37). Our data confirm that the ability of TRAF1 to bind to the CD30 receptor is also confined to the carboxy-terminal TRAF domain as demonstrated by the smallest TRAF1 clone isolated in the yeast two-hybrid screen. Overexpression of a truncated CRAF1 protein containing the CD40-binding domain has been shown to block the ability of CD40 to induce CD23 in transfected cells (28). Deletion of the NH2-terminal 87 amino acids of TRAF2 abolishes the ability of TRAF2 type 2 and CD40 to induce NF-κB in cotransfection assays without affecting its ability to bind TNFR type 2 or CD40 (37). Thus, the NH2 termini of TRAF proteins appear to be involved in signal transduction. The NH2 terminus of TRAF1 is the most divergent in the TRAF family, suggesting that TRAF1 may have signaling properties that are different than TRAF2 or CRAF1. Furthermore, TRAF1 has a more restricted tissue distribution relative to TRAF2 or CRAF1. Like CD30, TRAF1 appears to be expressed primarily in lymphoid tissues (27, 32).

The data presented here and elsewhere (27–31, 37) indicate that members of the TNF receptor family have distinct TRAF binding properties. We have found that CD30 can associate directly with TRAF1, TRAF2, and CRAF1. In contrast, TNFR type 2 has been shown to associate directly only with TRAF2 (27), whereas CD40 has been shown to associate with both CRAF1 and TRAF2 (37). The differential abilities of the TNF receptor family to bind to TRAF proteins is reminiscent of cytokine receptor signaling through the Jak/STAT signal transduction pathway (48). The ability of cytokine receptors to bind different combinations of Jaks and STATs allows these receptors to send specific signals. Thus, by binding different combinations of TRAF proteins, TNF receptor proteins may be able to achieve signaling specificity while using an overlapping signal transduction pathway. The analogy to Jak/STAT pathway is further supported by the identification of a fourth member of the TRAF family, CART1 (49). CART1 was identified by its differential expression in breast carcinoma cells and was found to localize to the nucleus of these cells, suggesting that TRAFs may be latent cytoplasmic transcription factors (49). TRAF2 and TRAF1 were recently shown to bind to c-IAP1 and c-IAP2 (36), which belong to the recently identified family of cellular homologues of the baculovirus IAP protein (36, 50–52). It is possible that the cellular IAP proteins may be transcription factors as well. In addition to the differing abilities to bind TRAF proteins, our observation that CD30 contains two non-identical binding sites for TRAF proteins adds further complexity to the possible CD30-TRAF protein signaling complexes that assemble in cells. Because CD30, like other TNF-related receptors, is likely to trimerize in response to ligand binding and each receptor contains two binding sites for TRAF proteins, the composition of CD30 signaling complexes could be quite divergent in different cell types or under different conditions of stimulation.

It will be important to determine the roles played by the individual TRAF proteins during CD30 signal transduction. In addition, there may be other factors involved in CD30 signal transduction. The TRAF-binding domain was localized to the COOH-terminal 36 amino acids of the CD30 cytoplasmic domain, which is 188 amino acids long (16). Therefore, it is possible that the remaining 153 amino acids of the CD30 cytoplasmic domain have been retained during evolution because they also contribute to the assembly of a CD30 signaling complex. To address these issues, the signaling properties of a CD30 receptor containing the minimal TRAF-binding domain will need to be compared with the wild type receptor tail.

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