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Induction of Nuclear Factor κB by the CD30 Receptor Is Mediated by TRAF1 and TRAF2

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CD30 is a lymphoid cell-specific surface receptor which was originally identified as an antigen expressed on Hodgkin's lymphoma cells. Activation of CD30 induces the nuclear factor κB (NF-κB) transcription factor. In this study, we define the domains in CD30 which are required for NF-κB activation. Two separate elements of the cytoplasmic domain which were capable of inducing NF-κB independently of one another were identified. The first domain (domain 1) mapped to a ~120-amino-acid sequence in the membrane-proximal region of the CD30 cytoplasmic tail, between residues 410 and 531. A second, more carboxy-terminal region (domain 2) was identified between residues 553 and 595. Domain 2 contains two 5- to 10-amino-acid elements which can mediate the binding of CD30 to members of the tumor necrosis factor receptor-associated factor (TRAF) family of signal transducing proteins. Coexpression of CD30 with TRAF1 or TRAF2 but not TRAF3 augmented NF-κB activation through domain 2 but not domain 1. NF-κB induction through domain 2 was inhibited by coexpression of either full-length TRAF3 or dominant negative forms of TRAF1 or TRAF2. In contrast, NF-κB induction by domain 1 was not affected by alterations in TRAF protein levels. Together, these data support a model in which CD30 can induce NF-κB by both TRAF-dependent and -independent mechanisms. TRAF-dependent induction of NF-κB appears to be regulated by the relative levels of individual TRAF proteins in the cell.

The tumor necrosis factor (TNF) receptor (TNFR) superfamily (2, 4, 38) is comprised of a number of cell surface receptors which includes the TNFR types 1 and 2 (TNFR1 and TNFR2), the low-affinity nerve growth factor receptor, CD30, CD40, Fas (Apo-I/CD95), and lymphotoxin-α. Mutations and/or aberrant expression of these receptors and their ligands have been found in a diverse number of malignancies and immunological deficiencies (18).

CD30 was first identified as an immunological marker expressed in Hodgkin's lymphoma cells (15, 37, 41). Both membrane-bound and soluble forms of CD30 have been identified, and levels of soluble CD30 have recently been shown to correlate with disease progression in anaplastic large-cell lymphoma (29). CD30 is normally expressed on activated and memory T cells (14) and has also been detected in virus-transformed T- and B-cell lines. Targeted disruption of the murine CD30 gene (6) has implicated a role for this receptor in thymic negative selection and cell death (1). Activation of CD30, either by direct interaction with its physiologic ligand or by cross-linking with CD30-specific antibodies, has been reported to augment T-cell receptor-mediated proliferation (39). Conversely, activation of CD30 has been shown to induce apoptosis in T-cell hybridoma lines (23). Stimulation of CD30 has recently been shown to induce the nuclear translocation of the p50 and RelA members of the nuclear factor κB (NF-κB) transcription factor family (3, 5) and, through this mechanism, to activate human immunodeficiency virus (HIV) gene expression (25).

The signal transduction pathways utilized by CD30 are not well understood. Sequence comparison of the cytoplasmic tail of CD30 does not reveal obvious homology to the cytoplasmic domains of other members of the TNFR superfamily, such as the death domains identified in Fas and TNFR1. Recently, however, three members of the TNFR-associated factor (TRAF) family of signal-transducing molecules, TRAF1, (27, 34), TRAF2, (34, 40), and TRAF3 (8, 20, 27, 36), have been shown to bind to the cytoplasmic tail of CD30 (16, 23).

In this report, we identify the elements in the cytoplasmic tail of CD30 which are required for the induction of NF-κB. Two major domains were found to transduce an activation signal. Domain 1 is a ~120-residue amino-terminal region which weakly induces NF-κB. Domain 2 maps to the carboxy-terminal ~42 amino acids (residues 554 to 595) and correlates with two sites which can bind TRAF proteins. The effects of domain 2 on NF-κB induction were further examined by coexpression of both wild-type and dominant mutant TRAFs. These findings suggest a model in which both TRAF1 and TRAF2 but not TRAF3 can mediate CD30-induced NF-κB activation.

MATERIALS AND METHODS

Cell culture and biological reagents. Human embryonic kidney 293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml and grown at 37°C under 5% CO_2. Cells were transfected by the calcium phosphate procedure as previously described (31). Human recombinant TNF-α was purchased from Boehringer Mannheim.

Plasmids. CD28/CD30 chimeras were constructed by PCR amplification of the cytoplasmic tail of human CD30 (13) from the previously described yeast two-hybrid CD30 bait constructs (16). The ΔPEQET, Δ9, Δ19, and Δ36 mutants have been described previously (16) and are referred to in this report by their residue numbers. Additional deletion constructs were generated by PCR with primers complementary to CD30 sequences but incorporating stop codons as indicated. All PCRs were performed with *Pfu* DNA polymerase (Stratagene). PCR products were cloned into the *Xho*I site at residue 180 of a modified murine CD28 clone (17) in the eukaryotic expression vector pcDNA3 (Invitrogen). CD28Δtail was generated by digestion of the wild-type CD28 plasmid with *Xho*I and *Xba*I,

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filling in with Klenow DNA polymerase, and religation. All PCR-derived products were verified by DNA sequence analysis.

The isolation of full-length cDNAs encoding human TRAF1, TRAF2, and TRAF3 has been described previously (16). Expression vectors were constructed by cloning *Xho*I fragments containing the entire coding sequence for each TRAF protein into the *Xho*I site of pcDNA3. Truncated versions of TRAF1, TRAF2, and TRAF3 were generated by PCR with primers encoding initiation methionines and incorporating consensus Kozak sequences (22) at the appropriate position to generate amino-terminal truncations, as described below. PCR products were subcloned into pcDNA3 and verified by DNA sequencing and in vitro translation.

The β-actin–β-galactosidase plasmid (26) used for transfection efficiency standardization was a kind gift of J. Yuan, Massachusetts General Hospital. The κB-responsive luciferase plasmid and parental control plasmid (21) were kind gifts of H. Singh, The University of Chicago.

Luciferase assays. For luciferase assays, six-well plates were seeded with 5×10^5 cells per well in 2 ml of medium and transfected with 50 ng each of an internal β-galactosidase transfection efficiency control plasmid and either a κB-responsive luciferase reporter plasmid containing two canonical κB sites (21) or a control plasmid lacking κB sites. The amount of DNA was kept constant for each transfectant by the addition of pcDNA3. Cells were harvested 24 to 36 h after transfection, essentially as described previously (7). Briefly, cells were washed once in phosphate-buffered saline and lysed in 0.5 ml of reporter lysis buffer (Promega) for 15 min at room temperature. Luciferase assays were performed on 100-μl aliquots of lysate and analyzed by luminometry. β-Galactosidase reactions were performed exactly as previously described (7) on 20 μl of cell lysate, and luciferase data were normalized to account for variations in transfection efficiency. For luciferase experiments, cells were transfected in triplicate and the data shown are representative of at least two experiments.

EMSA. Cells were transfected with 5 μg of CD28/CD30 chimeric plasmid and 5 μg of TRAF expression vectors, unless otherwise specified. The amount of DNA was kept constant for each transfectant by the addition of pcDNA3. Nuclear extracts were prepared as previously described (30), and electrophoretic mobility shift assay (EMSA) analysis was performed (11) with double-stranded oligonucleotides encompassing a single canonical κB site (GGGACTTTCC). Competition analyses were performed with unlabeled oligonucleotide containing either a canonical κB site or a single point mutation (GCGACTTTCC) previously shown to abolish κB-specific binding (28).

RESULTS

The carboxy-terminal 186 residues of CD30 can transduce signals which activate NF-κB. To identify the elements in CD30 which are required for signal transduction, we used a chimeric construct in which the extracellular and transmembrane domains of murine CD28 were fused to the cytoplasmic domain (residues 410 to 595) of human CD30. Since CD28 naturally exists as a homodimer and dimerization of other members of the TNFR family has been shown to be sufficient for the induction of NF-κB (7), we reasoned that ectopic expression of a CD28/CD30 chimera would constitutively transduce CD30-specific signals, obviating the requirement for activation by ligand or agonistic antibodies. As shown in Fig. 1A, transient transfection of the CD28-CD30 chimera in the high-transfection-efficiency human embryonic kidney line, 293, produced a ca. eightfold induction of a κB-responsive luciferase reporter plasmid. In contrast, transfections of empty vector, a control vector encoding a truncated CD28 molecule lacking its own cytoplasmic tail, or a vector encoding wild-type CD28, were all unable to induce the activation of NF-κB (Fig. 1A and data not shown). The addition of a cross-linking CD28 monoclonal antibody to transfected cells resulted in only a slight further augmentation of NF-κB induction (data not shown). Flow cytometric analysis of transfected cells revealed no significant differences in the levels of protein expressed by the different vectors (data not shown). These data indicate that the presence of the cytoplasmic tail of CD30 in the CD28 chimera was sufficient to mediate signals which resulted in the activation of NF-κB.

The CD30-induced activation of NF-κB was also observed by EMSA analysis of nuclear extracts prepared from transfected 293 cells (Fig. 1B). The mobility of this nucleoprotein complex was indistinguishable from that of the major NF-κB

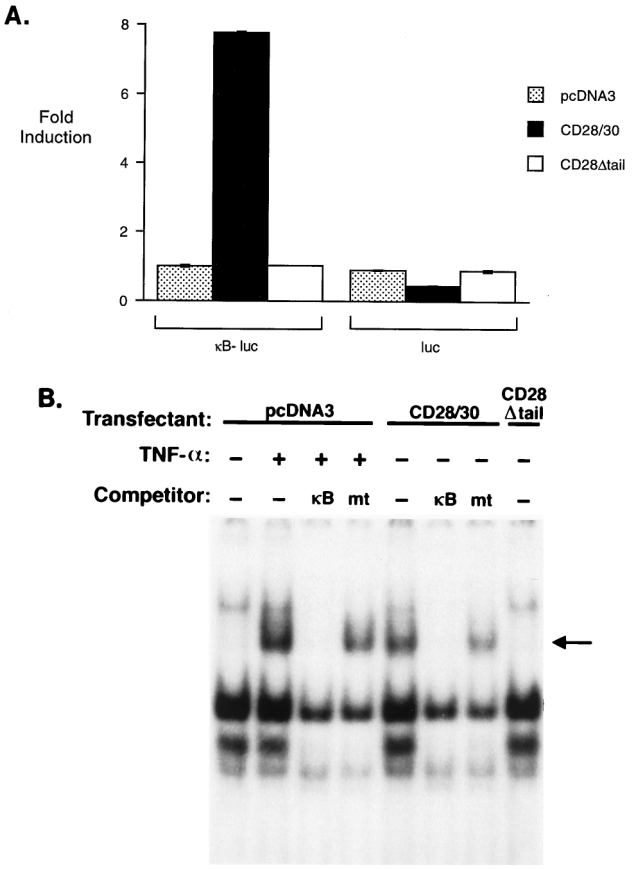

FIG. 1. Induction of NF-κB by CD30. (A) 293 cells were transfected in triplicate with the indicated plasmids together with a luciferase reporter plasmid containing two tandem repeats of the κB element (κB-luc) or the parent plasmid lacking the κB elements (luc) and analyzed for luciferase activity 36 h following transfection as described in Materials and Methods. The data shown are normalized for transfection efficiency to a β-galactosidase control plasmid driven by the β-actin promoter (26), which was included in the transfection, and are presented as fold inductions relative to transfection of pcDNA3 alone. The data are representative of three independent experiments. (B) 293 cells were transfected with 5 μg of pcDNA3, CD28/30 chimeric expression vector, or a CD28 vector lacking a cytoplasmic tail (CD28Δtail), as indicated. The cells were treated with recombinant human TNF-α (200 U/ml) where indicated 1 h prior to harvest. Nuclear extracts were prepared 48 h following transfection, and EMSA analysis was performed as described in Materials and Methods. Competition reactions were performed with 20 ng of unlabelled wild-type (κB) or mutant (mt) double-stranded oligonucleotide as described in the text. The position of the arrow indicates the mobility of the inducible, κB-specific nucleoprotein complex.

species induced in 293 cells by treatment with TNF-α, an effect which has previously been shown to be mediated by TNFR1 (33). To confirm the specificity of the CD30-induced complex, parallel EMSAs were performed in the presence of an ~50-fold excess of double-stranded oligonucleotide encompassing either the canonical κB site or a single-base-pair mutant previously shown to abolish NF-κB binding (11). The addition of wild-type competitor DNA abrogated the induced complex, while addition of the mutated form had little effect (Fig. 1B), confirming that the induced complex contained NF-κB.

Two separate domains in the cytoplasmic tail of CD30 mediate the induction of NF-κB. To define the sequences in the cytoplasmic domain of CD30 required for the activation of NF-κB, plasmids which resulted in the progressive deletion of residues in the carboxy terminus of CD30 were generated, as shown in Fig. 2A. Removal of the carboxy-terminal 19 amino acids (vector 410–576) resulted in a slight decrease in NF-κB

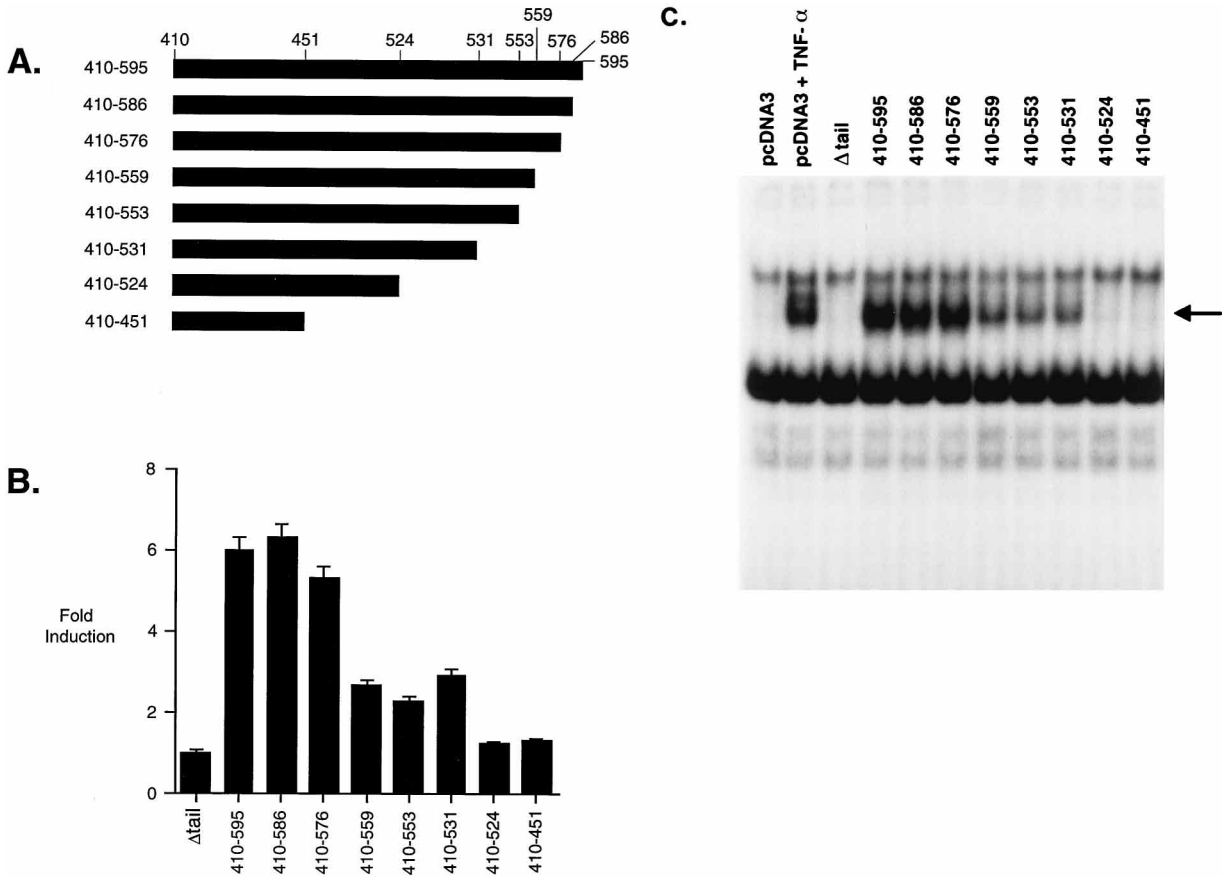

FIG. 2. Carboxy-terminal deletion analysis of the cytoplasmic tail of CD30. (A) Schematic representation of carboxy-terminal deletion constructs. (B) Luciferase analysis of lysates prepared from transfected cells. 293 cells were transfected with 50 ng each of κB luciferase plasmid, β-galactosidase plasmid, and the indicated deletion construct, and lysates were analysed for luciferase activity as described in the legend to Fig. 1. The data are representative of two independent experiments. (C) EMSA analysis of nuclear extracts prepared from transfected cells. A 5-μg sample of each of the indicated plasmids was transfected into 293 cells, and the cells were harvested 48 h later for EMSA analysis as described in the legend to Fig. 1. The position of the arrow indicates the mobility of the inducible, κB-specific nucleoprotein complex.

activity, as judged by luciferase reporter gene analysis (Fig. 2B). This activity was reduced significantly with a vector lacking the carboxy-terminal 36 residues (410–559), but a residual level of NF-κB activation remained in transfectants of subsequent deletions until the elimination of the carboxy-terminal 71 acids (vector 410–524). This effect was also observed qualitatively by EMSA analysis (Fig. 2C), suggesting that residues between 560 and 595, as well as those between 410 and 531, play a role in CD30 signaling.

To further delineate the elements in CD30 required for signaling, progressive deletions were generated at the amino terminus of the cytoplasmic tail (Fig. 3A). Deletion of residues 410 to 553 still resulted in strong induction of κB-dependent luciferase expression, revealing that the carboxy-terminal 42 amino acids alone (residues 554 to 595) were capable of independently inducing an NF-κB signal (Fig. 3B). As demonstrated in Fig. 2, sequences between 410 and 531 can also lead to the induction of NF-κB. However, residues 461 to 531 (Fig. 3B) or progressive deletions into this sequence (data not shown) were unable to activate NF-κB. Taken together, these studies demonstrate that at least two separable elements in the cytoplasmic tail of CD30, one between residues 410 and 531, which we have designated domain 1, and the second between 554 and 595, which we have named domain 2, are independently capable of transducing signals which lead to the induction of NF-κB (Fig. 4A).

Activation of NF-κB by CD30 is mediated by the TRAF family of signal transduction proteins. We have previously reported the isolation of three members of the TRAF family as factors which bind directly to sequences within domain 2 (16). Although activation of NF-κB by two other members of the TNFR superfamily, TNFR2 and CD40, is mediated by TRAF2 (7, 33), the TRAF binding properties of CD30 differ significantly from those of TNFR2 or CD40. We have previously identified two elements in domain 2, designated elements 2A and 2B, which can bind TRAFs (Fig. 4A; also see Fig. 7). Element 2A (amino acids 561 to 565, residues PEQET) binds TRAF1, TRAF2, and TRAF3, while element 2B (amino acids 577 to 586, residues LSVEEEGKED), binds TRAF1 and TRAF2 but not TRAF3 (16). To determine which of these elements were involved in mediating NF-κB activation, the properties of CD28/30 chimeras which lacked element 2A, 2B, or both were compared to those of chimeras containing the wild-type CD30 cytoplasmic tail. As shown in Fig. 4B, removal of either element 2A or 2B resulted in only a slight decrease in NF-κB induction, while removal of both elements reduced the level of NF-κB activation to that observed with domain 1 alone. Similar results were obtained by EMSA analysis (data not shown). This suggests that either 2A or 2B is sufficient to mediate NF-κB activation.

To examine the role of the TRAF proteins in CD30 signaling, 293 cells were cotransfected with CD28/CD30 chimeras

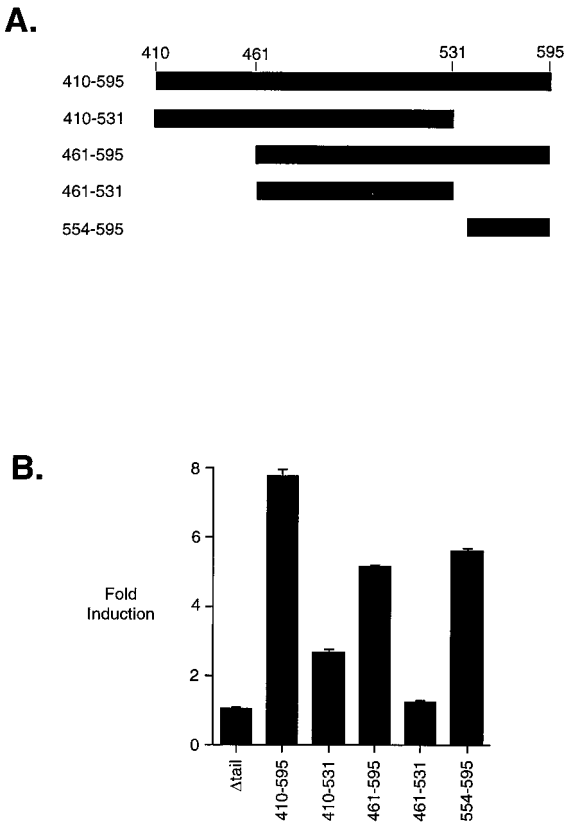

FIG. 3. Amino-terminal and internal deletion analysis of the cytoplasmic tail of CD30. (A) Schematic representation of deletion constructs. (B) Luciferase analysis of transfected constructs. 293 cells were transfected with 50 ng each of κB luciferase plasmid, β-galactosidase plasmid, and the indicated deletion constructs and analyzed for luciferase activity as described in Materials and Methods. The data are representative of two independent experiments.

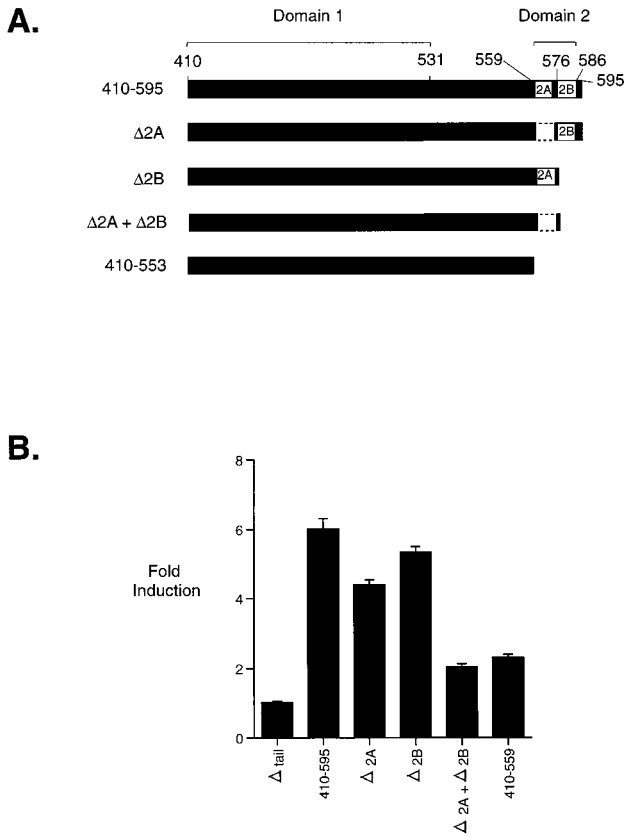

FIG. 4. Deletion analysis of elements 2A and 2B. (A) Schematic representation of deletion constructs. (B) Luciferase analysis of transfected constructs. 293 cells were transfected with 50 ng each of κB luciferase plasmid, β-galactosidase plasmid, and CD28 plasmids lacking a cytoplasmic tail (Δtail), containing the entire CD30 cytoplasmic tail (410–595), lacking the PEQET sequence (Δ2A), lacking the carboxy-terminal 19 residues (Δ2B), lacking both the PEQET and the carboxy-terminal 19 residues (Δ2A + Δ2B), or lacking the carboxy-terminal 36 residues (410–559). Lysates were prepared and analyzed for luciferase activity 36 h after transfection as described in Materials and Methods and in the legend to Fig. 1. The data are representative of two independent experiments.

along with expression vectors encoding TRAF1, TRAF2, or TRAF3. Coexpression of TRAF1 or TRAF2 augmented CD30-dependent activation of NF-κB (Fig. 5A), while TRAF3 was found to inhibit. Expression of TRAF1 or TRAF2 in the absence of the CD30 cytoplasmic tail did not result in activation of NF-κB under these conditions (Fig. 5).

To further define the roles of the TRAF proteins in signaling events mediated by CD30, amino-terminally truncated versions of TRAF1, TRAF2, and TRAF3 were used. Previous studies have shown that ectopic expression of an amino-terminally truncated form of TRAF2, which lacks the ring finger domain, exerts a dominant negative effect on TRAF2-dependent NF-κB activation (33). To determine whether dominant TRAF proteins could inhibit CD30 signaling, we constructed a similar version of TRAF2 to that previously described (33), as well as a truncated form of TRAF3 which also lacks the amino-terminal ring finger. Although TRAF1 does not contain a ring finger domain, an amino-terminally truncated version of TRAF1 was constructed which consisted of only the TRAF domain. As shown in Fig. 5A, coexpression of either truncated TRAF1 or TRAF2 with CD30 was able to significantly inhibit NF-κB. Coexpression of truncated TRAF3 inhibited NF-κB induction of CD30 to the same extent as did wild-type TRAF3.

To examine the domains in CD30 which are responsive to TRAF1, TRAF2, and TRAF3, coexpression experiments were performed with either domain 1- or domain 2-containing chimeras. Cotransfection of the TRAF vectors with the CD28/CD30 chimeric construct containing only domain 2 essentially recapitulated the results obtained with the full-length CD30 tail (Fig. 5C). In contrast, expression of wild-type or truncated TRAF constructs had no significant effect on the NF-κB signal generated by domain 1 (Fig. 5B). These findings suggest that domain 2 induces NF-κB through the TRAF pathway whereas domain 1 induces NF-κB by a mechanism which is not directly dependent on TRAF1, TRAF2, or TRAF3.

Since the ability of TRAF1 to augment NF-κB has not been previously reported, we investigated this effect further (Fig. 6). Full-length TRAF1 reproducibly enhanced NF-κB-dependent transcription initiated by transfection of the CD28/CD30 chimera. We have previously shown that the carboxy terminus of TRAF1, which contains the TRAF domain, is necessary and sufficient to mediate binding to CD30 (16). Therefore, the effects of this carboxy-terminal domain of TRAF1, TRAF1 (184–417), on NF-κB induction, were compared to those of the amino terminus of TRAF1 (residues 1 to 183). As shown in Fig. 6, transfection of TRAF1 (1–183) had no significant effect on either basal or CD30-induced NF-κB levels, while TRAF1 (184–417), which contains the TRAF domain, reproducibly inhibited CD30-mediated signaling.

The experiments described above provide strong evidence

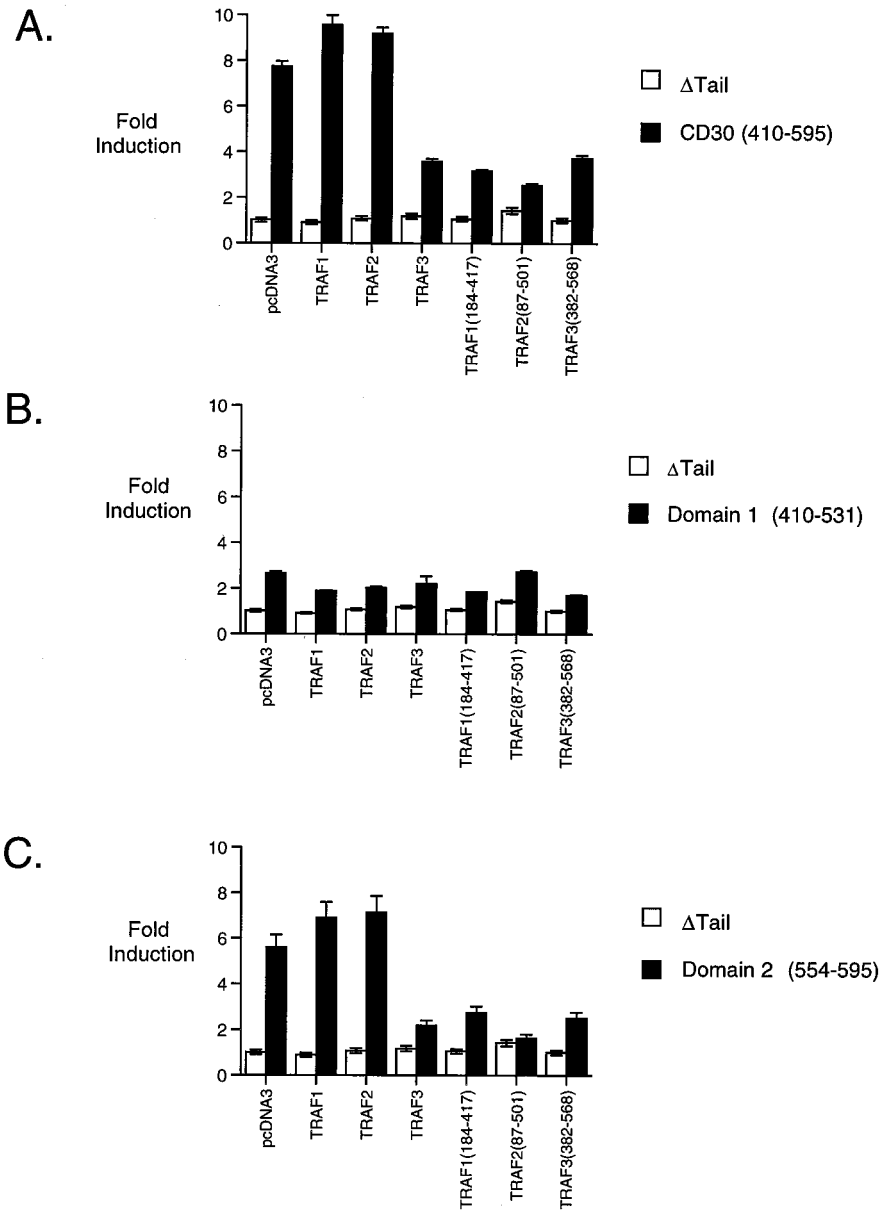

FIG. 5. Involvement of TRAFs in NF-κB induction mediated by different domains of CD30. The indicated TRAF expression vectors (100 ng) or a pcDNA3 control were cotransfected into 293 cells with 100 ng of either the CD28/CD30 chimera or a CD28 vector lacking a cytoplasmic tail (CD28Δtail) as indicated, together with 50 ng each of κB luciferase reporter plasmid and the β-galactosidase transfection control plasmid. The cells were harvested 36 h following transfection and analyzed by the luciferase assay as described in Materials and Methods. The data are representative of two independent experiments.

for the involvement of both TRAF1 and TRAF2 in CD30 signal transduction. To further examine the effects of these proteins on NF-κB induction, EMSA analysis was performed on nuclear extracts prepared from 293 cells which had been transfected with different combinations of TRAF1 and TRAF2, with and without CD28/CD30 chimeric constructs. As shown in Fig. 7, overexpression of TRAF1 in the absence of CD30 did not induce NF-κB, a finding which is consistent with the data shown in Fig. 5. Similarly, in the absence of CD30, overexpression of TRAF2 did not result in NF-κB induction under these conditions (Fig. 5 and data not shown). However, TRAF1 was found to augment NF-κB induced both by full-length CD30 (amino acids 410 to 595) and by domain 2 (amino acids 554 to 595). Moreover, induction of NF-κB by domain 2 was augmented by coexpression of TRAF2, and transfection of both TRAF1 and TRAF2 along with the domain 2 vector resulted in a more potent activation, suggesting that TRAF1 and TRAF2 can cooperate in NF-κB induction. In contrast, full-length TRAF3 suppressed NF-κB induction through the CD30 cytoplasmic tail.

DISCUSSION

Like many other members of the TNF receptor superfamily, CD30 has been implicated in lymphocyte regulation (2, 15). Activation of CD30 has been reported to induce a variety of effects, which range from proliferation to apoptosis, depending on cell type (14). The recent finding that CD30 null mice have

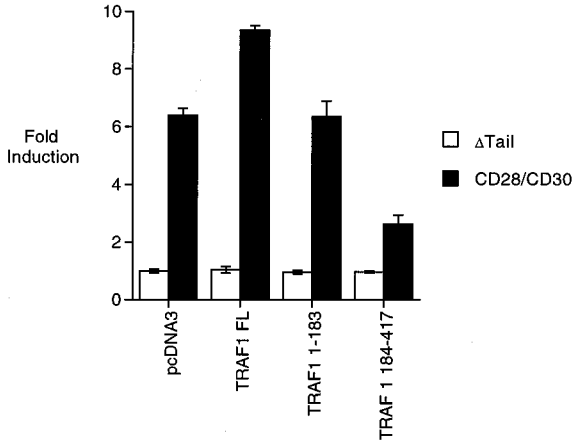

FIG. 6. Deletion analysis of TRAF1. Expression vectors (100 ng) encoding the indicated residues of TRAF1 or a pcDNA3 control were cotransfected into 293 cells with 100 ng of either the CD28/CD30 chimera or a CD28 vector lacking a cytoplasmic tail (CD28Δtail) as indicated, together with 50 ng each of κB luciferase reporter plasmid and β-galactosidase transfection control plasmid. The cells were harvested 36 h following transfection and analyzed by the luciferase assay as described in Materials and Methods. The data are representative of three independent experiments.

a deficiency in negative selection of thymocytes (1) implies that CD30 can play an inhibitory role in cell survival. In contrast, the high levels of CD30 expressed in Hodgkin's lymphoma cells (41) suggest that CD30 may function to enhance cell survival or proliferation. CD30 surface expression is induced late in lymphocyte activation, suggesting that it does not play a role in the primary response to antigenic signals but, rather, that it performs a modulatory function at a later stage. The recent findings that activation of CD30 can induce the expression of latent HIV (25) through NF-κB (5) suggest that it may play a

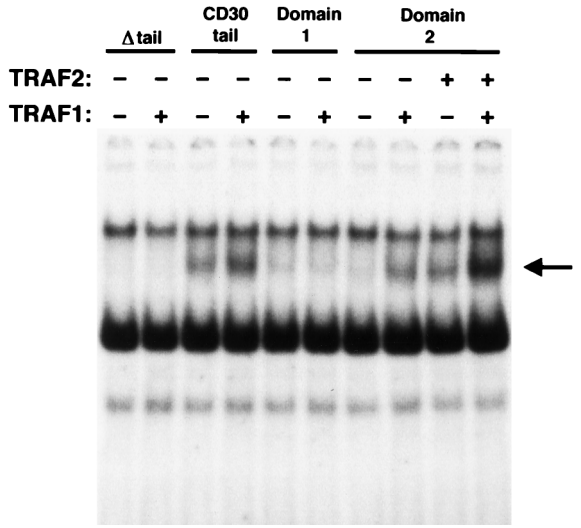

FIG. 7. Induction of NF-κB by CD30 and TRAF1. Samples (5 μg) of the indicated expression vectors or a pcDNA3 control were cotransfected with 5 μg of CD28 expression vectors lacking a cytoplasmic domain (Δtail) or containing full-length CD30 or domain 1 or domain 2 of CD30 as indicated. Nuclear extracts were prepared 48 h after transfection and analyzed by EMSA as described in Materials and Methods and in the legend to Fig. 1. Transfection of TRAF2 with CD28Δtail did not induce NF-κB under these conditions (data not shown). The position of the arrow indicates the mobility of the inducible, κB-specific nucleoprotein complex.

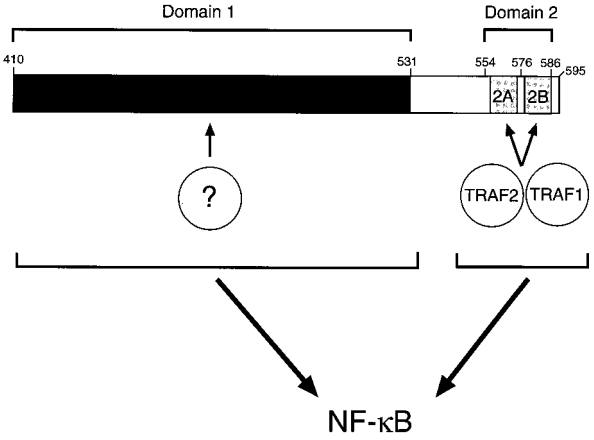

FIG. 8. Schematic representation and nomenclature of the signaling domains identified in the cytoplasmic tail of CD30. See the text for details.

role in the progression from clinical latency to AIDS in HIV-infected patients.

The studies described here reveal that the carboxy-terminal 186 residues of CD30 can induce at least two signal transduction pathways which can independently lead to the activation of NF-κB (Fig. 8). Domain 1 is contained within a ~120-amino-acid sequence proximal to the cytoplasmic membrane, while domain 2 is encompassed by a 42-amino-acid sequence at the carboxy terminus of the protein. Further deletions into domain 1 abrogated its ability to induce NF-κB, suggesting that the entire sequence is required for signaling. While this domain is larger than expected for a TRAF-binding domain (8, 20), it is not dissimilar to the size of the death domains located in the cytoplasmic tails of two other members of the TNFR superfamily, TNFR1 and Fas (9, 42). In addition to inducing apoptosis, the death domain of TNFR1 can effect NF-κB activation through its interaction with TRADD (19). Inspection of domain 1 of CD30 (12) has revealed a limited homology to the consensus death domain sequence (9), and so it is possible that domain 1 represents a death domain capable of exerting its effects through TRADD or TRADD-like factors.

Domain 2 is contained within the carboxy-terminal ~42 amino acids of CD30 and can be further subdivided into two smaller domains, which we have designated elements 2A and 2B. Both of these elements can bind TRAF1 and TRAF2, while only element 2A can bind TRAF3 (16). Both elements appear to play a role in NF-κB signaling (Fig. 4B).

The identification of two separable signaling domains in CD30 may provide an explanation for the disparate effects of CD30 which have been observed. Activation of CD30 in different cell types could lead to diverse signaling events, depending on the availability of downstream signaling intermediates. Thus, stimulation through CD30 could result in proliferative signals, possibly through TRAF2-induced NF-κB in a manner which has been described for TNFR2 (34, 43), but could alternatively induce apoptosis, as described for TNFR1 (42). The decision of which pathway the cell takes may therefore be determined by the endogenous levels of TRAF/TRADD factors in the cell. Moreover, the recent report that TRAF2 and TRADD can interact directly (19) leads to the possibility of signaling cross talk between CD30 and other members of the TNFR superfamily such as TNFR1, thus increasing the potential diversity of effects.

The experiments presented in this report demonstrate that activation of NF-κB through domain 2 can be mediated by the

TRAF family of proteins. Of these, TRAF1, TRAF2, and TRAF3 have all been shown to interact directly with domain 2 (16, 23) and TRAF2 has previously been shown to mediate CD40- and TNFR2-induced NF-κB activation (7, 33), but TRAF1 has not. Here we show that TRAF1 can lead to the induction of NF-κB through domain 2 (Fig. 5), and that TRAF1 can function cooperatively with TRAF2 to produce a maximal induction (Fig. 7).

Expression of transfected TRAF2 in the absence of CD30 did not result in a significant induction of NF-κB at the levels used in these experiments, (Fig. 5 and 7). Therefore, the presence of TRAF2 in lysates of transfected cells was confirmed by Western blot analysis (12). This finding is consistent with the studies described for CD40 by Cheng and Baltimore (7). Transfection of large amounts of TRAF2 expression vector can result in the induction of NF-κB (12), but this could be due to indirect effects such as cross-linking of endogenous receptors.

Previous experiments have shown that a version of TRAF2 lacking the amino-terminal ring finger can function as a dominant inhibitor of NF-κB induction, and this has led to the suggestion that the ring finger plays an important role in NF-κB signaling. Since TRAF1 lacks a ring finger, it might not be expected to participate in the NF-κB signaling pathway, but our data demonstrate that removal of the amino terminus of TRAF1 also results in the generation of a dominant negative version of the molecule (Fig. 5). This suggests that the ring finger of TRAF2 itself is not the only requirement for TRAF-mediated signaling.

The mechanism of TRAF1- and TRAF2-mediated signaling is currently unclear. Recent reports have shown that both proteins can interact with TANK/I-TRAF, a novel protein which has been described as both augmenting (7) and inhibiting (35) TRAF2-mediated NF-κB induction. However, TRAF3 can also bind to TANK/I-TRAF (7, 35), suggesting either that TRAF3 interacts with TANK/I-TRAF in a different conformation which does not lead to NF-κB induction or that additional factors are involved in NF-κB induction. Other candidate factors which may play a role in this pathway are the recently described mammalian homologs of the baculovirus IAP protein (10, 24, 32, 44).

We have previously shown that the TRAF proteins can bind to two 5- to 10-amino-acid elements in CD30, both of which are located in domain 2 (16). The first of these (Fig. 8, element 2A) encompasses the sequence PEQET (residues 561 to 565) and has been shown in yeast two-hybrid experiments to bind TRAF1, TRAF2, and TRAF3. Element 2B (Fig. 8) is located between residues 577 and 586 and has been shown to bind TRAF1 and TRAF2 but not TRAF3 (16). Coexpression of TRAF3 was found to inhibit signaling by domain 2 (Fig. 5). This inhibition occurred with both wild-type TRAF3 and a version lacking the ring finger (Fig. 5). It is possible that TRAF3 competes with TRAF2 for binding to element 2A. However, a downstream role for TRAF3, for example by binding to TANK/I-TRAF, cannot be excluded.

Our findings suggest that activation of CD30 can initiate a complex set of intracellular signals which could potentially lead to changes in gene expression, proliferation, and apoptosis. Further, the identification of multiple signaling elements may reflect the cell type specificity of CD30, and the novel observation that NF-κB can be induced through TRAF1 raises the possibility that TRAF1 is more widely involved in TNFR superfamily signal transduction than had previously been thought.

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