Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6721–6725, June 1996
Biochemistry

The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation

(tumor necrosis factor receptors/CD40/signal transduction)

HO YEONG SONG, MIKE ROTHE, AND DAVID V. GOEDDEL*

Tularik, Inc., 270 East Grand Avenue, South San Francisco, CA 94080

Contributed by David V. Goeddel, March 13, 1996

ABSTRACT **TRAF1 and TRAF2 form an oligomeric complex that associates with the cytoplasmic domains of various members of the tumor necrosis factor (TNF) receptor superfamily. TRAF2 action is required for activation of the transcription factor NF-κB triggered by TNF and the CD40 ligand. Here we show that TRAF1 and TRAF2 interact with A20, a zinc finger protein, whose expression is induced by agents that activate NF-κB. Mutational analysis revealed that the N-terminal half of A20 interacts with the conserved C-terminal TRAF domain of TRAF1 and TRAF2. In cotransfection experiments, A20 blocked TRAF2-mediated NF-κB activation. A20 also inhibited TNF and IL-1-induced NF-κB activation, suggesting that it may inhibit NF-κB activation signaled by diverse stimuli. The ability of A20 to block NF-κB activation was mapped to its C-terminal zinc finger domain. Thus, A20 is composed of two functionally distinct domains, an N-terminal TRAF binding domain that recruits A20 to the TRAF2-TRAF1 complex and a C-terminal domain that mediates inhibition of NF-κB activation. Our findings suggest a possible molecular mechanism that could explain A20's ability to negatively regulate its own TNF-inducible expression.**

Tumor necrosis factor (TNF) is a cytokine that is produced by activated macrophages and plays an important role in the regulation of immune and inflammatory responses (1). TNF signaling is mediated by cell surface receptors of 55 kDa (TNFR1) and 75 kDa (TNFR2) that are present on most cell types (2, 3). The intracellular domains of TNFR1 and TNFR2 are unrelated in primary amino acid sequence, suggesting they use distinct signaling mechanisms (2, 3). TNFR1 has been regarded as the major signal transducer for many TNF functions, including cytotoxicity, whereas TNFR2 signals a more limited number of responses (2, 3). However, both receptors are capable of independently activating the transcription factor NF-κB (4, 5). Searches for TNFR-associated factors have resulted in cloning of TRADD (6), which interacts with TNFR1, and TRAF1 and TRAF2, which associate with TNFR2 (5).

TNFR1 contains a so-called "death domain" of ≈80 residues near its C terminus, which is required for TNF-induced cytotoxicity (7) and receptor oligomerization (8, 9). TRADD contains a region at its C terminus that shares homology with the death domain of TNFR1 (6). The interaction of the death domains of TRADD and TNFR1 is TNF-dependent, suggesting TNFR1 oligomerization is a prerequisite for the recruitment of TRADD to the TNFR1 complex (10). When overexpressed, TRADD induces apoptosis and activates NF-κB (6), which are hallmark activities signaled by TNFR1.

TRAF proteins are a family of signal transducers having a C-terminal homology region termed the "TRAF domain" (5). In addition to TRAF1 and TRAF2, which interact with each other and form a complex with TNFR2, two other members of this family, TRAF3 and TRAF4 (CART1), have been identified. TRAF3, which is also called CD40bp (11), CRAF-1 (12), LAP1 (13), and CAP1 (14), is structurally similar to TRAF2. TRAF3 interacts with the intracellular domain of CD40 and may be important for certain CD40-mediated signal transduction pathways (12). TRAF3 also interacts with the Epstein–Barr virus-encoded protein LMP1, which is crucial for Epstein–Barr virus-induced B cell transformation (13). TRAF4 (CART1) is the most recently cloned TRAF family member (15). In contrast to TRAF2 and TRAF3, which are ubiquitously expressed and localized in the cytoplasm (5, 13), TRAF4 is a nuclear protein that was found to be predominantly expressed in breast carcinoma (15). This raises the possibility that TRAF4 may belong to a subfamily of TRAFs that function as nuclear-signaling or transcription factors.

TRAF2 can interact with both TNFR2 and CD40, and it acts as a common mediator for NF-κB activation through these two receptors (16). TRAF2 can also be recruited to TNFR1 through its interaction with TRADD (10). A dominant negative TRAF2 blocked TNFR1-mediated NF-κB activation in 293 cells, suggesting that TRAF2 is also involved in the TNFR1-induced NF-κB activation pathway. In contrast to TRAF2, neither TRAF1 nor TRAF3 can activate NF-κB in cotransfection experiments (16).

TRAF2 consists of at least three domains (5, 17). The N-terminal RING finger motif (18) is required for NF-κB activation by TRAF2 (16). The central cysteine-rich domain consists of five zinc finger-like structures. The C-terminal TRAF domain (residues 272–501) can be further divided into two subdomains. The TRAF-N subdomain (residues 272–355) comprises a predicted coil–coil structure, which may be involved in oligomerization of TRAFs (5, 16). This domain is also responsible for interaction with cellular inhibitors of apoptosis proteins that associate with the TNFR2–TRAF complex (19). The TRAF-C subdomain (residues 356–501) is required for binding to TNFR2 (19).

In this study we used the yeast two-hybrid system to search for TRAF2-interacting proteins that might participate in NF-κB activation signaled by TRAF2. We identified A20, a TNF-inducible zinc finger protein, that also interacts with TRAF1. Overexpression of A20 blocks NF-κB activation by several stimuli, including TRAF2. This negative feedback regulation of TNF-induced NF-κB activation by A20 is probably mediated by A20's association with the TRAF2–TRAF1 complex.

MATERIALS AND METHODS

Cell Culture and Reagents. The human embryonic kidney 293 cell line and the murine interleukin 2-dependent CT6 cell line were maintained as described (5). TNF and interleukin 1 (IL-1) were provided by Genentech. The monoclonal anti-Flag antibody (M2) was obtained from IBI, and the monoclonal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-1, interleukin 1; TNF, tumor necrosis factor.
*To whom reprint requests should be addressed.

anti-human A20 antibody was provided by V. Dixit (University of Michigan). The polyclonal anti-mouse TRAF1 antibody was raised using glutathione *S*-transferase-TRAF1 as antigen. The rabbit anti-murine TNFR2 antibody was described (5). For Western blot analysis, anti-mouse IgG antibody conjugated with horseradish peroxidase and enhanced chemiluminescence (ECL) kit (Amersham) were used.

Expression Vectors. Mammalian expression vectors for TRADD, TRAF1, TRAF2, TNFR2, and CD40 have been described (6, 16). Flag epitope-tagged TRAF1 and TRAF2 expression vectors have been described (19). The human A20 expression plasmid was provided by V. Dixit. Truncated A20 expression vectors for the N-terminal half (amino acids 1–386) and the C-terminal half (amino acids 387–790) of A20 were prepared by PCR. The NF-κB reporter plasmid, pELAM-luc, and the β-galactosidase control plasmid, pCMV-βGal, were described (10).

Yeast Two-Hybrid System. Vectors and procedures for the two-hybrid library screening were as described (5). The mouse peripheral lymph node two-hybrid cDNA library was supplied by L. Lasky (Genentech). Deletion constructs for two-hybrid mapping were made by PCR. Yeast strain Y190 was used for library screening and retransformation experiments. We followed the Matchmaker Two Hybrid System protocol (Clontech) for transformation and plasmid DNA rescue from yeast.

Transfection and Coimmunoprecipitation. The calcium phosphate precipitation method was used for transfections (20). For coimmunoprecipitations, 2×10^6 cells per 100-mm plate were transfected with 5 μg of appropriate expression vectors. Twenty-four hours after transfection, the cells were washed once with cold PBS, lysed in 500 μl of lysis buffer containing 50 mM Hepes (pH 7.6), 250 mM NaCl, 0.1% Nonidet P-40, and 5 mM EDTA. The lysate was cleared by centrifugation and incubated with 1 μl of M2 anti-Flag antibody or 5 μl of anti-human A20 antibody and 20 μl of protein G-agarose slurry (Oncogene Science) for 4 hr. Immunoprecipitates were washed 5 times with lysis buffer containing 1 M NaCl. Coprecipitating proteins were separated by SDS/PAGE and analyzed by Western blot analysis with the anti-human A20 antibody.

For endogenous coimmunoprecipitations, $\approx 1 \times 10^8$ 293 cells were lysed in 3 ml of lysis buffer. The cleared lysate was incubated with 3 μl of rabbit sera against GST-TRAF1. Proteins coprecipitated by the beads were separated by SDS/PAGE and subjected to Western blotting with anti-A20 antibody.

Reporter Gene Assays. 293 cells were seeded in six-well plates at 2×10^5 cells per well 24 hr before transfection. Transfections were performed as described above. Each transfection contained 0.5 μg of pELAM-luc, 0.5 μg of pRSV-βGal (6), and various amounts of the appropriate expression vectors. Twenty-four to 36 hr after transfection, cells were lysed in 200 μl of lysis buffer (Luciferase Assay Kit, Promega). Lysate (20 μl) was mixed with 100 μl of luciferase assay reagent. Luciferase activity was measured in a model 20e luminometer (Turner Designs, Palo Alto, CA). β-Galactosidase activity was measured to normalize transfection efficiencies as described (6).

RESULTS

Identification of A20 as a TRAF2-Interacting Protein. To identify TRAF2-interacting proteins, we used the yeast two-hybrid system (21) to screen a mouse peripheral lymph node cDNA library. Forty-one His$^+$ and LacZ$^+$ colonies were analyzed. Based on restriction mapping and sequencing, several distinct groups of cDNA clones were isolated. The two major classes of clones encoded TRAF2 and TRAF1, confirming our earlier observation that TRAF2 can form homodimers and interact with TRAF1 (5). One clone, S-21, contained a 4.5-kb cDNA insert encoding a nearly full-length murine A20 protein (22) lacking only eight amino acids from its N terminus.

A20 was originally identified as a TNF-inducible zinc finger protein of 80 kDa (23). It has been reported that A20 can protect several cell lines from TNF-induced cytotoxicity (24) and can induce antiapoptotic activity in B cells following CD40 activation (25). A20 was also shown to be constitutively expressed at high level in lymphoid tissues, suggesting that A20 may have an important function in lymphoid systems (22).

Retransformation assays performed with the A20 clone S-21 confirmed that the interaction between A20 and TRAF2 was specific. We then performed two-hybrid deletion mapping analysis on TRAF2 to delineate the domains required for interaction with A20. The N-terminal RING and central zinc finger domains of TRAF2 were not required for binding to A20, because TRAF2$_{(87–501)}$ and TRAF2$_{(264–501)}$ mutants interacted strongly with A20, whereas a TRAF2$_{(1–86)}$ mutant failed to interact (Table 1). TRAF2$_{(1–358)}$, which lacks the TRAF-C domain, also interacts with A20. This demonstrates that the TRAF-N domain is probably important for interaction with A20.

We next tested whether TRAF1 and TRAF3 could also interact with A20 in the two-hybrid system. The interaction between TRAF1 and A20 was as strong as that observed between TRAF2 and A20. In contrast, TRAF3 did not interact with A20 in the two-hybrid system (Table 1). This result is consistent with A20 interacting with the TRAF-N domain of TRAF1 and TRAF2, because this domain is highly conserved between TRAF1 and TRAF2 but poorly conserved in TRAF3.

Sequence analysis of A20 had previously revealed two distinct structural domains (22, 23). We have designated the N-terminal half (residues 1–386), the "N domain," and the C-terminal half (residues 387–790), the "Zn domain." The N domain shows no obvious homology to any known proteins. The Zn domain contains seven zinc fingers, the majority having the structure Cys-X4-Cys-X11-Cys-X2-Cys. Based on the two-hybrid deletion mapping, the N domain of A20 is sufficient for interaction with both TRAF1 and TRAF2 (Table 1), whereas the Zn domain does not interact with either TRAF1 or TRAF2 (Table 1). However, the Zn domain mediates A20 self-association (Table 1).

Table 1. Interactions between A20 and TRAFs

DNA-binding domain hybrid	Activation domain hybrid	Interaction
Vector	A20	−
TNFR2	A20	−
TRAF1	A20	+
TRAF2	A20	+
TRAF2$_{(1–358)}$	A20	+
TRAF2$_{(1–86)}$	A20	−
TRAF2$_{(87–501)}$	A20	+
TRAF2$_{(264–501)}$	A20	+
TRAF3	A20	−
A20	Vector	−
A20	A20	+
A20$_{(1–386)}$	A20	−
A20$_{(1–386)}$	TRAF1	+
A20$_{(1–386)}$	TRAF2	+
A20$_{(387–790)}$	A20	+
A20$_{(387–790)}$	TRAF1	−
A20$_{(387–790)}$	TRAF2	−

Yeast Y190 cells were cotransformed with expression vectors encoding various Gal4 activation domain- and Gal4-binding domain-fusion proteins as indicated. Filter assays for β-galactosidase activity were performed to detect interactions between fusion proteins. Plus signs indicate blue color developed within 2 hr of the assay, and minus signs indicate no color development within 24 hr.

Association of A20 with the TRAF1–TRAF2 Complex in Mammalian Cells. To confirm the interaction of A20 with TRAF1 and TRAF2 in mammalian cells, expression vectors encoding Flag epitope-tagged TRAF1 and TRAF2 were transiently cotransfected with an A20 expression vector in human 293 cells. Extracts from transfected cells were immunoprecipitated with an anti-Flag antibody, followed by immunoblotting with an anti-A20 antibody. The A20 protein was coprecipitated with TRAF1 but not with TRAF2 (Fig. 1*A*). When TRAF1, TRAF2, and A20 were coexpressed, significantly more A20 was coprecipitated than when TRAF1 and A20 were coexpressed (Fig. 1*A*, lane 6). These data suggest that A20 may interact with TRAF2–TRAF1 heterocomplex in mammalian cells.

We also looked for association between endogenous TRAF1 and A20 in nontransfected cells. Lysates from 293 cells were incubated with an anti-TRAF1 polyclonal antibody to precipitate the endogenous TRAF1 complex. Subsequent Western blotting of the TRAF1-associated complex with anti-A20 antibody showed that a 90-kDa band corresponding to A20 coprecipitated with TRAF1 (Fig. 1*B*), suggesting a physiological association between TRAF1 and A20.

Induction of A20 mRNA by TNFR2. In addition to TNF, several other proteins that can trigger NF-κB activation, such as IL-1, CD40, and Epstein–Barr virus-encoded LMP1 also induce A20 expression (22, 25). A study of the A20 promoter region revealed that induction of A20 expression by these agents was mediated by two κB elements (26), which are the recognition sequences for the transcription factor NF-κB. Induction of NF-κB by TNF is independently signaled by both TNF receptors (4, 5). To examine if the TRAF-2-interacting TNFR2 can induce A20 expression, we selected murine CT6 cells, which do not contain TNF-R1 (5). Following treatment with agonistic anti-mTNFR2 antibodies, A20 mRNA levels were increased ≈5-fold as determined by Northern blot analysis (Fig. 2). Thus, TNFR2 activation can mediate A20 expression, presumably via TRAF2-induced NF-κB activation.

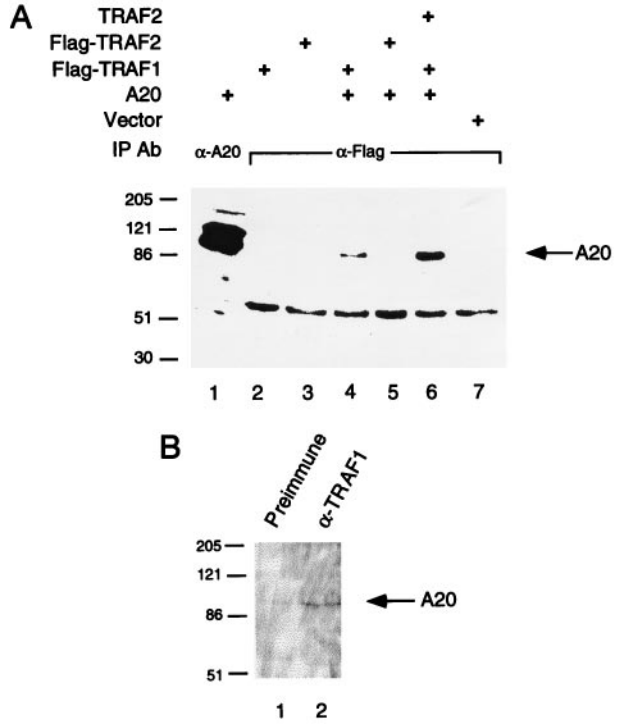

FIG. 1. Interaction of A20 with TRAF1 and TRAF2 in mammalian cells. (*A*) Interaction between TRAFs and A20 in cotransfection assays. 293 cells (2×10^6) were transiently transfected with the indicated combinations of expression vectors for A20, TRAF2, and Flag epitope-tagged TRAF1 and TRAF2. After 24 hr, lysates were prepared and immunoprecipitated with an anti-human A20 monoclonal antibody (lane 1) or an anti-Flag monoclonal antibody (lanes 2–7). Coprecipitating A20 was detected by Western blotting with the A20 antibody. (*B*) Interaction between endogenous TRAF1 and A20. Lysates from 1×10^8 293 cells were incubated with the polyclonal anti-TRAF1 antibody for 4 hr. Coprecipitating A20 was detected by Western blotting with the A20 antibody. The arrow indicates the 90-kDa A20 protein that specifically associated with endogenous TRAF1.

A20 Inhibits TRAF2-Mediated NF-κB Activation. When overexpressed, TRAF2, but not TRAF1 or TRAF3, potently activates NF-κB in both 293 and CT6 cells (16). Because A20 interacts with TRAF1, which forms a complex with TRAF2, we investigated the effect of A20 expression on TRAF2-mediated NF-κB activation. TRAF2 overexpression in 293 cells strongly activated an NF-κB-dependent reporter gene (230-fold induction with 1 μg of TRAF2 expression vector; Fig. 3). However, coexpression of A20 blocked TRAF2-mediated NF-κB activation in a dose-dependent manner, with nearly complete inhibition observed with 1 μg of A20 expression vector. Because NF-κB activation by TNFR2 and CD40 is mediated by TRAF2 (16), we examined whether A20 could also block NF-κB activation following TNFR2 and CD40 overexpression. A20 strongly inhibited NF-κB activation induced by overexpression of TNFR2 and CD40 in 293 cells (Fig. 4), confirming its role as an inhibitor of TRAF2-mediated NF-κB activation.

A20 Inhibits TNF and IL-1-Induced NF-κB Activation in 293 Cells. In nontransfected human 293 cells, NF-κB activation by TNF is mediated by TNFR1 exclusively (16). We reasoned that A20 would also block TNF-induced NF-κB in 293 cells because of TRAF2's involvement in NF-κB activation by TNFR1 (10). TNF treatment of 293 cells led to a 40-fold increase in NF-κB reporter gene activity (Fig. 5*A*). Coexpression of A20 abolished TNF-mediated NF-κB activation in 293 cells. Previous studies have shown that overexpression of TRADD, a signal transducer for TNFR1, activates NF-κB in 293 cells (6). We found that coexpression of A20 also strongly inhibited TRADD-induced NF-κB activation (Fig. 4, lane 4). These data suggest that A20 is a negative regulator of the TNFR1-mediated NF-κB activation pathway.

We next examined the effect of A20 on the IL-1-induced NF-κB activation pathway. IL-1 activates NF-κB using a pathway distinct from TNF in 293 cells (10). Following IL-1 treatment, we observed a 7-fold induction in NF-κB activity (Fig. 5*B*). Expression of A20 also greatly reduced IL-1-induced

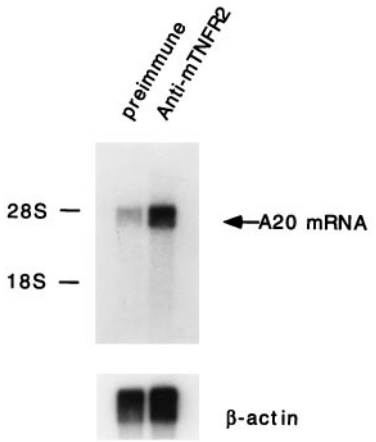

FIG. 2. Induction of A20 mRNA by TNFR2 activation in murine CT6 cells. CT6 cells were treated with agonistic anti-murine TNFR2 antibodies for 4 hr, and MRNA was isolated. Northern blotting was performed with murine A20 cDNA as probe. The arrow indicates the 4.5-kb A20 mRNA. The blot was stripped and reprobed with a β-actin cDNA probe (*Lower*).

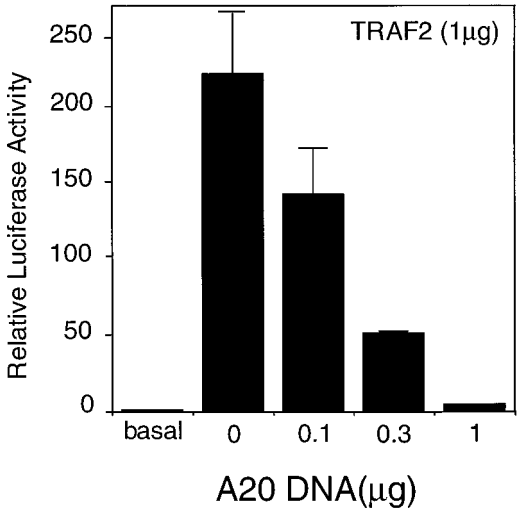

FIG. 3. Inhibition of TRAF2-mediated NF-κB activation by A20 overexpression. 293 cells were transiently transfected with a TRAF2 expression vector (1 μg) and increasing amounts of the A20 expression vector as indicated. The NF-κB reporter plasmid pELAM-luc (0.5 μg), and the β-galactosidase expression plasmid, pRSV-βGal (0.5 μg), were included in each transfection. After 24 hr, luciferase activities were determined and normalized on the basis of β-galactosidase activities. The values indicated represent normalized luciferase activities relative to the control transfection using the reporter plasmid and an empty expression vector and are shown as mean ± SEM for experiments performed in duplicate.

NF-κB activity. These results suggest that A20 may be an inhibitor of NF-κB activation triggered by divergent stimuli.

The Zn Domain of A20 Inhibits NF-κB Activation Pathways. A20 comprises two structurally distinct regions, an N domain that binds TRAF1 and TRAF2, and a C-terminal Zn domain. To investigate whether the NF-κB inhibitory activity of A20 could be mapped to either of these domains, we expressed these two domains individually in 293 cells and determined their function as described above. NF-κB activation by TRAF2 was significantly, but not completely, inhibited by coexpression of the N-terminal 386 amino acids of A20. However, this N domain had little or no effect on NF-κB activation triggered by overexpression of TNFR2, CD40, and TRADD or TNF treatment (Fig. 4). When the Zn domain (amino acids 387–790) was coexpressed, NF-κB activation was completely blocked in all experiments (Fig. 4). These data suggest that the two domains of A20 have distinct functions. The N domain recruits A20 to the TRAF2–TRAF1 complex, whereas the Zn domain exhibits NF-κB inhibitory activity in response to diverse stimuli.

DISCUSSION

NF-κB activity is negatively regulated by IκB, which sequesters latent NF-κB in an inactive form in the cytoplasm (27). Following TNF stimulation, IκB is rapidly phosphorylated on serines 32 and 36, targeting it for degradation by the ubiquitin-proteosome pathway (28). NF-κB then translocates from the cytoplasm into the nucleus, where it induces the expression of a number of genes involved in the inflammatory response.

TRAF2 appears to be critically involved in NF-κB activation mediated by TNF and CD40, because a mutant TRAF2 lacking the N-terminal RING finger can block these activities (10, 16). The sequence of TRAF2 does not reveal any clues as to how it activates the NF-κB pathway. Presumably, TRAF2 functions as an adaptor and/or regulatory protein that controls the activation of other key effector proteins in NF-κB activation pathways, perhaps even the kinase that phosphorylates IκB. In this study, we set out to identify TRAF2-interacting proteins involved in activation of NF-κB. Using the yeast two-hybrid system, we identified A20, a TNF-inducible zinc finger protein and showed that it associates with the TRAF2–TRAF1 complex. However, rather than being a positive effector protein, we found that A20 is a potent inhibitor of NF-κB activation. Very recently, Jäättelä *et al.* (29) also showed that A20 overexpression could inhibit NF-κB and AP-1 reporter activities induced by both TNF and IL-1. Our work suggests a possible mechanism by which this inhibition could occur.

Although both TRAF2 and TRAF1 interact strongly with A20 in two-hybrid assays and the A20 binding site was mapped to the TRAF domain of TRAF2, we could not confirm direct TRAF2–A20 interaction in human cells. In contrast, the interaction of TRAF1 with A20 was observed in both transfected and nontransfected cell lines. Therefore, it appears that the ability of A20 to block TNF and TRAF2-mediated NF-κB activation may take place indirectly through the formation of a TRAF2–TRAF1–A20 complex. Evidence for such a TRAF2–TRAF1–A20 complex was seen in coimmunoprecipitation assays where A20 was found to interact with TRAF2–TRAF1 better than with TRAF1 alone (Fig. 1*A*).

Whereas the function of TRAF2 as a mediator of NF-κB activation in TNF and CD40 signaling has been established

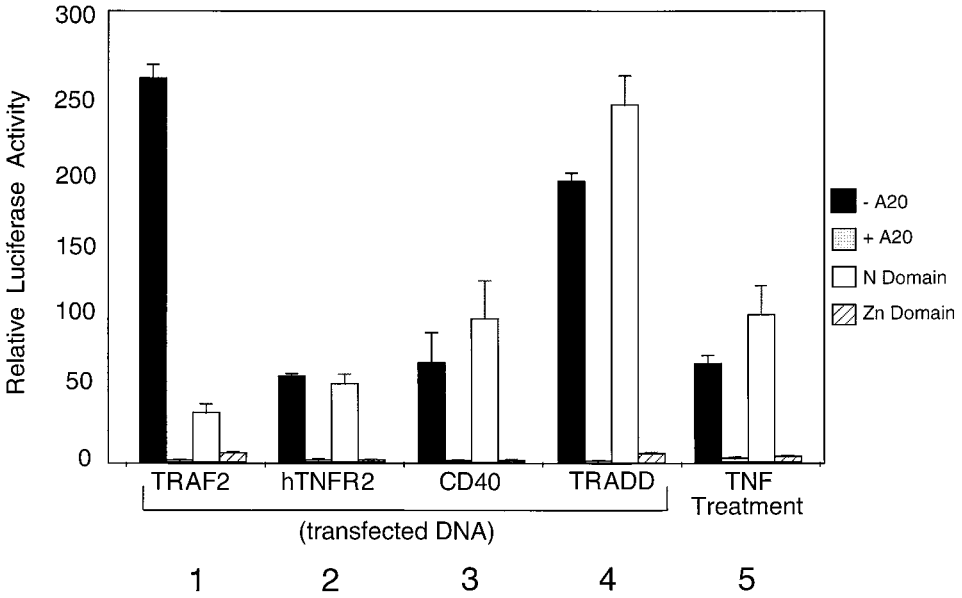

FIG. 4. Inhibition of NF-κB activation by full-length and truncated A20 proteins. 293 cells were transiently transfected with expression vectors for TRAF2 (1 μg), hTNFR2 (1 μg), CD40 (1 μg), or TRADD (1 μg) alone (filled bars), with wild-type A20 (1 μg) (shaded bars), with the A20 N domain (1 μg) (open bars), or with Zn domain (1 μg) (hatched bars). In lane 5, cells were transfected with an empty expression plasmid for 24 hr, then treated with TNF (100 ng/ml) for 6 hr. The values indicated represent normalized luciferase activities relative to control transfections and are shown as mean ± SEM based on duplicate assays.

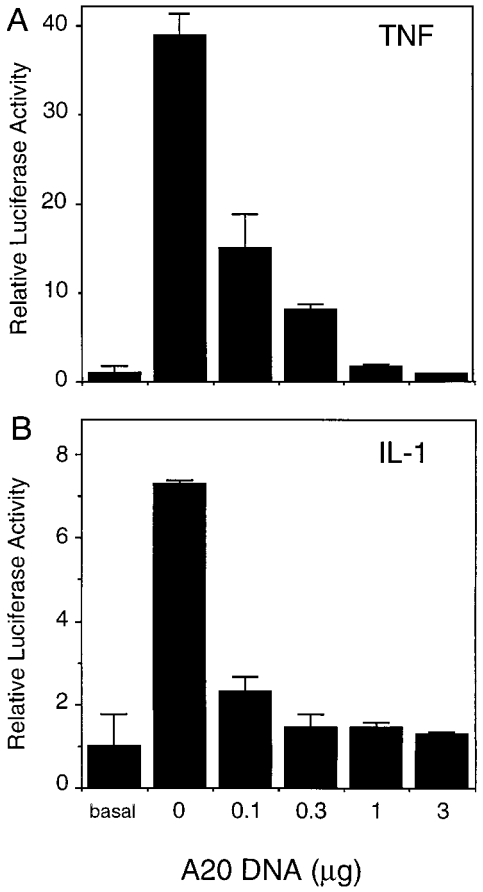

FIG. 5. Inhibition of TNF- and IL-1-induced NF-κB activation by A20 overexpression. 293 cells were transiently transfected with increasing amounts of the A20 expression vector as indicated. Thirty-six hours after transfection, cells were treated for 6 hr with 100 ng of TNF per ml (*A*) or 20 ng of IL-1 per ml (*B*). The values indicated represent normalized luciferase activities relative to the same cells without cytokine treatment and are shown as mean ± SEM based on duplicate assays.

(10, 16), the role played by TRAF1 has been unclear. Although TRAF1 shares a significant C-terminal sequence identity with TRAF2, it lacks the RING and zinc finger domains at its N-terminus, which are essential for NF-κB activation by TRAF2 (16). We recently showed that both TRAF1 and TRAF2 are required to recruit cellular inhibitor of apoptosis proteins to TNFR2 (19). Here we demonstrate that TRAF1 allows TRAF2 to associate with A20 in mammalian cells.

A20 has been shown to negatively regulate expression from its own promoter (26). Our results suggest a possible explanation for this negative feedback mechanism. The activation of NF-κB by TNFR1, TNFR2, and CD40 is a TRAF2-dependent process that leads to induction of A20 gene expression. The newly synthesized A20 can then associate with the TRAF2–TRAF1 complex to shut off the signaling cascade, subsequently resulting in down-regulation of A20 synthesis. Thus, A20 may play a role analagous to IκB, but at an earlier stage in the NF-κB activation pathway. Furthermore, the ability of A20 to inhibit TRAF2-mediated NF-kB activation might be dependent on the presence of TRAF1.

We found that the N-terminal domain of A20 is required for TRAF interaction, whereas the C terminal zinc finger domain inhibits signal transduction. These results suggest that the function of the N domain may be to recruit the inhibitory Zn domain to the NF-κB activation cascade. Under normal conditions, such recruitment might be a mechanism to increase the concentration of A20 at the appropriate target. Enforced overexpression of the Zn domain alone could bypass this recruitment requirement by greatly increasing the overall concentration of this inhibitory domain.

We and others (29) have shown that A20 can also inhibit IL-1-induced NF-κB activation. The ability of A20 to block NF-κB activation is therefore not limited to TRAF2-mediated pathways, because TRAF2 is not involved in IL-1 signaling (10). Thus, if our model for TRAF2 inhibition by A20 is correct, inhibition of IL-1 signaling might be due to association of the overexpressed A20 inhibitory domain with a yet unknown effector protein that is shared by the TRAF2-dependent and -independent NF-κB activation pathways. The identity of this putative positive regulator of NF-κB activity remains an important goal.

We thank Dr. V. Dixit for providing the human A20 expression vector and the anti-A20 monoclonal antibody and Dr. L. Lasky for the mouse peripheral lymph node two-hybrid cDNA library. We also thank K. Williamson for DNA sequencing and Drs. H.-B. Shu, H. Hsu, P. Baeuerle, and V. Dixit for critical review of the manuscript.

1. Beutler, B. & Cerami, A. (1988) *Annu. Rev. Biochem.* **57,** 505–518.
2. Tartaglia, L. A. & Goeddel, D. V. (1992) *Immunol. Today* **13,** 151–153.
3. Vandenabeele, P., Declercq, W., Beyaert, R. & Fiers, W. (1995) *Trends Cell Biol.* **5,** 392–399.
4. Kruppa, G., Thoma, B., Machleidt, T., Wiegmann, K. & Krönke, M. (1992) *J. Immunol.* **148,** 3152–3157.
5. Rothe, M., Wong, S. C., Henzel, W. J. & Goeddel, D. V. (1994) *Cell* **78,** 681–692.
6. Hsu, H., Xiong, J. & Goeddel, D. V. (1995) *Cell* **81,** 495–504.
7. Tartaglia, L. A., Ayres, T. M., Wong, G. H. W. & Goeddel, D. V. (1993) *Cell* **74,** 845–853.
8. Boldin, M. P., Mett, I. L., Varfolomeev, E. E., Chumakov, I., Shemer-Avni, Y., Camonis, J. H. & Wallach, D. (1995) *J. Biol. Chem.* **270,** 387–391.
9. Song, H. Y., Dunbar, J. D. & Donner, D. B. (1994) *J. Biol. Chem.* **269,** 22492–22495.
10. Hsu, H, Shu, H.-B., Pan, M.-G. & Goeddel, D. V. (1996) *Cell* **84,** 299–308.
11. Hu, H. M., O'Rourke, K., Boguski, M. S. & Dixit, V. M. (1994) *J. Biol. Chem.* **269,** 30069–30072.
12. Cheng, G., Cleary, A. M., Ye, Z.-s., Hong, D. I., Lederman, S. & Baltimore, D. (1995) *Science* **267,** 1494–1498.
13. Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArsdale, T., Ware, C. & Kieff, E. (1995) *Cell* **80,** 389–399.
14. Sato, T., Irie, S. & Reed, J. C. (1995) *FEBS Lett.* **358,** 113–118.
15. Régnier, C., Tomasetto, C., Moog-Lutz, C., Chenard, M.-P., Wendling, C., Basset, P. & Rio, M.-C. (1995) *J. Biol. Chem.* **270,** 25715–25721.
16. Rothe, M., Sarma, V., Dixit, V. M. & Goeddel, D. V. (1995) *Science* **269,** 1424–1427.
17. Song, H. Y. & Donner, D. B. (1995) *Biochem. J.* **809,** 825–829.
18. Freemont, P. S. (1993) *Ann. N.Y. Acad. Sci.* **684,** 174–192.
19. Rothe, M., Pan, M.-G., Henzel, W. A., Ayres, T. M. & Goeddel, D. V. (1995) *Cell* **83,** 1243–1252.
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) *Curr. Prot. Mol. Biol.* **1,** 911–913.
21. Fields, S. & Song, O. (1989) *Nature (London)* **340,** 245–246.
22. Tewari, M., Wolf, F. W., Seldin, M. F., O'Shea, K. S., Dixit, V. M. & Turka, L. A. (1995) *J. Immunol.* **154,** 1699–1706.
23. Opipari, A. W., Boguski, M. S. & Dixit, V. M. (1990) *J. Biol. Chem.* **265,** 14705–14708.
24. Opipari, A. W., Hu, H. M., Yabkowitz, R. & Dixit, V. M. (1992) *J. Biol. Chem.* **267,** 12424–12427.
25. Sarma, V., Lin, Z., Clark, L., Rust, B. M., Tewari, M., Noelle, R. J. & Dixit, V. M. (1995) *J. Biol. Chem.* **270,** 12343–12346.
26. Krikos, A., Laherty, C. D. & Dixit, V. M. (1992) *J. Biol. Chem.* **267,** 17971–17976.
27. Lenardo, M. & Baltimore, D. (1989) *Cell* **58,** 227–229.
28. Thanos, D. & Maniatis, T. (1995) *Cell* **80,** 529–532.
29. Jäättelä, M., Mouritzen, H., Elling, F. & Bastholm, L. (1996) *J. Immunol.* **156,** 1166–1173.