Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 1390–1395, February 1997
Immunology

CD30 induction of human immunodeficiency virus gene transcription is mediated by TRAF2

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Communicated by Louis Kunkel, Harvard Medical School, Boston, MA, December 17, 1996 (received for review September 4, 1996)

ABSTRACT **CD30 is a member of the tumor necrosis factor receptor (TNFR) superfamily expressed on activated T and B lymphocytes and natural killer cells. Ligation of CD30 was previously shown to induce NF-κB activation and HIV expression in chronically infected T lymphocytes. In this study, we report that two members of the TNFR-associated factor (TRAF) family of proteins, TRAF1 and TRAF2, independently bind to the intracellular domain of CD30 (CD30IC). Transient overexpression of TRAF2, but not TRAF1, induced NF-κB activation and HIV-1-long terminal repeat-driven transcription in the T cell line, KT3. Moreover, dominant negative mutants consisting of the TRAF domain of TRAF1 and TRAF2 inhibited CD30 induction of NF-κB activation and HIV-1 transcription. These results suggest that CD30 ligation may enhance the expression of HIV via TRAF-2-mediated activation of NF-κB.**

CD30 is a member of the tumor necrosis factor receptor (TNFR) superfamily (1) and was originally identified by mAb Ki-1 developed against Hodgkin and Reed-Sternberg cells (2). CD30 is also expressed on activated T and B lymphocytes, on activated natural killer cells, and on a variety of transformed lymphocytes (3–5). These include non-Hodgkin (Burkitt) lymphomas, large anaplastic lymphomas, cutaneous T cell lymphomas, T lymphocytes transformed with human T cell lymphotrophic viruses type I and II, and B lymphocytes transformed with Epstein–Barr virus (3, 5–8).

The CD30 ligand (CD30L) is a type II membrane glycoprotein that belongs to the TNF superfamily (9). It is predominantly expressed on activated T lymphocytes and monocytes/macrophages, but is also present on granulocytes and some Burkitt lymphoma cell lines (5, 9). CD30–CD30L interaction results in a variety of effects in B cells, including growth and differentiation (10). In T cell lymphomas, CD30L induces apoptosis, which is dependent on concomittant T cell antigen receptor/CD3 cross-linking (11). Furthermore, CD30 ligation plays a role in negative selection of T cells that is impaired in CD30-deficient mice (12). CD30 ligation in human T cell lines induces the NF-κB transcription factor and the expression of a number of genes regulated by NF-κB—e.g., interleukin (IL)-6, TNF, lymphotoxin (LT)-α, and ICAM-1 (13, 14). An important consequence of CD30 ligation is the induction of HIV gene expression in chronically infected T lymphocytes (15). This has been shown to be dependent on activation of the NF-κB binding to recognition sequences in the long terminal repeat (LTR) region of HIV-1.

TNFR-associated factors (TRAFs) belong to a recently described family of proteins that bind to the cytoplasmic domain of the TNFR family members (16). There are five known members of the TRAF family: TRAF1, TRAF2, TRAF3, TRAF4 (CART1), and TRAF5 (17–21). TRAF-1 and TRAF-2 were found to associate with the intracellular domain of TNFR2 (16). However, TRAF1 is indirectly associated with TNFR2 via dimerization with TRAF2, whereas TRAF2 and TRAF3 bind directly to CD40 (18–20, 22). TRAF3 is also associated with the lymphotoxin-β receptor and TNFR2 (20). Recently, it was shown that TRAF5 binds to the lymphotoxin-β receptor (21). TRAF4 was cloned from a breast carcinoma cell line and is the only TRAF that has been localized to the nucleus and may not be associated with a surface receptor (17).

TRAF proteins share a conserved C-terminal TRAF domain (≈230 aa), which is composed of two structural subdomains TRAF-N and TRAF-C. The TRAF-C domain is responsible for oligomerization and binding to the receptors (19). The TRAF-N domains from TRAF1 and TRAF2 have been shown to mediate the binding to members of the family of inhibitor of apoptosis proteins (23). In addition to the C-terminal TRAF domain, TRAF2, TRAF3, TRAF4, and TRAF5, but not TRAF1, contain an N-terminal ring finger and zinc fingers. Functional analysis demonstrated that TRAF2 is a common signal transducer for TNFR2 and CD40, which mediates activation of the transcription factor NF-κB (22). It was recently reported that TRAF-2 binds TRADD, the TNFR1-associated death domain containing protein, and probably is recruited for NF-κB activation through TNFR1 (24).

In this paper, we have examined the molecular mechanisms of HIV gene induction by CD30 ligation. We show that the intracellular domain of CD30 is associated with TRAF1 and TRAF2. Each of these proteins binds independently to the same region (aa 553–595) of CD30. Transient overexpression of TRAF2, but not TRAF1, resulted in NF-κB nuclear translocation and induction of HIV-1-LTR-driven transcription. Moreover, overexpression of dominant negative mutants of TRAF1 or TRAF2 (ΔTRAF1 or ΔTRAF2) consisting of the TRAF domains of these proteins inhibited CD30-induced NF-κB activation and HIV transcription. These results suggest that CD30 ligation may enhance the progression of HIV infection via TRAF2-mediated activation of NF-κB.

MATERIALS AND METHODS

Yeast Two-Hybrid System. To screen for the proteins that are able to interact with the intracellular region of CD30 we constructed a fusion protein, where the region spanning the intracellular domain of human CD30 (CD30IC) (1) and encoding for aa 413–595 was fused with the DNA-binding domain of the yeast Gal4 transcriptional factor. DNA corresponding to the CD30IC was obtained by reverse transcription

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0027-8424/97/941390-6$2.00/0
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Abbreviations: CD30IC, CD30 intracellular domain; CD30L, CD30 ligand; TNFR, tumor necrosis factor receptor; TRAF, TNFR-associated factor; LTR, long terminal repeat; GST, glutathione *S*-transferase; EMSA, electrophoretic mobility-shift assay.

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and subsequent PCR of total cellular RNA from the KT-3 cell line using the sense (GGGCCAGAAGCTCCACCTGTGCTAC) and antisense primers (GGAATTCACTTTCCAGAGGCAGCATGTG). cDNA was prepared from the KT-3 cell RNA by reverse transcription with Superscript II (GIBCO/BRL) and PCR was performed with *Taq* polymerase from Boehringer Mannheim (35 cycles: 1 min at 94°C, 1 min at 60°C, 1 min at 72°C). A 0.6-kb PCR product was subcloned into the pCRII vector (Invitrogen) and sequenced. This DNA was then subcloned into the *Eco*RI-digested "bait" vector pGBT9 (CLONTECH).

mRNA from the KT-3 cell line was used to construct a cDNA directional oligo(dT) library in the vector pGAD424 (CLONTECH), encoding the Gal4 activation domain. The IL-6-dependent T lymphoma cell line (KT-3) was kindly provided by S. Konda (Kanazawa Medical University, Ishikawa, Japan) (25, 26). Two-hybrid screening was performed essentially as recommended by the manufacturer (Matchmaker Two-Hybrid System Protocol; CLONTECH).

Coprecipitation with Glutathione *S*-Transferase (GST) Fusion Proteins and Western Blotting. DNA corresponding to the CD30IC, which was obtained by reverse transcription–PCR as described above, was subcloned into the *Eco*RI site of GST expression vector pGEX-4T-1 (Pharmacia). GST-FasIC and GST-CD40IC expression vectors were constructed in pGEX-2TK (Pharmacia). Vectors were transformed into *Escherichia coli* strain BL21 and fusion proteins were prepared on GST–Sepharose beads (Pharmacia) according to the manufacturer's recommendations.

Five million KT-3 cells were lysed in lysis buffer (0.5% Nonidet P-40/150 mM NaCl/10 mM KCl/0.5 mM DTT/0.2 mM phenylmethylsulfonyl fluoride/10 μM leupeptin/100 ng/ml pepstatin/100 ng/ml chymostatin/1 μM antipain) and debris was removed by centrifugation at 14,000 rpm for 10 min. The supernatant was precleared with GST–Sepharose beads for 2 hr at 4°C and incubated with the respective GST fusion protein-Sepharose beads for 4 hr at 4°C. Beads were washed twice in the lysis buffer, bound proteins were resolved on the 12% precast gels (Bio-Rad) and transferred onto BioTrace nitrocellulose membrane (Gelman). Membranes were blocked with blotting solution (2% BSA/0.2% gelatin in PBS) for 2 hr at 37°C and incubated with rabbit antibodies to C-terminal peptides of TRAF1, TRAF2, and TRAF3 (Santa Cruz Biotechnology). After washing, the membranes were incubated with protein A-conjugated to horseradish peroxidase (Bio-Rad). Positive bands were detected by using ECL reagents (Amersham/Life Sciences).

For detection of the expression of TRAF1 and TRAF2 proteins by Western blot analysis, KT-3 and Jurkat E6.1 cells were lysed in lysis buffer for 30 min on ice. Debris was removed by centrifugation at 14,000 rpm for 10 min and protein concentration was estimated by the bicinchoninic acid (BCA) protein assay kit (Pierce). Proteins (50 μg per lane) were resolved on the 12% precast gels (Bio-Rad), transferred onto BioTrace nitrocellulose membrane (Gelman) and Western blot analysis was performed essentially as described above.

Cell Transfection and Stimulation. cDNAs for truncated ΔTRAF1 and ΔTRAF2, and full-length TRAF1 and TRAF2 proteins, were obtained by PCR amplification using Pfu polymerase (Stratagene). This was followed by incubation with *Taq* polymerase (Boehringer Mannheim) at 72°C for 15 min to allow for the addition of single, 3′A overhang to enable direct cloning into the pCRII vector (Invitrogen). All constructs were confirmed by restriction enzyme analysis and DNA sequencing. For expression experiments, DNA fragments were subcloned into pcDNA3 expression vector (Invitrogen).

Anti-CD30 mAb (C10) was kindly provided by Eckhard R. Podack (University of Miami) as ascites (27) and purified by using protein G-Sepharose (Pharmacia). Anti-CD30 mAbs Ki1 and BerH2 were purchased from Dako. Isotype control antibodies were purchased from Sigma. CD30 expression on KT-3 cells was detected with C10, Ki1, and BerH2 mAbs. KT-3 cells were maintained in the presence of recombinant IL-6 (2 ng/ml) (R & D Systems) and were transfected with the pcDNA3 expression vectors using the CellPhect transfection kit from Pharmacia. Following transfection, KT-3 cells were resuspended in the absence of IL-6 in medium containing 1% low endotoxin fetal calf serum (Intergen, Purchase, NY) and left for 4 hr, then were transferred to wells coated with C10 mAb or isotype control and harvested for analysis 20 hr later. The 24-well plates were coated with C10 mAb or isotype control immunoglobulins (10 μg/ml) in PBS overnight at 37°C.

Electrophoretic Mobility-Shift Assay (EMSA). Single-stranded oligonucleotides were 5′ end-labeled with [γ-^{32}P]ATP, annealed, and purified by PAGE. The double-stranded oligonucleotide (TCGCTGGGGACTTTCCAGGGA) used in these experiments corresponded to the proximal NF-κB site of the HIV-1-LTR (28). Nuclear extracts were prepared essentially as described by Tsytsykova *et al.* (29). Briefly, 10^7 cells were washed twice with ice-cold PBS, resuspended in cold 10 mM Hepes buffer (pH 7.9) containing 0.01% Nonidet P-40, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and protease inhibitor cocktail, and incubated for 10 min on ice. Nuclei were pelleted in a microcentrifuge for 10 sec and resuspended in ice-cold 20 mM Hepes buffer (pH 7.9) containing 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, and protease inhibitor cocktail. After incubation on ice for 20 min, debris was removed by centrifugation at 14,000 rpm for 10 min at 4°C. The supernatants were aliquoted and frozen at −80°C. The protein concentration was estimated by the BCA protein assay kit (Pierce).

For each reaction, 1×10^3 cpm ($\approx$0.1 ng) of radiolabeled oligonucleotide probe was incubated with 1–5 μg of nuclear extract in 20 μl of binding buffer for 30 min on ice or at room temperature. Samples were run on 5% polyacrylamide gel in 1 $\times$ TBE (89 mM Tris-borate/89 mM boric acid/2 mM EDTA). Unlabeled competitors were added in 100-fold molar excess. Where indicated, the nuclear extracts were preincubated with the respective antibodies for 30 min before addition of radiolabeled oligonucleotides. Rabbit anti-p50 subunit of NF-κB antibodies were a kind gift from A. Israël (Institute Pasteur, Paris) and anti-p65 antibodies were a kind gift of N. Rice (Frederick Cancer Institute, Frederick, MD). Normal rabbit serum was purchased from Sigma.

Luciferase Reporter Gene Assay. HIV-1-LTR-pGL3 was constructed using the pGL3-basic vector (Promega) and the HIV-1-LTR 1.1-kb *Eco*RI–*Hin*dIII fragment from the pBENN-CAT vector obtained from Barbara K. Felber and George N. Pavlakis (AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health) (30). KT-3 cells were cotransfected with pcDNA3-TRAF expression vectors and HIV-1-LTR-pGL3. Firefly luciferase activity was measured by the luciferase assay kit (Promega) and expressed as relative light intensity per 1 μg of protein. Protein concentration was estimated by the BCA protein assay kit (Pierce). Efficiency of transfection was verified by cotransfection with a pCAT-control vector (Promega).

RESULTS

TRAF1 and TRAF2 Independently Associate with the Intracellular Domain of CD30. To obtain cDNAs that encode proteins that associate with the intracellular region of CD30, we used the yeast two-hybrid to screen a cDNA library prepared from the CD30$^+$ KT-3 T cell line. The bait plasmid consisted of cDNA encoding the last 182 of the 186 aa of the CD30 intracellular domain fused to the GAL4 DNA-binding domain. Of 1×10^6 yeast transformants, 15 colonies grew on leucine/tryptophan/histidine-deficient selection plates. All of

them were strongly positive for the expression of β-galactosidase activity. Sequence analysis of DNA from these clones revealed that they encoded for the TRAF family members, TRAF1 or TRAF2 (Table 1). The shortest of the cDNA clones, which were obtained for TRAF1 and TRAF2 in our two-hybrid system screen, encoded for the C-terminal TRAF domains of these proteins (aa 170–416 for TRAF1 and aa 292–501 for TRAF2). These results suggest that TRAF1 and TRAF2 proteins bind directly and independently to CD30 via their TRAF domains.

To confirm the interaction of CD30 with TRAF1 and TRAF2, we expressed the intracellular regions of CD30, Fas, and CD40 as GST fusion bacterial proteins, immobilized them on glutathione–Sepharose beads, and used them to precipitate proteins from the lysates of KT-3 cells. Bound proteins were resolved by gel electrophoresis and subsequently immunoblotted with antisera to TRAF1, TRAF2, and TRAF3. As shown in Fig. 1, GST-CD30IC, but not GST alone or GST-FasIC, bound TRAF1 and TRAF2. In our hands, there was no detectable binding of TRAF3 to GST-CD30IC (data not shown). As previously reported, GST-CD40IC bound TRAF2 and TRAF3, but no detectable amount of TRAF1 binding was observed. These results confirm those obtained with the two-hybrid system and indicate that the cytoplasmic region of CD30 binds TRAF1 or TRAF2.

To map the region of CD30 involved in binding to TRAF1 or TRAF2, we prepared several truncated forms of CD30IC as fusion proteins with the GAL4 DNA-binding domain and examined their ability to interact with TRAF1 or TRAF2 in the yeast two-hybrid system. Table 2 shows that TRAF1 and TRAF2 binding was localized to the same region of CD30IC, which consisted of the C-terminal 43 aa of CD30 (aa 553–595).

TRAF2 Mediates Activation of NF-κB Induced by CD30. To assess the role of TRAF proteins in CD30-mediated induction of NF-κB, truncated and full-length TRAF proteins were transiently overexpressed in KT-3 cells, and NF-κB activity was examined by EMSA using a radiolabeled 21-bp long oligonucleotide corresponding to the proximal NF-κB site of HIV-1-LTR (28). ΔTRAF1 (aa 169–416) and ΔTRAF2 (aa 270–501) encoded only for the TRAF domains of these proteins. These mutants retained their capacity to interact with CD30 in the yeast two-hybrid system (data not shown) but should function as dominant negatives, as has been previously demonstrated for TRAF2, TRAF3, and TRAF5 (21, 22). Analysis of lysates from transfected cells by Western blotting and subsequent densitometry revealed a 5- to 7-fold increase over baseline in the level of expression of TRAF1 or TRAF2 in cells transfected with full-length constructs. The level of expression of ΔTRAF1 or ΔTRAF2 was also 5- to 7-fold higher than that of native proteins (data not shown).

Fig. 2*A* shows that anti-CD30 mAb (C10), but not an isotype control, induced NF-κB activation in the nuclear extracts of KT-3 cells (lanes 2 and 3). Cells transiently transfected with ΔTRAF1 or ΔTRAF2 showed an increased baseline of NF-κB activity (lanes 4 and 7). However, CD30 ligation failed to increase NF-κB binding in these transfectants (lanes 5 and 8). Similar results were obtained in cells transfected with full-length TRAF1 (lanes 10 and 11). In contrast, transient overexpression of full-length TRAF2 in addition to increasing baseline NF-κB activity (lanes 14 and 15), markedly enhanced the induction of NF-κB binding by anti-CD30.

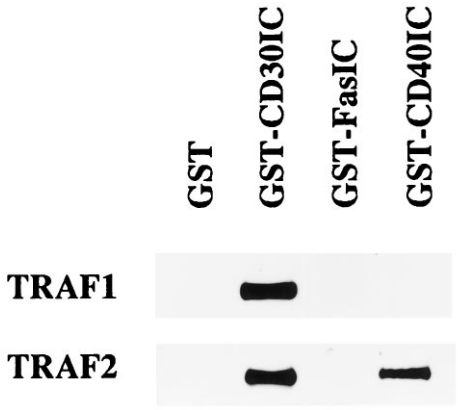

FIG. 1. Association of the TRAF proteins with the intracellular domain of CD30. Lysates from KT-3 cells were incubated with the GST fusion protein beads, including fusion proteins of the intracellular domains of CD30, Fas, and CD40, as indicated. Proteins were resolved on SDS/12% polyacrylamide gel and Western blotted with rabbit polyclonal antisera directed against TRAF1 and TRAF2.

It is unlikely that TRAF1 functions upstream of TRAF2 and that the failure of overexpression of TRAF1 to induce NF-κB and HIV-1 gene expression is due to the lack of sufficient amounts of TRAF2 in KT-3 cells. Jurkat E6.1 T cells express TRAF2 but no detectable TRAF1 protein by Western blotting (Fig. 3*A*) and there is no detectable binding of TRAF1 following precipitation of CD30-binding proteins with GST-CD30 from these cells (data not shown); yet ligation of CD30 induces NF-κB nuclear translocation in Jurkat E6.1 T cells (Fig. 3*B*).

Fig. 2*B* shows the analysis of the NF-κB-binding nuclear complex induced by CD30 signaling in KT-3 cells. The complex was competed away by a 100-fold molar excess of the unlabeled NF-κB oligonucleotide itself, but not by a 100-fold molar excess of the irrelevant AP-1 consensus oligonucleotide (lanes 4 and 5, respectively). Addition of rabbit polyclonal anti-p65 and anti-p50 NF-κB antibodies (lanes 6 and 7), but not of normal rabbit serum (lane 8), to the nuclear extracts 30 min prior to incubation with the radiolabeled NF-κB oligonucleotide, supershifted the complex induced by CD30.

Taken together, these data show that CD30 induces NF-κB-binding activity in human T cells, and this induction is mediated by TRAF2, but not TRAF1.

CD30-Dependent Induction of NF-κB Through TRAF2 Activates HIV-1-LTR-Driven Transcription in T Cells. To determine whether CD30 signaling induces NF-κB-dependent transcriptional activity in T cells, we placed the LTR of HIV-1 upstream of the luciferase gene in the pGL3 reporter vector. HIV-1-LTR-pGL3 was cotransfected with the pcDNA3 empty vector or with the pcDNA3 vectors containing full-size or truncated cDNAs of TRAF1 and TRAF2, identical to those used for EMSA experiments. Fig. 4 shows that anti-CD30 mAb induces luciferase gene expression driven by the HIV-1-LTR. In agreement with the results of EMSA, expression of ΔTRAF1 and ΔTRAF2 and full-length TRAF1 slightly in-

Table 1. cDNAs encoding proteins interacting with the intracellular domain of CD30 in the two-hybrid system.

cDNA	No. of clones	Amino acids encoded by cDNA
TRAF1	2	62–416
TRAF1	4	142–416
TRAF1	5	170–416
TRAF2	4	292–501

Table 2. Localization of the TRAF-binding domain within the CD30 intracellular domain.

Truncations of CD301C		Interaction with TRAF1	Interaction with TRAF2
414	595	+	+
414	538	−	−
414	500	−	−
552	595	+	+

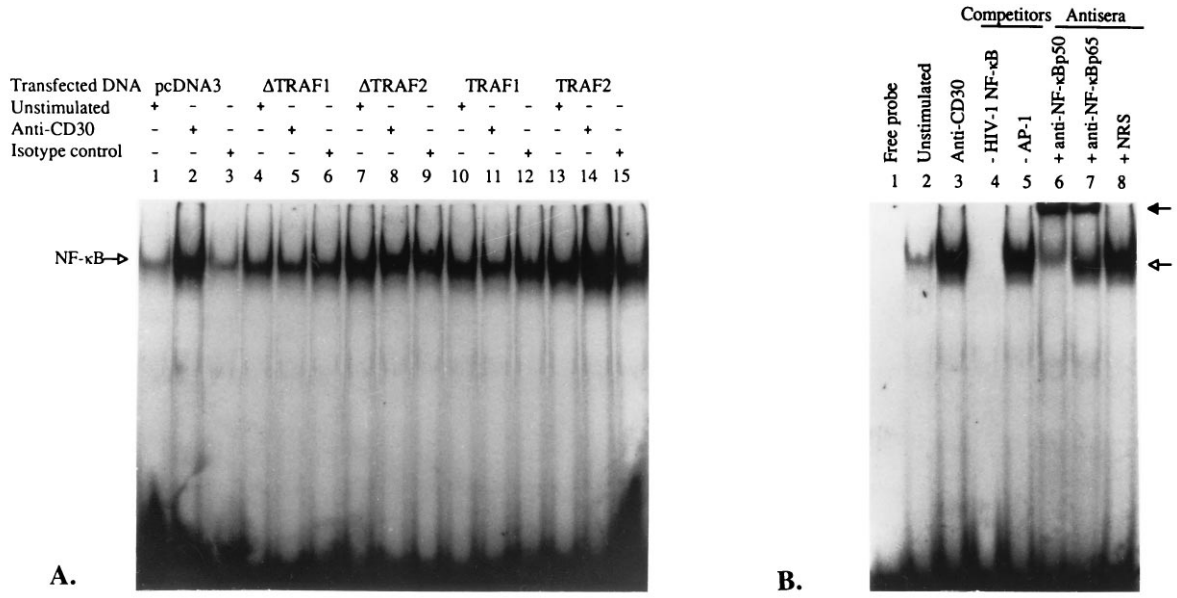

FIG. 2. Induction of NF-κB in KT-3 cells by CD30. (*A*) KT-3 cells were transiently transfected with pcDNA3-based vectors for expression of full-length (TRAF1 or TRAF2) and truncated (ΔTRAF1 or ΔTRAF2) proteins, as indicated. After overnight incubation, KT-3 cells were stimulated by anti-CD30 mAb (C10) for 1 hr, and nuclear extracts were prepared. EMSAs were performed as described. Similar results were obtained in three independent experiments. (*B*) Analysis of the NF-κB-binding complex induced by anti-CD30 mAb. Unlabeled oligonucleotide competitors were used at 100-fold molar excess. For supershift analysis, nuclear extracts from KT-3 cells were preincubated with antiserum to p50, p65, and normal rabbit serum (NRS). Similar results were obtained in three independent experiments. The open arrow denotes the NF-κB complexes.

creased baseline luciferase activity, but inhibited anti-CD30 induction of luciferase gene expression. Overexpression of full-size TRAF2 caused a marked induction of HIV-1-LTR-driven luciferase gene expression, which was further enhanced by anti-CD30 antibody.

DISCUSSION

In this study we show that TRAF1 and TRAF2 proteins directly associate with the intracellular domain of CD30, and that TRAF2, but not TRAF1, mediates CD30 induction of NF-κB activation and HIV-LTR driven transcription in T cells.

Using CD30IC as a bait in the yeast two-hybrid system we isolated cDNA clones that encoded for TRAF1 or TRAF2 proteins (Table 1). Interaction of TRAF1 or TRAF2 proteins with CD30 was confirmed by precipitation of these proteins from cell lysates using a GST-CD30IC fusion protein (Fig. 1). The TRAF domains of TRAF1 or TRAF2 were sufficient for CD30 binding (Table 1). This was consistent with previous data showing that TRAF proteins bind to members of the TNFR family via their C-terminal TRAF domain (19–23, 31). TRAF1 or TRAF2 bound directly (Table 1) and independently of each other to the CD30 cytoplasmic region. To date, CD30 is the only member of the TNFR family that interacts directly with TRAF-1. It was previously shown that, whereas TNFR2 associates with TRAF1 and TRAF2, only TRAF2 contacts TNFR2 directly, whereas TRAF1 interacts with TNFR2 indirectly through heterotrimer formation with TRAF2 (23).

As this work was in progress, two other groups demonstrated binding of TRAF1 and TRAF2 to CD30 (11, 32). In addition to TRAF1 and TRAF2, Gedrich *et al.* (32) isolated TRAF3 in their yeast two-hybrid system screen for CD30-associated proteins. However, the affinity of the TRAF3 binding to CD30 appeared to be weak according to the results of β-galactosidase assays. This may explain why we could not detect TRAF-3 by the yeast two-hybrid screen or by Western blotting of proteins precipitated with GST-CD30IC.

We have used a series of deletion mutants of CD30IC and mapped the region that interacts with TRAF1 or TRAF2 to the C-terminal 53 aa of CD30 (Table 2). This region contains the motif PXQET/D (aa 561–565, PEQET), which has been shown to be important for TRAF3 binding to CD40IC (aa 230–234, PVQET) (31, 32), and which is conserved in several members of the TNFR family including CD30, CD40, lymphotoxin-β receptor, OX40, and CD27. Lee *et al.* (11) mapped the region involved in binding of TRAF1 and TRAF2 to the C-terminal 66 aa of CD30IC, which contains the PEQET motif. Gedrich *et al.* (32) mapped the TRAF interacting region to the last 36 aa of CD30IC, and showed that in addition to the PXQET/D motif, a more C-terminal sequence EEEGKE (aa 580–585) is involved in the binding of TRAF1 or TRAF2 proteins to CD30. Taken together these data suggest that TRAF1 and TRAF2 bind to the same region of CD30.

Transient overexpression of TRAF2, but not of TRAF1, induced nuclear translocation of an NF-κB transcriptional complex, which contained p50 and p65 subunits (Fig. 2) and induced HIV-1-LTR-driven luciferase gene expression in KT-3 cells (Fig. 4). These data suggest that TRAF2, but not TRAF1, mediates CD30-dependent induction of NF-κB activation and HIV-LTR-driven transcription. This is consistent with the observation that CD30 ligation induces NF-κB activity in Jurkat E6.1 T cells, which express TRAF2 but no detectable TRAF1 (Fig. 3).

Overexpression of truncated mutants, ΔTRAF1 or ΔTRAF2, inhibited CD30-induced NF-κB activation and HIV-1-LTR-driven transcription. This is due to the competition of these mutants with native TRAF proteins for binding to CD30. Both ΔTRAF1 and ΔTRAF2 caused a modest degree of NF-κB activation and HIV-1-LTR-driven transcription. As has been previously suggested (22), multimeric complexes between truncated and native proteins may account for this increased baseline activity. Although, the mechanism of activation of NF-κB by TRAF2 is not well understood, it has been recently shown that TRAF2 binds to the cytoplasmic protein TANK, which serves as a co-inducer of NF-κB activation by TRAF2 (31). TRAF2 binds TANK via the same TRAF domain used for binding members of the TNFR family. Moreover, the region of TANK that binds TRAF proteins contains a sequence that is homologous to the PXQET/D motif. Thus, more than one mechanism may be involved in

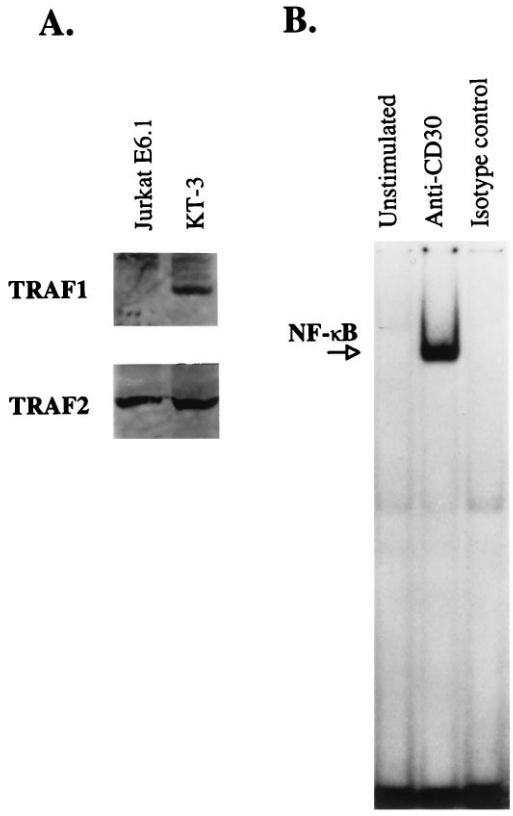

FIG. 3. Induction of NF-κB in Jurkat E6.1 cells by CD30. (*A*) Lysates from Jurkat E6.1 and KT-3 cells were resolved on SDS/12% polyacrylamide gel and Western blotted with rabbit polyclonal antisera directed against TRAF1 and TRAF2. (*B*) Analysis of the NF-κB-binding complex induced by anti-CD30 mAb in Jurkat E6.1 cells. Jurkat E6.1 cells were left unstimulated or stimulated by anti-CD30 mAb (C10) or isotype control antibody for 1 hr. Nuclear extracts were prepared and EMSA was performed as described. Similar results were obtained in three independent experiments. The open arrow denotes the NF-κB complexes.

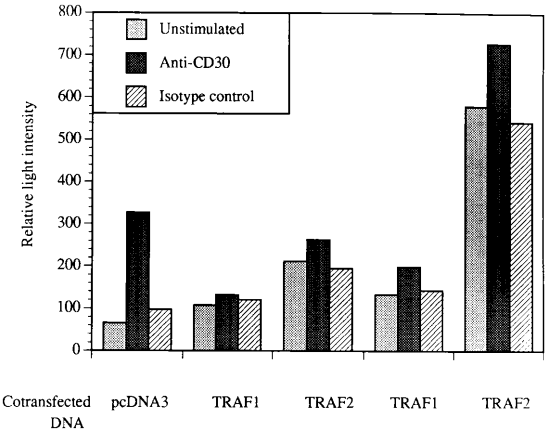

FIG. 4. Effect of full-length and truncated mutants of TRAF1 or TRAF2 on CD30 induction of HIV-1-LTR-driven transcription in T cells. KT-3 cells were transiently cotransfected with HIV-1-LTR-driven pGL3 luciferase reporter vector and pcDNA3-based vectors for expression of TRAF proteins. Four hours later, KT-3 cells were stimulated by anti-CD30 mAb (C10) as described. After 20 hr, cells were collected, lysed, and luciferase activity was measured. Luciferase activity was normalized to the protein concentration in the lysate and expressed as relative light intensity per 1 μg of protein. The open and closed arrows denote the NF-κB and supershifted complexes, respectively. The experiment shown is a representative of four independent experiments.

inhibition of CD30 activation of NF-κB and HIV gene transcription by truncated mutants ΔTRAF1 or ΔTRAF2. These mutants may not only compete for the binding of endogenous TRAF2 to CD30 but may also compete for TANK binding by TRAF2.

Given the extensively documented role of NF-κB in HIV gene expression, our observations strongly suggest that CD30 ligation activates TRAF2 and subsequently NF-κB to result in induction of HIV gene transcription. It is possible that CD30 activation of HIV expression is not restricted to T lymphocytes. A variety of cells including T and B lymphocytes, monocytes, and natural killer cells are able to express both CD30 and/or CD30L. Interaction between CD30 and its ligand may be especially facilitated in lymphoid organs, such as lymph nodes, which are known to harbor large numbers of HIV-infected cells. It has been shown that levels of circulating soluble CD30 (sCD30) are increased in patients infected with HIV (33, 34). This may potentially inhibit the interaction of CD30L and cell surface CD30. However, circulating sCD30 may not be able to interfere with CD30L–CD30 interactions in solid lymphoid organs.

The role of TRAF2 in CD30-mediated HIV gene expression demonstrated in this study suggests that pharmacologic agents that interfere with CD30-TRAF2 interaction or with TRAF2 activity may be useful in the treatment of HIV infection.

We thank Dr. Eckhard R. Podack for providing anti-CD30 mAb (C10). This work was supported by National Institutes of Health Grants AI-31541 and AR-43985 and grants from Baxter Healthcare and Alpha Therapeutics Co. D.A.W. was a Howard Hughes Medical Institute Physician Postdoctoral Fellow.

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