Innovative, Intuitive, Flexible.
Luminex Flow Cytometry Solutions
with Guava® and Amnis® Systems
Learn More >

Luminex
complexity simplified.

This information is current as of January 26, 2022.

# Cutting Edge: Contrasting Roles of TNF Receptor-Associated Factor 2 (TRAF2) and TRAF3 in CD40-Activated B Lymphocyte Differentiation

Bruce S. Hostager and Gail A. Bishop

*J Immunol* 1999; 162:6307-6311; ;
http://www.jimmunol.org/content/162/11/6307

---

**References** This article **cites 27 articles**, 15 of which you can access for free at:
http://www.jimmunol.org/content/162/11/6307.full#ref-list-1

**Why *The JI*?** Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 1999 by The American Association of
Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022

CUTTING EDGE

# Cutting Edge: Contrasting Roles of TNF Receptor-Associated Factor 2 (TRAF2) and TRAF3 in CD40-Activated B Lymphocyte Differentiation[1]

Bruce S. Hostager* and Gail A. Bishop[2]*†‡

**In B lymphocytes, CD40 signals contribute to the activation of Ab secretion, isotype switching, T cell costimulation, and immunological memory. TRAF proteins appear to be important components of the CD40 signal transduction complex, but their roles in the activation of B cell effector functions are poorly understood. We examined the contributions of TNF receptor-associated factor 2 (TRAF2) and TRAF3 to CD40-activated differentiation in mouse B cells transfected with inducible TRAF and dominant-negative TRAF cDNAs. We find that binding of TRAF2 and TRAF3 to CD40 is not required for the induction of Ab secretion, but that both TRAF molecules can regulate the activation process. We demonstrate a negative regulatory role for TRAF3 and that this activity is dependent on the availability of an intact TRAF3-binding site in the cytoplasmic domain of CD40. In contrast, TRAF2 appears to play a positive role in B cell differentiation, and this activity is apparent even when its binding site on CD40 is disrupted.** ***The Journal of Immunology,*** **1999, 162: 6307–6311.**

Engagement of CD40 by its ligand on activated T cells initiates signals in B lymphocytes that contribute to the activation of cell proliferation, differentiation, isotype switching, enhanced Ag presentation, and other events necessary for an efficient humoral immune response (1). The CD40 signal transduction pathway is only partially characterized but appears to involve several members of the TNF receptor-associated factor (TRAF)[3] family of proteins. TRAF2, -3, -5, and -6 all potentially interact with CD40 (2–5), but their roles in CD40 signaling are unclear. TRAF2 (2) and TRAF6 (5) can induce the activation of NF-$\kappa$B when transiently overexpressed in the transformed human kidney cell line 293. In B cell lines, however, mutant CD40 molecules that cannot bind TRAF2 remain able to activate NF-$\kappa$B (6). In addition, lymphocytes from mice expressing a "dominant-negative" TRAF2 (DNTRAF2) transgene do not display defects in CD40- or TNF receptor-induced NF-$\kappa$B activation (7), and fibroblast cell lines isolated from TRAF2-deficient mouse embryos (8) exhibit only slight delays in TNF-induced NF-$\kappa$B activation. However, lymphocytes from DNTRAF2-transgenic mice and TRAF2-deficient fibroblasts do display defects in the activation of c-Jun $NH_2$-terminal kinase mediated by TNFR family members.

The contribution of TRAF3 to CD40 signaling is more enigmatic. Mice deficient in TRAF3 die shortly after birth (9). However, B cells from these mice appear to up-regulate CD23 and proliferate normally in response to CD40 engagement. Fetal liver cells from TRAF3-deficient mice have been used to reconstitute the immune systems of irradiated wild-type mice. Immune responses in the reconstituted mice appear grossly normal, although there is a defect in isotype switching in response to T-dependent Ags. Experiments with TRAF3 in epithelial cell lines suggest that TRAF3 can inhibit CD40-mediated NF-$\kappa$B activation (2, 10).

Although transiently transfected epithelial cell lines have been useful in the preliminary characterization of the TRAF proteins, functional studies have been limited to examining the effects of TRAF (or DNTRAF) overproduction on proximal signaling events, such as NF-$\kappa$B activation. To examine the roles of TRAFs in hemopoietic cells, and specifically their roles in B lymphocyte effector functions, we have used inducible expression vectors to stably transfect mouse B cell lines with wild-type and mutant TRAF molecules. We find that the binding of TRAF2 or TRAF3 is unnecessary for the ability of CD40 to activate Ab secretion. However, both TRAF molecules can strongly modulate this CD40-mediated effector function, and they appear to exert their effects at separate steps in the activation process.

## Materials and Methods

*DNA constructs*

pEFLacR is a modification of p3′SS (Stratagene, La Jolla, CA) in which we replaced the F9-1 promoter with the EF-1$\alpha$ promoter from pEF-BOS (11), to achieve higher levels of Lac repressor (LacR) expression. Human TRAF3 cDNA was prepared from the RAJI B cell line and amplified by PCR using primers containing additional sequence coding for a carboxyl-terminal HA peptide tag. A similarly tagged mouse TRAF3 was prepared from M12.4.1 cDNA. The human TRAF3 construct was used to prepare DNTRAF3 (amino acids 298–568), similar to a mutant molecule previously reported (12). cDNA constructs coding for mouse and human TRAF2

Departments of *Microbiology and †Internal Medicine, University of Iowa, and ‡VA Medical Center, Iowa City, IA 52242

Received for publication February 26, 1999. Accepted for publication March 29, 1999.

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

[1] This work was supported by an Arthritis Foundation Postdoctoral Fellowship awarded to B.S.H., grants to G.A.B. from the National Institutes of Health (AI28847, CA66570), and the Veterans Administration (Merit Review 383). Core support was provided by National Institutes of Health Grant DK25295 to the University of Iowa Diabetes and Endocrinology Research Center.

[2] Address correspondence and reprint requests to Dr. Gail A. Bishop, University of Iowa, Department of Microbiology, 3–570 BSB, Iowa City, IA 52242. E-mail address: gail-bishop@uiowa.edu

[3] Abbreviations used in this paper: DN, dominant-negative; LacR, lac repressor protein; TRAF, TNF receptor-associated factor; BCR, B cell Ag receptor; IPTG, isopropyl $\beta$-D-thiogalactopyranoside.

Copyright © 1999 by The American Association of Immunologists

0022-1767/99/$02.00

Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022

were prepared from M12.4.1 and Ramos cells, respectively. Both TRAF2 constructs contained $NH_2$-terminal FLAG epitope tags. FLAG-tagged mouse DNTRAF2 (2) was provided by Dr. Vishva Dixit (University of Michigan, Ann Arbor, MI). All TRAF cDNA constructs used in stable transfections were subcloned into the inducible expression vector pOPRSVI (Stratagene). cDNA constructs used in 293T transient expression assays were subcloned into the pRSV.5(neo) or pRSV.5(hyg) expression vectors (13). Human CD40 constructs were previously reported (14), and for some transfections they were subcloned into an expression vector coding for zeomycin resistance (Invitrogen, Carlsbad, CA).

### *Cell lines*

The mouse B cell line CH12.LX has been described (15). CH12.LacR cell lines were produced by stably transfecting CH12.LX cells with p3′SS or pEFLacR. *Spodoptera frugiperda* (*Sf*9) cells expressing mCD40L were prepared as previously described (14). The human kidney cell line 293T (16) was a gift of Dr. Vishva Dixit. Human Ramos B cells were from the American Type Culture Collection (ATCC, Manassas, VA).

### *Transfections*

Stable transfections of mouse B cell lines were conducted using electroporation as described (14). Transient transfection of 293T cells was accomplished using a calcium phosphate-based method (17).

### *Immunoprecipitations and Western blotting*

Transfected 293T cells were removed from plates with 5 mM EDTA in serum-free DMEM. Cells were suspended in buffer (5.4 mM KCl, 137 mM NaCl, 4.2 mM $NaHCO_3$, pH 8.1) and surface-biotinylated with sulfo-NHS-biotin (Pierce Chemical, Rockford, IL). Cells were washed once in complete culture medium and then resuspended in low salt lysis buffer (1% Triton X-100, 20 mM Tris (pH 8.0), 137 mM NaCl, with protease and phosphatase inhibitors) and incubated for 30 min on ice. Cleared supernatants were incubated for 2 h at 4°C (on rotator) with protein G-agarose beads (Sigma Chemical, St. Louis, MO) preconjugated with anti-hCD40 mAb. Beads were washed four times with modified lysis buffer containing 400 mM NaCl and then once with low salt lysis buffer. Immunoprecipitation from B cell lysates was performed essentially as above, except that cell lysates were prepared by resuspending $5 \times 10^6$ cells in 500 $\mu$l low salt lysis buffer and incubating for 15 min at 37°C. Immunoprecipitated proteins were separated by SDS-PAGE and then electroblotted onto nitrocellulose membranes. A chemiluminescent substrate (Pierce) was used to detect HRP-labeled Abs and streptavidin-HRP (Sigma) on Western blots.

### *Ab secretion assays*

Ab secretion assays were performed as described (18), except that cells were preincubated for 24 h in 96-well flat bottom plates with 100 $\mu$M isopropyl $\beta$-D-thiogalactopyranoside (IPTG) to induce production of TRAF proteins (150 $\mu$l and 1500 cells/well). Stimuli were then added (in a volume of 50 $\mu$l), and the cells were incubated for an additional 48 h. A 0.1% suspension of sheep RBC was used as Ag, anti-mCD40 Ab was used at a final concentration of 1 $\mu$g/ml, anti-hCD40 at 5 $\mu$g/ml, LPS (*Escherichia coli* serotype 055:B5, Sigma) at 50 $\mu$g/ml, and CD40L cells were used at a ratio of 1 CD40L-expressing *Sf*9 cell/4 B cells. CH12.LX cells produce IgM specific for phosphatidylcholine, an Ag on the surface of sheep RBC allowing detection of Ab-secreting cells by their ability to form lytic plaques on a lawn of RBC in the presence of complement. Results are presented as the ratio of plaque-forming cells to viable cells recovered from each culture.

### *Abs*

mAb to mouse CD40 (clone 1C10, rat IgG2a) (19) was kindly provided by Dr. Frances Lund, The Trudeau Institute, Saranac Lake, NY. MOPC-21 (mouse IgG1) was from Sigma. Ab to human CD40 (clone G28-5, ATCC) was purified from cell culture supernatants. Polyclonal goat anti-rabbit Ab conjugated to HRP was from Bio-Rad (Hercules, CA). Polyclonal rabbit Abs to TRAF2 and TRAF3 were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-HA mAb was from Berkeley Antibody (Richmond, CA), and anti-FLAG mAb was from Sigma.

## Results and Discussion

### *CD40-TRAF2/3 binding is not required for the induction of Ab secretion*

Stimulation of the mouse B cell line CH12.LX with anti-CD40 Abs or CD40L results in the induction of Ab secretion as measured by the increased frequency of differentiated, Ab-forming cells (20). We previously utilized CH12.LX to characterize regions of the cytoplasmic domain of CD40 important for signaling (14). A mutant CD40 molecule containing a threonine to alanine substitution at position 234 ($CD40A^{234}$) was fully able to activate differentiation/Ab secretion, although this mutant is reported to have no TRAF3-binding activity (3). This suggests that TRAF3 binding is not required for CD40-mediated Ab secretion. The observation that TRAF2 and TRAF3 appear to bind the same site on CD40 (21, 22) predicts that $CD40A^{234}$ lacks TRAF2 binding activity as well. To confirm that $CD40A^{234}$ fails to bind TRAF2 and TRAF3, 293T cells were transiently transfected with TRAF2 or TRAF3 and wild-type hCD40 or $hCD40A^{234}$. CD40 immunoprecipitates from cell lysates were tested for coprecipitation of the TRAFs. As a negative control, we used a CD40 molecule truncated by 32 amino acids (hCD40Δ32), which lacks the PXQXT minimal TRAF-binding motif (21–23) (Fig. 1*A*). We also tested a CD40 molecule truncated by 22 amino acids (hCD40Δ22), the functional activity of which is very similar to that of $hCD40A^{234}$ (14), suggesting that it may also fail to bind TRAF2 and TRAF3. Like $hCD40A^{234}$, hCD40Δ22 cannot induce up-regulation of B7–1 but can stimulate Ab secretion. Although this molecule retains the minimal TRAF binding motif, it appears that amino acid residues adjacent to this sequence may also effect TRAF binding (23). Fig. 1 shows that both TRAFs coprecipitated with full-length CD40, and to a very limited extent with $hCD40A^{234}$ (seen on long exposures, not shown). The TRAFs did not coprecipitate with hCD40Δ32 or hCD40Δ22. Together, the data in Fig. 1 and our previous functional experiments (14) indicate that CD40 can activate Ab secretion in CH12.LX cells in the absence of TRAF2 or TRAF3 binding.

### *Effects of Inducible TRAF2 and TRAF3 on CD40-activated Ab secretion*

Although not absolutely required for CD40-induced Ab secretion, TRAF2 and TRAF3 may still regulate this effector function. To test this hypothesis, we examined the effects of inducible TRAF2 and TRAF3 expression on CD40-activated Ab secretion. CH12.LX cells were first stably transfected with a plasmid coding for LacR protein, generating the cell line CH12.Lac. CH12.Lac cells were then transfected with plasmids encoding TRAF constructs behind a promoter containing LacR-binding sites. TRAF expression was therefore inducible with IPTG (a LacR inactivator) (Fig. 2). Thus, cells could be transfected with cDNA constructs coding for proteins that might interfere with cell growth, and the effects of high (induced) and low (basal) TRAF expression could be compared in the same transfectant.

In the absence of IPTG, CH12.Lac cells transfected with an inducible human TRAF3 construct (CH12.T3 cells) differentiated into Ab-secreting cells in response to CD40 ligand (Fig. 3*A*). However, IPTG-induced overexpression of TRAF3 in CH12.T3 almost completely abrogated CD40-stimulated differentiation. IPTG did not inhibit CD40-stimulated differentiation of CH12.Lac. Induced overexpression of a mouse TRAF3 construct had the same effect (data not shown). Although B cell Ag receptor (BCR) signaling alone cannot activate Ab secretion by CH12.LX cells, CD40-mediated Ab secretion can be significantly enhanced by BCR costimulation (20). However, even this costimulation could not overcome the inhibitory activity of inducible TRAF3 (Fig. 3*A*). In spite of the dramatic inhibitory effects of TRAF3, its induction had no detectable effects on cell viability. Also, the results of the Ab secretion assays were normalized to the number of viable cells recovered following stimulation. In contrast to the effects of TRAF3,

Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022

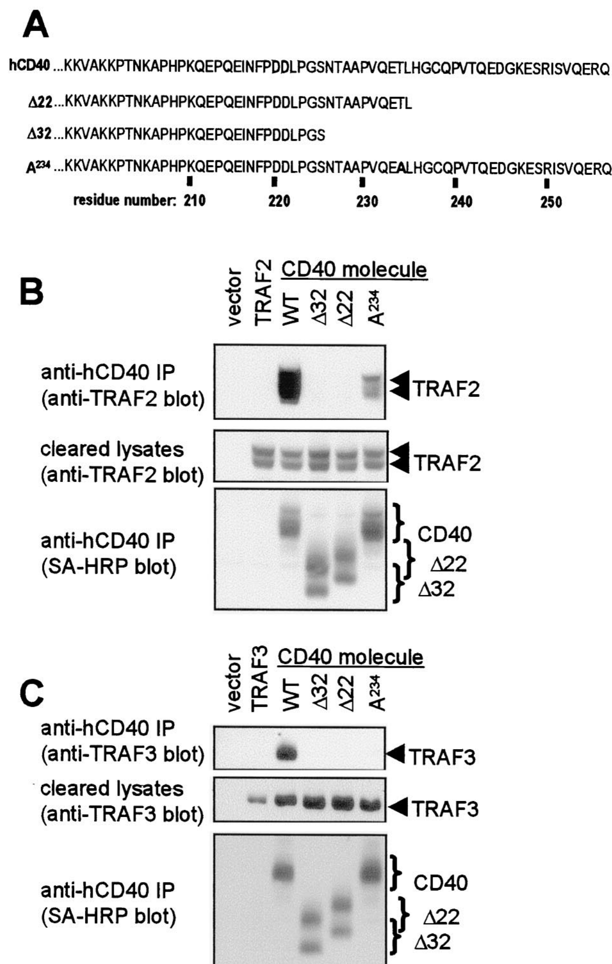


**FIGURE 1.** TRAF2 and TRAF3 do not bind truncated hCD40. *A*, Cytoplasmic domains of hCD40 molecules. 293T cells were transiently transfected with human TRAF2 (*B*) or human TRAF3 (*C*) and hCD40, hCD40Δ32, hCD40Δ22, or hCD40A$^{234}$. hCD40 immunoprecipitations were conducted as in *Materials and Methods.* Western blots of preimmunoprecipitation lysates demonstrate similar TRAF production in each of the transfected cultures. Reprobing the blots with streptavidin-HRP demonstrated that similar amounts of CD40 were in each lane. Similar results were obtained in two other experiments and also using mouse TRAF2 (not shown).

inducible mouse TRAF2 increased the frequency of Ab-secreting cells in CD40-stimulated cultures (Fig. 3*B*).

To test the specificity of the effects of induced TRAF2 and TRAF3, we examined their effects on LPS-stimulated Ab secretion. LPS is a potent activator of Ab secretion in freshly isolated B cells and CH12.LX (15). The effects of TRAF3 overexpression were specific for CD40 signaling, as inducible TRAF3 did not affect LPS-stimulated Ab secretion (Fig. 4*A*). The coengagement of class II MHC and BCR can also activate Ab secretion in CH12.LX cells (15), but this stimulus was also unaffected by TRAF3 overexpression (not shown). Interestingly, overexpression of TRAF2 augmented Ab secretion stimulated by LPS (Fig. 4*B*) and consistently increased the basal frequency of Ab secreting cells by ~2-fold, whereas the induced expression of TRAF3 did not appear to alter basal differentiation.

### *Effects of DNTRAF molecules on CD40-activated Ab secretion*

Various DNTRAF molecules appear to interfere with CD40 signaling (2, 12, 24). These molecules lack portions of the $NH_2$-terminal zinc ring or zinc finger domains, yet retain the ability to bind CD40. On the basis of the results of our experiments with wild-type TRAF2 and TRAF3, we predicted that DNTRAF2 would inhibit and DNTRAF3 would augment CD40-activated Ab secretion. Indeed, induced expression of DNTRAF2 significantly inhibited Ab secretion stimulated by either LPS or anti-CD40 mAb (Fig. 4*C*). DNTRAF2 also consistently inhibited basal (unstimulated) Ab secretion by ~50%.

Because inducible TRAF3 inhibited CD40-mediated Ab secretion, it seemed possible that DNTRAF3 would augment Ab secretion by interfering with the activity of endogenous TRAF3. However, the expression of DNTRAF3 was also inhibitory (Fig. 4*D*), suggesting that the physical association of TRAF3 with CD40 mediates its negative regulatory function, possibly by altering the stoichiometry of CD40-associated proteins. The effects of DNTRAF3 were specific for CD40 signaling, given that LPS-stimulated Ab secretion was unaffected.

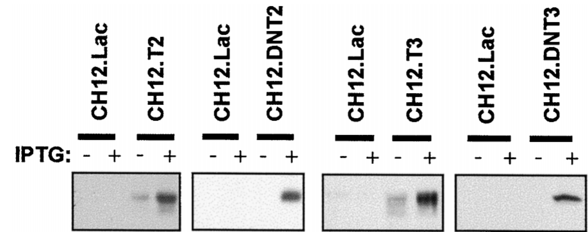


**FIGURE 2.** Inducible expression of TRAF2 and TRAF3 in transfected CH12.Lac cells. Cells were incubated with or without 100 μM IPTG for 48 h. Epitope-tagged TRAF proteins were immunoprecipitated from cell lysates using anti-FLAG mAb (TRAF2) or anti-HA (TRAF3). TRAF molecules (TRAF2 (T2) ~56kDa, DNTRAF2 (DNT2) ~47 kDa, TRAF3 (T3) ~63 kDa, DNTRAF3 (DNT3) ~19 kDa) were detected on Western blots with rabbit polyclonal antisera.

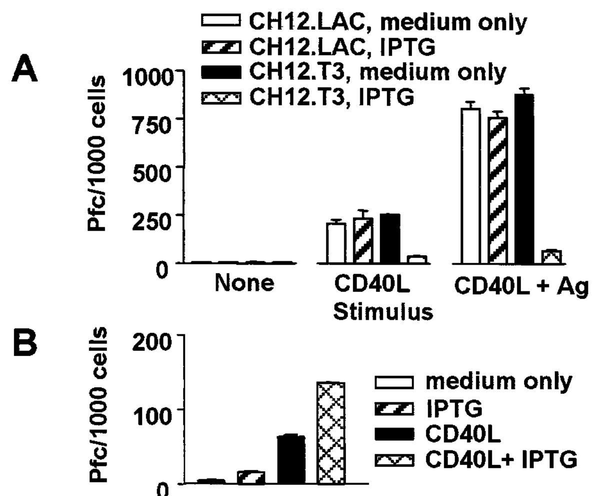


**FIGURE 3.** Induced expression of TRAF3 inhibits, and TRAF2 augments, CD40-mediated Ab secretion. *A*, CH12.Lac and CH12.T3 cells, ± IPTG were stimulated with CD40L and Ag as described in *Materials and Methods*. The number of plaque-forming cells (PFC) per thousand viable cells recovered is indicated. Results are the mean ± SE of replicate samples. Similar results were obtained in two additional experiments, in one of which different clones were tested. *B*, CH12.T2 cells were stimulated with CD40L as in *Materials and Methods.* Similar results were obtained in two additional experiments, and three additional experiments with a second CH12.T2 clone.

Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022

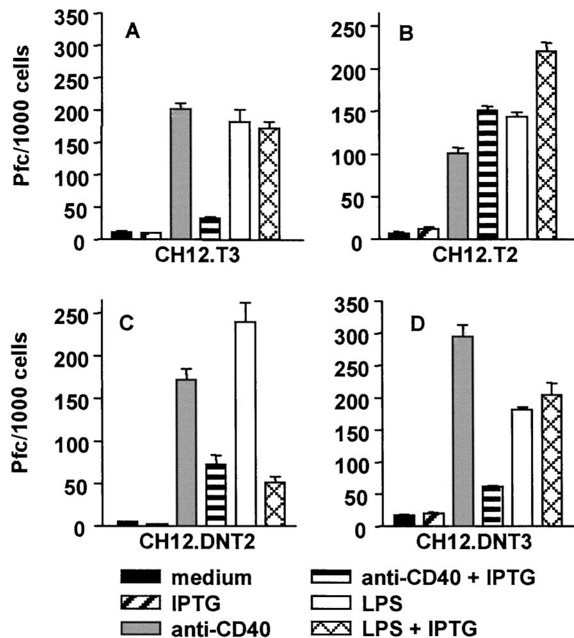


**FIGURE 4.** Effects of inducible TRAF and DNTRAF molecules on Ab secretion stimulated by anti-CD40 or LPS. Induction of TRAF3 (*A*), TRAF2 (*B*), DNTRAF2 (*C*), or DNTRAF3 (*D*), and stimuli were as in *Materials and Methods*. Similar results were obtained in a second experiment for each molecule.

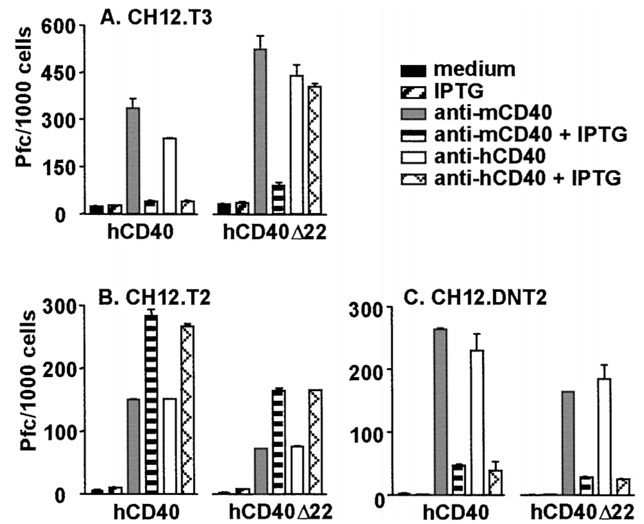


**FIGURE 5.** Effects of inducible TRAF2 and TRAF3 on Ab secretion stimulated by truncated CD40. CH12.T3 (*A*), CH12.T2 (*B*), and CH12.DNT2 (*C*) were stably transfected with either full-length hCD40 or hCD40Δ22. IPTG and anti-CD40 mAbs were added as in *Materials and Methods.* Similar results were obtained in two additional CH12.T3 experiments, a second CH12.T2 experiment, and a second CH12.DNT2 experiment.

*Effects of inducible TRAFs on hCD40Δ22-activated Ab secretion*

Because LPS-stimulated Ab secretion can be modified by inducible TRAF2, it is possible that the TRAFs may also exert their effects without binding CD40. To test this, CH12.T2, CH12.DNT2 and CH12.T3 were transfected with wild-type hCD40 or hCD40Δ22 (expression confirmed by flow cytometry, not shown) and were tested in Ab secretion assays. Mouse and human CD40 have highly homologous cytoplasmic domains and have indistinguishable function in mouse B cell lines (6, 14). Species-specific mAbs allow stimulation of cells through either the transfected hCD40, or the endogenous mCD40 as a control. Inducible TRAF3 and DNTRAF2 inhibited the activation of Ab secretion mediated by either hCD40 or mCD40 (Fig. 5, *A* and *C*). However, Ab secretion stimulated by CD40Δ22 was not affected by inducible TRAF3 (Fig. 5*A*). Thus, an intact TRAF2/3-binding site on CD40 is required for TRAF3 to regulate Ab secretion. Interestingly, inducible TRAF2 was able to enhance (and DNTRAF2 inhibited) basal Ab secretion as well as secretion mediated by either hCD40 or hCD40Δ22 (Fig. 5, *B* and *C*). These observations indicate that overexpression of TRAF2 may bypass early steps in CD40 signaling or augment signaling through other cell surface receptors that utilize TRAF2, such as the TNF receptors (25), and CD27 (26). We are currently examining potential contributions of these receptors to the activation of Ab secretion in CH12.LX cells.

Although TRAF2 and TRAF3 share a binding site on CD40, competition for this site may not explain the ability of TRAF3 to inhibit signaling. Clearly, CD40Δ22 remains able to stimulate Ab secretion even though it cannot bind TRAF2. Alternatively, we suggest that TRAF3 may interfere with the binding of other signaling proteins to CD40 or may serve to recruit inhibitory molecules. Potentially, TRAF3 disrupts interactions of CD40 with TRAF6, the binding site of which on CD40 lies between the membrane and the TRAF2/3 site, and the role of which in Ab secretion remains to be tested.

With notable exceptions (7, 9, 24, 27, 28), much of the functional characterization of TRAF2 and TRAF3 has been conducted in transiently transfected epithelial cell lines. We demonstrate that inducible vector systems can be used to stably transfect lymphocyte cell lines with TRAF cDNA constructs, facilitating the examination of the roles of TRAFs in lymphocyte effector functions. Our results demonstrate that the roles played by TRAF molecules in CD40-stimulated Ab secretion are complex, potentially contributing to both positive and negative regulation, and suggest the potential for the cellular regulation of CD40 signaling through modulation of TRAF expression.

## Acknowledgments

We thank Luis Ramirez for technical assistance and Dr. John Harty for critical review of the manuscript.

## References


1. Foy, T. M., A. Aruffo, J. Bajorath, J. E. Buhlmann, and R. J. Noelle. 1996. Immune regulation by CD40 and its ligand GP39. *Annu. Rev. Immunol. 14:591.*
2. Rothe, M., V. Sarma, V. W. Dixit, and D. V. Goeddel. 1995. TRAF2-mediated activation of NF-κB by TNF receptor 2 and CD40. *Science 269:1424.*
3. Hu, H. M., K. O'Rourke, M. S. Boguski, and V. M. Dixit. 1994. A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J. Biol. Chem. 269:30069.*
4. Ishida, T., T. Tojo, T. Aoki, N. Kobayashi, T. Ohishi, T. Watanabe, T. Yamamoto, and J. Inoue. 1996. TRAF5, a novel TNF receptor-associated factor family protein, mediates CD40 signaling. *Proc. Natl. Acad. Sci. USA 93: 9437.*
5. Ishida, T., S. Mizushima, S. Azuma, N. Kobayashi, T. Tojo, K. Suzuki, S. Aizawa, T. Watanabe, G. Mosialos, E. Kieff, T. Yamamoto, and J. Inoue. 1996. Identification of TRAF6, a novel tumor necrosis factor receptor-associated factor protein that mediates signaling from an amino-terminal domain of the CD40 cytoplasmic region. *J. Biol. Chem. 271:28745.*
6. Hsing, Y., B. S. Hostager, and G. A. Bishop. 1997. Characterization of CD40 signaling determinants regulating NF-κB activation in B lymphocytes. *J. Immunol. 159:4898.*
7. Lee, S. Y., A. Reichlin, A. Santana, K. A. Sokol, M. C. Nussenzweig, and Y. Choi. 1997. TRAF2 is essential for JNK but not NF-κB activation and regulates proliferation and survival. *Immunity 7:703.*
8. Yeh, W. C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J. L. Delapompa, D. Ferrick, B. Hum, N. Iscove, P. Ohashi, M. Rothe, D. V. Goeddel, and T. W. Mak. 1997. Early lethality, functional NF-κB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity 7:715.*


Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022

9. Xu, Y., G. Cheng, and D. Baltimore. 1996. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity 5:407.*
10. Miller, W. E., G. Mosialos, E. Kieff, and N. Raab-Traub. 1997. Epstein-Barr virus LMP1 induction of the epidermal growth factor receptor is mediated through a TRAF signaling pathway distinct from NF-κB activation. *J. Virol. 71:586.*
11. Mizushima, S., and S. Nagata. 1990. pEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Res. 18:5322.*
12. Cheng, G., A. M. Cleary, Z. S. Ye, D. I. Hong, S. Lederman, and D. Baltimore. 1995. Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science 267:1494.*
13. Long, E. O., B.-S. Rosen, D. R. Karp, M. Malnati, R. P. Sekaly, and D. Jaraquemada. 1991. Efficient cDNA expression vectors for stable and transient expression of HLA-DR in transfected fibroblast and lymphoid cells. *Hum. Immunol. 31:229.*
14. Hostager, B. S., Y. Hsing, D. E. Harms, and G. A. Bishop. 1996. Different CD40-mediated signaling events require distinct CD40 structural features. *J. Immunol. 157:1047.*
15. Bishop, G. A., and G. Haughton. 1986. Induced differentiation of a transformed clone of Ly-1$^+$ B cells by clonal T cells and antigen. *Proc. Natl. Acad. Sci. USA 83:7410.*
16. DuBridge, R. B., P. Tang, H. C. Hsia, P. Leong, J. H. Miller, and J. H. Calos. 1987. Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol. Cell. Biol. 7:379.*
17. Kingston, R. E. 1995. Introduction of DNA into mammalian cells. In *Current Protocols in Molecular Biology.* K. Janssen, ed. John Wiley and Sons, Brooklyn, NY.
18. Bishop, G. A. 1991. Requirements of class II-mediated B cell differentiation for class II crosslinking and cAMP. *J. Immunol. 147:1107.*
19. Heath, A. W., W. W. Wu, and M. C. Howard. 1994. Monoclonal antibodies to murine CD40 define two distinct functional epitopes. *Eur. J. Immunol. 24:1828.*
20. Bishop, G. A., W. D. Warren, and M. T. Berton. 1995. Signaling via MHC class II molecules and antigen receptors enhances the B cell response to gp39/CD40 ligand. *Eur. J. Immunol. 25:1230.*
21. Pullen, S. S., H. G. Miller, D. S. Everdeen, T. T. A. Dang, J. J. Crute, and M. R. Kehry. 1998. CD40-tumor necrosis factor receptor-associated factor (TRAF) interactions: regulation of CD40 signaling through multiple TRAF binding sites and TRAF hetero-oligomerization. *Biochemistry 37:11836.*
22. Boucher, L. M., L. E. Marengere, Y. Lu, S. Thukral, and T. W. Mak. 1997. Binding sites of cytoplasmic effectors TRAF1, 2, and 3 on CD30 and other members of the TNF receptor superfamily. *Biochem. Biophys. Res. Commun. 233:592.*
23. Cheng, G., and D. Baltimore. 1996. TANK, a co-inducer with TRAF2 of TNF- and CD40L-mediated NF-κB activation. *Genes Dev. 10:963.*
24. Grammer, A. C., J. L. Swantek, R. D. McFarland, Y. Miura, T. Geppert, and P. E. Lipsky. 1998. TRAF3 signaling mediates activation of p38 and Jun N-terminal kinase, cytokine secretion, and Ig production following ligation of CD40 on human B cells. *J. Immunol. 161:1183.*
25. Smith, C. A., T. Farrah, and R. G. Goodwin. 1994. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell 76:959.*
26. Maurer, D., w. Holter, O. Majdic, G. F. Fischer, and W. Knapp. 1991. CD27 expression by a distinct subpopulation of human B lymphocytes. *Eur. J. Immunol. 20:2679.*
27. Devergne, O., E. Hatzivassiliou, K. M. Izumi, K. M. Kaye, M. F. Kleijnen, E. Kieff, and G. Mosialos. 1996. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-κB activation. *Mol. Cell. Biol. 16:7098.*
28. Kuhné, M. R., M. Robbins, J. E. Hambor, M. F. Mackey, Y. Kosaka, T. Nishimura, J. P. Gigley, R. J. Noelle, and D. M. Calderhead. 1997. Assembly and regulation of the CD40 receptor complex in human B cells. *J. Exp. Med. 186:337.*

Downloaded from http://www.jimmunol.org/ by guest on January 26, 2022