Tumor Necrosis Factor Receptor (TNFR)-associated Factor 5 Is a Critical Intermediate of Costimulatory Signaling Pathways Triggered by Glucocorticoid-induced TNFR in T Cells*

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Tumor necrosis factor receptor (TNFR) family members such as glucocorticoid-induced TNFR (GITR) control T cell activation, differentiation, and effector functions. Importantly, GITR functions as a pivotal regulator of physiologic and pathologic immune responses by abrogating the suppressive effects of T regulatory cells and costimulating T effector cells. However, the molecular mechanisms underlying GITR-triggered signal transduction pathways remain unclear. Interestingly, GITR-induced stimulation of TNFR-associated factor (TRAF) 5-deficient T cells resulted in decreased activation of nuclear factor κB as well as the mitogen-activated protein kinases p38 and extracellular signal-regulated protein kinase, whereas activation of c-Jun N-terminal kinase was less affected. Consistent with impaired signaling, costimulatory effects of GITR were diminished in TRAF5−/− T cells. In sum, our studies indicate that TRAF5 plays a crucial role in GITR-induced signaling pathways that augment T cell activation.

GITR, which is expressed at low levels on naïve T effector cells and at elevated levels on CD4+CD25+ T regulatory cells and activated T effector cells, provides costimulatory signals that enhance T cell proliferation, survival, and cytokine production (1–4). GITR-induced signaling renders T effector cells refractory to suppression by T regulatory cells and regulates T cell-mediated pathologies in animal models, such as autoimmune gastritis, type I diabetes, and autoimmune encephalomyelitis (5–8). Although the importance of GITR in modulating immune responses has become apparent, relatively little is known about the molecular mechanisms of GITR-induced signaling (for review, see Ref. 9).

TRAFs are adapter proteins that transmit extracellular signals upon recruitment to the cytoplasmic domains of tumor necrosis factor receptors (TNFRs) where they serve as foci for the induction of molecular events including nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) activation (10, 11). TRAF5 has been implicated in signaling pathways triggered by several TNFRs, where it can serve as a surrogate for TRAF2 as evidenced by the redundancy of the two TRAFs in TNFα-mediated NF-κB activation (12). In contrast to the postnatal lethality and profound disruption of immune homeostasis observed in mice lacking TRAF2, TRAF5-deficient mice generated independently by us and others lack gross abnormalities (13,14). Despite inconspicuous defects in signaling triggered by CD27 and CD40, TRAF5-deficient lymphocytes display reduced responsiveness to stimulation by these TNFRs (13). TRAF5 has also been implicated in limiting the induction of T helper type 2 responses likely triggered by Ox40 (16). Given the predominant function of TRAF2 and the mild phenotype of TRAF5-deficient mice, a clear role for TRAF5 in TNFR-induced signaling has yet to emerge.

GITR activates all three subfamilies of MAPK and the canonical NF-κB pathway and promotes survival during early stages of T cell activation (4). Our recent results indicate that GITR employs TRAFs as signaling intermediates in a manner distinct from other TNFRs. Specifically, TRAF4, the orphan member of the TRAF family, enhances NF-κB activation triggered by GITR (17). Intriguingly, TRAF2 negatively regulates GITR-induced NF-κB activation, implying disparate functions of TRAF5 and TRAF2 downstream of GITR (9, 19). Co-immunoprecipitation analysis of transfected human embryonic kidney 293 (HEK293) cells did not reveal interactions between TRAF5 and the human homologue of GITR, termed activation-inducible TNFR, arguing that TRAF5 is not engaged in GITR-mediated signaling (20). However, more recently published data suggest a weak interaction between GITR and TRAF5 (21). Further, despite weak or transient interaction between GITR and TRAF2 as well as the apparent lack of interaction between GITR and TRAF4, both TRAFs are critical for regulating GITR-induced NF-κB activation (9, 17, 19). To define further the molecular mechanisms of GITR-induced signaling, we analyzed the impact of TRAF5 deficiency on signaling pathways and T cell activation after GITR cross-linking. These studies revealed that TRAF5 is crucial for activation of NF-κB and specific subsets of MAPK as well as T cell costimulation triggered by GITR.

EXPERIMENTAL PROCEDURES

Animals—To generate TRAF5−/− mice, a 6-kb KpnI-HindIII fragment containing exons I and II of TRAF5 was replaced with a neomycin resistance cassette placed in reverse transcriptional orientation in a targeting construct. R1 embryonic stem cells electroporated with this construct were selected and maintained as previously described (22). Multiple isolated clones were injected and chimeras were bred to C57BL/6 mice purchased from Jackson Laboratories. The mutant allele was backcrossed for more than 10 generations to the DO11.10 and C57BL/6

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‡The abbreviations used are: GITR, glucocorticoid-induced TNFR; TNFR, tumor necrosis factor receptor; MAPK, mitogen-activated protein kinase; HEK, human embryonic kidney; Ab, antibody; IL, interleukin; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase.

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backgrounds, respectively. DO11.10 mice expressing a T cell receptor transgene specific for a peptide of ovalbumin (OVA323–339) presented on I-A<sup>d</sup> were a gift from Dr. K. Murphy (23). All mice were housed under specific pathogen-free conditions in accordance with the guidelines of the Animal Studies Committee of Washington University.

Antibodies—Abs specific for phosphorylated and total extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 were purchased from Cell Signaling Technology. Polyclonal anti-I<sub>κB</sub>α (BD Biosciences), polyclonal anti-TRAF5 (C19; Santa Cruz Biotechnology), monoclonal anti-actin (C4; Chemicon International), and polyclonal secondary Abs coupled to horseradish peroxidase (Santa Cruz Biotechnology) were used for Western blotting. Anti-CD3 Ab and control, GITR-specific, or CD28-specific Abs (5 μg/ml) were treated with GITR-specific or control Ab (5 μg/ml) at 37 °C for indicated times. MAPK and NF-κB activation were measured as described previously (4). NF-κB activity was also quantified by analyzing the relative luciferase units from the reporter plasmid containing two canonical NF-κB sites with units from a similar reporter plasmid containing only a minimal promoter.

Immunoblot Analysis—DO11.10 splenocytes were isolated and activated as described previously (4). Activated DO11.10 splenocytes (5 × 10<sup>6</sup> cells) from TRAF5<sup>−/−</sup>, TRAF5<sup>+/−</sup>, or TRAF5<sup>+/+</sup> littermates were treated with GITR-specific or control Ab (5 μg/ml) at 37 °C for indicated times. MAPK and NF-κB activation were measured as described previously (4). NF-κB activation was also quantified by analyzing the relative I<sub>κB</sub>α levels in cells. To normalize cellular levels of I<sub>κB</sub>α to actin or other protein loading controls, short exposures of Western blots of three independent experiments were scanned and analyzed using ImageJ software (version 1.34n, Wayne Rasband, National Institutes of Health).

Proliferation Assays and Cytokine Production—TRAF5-deficient or -sufficient cells (10<sup>5</sup>) were plated in triplicate in 96-well plates and treated with the indicated concentrations of OVA323–339 or soluble anti-CD3 Ab and control, GITR-specific, or CD28-specific Abs (5 μg/ml).

FIGURE 1. Generation of TRAF5-deficient mice. A, the genomic organization of the trAF5 gene, targeting construct, recombinant allele, and probe used for Southern blot analysis. Restriction sites: H, HindIII; R, EcoRI; K, KpnI; and X, XhoI. B, Southern blot analysis of EcoRI-digested genomic DNA from TRAF5 mutant mice where the 15-kb fragment indicates the wild type allele and the 8-kb fragment denotes the recombinant allele. C, Western blot analysis of TRAF5 expression in antigen-activated splenocytes from DO11.10 TRAF5<sup>−/−</sup> and TRAF5<sup>+/+</sup> mice. Actin served as protein loading control.

FIGURE 2. TRAF5 deficiency does not cause inherent defects in T cell activation. Naive DO11.10 splenocytes of the indicated genotypes were cultured with 0.3 μg OVA<sub>323–339</sub> peptide for 3 days and rested for 18 h. A, GITR surface expression was analyzed by flow cytometry. The dotted line indicates isotype control staining. GITR expression is depicted by the gray shaded line for TRAF5<sup>−/−</sup> T cells and the dark line for TRAF5<sup>+/+</sup> T cells. B, activated T cells were double stained for CD4 and CD25 prior to flow cytometric analysis. Similar expression patterns were observed in two additional experiments.
TRAF5 Positively Regulates NF-κB Activation Triggered by GITR—We have previously shown that GITR employs TRAF2 as a negative regulator of NF-κB activation (19). Therefore, we hypothesized that other TRAFs are involved in activating this pathway downstream of GITR. IκBα is a crucial regulator of NF-κB activation (for review, see Refs. 25–27). Because IκBα degradation has been firmly established as a critical molecular event in the canonical NF-κB activation pathways and IκBα itself is a transcriptional target of NF-κB, we have used cellular IκBα levels as surrogate markers for NF-κB activation similar to our previous studies (4, 19). DO11.10 T cells were activated with cognate antigen (OV A323–339) to augment GITR expression. GITR cross-linking on activated DO11.10 T cells from TRAF5−/−, TRAF5+/+, and TRAF5+/− littermates revealed significantly diminished NF-κB activation, monitored by IκBα degradation, in the absence of TRAF5−/− (Fig. 3A). To quantify these results, the levels of IκBα were normalized to actin or other protein loading controls using ImageJ software (National Institutes of Health) after scanning short exposures of Western blots from three individual experiments. These analyses confirmed a statistically significant reduction of NF-κB activation in TRAF5-deficient T cells compared with TRAF5-sufficient controls (Fig. 3B and data not shown). Although TRAF5 heterozygous T cells exhibited a delay and
reduced magnitude in IκBα degradation compared with control cells in some experiments, the differences to TRAF5−/− cells were not significant. Interestingly, the lack of TRAF5 resulted in increased levels of IκBα in response to GITR-induced signaling, suggesting that TRAF5-independent pathways play also a role in regulating transcription or stability of IκBα. Taken together, these results argue that TRAF5 is a critical regulator of GITR-induced NF-κB activation but that one copy of TRAF5 in the genome is sufficient to control downstream signaling events.

To investigate further the role of TRAF5 in GITR-induced NF-κB activation, HEK293 cells were transiently transfected with expression constructs of GITR ligand and full-length GITR as well as TRAF5 similar to our published studies of TRAF4 and TRAF2 (17, 19). Consistent with these previous results, co-expression of GITR and GITR ligand triggered NF-κB activation in HEK293 cells (Fig. 3C). However, in contrast to TRAF2 and TRAF4, exogenous expression of TRAF5 was sufficient to activate NF-κB in HEK293 cells. Furthermore, GITR-induced NF-κB activation in the presence of exogenous TRAF5 was not altered beyond the level triggered by either stimulus alone. Decreasing TRAF5 expression by titrating the TRAF5 expression construct produced similar findings, suggesting that exogenous expression of TRAF5 causes maximal NF-κB activation that masks any augmentation of GITR-induced NF-κB activation (data not shown). As a control, HEK293 cells were co-transfected with GITR, GITR ligand, and TRAF4, which resulted in GITR-induced NF-κB activation as previously described (17). In sum, these findings indicate that TRAFs fulfill distinct functions downstream of GITR and suggest distinct regulatory mechanisms for diverse TRAF-mediated events.

**TRAFF5 Is Critical for GITR-induced Activation of p38 and ERK**—Previously, we have observed that GITR cross-linking triggers activation of all three MAPK subsets (4). To determine whether TRAF5 regulates GITR-triggered MAPK activation, MAPK phosphorylation was monitored upon GITR cross-linking on activated T cells. Similar to the canonical NF-κB pathway, GITR-induced activation of p38, ERK1, and ERK2 was significantly reduced in TRAF5−/− DO11.10 T cells (Fig. 4). Interestingly, in contrast to p38 and ERK, JNK phosphorylation was less affected by TRAF5 levels, arguing that TRAF5-independent pathways mediate JNK activation triggered by GITR. The finding that TRAF5 is pivotal for GITR-induced activation of p38 and ERK suggests that these MAPKs along with NF-κB orchestrate GITR-induced T cell costimulation.

**TRAFF5-dependent Signaling Mediates GITR-induced Costimulation of T Cells**—We have demonstrated recently that GITR-induced activation of NF-κB and MAPK is associated with enhanced antigen-dependent T cell proliferation (4). To determine the role of TRAF5 in GITR-induced cellular events, activated DO11.10 T cells were treated with suboptimal concentrations of OVA_{323–339} in the presence of control, GITR-, or CD28-specific Abs. Consistent with reduced activation of NF-κB, p38, and ERK, TRAF5 deficiency resulted in diminished GITR-induced enhancement of antigen-dependent T cell proliferation (Fig. 5A). Proliferation of T cells treated with isotype control Abs of irrelevant specificity or CD28-specific Abs as controls was unaffected by TRAF5 deficiency. However, TRAF5 deficiency did not completely ablate GITR-induced costimulation, suggesting residual TRAF5-independent effects downstream of GITR.
GITR differentially regulates cellular events depending on the activation state of T cells (4). Therefore, we analyzed the effects of TRAF5 on GITR-induced T cell proliferation by stimulating naive splenocytes from TRAF5−/− or TRAF5+/+ C57BL/6 mice. Consistent with previous studies, TRAF5 deficiency did not significantly affect the number or percentage of CD4+ and CD8+ T cells or GITR surface expression (Ref. 13 and data not shown). However, the lack of TRAF5 resulted in diminished T cell proliferation induced by CD3- and GITR-specific Abs at 48 and 72 h (Fig. 5B). Stimulation of naive D011.10 T cells with their specific antigen in the presence of control or GITR-specific Abs yielded analogous results (data not shown). Consistent with GITR-induced NF-κB and MAPK activation not being completely abolist in TRAF5−/− T cells, GITR-induced costimulation was not eliminated entirely in TRAF5−/− T cells, suggesting that TRAF5-independent pathways contribute to the costimulatory effects of GITR. These observations highlight the importance of TRAF5 in GITR-induced costimulatory pathways but indicate that TRAF5-independent mechanisms can also play a role downstream of GITR in T cells.

Besides augmenting proliferation, GITR-induced signaling enhances the production of IL-2, which is a cardinal feature of costimulation (3, 4, 8). To define the role of TRAF5 in this event, IL-2 levels in supernatants of TRAF5−/− and TRAF5+/+ splenocytes stimulated with CD3-specific Ab and control or GITR-specific Abs for 72 h were measured by enzyme-linked immunosorbent assay. Whereas TRAF5 deficiency did not alter IL-2 production caused by T cell receptor signaling alone, increased IL-2 levels induced by GITR in wild type cells were absent in TRAF5−/− cells (Fig. 5C). Given that IL-2 is an NF-κB-dependent gene product, this result confirms the importance of TRAF5 in activating NF-κB downstream of GITR. Taken together, our analyses revealed that TRAF5 is a pivotal signaling intermediate that regulates T cell costimulation induced by GITR.

**DISCUSSION**

GITR functions as a costimulatory receptor augmenting T cell activation and rendering T effector cells refractory to suppression by T regulatory cells (2–4, 8). However, the molecular mechanisms of GITR-induced signaling remain enigmatic. TRAFs provide the platform for convergent and divergent signaling pathways that control NF-κB and MAPK activation triggered by TNFRs (10, 11). Studies using transfected cell lines have suggested that TRAF5 regulate signaling triggered by GITR and its human homologue activation-inducible TNFR (17, 19, 20, 28). Further, TRAF5 contributes to the activation of NF-κB and MAPKs induced by various TNFRs (11). Therefore, we hypothesized that TRAF5 contributes to GITR-induced signaling culminating in T cell costimulation.

In this report, we used TRAF5−/− T cells to determine the function of TRAF5 in GITR-induced signal transduction and costimulation. TRAF5 deficiency impeded GITR-induced activation of NF-κB, p38, and ERK but had less impact on JNK activation in activated T cells (Figs. 3 and 4). The defects in GITR-induced signaling were not due to an inherent defect in T cell function as the expression of GITR and CD25, which are markers of T cell activation, were similar between TRAF5−/− and TRAF5+/+ cells (Fig. 2). MAPKs and NF-κB globally influence activation, differentiation, and survival of T cells (29). Consistently, reduced activation of p38, ERK, and NF-κB caused by TRAF5 deficiency compromised the ability of GITR to augment proliferation of naive and activated T cells (Fig. 5, A and B). TNFRs recruit multiple TRAFs as well as other adapter molecules to execute downstream signaling events, which is consistent with our observation that GITR triggers TRAF5-dependent and -independent pathways (10, 11). Attempts to dissect TRAF5-independent effects through the use of the JNK inhibitor SP600125 were unsuccessful because the concentration of the inhibitor needed to reduce GITR-induced JNK activation eliminated the primary, TCR-mediated proliferative stimulus (data not shown). TRAF5 was also critical for IL-2 production triggered by GITR, confirming the role of TRAF5 in GITR-induced NF-κB activation that regulates T cell effector functions (Fig. 5C).

These findings differ from the impact of TRAF5 on specific signaling events triggered by other TNFRs. Without overt defects in JNK and NF-κB activation, TRAF5 deficiency causes impaired lymphocyte activation mediated by CD40 and CD27 as well as osteoclastogenesis induced by TNFα and receptor activator of NF-κB ligand (RANKL) (13, 18). A likely explanation for the difference between these TNFRs and GITR regarding TRAF5-dependent signaling is that the contributions of individual TRAFs to signaling events induced by distinct TNFRs are not uniform. For instance, Kim et al. (15) reported recently a non-redundant role for TRAF2 in NF-κB activation triggered by lymphotxin β receptor, whereas Tada et al. (12) showed that TRAF5 compensates for TRAF2 deficiency in NF-κB activation induced by receptors of TNFα. Supporting this hypothesis, we have observed distinctive aspects of TRAF-mediated signaling triggered by GITR in comparison with other TNFRs. In particular, GITR-induced NF-κB activation is inhibited by TRAF2 and augmented by TRAF4 (17, 19). To support further a direct role of TRAF5 in GITR-induced signaling and minimize the variables controlling signaling events mediated by TRAF5, we attempted studies with exogenous TRAF5 in HEK293 cells (Fig. 3C). Although we and others have used this system successfully to define the function of other TRAFs, studies with exogenous TRAF5 were inconclusive due to NF-κB activation triggered by overexpression of TRAF5 even in the absence of GITR-induced signaling. Whether this is due to difference in expression levels or intrinsic properties of the distinct TRAFs requires further investigation.

In conclusion, the finding that TRAF5 deficiency results in reduced activation of NF-κB and distinct MAPKs in T cells argues that TRAF5 functions as proximal signaling intermediate linking GITR with downstream signaling events such as activation of NF-κB, p38, and ERK that are associated with T cell activation, differentiation, and effector functions. Because GITR-induced signaling controls T cell-mediated autoimmune responses, TRAF5-dependent pathways may be attractive targets for therapeutic approaches directed at treating autoimmune disorders.

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**REFERENCES**

1. McHugh, R. S., Whitters, M. J., Piccirillo, C. A., Young, D. A., Shevach, E. M., Collins, M., and Byrne, M. C. (2002) *Immunity* 16, 311–323
2. Tone, M., Tone, Y., Adams, E., Yates, S. F., Frewin, M. R., Cobbold, S. P., and Waldmann, H. (2003) *Proc. Natl. Acad. Sci. U. S. A.* 100, 15059–15064
3. Ronchetti, S., Zollo, O., Bruscoli, S., Agostini, M., Bianchini, R., Nocentini, G., Ayroldi, E., and Riccardi, C. (2006) *Eur. J. Immunol.* 36, 613–622
4. Esparza, E. M., and Arch, R. H. (2005) *J. Immunol.* 174, 7869–7874
5. Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y., and Sakaguchi, S. (2002) *Nat. Immunol.* 22, 135–142
6. Suri, A., Shimizu, J., Katz, J. D., Sakaguchi, S., Unanue, E. R., and Kanagawa, O. (2004) *Eur. J. Immunol.* 34, 447–454
7. Kohn, A. P., Williams, J. S., and Miller, S. D. (2004) *J. Immunol.* 172, 4686–4690
8. Stephens, G. L., McHugh, R. S., Whitters, M. J., Young, D. A., Luxenberg, D., Carrero, B. M., Collins, M., and Shevach, E. M. (2004) *J. Immunol.* 173, 5008–5020
9. Esparza, E. M., and Arch, R. H. (2006) *Front. Biosci.* 11, 1448–1465
10. Arch, R. H., Gedrich, R. W., and Thompson, C. B. (1998) *Genes Dev.* 12, 2821–2830
11. Dempsey, P. W., Doyle, S. E., He, J. Q., and Cheng, G. (2003) *Cytokine Growth Factor"
12. Tada, K., Okazaki, T., Sakon, S., Kobayai, T., Kurosawa, K., Yamaoka, S., Hashimoto, H., Mak, T. W., Yagita, H., Okumura, K., Yeh, W. C., and Nakano, H. (2001) J. Biol. Chem. 276, 36530–36534
13. Nakano, H., Sakon, S., Koseki, H., Takemori, T., Tada, K., Matsumoto, M., Matsumoto, I., Sakai, T., Shirasawa, T., Akiha, H., Kobata, T., Santee, S. M., Ware, C. F., Bennett, P. D., Taniguchi, M., Yagita, H., and Okumura, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9803–9808
14. Yeh, W. C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J. L., Ferrick, D., Hum, B., Iscove, N., Ohashi, P., Rothe, M., Goeddel, D. V., and Mak, T. W. (1997) Immunity 7, 715–725
15. Kim, Y. S., Nedospasov, S. A., and Liu, Z. G. (2005) Mol. Cell. Biol. 25, 2130–2137
16. So, T., Salek-Ardakani, S., Nakano, H., Ware, C. F., and Croft, M. (2004) J. Immunol. 172, 4292–4297
17. Esparza, E. M., and Arch, R. H. (2004) Cell. Mol. Life Sci. 61, 3087–3092
18. Kanazawa, K., Azuma, Y., Nakano, H., and Kudo, A. (2003) J. Bone Miner. Res. 18, 443–450
19. Esparza, E. M., and Arch, R. H. (2005) J. Immunol. 174, 7875–7882
20. Kwon, B., Yu, K. Y., Ni, J., Yu, G. L., Jang, I. K., Kim, Y. J., Xing, L., Liu, D., Wang, S. X., and Kwon, B. S. (1999) J. Biol. Chem. 274, 6056–6061
21. Hauer, J., Puschner, S., Ramakrishnan, P., Simon, U., Bongers, M., Federle, C., and Engelmann, H. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 2874–2879
22. Shiels, H., Li, X., Schumacker, P. T., Maltepe, E., Padrid, P. A., Sperling, A., Thompson, C. B., and Lindsten, T. (2000) Am. J. Pathol. 157, 679–688
23. Murphy, K. M., Heimberger, A. B., and Loh, D. Y. (1990) Science 250, 1720–1723
24. Arch, R. H., and Thompson, C. B. (1998) Mol. Cell. Biol. 18, 558–565
25. Baldwin, A. S., Jr. (1996) Annu. Rev. Immunol. 14, 649–683
26. Ghosh, S., and Karin, M. (2002) Cell 109, (suppl.) S81–S96
27. Rothwarf, D. M., and Karin, M. (1999) Sci. STKE 1999, RE1
28. Gurney, A. L., Marsters, S. A., Huang, R. M., Pitti, R. M., Mark, D. T., Baldwin, D. T., Gray, A. M., Dowd, A. D., Brush, A. D., Heldens, A. D., Schow, A. D., Goddard, A. D., Wood, W. I., Baker, K. P., Godowski, P. J., and Ashkenazi, A. (1999) Curr. Biol. 9, 215–218
29. Arch, R. H., and Thompson, C. B. (1999) Annu. Rev. Cell Dev. Biol. 15, 113–140

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