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Glucocorticoid-Induced TNF Receptor Functions as a Costimulatory Receptor That Promotes Survival in Early Phases of T Cell Activation[1]

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Glucocorticoid-induced TNFR (GITR) is a member of the TNFR family that can inhibit the suppressive function of regulatory T cells and promote the survival and activation of T cells. However, little is known about the molecular mechanisms regulating T cell survival and activation downstream of GITR. To gain further insight into the cellular events and signaling pathways triggered by GITR, survival, proliferation, and cytokine production as well as activation of MAPKs and NF-κB were monitored after cross-linking of the receptor on naive and activated T cells. GITR cross-linking provided costimulation of naive and activated T cells and resulted in activation of MAPKs and NF-κB. Although GITR-induced signaling pathways augmented the survival of naive T cells, they were not sufficient to inhibit activation-induced cell death triggered by CD3 cross-linking of activated T cells. Differences in the contributions of GITR to cell survival between naive and activated T cells suggest that the receptor triggers specific pathways depending on the activation state of the T cell. ***The Journal of Immunology,*** **2005, 174: 7869–7874.**

The TNFR-related protein glucocorticoid-induced TNFR (GITR)[3] is highly expressed on regulatory T (T_{reg}) cells and has been suggested to be a marker for these lymphocytes (1). In addition, GITR is expressed at low levels on naive T cells, and GITR expression is up-regulated upon activation (2). GITR has been suggested to regulate survival, proliferation, and effector function(s) of T cell subsets (1–6). In particular, GITR has been implicated in inhibiting the function of $CD4^+/CD25^+$ T_{reg} cells that control immune effector cells. Treating T_{reg} cells with GITR ligand (GITR-L) or Ab specific for GITR abrogates the suppressive function of T_{reg} cells in vitro (1, 4, 5). Agonistic Ab specific for GITR breaks peripheral tolerance without eliminating T_{reg} cells (5). Further, depleting $GITR^{high}$ cells before adoptive transfer of T cells into nude mice results in autoimmune gastritis in the host (7). Taken together, these studies indicate that GITR plays a crucial role in regulating autoimmune responses.

The development of lymphocytes, including T cells, is grossly normal in GITR-deficient mice (3). However, T cells from GITR-deficient mice display an exaggerated activation phenotype when stimulated with CD3-specific Ab. Compared with GITR-sufficient controls, $GITR^{-/-}$ T cells express higher levels of IL-2, the high affinity IL-2R (CD25), and Fas (CD95), which results in increased proliferative responses and increased susceptibility to activation-induced cell death (AICD). Consistently, lowering GITR expression by antisense mRNA sensitizes cells to cell death, whereas overexpression of GITR increases the resistance of T cell clones to apoptosis induced by anti-CD3 Ab (2). However, recent reports suggest that GITR can increase AICD and potentially trigger cell death through its interaction with the proapoptotic protein Siva (8, 9). These studies point to GITR as a receptor that regulates T cell survival, but the molecular mechanisms triggered by GITR are poorly understood.

The data presented in this study indicate that GITR promotes proliferation, IL-2 production, and survival of naive T cells as well as costimulates previously activated T cells. Furthermore, GITR triggers the activation of three subsets of MAPKs and NF-κB. However, these GITR-induced events are insufficient to directly inhibit AICD of primary T cells.

Materials and Methods

Animals

BALB/c mice were purchased from The Jackson Laboratory. DO11.10 mice transgenic for a TCR specific for residues 323–339 of OVA ($OVA_{323–339}$) were a kind gift from Dr. K. Murphy (Washington University School of Medicine, St. Louis, MO). All mice were kept at a specific pathogen-free facility in accordance with the guidelines of the animal studies committee of Washington University.

Antibodies

Polyclonal anti-IκBα (BD Biosciences), monoclonal anti-actin (C4; Chemicon International), and HRP-conjugated polyclonal secondary Abs (Santa Cruz Biotechnology) were used for Western blot analysis. Abs specific for phosphorylated and total ERK, JNK, and p38 as well as MAPK-specific in vitro kinase kits were purchased from Cell Signaling Technology. Anti-CD3ε (145-2C11), anti-CD4 (H129.1), anti-CD25 (PC61), anti-I-A^d (AMS-32.1), anti-B220 (RA3-6B2), anti-CD28 (PV.1), CTLA-4-Ig, anti-rat IgG1 (RG11/39.4), anti-rat IgG2a (RG7/1.30), and control rat IgG2a (R35-95) Abs were purchased from BD Biosciences. Anti-GITR-L Ab (YGL 386.2.2) was a kind gift of Dr. H. Waldmann (University of Oxford, Oxford, U.K.) and has been characterized previously (4). Anti-GITR Abs (BAF524 and 108619), control goat Ab, GITR-L, and anti-His mAb were purchased from R&D Systems. For flow cytometry, Abs were directly conjugated with FITC or PE as indicated or were biotinylated for detection with FITC-streptavidin (BD Biosciences).

Cell death assay

CD3-specific Ab was immobilized in 96-well plates at the indicated concentrations in PBS at 4°C overnight. Activated DO11.10 or BALB/c T cells

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[3] Abbreviations used in this paper: GITR, glucocorticoid-induced TNFR; AICD, activation-induced cell death; GITR-L, GITR ligand; PI, propidium iodide; TRAF, TNFR-associated factor; T_{reg}, regulatory T cell.

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(1.5×10^5/well) were stimulated with plate-bound anti-CD3 Ab and soluble anti-GITR or control Ab (5 μg/ml). After the times indicated in the figures, cell viability was determined by flow cytometry of 10^4 events on the basis of cell size and propidium iodide (PI) staining (0.25 μg/ml) or after staining with annexin V and PI according to the manufacturer's protocol (Trevigen).

Cytokine production

DO11.10 splenocytes (2.5×10^6/well) were plated in 12-well plates with 0.1 μM $OVA_{323–336}$ and the indicated Abs. Supernatants were harvested after 72 h and assayed for IL-2 levels using an ELISA kit (BD Biosciences).

Flow cytometric analysis (FACS) of surface expression of lymphocyte markers

Naive and in vitro activated lymphocytes (2.5×10^5 cells) were stained for 30 min at 4°C in 50 μl with the indicated Abs or appropriate isotype controls. The stained cells were washed three times and resuspended in PBS. Analysis was performed on gated live cells using FACScan or FACSCalibur flow cytometers (BD Biosciences) and WinMDI software (Joe Trotter).

In vitro activation of lymphocytes

Spleens were removed from DO11.10 or BALB/c mice and mechanically disrupted into a single cell suspension. Erythrocytes were removed by lysis in ACK buffer (150 mM KCl, 10 mM $KHCO_3$, and 0.1 mM EDTA, pH 7.2). Five million lymphocytes per well were plated on six-well plates in lymphocyte medium (RPMI 1640 supplemented with 10% FBS, 4 mM L-glutamine, 10 mM HEPES, 100 mM nonessential amino acids, 57 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin). DO11.10 T cells were stimulated with 0.3 μM $OVA_{323–339}$, whereas BALB/c T cells were activated by plate-bound CD3-specific (1 μg/ml) and soluble CD28-specific Abs (1 μg/ml) for 3 days. Activated lymphocytes were washed with PBS after primary stimulation and rested for 18 h in lymphocyte medium at 37°C. For subsequent analysis, live cells were isolated by centrifugation in Ficoll-Paque (Amersham Biosciences).

MAPK phosphorylation and in vitro kinase assays

Activated DO11.10 splenocytes (5×10^6 cells for detection of MAPK phosphorylation, 10^7 cells for in vitro kinase assay) were treated with GITR-specific or control Ab (5 μg/ml) at 37°C for the indicated times and handled according to the manufacturer's specifications (Cell Signaling Technology), except that secondary Abs used to detect phosphorylated or total protein levels were obtained from Santa Cruz Biotechnology. Chemiluminescence was performed with reagents from Cell Signaling Technology or Amersham Biosciences.

Proliferation assays

Naive or activated cells (10^5) were plated in triplicate per condition in 96-well plates. When indicated, BALB/c splenocytes irradiated at 2000 rad were used as accessory cells. One microcurie per well of [^{3}H]thymidine (ICN Biomedical) was added 8 h before the indicated time point.

Results

GITR does not directly prevent AICD

Studies of T cell lines transfected with GITR and GITR-deficient primary T cells activated by Con A suggested that the receptor modulates AICD (2, 3, 10). To define the molecular mechanism of GITR-induced inhibition of AICD under physiologic conditions, naive DO11.10 splenocytes were activated with Ag ($OVA_{323–339}$) in vitro. Upon Ag stimulation, the vast majority of cells in the culture were activated $CD4^+$ T cells that expressed high levels of GITR and CD25 (data not shown). Subsequently, activated lymphocytes were rested for 18 h before treatment with plate-bound Ab specific for CD3 to cause AICD. Analysis of cell size and PI staining by FACS revealed a significant decline in cell survival in response to CD3 stimulation (data not shown). In contrast to published studies that analyzed the effects of GITR overexpression, incubation of activated T cells expressing physiologic levels of GITR with GITR-specific Ab did not promote cell survival in comparison to control Ab, as indicated by similar percentages of PI^- cells with a forward scatter typical for live cells in cultures 18 h post-restimulation. However, PI stains cells at a relatively late stage of apoptosis and does not distinguish between apoptotic and necrotic cells. To examine whether GITR stimulation affects earlier events, apoptosis was measured by double staining with annexin V and PI. Annexin V interacts with phosphatidylserine, which becomes exposed on the cell surface early during apoptosis while the cell membranes are still intact, i.e., the cells are PI^-. Consistent with our previous data, incubation with GITR-specific Ab did not prevent early apoptotic events between 3 and 13 h post-restimulation of the cells with low or high concentrations of anti-CD3 Ab (Fig. 1*A*). At the 13 h time point, the apparent decrease in the percentage of early apoptotic events was the result of the increased percentage of late (annexin V^+/PI^+) apoptotic events (data not shown). To rule out that stimulation of T cells with GITR-specific Ab failed to block antiapoptotic events due to the rapid induction of proapoptotic pathways caused by CD3-specific Ab, activated T cells were incubated with anti-GITR or control Ab for 2 h before and continuously during restimulation by CD3 cross-linking. Consistent with our earlier results, this treatment was also insufficient to protect the T cells from AICD (Fig. 1*B*).

DO11.10 mice are an established TCR transgenic strain, and we have shown previously that stimulation with $OVA_{323–339}$ results in characteristic cellular events similar to those observed in activated T cells isolated from control mice lacking a TCR transgene (11). Consistent with our studies of activated DO11.10 T cells, activated BALB/c T cells were not protected against CD3-induced AICD by GITR-specific Ab even when additional cross-linking was provided by an isotype-specific Ab (Fig. 1*C*). Indicative of differences in the costimulatory pathways, CD28 cross-linking afforded significant protection from AICD under these experimental conditions. Taken together, the data indicate that GITR does not directly inhibit AICD.

GITR functions as a costimulatory receptor on T cells

GITR along with other members of the TNFR family, including Ox40, CD30, 4-1BB, and CD27, has been suggested to promote T cell activation similar to CD28 (12, 13). Hallmarks of costimulation include increased proliferation, cytokine production, and cell survival. To determine whether GITR costimulates activated T cells under conditions used to study AICD, activated DO11.10 T cells were treated with cognate Ag in the presence of GITR-specific or control Abs. GITR cross-linking using a specific Ab was sufficient to enhance Ag-dependent proliferation of previously activated T cells (Fig. 2*A*). Although the effect was less pronounced, GITR cross-linking also augmented T cell proliferation in the presence of exogenous APCs, which can costimulate via CD80/CD86 and other surface molecules. Furthermore, GITR-L expression was not detected on activated DO11.10 splenocytes, arguing that residual APCs, present as $<4\%$ $B220^+$ cells and expressing adequate levels of $I\text{-}A^d$, provide suboptimal costimulation via GITR to T cells under conditions used to analyze AICD (data not shown). These results demonstrate that GITR can trigger costimulatory signals in previously activated T cells.

CD28 is a major costimulatory receptor on naive T cells that promotes the expression of cytokines and drives cell cycle progression (14). To define the impact of CD28 on GITR-induced costimulation of T cells, naive DO11.10 splenocytes were treated with $OVA_{323–339}$ and GITR-specific or control Ab in the absence or the presence of CTLA-4-Ig, which blocks the interaction of CD80/CD86 on APCs with CD28 on T cells (15). Reducing CD28 signaling resulted in overall decreased IL-2 production and proliferation (Fig. 2, *B* and *C*). However, confirming its costimulatory function, GITR augmented IL-2 production and proliferation of

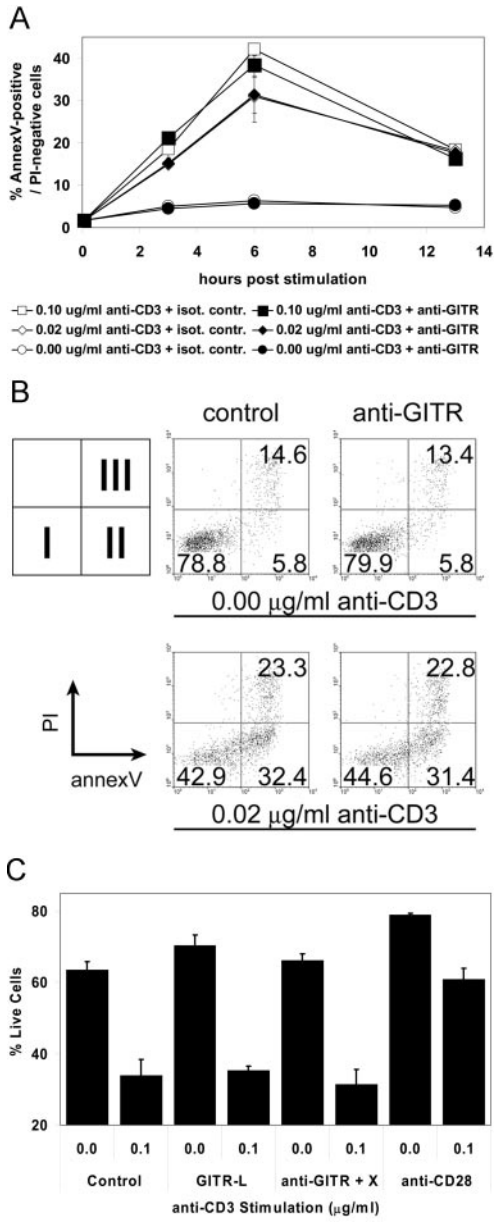

FIGURE 1. GITR stimulation does not protect T cells from AICD. *A*, Naive DO11.10 splenocytes were cultured with 0.3 μM $OVA_{323–339}$ peptide for 3 days and rested for 18 h. Cells were stained with the indicated Abs and analyzed by FACS. Activated T cells were restimulated with plate-bound anti-CD3 Ab at the indicated concentrations and soluble GITR-specific or control Ab (5 μg/ml). At the indicated times, cells were stained with annexin V and PI and analyzed by flow cytometry. Flow cytometric analysis was performed to determine the percentages of early apoptotic (annexin V^+/PI^-) cells. Error bars indicate the SD of triplicate samples in a representative of three independent experiments. *B*, Activated T cells were incubated with anti-GITR or control Abs for 2 h before and continuous with restimulation with anti-CD3 Ab. The percentages of live (I), early apoptotic (II), and late apoptotic/necrotic (III) cells were determined 6 h post-restimulation of activated T cells with CD3-specific Ab, as described above. Shown are representative FACS plots of triplicate samples of three independent experiments. *C*, Primary stimulation of BALB/c splenocytes was provided by plate-bound anti-CD3 Ab (1 μg/ml) with soluble anti-CD28 Ab (1 μg/ml) for 3 days, and activated cells were rested for 18 h. Costimulatory signals were provided by soluble GITR-L (0.5 μg/ml) with cross-linking anti-His Ab (5 μg/ml), anti-GITR Ab (5 μg/ml), cross-linking secondary Ab (5 μg/ml; indicated as X), or anti-CD28 Ab (5 μg/ml) for 2 h before and continuous with restimulation with plate-bound anti-CD3 Ab. Twenty-four hours later, cells were stained with annexin V and PI and were analyzed by FACS. Live cells were counted as annexin V^-/PI^- events.

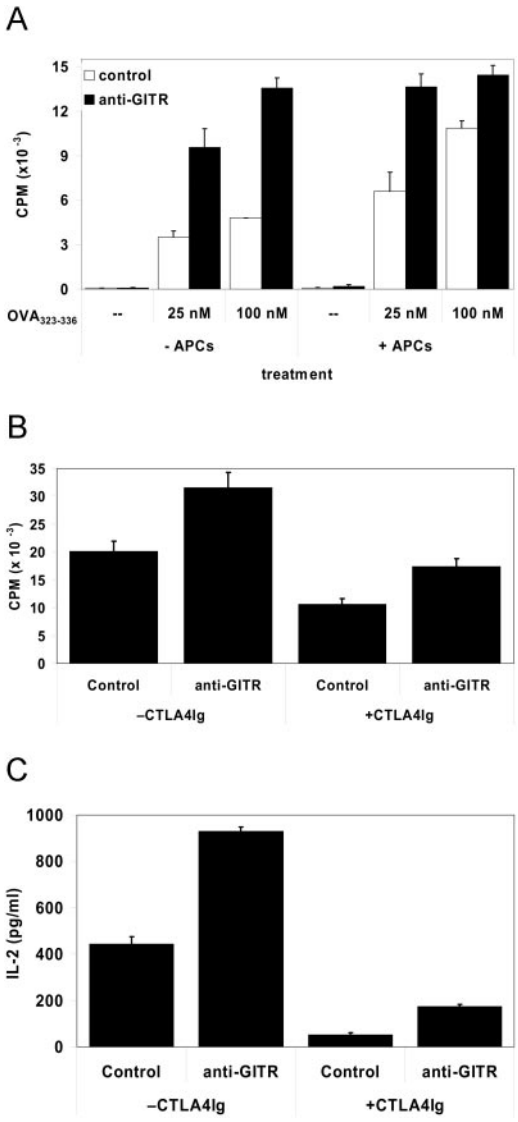

FIGURE 2. Costimulatory effects of GITR on DO11.10 T cells. *A*, Ag-activated DO11.10 T cells were restimulated with the indicated concentrations of $OVA_{323–339}$ with GITR-specific or control Abs (5 μg/ml) in the absence or the presence of exogenous irradiated APCs. Proliferation was determined 48 h post-restimulation. *B*, Naive DO11.10 splenocytes were stimulated with 0.1 μM $OVA_{323–339}$ with anti-GITR or control Ab (5 μg/ml) in the absence or the presence of CTLA-4-Ig (5 μg/ml). IL-2 production was determined at 72 h from culture supernatants by ELISA. *C*, Parallel with *B*, proliferation was measured at 72 h. All assays were performed in triplicate samples. Shown are average results and SD of one representative of three independent experiments.

naive DO11.10 T cells activated by Ag even in the presence of CTLA-4-Ig.

Consistently, cross-linking of GITR with GITR-L or GITR-specific Ab also promoted the proliferation of splenocytes from naive BALB/c mice after treatment of cells with soluble or plate-bound CD3-specific Ab (Fig. 3*A* and data not shown). Reducing CD28 costimulation triggered by CD80/CD86 on APCs present in the cultures of nontransgenic T cells using CTLA-4-Ig did not interfere with the enhancement of proliferative responses due to GITR cross-linking with GITR-L or GITR-specific Ab (data not shown). CD28-specific Ab, which provided costimulation in the presence of CTLA-4-Ig, was included as a positive control in these studies. In contrast to activated T cells that were not protected against

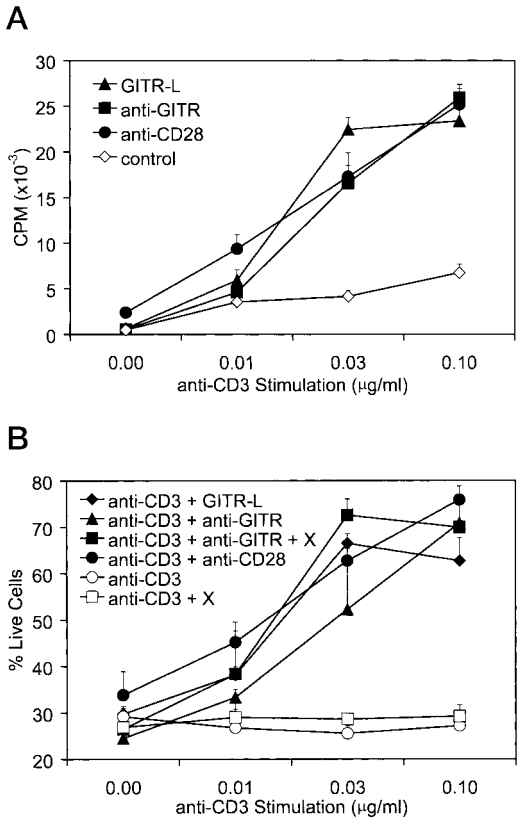

FIGURE 3. GITR augments proliferation and survival at early phases of T cell activation. *A*, Naive BALB/c splenocytes were stimulated with soluble anti-CD3 Ab at the indicated concentrations in the presence of soluble GITR-L (0.5 μg/ml) with cross-linking anti-His Ab (5 μg/ml) or Abs (5 μg/ml) specific for CD28, GITR, or control. X, isotype-specific secondary Ab used to cross-link the anti-GITR Ab. Proliferation was determined at 72 h. *B*, Parallel with *A*, cells were stained with PI at 72 h and analyzed by flow cytometry. Live cells were assigned on the basis of cell size and exclusion of PI.

AICD by GITR cross-linking, naive T cells showed increased survival in response to GITR cross-linking, similar to CD28 cross-linking during primary stimulation (Fig. 3*B*). However, it is currently unclear whether this increased percentage of live cells is due to decreased apoptosis and/or proliferative expansion. These findings suggest that GITR plays a role in promoting cell survival of primary T cells during their initial stimulation, but is not sufficient to protect against AICD in the late phases of immune responses.

GITR triggers activation of NF-κB and MAPKs

To characterize signaling pathways that may contribute to GITR-induced cellular effects, we analyzed the activation of NF-κB and MAPKs. NF-κB is a critical regulator of cell survival and apoptosis, which is activated by a variety of TNFR family members, including GITR (4, 16). Dimeric NF-κB molecules are sequestered in the cytoplasm by IκBs, which are degraded in response to stimuli that cause NF-κB activation and are resynthesized as NF-κB-dependent gene products (17). To study NF-κB activation under the conditions used to analyze AICD, IκBα levels were measured by Western blot of lysates of activated DO11.10 splenocytes that had been restimulated with GITR-specific or control Abs. Typical of TNFR-induced NF-κB activation, IκBα levels initially decreased and later increased in primary T cells stimulated with anti-GITR Ab compared with the negative controls (Fig. 4 and data not shown). Combined with the analyses of cell viability of activated

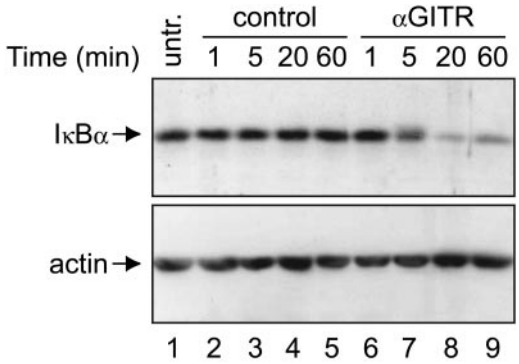

FIGURE 4. GITR induces NF-κB activation. Parallel to the analyses of AICD in Fig. 1, IκBα levels were measured by Western blot of lysates from activated DO11.10 T cells stimulated with GITR-specific or control Abs. Membranes were subsequently stripped and probed for actin to determine loading of total protein.

T cells presented above, these results indicate that the net effect of GITR-induced NF-κB activation neither promotes nor inhibits AICD.

Similar to NF-κB, MAPKs play crucial roles in regulating cell survival and apoptosis and can be activated by a variety of TNFR family members (16, 18). In response to GITR-induced signaling in primary T cells, we monitored MAPK activation by Western blot using Abs specific for phosphorylated forms of the three MAPK families. After treatment of activated DO11.10 splenocytes with GITR-specific Ab, activation of ERKs, JNKs, and p38 was increased, and peak phosphorylation levels were observed 20 min post-GITR cross-linking (Fig. 5*A*). JNK2 was the predominant JNK protein expressed and phosphorylated in DO11.10 lymphocytes, whereas only minute expression and phosphorylation of JNK1 were detected. Although ERK1 expression was substantially lower than ERK2 expression, the phosphorylation of both MAPKs reached similar magnitudes. However, ERK2 activation occurred much earlier and was more prolonged than ERK1 activation. Similar to ERK2, phosphorylation of p38 was also evident at 1 min poststimulation and was still present at 1 h poststimulation. To verify that phosphorylation of MAPKs correlates with increased kinase activity, in vitro kinase assays for p38, JNKs, and ERKs were performed. Compared with control Ab, GITR-specific Ab induced phosphorylation of the p38 substrate ATF2 (Fig. 5*B*). Similar results were obtained with the respective substrates of JNK and ERK, c-Jun and Elk1 (data not shown). Taken together, our results indicate that GITR can trigger the activation of all three MAPK subfamilies. However, similar to NF-κB activation and other costimulatory events triggered by GITR-induced signaling pathways, the overall impact of increased MAPK activation was insufficient to affect AICD.

Discussion

GITR belongs to a subset of TNFR family members that lack a cytoplasmic death domain and regulate activation, differentiation, and survival of lymphocytes (for review, see Refs. 2 and 16). GITR has been identified as a marker of natural $CD4^+CD25^+$ T_{reg} cells that regulate immune responses to self Ags (1, 5). Recently, GITR has been suggested to function as a costimulatory molecule, promoting activation of naive T cells (4). However, the molecular events triggered by GITR are not well understood.

TNFR-related proteins lacking a death domain have been implicated in pathways promoting cell survival via activation of NF-κB (16). Consistently, GITR is thought to inhibit cell death

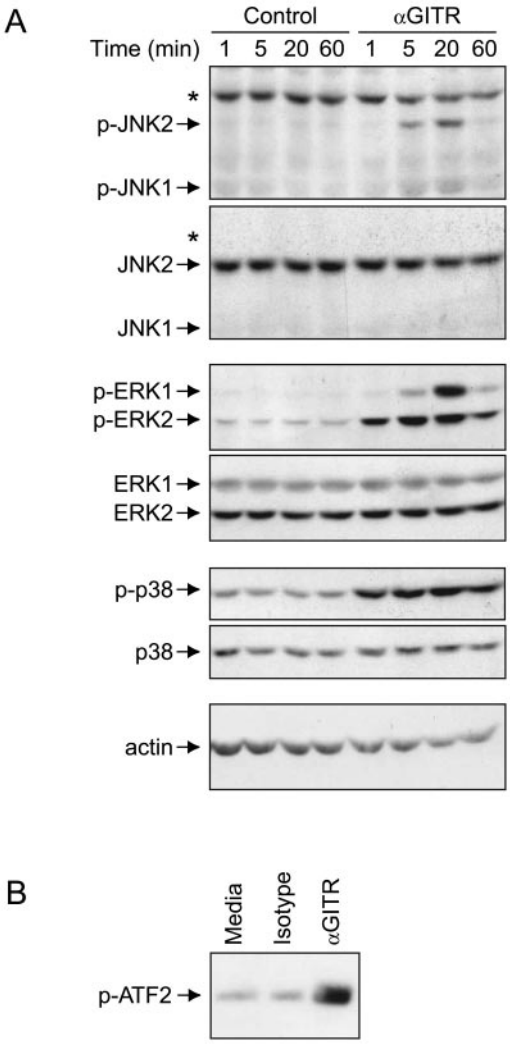

FIGURE 5. Signaling pathways triggered by GITR lead to activation of MAPKs. *A*, Western blot for phosphorylated forms of MAPKs was performed from lysates of activated DO11.10 splenocytes restimulated with GITR-specific or control Ab (5 μg/ml) for the indicated time points. Membranes were subsequently stripped and probed for total MAPK protein levels. As an additional loading control, the membrane was reprobed with Ab specific for actin. *, A nonspecific band detected by the Ab specific for phospho-JNK that is not detected by the pan-JNK Ab. *B*, In vitro kinase assay for p38 was performed from lysates of activated DO11.10 splenocytes stimulated with GITR-specific or control Ab for 20 min. Phosphorylation of ATF-2, a specific substrate of p38, was detected by Western blot.

pathways in lymphocytes, and GITR-deficient T cells have been shown to be more sensitive to AICD, a form of apoptosis that controls lymphocyte homeostasis (2, 3, 10). However, it is unclear whether GITR activates antiapoptotic signaling pathways directly or affects cell survival indirectly via the production of cytokines such as IL-2 and receptors such as IL-2R and Fas that control pro- and antiapoptotic pathways. To test the role of GITR-induced signaling events in AICD, activated T cells were restimulated with CD3-specific Ab and either GITR-specific or control Ab. In contrast to studies of cells deficient in or transfected with GITR, cross-linking of endogenous GITR did not protect activated primary lymphocytes from AICD (Fig. 1). Consistent with previously published findings, GITR cross-linking with specific Ab or engagement by its ligand did result in increased proliferation, IL-2 production, and survival of T cells during primary stimulation as well as augmented the proliferation of activated T cells (Figs. 2 and 3). No differences in the rate of AICD were detected despite activation of the canonical NF-κB pathway and MAPKs (Figs. 4 and 5). Therefore, the experimental conditions used to study the effects of GITR-induced events were sufficient to trigger signaling pathways and cellular functions in T cells. Importantly, these findings indicate that the contribution of GITR to cell survival pathways depends upon the stage of T cell activation and stimulus used to induce cell death.

Our data suggest that the effects of GITR-induced signal transduction are regulated by additional signals that result in differences in the survival of naive and activated T cells. Wajant (19) discusses how NF-κB activation leads to coordinated expression of prosurvival proteins, including members of the Bcl-2 family and proapoptotic gene products, such as TRAIL. By establishing the balance between cell survival and apoptosis through changes in gene expression, NF-κB promotes cellular expansion during the acute phase of an immune response, yet provides regulatory mechanisms to control inflammation. Beyond controlling NF-κB activity, GITR has been shown to interact with the proapoptotic protein Siva, which may initiate cell death pathways and could further counterbalance the cytoprotective effects of NF-κB (8). As mentioned by Riccardi et al. (20), adapter proteins that interact with GITR could potentially regulate cellular events triggered by the receptor, such as regulation of T cell survival. Recently, we have shown that TNFR-associated factor 2 (TRAF2) interacts with GITR in T cells and functions as a negative regulator of NF-κB activation induced by the receptor (21). Moreover, TRAF2 can regulate the production of reactive oxygen species induced by TNFR-related proteins, which can shift the balance between cell survival and death (22). Because TRAF2 is a central regulator of cell survival and apoptotic pathways triggered by TNFR-related proteins, it is intriguing to speculate that TRAF2-mediated signaling is involved in deciding the ultimate fate of T cells that receive stimulation through GITR (23). These possibilities and whether GITR plays distinct roles in effector T cells and T_{reg} cells need to be further addressed to establish the role of GITR in T cell survival.

Distinct MAPK family members, such as p38 and JNK, can be activated by TNFR-related proteins and regulate lymphocyte functions, survival, and apoptosis (16, 24). Western blot analysis and in vitro kinase assays indicated that GITR can induce activation of the MAPK subfamilies, JNK, ERK, and p38 (Fig. 5). Although quantitative differences in phosphorylation were detected due to dissimilar expression levels of the distinct MAPKs in lymphocytes, activation of all three kinase subfamilies peaked 20 min after stimulation by GITR cross-linking. These results suggest a role for MAPKs in regulating the GITR-induced responses of effector T cells and/or T_{reg} cells.

In summary, GITR promotes the activation, survival, and cytokine production of T cells. Furthermore, GITR can induce the activation of NF-κB and three subfamilies of MAPKs, ERKs, JNKs, and p38. However, costimulatory signaling events triggered by GITR do not directly inhibit AICD. Understanding the molecular details of cell survival pathways triggered by GITR in T cells will provide further insights into the mechanisms involved in physiologic and pathologic immune responses.

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Disclosures

The authors have no financial conflict of interest.

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