Signaling triggered by glucocorticoid-induced tumor necrosis factor receptor family-related gene: Regulation at the interface between regulatory T cells and immune effector cells

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1. ABSTRACT

Mammals and other higher vertebrates have developed an adaptive immune system to defy effectively countless pathogens and cancerous cells encountered during the lifetime of an individual. B and T lymphocytes, which are essential in orchestrating adaptive immune responses, express surface receptors specific for foreign and abnormal self-antigens. Genesis of this antigen receptor repertoire poses significant risks for autoimmunity caused by self-reactive lymphocytes. Therefore, organisms with adaptive immune systems have evolved central and peripheral tolerance mechanisms. In peripheral tissues, regulatory T (T<sub>reg</sub>) cells function in a dominant, cell-extrinsic manner to limit inflammatory responses and autoimmune disorders. To tap the potential clinical utility of these specialized lymphocytes, advances have been made in understanding how T<sub>reg</sub> cell-mediated suppression of immune effector cells is achieved and regulated. Importantly, signaling induced by a recently identified member of the tumor necrosis factor receptor (TNFR) family, termed glucocorticoid-induced TNFR family-related gene (GITR), abrogates the suppressive effects of T<sub>reg</sub> cells. GITR plays a pivotal role in controlling T cell-mediated responses in experimental models of organ-specific autoimmunity, chronic infection, and anti-tumor immunity. These findings highlight the importance of elucidating the molecular underpinnings of GITR-induced signaling. We propose that GITR employs adapter proteins, including TNFR-associated factors (TRAFs), to regulate diverse signaling pathways and transcriptional programs that control the interplay between T<sub>reg</sub> cells and immune effector cells.

2. BRIEF HISTORY OF NATURAL T<sub>REG</sub> CELLS

Higher vertebrates including humans evolved an adaptive immune system to handle with more efficiency the threats of parasitic microorganisms and malignant cells. The random generation of antigen receptors expressed on lymphocytes is a cardinal feature of the adaptive immune system. However, the diverse specificity of the antigen receptor repertoire designed to counter the broad variety and rapid mutation rates of virulent pathogens is interwoven with the evolutionary burden of potential self-reactivity and the development of autoimmune disorders (for review, see 1). Clonal anergy and deletion by apoptosis during lymphocyte development are cell-intrinsic, recessive mechanisms that maintain immunologic self-tolerance (2-5). Despite these safeguards, a significant percentage of self-reactive lymphocytes are functionally competent in peripheral tissues of healthy individuals (6). Consequently, immunization with self antigen and potent adjuvant can elicit autoimmune responses in otherwise normal subjects (7). Therefore, additional regulatory pathways are vital for preserving self tolerance during immune responses.

Early evidence implied the existence of T cells specialized to maintain self-tolerance. For instance, mice thymectomized between postnatal days two and four develop organ-specific autoimmune disease that is prevented by reconstituting syngeneic T cells from adult mice (8). Heterogeneous lymphocyte populations, such as CD<sub>5</sub><sup>high</sup> or CD45RB<sup>low</sup> and CD45RC<sup>low</sup> T cells, respectively, exhibit suppressor activity (9-13). But these markers are expressed too widely to define T<sub>reg</sub> cells. The
better operational definition of naïve lymphocytes enriched for suppressor activity is CD4+ T cells constitutively expressing the α subunit of the interleukin (IL)-2 receptor (CD25), which constitute 5-10% of total CD4+ T cells in normal individuals (14,15). Natural CD4+ CD25+ Treg cells that develop in the thymus have been distinguished from adaptive Treg cells, such as IL-10-producing Tr̄e1 cells, that gain suppressive functions in the periphery during the course of an immune response (16). In this review article we will focus on natural Treg cells and discuss the current models of how these specialized T cells operate and are influenced by GITR-induced signaling.

CD4+ CD25+ Treg cells are thought to be antigen experienced, but resting (for review, see 17). Expression of a diverse T cell receptor (TCR) repertoire on the surface of mouse and human Treg cells suggests that they have undergone normal thymic selection (18-20). Studies with mice expressing a TCR transgene specific for an epitope of influenza hemagglutinin (HA) crossed to mice that express high or low affinity HA peptides have revealed that Treg cells recognize peptides with relatively high affinity (21). This mode of selection in combination with negative selection of T cells recognizing peptides with very high affinity or avidity assure the generation of Treg cells specific for self-antigen while eliminating potentially pathogenic autoreactive T cells. Additional data suggest that antigenic stimulation in the periphery is required for the maintenance of Treg cells and to regulate their suppressive function (22-25). However, more detailed analyses are required to define the consequences of antigen stimulation of Treg cells. At least in vitro, Treg cell-mediated suppression depends on cell-cell contact. Therefore, it is conceivable that Treg cells are exposed to antigen at the site of an inflammatory response. This notion is supported by the finding that Treg cells express a unique pattern of chemokine receptors, arguing that the balance of T eff and Treg cells at the site of inflammation and their affinity for peptide/major histocompatibility complex (MHC) II complexes play critical roles in determining the outcome of immune responses against self and foreign antigens (26,27).

3. MOLECULAR DEFINITION OF TREG CELL FUNCTION

Most cell surface proteins used as markers of Treg cells including CD25, cytotoxic T lymphocyte antigen (CTLA)-4, lymphocyte activation gene (LAG)-3 and GITR are also up-regulated upon lymphocyte activation (for review, see 28). The most-specific molecular definition for natural Treg cells is the expression of the forkhead-box transcription factor FoxP3 (29). Treg cells isolated from naïve mice and sorted on the basis of high CD4 and CD25 surface levels express elevated levels of FoxP3, whereas FoxP3 expression is low or undetectable in CD4+ CD25- effector T (T eff) cells (30-32). However, the nuclear localization of FoxP3 has limited its usefulness as a Treg cell marker in cell sorting experiments (33).

3.1. FoxP3 is an essential marker of Treg cells

Mutations in FoxP3 cause the fatal recessive disorder “immunodysregulation, polyendocrinopathy and enteropathy, X-linked syndrome” (IPEX) in children (34-36). Consistently, scurfy mice with a mutation in the mouse ortholog of FoxP3, termed scurfy, resemble mice genetically deficient in FoxP3, which lack natural Treg cells and display an IPEX-like syndrome (30-32,37). Adoptive transfer of FoxP3-sufficient Treg cells is sufficient to reverse the phenotype caused by disruptions of FoxP3 function. Furthermore, ectopic expression of FoxP3 in T eff cells confers a Treg cell-like phenotype, suggesting that the transcription factor instructs Treg cell development (31,32). Studies of mice expressing a genetic knock-in allele encoding green fluorescent protein (GFP) fused to FoxP3 revealed that CD4+ CD25+ FoxP3+ T cells can suppress T eff cell proliferation as effectively as CD4+ CD25+ FoxP3- T cells (38). These findings establish that expression of FoxP3, in contrast to CD25, differentiates natural Treg cells from other T cell subsets. Taken together, FoxP3 is an essential component of the genetic program that specifies the development of the natural Treg cell lineage.

3.2. IL-2 is a critical regulatory cytokine for Treg cells

To analyze the mechanism of action of Treg cells, Thornton and Shevach established an in vitro assay to approximate the suppressive function of Treg cells in vivo (39). When Treg cells are removed from T cell populations, the remaining T eff cells respond more briskly to TCR stimulation. Reconstitution of Treg cells into effector cell populations – typically CD4+ CD25+ T eff cells – dampens T eff cells proliferative responses (15). TCR-stimulated Treg cells suppress T cell activation in an antigen-nonspecific fashion that requires close proximity of Treg cells to the suppressed population (39,40). In contrast to T eff cells and typical of anergic lymphocytes, TCR stimulation does not trigger proliferation of Treg cells in vitro, unless the cultures are supplemented with high concentrations of IL-2 (39). In addition to high concentrations of IL-2, strong costimulatory signals triggered by CD28 obviate Treg cell-mediated suppression of responder cells. Treg cells subtype IL-2 production by T eff cells, which primes Treg cell suppressive activity to quell additional IL-2 synthesis required for effector cell proliferation (41). Given their constitutive CD25 expression, Treg cells have been suggested to competitively consume IL-2 required for T eff cell proliferation (42). These observations could explain the apparent contact-dependence and supra-physiologic ratios of Treg cells needed to observe suppression in vitro. However, IL-2 consumption cannot be the only mechanism of suppression as CD4+ CD25+ T cells also exhibit suppressive properties (43). Overall, Treg cell-mediated suppression in vitro targets IL-2 and requires high ratios of Treg cells to effector cells.

Although instrumental in studies of Treg cell function, the in vitro suppressor assay does not always recapitulate the in vivo dynamics of Treg cells. For instance, inhibitory cytokines such as IL-10 and TGF-β are expressed at high levels by Treg cells and are critical for their suppressive function in animal model systems, yet blockade of these pathways in vitro yielded conflicting findings (39,44-49). In contrast to their apparent
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anergic phenotype in vitro, T<sub>reg</sub> cells can proliferate in lymphopenic hosts, in mice deficient in T<sub>reg</sub> cells, or after antigenic stimulation (44,50-53). Genetic deficiencies in IL-2 or the α and β chains of the IL-2 receptor produce a fatal lymphoproliferative disorder with autoimmune manifestations, such as inflammatory bowel disease (54-56). These results are consistent with IL-2-induced signaling being essential for the development and maintenance of T<sub>reg</sub> cells. However, adoptive transfer of wild-type T<sub>reg</sub> cells is sufficient to inhibit the induction of lethal autoimmunity in mice lacking IL-2Rβ (53). Therefore, suppression of IL-2 transcription or competitive consumption of IL-2 does not fully account for T<sub>reg</sub> cell-mediated suppression in vivo. The extracellular milieu, homing of proliferating T<sub>reg</sub> cells to sites of inflammation, and additional cell types found in inflamed tissues are pivotal for this mode of immune regulation.

3.3. CTLA-4 on T<sub>reg</sub> cells controls APC functions via reverse signaling

The observations that CTLA-4 is constitutively expressed on T<sub>reg</sub> cells and mice deficient in CTLA-4 or treated with a non-depleting Ab specific for CTLA-4 die of a lymphoproliferative disorder imply that CTLA-4 is crucial for T<sub>reg</sub> cell function (57-61). Supporting this notion, adoptive co-transfer of CTLA-4-sufficient and -deficient bone marrow cells or splenocytes prevents lymphoproliferative expansion of CTLA-4<sup>−/−</sup> T cells in the host, suggesting that uncontrolled proliferation of CTLA-4-deficient T cells is, at least in part, due to the lack of extrinsic inhibitory mechanisms (62,63). Consistent with these findings, CTLA-4-deficient cells have reduced suppressive effects and treatment with CTLA-4-specific Ab has been shown to abrogate T<sub>reg</sub> cell-mediated suppression of T<sub>eff</sub> cells in vitro (61). CTLA-4-expressed on T<sub>reg</sub> cells induces reverse signaling through CD80 and CD86 that up-regulates indoleamine 2,3-dioxygenase in antigen presenting cells (APCs) to reduce levels of free tryptophane required for T<sub>eff</sub> cell activation (64). On the other hand, T<sub>reg</sub> cell-mediated suppression occurs in APC-free culture conditions, indicating that the inhibitory effects of T<sub>reg</sub> cells extend beyond APCs (65). In contrast to the studies in mice, recent data have indicated that interference with CTLA-4-induced signaling of human CD4<sup>+</sup> CD25<sup>+</sup> T<sub>reg</sub> cells is not sufficient to abrogate their suppressive activity, suggesting alternative mechanisms of suppression (61,66). Consistent with the finding that CTLA-4 is not required for the T<sub>reg</sub> cell survival or activation, T<sub>reg</sub> cells can be isolated from CTLA-4-deficient mice (41,67). However, the suppressive mechanism of CTLA-4<sup>−/−</sup> T<sub>reg</sub> cells is quantitatively different and, in contrast to CTLA-4<sup>−/−</sup> T<sub>reg</sub> cells, seems to depend on TGF-β (68). This is supported by published evidence that implicates a role for CTLA-4 on T<sub>reg</sub> cells in controlling autoimmune T cell immunity but argues that T<sub>reg</sub> cell-mediated suppression does not fully account for the attenuating effects of CTLA-4 (69,70). Hence, CTLA-4 regulates adaptive immune responses by influencing the activities of various immune cells, including T<sub>eff</sub> and T<sub>reg</sub> cells.

4. GITR SHIFTS THE BALANCE OF T<sub>REG</sub> CELL-MEDIATED SUPPRESSION

During adaptive immune responses, antigen is presented to T cells in the MHC on the surface of APCs (for review, see 71,72). However, this engagement of the TCR, in and of itself, is not enough to activate T cells. Besides receptors of the immunoglobulin family, GITR and other TNFRs provide secondary signals that are integrated with the primary TCR stimulus to regulate diverse aspects of T cell function (73-75). These include lymphocyte expansion, and survival of antigen-specific T cells; differentiation into T helper subsets and memory cells; and effector functions that orchestrate the action of other inflammatory cells. Yet given the plethora of TNFRs and other receptors expressed on T cells, why would studying GITR-induced signaling increase our understanding of how the immune system operates? In this and subsequent sections, we will open a discourse addressing this question.

Accessory receptors among the same class operate under different conditions and yield distinct outcomes. TNFRs control the balance between lymphocyte survival and apoptosis (76). GITR and other TNFRs that lack a death domain in their intracellular region have been implicated in promoting T cell survival (77). As a means of attenuating immune responses, death domain-containing TNFRs can trigger apoptotic pathways to eliminate unwarranted T cells (78). Moreover, different regulatory mechanisms control the expression of related TNFRs, such as Ox40 and CD30, during the course of lymphocyte activation, suggesting that the receptors fulfill diverse functions (79). The incorporation of these diverse molecular events in T cells is essential for the maintenance of immune homeostasis. Individual accessory receptors impact the entire web of signaling events taking place within T cells. Therefore, grasping the intricacies of signaling triggered by GITR will provide insights into why GITR has been selected for and how the receptor is specialized in modulating immune responses.

Around the time that Sakaguchi and his colleagues sparked renewed interest in the suppressive effects of T<sub>reg</sub> cells, GITR was identified as a glucocorticoid-induced gene in a mouse T cell hybridoma (80). The immunologic function of GITR became apparent from microarray analysis of genes differentially expressed in T<sub>reg</sub> cells (81). Independently, a panel of Abs were screened for the ability to abrogate T<sub>reg</sub> cell-mediated inhibition as a means to characterize the molecular basis of T<sub>reg</sub> cell function (82). These complementary approaches revealed that GITR is highly expressed on T<sub>reg</sub> cells. When cross-linked with agonistic Abs in vitro or in vivo, GITR attenuates T<sub>reg</sub> cell-mediated suppression of T<sub>eff</sub> cell proliferation and abrogates peripheral immunological tolerance without eliminating T<sub>reg</sub> cells (82). Curiously, complete elimination of T<sub>reg</sub> cell function produces a wider spectrum of organ-specific pathology than the predominant autoimmune gastritis triggered by GITR stimulation in nude mice (82,83). The difference implies that the effect of GITR on T<sub>reg</sub> cell-mediated suppression is more selective than gross ablation of T<sub>reg</sub> cells. Alternatively, GITR-
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Figure 1. GITR exerts functional effects on T\textsubscript{reg} and immune effector cells. GITR is expressed on both T\textsubscript{eff} and T\textsubscript{reg} cells. Despite intensive research, the cellular and molecular events triggered by GITR in the two subsets of T cells has not been fully elucidated. The current notion is that GITR functions as costimulatory receptor on T\textsubscript{eff} cells and abrogates the suppressive function of T\textsubscript{reg} cells. Thereby, GITR promotes T cell effector functions, which are pivotal for successful immune responses but can also lead to autoimmune disorders. Whether this is due to GITR-induced proliferation of T cells, which is critical for T\textsubscript{eff} cell expansion but results in reduced suppression by T\textsubscript{reg} cells, or distinct signaling events in the two T cell types awaits clarification.

Induced signaling in cells other than T\textsubscript{reg} cells may direct immune responses specifically against gastric parietal cells. Nevertheless, GITR stimulation heightens inflammatory responses in experimental models of autoimmune encephalomyelitis and diabetes, indicating that GITR-induced signaling regulates immunologic tolerance (84,85). Hence, GITR is a crucial surface receptor in the regulation of T\textsubscript{reg} cell-mediated suppression of immune effector cells.

Given its restricted expression pattern on naïve T cells, GITR has been suggested to be a marker for T\textsubscript{reg} cells (81). This notion is supported by the observation that depletion of GITR\textsuperscript{high} cells before adoptive transfer of T cells into nude mice results in autoimmune gastritis in the host (82,86). Besides GITR, a select few TNFR family members including Ox40 and 4-1BB are preferentially expressed on T\textsubscript{reg} cells in naïve mice. Little is known about the role of these TNFRs in regulating T\textsubscript{reg} cell function, but their impact on the interplay between T\textsubscript{reg} cells and immune effector cells differs from GITR (81,87-89).

The mechanism of action for GITR-triggered signaling is currently controversial (Figure 1). GITR may attenuate T\textsubscript{reg} cells directly or activate T\textsubscript{eff} cells to overcome inhibition (for review, see 29). Using co-culture of mouse T\textsubscript{reg} cells with rat responder cells that are not stimulated by the agonistic Ab specific for mouse GITR-L by Stephens et al. revealed low levels of GITR-L on freshly isolated CD11c\textsuperscript{+} dendritic cells (DCs) and elevated GITR-L expression on splenic CD11c\textsuperscript{low} B220\textsuperscript{+} plasmacytoid DCs (94). In addition, the protein was detected on splenic as well as subsets of peritoneal B cells and DN thymocytes. While GITR-L could not be detected on more mature thymocytes or unstimulated T cells isolated from lymph nodes, its expression was induced after activation of both CD4 and CD8 T cells by soluble CD3-specific Ab. Similarly, activation of B cells resulted in an initial increase of GITR-L surface expression. Surface expression of GITR-L, however, was transient under these conditions and reached levels below those found on unstimulated cells after 48 to 60 hours of stimulation. Interestingly, signaling triggered by Toll-like receptors (TLRs) negatively regulates GITR-L expression on APCs. Taken together, the expression profile of GITR-L suggests that GITR-induced signaling plays a role in the early steps of thymocyte maturation and during the early phases of adaptive immune responses.

The ligands of mouse GITR and its human ortholog, termed activation-induced TNFR (AICTR) – GITR-L and AICTR-L, respectively – have recently been identified by several groups (90-93). While AICTR-L expression was first described in an endothelial cell line, GITR-L expression was found in dendritic cells. More detailed subsequent studies using an Ab specific for GITR-L revealed low levels of GITR-L on freshly isolated CD11c\textsuperscript{+} dendritic cells (DCs) and elevated GITR-L expression on splenic CD11c\textsuperscript{low} B220\textsuperscript{+} plasmacytoid DCs (94). In addition, the protein was detected on splenic as well as subsets of peritoneal B cells and DN thymocytes. While GITR-L could not be detected on more mature thymocytes or unstimulated T cells isolated from lymph nodes, its expression was induced after activation of both CD4 and CD8 T cells by soluble CD3-specific Ab. Similarly, activation of B cells resulted in an initial increase of GITR-L surface expression. Surface expression of GITR-L, however, was transient under these conditions and reached levels below those found on unstimulated cells after 48 to 60 hours of stimulation. Interestingly, signaling triggered by Toll-like receptors (TLRs) negatively regulates GITR-L expression on APCs. Taken together, the expression profile of GITR-L suggests that GITR-induced signaling plays a role in the early steps of thymocyte maturation and during the early phases of adaptive immune responses.

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Survival during early stages of activation (97,98,100). In agreement with the T<sub>eff</sub> cell population being a physiologic target, GITR stimulation of wild-type T<sub>eff</sub> cells interferes with T<sub>reg</sub> cell-mediated suppression irrespective of GITR expression on T<sub>reg</sub> cells (94). Importantly, GITR ligation on GITR<sup>−/−</sup> T<sub>reg</sub> cells in co-culture with GITR-deficient T<sub>eff</sub> cells did not abrogate suppression. Taken together, these findings suggest multiple sites of action for GITR, which influences the properties of T<sub>reg</sub> cells and T<sub>eff</sub> cells and thus enhances T cell immunity.

How does GITR induce abrogation of T<sub>reg</sub> cell-mediated suppression and trigger costimulatory effects in T cells? GITR may costimulate through regulation of nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs), which are known to exert pleiotropic influence over T cell activation, differentiation, and effector function (102,103). Perhaps GITR-induced signaling allows re-engagement of mitogenic pathways in T<sub>eff</sub> cells during co-culture with T<sub>reg</sub> cells. This notion would be consistent with the finding that T<sub>reg</sub> cell-mediated suppression of T<sub>eff</sub> cells is reversible after separation of the co-cultured T<sub>reg</sub> cells and proper antigen presentation (44,104). T<sub>reg</sub> cells uncouple the IL-2 signaling pathway in T<sub>eff</sub> cells leading to incomplete activation that results in an aergic phenotype (105). IL-2 blockade eliminates the ability of GITR to abrogate T<sub>reg</sub> cell-mediated inhibition, suggesting that IL-2 production induced by GITR in T<sub>eff</sub> cells is vital to overcome suppression (99). Of note, GITR stimulation also preferentially increases IL-10 production, although the significance of this immunomodulatory cytokine on GITR function has not been established (98). These data suggest that cellular events triggered by GITR target cytokine-induced signaling, which allows T<sub>eff</sub> cells to circumvent T<sub>reg</sub> cell-mediated suppression.

Besides its role in T<sub>eff</sub> Cells, GITR exerts potent cellular effects on T<sub>reg</sub> cells as evidenced by the observation that T<sub>reg</sub> cells pre-treated with an agonistic GITR-specific Ab were no longer effective in inhibiting T<sub>eff</sub> cell proliferation (88). Granzymes, a family of pro-apoptotic proteases, have been recently identified as mediators in T<sub>reg</sub> cell-mediated suppression. Large numbers of granzyme-filled granules are found in natural and adaptive human T<sub>reg</sub> cells capable of perforin-dependent autologous killing of activated T cells (106,107). Granzyme B is preferentially expressed at high levels in mouse T<sub>reg</sub> cells and is reduced upon GITR crosslinking (81,95). Confirming the role of granzymes in T<sub>reg</sub> cell-mediated suppression, granzyme B deficiency impairs the suppressive capacity of T<sub>reg</sub> cells (95). Perhaps GITR regulates signaling pathways and transcriptional programs that influence the granzyme profile and/or transport of pre-formed granzyme-containing vesicles in T<sub>reg</sub> cells. Besides elucidating granzyme transcriptional regulation, comparative microarray analysis of sorted T cell populations after GITR stimulation may uncover other effector proteins that account for the effects of GITR on T cell tolerance. Taken together, GITR may directly regulate cellular events in T<sub>reg</sub> cells that control the activation of T<sub>eff</sub> cells.

GITR-deficient mice that were generated in the laboratories of Riccardi and Pandolfi develop normally without any overt signs of autoimmunity, suggesting that GITR is not essential for Treg development and function or that compensatory mechanisms exist (108). Based on the ability of GITR to costimulate T<sub>eff</sub> cells and dampen T<sub>reg</sub> cell-mediated suppression, a reasonable prediction is that T cells lacking GITR respond less briskly to antigen receptor stimulation. Paradoxically, GITR-deficient T cells are hyper-responsive to TCR stimulation, implying that GITR is involved in uncharted aspects of immune regulation; possibilities include a role for the receptor in controlling the potency or frequency of T<sub>reg</sub> cells (108). Although GITR expression on T<sub>reg</sub> cells is not required for their suppressive function, T<sub>reg</sub> cell survival may depend on GITR and, thus, account for the paradoxical phenotype of GITR-deficient mice (94,97). Follow-up studies of GITR<sup>−/−</sup> T cells by Shevach and his colleagues have revealed the opposite trend; namely, impaired proliferation after TCR stimulation of GITR<sup>−/−</sup> T<sub>eff</sub> cells in the presence of physiologic number of T<sub>reg</sub> cells (94). Whether this discrepancy is due to differences in mitogenic stimuli or other experimental conditions remains to be determined. Interestingly, the TNFR family member herpes virus entry mediator (HVEM) was recently discovered as the physiologic ligand for B and T cell attenuator (BTLA), an immunoglobulin family receptor that provides inhibitory signals to lymphocytes (109). Therefore, one intriguing hypothesis is that GITR functions in a manner similar to HVEM by initiating signals triggered by a surface molecule that attenuates T cell responses. However, it remains to be determined whether the hyper-responsive phenotype of GITR<sup>−/−</sup> T cells is the result of such an effect of GITR. In total, genetic analyses argue that GITR plays a pivotal role in adaptive immune responses by controlling the activation potential of T cells.

GITR was described initially as a promoter of T cell survival, similar to other TNFRs lacking a death domain in their cytoplasmic domain (80). Reducing GITR expression by antisense mRNA predisposes T cell clones to apoptosis induced by anti-CD3 Ab, whereas GITR over-expression confers resistance to apoptosis (80,110). Further, T cells lacking GITR are more prone to activation-induced cell death (AICD), implying that GITR sustains T cell viability at later stages of T cell activation (108). However, GITR-deficient in comparison to –sufficient T cells produce elevated levels of IL-2, CD25, and Fas (CD95), which are well-characterized regulators of pro- and anti-apoptotic pathways (108). Hence, it is unclear whether the contribution of GITR to T cell survival is due to a direct signaling event or secondary to altered levels of proteins known to regulate cell survival. In addition, the argument has been made that GITR increases AICD and potentially initiates cell death pathways through interaction with the death domain-containing protein Siva (111,112). The potential role of GITR in activating cell death pathways could explain the observation that low levels of GITR stimulation foster allogeneic immune responses, while high amounts of GITR signaling attenuate them (88). Perhaps the actions of GITR resemble those of CD30, a
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TNFR lacking a death domain and sensitizes cells to TNF-α-induced apoptosis (113). Recently, T cells expressing physiologic quantities of GITR were used to revisit the issue of GITR in T cell survival (100). In contrast with studies using GITR-deficient or –transfected T cell lines, crosslinking of GITR expressed at physiologic levels on T cells does not prevent AICD despite activation of downstream signaling pathways triggered by the receptor (80,100,108). Although GITR is dispensable in preventing apoptosis of activated T cells, GITR fosters the survival of naïve T cells during early stages of activation (100). Therefore, the involvement of GITR in T cell survival pathways depends upon the activation state of T cells and the conditions used to activate and trigger cell death.

In peripheral tissues, T<sub>reg</sub> cells can persist quiescently for extended durations and are known to be highly resistant to apoptotic pathways (43,44,50,114). As GITR is constitutively expressed on T<sub>reg</sub> cells, it has been proposed that GITR is vital to the survival of this population (77). Indeed, reduced percentages of T<sub>reg</sub> cells in peripheral lymphoid organs of GITR-deficient mice have been described, but this finding was not reproduced in an independent study (94,97). Decreased apoptosis was seen in T<sub>reg</sub> cells treated with CD3- and GITR-specific Abs compared to anti-CD3 treatment alone, consistent with GITR promoting the survival of T<sub>reg</sub> cells (97). However, the propensity of GITR to trigger cell division in T<sub>reg</sub> cells complicates interpretation of this data. It is unclear whether the increased percentage of live T<sub>reg</sub> cells caused by GITR stimulation is due to decreased apoptosis and/or proliferative expansion. Therefore, determination of the cytoprotective role of GITR in T<sub>reg</sub> cells awaits further investigation.

GITR initiates diverse signaling pathways and transcriptional programs that control the interplay between T<sub>reg</sub> cells and immune effector cells. On the molecular level, GITR regulates the activity of MAPKs and NF-κB, which are pleiotropic effector proteins (102,103). These and other molecular events induced by GITR influence T cell activation, survival, and effector functions that orchestrate adaptive immune responses. While the importance of GITR in T cell biology has been appreciated, more detailed analyses of GITR-induced signal transduction pathways in human and mouse T cells are required to provide insights into the molecular basis of how GITR shapes pivotal facets of inflammation.

5. TRAFS LINK TNFRS TO DOWNSTREAM SIGNALING EVENTS

TNFR family members lack inherent enzymatic activity associated with their cytoplasmic domains (115). Through interactions with adapter proteins, the cytoplasmic domains of TNFRs serve as foci for the assembly of protein complexes that transmit extracellular signals (116). TRAFs are one such family of adapter proteins that are recruited directly or indirectly to TNFRs and regulate downstream signaling events including NF-κB and c-Jun N-terminal kinase (JNK) activation by mediating protein-protein interactions via their conserved C-terminal TRAF domain (117-119). For instance, TRAFs directly recruit the inhibitor of κB (IκB) kinase (IKK) signalosome to signaling complexes containing TNFRs in cooperation with receptor interacting protein (RIP) (120). Further, TRAFs interact with initiator kinases of other downstream signaling cascades, including NF-κB-inducing kinase (NIK), MAPK/extracellular signal-regulated kinase (ERK) kinase kinase (MEKK) 1, MEKK3, apoptosis signal-regulating kinase (ASK) 1, and germinal center kinase-related kinase (GCKR) (121-124). Consistent with the presence of N-terminal RING finger motifs, TRAFs can function as E3 ubiquitin ligases and are substrates of ubiquitination themselves (123,125-129). The roles of ubiquitination and other potential post-translational modifications of TRAFs and their interacting partners have not been clearly defined, but may provide specificity in TRAF-mediated regulation of downstream signaling events.

TRAF1, which is expressed in activated and transformed lymphocytes, dendritic cells, and epithelial cells, is thought to attenuate molecular events induced by TNFRs (130-132). TRAF2, which was identified alongside TRAF1 in TNFR-II-containing complexes, is ubiquitously expressed (117,133). Although genetic studies argue that TRAF2 augments signaling induced by TNFRs, TRAF2 inhibits activation of the alternative NF-κB pathway and represses T helper type 2 responses (134-137). These data raise the intriguing possibility that TRAF2 differentially regulates signaling pathways. Despite structural homology to TRAF2, TRAF3 in general inhibits molecular events triggered by TNFRs (138,139). Similar to mice lacking TRAF2, TRAF3-deficient mice runt and die shortly after birth (134,140). TRAF4 is the most divergent and least characterized TRAF (141). TRAF4-deficient mice are viable, but exhibit developmental defects in the trachea, axial skeleton, and closure of the neural tube (142,143). Although pivotal in these developmental processes and implicated in TNF-α-induced MAPK activation by oxidative pathways, TRAF4-mediated signaling induced by TNFRs remains to be defined (144,145). TRAF5 is structurally and functionally related to TRAF2 as evidenced by their redundancy in TNF-α-mediated NF-κB activation (146). However, consistent with the restricted expression of TRAF5 in lung, thymus, spleen and kidney, TRAF5<sup>−/−</sup> mice do not exhibit the severe wasting syndrome observed in mice lacking TRAF2 (134,147,148). Although TRAF5 subtly influences immune responses triggered by TNFRs, specific TRAF5-dependent signaling pathways have not been observed to date (148,149). TRAF6 elaborates signaling pathways induced by TNFRs as well as receptors of the IL-1R/TLR families (150). Consistent with these findings, TRAF6<sup>−/−</sup> mice develop osteopetrosis and exhibit impaired signaling downstream of IL-1R, TLR, and CD40 (151). Further, TRAF6 has been implicated in NF-κB activation induced by the TCR, but the physiological significance of this observation has not been ascertained (152).

6. TRAFS AS MEDIATORS OF GITR-INDUCED SIGNALING

Initial studies of TRAFs in GITR-induced signaling centered on the human receptor AITR. AITR
TABLE 1. The evolving paradigm of TRAF-mediated signaling triggered by GITR

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Previous View</th>
<th>Present View</th>
<th>Issues that need to be addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GITR</td>
<td>Molecular</td>
<td>AITR induces NF-κB activation.</td>
<td>GITR induces NF-κB and MAPK activation.</td>
<td>Impact on other signaling events.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Sites of action.</td>
</tr>
<tr>
<td>TRAF1</td>
<td>Molecular</td>
<td>Inhibits AITR-induced NF-κB activation.</td>
<td>Inhibits GITR-induced NF-κB activation.</td>
<td>Impact on other signaling events.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Sites of action.</td>
</tr>
<tr>
<td>TRAF2</td>
<td>Molecular</td>
<td>Dominant-negative mutant inhibits AITR-induced NF-κB activation, suggesting an activating role.</td>
<td>Inhibitory role in GITR-induced NF-κB activation. Antagonizes TRAF4-mediated signaling triggered by GITR.</td>
<td>Differences in molecular events induced by GITR and other TNFRs.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Role in other aspects of signaling.</td>
</tr>
<tr>
<td>TRAF3</td>
<td>Molecular</td>
<td>Inhibits AITR-induced NF-κB activation.</td>
<td>Inhibits GITR-induced NF-κB activation.</td>
<td>Impact on other signaling events.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Sites of action.</td>
</tr>
<tr>
<td>TRAF4</td>
<td>Molecular</td>
<td>No function in GITR-induced signaling.</td>
<td>Augments GITR-induced NF-κB activation. Antagonizes TRAF4-mediated signaling triggered by GITR.</td>
<td>Impact on other signaling events.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Sites of action.</td>
</tr>
<tr>
<td>TRAF5</td>
<td>Molecular</td>
<td>No function in GITR-induced signaling.</td>
<td>Critical for activation of p38, ERK, and NF-κB. Dispensable for JNK activation.</td>
<td>Biochemical link from GITR to signaling effectors. Potential cross-talk with other TRAFs.</td>
</tr>
<tr>
<td>Cellular</td>
<td>Not determined.</td>
<td></td>
<td></td>
<td>Sites of action.</td>
</tr>
</tbody>
</table>

Summary of TRAF-mediated events triggered by GITR. Studies of GITR and AITR have revealed novel aspects of TRAF-mediated signaling events. Additional experiments are necessary to characterize the impact of the individual TRAFs on GITR-mediated events in vivo. See text for further details and references.

binds to TRAF1, TRAF2 and TRAF3 and functional studies suggest a role of TRAFs in AITR-induced events (90). However, the mechanisms by which TRAFs affect GITR-induced molecular and cellular events have not been fully elucidated.

6.1. TRAF2 inhibits GITR-induced NF-κB activation

TRAFs are recruited to the cytoplasmic domain of GITR like other TNFRs (90,153). Further, certain features of GITR-induced signaling, such as NF-κB and MAPK activation, are analogous to pathways triggered by other TNFRs (Table 1 and references 75,91,93,96-100). Mutational analyses revealed that the ability of the receptor to recruit TRAFs correlates with GITR-induced NF-κB activation (153). A20, an NF-κB-dependent gene product that regulates TRAF-mediated signaling triggered by TNFRs, attenuates GITR-induced NF-κB activation (113,153-155). Transfection of TRAF1 and TRAF3 revealed that these TRAFs play their customary inhibitory roles in NF-κB activation induced by GITR and AITR (E.M. Espanza and R.H. Arch, unpublished observation and references 90,156). These parallels of signal transduction triggered by GITR and other TNFRs could underlie the common costimulatory function of several TNFR family members.

Despite similarities in signaling pathways among GITR and other TNFRs, analysis of TRAF-mediated events triggered by GITR discerned intriguing differences. For instance, TRAF2 inhibits GITR-induced NF-κB activation, which contrasts with the role of TRAF2 in augmenting signaling events triggered by other TNFRs, including AITR in human cells (Table 1 and references 110,134,135,153). The molecular differences in signaling pathways triggered by GITR, AITR and other TNFRs remain to be elucidated.

The mechanism underlying the distinct utilization of TRAF2 by GITR as a negative regulator of NF-κB activation may entail serine phosphorylation, K48-, and K63-linked polyubiquitination. These post-translational modifications regulate TRAF2 function by influencing receptor interactions, protein stability, and the E3 ubiquitin ligase activity of the adapter protein (123,127-129,157,158). TNFRs trigger the assembly of protein complexes to activate downstream events. Perhaps GITR fails to engage potentiating factors, such as protein kinases or components of the ubiquitin-conjugating apparatus, required for TRAF2 to activate NF-κB. Alternatively, regulatory proteins such as A20 and CYLD, which inhibit NF-κB activation triggered by TNFRs through their ubiquitin-editing domains, or protein phosphatases may be recruited to GITR-induced complexes to alter the mode of TRAF2-mediated signaling (159-161). Besides post-translational modifications, GITR may regulate TRAF2 localization such that critical components of the NF-κB activating machinery are present in suboptimal ratios or sequestered to be rendered inactive. Supporting this hypothesis, interaction with the cytoplasmic domain of GITR translocates TRAF2 to the detergent-insoluble fraction of cell lysates (113,153). Moreover, TRAF2 localization shifts from the cytoplasm to the plasma membrane upon ligand engagement of GITR (J.L. Yen and R.H. Arch, unpublished observation). Comparative analysis of post-translational modifications and sub-cellular localization of TRAF2 induced by GITR versus other TNFR-related proteins is necessary to delineate the unique role of TRAF2 in GITR-induced signaling.
both JNK activation and cell death, arguing that NF-κB antagonizes the JNK pathway as a means to fulfill its anti-apoptotic function. Further, NF-κB-dependent transcription regulates the duration of TNF-α-induced JNK activation that is dependent on the accumulation of reactive oxygen species (ROS) (164). ROS are themselves products of TRAF2-mediated signaling triggered by TNFRs (165). Suggested by the tightly linked nature of the JNK, NF-κB and ROS pathways, the inhibitory effects of TRAF2 on GITR-induced NF-κB activation may have wide-range consequences on JNK activation, ROS production, and other pathways that decide the fate of Treg cells and immune effector cells.

6.2. TRAF4 functions as distal signaling intermediate downstream of GITR

In addition to this newly characterized function of TRAF2, GITR employs the orphan TRAF family member TRAF4 as a mediator of NF-κB activation (Table 1 and reference 166). Consistent with the inhibitory function of A20 on TRAF-mediated signaling, expression of A20 abrogates the ability of TRAF4 to increase NF-κB activation. In contrast to other TRAFs, TRAF4 does not seem to interact with GITR (153). This suggests that TRAF4 augments NF-κB activation via a different mechanism than other TRAFs, which require receptor binding. TRAF4-mediated enhancement of GITR-induced NF-κB activation depends on TRAF-interacting residues in the cytoplasmic domain of GITR, arguing that other TRAFs or adapter proteins are required for TRAF4 function (166). TRAF4 contains two nuclear localization sequences, but can also be detected in the cytoplasm (167,168). These findings imply that shuttling of TRAF4 between the cytoplasm and the nucleus may underlie its ability to increase NF-κB activation triggered by GITR. Intriguingly, TRAF4 antagonizes the inhibitory effects of TRAF2 on NF-κB activation triggered by GITR (Figure 2 and reference 166). Moreover, TRAF4 relocalizes TRAF2 to the detergent-insoluble fraction of lysates from transfected HEK293 cells, which resembles a mode of regulation used by A20 and TRAF1 to limit TRAF2 signaling (R.H. Arch, unpublished observation and references 113,169). Our interpretation is that TRAF4 relieves inhibition by signaling attenuators, such as TRAF2, as a mechanism to augment GITR-induced NF-κB activation. To define further the function of TRAF4, it will be interesting to investigate whether TRAF4 can also regulate AITR-induced signaling in human cells or whether the effects of TRAF4 are restricted to GITR-induced signaling in the mouse.

Although originally identified as a nuclear protein, TRAF4 localizes in both overlapping and distinct cytoplasmic compartments with TRAF2 in thymocytes, splenocytes, and transfected HEK293 cells (E.M. Esparza, J.L. Yen, and R.H. Arch, unpublished observations and reference 167). This localization pattern is consistent with these TRAFs exhibiting functional interplay in the context of GITR signaling as well as controlling distinct downstream signaling effectors. For instance, TRAF4 interacts with p47phox, a regulatory component of the

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**Figure 2.** Newly described facets of TRAF-mediated signaling triggered by GITR. GITR directly recruits TRAF2, TRAF5, and other TRAFs, which may cross-talk through competition for overlapping receptor-binding sites and/or other protein-protein interactions. TRAF2 inhibits GITR-induced NF-κB activation. Furthermore, TRAF4 augments NF-κB activation triggered by GITR without requiring direct interaction with the receptor. The effects of TRAF2- and TRAF4-mediated signaling counteract each other in regulating GITR-induced NF-κB activation. Little is known about the role of TRAF5 in GITR-induced activation of NF-κB and MAPKs. We propose that TRAF5 links TRAF4 into proximal GITR-induced signaling events. Collectively, TRAFs function as integrative platforms regulating diverse signaling pathways triggered by GITR that exert pleiotropic effects on T cell function and thereby control adaptive immune responses.

Little is known regarding the impact of TRAF2 on signaling pathways triggered by GITR beyond regulating NF-κB activation. TRAF2 deficiency abolishes JNK activation induced by TNF-α (134). TRAF2 ubiquitination induced by TNF-α is necessary for relocalization of TRAF2 to the detergent-insoluble fraction that initiates only JNK activation (128). These findings indicate that TRAF2-mediated JNK activation can be mechanistically differentiated from NF-κB and p38 activation. In cells defective in NF-κB activation, prolonged JNK activation during TNF-α stimulation promotes apoptosis (162,163). Expression of the NF-κB-dependent gene products XIAP and GADD45β oppose...
6.3. Implications for TRAF5 and TRAF6 in Treg cell function and development

A recent study by Hauer et al. described an interaction between GITR and TRAF5, implying that the adapter protein functions as a proximal link for GITR to transmit downstream signaling events (Table 1 and reference 156). As distinct TRAFs bind overlapping sites in the cytoplasmic domains of TNFRs, TRAF5 may be recruited to the TRAF-interacting sites required for GITR-induced NF-κB activation (153). Additionally, TRAF5 may compete for contact surfaces of GITR and/or hetero-oligomerize with other TRAFs, providing additional means of cross-talk among TRAF-mediated pathways (Figure 2). Given the homology of TRAF5 to TRAF2, it would be intriguing to determine what role TRAF5 plays in GITR-induced signaling. Perhaps TRAF5 functions as an E3 ubiquitin ligase, whose activity is regulated by GITR crosslinking. Alternatively, TRAF5, through its interactions with enzymatic proteins and scaffold molecules, likely serves as an adapter protein involved in the assembly and disassembly of GITR-induced signaling complexes.

Thus far, the impact of TRAF6 on GITR-induced pathways remains unclear (Table 1). AITR-induced NF-κB activation is unaffected by a dominant negative mutant of TRAF6 (110). But Treg cells selectively express TLR4, -5, -7, and -8 and proliferate in response to LPS, which correlates with reduced suppressor capacity (170). As TRAF6 mediates certain aspects of TLR-induced signaling, TRAF6 may be involved in integrating molecular events triggered by TLRs in Treg cells. Besides regulating signaling within Treg cells, TRAF6 is critical for their development and preserving immunologic tolerance. Cortical and medullary thymic epithelial cells (cTECs and mTECs, respectively) orchestrate positive and negative selection of T cells as well as the generation of Treg cells, which are crucial for central and peripheral tolerance mechanisms (171,172). TRAF6 deficiency impairs the organization and maturation of mTECs (173). Importantly, Treg cells fail to develop in the disordered thymus of TRAF6-deficient mice and autoimmunity ensues after transplantation of TRAF6−/− thymic stoma into nude mice (173). These findings illustrate the importance of TRAF6 in maintaining self-tolerance.

6.4 TRAFs are pivotal intermediates of GITR-induced signaling and other pathways that influence Treg cell function

Similar to their function downstream of other TNFRs, TRAF1 and TRAF3 inhibit GITR-induced NF-κB activation. TRAF2, however, plays a novel inhibitory role in GITR-induced NF-κB activation. Interestingly, although TRAF4 augments GITR-induced NF-κB activation and antagonizes the effects of TRAF2, it acts at a more distal stage of GITR-induced signaling than other TRAFs. GITR interacts with TRAF5, implying that this adapter protein transmits signals induced by GITR. TRAF6 is essential in maintaining immunologic tolerance and promoting the development of Treg cells but does not seem to play a role in GITR-induced events. The findings that TRAF-mediated pathways intersect in regulating GITR-induced NF-κB activation highlight the importance of cross-talk among distinct TRAFs in controlling the outcome of GITR-induced signaling. The interconnection among convergent and divergent branches of TRAF-mediated signaling argues that GITR-induced signaling has far-reaching impact on the entire signaling network in T cells.

7. OTHER SIGNALING INTERMEDIATES DOWNSTREAM OF GITR

Besides TRAFs, other signaling molecules have been implicated in GITR-induced signal transduction. For instance, GITR interacts with Siva, a death-domain containing protein that induces caspase-dependent apoptosis of T cells (111,174). Moreover, GITR has been implicated as a weak inducer of the noncanonical NF-κB pathway, consistent with the observation that AITR-induced NF-κB activation is inhibited by the dominant-negative mutant of NIK (110,156). A20 impedes GITR-induced NF-κB activation, illustrating how modifiers of TRAF-mediated signaling provide additional means of regulating molecular events triggered by GITR (153,166). The cytoplasmic domain of GITR contains a putative serine phosphorylation site, implying that phosphoserine-specific adapter proteins are involved in GITR-induced signaling (80). Moreover, splice variants of GITR have been identified with distinct cytoplasmic domains, one of which may bind the Src-related kinase p56Lck through a CXC motif (175). Further genetic, biochemical, and functional analysis should broaden our understanding of these and other signaling mechanisms used by GITR to control T cell function.

8. CONCLUSIONS AND PERSPECTIVES

The immune system of humans and other mammals consists of a network of regulatory and effector cells that defend the body against cancer cells and invading pathogens (72). Specialized in regulating diverse aspects of the immune response, Treg cells illustrate the theoretical principle articulated by Paul Ehrlich that the immune system should not damage the organism it was designed to protect (176). Treg cells have evolved to finely tune inflammatory responses, safeguard against autoimmunity, and facilitate induction of immunological memory against invading pathogens (177,178). Disturbances in the equilibrium between Treg cells and immune effector cells have been linked to autoimmune responses and inefficient clearance of pathogens and cancerous cells (29,179). Autoimmunity occurs when the function of Treg cells is compromised as evidenced by the development of detrimental IPEX in children (35,36). Treg cell-mediated suppression is a prime target for infectious agents that subvert the immune system to avoid elimination. Excessive suppression mediated by Treg cells facilitates chronic infections and instigates disease reactivation that
fosters transmission (179). Bypassing T<sub>reg</sub> cell-mediated inhibition is a prerequisite to mount effector responses that clear infections (180). However, complete elimination of suppression is maladaptive in cases where T<sub>reg</sub> cells allow pathogen persistence, which promotes generation and maintenance of memory cells that defend against subsequent infections (181). Given that many tumor-associated antigens are derived from normal self-constituents, T<sub>reg</sub> cells designed to maintain self-tolerance accumulate around cancerous sites and hinder the activation and expansion of tumor-specific T<sub>eff</sub> cells (182-184). While depletion of T<sub>reg</sub> cells or other immunomodulatory strategies, such as CTLA-4 blockade, can provoke ardent immune responses to cancerous cells, these treatment may also impact non-specifically effector immune responses (185,186). Given the potential side effects of autoimmune and disrupting beneficial immune responses, more selective methods of manipulating T<sub>reg</sub> cell-mediated suppression are needed before they can be applied in clinical applications for humans.

GITR-induced signaling represents an avenue to manipulate immune responses by altering the dynamics between T<sub>reg</sub> cells and immune effector cells. As proof of principle, GITR stimulation facilitates the clearance of chronic viral infection and anti-tumorigenic immune responses in experimental model systems (187,188). Moreover, molecular events triggered by GITR have been implicated in reversing the decline in immune effector functions due to aging (189). Conversely, interference with GITR-induced signaling may promote engraftment of transplanted organs and remedy immune-mediated disorders. Insights into the distinctive utilization of TRAFs and other intermediates of GITR-induced signaling have furthered our understanding of the molecular events triggered by this receptor. Specifically, TRAFs and other adapter proteins integrate GITR-induced signaling that control T cell function and can be targeted to modulate immune responses. Similarities and differences in the function of mouse and human T<sub>reg</sub> cells as well as signaling pathways triggered by GITR in mouse and AITR in human cells demonstrate the importance of studying the mechanisms controlling the function of T<sub>reg</sub> cells. Application of principles learned from GITR-induced signaling may provide the mechanistic underpinnings for the development of innovative therapeutic strategies that adjust the equilibrium between T<sub>reg</sub> cells and immune effector cells to achieve the desired clinical goal.

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death domain, Siva-2, similarly induce apoptosis in T lymphocytes via a caspase-dependent mitochondrial pathway. 


Abbreviations: AICD – activation-induced cell death; AITR – activation-induced TNFR; APC – antigen presenting cell; ASK – apoptosis signal-regulating kinase; BTLA – B and T cell attenuator; cTEC – cortical thymic epithelial cell; CTLA-4 – cytotoxic T lymphocyte antigen; DC – dendritic cell; ERK – extracellular signal-regulated kinase; GCKR – germinal center kinase-related kinase; GFP – green fluorescent protein; GITR – glucocorticoid-induced TNFR family-related gene; HA – hemagglutinin; HVEM – Herpes virus entry mediator; IKK – IκB kinase; IL – interleukin; IPEX – immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; IκB – inhibitor of IκB; JNK – c-Jun N-terminal kinase; LAG – lymphocyte activation gene; MAPK – mitogen-activated protein kinase; MEKK – MAPK/ERK kinase kinase; MHC – major histocompatibility complex; mTEC – medullary TEC; NF-κB – nuclear factor κB; NIK – NF-κB-inducing kinase; RIP – receptor-interacting protein; ROS – reactive oxygen species; TCR – T cell receptor; T_{eff} cell – effector T cell; TLR – Toll-like receptor; TNF – tumor necrosis factor; TNFR – TNF receptor; TRAF – TNF-associated factor; T_{reg} cell – regulatory T cell

Key Words: Immune Response, Signal Transduction, T cells, Tumor necrosis factor receptor (TNFR), Cytokine, TNFR-associated factor (TRAF), Inhibition, Activation, Glucocorticoid-induced TNFR family-related gene (GITR), NF-kappaB, Signaling, Review

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