[Frontiers in Bioscience 11, 1448-1465, May 1, 2006]

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

Edward M. Esparza and Robert H. Arch

Departments of Medicine and Pathology & Immunology, Washington University, School of Medicine, Saint Louis, MO 63110

TABLE OF CONTENTS

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_{reg}) 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_{reg} 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_{reg} 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_{reg} cells and immune effector cells.

2. BRIEF HISTORY OF NATURAL T_{REG} 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 $CD5^{high}$ or $CD45RB^{low}$ and $CD45RC^{low}$ T cells, respectively, exhibit suppressor activity (9-13). But these markers are expressed too widely to define T_{reg} 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 $CD4^+$ $CD25^+$ T_{reg} cells that develop in the thymus have been distinguished from adaptive T_{reg} cells, such as IL-10-producing T_R1 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 T_{reg} cells and discuss the current models of how these specialized T cells operate and are influenced by GITR-induced signaling.

$CD4^+$ $CD25^+$ T_{reg} 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 T_{reg} 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 T_{reg} 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 T_{reg} 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 T_{reg} cells and to regulate their suppressive function (22-25). However, more detailed analyses are required to define the consequences of antigen stimulation of T_{reg} cells. At least *in vitro*, T_{reg} cell-mediated suppression depends on cell-cell contact. Therefore, it is conceivable that T_{reg} cells are exposed to antigen at the site of an inflammatory response. This notion is supported by the finding that T_{reg} cells express a unique pattern of chemokine receptors, arguing that the balance of T_{eff} and T_{reg} 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 T_{REG} CELL FUNCTION

Most cell surface proteins used as markers of T_{reg} 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 T_{reg} cells is the expression of the forkhead-box transcription factor FoxP3 (29). T_{reg} 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 T_{reg} cell marker in cell sorting experiments (33).

3.1. FoxP3 is an essential marker of T_{reg} 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 scurfin, resemble mice genetically deficient in FoxP3, which lack natural T_{reg} cells and display an IPEX-like syndrome (30-32,37). Adoptive transfer of FoxP3-sufficient T_{reg} cells is sufficient to reverse the phenotype caused by disruptions of FoxP3 function. Furthermore, ectopic expression of FoxP3 in T_{eff} cells confers a T_{reg} cell-like phenotype, suggesting that the transcription factor instructs T_{reg} 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 T_{reg} cells from other T cell subsets. Taken together, FoxP3 is an essential component of the genetic program that specifies the development of the natural T_{reg} cell lineage.

3.2. IL-2 is a critical regulatory cytokine for T_{reg} cells

To analyze the mechanism of action of T_{reg} cells, Thornton and Shevach established an *in vitro* assay to approximate the suppressive function of T_{reg} cells *in vivo* (39). When T_{reg} cells are removed from T cell populations, the remaining T_{eff} cells respond more briskly to TCR stimulation. Reconstitution of T_{reg} cells into effector cell populations – typically $CD4^+$ $CD25^-$ T_{eff} cells – dampens T_{eff} cells proliferative responses (15). TCR-stimulated T_{reg} cells suppress T cell activation in an antigen-nonspecific fashion that requires close proximity of T_{reg} 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 T_{reg} 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 T_{reg} cell-mediated suppression of responder cells. T_{reg} cells subvert IL-2 production by T_{eff} cells, which primes T_{reg} cell suppressive activity to quell additional IL-2 synthesis required for effector cell proliferation (41). Given their constitutive CD25 expression, T_{reg} 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 T_{reg} 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, T_{reg} cell-mediated suppression *in vitro* targets IL-2 and requires high ratios of T_{reg} cells to effector cells.

Although instrumental in studies of T_{reg} cell function, the *in vitro* suppressor assay does not always recapitulate the *in vivo* dynamics of T_{reg} cells. For instance, inhibitory cytokines such as IL-10 and TGF-β are expressed at high levels by T_{reg} 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

anergic phenotype *in vitro*, T_{reg} cells can proliferate in lymphopenic hosts, in mice deficient in T_{reg} 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 disease 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_{reg} cells. However, adoptive transfer of wild-type T_{reg} 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_{reg} cell-mediated suppression *in vivo*. The extracellular milieu, homing of proliferating T_{reg} 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_{reg} cells controls APC functions via reverse signaling

The observations that CTLA-4 is constitutively expressed on T_{reg} 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_{reg} 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-$4^{-/-}$ 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_{reg} cell-mediated suppression of T_{eff} cells *in vitro* (61). CTLA-4 expressed on T_{reg} 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_{eff} cell activation (64). On the other hand, T_{reg} cell-mediated suppression occurs in APC-free culture conditions, indicating that the inhibitory effects of T_{reg} 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 $CD4^+$ $CD25^+$ T_{reg} 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_{reg} cell survival or activation, T_{reg} cells can be isolated from CTLA-4-deficient mice (41,67). However, the suppressive mechanism of CTLA-$4^{-/-}$ T_{reg} cells is quantitatively different and, in contrast to CTLA-4^+ T_{reg} cells, seems to depend on TGF-β (68). This is supported by published evidence that implicates a role for CTLA-4 on T_{reg} cells in controlling autoreactive T cell immunity but argues that T_{reg} 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_{eff} and T_{reg} cells.

4. GITR SHIFTS THE BALANCE OF T_{REG} 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 activation, 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_{reg} 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_{reg} cells (81). Independently, a panel of Abs were screened for the ability to abrogate T_{reg} cell-mediated inhibition as a means to characterize the molecular basis of T_{reg} cell function (82). These complementary approaches revealed that GITR is highly expressed on T_{reg} cells. When cross-linked with agonistic Abs *in vitro* or *in vivo*, GITR attenuates T_{reg} cell-mediated suppression of T_{eff} cell proliferation and abrogates peripheral immunological tolerance without eliminating T_{reg} cells (82). Curiously, complete elimination of T_{reg} 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_{reg} cell-mediated suppression is more selective than gross ablation of T_{reg} cells. Alternatively, GITR-

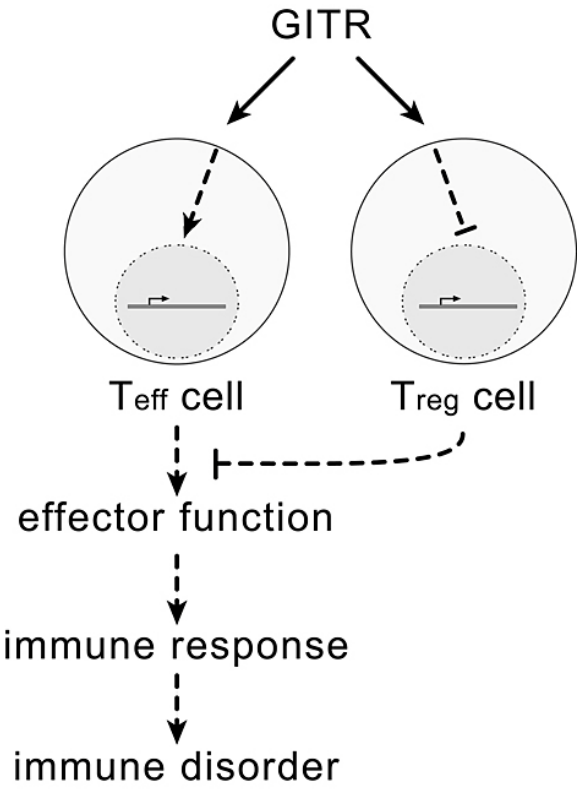

Figure 1. GITR exerts functional effects on T_{reg} cells and immune effector cells. GITR is expressed on both T_{eff} and T_{reg} cells. Despite intensive research, the cellular and molecular events triggered by GITR in the two subsets of T cells has not been fully elucidated. The current notion is that GITR functions as costimulatory receptor on T_{eff} cells and abrogates the suppressive function of T_{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_{eff} cell expansion but results in reduced suppression by T_{reg} cells, or distinct signaling events in the two T cell types awaits clarification.

induced signaling in cells other than T_{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_{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_{reg} cells (81). This notion is supported by the observation that depletion of $GITR^{high}$ 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_{reg} cells in naïve mice. Little is known about the role of these TNFRs in regulating T_{reg} cell function, but their impact on the interplay between T_{reg} cells and immune effector cells differs from GITR (81,87-89). For example, GITR but neither Ox40 nor 4-1BB can trigger proliferation of T_{reg} cells, which is even more pronounced in the presence of T_{eff} cells (88,89). Moreover, ectopic expression of FoxP3 in T_{eff} cells not only induces a functional T_{reg} cell phenotype as described above, but also causes constitutive expression of GITR in the absence of TCR stimulation (31,32). These findings suggest that FoxP3 targets GITR expression as part of a specialized transcriptional program associated with instruction of the T_{reg} cell lineage.

The ligands of mouse GITR and its human ortholog, termed activation-induced TNFR (AITR) – GITR-L and AITR-L, respectively – have recently been identified by several groups (90-93). While AITR-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 by Stephens *et al.* revealed low levels of GITR-L on freshly isolated $CD11c^{+}$ dendritic cells (DCs) and elevated GITR-L expression on splenic $CD11c^{low}$ $B220^{+}$ 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 mechanism of action for GITR-triggered signaling is currently controversial (Figure 1). GITR may attenuate T_{reg} cells directly or activate T_{eff} cells to overcome inhibition (for review, see 29). Using co-culture of mouse T_{reg} cells with rat responder cells that are not stimulated by the agonistic Ab specific for mouse GITR, initial reports concluded that GITR-induced signaling in T_{reg} cells directly abrogates their suppressive capacity (82). Consistent with this notion, GITR stimulation of sorted T_{reg} cells alters expression of suppressive mediators, such as granzymes (95). However, low levels of GITR are expressed on naïve T_{eff} cells, which increase upon lymphocyte activation (81,82,96-100). Consistent with the increased GITR expression on activated T_{eff} cells, the promoter of GITR contains multiple binding sites for transcription factors that orchestrate T cell effector responses (101). GITR ligation augments T_{eff} cell proliferation triggered by sub-optimal TCR agonists (96-100). GITR also promotes other hallmarks of costimulation including cytokine production and T cell

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

How does GITR induce abrogation of T_{reg} 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_{eff} cells during co-culture with T_{reg} cells. This notion would be consistent with the finding that T_{reg} cell-mediated suppression of T_{eff} cells is reversible after separation of the co-cultured T_{eff} cells and proper antigen presentation (44,104). T_{reg} cells uncouple the IL-2 signaling pathway in T_{eff} cells leading to incomplete activation that results in an anergic phenotype (105). IL-2 blockade eliminates the ability of GITR to abrogate T_{reg} cell-mediated inhibition, suggesting that IL-2 production induced by GITR in T_{eff} 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_{eff} cells to circumvent T_{reg} cell-mediated suppression.

Besides its role in T_{eff} cells, GITR exerts potent cellular effects on T_{reg} cells as evidenced by the observation that T_{reg} cells pre-treated with an agonistic GITR-specific Ab were no longer effective in inhibiting T_{eff} cell proliferation (88). Granzymes, a family of pro-apoptotic proteases, have been recently identified as mediators in T_{reg} cell-mediated suppression. Large numbers of granzyme-filled granules are found in natural and adaptive human T_{reg} cells capable of perforin-dependent autologous killing of activated T cells (106,107). Granzyme B is preferentially expressed at high levels in mouse T_{reg} cells and is reduced upon GITR crosslinking (81,95). Confirming the role of granzymes in T_{reg} cell-mediated suppression, granzyme B deficiency impairs the suppressive capacity of T_{reg} 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_{reg} 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_{reg} cells that control the activation of T_{eff} 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_{eff} cells and dampen T_{reg} 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_{reg} cells (108). Although GITR expression on T_{reg} cells is not required for their suppressive function, T_{reg} cell survival may depend on GITR and, thus, account for the paradoxical phenotype of GITR-deficient mice (94,97). Follow-up studies of $GITR^{-/-}$ T cells by Shevach and his colleagues have revealed the opposite trend; namely, impaired proliferation after TCR stimulation of $GITR^{-/-}$ T_{eff} cells in the presence of physiologic number of T_{reg} 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 $GITR^{-/-}$ 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

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_{reg} 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_{reg} cells, it has been proposed that GITR is vital to the survival of this population (77). Indeed, reduced percentages of T_{reg} 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_{reg} cells treated with CD3- and GITR-specific Abs compared to anti-CD3 treatment alone, consistent with GITR promoting the survival of T_{reg} cells (97). However, the propensity of GITR to trigger cell division in T_{reg} cells complicates interpretation of this data. It is unclear whether the increased percentage of live T_{reg} cells caused by GITR stimulation is due to decreased apoptosis and/or proliferative expansion. Therefore, determination of the cytoprotective role of GITR in T_{reg} cells awaits further investigation.

GITR initiates diverse signaling pathways and transcriptional programs that control the interplay between T_{reg} 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, $TRAF5^{-/-}$ 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, $TRAF6^{-/-}$ 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

Protein	Function	Previous View	Present View	Issues that need to be addressed
GITR	Molecular	AITR induces NF-κB activation.	GITR induces NF-κB and MAPK activation.	Impact on other signaling events.
	Cellular	May inhibit AICD. Abrogates suppressive effects of T_{reg} cells.	Does not inhibit AICD. Promotes T cell survival in early activation phase. Enhances activation of T_{eff} cells.	Sites of action. Mechanism of regulating immunologic tolerance.
TRAF1	Molecular	Inhibits AITR-induced NF-κB activation.	Inhibits GITR-induced NF-κB activation.	Impact on other signaling events.
	Cellular	Not determined.	Not determined.	Sites of action
TRAF2	Molecular	Dominant-negative mutant inhibits AITR-induced NF-κB activation, suggesting an activating role.	Inhibitory role in GITR-induced NF-κB activation. Antagonizes TRAF4–mediated signaling triggered by GITR.	Differences in molecular events induced by GITR and other TNFRs. Role in other aspects of signaling.
	Cellular	Not determined.	Not determined.	*In vivo* mechanisms and sites of action.
TRAF3	Molecular	Inhibits AITR-induced NF-κB activation.	Inhibits GITR-induced NF-κB activation.	Impact on other signaling events.
	Cellular	Not determined.	Not determined.	*In vivo* mechanisms and sites of action.
TRAF4	Molecular	No function in GITR-induced signaling.	Augments GITR-induced NF-κB activation. Antagonizes TRAF2-mediated signaling triggered by GITR.	Impact on other signaling events.
	Cellular	Not determined.	Not determined.	*In vivo* mechanisms and sites of action.
TRAF5	Molecular	No function in GITR-induced signaling.	Critical for activation of p38, ERK, and NF-κB. Dispensable for JNK activation.	Biochemical link from GITR to signaling effectors. Potential cross-talk with other TRAFs.
	Cellular	Not determined.	Enhances GITR-induced T cell activation.	*In vivo* impact and sites of action.
TRAF6	Molecular	Not determined.	Does not play a role downstream of GITR.	Not applicable.
	Cellular	Not determined.	Not applicable.	Not applicable.

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. Esparza 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.

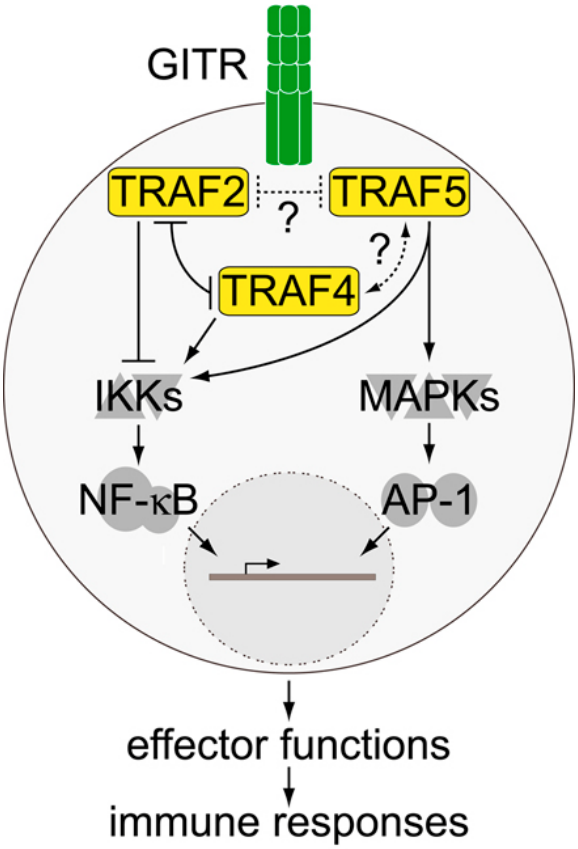

Figure 2. Newly described facets of TRAF-mediated signaling triggered by GITR. GITR directly recruits TRAF2, TRAF5, and other TRAFs, which may cross-talk through competition for overlapping receptor-binding sites and/or other protein-protein interactions. TRAF2 inhibits GITR-induced NF-κB activation. 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 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 T_{reg} 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 $p47^{phox}$, a regulatory component of the

NADPH oxidase complex that is a potent source of ROS (144,145). The interaction between TRAF4 and $p47^{phox}$ regulates TNF-α-induced ERK and JNK activation (144,145). As a result, TRAF4 may be involved in other divergent branches of GITR-induced signaling.

6.3. Implications for TRAF5 and TRAF6 in T_{reg} 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 T_{reg} 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 T_{reg} cells. Besides regulating signaling within T_{reg} 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 T_{reg} cells, which are crucial for central and peripheral tolerance mechanisms (171,172). TRAF6 deficiency impairs the organization and maturation of mTECs (173). Importantly, T_{reg} 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 T_{reg} 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 T_{reg} 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 $p56^{lck}$ 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, T_{reg} cells illustrate the theoretical principle articulated by Paul Ehrlich that the immune system should not damage the organism it was designed to protect (176). T_{reg} 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 T_{reg} 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 T_{reg} cells is compromised as evidenced by the development of detrimental IPEX in children (35,36). T_{reg} cell-mediated suppression is a prime target for infectious agents that subvert the immune system to avoid elimination. Excessive suppression mediated by T_{reg} cells facilitates chronic infections and instigates disease reactivation that

fosters transmission (179). Bypassing T_{reg} 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_{reg} 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_{reg} cells designed to maintain self-tolerance accumulate around cancerous sites and hinder the activation and expansion of tumor-specific T_{eff} cells (182-184). While depletion of T_{reg} 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 (69,185,186). Given the potential side effects of autoimmunity and disrupting beneficial immune responses, more selective methods of manipulating T_{reg} 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_{reg} 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_{reg} 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_{reg} 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_{reg} cells and immune effector cells to achieve the desired clinical goal.

9. ACKNOWLEDGEMENTS

We apologize to all colleagues who have contributed to our understanding of T_{reg} cell biology and GITR-induced signaling but whose work could not be cited due to space limitations. Research of the authors was supported in part by grants of the National Institute of Health (NIH) [5 RO1 HL67312-02 and T32 HL007317] and an Investigator Award of the Cancer Research Institute [RHA].

10. REFERENCES

1.Goodnow, C. C., J. Sprent, B. F. de St Groth & C. G. Vinuesa: Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 435(7042), 590-597 (2005)

2.Goodnow, C. C., J. Crosbie, S. Adelstein, T. B. Lavoie, S. J. Smith-Gill, R. A. Brink, H. Pritchard-Briscoe, J. S. Wotherspoon, R. H. Loblay, K. Raphael, R. J. Trent & A. Basten: Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* 334(6184), 676-682 (1988)

3.Hartley, S. B., J. Crosbie, R. Brink, A. B. Kantor, A. Basten & C. C. Goodnow: Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature* 353(6346), 765-769 (1991)

4.Kisielow, P., H. Blüthmann, U. D. Staerz, M. Steinmetz & H. von Boehmer: Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature* 333(6175), 742-746 (1988)

5.Surh, C. D. & J. Sprent: T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature* 372(6501), 100-103 (1994)

6.Anderton, S. M. & D. C. Wraith: Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol* 2(7), 487-498 (2002)

7.Bernard, C. C. & P. R. Carnegie: Experimental autoimmune encephalomyelitis in mice: immunologic response to mouse spinal cord and myelin basic proteins. *J Immunol* 114(5), 1537-1540. (1975)

8.Nishizuka, Y. & T. Sakakura: Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science* 166(906), 753-755 (1969)

9.Sakaguchi, S., K. Fukuma, K. Kuribayashi & T. Masuda: Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J Exp Med* 161(1), 72-87 (1985)

10.Powrie, F. & D. Mason: $OX\text{-}22^{high}$ $CD4^+$ T cells induce wasting disease with multiple organ pathology: prevention by the $OX\text{-}22^{low}$ subset. *J Exp Med* 172(6), 1701-1708 (1990)

11.Fowell, D. & D. Mason: Evidence that the T cell repertoire of normal rats contains cells with the potential to cause diabetes. Characterization of the $CD4^+$ T cell subset that inhibits this autoimmune potential. *J Exp Med* 177(3), 627-636 (1993)

12.Powrie, F., M. W. Leach, S. Mauze, L. B. Caddle & R. L. Coffman: Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* 5(11), 1461-1471 (1993)

13.Xystrakis, E., I. Bernard, A. S. Dejean, T. Alsaati, P. Druet & A. Saoudi: Alloreactive CD4 T lymphocytes responsible for acute and chronic graft-versus-host disease are contained within the $CD45RC^{high}$ but not the $CD45RC^{low}$ subset. *Eur J Immunol* 34(2), 408-417 (2004)

14.Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh & M. Toda: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25) Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 155(3), 1151-1164. (1995)

15.Shevach, E. M.: $CD4^+$ $CD25^+$ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2(6), 389-400. (2002)

16.Bluestone, J. A. & A. K. Abbas: Natural versus adaptive regulatory T cells. *Nat Rev Immunol* 3(3), 253-257. (2003)

17.Asseman, C. & M. von Herrath: About $CD4^{pos}$ $CD25^{pos}$ regulatory cells. *Autoimmun Rev* 1(4), 190-197 (2002)

18.Buenafe, A. C., L. Tsaknaridis, L. Spencer, K. S. Hicks, R. H. McMahan, L. Watson, N. E. Culbertson, D. Latocha, K. Wegmann, T. Finn, R. Bartholomew, G. G. Burrows, R. Whitham, D. N. Bourdette, R. E. Jones, H. Offner, Y. K. Chou & A. A. Vandenbark: Specificity of regulatory $CD4^+CD25^+$ T cells for self-T cell receptor determinants. *J Neurosci Res* 76(1), 129-140 (2004)

19.Fujishima, M., M. Hirokawa, N. Fujishima & K. Sawada: TCRαβ repertoire diversity of human naturally occurring $CD4^+CD25^+$ regulatory T cells. *Immunol Lett* 99(2), 193-197 (2005)

20.Hsieh, C. S., Y. Liang, A. J. Tyznik, S. G. Self, D. Liggitt & A. Y. Rudensky: Recognition of the peripheral self by naturally arising $CD25^+$ $CD4^+$ T cell receptors. *Immunity* 21(2), 267-277 (2004)

21.Jordan, M. S., A. Boesteanu, A. J. Reed, A. L. Petrone, A. E. Holenbeck, M. A. Lerman, A. Naji & A. J. Caton: Thymic selection of $CD4^+CD25^+$ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2(4), 301-306 (2001)

22.Seddon, B. & D. Mason: Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J Exp Med* 189(5), 877-882 (1999)

23.Salomon, B., D. J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe & J. A. Bluestone: B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12(4), 431-440 (2000)

24.Karim, M., G. Feng, K. J. Wood & A. R. Bushell: $CD25^+CD4^+$ regulatory T cells generated by exposure to a model protein antigen prevent allograft rejection: antigen-specific reactivation *in vivo* is critical for bystander regulation. *Blood* 105(12), 4871-4877 (2005)

25.Voo, K. S., G. Peng, Z. Guo, T. Fu, Y. Li, L. Frappier & R. F. Wang: Functional characterization of EBV-encoded nuclear antigen 1-specific $CD4^+$ helper and regulatory T cells elicited by *in vitro* peptide stimulation. *Cancer Res* 65(4), 1577-1586 (2005)

26.Colantonio, L., A. Iellem, F. Sinigaglia & D. D'Ambrosio: Skin-homing CLA^+ T cells and regulatory $CD25^+$ T cells represent major subsets of human peripheral blood memory T cells migrating in response to CCL1/I-309. *Eur J Immunol* 32(12), 3506-3514 (2002)

27.Iellem, A., M. Mariani, R. Lang, H. Recalde, P. Panina-Bordignon, F. Sinigaglia & D. D'Ambrosio: Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by $CD4^+CD25^+$ regulatory T cells. *J Exp Med* 194(6), 847-853 (2001)

28.Arch, R. H.: Function of tumor necrosis factor receptor (TNFR) family members on regulatory T cells. *Immunol. Res.*, *in press* (2005)

29.Sakaguchi, S.: Naturally arising Foxp3-expressing $CD25^+CD4^+$ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 6(4), 345-352 (2005)

30.Khattri, R., T. Cox, S. A. Yasayko & F. Ramsdell: An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat Immunol* 4(4), 337-342. (2003)

31.Hori, S., T. Nomura & S. Sakaguchi: Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299(5609), 1057-1061. (2003)

32.Fontenot, J. D., M. A. Gavin & A. Y. Rudensky: Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat Immunol* 4(4), 330-336. (2003)

33.Schubert, L. A., E. Jeffery, Y. Zhang, F. Ramsdell & S. F. Ziegler: Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem* 276(40), 37672-37679 (2001)

34.Chatila, T. A., F. Blaeser, N. Ho, H. M. Lederman, C. Voulgaropoulos, C. Helms & A. M. Bowcock: JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J Clin Invest* 106(12), R75-81 (2000)

35.Bennett, C. L., J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance & H. D. Ochs: The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 27(1), 20-21 (2001)

36.Wildin, R. S., F. Ramsdell, J. Peake, F. Faravelli, J. L. Casanova, N. Buist, E. Levy-Lahad, M. Mazzella, O. Goulet, L. Perroni, F. D. Bricarelli, G. Byrne, M. McEuen, S. Proll, M. Appleby & M. E. Brunkow: X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 27(1), 18-20 (2001)

37.Brunkow, M. E., E. W. Jeffery, K. A. Hjerrild, B. Paeper, L. B. Clark, S. A. Yasayko, J. E. Wilkinson, D. Galas, S. F. Ziegler & F. Ramsdell: Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal

lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 27(1), 68-73 (2001)

38.Fontenot, J. D., J. P. Rasmussen, L. M. Williams, J. L. Dooley, A. G. Farr & A. Y. Rudensky: Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* 22(3), 329-341 (2005)

39.Thornton, A. M. & E. M. Shevach: $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 188(2), 287-296 (1998)

40.Thornton, A. M. & E. M. Shevach: Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific. *J Immunol* 164(1), 183-190 (2000)

41.Thornton, A. M., C. A. Piccirillo & E. M. Shevach: Activation requirements for the induction of $CD4^+CD25^+$ T cell suppressor function. *Eur J Immunol* 34(2), 366-376 (2004)

42.de la Rosa, M., S. Rutz, H. Dorninger & A. Scheffold: Interleukin-2 is essential for $CD4^+CD25^+$ regulatory T cell function. *Eur J Immunol* 34(9), 2480-2488 (2004)

43.Apostolou, I., A. Sarukhan, L. Klein & H. Von Boehmer: Origin of regulatory T cells with known specificity for antigen. *Nat Immunol* 3(8), 756-763. (2002)

44.Klein, L., K. Khazaie & H. von Boehmer: *In vivo* dynamics of antigen-specific regulatory T cells not predicted from behavior *in vitro*. *Proc Natl Acad Sci U S A* 100(15), 8886-8891 (2003)

45.Piccirillo, C. A., J. J. Letterio, A. M. Thornton, R. S. McHugh, M. Mamura, H. Mizuhara & E. M. Shevach: $CD4^+CD25^+$ regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J Exp Med* 196(2), 237-246 (2002)

46.Nakamura, K., A. Kitani & W. Strober: Cell contact-dependent immunosuppression by $CD4^+CD25^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor β. *J Exp Med* 194(5), 629-644 (2001)

47.Annacker, O., R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano & A. Bandeira: $CD25^+$ $CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J Immunol* 166(5), 3008-3018 (2001)

48.Chen, M. L., M. J. Pittet, L. Gorelik, R. A. Flavell, R. Weissleder, H. von Boehmer & K. Khazaie: Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-β signals *in vivo*. *Proc Natl Acad Sci U S A* 102(2), 419-424 (2005)

49.Green, E. A., L. Gorelik, C. M. McGregor, E. H. Tran & R. A. Flavell: $CD4^+CD25^+$ T regulatory cells control anti-islet $CD8^+$ T cells through TGF-β-TGF-β receptor interactions in type 1 diabetes. *Proc Natl Acad Sci U S A* 100(19), 10878-10883 (2003)

50.Fisson, S., G. Darrasse-Jeze, E. Litvinova, F. Septier, D. Klatzmann, R. Liblau & B. L. Salomon: Continuous activation of autoreactive $CD4^+$ $CD25^+$ regulatory T cells in the steady state. *J Exp Med* 198(5), 737-746 (2003)

51.Gavin, M. A., S. R. Clarke, E. Negrou, A. Gallegos & A. Rudensky: Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells *in vivo*. *Nat Immunol* 3(1), 33-41 (2002)

52.Walker, L. S., A. Chodos, M. Eggena, H. Dooms & A. K. Abbas: Antigen-dependent proliferation of $CD4^+$ $CD25^+$ regulatory T cells *in vivo*. *J Exp Med* 198(2), 249-258 (2003)

53.Malek, T. R., A. Yu, V. Vincek, P. Scibelli & L. Kong: CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the nonredundant function of IL-2. *Immunity* 17(2), 167-178 (2002)

54.Kundig, T. M., H. Schorle, M. F. Bachmann, H. Hengartner, R. M. Zinkernagel & I. Horak: Immune responses in interleukin-2-deficient mice. *Science* 262(5136), 1059-1061 (1993)

55.Willerford, D. M., J. Chen, J. A. Ferry, L. Davidson, A. Ma & F. W. Alt: Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3(4), 521-530 (1995)

56.Suzuki, H., T. M. Kundig, C. Furlonger, A. Wakeham, E. Timms, T. Matsuyama, R. Schmits, J. J. Simard, P. S. Ohashi, H. Griesser & *et al.*: Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science* 268(5216), 1472-1476 (1995)

57.Tivol, E. A., F. Borriello, A. N. Schweitzer, W. P. Lynch, J. A. Bluestone & A. H. Sharpe: Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3(5), 541-547. (1995)

58.Waterhouse, P., J. M. Penninger, E. Timms, A. Wakeham, A. Shahinian, K. P. Lee, C. B. Thompson, H. Griesser & T. W. Mak: Lymphoproliferative disorders with early lethality in mice deficient in *Ctla-4*. *Science* 270(5238), 985-988. (1995)

59.Bachmann, M. F., G. Kohler, B. Ecabert, T. W. Mak & M. Kopf: Cutting edge: lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. *J Immunol* 163(3), 1128-1131. (1999)

60.Read, S., V. Malmstrom & F. Powrie: CTLA-4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J Exp Med* 192(2), 295-302. (2000)

61.Takahashi, T., T. Tagami, S. Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, T. W. Mak & S. Sakaguchi:

Immunologic self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 192(2), 303-310. (2000)

62.Bachmann, M. F., G. Kohler, B. Ecabert, T. W. Mak & M. Kopf: Cutting edge: lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. *J Immunol* 163(3), 1128-1131 (1999)

63.Tivol, E. A. & J. Gorski: Re-establishing peripheral tolerance in the absence of CTLA-4: complementation by wild-type T cells points to an indirect role for CTLA-4. *J Immunol* 169(4), 1852-1858 (2002)

64.Fallarino, F., U. Grohmann, K. W. Hwang, C. Orabona, C. Vacca, R. Bianchi, M. L. Belladonna, M. C. Fioretti, M. L. Alegre & P. Puccetti: Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 4(12), 1206-1212 (2003)

65.Piccirillo, C. A. & E. M. Shevach: Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. *J Immunol* 167(3), 1137-1140 (2001)

66.Levings, M. K., R. Sangregorio & M. G. Roncarolo: Human $CD25^+CD4^+$ T regulatory cells suppress naive and memory T cell proliferation and can be expanded *in vitro* without loss of function. *J Exp Med* 193(11), 1295-1302. (2001)

67.Tang, E. D., C. Y. Wang, Y. Xiong & K. L. Guan: A role for NF-κB essential modifier/IκB kinase-γ (NEMO/IKKγ) ubiquitination in the activation of the IκB kinase complex by tumor necrosis factor-α. *J Biol Chem* 278(39), 37297-37305 (2003)

68.Tang, Q., E. K. Boden, K. J. Henriksen, H. Bour-Jordan, M. Bi & J. A. Bluestone: Distinct roles of CTLA-4 and TGF-β in $CD4^+CD25^+$ regulatory T cell function. *Eur J Immunol* 34(11), 2996-3005 (2004)

69.Sutmuller, R. P., L. M. van Duivenvoorde, A. van Elsas, T. N. Schumacher, M. E. Wildenberg, J. P. Allison, R. E. Toes, R. Offringa & C. J. Melief: Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of $CD25^+$ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 194(6), 823-832 (2001)

70.Eggena, M. P., L. S. Walker, V. Nagabhushanam, L. Barron, A. Chodos & A. K. Abbas: Cooperative roles of CTLA-4 and regulatory T cells in tolerance to an islet cell antigen. *J Exp Med* 199(12), 1725-1730 (2004)

71.Green, J. M. & C. B. Thompson: Modulation of T cell proliferative response by accessory cell interactions. *Immunol Res* 13(4), 234-243 (1994)

72.Parkin, J. & B. Cohen: An overview of the immune system. *Lancet* 357(9270), 1777-1789 (2001)

73.Frauwirth, K. A. & C. B. Thompson: Activation and inhibition of lymphocytes by costimulation. *J Clin Invest* 109(3), 295-299 (2002)

74.Croft, M.: Co-stimulatory members of the TNFR family: keys to effective T-cell immunity? *Nat Rev Immunol* 3(8), 609-620 (2003)

75.Watts, T. H.: TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 23, 23-68 (2005)

76.Arch, R. H. & C. B. Thompson: Lymphocyte survival–the struggle against death. *Annu Rev Cell Dev Biol* 15, 113-140 (1999)

77.Nocentini, G. & C. Riccardi: GITR: a multifaceted regulator of immunity belonging to the tumor necrosis factor receptor superfamily. *Eur J Immunol* 35(4), 1016-1022 (2005)

78.Chan, K. F., M. R. Siegel & J. M. Lenardo: Signaling by the TNF receptor superfamily and T cell homeostasis. *Immunity* 13(4), 419-422 (2000)

79.Toennies, H. M., J. M. Green & R. H. Arch: Expression of CD30 and Ox40 on T lymphocyte subsets is controlled by distinct regulatory mechanisms. *J Leukoc Biol* 75(2), 350-357 (2004)

80.Nocentini, G., L. Giunchi, S. Ronchetti, L. T. Krausz, A. Bartoli, R. Moraca, G. Migliorati & C. Riccardi: A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc Natl Acad Sci U S A* 94(12), 6216-6221. (1997)

81.McHugh, R. S., M. J. Whitters, C. A. Piccirillo, D. A. Young, E. M. Shevach, M. Collins & M. C. Byrne: $CD4^+CD25^+$ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 16(2), 311-323. (2002)

82.Shimizu, J., S. Yamazaki, T. Takahashi, Y. Ishida & S. Sakaguchi: Stimulation of $CD25^+CD4^+$ regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 22, 135-142 (2002)

83.Sakaguchi, S.: Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 22, 531-562 (2004)

84.Kohm, A. P., J. S. Williams & S. D. Miller: Cutting Edge: Ligation of the glucocorticoid-induced TNF receptor enhances autoreactive $CD4^+$ T cell activation and experimental autoimmune encephalomyelitis. *J Immunol* 172(8), 4686-4690 (2004)

85.Suri, A., J. Shimizu, J. D. Katz, S. Sakaguchi, E. R. Unanue & O. Kanagawa: Regulation of autoimmune diabetes by non-islet-specific T cells - a role for the glucocorticoid-induced TNF receptor. *Eur J Immunol* 34(2), 447-454 (2004)

86.Uraushihara, K., T. Kanai, K. Ko, T. Totsuka, S. Makita, R. Iiyama, T. Nakamura & M. Watanabe: Regulation of murine inflammatory bowel disease by $CD25^+$ and $CD25^-$ $CD4^+$ glucocorticoid-induced TNF receptor family-related gene$^+$ regulatory T cells. *J Immunol* 171(2), 708-716 (2003)

87.Takeda, I., S. Ine, N. Killeen, L. C. Ndhlovu, K. Murata, S. Satomi, K. Sugamura & N. Ishii: Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. *J Immunol* 172(6), 3580-3589 (2004)

88.Valzasina, B., C. Guiducci, H. Dislich, N. Killeen, A. D. Weinberg & M. P. Colombo: Triggering of OX40 (CD134) on $CD4^+CD25^+$ T cells blocks their inhibitory activity: a novel regulatory role for OX40 and its comparison with GITR. *Blood* 105(7), 2845-2851 (2005)

89.Choi, B. K., J. S. Bae, E. M. Choi, W. J. Kang, S. Sakaguchi, D. S. Vinay & B. S. Kwon: 4-1BB-dependent inhibition of immunosuppression by activated $CD4^+CD25^+$ T cells. *J Leukoc Biol* 75(5), 785-791 (2004)

90.Kwon, B., K. Y. Yu, J. Ni, G. L. Yu, I. K. Jang, Y. J. Kim, L. Xing, D. Liu, S. X. Wang & B. S. Kwon: Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand. *J Biol Chem* 274(10), 6056-6061. (1999)

91.Yu, K. Y., H. S. Kim, S. Y. Song, S. S. Min, J. J. Jeong & B. S. Youn: Identification of a ligand for glucocorticoid-induced tumor necrosis factor receptor constitutively expressed in dendritic cells. *Biochem Biophys Res Commun* 310(2), 433-438 (2003)

92.Tone, M., Y. Tone, E. Adams, S. F. Yates, M. R. Frewin, S. P. Cobbold & H. Waldmann: Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc Natl Acad Sci U S A* 100(25), 15059-15064 (2003)

93.Kim, J. D., B. K. Choi, J. S. Bae, U. H. Lee, I. S. Han, H. W. Lee, B. S. Youn, D. S. Vinay & B. S. Kwon: Cloning and characterization of GITR ligand. *Genes Immun* 4(8), 564-569 (2003)

94.Stephens, G. L., R. S. McHugh, M. J. Whitters, D. A. Young, D. Luxenberg, B. M. Carreno, M. Collins & E. M. Shevach: Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by $CD4^+CD25^+$ T cells. *J Immunol* 173(8), 5008-5020 (2004)

95.Gondek, D. C., L. F. Lu, S. A. Quezada, S. Sakaguchi & R. J. Noelle: Cutting edge: contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J Immunol* 174(4), 1783-1786 (2005)

96.Tone, M., Y. Tone, E. Adams, S. F. Yates, M. R. Frewin, S. P. Cobbold & H. Waldmann: Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc Natl Acad Sci U S A* 100(25), 15059-15064 (2003)

97.Ronchetti, S., O. Zollo, S. Bruscoli, M. Agostini, R. Bianchini, G. Nocentini, E. Ayroldi & C. Riccardi: Frontline: GITR, a member of the TNF receptor superfamily, is costimulatory to mouse T lymphocyte subpopulations. *Eur J Immunol* 34(3), 613-622 (2004)

98.Kanamaru, F., P. Youngnak, M. Hashiguchi, T. Nishioka, T. Takahashi, S. Sakaguchi, I. Ishikawa & M. Azuma: Costimulation via glucocorticoid-induced TNF receptor in both conventional and $CD25^+$ regulatory $CD4^+$ T cells. *J Immunol* 172(12), 7306-7314 (2004)

99.Ji, H. B., G. Liao, W. A. Faubion, A. C. Abadia-Molina, C. Cozzo, F. S. Laroux, A. Caton & C. Terhorst: Cutting Edge: The natural ligand for glucocorticoid-induced TNF receptor-related protein abrogates regulatory T cell suppression. *J Immunol* 172(10), 5823-5827 (2004)

100.Esparza, E. M. & R. H. Arch: Glucocorticoid-induced TNF receptor functions as a costimulatory receptor that promotes survival in early phases of T cell activation. *J Immunol* 174(12), 7869-7874 (2005)

101.Nocentini, G., A. Bartoli, S. Ronchetti, L. Giunchi, A. Cupelli, D. Delfino, G. Migliorati & C. Riccardi: Gene structure and chromosomal assignment of mouse GITR, a member of the tumor necrosis factor/nerve growth factor receptor family. *DNA Cell Biol* 19(4), 205-217. (2000)

102.Li, Q. & I. M. Verma: NF-κB regulation in the immune system. *Nat Rev Immunol* 2(10), 725-734. (2002)

103.Dong, C., R. J. Davis & R. A. Flavell: MAP kinases in the immune response. *Annu Rev Immunol* 20, 55-72 (2002)

104.Jordan, M. S., M. P. Riley, H. von Boehmer & A. J. Caton: Anergy and suppression regulate $CD4^+$ T cell responses to a self peptide. *Eur J Immunol* 30(1), 136-144 (2000)

105.Duthoit, C. T., D. J. Mekala, R. S. Alli & T. L. Geiger: Uncoupling of IL-2 signaling from cell cycle progression in naive $CD4^+$ T cells by regulatory $CD4^+CD25^+$ T lymphocytes. *J Immunol* 174(1), 155-163 (2005)

106.Grossman, W. J., J. W. Verbsky, W. Barchet, M. Colonna, J. P. Atkinson & T. J. Ley: Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 21(4), 589-601 (2004)

107.Grossman, W. J., J. W. Verbsky, B. L. Tollefsen, C. Kemper, J. P. Atkinson & T. J. Ley: Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 104(9), 2840-2848 (2004)

108.Ronchetti, S., G. Nocentini, C. Riccardi & P. P. Pandolfi: Role of GITR in activation response of T lymphocytes. *Blood* 100(1), 350-352. (2002)

109.Sedy, J. R., M. Gavrieli, K. G. Potter, M. A. Hurchla, R. C. Lindsley, K. Hildner, S. Scheu, K. Pfeffer, C. F. Ware, T. L. Murphy & K. M. Murphy: B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol* 6(1), 90-98 (2005)

110.Gurney, A. L., S. A. Marsters, R. M. Huang, R. M. Pitti, D. T. Mark, D. T. Baldwin, A. M. Gray, A. D. Dowd, A. D. Brush, A. D. Heldens, A. D. Schow, A. D. Goddard, W. I. Wood, K. P. Baker, P. J. Godowski & A. Ashkenazi: Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr Biol* 9(4), 215-218. (1999)

111.Spinicelli, S., G. Nocentini, S. Ronchetti, L. T. Krausz, R. Bianchini & C. Riccardi: GITR interacts with the pro-apoptotic protein Siva and induces apoptosis. *Cell Death Differ* 9(12), 1382-1384. (2002)

112.Muriglan, S. J., T. Ramirez-Montagut, O. Alpdogan, T. W. Van Huystee, J. M. Eng, V. M. Hubbard, A. A. Kochman, K. H. Tjoe, C. Riccardi, P. P. Pandolfi, S. Sakaguchi, A. N. Houghton & M. R. Van Den Brink: GITR activation induces an opposite effect on alloreactive $CD4^+$ and $CD8^+$ T cells in graft-versus-host disease. *J Exp Med* 200(2), 149-157 (2004)

113.Arch, R. H., R. W. Gedrich & C. B. Thompson: Translocation of TRAF proteins regulates apoptotic threshold of cells. *Biochem Biophys Res Commun* 272(3), 936-945. (2000)

114.Papiernik, M., M. L. de Moraes, C. Pontoux, F. Vasseur & C. Penit: Regulatory CD4 T cells: expression of IL-2Rα chain, resistance to clonal deletion and IL-2 dependency. *Int Immunol* 10(4), 371-378 (1998)

115.Aggarwal, B. B.: Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3(9), 745-756 (2003)

116.Dempsey, P. W., S. E. Doyle, J. Q. He & G. Cheng: The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev* 14(3-4), 193-209 (2003)

117.Arch, R. H., R. W. Gedrich & C. B. Thompson: Tumor necrosis factor receptor-associated factors (TRAFs)–a family of adapter proteins that regulates life and death. *Genes Dev* 12(18), 2821-2830. (1998)

118.Wajant, H., F. Henkler & P. Scheurich: The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal* 13(6), 389-400 (2001)

119.Chung, J. Y., Y. C. Park, H. Ye & H. Wu: All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci* 115(Pt 4), 679-688. (2002)

120.Devin, A., A. Cook, Y. Lin, Y. Rodriguez, M. Kelliher & Z. Liu: The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* 12(4), 419-429. (2000)

121.Baud, V., Z. G. Liu, B. Bennett, N. Suzuki, Y. Xia & M. Karin: Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. *Genes Dev* 13(10), 1297-1308. (1999)

122.Yang, J., Y. Lin, Z. Guo, J. Cheng, J. Huang, L. Deng, W. Liao, Z. Chen, Z. Liu & B. Su: The essential role of MEKK3 in TNF-induced NF-κB activation. *Nat Immunol* 2(7), 620-624. (2001)

123.Shi, C. S. & J. H. Kehrl: Tumor Necrosis Factor (TNF)-induced germinal center kinase-related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13-Uev1A/TNF receptor-associated factor 2 (TRAF2) *J Biol Chem* 278(17), 15429-15434. (2003)

124.Cannons, J. L., K. P. Hoeflich, J. R. Woodgett & T. H. Watts: Role of the stress kinase pathway in signaling via the T cell costimulatory receptor 4-1BB. *J Immunol* 163(6), 2990-2998 (1999)

125.Deng, L., C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart & Z. J. Chen: Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103(2), 351-361 (2000)

126.Wang, C., L. Deng, M. Hong, G. R. Akkaraju, J. Inoue & Z. J. Chen: TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 412(6844), 346-351 (2001)

127.Li, X., Y. Yang & J. D. Ashwell: TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* 416(6878), 345-347. (2002)

128.Habelhah, H., S. Takahashi, S. G. Cho, T. Kadoya, T. Watanabe & Z. Ronai: Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-κB. *EMBO J* 23(2), 322-332 (2004)

129.Brown, K. D., B. S. Hostager & G. A. Bishop: Regulation of TRAF2 signaling by self-induced degradation. *J Biol Chem* 277(22), 19433-19438. (2002)

130.Zapata, J. M., M. Krajewska, S. Krajewski, S. Kitada, K. Welsh, A. Monks, N. McCloskey, J. Gordon, T. J. Kipps, R. D. Gascoyne, A. Shabaik & J. C. Reed: TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol* 165(9), 5084-5096 (2000)

131.Durkop, H., H. D. Foss, G. Demel, H. Klotzbach, C. Hahn & H. Stein: Tumor necrosis factor receptor-

associated factor 1 is overexpressed in Reed-Sternberg cells of Hodgkin's disease and Epstein-Barr virus-transformed lymphoid cells. *Blood* 93(2), 617-623 (1999)

132.Tsitsikov, E. N., D. Laouini, I. F. Dunn, T. Y. Sannikova, L. Davidson, F. W. Alt & R. S. Geha: TRAF1 is a negative regulator of TNF signaling: Enhanced TNF signaling in TRAF1-deficient mice. *Immunity* 15(4), 647-657. (2001)

133.Rothe, M., S. C. Wong, W. J. Henzel & D. V. Goeddel: A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* 78(4), 681-692. (1994)

134.Yeh, W. C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J. L. de la Pompa, D. Ferrick, B. Hum, N. Iscove, P. Ohashi, M. Rothe, D. V. Goeddel & T. W. Mak: Early lethality, functional NF-κB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* 7(5), 715-725. (1997)

135.Nguyen, L. T., G. S. Duncan, C. Mirtsos, M. Ng, D. E. Speiser, A. Shahinian, M. W. Marino, T. W. Mak, P. S. Ohashi & W. C. Yeh: TRAF2 deficiency results in hyperactivity of certain TNFR1 signals and impairment of CD40-mediated responses. *Immunity* 11(3), 379-389. (1999)

136.Grech, A. P., M. Amesbury, T. Chan, S. Gardam, A. Basten & R. Brink: TRAF2 differentially regulates the canonical and noncanonical pathways of NF-κB activation in mature B cells. *Immunity* 21(5), 629-642 (2004)

137.Lieberson, R., K. A. Mowen, K. D. McBride, V. Leautaud, X. Zhang, W. K. Suh, L. Wu & L. H. Glimcher: Tumor necrosis factor receptor-associated factor (TRAF)2 represses the T helper cell type 2 response through interaction with NFAT-interacting protein (NIP45) *J Exp Med* 194(1), 89-98. (2001)

138.Takaori-Kondo, A., T. Hori, K. Fukunaga, R. Morita, S. Kawamata & T. Uchiyama: Both amino- and carboxyl-terminal domains of TRAF3 negatively regulate NF-κB activation induced by OX40 signaling. *Biochem Biophys Res Commun* 272(3), 856-863 (2000)

139.Xu, L. G. & H. B. Shu: TNFR-associated factor-3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF-κB activation and IL-10 production. *J Immunol* 169(12), 6883-6889. (2002)

140.Xu, Y., G. Cheng & D. Baltimore: Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* 5(5), 407-415. (1996)

141.Grech, A., R. Quinn, D. Srinivasan, X. Badoux & R. Brink: Complete structural characterisation of the mammalian and *Drosophila* TRAF genes: implications for TRAF evolution and the role of RING finger splice variants. *Mol Immunol* 37(12-13), 721-734 (2000)

142.Shiels, H., X. Li, P. T. Schumacker, E. Maltepe, P. A. Padrid, A. Sperling, C. B. Thompson & T. Lindsten: TRAF4 deficiency leads to tracheal malformation with resulting alterations in air flow to the lungs. *Am J Pathol* 157(2), 679-688 (2000)

143.Régnier, C. H., R. Masson, V. Kedinger, J. Textoris, I. Stoll, M. P. Chenard, A. Dierich, C. Tomasetto & M. C. Rio: Impaired neural tube closure, axial skeleton malformations, and tracheal ring disruption in TRAF4-deficient mice. *Proc Natl Acad Sci U S A* 99(8), 5585-5590 (2002)

144.Xu, Y. C., R. F. Wu, Y. Gu, Y. S. Yang, M. C. Yang, F. E. Nwariaku & L. S. Terada: Involvement of TRAF4 in oxidative activation of c-Jun N-terminal kinase. *J Biol Chem* 277(31), 28051-28057. (2002)

145.Li, J. M., L. M. Fan, M. R. Christie & A. M. Shah: Acute tumor necrosis factor alpha signaling via NADPH oxidase in microvascular endothelial cells: role of $p47^{phox}$ phosphorylation and binding to TRAF4. *Mol Cell Biol* 25(6), 2320-2330 (2005)

146.Tada, K., T. Okazaki, S. Sakon, T. Kobarai, K. Kurosawa, S. Yamaoka, H. Hashimoto, T. W. Mak, H. Yagita, K. Okumura, W. C. Yeh & H. Nakano: Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-κB activation and protection from cell death. *J Biol Chem* 276(39), 36530-36534. (2001)

147.Ishida, T. K., T. Tojo, T. Aoki, N. Kobayashi, T. Ohishi, T. Watanabe, T. Yamamoto & J. Inoue: TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proc Natl Acad Sci U S A* 93(18), 9437-9442 (1996)

148.Nakano, H., S. Sakon, H. Koseki, T. Takemori, K. Tada, M. Matsumoto, E. Munechika, T. Sakai, T. Shirasawa, H. Akiba, T. Kobata, S. M. Santee, C. F. Ware, P. D. Rennert, M. Taniguchi, H. Yagita & K. Okumura: Targeted disruption of *Traf5* gene causes defects in CD40- and CD27- mediated lymphocyte activation. *Proc Natl Acad Sci U S A* 96(17), 9803-9808. (1999)

149.So, T., S. Salek-Ardakani, H. Nakano, C. F. Ware & M. Croft: TNF receptor-associated factor 5 limits the induction of Th2 immune responses. *J Immunol* 172(7), 4292-4297 (2004)

150.Kobayashi, T., M. C. Walsh & Y. Choi: The role of TRAF6 in signal transduction and the immune response. *Microbes Infect* 6(14), 1333-1338 (2004)

151.Lomaga, M. A., W. C. Yeh, I. Sarosi, G. S. Duncan, C. Furlonger, A. Ho, S. Morony, C. Capparelli, G. Van, S. Kaufman, A. van der Heiden, A. Itie, A. Wakeham, W. Khoo, T. Sasaki, Z. Cao, J. M. Penninger, C. J. Paige, D. L. Lacey, C. R. Dunstan, W. J. Boyle, D. V. Goeddel & T. W. Mak: TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* 13(8), 1015-1024. (1999)

152.Sun, L., L. Deng, C. K. Ea, Z. P. Xia & Z. J. Chen: The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell* 14(3), 289-301 (2004)

153.Esparza, E. M. & R. H. Arch: Glucocorticoid-induced TNF receptor, a costimulatory receptor on naive and activated T cells, uses TNF receptor-associated factor 2 in a novel fashion as an inhibitor of NF-κB activation. *J Immunol* 174(12), 7875-7882 (2005)

154.Krikos, A., C. D. Laherty & V. M. Dixit: Transcriptional activation of the tumor necrosis factor α-inducible zinc finger protein, A20, is mediated by κB elements. *J Biol Chem* 267(25), 17971-17976. (1992)

155.Song, H. Y., M. Rothe & D. V. Goeddel: The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation. *Proc Natl Acad Sci U S A* 93(13), 6721-6725. (1996)

156.Hauer, J., S. Puschner, P. Ramakrishnan, U. Simon, M. Bongers, C. Federle & H. Engelmann: TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-κB pathway by TRAF-binding TNFRs. *Proc Natl Acad Sci U S A* 102(8), 2874-2879 (2005)

157.Chaudhuri, A., S. Orme, T. Vo, W. Wang & B. J. Cherayil: Phosphorylation of TRAF2 inhibits binding to the CD40 cytoplasmic domain. *Biochem Biophys Res Commun* 256(3), 620-625 (1999)

158.Habelhah, H., I. J. Frew, A. Laine, P. W. Janes, F. Relaix, D. Sassoon, D. D. Bowtell & Z. Ronai: Stress-induced decrease in TRAF2 stability is mediated by Siah2. *EMBO J* 21(21), 5756-5765 (2002)

159.Wertz, I. E., K. M. O'Rourke, H. Zhou, M. Eby, L. Aravind, S. Seshagiri, P. Wu, C. Wiesmann, R. Baker, D. L. Boone, A. Ma, E. V. Koonin & V. M. Dixit: De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. *Nature* 430(7000), 694-699 (2004)

160.Kovalenko, A., C. Chable-Bessia, G. Cantarella, A. Israel, D. Wallach & G. Courtois: The tumour suppressor CYLD negatively regulates NF-κB signalling by deubiquitination. *Nature* 424(6950), 801-805 (2003)

161.Trompouki, E., E. Hatzivassiliou, T. Tsichritzis, H. Farmer, A. Ashworth & G. Mosialos: CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activation by TNFR family members. *Nature* 424(6950), 793-796 (2003)

162.Tang, G., Y. Minemoto, B. Dibling, N. H. Purcell, Z. Li, M. Karin & A. Lin: Inhibition of JNK activation through NF-κB target genes. *Nature* 414(6861), 313-317 (2001)

163.De Smaele, E., F. Zazzeroni, S. Papa, D. U. Nguyen, R. Jin, J. Jones, R. Cong & G. Franzoso: Induction of gadd45β by NF-κB downregulates pro-apoptotic JNK signalling. *Nature* 414(6861), 308-313 (2001)

164.Sakon, S., X. Xue, M. Takekawa, T. Sasazuki, T. Okazaki, Y. Kojima, J. H. Piao, H. Yagita, K. Okumura, T. Doi & H. Nakano: NF-κB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *EMBO J* 22(15), 3898-3909 (2003)

165.Chandel, N. S., P. T. Schumacker & R. H. Arch: Reactive oxygen species are downstream products of TRAF-mediated signal transduction. *J Biol Chem* 276(46), 42728-42736. (2001)

166.Esparza, E. M. & R. H. Arch: TRAF4 functions as an intermediate of GITR-induced NF-κB activation. *Cell Mol Life Sci* 61(24), 3087-3092 (2004)

167.Régnier, C. H., C. Tomasetto, C. Moog-Lutz, M. P. Chenard, C. Wendling, P. Basset & M. C. Rio: Presence of a new conserved domain in CART1, a novel member of the tumor necrosis factor receptor-associated protein family, which is expressed in breast carcinoma. *J Biol Chem* 270(43), 25715-25721 (1995)

168.Glauner, H., D. Siegmund, H. Motejadded, P. Scheurich, F. Henkler, O. Janssen & H. Wajant: Intracellular localization and transcriptional regulation of tumor necrosis factor (TNF) receptor-associated factor 4 (TRAF4) *Eur J Biochem* 269(19), 4819-4829 (2002)

169.Arron, J. R., Y. Pewzner-Jung, M. C. Walsh, T. Kobayashi & Y. Choi: Regulation of the subcellular localization of tumor necrosis factor receptor-associated factor (TRAF)2 by TRAF1 reveals mechanisms of TRAF2 signaling. *J Exp Med* 196(7), 923-934. (2002)

170.Caramalho, I., T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury & J. Demengeot: Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 197(4), 403-411 (2003)

171.Kyewski, B. & J. Derbinski: Self-representation in the thymus: an extended view. *Nat Rev Immunol* 4(9), 688-698 (2004)

172.Bensinger, S. J., A. Bandeira, M. S. Jordan, A. J. Caton & T. M. Laufer: Major histocompatibility complex class II-positive cortical epithelium mediates the selection of $CD4^+25^+$ immunoregulatory T cells. *J Exp Med* 194(4), 427-438 (2001)

173.Akiyama, T., S. Maeda, S. Yamane, K. Ogino, M. Kasai, F. Kajiura, M. Matsumoto & J. Inoue: Dependence of self-tolerance on TRAF6-directed development of thymic stroma. *Science* 308(5719), 248-251 (2005)

174.Py, B., C. Slomianny, P. Auberger, P. X. Petit & S. Benichou: Siva-1 and an alternative splice form lacking the

death domain, Siva-2, similarly induce apoptosis in T lymphocytes via a caspase-dependent mitochondrial pathway. *J Immunol* 172(7), 4008-4017 (2004)

175.Nocentini, G., S. Ronchetti, A. Bartoli, S. Spinicelli, D. Delfino, L. Brunetti, G. Migliorati & C. Riccardi: Identification of three novel mRNA splice variants of GITR. *Cell Death Differ* 7(4), 408-410. (2000)

176.Ehrlich, P.: Collected Studies on Immunity. J. Wiley & Sons, New York (1910)

177.Sakaguchi, S.: Control of immune responses by naturally arising $CD4^+$ regulatory T cells that express toll-like receptors. *J Exp Med* 197(4), 397-401 (2003)

178.Belkaid, Y.: The role of $CD4^+CD25^+$ regulatory T cells in Leishmania infection. *Expert Opin Biol Ther* 3(6), 875-885 (2003)

179.Belkaid, Y. & B. T. Rouse: Natural regulatory T cells in infectious disease. *Nat Immunol* 6(4), 353-360 (2005)

180.von Boehmer, H.: Mechanisms of suppression by suppressor T cells. *Nat Immunol* 6(4), 338-344 (2005)

181.Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach & D. L. Sacks: $CD4^+CD25^+$ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* 420(6915), 502-507 (2002)

182.Wang, H. Y., D. A. Lee, G. Peng, Z. Guo, Y. Li, Y. Kiniwa, E. M. Shevach & R. F. Wang: Tumor-specific human $CD4^+$ regulatory T cells and their ligands: implications for immunotherapy. *Immunity* 20(1), 107-118 (2004)

183.Ormandy, L. A., T. Hillemann, H. Wedemeyer, M. P. Manns, T. F. Greten & F. Korangy: Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 65(6), 2457-2464 (2005)

184.Schaefer, C., G. G. Kim, A. Albers, K. Hoermann, E. N. Myers & T. L. Whiteside: Characteristics of CD4+CD25+ regulatory T cells in the peripheral circulation of patients with head and neck cancer. *Br J Cancer* 92(5), 913-920 (2005)

185.Shimizu, J., S. Yamazaki & S. Sakaguchi: Induction of tumor immunity by removing $CD25^+CD4^+$ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 163(10), 5211-5218 (1999)

186.Onizuka, S., I. Tawara, J. Shimizu, S. Sakaguchi, T. Fujita & E. Nakayama: Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. *Cancer Res* 59(13), 3128-3133 (1999)

187.Dittmer, U., H. He, R. J. Messer, S. Schimmer, A. R. Olbrich, C. Ohlen, P. D. Greenberg, I. M. Stromnes, M. Iwashiro, S. Sakaguchi, L. H. Evans, K. E. Peterson, G. Yang & K. J. Hasenkrug: Functional impairment of $CD8^+$ T cells by regulatory T cells during persistent retroviral infection. *Immunity* 20(3), 293-303 (2004)

188.Calmels, B., S. Paul, N. Futin, C. Ledoux, F. Stoeckel & B. Acres: Bypassing tumor-associated immune suppression with recombinant adenovirus constructs expressing membrane bound or secreted GITR-L. *Cancer Gene Ther* 12(2), 198-205 (2005)

189.Shimizu, J. & E. Moriizumi: $CD4^+$ $CD25^-$ T Cells in aged mice are hyporesponsive and exhibit suppressive activity. *J Immunol* 170(4), 1675-1682. (2003)

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 κB; 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 – TNFR-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

Send correspondence to: Dr Robert H. Arch, Washington University, School of Medicine, Campus Box 8052, 660 S. Euclid Ave., St. Louis, MO 63110, Tel:314-747-4681, Fax: 314-362-9010, E-mail:arch@wustl.edu

http://www.bioscience.org/current/vol11.htm