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Are T_{regs} defective in Type 1 Diabetes and can we fix them?

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Abstract

Regulatory T cells (T_{regs}) are critical regulators of peripheral immune tolerance. T_{reg} insufficiency can lead to autoimmune disorders, including Type 1 Diabetes (T1D). Increasing evidence in mouse models of T1D, as well as other autoimmune disorders, suggest that there are defects in T_{reg}-mediated suppression. Indeed, while T_{reg} frequency in the peripheral blood of T1D patients is unaltered, their suppressive abilities are diminished compared to T_{regs} in healthy controls. Although expression of the transcription factor Foxp3 is a prerequisite for T_{reg} development and function, there are many additional factors that can alter their stability, survival and function. Much has been learned in other model systems, such as tumors, about the mechanism and pathways that control T_{reg} stability and function. This review poses the question: can we use these findings to develop new therapeutic approaches that might boost T_{reg} stability, survival and/or function in T1D, and possibly other autoimmune disorders?

Introduction

T1D, also known as Juvenile Diabetes, is a chronic autoimmune disorder where a targeted immune response by both T and B cells leads to destruction of insulin producing β-cells in the islets of the pancreas (1). T1D is one of the most common chronic diseases of children. Around 70,000 children are diagnosed with T1D each year, a number that is rising by 3-5% each year in developing countries (2). Defects in the control of effector populations is a common culprit in many autoimmune disorders including T1D (3), and this may be due to dysfunctions in T_{reg}-mediated suppression.

T_{regs} are either generated within the thymus, known as thymic-derived T_{regs} (tT_{regs}), or in the periphery, known as peripherally-derived T_{regs} (pT_{regs}), where pT_{reg} generation requires TGFβ for their differentiation (4, 5). While pT_{regs} have been shown to play an important role at mucosal sites and at the fetal-maternal interface (6, 7), we will be focusing on tT_{regs} as they are the dominant regulatory population that are impacted in T1D. tT_{regs} arise in the thymus upon high-affinity T cell receptor (TCR) signals to self-antigens and have a diverse repertoire (8, 9), suggesting that they have broad antigen specificity. tTregs are typically found in lymphoid tissues and can traffic to peripheral tissues during times of inflammation.

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T_{regs} express the transcription factor forkhead box 3 (Foxp3), which is required for their development and function. In the absence of functional Foxp3, humans succumb to a lymphoproliferative disorder known as immunodysregulation polyendocrinopathyenteropathy X-linked syndrome (IPEX). Scurfy mice, which have a point mutation in *Foxp3,* develop a similar phenotype and succumb to disease early in life (10, 11). Bone marrow transplantation in IPEX patients and adoptive transfer of $Foxp3^+$ T_{regs} or T cell-enriched splenocytes into $Foxp3^{-/-}$ or Scurfy mice restores normal immune homeostasis, supporting the necessity for T_{regs} in preventing autoimmune responses (12, 13). pT_{regs} arising from $CD4^+Foxp3^-$ splenocytes have also been suggested to play a role in immune homeostasis as their TCR repertoire is non-overlapping with tT_{regs} (14, 15). Of note, splenocyte transfer may also limit the expansion of recipient diabetogenic T cells independently of any impact of tT_{regs} and pT_{regs} (16). T_{regs} can suppress immune responses by both cell-cell (CTLA-4, Granzyme B) and soluble factor- (TGFβ, IL-10, IL-35, adenosine) mediated mechanisms (17, 18). These effector functions may become deficient upon T_{reg} instability, which may lead to the development of autoimmunity, in this case T1D.

A two-checkpoint hypothesis has been suggested in the progression of T1D from insulitis to overt diabetes where T_{regs} play a central role at these checkpoints based on studies performed in mice (19). During the first checkpoint, autoreactive T cells begin entering the islet but are still under T_{reg}-mediated control and therefore insulitic. The transition from insulitis to overt diabetes occurs when T_{regs} lose their ability to suppress effector cell responses. Is the loss of stability in T_{regs} a factor in T1D progression from insulitis to overt diabetes? While many factors, including genetics and environment, contribute to the development of T1D, this review will focus on the failure of T_{regs} to control autoreactive T cells and how this may relate to T_{reg} instability. This review will summarize the contributions from other models in understanding what factors are important for T_{reg} stability (Fig. 1). Can we use what has been learned toward stabilizing T_{regs} in T1D?

Loss of T_{reg} phenotype and function in T1D and autoimmune diabetes

While the majority of studies have reported no differences in the frequency of T_{regs} in peripheral blood isolated from T1D patients, defects in T_{reg} phenotype and suppressive capacity have been reported (20-24). Unfortunately, the majority of data obtained from T1D patients is from peripheral blood, due to the feasibility of obtaining pancreas samples from T1D patients. Therefore, whether T_{regs} are actively playing a role in limiting β cell destruction or have an altered phenotype of function in the islets during the disease course is unknown. Thus, mouse models of TID have been employed to investigate disease progression in the islet microenvironment.

The most commonly used model for T1D is the non-obese diabetic (NOD) mouse. NOD mice spontaneously develop autoimmune diabetes starting at ~10 weeks of age in females and with increasing incidence over time until ~25 weeks (25). Both diabetes onset and progression is delayed in male NOD mice. Diabetes incidence in females and males is usually ~80% and ~30%, respectively. This may be due to differences in the gut microbiome between females and males, due to hormonal differences (26). Other environmental factors, including housing conditions and diet, can also affect the development of autoimmune diabetes (25). Genetic analyses have uncovered susceptibility loci in NOD mice that are known as the insulin-dependent diabetes (*Idd*) loci. Over 40 *Idd* loci have been identified with the major histocompatibility complex (MHC) exhibiting the highest linkage with T1D incidence, (25, 27). The NOD mouse shares many similarities to T1D in humans, but with some notable differences (25). Nevertheless, the NOD mouse has proven to be a useful model to study the role of T_{regs} in autoimmune diabetes.

T_{reg} modulation studies have highlighted their importance in limiting autoimmune diabetes and controlling immune responses in the islet, despite some contradictory observations. Whereas T_{reg} depletion using anti-CD25 (PC61) has been shown to accelerate autoimmune diabetes development in several studies (28-30), one group observed complete protection from the development of autoimmune diabetes (31), perhaps due to the depletion of activated diabetogenic $CD25^+$ effector T cells in addition to $CD25^+$ T_{regs} as a consequence of late initiation of PC61 treatment (>9weeks). However, mice that lack T_{regs}, due to *Foxp3* deficiency rapidly develop autoimmune diabetes (32). Indeed, temporal depletion of T_{regs}, due to diphtheria toxin (DT) treatment of *Foxp3*-DTR (diphtheria toxin receptor) mice showed strong immune infiltrates in the pancreas two weeks after DT treatment (33). Of note, NOD.*Foxp3*-DTR mice (*Foxp3* bacterial artificial chromosome (BAC) Tg, DEREG mouse model) do not develop diabetes at an accelerated rate (34). These conflicting observations with two independently generated BAC Tg NOD.*Foxp3*-DTR strains may be due to differences in expression and deletional efficiency and warrant further investigation. Interestingly, mice expressing the BDC2.5 TCR transgene (expressed on $CD4^+$ T cells specific for the islet antigen chromogranin A), which are immunocompetent only develop insulitis (35). However, when the BDC2.5 TCR transgene is expressed on a $Rag^{-/-}$ background, in which $CD4^+$ effector T cells develop but T_{regs} do not, mice succumb to diabetes rapidly. Indeed, diphtheria toxin treatment of NOD.*Foxp3*-DTR mice crossed to BDC2.5 Tg also rapidly develops diabetes (33). Collectively, these studies suggest that diabetes onset may be associated with decreased T_{reg} numbers or function.

If humans and mice are not T_{reg} deficient, why do they succumb to T1D and autoimmune diabetes, respectively? What is affecting their functionality? Interestingly, islet infiltrating T_{regs} in mouse models still express high levels of Foxp3, but have decreased expression of the high affinity IL-2 receptor CD25 and survival factor Bcl2 (36). Likewise in T1D patients, T_{regs} found in PBMCs have low expression of another T_{reg}-associated marker GITR (37), which will be further discussed later in the review. In children with T1D, a higher proportion of T_{regs} produce the pro-inflammatory cytokines IL-12 and IL-18, which are also found at increase levels in serum, compared to healthy controls (23). Consequently, this altered T_{reg} phenotype has been implicated in T1D pathogenesis. Thus, if altered T_{reg} numbers and/or function are primary contributors to the development of T1D, boosting either parameter *in vivo* may provide a therapeutic opportunity.

Boosting T_{regs} in mice and humans

Pharmacological-based therapy

Inducing T_{reg} proliferation via multiple pharmacological methods has been proposed and attempted in both the NOD mouse model and in clinical trials. IL-2, which is important for maintenance of T_{regs}, has been a potential target for T_{reg} therapy (38) (Fig. 1). While high dose IL-2 has been used as a therapeutic approach in the treatment of melanoma and renal cancers, low dose IL-2 in NOD mice can reverse established disease by increasing T_{reg} numbers and function (36, 39). IL-2/anti-IL-2 Ab complexes have also been used to preferentially promote T_{reg} expansion (40). Modulation of mTOR activity with rapamycin has been shown to promote T_{reg} expansion, survival and function (41). Although no difference in T_{reg} number, proliferation or cytokine production was seen with rapamycin therapy prior to islet transplantation, T_{regs} do have increased suppressive capabilities (42). A combinational therapy has also been assessed with the use of IL-2/anti-IL-2 Ab complexes in combination with rapamycin and islet antigen peptide treatment. T_{reg} expansion was observed and mice protected from diabetes development in both spontaneous and induced models of diabetes (43).

Non-activating, non-FcR binding CD3 antibodies may currently be the most promising treatment for T1D. More than 8 clinical trials have targeted this approach, with five of which are using teplizumab, a humanized non-FcR-binding anti-CD3 monoclonal antibody (44). C-peptide is a byproduct of insulin production and is produced at equimolar concentrations and thus can be used to determine the amount of insulin produced by β-cells. Short-term treatment of younger individuals and recent onset patients with teplizumab has shown promising results in four-year follow-up studies, based on C-peptide levels, with limited toxicity (45-48). While its mechanism of action is currently unclear, a two-fold tolerance induction has been suggested through depletion of pathogenic T cells and preservation of T_{regs} and their function (49, 50). Although the mechanisms of action of all these therapeutic approaches is different, in all cases the common denominator is increased T_{reg} number and function.

Cell-based therapy

As T_{reg} insufficiency may be a key driver of T1D and autoimmune diabetes, increasing the number of T_{regs} in circulation may overcome this deficiency. Repeated T_{reg} adoptive transfer into neonatal NOD mice can delay the onset of autoimmune diabetes (51), suggesting that T_{reg} number or functionality may be deficient in NOD mice over time thereby requiring supplementation. Adoptive transfer of pre-diabetic NOD splenocytes or BDC2.5 TCR Tg T_{eff} cells into immunodeficient NOD mice develop autoimmune diabetes ~14 days post-transfer. Interestingly, disease can be prevented following co-transfer with $>10^6$ polyclonal T_{regs} or as few as 5×10^4 BDC2.5 TCR Tg T_{regs} (34). Adoptive transfer of a low number of DC-expanded BDC2.5 TCR Tg T_{regs} into pre-diabetic NOD mice also blocks diabetes development and can rescue mice with overt diabetes (52). While low numbers of antigen-specific T_{regs} are able to reverse autoimmune diabetes, adoptive transfer of ten-fold more polyclonal T_{regs} is not as effective in treating NOD mice therapeutically (53), suggesting that specificity for β cell antigens is critically important for optimal T_{reg} functionality.

In vitro-expanded polyclonal T_{regs} are currently in clinical trials as a promising alternative to pharmacological-based therapies. Phase 1 clinical trials have been performed in both children and adults with no safety concerns thus far (54-56). Interestingly, some potential efficacy has been observed in children at 4-5 week follow-up based on C-peptide levels.

However, while C-peptide levels were increased initially at one- and two-year follow-ups, they declined over time. Approximately 25% of the transferred T_{regs} with a naïve/memory-like phenotype were still present in patients at one-year follow-up based on deuterium incorporation. A similar trial has also been conducted in Poland with promising results. At a one-year follow-up of 12 children with T1D, increased C-peptide levels and diminished use of insulin was observed in 8 of 12 patients and, remarkably, complete insulin independence was achieved in 2 of 12 patients (55). Whether these observations are durable and can be replicated in Phase 2 clinical trials remains to be determined.

While these initial observations are encouraging, the key challenge is likely to focus on understanding what the primary limitations are for successful, durable responses and can these be overcome with [i] increased T_{reg} numbers, [ii] islet antigen specificity, and/or [iii] approaches that increased stability, survival, functionality and longevity. There is a growing consensus that future clinical trails need to focus on the development of T_{regs} with β cell antigen-specificity to maximize [i] islet homing and therapeutic index, and [ii] retention of T_{regs} over time to endure a durable response. Also, is the adoptive transfer of more T_{regs} the only viable therapeutic approach or could the T_{regs} that are already present in the patient be 'reinvigorated'? Clearly, gaining a greater understanding of the mechanisms and factors that control T_{reg} stability and function will greatly inform future clinical development.

Loss of T_{reg} stability and function

What is T_{reg} stability, how does this differ from plasticity, and what drives instability? T_{reg} plasticity and stability have been used interchangeably in the past, but represent two distinct T_{reg} fates. A stable T_{reg} expresses the transcription factor Foxp3, is suppressive, produces anti-inflammatory cytokines (such as IL-10 and IL-35), and a minimal amount of effector cytokines (eg. IFNγ, TNFα, IL-2) (57). When T_{regs} exhibit plasticity, they still express Foxp3 and remain functionally suppressive but gain distinct migratory and functional programs that can enhance their capacity to suppress certain Th subsets (58). In contrast, destabilized T_{regs} lose their suppressive abilities and gained effector functions, while either retaining Foxp3 expression (59) and eventually losing Foxp3 expression and becoming pathogenic "ex-T_{regs}" in inflammatory environments (60). In both the NOD mice and humans with T1D, T_{regs} are identified based on Foxp3 expression, yet exhibit defective suppressive activity suggesting that they may be destabilized. Further analysis of T_{reg} stability has recently been extended to include epigenetic modifications by assessing the methylation pattern of the conserved noncoding sequence 2 (CNS2 or TSDR) in the 5' UTR of the *Foxp3* gene, where tT_{regs} are demethylated at this locus and loss of stability has been associated with re-methylation at this locus (61). *Foxp3* CNS2 hypomethylation appears to be important for the binding of key transcription factors including NF-kB, CREB/ATF, Ets1 and STAT5 (62-64). Methylation studies have expanded to other T_{reg} associated genes *Il2ra* (CD25), Ikzf4 (Eos), *Ctla4* (cytotoxic T-lymphocyte-associated protein 4), and *Tnfrsf18* (GITR), which are also hypomethylated (65).

In addition to epigenetic modifications of target genes, other mechanisms including microRNAs (miRNAs) may also modulate disease development. These short non-coding RNAs are transcribed and processed via the RNAses Drosha and Dicer to generate mature miRNAs that silence genes either through repressing translation or accelerating transcript degradation (66). Mice with a T_{reg}-restricted deletion of Dicer or Drosha possess unstable T_{regs} with poor suppressive ability, diminished expression of T_{reg}-associated molecules, increased effector cytokines, and succumb to a Scurfy-like disease (67-69). Similar results have also been seen upon gene silencing of miR-126 in a breast cancer tumor model leading to increased anti-tumor immunity by altering activation of the PI3K/AKT pathway (70). While miR-155 is not necessary for T_{reg} homeostasis or its suppressive function, its role in downregulating SOCS1, which increases responsiveness of STAT5, can make T_{regs} better responders to IL-2, even under suboptimal conditions (71). T_{reg}-specific ablation of the miR-17-92 cluster results in exacerbated EAE with decreased IL-10 producing T_{regs} (72), but is not required for thymic generation of T_{regs} (73). miR-10a is selectively expressed in T_{regs} and expression has been correlated to autoimmune disease susceptibility as the autoimmune-resistant C57BL/6 strain expresses high levels of miR-10a while the autoimmune-susceptible NOD strain expresses lower levels (74). These studies suggest that certain miRNAs may be important in maintaining T_{reg} stability and function. Indeed, miR-342, miR-191, and miR-510 are differentially expressed in T_{regs} of patients with T1D, but whether these are biomarkers or contribute to disease still needs to be further elucidated (75).

Understanding the factors and pathways that control T_{reg} stability would clearly facilitate their therapeutic utilization in T1D, as well as other autoimmune and inflammatory diseases, and potentially in transplantation. While Foxp3 is the master transcription factor that is required for T_{reg} development and functionality, a variety of external signals from cytokines and surface receptors, via intracellular signaling molecules impinge on T_{regs} and impact their stability.

Factors that impact T_{reg} stability

Cytokines

Several cytokines have a substantive impact on T_{reg} development and function (Fig. 1). IL-2, produced by effector T cells, is necessary for the maintenance and function of T_{regs}, as they do not make their own autocrine IL-2 (76-78). The majority of T_{regs} express the high affinity IL-2 receptor (*Il2ra*, CD25) that signals via STAT5 (79). Genetically manipulated mice deficient in *Il2* or *Il2ra* phenocopy *Foxp3*-deficient or T_{reg}-ablated mice, yet still harbor T cells that express diminished levels of Foxp3 (80, 81). Humans with CD25 deficiency also have many of the same symptoms as seen in patients with IPEX (82). IL-2 reverses anergic, non-proliferative phenotype of T_{regs} *in vitro* and promotes their capacity to suppress immune responses (83). IL-2 withdrawal has been shown *in vitro* to limit T_{reg} suppressive ability (84). Under sub-optimal IL-2 conditions, the CNS2 element sustains Foxp3 expression, while in its absence, actively proliferating T_{regs} lose Foxp3 expression at an accelerated rate (85). Genome wide association studies have identified IL-2 pathway polymorphisms in both T1D (*Il2ra)* and autoimmune diabetes (*Il2)* (86-88). Indeed, reduced IL-2 signaling, via pSTAT5 analysis, has been documented in T1D patients with diminished T_{reg} suppressive capabilities (89, 90). In NOD mice, T_{regs} have decreased Bcl2 and CD25 expression only in inflamed islets. This may be due to decreased levels of IL-2 in the islet as low-dose IL-2 treatment increases T_{reg} survival and protection (36). These studies highlight the importance of IL-2 in T_{reg} function and possible defects that might lead to the development of T1D.

Inflammatory environments have been shown to destabilize T_{regs} in many models due to their interaction with or production of pro-inflammatory cytokines. While several cytokines may destabilize T_{regs}, we will focus here on those that may be relevant to T1D. IFNγ is highly expressed in many inflammatory conditions and may limit T_{reg} function. Upon stimulation with IFNγ *in vitro*, T_{regs} downregulate CD25, lose Foxp3 expression, and exhibit limited expansion (91). Under high salt conditions, T_{regs} can begin to produce IFNγ and lose suppressive activity, which can be restored upon antibody blockade of IFNγ (92). Whether this T_{reg}-derived IFNγ acts on T_{regs} or T_{effs} still needs to be further elucidated (92). In T1D patients, increases in IFNγ^+Foxp3$^+$ T_{regs} has been observed in peripheral blood. These cells are predominantly hypermethylated at the CNS2 locus but still exhibit suppressive function (93).

T_{regs} constitutively express TNFRII, which upon signaling leads to diminished Foxp3 mRNA and protein levels, and reduced suppressive actvity. Not surprisingly, patients with active rheumatoid arthritis (RA) possess T_{regs} that express lower Foxp3 expression and suppressive ability, and this could be reversed with anti-TNF (infliximab) treatment (94). In contrast, others have shown the requirement for TNF signaling in the generation of functional T_{regs} within the thymus and their function in inflammatory settings. In colitis models, expression of TNFRII expression is critical for T_{reg} function (95, 96). Likewise, in NOD mice, TNF receptor deficiency protects mice from autoimmune diabetes and increases T_{reg} mediated suppression (97).

The role of IL-27 in T_{reg} stability has been quite conflicting. IL-27 has been shown to antagonize pT_{reg} generation (98), but has been shown to enhance tT_{reg} function in a T-cell mediated colitis model via a Lag3-mediated mechanism (99). In a tumor model, IL-27Rα-deficient mice have decreased T_{regs} in the tumor microenvironment suggesting IL-27 may act indirectly on T_{regs} via suppressing IL-2 generation by effector T cells (100). Nevertheless, the role of IL-27 specifically on T_{regs} has yet to be clarified. Increased IL-27 has been documented in autoimmune diabetes and blockade of IL-27 in NOD mice delays the onset of autoimmune diabetes (101).

Extensive studies still need to be performed to assess whether these cytokines directly impact T_{regs} before conclusions can be drawn regarding their role in modulating T_{reg} function in T1D and autoimmune diabetes.

Surface molecules

Several cell surface molecules have been shown to impact T_{reg} stability and function (Fig. 1). OX40 (*Tnfrsf4*, CD134) is part of the tumor necrosis factor superfamily receptors (TNFRs) and is expressed on T_{regs} (102), yet its role in T_{reg}-mediated suppression has led to conflicting results both *in vitro* (103-105) and *in vivo*. OX40 expression on T_{regs} may play a role in suppressing inflammatory responses *in vivo* as mice with a T_{reg}-restricted deficiency in OX40 retain Foxp3 expression yet develop gut inflammation in a T cell-mediated gut inflammation model (106). Indeed, use of an agonist anti-OX40 (OX86) protects NOD mice from the development of autoimmune diabetes (107). However, disease is inhibited in *Ox40l*$^{-/-}$ mice and neutralizing anti-OX40L-treated NOD mice (108). Whether T_{regs} are the primary subset responding to OX40L has not been fully addressed as $CD4^+$ and $CD8^+$ T cells also express OX40 during autoimmune diabetes progression (109).

GITR (*Tnfrsf18*, CD357) is another TNFR family member that is found at high levels on the surface of T_{regs} (102). Paradoxically, use of an agonist anti-GITR (DTA-1) undermines T_{reg}-mediated suppression and tolerance in tumor models. Both a decrease in T_{reg} frequency and expression of Foxp3 in intratumoral T_{regs} has been seen (110). This loss of Foxp3, and Helios, expression is mediated by the c-Jun N-terminal kinase (JNK) pathway. Treatment of lung allergy mice with a JNK inhibitor led to reversal of GITR-induced changes in phenotype and function; and therefore was rescued from disease (111). Indeed, accelerated development of autoimmune diabetes has also been seen using a different agonistic anti-GITR antibody (2F8) (112), suggesting that activation of this pathway may be detrimental to T_{reg} stability.

Cytotoxic T lymphocyte antigen 4 (*Ctla4*, CD152) is highly expressed on T_{regs} and has extensively been studied as an inhibitory molecule important for T cell homeostasis and tolerance (113). *Ctla4*$^{-/-}$ mice succumb to fatal lymphoproliferative disease (114), while T_{reg} numbers are increased (115, 116). Results from *in vivo* models of autoimmunity have been quite conflicting, where *Ctla4*$^{-/-}$ T_{regs} are suppressive in some instances but not in others (115, 117). *Ctla4* is a susceptibility gene in autoimmune diseases, including T1D, where many polymorphisms have been identified (118-120). Costimulation blockade using anti-CTLA4 (Abatacept) has recently been shown in phase II clinical trials to delay the progression of T1D (121), but whether T_{regs} are playing a direct role still needs to be assessed further.

Neuropilin-1 (Nrp1) is an important factor in axonal guidance during embryonic development, but its role in the immune system has only recently been appreciated. Nrp1 is highly expressed on tT_{regs} but is expressed at lower levels in pT_{regs} (122-124). A role for Nrp1 in promoting the stability, survival and function of T_{regs} has been suggested (125). Nrp1 on T_{regs} has been shown to interact with both Semaphorin-4a (Sema4a) and VEGF. Mice with a T_{reg}-specific Nrp1 deletion had substantially reduced tumor growth in multiple models suggesting that T_{reg}-mediated suppression of anti-tumor immunity has been lost (125, 126). Interestingly, these mice did not succumb to autoimmunity and inflammatory disease and the frequency of $Foxp3^+$ T_{regs} was not altered (125). Stabilization via the Nrp1:Sema4a pathway enhances expression of the survival factor Bcl2, effector molecules IL-10 and CD73, limits expression of lineage-associated transcription factors, including T-bet, IRF4 and RORγt, and the pro-inflammatory cytokine IFNγ (125). Boosting T_{reg} function by engaging the Nrp1:Sema4a pathway may be a possible therapeutic approach to stabilize T_{regs} *in vivo* or prior to adoptive transfer.

Intracellular signaling molecules

There are also several intracellular proteins that appear to modulate T_{reg} stability and function by dependently or indirectly modulating Foxp3 function or stability (Fig. 1). Eos (*Ikzf4*), a zinc-finger transcription factor, is a member of the Ikaros family of transcription factors and is highly expressed in T_{regs}. Eos interacts directly with Foxp3 and is necessary for gene silencing (*Il2*, *Ifng*, etc.) while maintaining expression of key T_{reg}-associated genes including *Ctla4* and GITR (127). Silencing of *Eos* using siRNA does not result in loss of Foxp3 expression but does result in the loss of T_{reg} suppression in a T cell-mediated colitis model and induction of effector cytokines, such as IFNγ and IL-2 (127). Downregulation of Eos expression is required for the reprograming of T_{regs} into helper-like cells that retain Foxp3 expression (128). These $Eos^{-}Foxp3^{+}$ T_{regs} (Eos-liable) exhibit reduced regulatory function and enhanced expression of CD40L, IL-2 and IL-17 (128). Of note, global deletion of Eos in mice does not affect the function or phenotype of T_{regs} *in vivo* and *in vitro,* but does result in the development of more severe EAE. This observation was attributed to the function of Eos in T_{eff} populations (129).

Helios (*Ikzf2*), another member of the Ikaros transcription factor family, was once thought to distinguish tT_{regs} from pT_{regs}, however, it now appears that Helios expression is highly dependent on antigen stimulation via the TCR (130, 131). While Helios does not form a complex with Foxp3 or bind to the *Foxp3* locus, Helios plays an indirect role in supporting T_{reg} stability (132, 133). In mice with a T_{reg}-specific Helios deficiency develop autoimmunity development and appear to possess unstable T_{regs} with diminished Foxp3 expression, increased effector cytokine expression and reduced suppressive activity (133). ChIP-Seq and pathway analysis of Helios targeting genes in T_{regs} highlighted deficiencies in the IL-2Ra/STAT5b pathway suggesting that Helios may be important in regulating IL-2 signaling and T_{reg} survival (133).

Foxo1 and Foxo3, which are also forkhead box transcription factors, pay a key role in maintaining Foxp3 expression in T_{regs} (134). Mice deficient in Foxo1 in T_{regs} succumb to a Scurfy-like phenotype by 5 weeks. This lymphoproliferative disease is not due to the loss of T_{reg} number but rather their loss of function (135, 136). This phenotype can be rescued by expression of $Foxo1^{AAA}$, where Foxo1 is insensitive to 14-3-3-mediated cytosolic restriction and is this confined to the nucleus where it can facilitate Foxp3 function. Autoimmunity is further exacerbated by dual deletion of Foxo1 and Foxo3 (136). Foxo1/3 binds directly to the *Foxp3* locus and controls promoter activity (136, 137).

The phosphatase, PTEN, has recently been shown to paly a pivotal role in mediating T_{reg} stability. PTEN is an upstream inhibitor of the PI(3)K-Akt pathway and therefore inhibits mechanistic target of rapamycin complex (mTORC)1 and mTORC2 activity (138). Upon genetic deletion of PTEN in T_{regs}, mice have increased levels of auto-antibodies, renal pathology and ongoing age-related autoimmunity. Nevertheless, T_{regs} are found in high numbers and readily proliferate compared to PTEN-sufficient T_{regs}. These T_{regs} are highly activated and express higher levels of ICOS, PD-1 and IFNγ, decreased levels of CD25, and have a higher proportion of “ex-T_{regs}” based on the use of lineage-tracing experiments (61, 139). The mechanism of T_{reg}-mediated loss of suppression is via upregulation of mTORC2 activity upon PTEN loss (139). Indeed, inhibition of mTOR in T_{regs} leads to heightened stability of Foxp3 expression (140) and T_{reg} specific loss of mTOR inhibitor tuberous sclerosis 1 (TSC1) results in loss of Foxp3 expression, suppressive functionality, and increased expression of IL-17 (141). Interestingly, Nrp1, which as discussed above promotes T_{reg} stability and function, has been shown to signal via PTEN that in turn limits Akt activity, reduces Foxo phosphorylation and thus nuclear exclusion, thereby promoting Foxp3 activity (125). Taken together, these observations provide a potential causal link between Nrp1, PTEN and Foxo in mediating T_{reg} stability and function.

Conclusions

In summary, many factors impinge on T_{regs} to either promote or undermine their stability, survival and function (Fig. 1). Some of these pathways are inherent, while others are induced or selectively utilized in inflammatory environments (59). We postulate that a primary driver of autoimmunity may be T_{reg} insufficiency caused by a failure to promote pathways that enforce their stability survival and function. In tumors, where T_{reg} activity is arguably at its most robust, T_{reg} stability is enforced by an Nrp1:PTEN:Foxo axis, and potentially other mechanisms, to prevent effective anti-tumor immunity. This also appears to protect T_{regs} from destabilizing forces that may be quite severe given the hostile intratumoral microenvironment, which is hypoxic, acidic, and nutrient and glucose starved. Thus, under normal circumstances T_{regs} seem to be well adapted to respond to cues from diverse microenvironments to maintain T_{reg} stability and function. However, we posit that genetic, environmental or contextual factors conspire to undermine these programs that ultimately leads to T_{reg} insufficiency and autoimmunity.

This hypothesis and the information outlined above raise several key questions. (1) Can we boost T_{regs} that are already present but appear to exhibit insufficiency? This could be achieved by developing therapeutics that promote utilization of the Nrp1:PTEN:Foxo axis. For example, Sema4a-Ig fusion proteins may act as Nrp1 agonists thereby promoting T_{reg} stability and function. Alternatively, intracellular delivery of therapies that promote Foxo stability and nuclear translocation may produce a similar T_{reg} stabilizing effect. (2) Can we inhibit pathways that lead to instability? While we need to gain a greater understanding of the factors that promote T_{reg} instability, approaches that limit the factors that are known to drive these processes may be beneficial. The use of blocking antibodies against cytokines that can destabilize T_{regs} may be useful in a manner analogous to TNFα blockade in RA. We could also develop antibodies to block OX40L from interacting with OX-40 on T_{regs}. (3) Is a combinatorial therapy possible and necessary? Given that there may be a two-fold defect in T_{reg} number and function in T1D, combinatorial therapy may be most useful. One could combine T_{reg} adoptive transfer with approaches that promote T_{reg} stability, prior to and/or following transfer. Of course, these approaches may also be combined with current therapies that are in clinical trials for T1D, such as teplizumab (non-FcR binding anti-CD3). Indeed, one might argue that as combinatorial approaches are the mainstay of effective cancer therapy it is likely that combinatorial approaches will be required for the treatment of T1D, with perhaps the inclusion of therapies that promote T_{regs} stability and function.

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References

1. Herold KC, Vignali DA, Cooke A, Bluestone JA. Type 1 diabetes: translating mechanistic observations into effective clinical outcomes. Nature reviews. Immunology. 2013; 13:243–256.
2. Burn P. Type 1 diabetes. Nat Rev Drug Discov. 2010; 9:187–188. [PubMed: 20190784]
3. Long SA, Buckner JH. CD4+FOXP3+ T regulatory cells in human autoimmunity: more than a numbers game. J Immunol. 2011; 187:2061–2066. [PubMed: 21856944]
4. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. Immunity. 2013; 38:414–423. [PubMed: 23521883]
5. Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, Jiang S, Kuchroo VK, Mathis D, Roncarolo MG, Rudensky A, Sakaguchi S, Shevach EM, Vignali DA, Ziegler SF. Regulatory T cells: recommendations to simplify the nomenclature. Nat Immunol. 2013; 14:307–308. [PubMed: 23507634]
6. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012; 482:395–399. [PubMed: 22318520]
7. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell. 2012; 150:29–38. [PubMed: 22770213]
8. Stritesky GL, Jameson SC, Hogquist KA. Selection of self-reactive T cells in the thymus. Annu Rev Immunol. 2012; 30:95–114. [PubMed: 22149933]
9. Pacholczyk R, Ignatowicz H, Kraj P, Ignatowicz L. Origin and T cell receptor diversity of Foxp3+CD4+CD25+ T cells. Immunity. 2006; 25:249–259. [PubMed: 16879995]
10. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001; 27:68–73. [PubMed: 11138001]
11. Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet. 2002; 39:537–545. [PubMed: 12161590]
12. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003; 4:330–336. [PubMed: 12612578]
13. Collison LW, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehg JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA. IL-35-mediated induction of a potent regulatory T cell population. Nat Immunol. 2010; 11:1093–1101. [PubMed: 20953201]
14. Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B, Ziegelbauer J, Yassai M, Li SH, Relland LM, Wise PM, Chen A, Zheng YQ, Simpson PM, Gorski J, Salzman NH, Hessner MJ, Chatila TA, Williams CB. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. Immunity. 2011; 35:109–122. [PubMed: 21723159]
15. Smyk-Pearson SK, Bakke AC, Held PK, Wildin RS. Rescue of the autoimmune scurfy mouse by partial bone marrow transplantation or by injection with T-enriched splenocytes. Clin Exp Immunol. 2003; 133:193–199. [PubMed: 12869024]
16. Chang X, Zheng P, Liu Y. Homeostatic proliferation in the mice with germline FoxP3 mutation and its contribution to fatal autoimmunity. J Immunol. 2008; 181:2399–2406. [PubMed: 18684929]
17. Collison LW, Vignali DA. In vitro Treg suppression assays. Methods Mol Biol. 2011; 707:21–37. [PubMed: 21287326]
18. Sojka DK, Huang YH, Fowell DJ. Mechanisms of regulatory T-cell suppression - a diverse arsenal for a moving target. Immunology. 2008; 124:13–22. [PubMed: 18346152]
19. Andre I, Gonzalez A, Wang B, Katz J, Benoist C, Mathis D. Checkpoints in the progression of autoimmune disease: lessons from diabetes models. Proc Natl Acad Sci U S A. 1996; 93:2260–2263. [PubMed: 8637860]

20. Brusko T, Wasserfall C, McGrail K, Schatz R, Viener HL, Schatz D, Haller M, Rockell J, Gottlieb P, Clare-Salzler M, Atkinson M. No alterations in the frequency of FOXP3+ regulatory T-cells in type 1 diabetes. Diabetes. 2007; 56:604–612. [PubMed: 17327427]
21. Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI. Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. Diabetes. 2005; 54:92–99. [PubMed: 15616015]
22. Brusko TM, Wasserfall CH, Clare-Salzler MJ, Schatz DA, Atkinson MA. Functional defects and the influence of age on the frequency of CD4+ CD25+ T-cells in type 1 diabetes. Diabetes. 2005; 54:1407–1414. [PubMed: 15855327]
23. Ryba-Stanislawowska M, Rybarczyk-Kapturska K, Mysliwiec M, Mysliwska J. Elevated levels of serum IL-12 and IL-18 are associated with lower frequencies of CD4(+)CD25 (high)FOXP3 (+) regulatory t cells in young patients with type 1 diabetes. Inflammation. 2014; 37:1513–1520. [PubMed: 24677179]
24. Haseda F, Imagawa A, Murase-Mishiba Y, Terasaki J, Hanafusa T. CD4(+) CD45RA(−) FoxP3high activated regulatory T cells are functionally impaired and related to residual insulin-secreting capacity in patients with type 1 diabetes. Clin Exp Immunol. 2013; 173:207–216. [PubMed: 23607886]
25. Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. Annu Rev Immunol. 2005; 23:447–485. [PubMed: 15771578]
26. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, von Bergen M, McCoy KD, Macpherson AJ, Danska JS. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013; 339:1084–1088. [PubMed: 23328391]
27. Driver JP, Serreze DV, Chen YG. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. Semin Immunopathol. 2011; 33:67–87. [PubMed: 20424843]
28. Mellanby RJ, Thomas D, Phillips JM, Cooke A. Diabetes in non-obese diabetic mice is not associated with quantitative changes in CD4+ CD25+ Foxp3+ regulatory T cells. Immunology. 2007; 121:15–28. [PubMed: 17428252]
29. Marino E, Villanueva J, Walters S, Liuwantara D, Mackay F, Grey ST. CD4(+)CD25(+) T-cells control autoimmunity in the absence of B-cells. Diabetes. 2009; 58:1568–1577. [PubMed: 19336675]
30. Billiard F, Lobry C, Darrasse-Jeze G, Waite J, Liu X, Mouquet H, DaNave A, Tait M, Idoyaga J, Leboeuf M, Kyratsous CA, Burton J, Kalter J, Klinakis A, Zhang W, Thurston G, Merad M, Steinman RM, Murphy AJ, Yancopoulos GD, Aifantis I, Skokos D. Dll4-Notch signaling in Flt3-independent dendritic cell development and autoimmunity in mice. J Exp Med. 2012; 209:1011–1028. [PubMed: 22547652]
31. Hubner MP, Shi Y, Torrero MN, Mueller E, Larson D, Soloviova K, Gondorf F, Hoerauf A, Killoran KE, Stocker JT, Davies SJ, Tarbell KV, Mitre E. Helminth protection against autoimmune diabetes in nonobese diabetic mice is independent of a type 2 immune shift and requires TGF-beta. J Immunol. 2012; 188:559–568. [PubMed: 22174447]
32. Chen Z, Herman AE, Matos M, Mathis D, Benoist C. Where CD4+CD25+ T reg cells impinge on autoimmune diabetes. The Journal of experimental medicine. 2005; 202:1387–1397. [PubMed: 16301745]
33. Feuerer M, Shen Y, Littman DR, Benoist C, Mathis D. How punctual ablation of regulatory T cells unleashes an autoimmune lesion within the pancreatic islets. Immunity. 2009; 31:654–664. [PubMed: 19818653]
34. Petzold C, Riewaldt J, Watts D, Sparwasser T, Schallenberg S, Kretschmer K. Foxp3(+) regulatory T cells in mouse models of type 1 diabetes. J Diabetes Res. 2013; 2013:940710. [PubMed: 23691523]
35. Gonzalez A, Andre-Schmutz I, Carnaud C, Mathis D, Benoist C. Damage control, rather than unresponsiveness, effected by protective DX5+ T cells in autoimmune diabetes. Nat Immunol. 2001; 2:1117–1125. [PubMed: 11713466]

36. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo CA, Salomon BL, Bluestone JA. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity. 2008; 28:687–697. [PubMed: 18468463]
37. Xufre C, Costa M, Roura-Mir C, Codina-Busqueta E, Usero L, Pizarro E, Obiols G, Jaraquemada D, Marti M. Low frequency of GITR+ T cells in ex vivo and in vitro expanded Treg cells from type 1 diabetic patients. Int Immunol. 2013; 25:563–574. [PubMed: 23929911]
38. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. J Exp Med. 2005; 201:723–735. [PubMed: 15753206]
39. Grinberg-Bleyer Y, Baeyens A, You S, Elhage R, Fourcade G, Gregoire S, Cagnard N, Carpentier W, Tang Q, Bluestone J, Chatenoud L, Klatzmann D, Salomon BL, Piaggio E. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. J Exp Med. 2010; 207:1871–1878. [PubMed: 20679400]
40. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science. 2006; 311:1924–1927. [PubMed: 16484453]
41. Chapman NM, Chi H. mTOR signaling, Tregs and immune modulation. Immunotherapy. 2014; 6:1295–1311. [PubMed: 25524385]
42. Monti P, Scirpoli M, Maffi P, Piemonti L, Secchi A, Bonifacio E, Roncarolo MG, Battaglia M. Rapamycin monotherapy in patients with type 1 diabetes modifies CD4+CD25+FOXP3+ regulatory T-cells. Diabetes. 2008; 57:2341–2347. [PubMed: 18559659]
43. Manirarora JN, Wei CH. Combination Therapy Using IL-2/IL-2 Monoclonal Antibody Complexes, Rapamycin, and Islet Autoantigen Peptides Increases Regulatory T Cell Frequency and Protects against Spontaneous and Induced Type 1 Diabetes in Nonobese Diabetic Mice. J Immunol. 2015; 195:5203–5214. [PubMed: 26482409]
44. Skyler JS. The compelling case for anti-CD3 in type 1 diabetes. Diabetes. 2013; 62:3656–3657. [PubMed: 24158991]
45. Keymeulen B, Walter M, Mathieu C, Kaufman L, Gorus F, Hilbrands R, Vandemeulebroucke E, Van de Velde U, Crenier L, De Block C, Candon S, Waldmann H, Ziegler AG, Chatenoud L, Pipeleers D. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. Diabetologia. 2010; 53:614–623. [PubMed: 20225393]
46. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med. 2002; 346:1692–1698. [PubMed: 12037148]
47. Herold KC, Gitelman SE, Willi SM, Gottlieb PA, Waldron-Lynch F, Devine L, Sherr J, Rosenthal SM, Adi S, Jalaludin MY, Michels AW, Dziura J, Bluestone JA. Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. Diabetologia. 2013; 56:391–400. [PubMed: 23086558]
48. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med. 2005; 352:2598–2608. [PubMed: 15972866]
49. Penaranda C, Tang Q, Bluestone JA. Anti-CD3 therapy promotes tolerance by selectively depleting pathogenic cells while preserving regulatory T cells. J Immunol. 2011; 187:2015–2022. [PubMed: 21742976]
50. Daifotis AG, Koenig S, Chatenoud L, Herold KC. Anti-CD3 clinical trials in type 1 diabetes mellitus. Clin Immunol. 2013; 149:268–278. [PubMed: 23726024]
51. Wu AJ, Hua H, Munson SH, McDevitt HO. Tumor necrosis factor-alpha regulation of CD4+CD25+ T cell levels in NOD mice. Proc Natl Acad Sci U S A. 2002; 99:12287–12292. [PubMed: 12221281]
52. Tarbell KV, Petit L, Zuo X, Toy P, Luo X, Mqadmi A, Yang H, Suthanthiran M, Mojsov S, Steinman RM. Dendritic cell-expanded, islet-specific CD4+ CD25+ CD62L+ regulatory T cells

restore normoglycemia in diabetic NOD mice. J Exp Med. 2007; 204:191–201. [PubMed: 17210729]

53. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med. 2004; 199:1467–1477. [PubMed: 15184500]
54. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, Wujtewicz MA, Witkowski P, Mlynarski W, Balcerska A, Mysliwska J, Trzonkowski P. Administration of CD4+CD25highCD127- regulatory T cells preserves beta-cell function in type 1 diabetes in children. Diabetes Care. 2012; 35:1817–1820. [PubMed: 22723342]
55. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Derkowska I, Juscinska J, Owczuk R, Szadkowska A, Witkowski P, Mlynarski W, Jarosz-Chobot P, Bossowski A, Siebert J, Trzonkowski P. Therapy of type 1 diabetes with CD4(+)CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets - results of one year follow-up. Clin Immunol. 2014; 153:23–30. [PubMed: 24704576]
56. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, Herold KC, Lares A, Lee MR, Li K, Liu W, Long SA, Masiello LM, Nguyen V, Putnam AL, Rieck M, Sayre PH, Tang Q. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med. 2015; 7:315ra189.
57. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–564. [PubMed: 22224781]
58. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. Nat Rev Immunol. 2013; 13:461–467. [PubMed: 23681097]
59. Overacre AE, Vignali DA. Treg stability: to be or not to be. Curr Opin Immunol. 2016; 39:39–43. [PubMed: 26774863]
60. Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martinez-Llordella M, Ashby M, Nakayama M, Rosenthal W, Bluestone JA. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol. 2009; 10:1000–1007. [PubMed: 19633673]
61. Huynh A, DuPage M, Priyadharshini B, Sage PT, Quiros J, Borges CM, Townamchai N, Gerriets VA, Rathmell JC, Sharpe AH, Bluestone JA, Turka LA. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. Nature immunology. 2015; 16:188–196. [PubMed: 25559257]
62. Kim HP, Leonard WJ. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. J Exp Med. 2007; 204:1543–1551. [PubMed: 17591856]
63. Mouly E, Chemin K, Nguyen HV, Chopin M, Mesnard L, Leite-de-Moraes M, Burlen-defranoux O, Bandeira A, Bories JC. The Ets-1 transcription factor controls the development and function of natural regulatory T cells. J Exp Med. 2010; 207:2113–2125. [PubMed: 20855499]
64. Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, Bellucci R, Raderschall E, Canning C, Soiffer RJ, Frank DA, Ritz J. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood. 2006; 108:1571–1579. [PubMed: 16645171]
65. Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, Osaki M, Tanaka Y, Yamashita R, Nakano N, Huehn J, Fehling HJ, Sparwasser T, Nakai K, Sakaguchi S. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity. 2012; 37:785–799. [PubMed: 23123060]
66. Stefani G, Slack FJ. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol. 2008; 9:219–230. [PubMed: 18270516]
67. Zhou X, Jeker LT, Fife BT, Zhu S, Anderson MS, McManus MT, Bluestone JA. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. J Exp Med. 2008; 205:1983–1991. [PubMed: 18725525]
68. Chong MM, Rasmussen JP, Rudensky AY, Littman DR. The RNAseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. J Exp Med. 2008; 205:2005–2017. [PubMed: 18725527]

69. Jeker LT, Zhou X, Blelloch R, Bluestone JA. DGCR8-mediated production of canonical microRNAs is critical for regulatory T cell function and stability. PLoS One. 2013; 8:e66282. [PubMed: 23741528]
70. Qin A, Wen Z, Zhou Y, Li Y, Li Y, Luo J, Ren T, Xu L. MicroRNA-126 regulates the induction and function of CD4(+) Foxp3(+) regulatory T cells through PI3K/AKT pathway. J Cell Mol Med. 2013; 17:252–264. [PubMed: 23301798]
71. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. 2009; 30:80–91. [PubMed: 19144316]
72. de Kouchkovsky D, Esensten JH, Rosenthal WL, Morar MM, Bluestone JA, Jeker LT. microRNA-17-92 regulates IL-10 production by regulatory T cells and control of experimental autoimmune encephalomyelitis. J Immunol. 2013; 191:1594–1605. [PubMed: 23858035]
73. Jiang S, Li C, Olive V, Lykken E, Feng F, Sevilla J, Wan Y, He L, Li QJ. Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. Blood. 2011; 118:5487–5497. [PubMed: 21972292]
74. Jeker LT, Zhou X, Gershberg K, de Kouchkovsky D, Morar MM, Stadthagen G, Lund AH, Bluestone JA. MicroRNA 10a marks regulatory T cells. PLoS One. 2012; 7:e36684. [PubMed: 22629323]
75. Hezova R, Slaby O, Faltejskova P, Mikulkova Z, Buresova I, Raja KR, Hodek J, Ovesna J, Michalek J. microRNA-342, microRNA-191 and microRNA-510 are differentially expressed in T regulatory cells of type 1 diabetic patients. Cell Immunol. 2010; 260:70–74. [PubMed: 19954774]
76. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol. 2005; 6:1142–1151. [PubMed: 16227984]
77. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005; 6:345–352. [PubMed: 15785760]
78. Chen Q, Kim YC, Laurence A, Punkosdy GA, Shevach EM. IL-2 controls the stability of Foxp3 expression in TGF-beta-induced Foxp3+ T cells in vivo. J Immunol. 2011; 186:6329–6337. [PubMed: 21525380]
79. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995; 155:1151–1164. [PubMed: 7636184]
80. Almeida AR, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J Immunol. 2002; 169:4850–4860. [PubMed: 12391195]
81. Bayer AL, Yu A, Adeegbe D, Malek TR. Essential role for interleukin-2 for CD4(+)CD25(+) T regulatory cell development during the neonatal period. J Exp Med. 2005; 201:769–777. [PubMed: 15753210]
82. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. J Allergy Clin Immunol. 2007; 119:482–487. [PubMed: 17196245]
83. Thornton AM, Piccirillo CA, Shevach EM. Activation requirements for the induction of CD4+CD25+ T cell suppressor function. Eur J Immunol. 2004; 34:366–376. [PubMed: 14768041]
84. Thornton AM, Donovan EE, Piccirillo CA, Shevach EM. Cutting edge: IL-2 is critically required for the in vitro activation of CD4+CD25+ T cell suppressor function. J Immunol. 2004; 172:6519–6523. [PubMed: 15153463]
85. Feng Y, Arvey A, Chinen T, van der Veeken J, Gasteiger G, Rudensky AY. Control of the inheritance of regulatory T cell identity by a cis element in the Foxp3 locus. Cell. 2014; 158:749–763. [PubMed: 25126783]
86. Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, Gonzalez-Munoz A, Clark J, Veijola R, Cubbon R, Chen SL, Rosa R, Cumiskey AM, Serreze DV, Gregory S, Rogers J, Lyons PA, Healy B, Smink LJ, Todd JA, Peterson LB, Wicker LS, Santamaria P. Interleukin-2 gene

variation impairs regulatory T cell function and causes autoimmunity. Nat Genet. 2007; 39:329–337. [PubMed: 17277778]

87. Wang J, Wicker LS, Santamaria P. IL-2 and its high-affinity receptor: genetic control of immunoregulation and autoimmunity. Semin Immunol. 2009; 21:363–371. [PubMed: 19447046]
88. Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, Jones R, Ring SM, McArdle W, Pembrey ME, Strachan DP, Dunger DB, Twells RC, Clayton DG, Todd JA. Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. Am J Hum Genet. 2005; 76:773–779. [PubMed: 15776395]
89. Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, Zhang ZY, Pihoker C, Sanda S, Greenbaum C, Buckner JH. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes. 2010; 59:407–415. [PubMed: 19875613]
90. Yang JH, Cutler AJ, Ferreira RC, Reading JL, Cooper NJ, Wallace C, Clarke P, Smyth DJ, Boyce CS, Gao GJ, Todd JA, Wicker LS, Tree TI. Natural Variation in Interleukin-2 Sensitivity Influences Regulatory T-Cell Frequency and Function in Individuals With Long-standing Type 1 Diabetes. Diabetes. 2015; 64:3891–3902. [PubMed: 26224887]
91. St Rose MC, Taylor RA, Bandyopadhyay S, Qui HZ, Hagymasi AT, Vella AT, Adler AJ. CD134/CD137 dual costimulation-elicited IFN-gamma maximizes effector T-cell function but limits Treg expansion. Immunol Cell Biol. 2013; 91:173–183. [PubMed: 23295363]
92. Hernandez AL, Kitz A, Wu C, Lowther DE, Rodriguez DM, Vudattu N, Deng S, Herold KC, Kuchroo VK, Kleinewietfeld M, Hafler DA. Sodium chloride inhibits the suppressive function of FOXP3+ regulatory T cells. J Clin Invest. 2015; 125:4212–4222. [PubMed: 26524592]
93. McClymont SA, Putnam AL, Lee MR, Esensten JH, Liu W, Hulme MA, Hoffmuller U, Baron U, Olek S, Bluestone JA, Brusko TM. Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. J Immunol. 2011; 186:3918–3926. [PubMed: 21368230]
94. Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. Blood. 2006; 108:253–261. [PubMed: 16537805]
95. Housley WJ, Adams CO, Nichols FC, Puddington L, Lingenheld EG, Zhu L, Rajan TV, Clark RB. Natural but not inducible regulatory T cells require TNF-alpha signaling for in vivo function. J Immunol. 2011; 186:6779–6787. [PubMed: 21572024]
96. Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T. cell phenotype in the inflammatory environment. J Immunol. 2013; 190:1076–1084. [PubMed: 23277487]
97. Chee J, Angstetra E, Mariana L, Graham KL, Carrington EM, Bluethmann H, Santamaria P, Allison J, Kay TW, Krishnamurthy B, Thomas HE. TNF receptor 1 deficiency increases regulatory T cell function in nonobese diabetic mice. J Immunol. 2011; 187:1702–1712. [PubMed: 21734073]
98. Huber M, Steinwald V, Guralnik A, Brustle A, Kleemann P, Rosenplanter C, Decker T, Lohoff M. IL-27 inhibits the development of regulatory T cells via STAT3. Int Immunol. 2008; 20:223–234. [PubMed: 18156621]
99. Do JS, Visperas A, Sanogo YO, Bechtel JJ, Dvorina N, Kim S, Jang E, Stohlman SA, Shen B, Fairchild RL, Baldwin Iii WM, Vignali DA, Min B. An IL-27/Lag3 axis enhances Foxp3(+) regulatory T cell-suppressive function and therapeutic efficacy. Mucosal Immunol. 2016; 9:137–145. [PubMed: 26013006]
100. Li MS, Liu Z, Liu JQ, Zhu X, Liu Z, Bai XF. The Yin and Yang aspects of IL-27 in induction of cancer-specific T-cell responses and immunotherapy. Immunotherapy. 2015; 7:191–200. [PubMed: 25713993]
101. Wang R, Han G, Wang J, Chen G, Xu R, Wang L, Li X, Shen B, Li Y. The pathogenic role of interleukin-27 in autoimmune diabetes. Cell Mol Life Sci. 2008; 65:3851–3860. [PubMed: 18931971]
102. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity. 2005; 22:329–341. [PubMed: 15780990]

103. Takeda I, Ine S, Killeen N, Ndhlovu LC, Murata K, Satomi S, Sugamura K, Ishii N. Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. J Immunol. 2004; 172:3580–3589. [PubMed: 15004159]

104. Valzasina B, Guiducci C, Dislich H, Killeen N, Weinberg AD, Colombo MP. 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. 2005; 105:2845–2851. [PubMed: 15591118]

105. Piconese S, Valzasina B, Colombo MP. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. J Exp Med. 2008; 205:825–839. [PubMed: 18362171]

106. Griseri T, Asquith M, Thompson C, Powrie F. OX40 is required for regulatory T cell-mediated control of colitis. J Exp Med. 2010; 207:699–709. [PubMed: 20368580]

107. Bresson D, Fousteri G, Manenkova Y, Croft M, von Herrath M. Antigen-specific prevention of type 1 diabetes in NOD mice is ameliorated by OX40 agonist treatment. Journal of autoimmunity. 2011; 37:342–351. [PubMed: 22063316]

108. Croft M. The role of TNF superfamily members in T-cell function and diseases. Nature reviews. Immunology. 2009; 9:271–285.

109. Pakala SV, Bansal-Pakala P, Halteman BS, Croft M. Prevention of diabetes in NOD mice at a late stage by targeting OX40/OX40 ligand interactions. European journal of immunology. 2004; 34:3039–3046. [PubMed: 15368274]

110. Cohen AD, Schaer DA, Liu C, Li Y, Hirschhorn-Cymmerman D, Kim SC, Diab A, Rizzuto G, Duan F, Perales MA, Merghoub T, Houghton AN, Wolchok JD. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. PLoS One. 2010; 5:e10436. [PubMed: 20454651]

111. Joetham A, Ohnishi H, Okamoto M, Takeda K, Schedel M, Domenico J, Dakhama A, Gelfand EW. Loss of T regulatory cell suppression following signaling through glucocorticoid-induced tumor necrosis receptor (GITR) is dependent on c-Jun N-terminal kinase activation. J Biol Chem. 2012; 287:17100–17108. [PubMed: 22461627]

112. You S, Poulton L, Cobbold S, Liu CP, Rosenzweig M, Ringler D, Lee WH, Segovia B, Bach JF, Waldmann H, Chatenoud L. Key role of the GITR/GITRLigand pathway in the development of murine autoimmune diabetes: a potential therapeutic target. PloS one. 2009; 4:e7848. [PubMed: 19936238]

113. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol. 2001; 19:225–252. [PubMed: 11244036]

114. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995; 3:541–547. [PubMed: 7584144]

115. Schmidt EM, Wang CJ, Ryan GA, Clough LE, Qureshi OS, Goodall M, Abbas AK, Sharpe AH, Sansom DM, Walker LS. Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. J Immunol. 2009; 182:274–282. [PubMed: 19109158]

116. Tai X, Van Laethem F, Pobezinsky L, Guinter T, Sharrow SO, Adams A, Granger L, Kruhlak M, Lindsten T, Thompson CB, Feigenbaum L, Singer A. Basis of CTLA-4 function in regulatory and conventional CD4(+) T cells. Blood. 2012; 119:5155–5163. [PubMed: 22403258]

117. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, Sharpe AH, Powrie F. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. J Immunol. 2006; 177:4376–4383. [PubMed: 16982872]

118. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003; 423:506–511. [PubMed: 12724780]

119. Scalapino KJ, Daikh DI. CTLA-4: a key regulatory point in the control of autoimmune disease. Immunol Rev. 2008; 223:143–155. [PubMed: 18613834]

120. Qu HQ, Bradfield JP, Grant SF, Hakonarson H, Polychronakos C, I. D. G. C. Type. Remapping the type I diabetes association of the CTLA4 locus. Genes Immun. 2009; 10(Suppl 1):S27–32. [PubMed: 19956097]

121. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Krischer JP, Skyler JS, G. Type 1 Diabetes TrialNet Abatacept Study. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet. 2011; 378:412–419. [PubMed: 21719096]

122. Bruder D, Probst-Kepper M, Westendorf AM, Geffers R, Beissert S, Loser K, von Boehmer H, Buer J, Hansen W. Neuropilin-1: a surface marker of regulatory T cells. Eur J Immunol. 2004; 34:623–630. [PubMed: 14991591]

123. Yadav M, Louvet C, Davini D, Gardner JM, Martinez-Llordella M, Bailey-Bucktrout S, Anthony BA, Sverdrup FM, Head R, Kuster DJ, Ruminski P, Weiss D, Von Schack D, Bluestone JA. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. J Exp Med. 2012; 209:1713–1722. S1711-1719. [PubMed: 22966003]

124. Weiss JM, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, Parkhurst CN, Xiong H, Dolpady J, Frey AB, Ruocco MG, Yang Y, Floess S, Huehn J, Oh S, Li MO, Niec RE, Rudensky AY, Dustin ML, Littman DR, Lafaille JJ. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. J Exp Med. 2012; 209:1723–1742. S1721. [PubMed: 22966001]

125. Delgoffe GM, Woo SR, Turnis ME, Gravano DM, Guy C, Overacre AE, Bettini ML, Vogel P, Finkelstein D, Bonnevier J, Workman CJ, Vignali DA. Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. Nature. 2013; 501:252–256. [PubMed: 23913274]

126. Hansen W, Hutzler M, Abel S, Alter C, Stockmann C, Kliche S, Albert J, Sparwasser T, Sakaguchi S, Westendorf AM, Schadendorf D, Buer J, Helfrich I. Neuropilin 1 deficiency on CD4+Foxp3+ regulatory T cells impairs mouse melanoma growth. J Exp Med. 2012; 209:2001–2016. [PubMed: 23045606]

127. Pan F, Yu H, Dang EV, Barbi J, Pan X, Grosso JF, Jinasena D, Sharma SM, McCadden EM, Getnet D, Drake CG, Liu JO, Ostrowski MC, Pardoll DM. Eos mediates Foxp3-dependent gene silencing in CD4+ regulatory T cells. Science. 2009; 325:1142–1146. [PubMed: 19696312]

128. Sharma MD, Huang L, Choi JH, Lee EJ, Wilson JM, Lemos H, Pan F, Blazar BR, Pardoll DM, Mellor AL, Shi H, Munn DH. An inherently bifunctional subset of Foxp3+ T helper cells is controlled by the transcription factor eos. Immunity. 2013; 38:998–1012. [PubMed: 23684987]

129. Rieder SA, Metidji A, Glass DD, Thornton AM, Ikeda T, Morgan BA, Shevach EM. Eos Is Redundant for Regulatory T Cell Function but Plays an Important Role in IL-2 and Th17 Production by CD4+ Conventional T Cells. J Immunol. 2015; 195:553–563. [PubMed: 26062998]

130. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, Shevach EM. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol. 2010; 184:3433–3441. [PubMed: 20181882]

131. Gottschalk RA, Corse E, Allison JP. Expression of Helios in peripherally induced Foxp3+ regulatory T cells. J Immunol. 2012; 188:976–980. [PubMed: 22198953]

132. Rudra D, deRoos P, Chaudhry A, Niec RE, Arvey A, Samstein RM, Leslie C, Shaffer SA, Goodlett DR, Rudensky AY. Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat Immunol. 2012; 13:1010–1019. [PubMed: 22922362]

133. Kim HJ, Barnitz RA, Kreslavsky T, Brown FD, Moffett H, Lemieux ME, Kaygusuz Y, Meissner T, Holderried TA, Chan S, Kastner P, Haining WN, Cantor H. Stable inhibitory activity of regulatory T cells requires the transcription factor Helios. Science. 2015; 350:334–339. [PubMed: 26472910]

134. Merkenschlager M, von Boehmer H. PI3 kinase signalling blocks Foxp3 expression by sequestering Foxo factors. J Exp Med. 2010; 207:1347–1350. [PubMed: 20603315]
135. Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV, Peng M, Chan P, Ma Q, Mo Y, Meijer D, Zhao K, Rudensky AY, Atwal G, Zhang MQ, Li MO. Novel Foxo1-dependent transcriptional programs control T(reg) cell function. Nature. 2012; 491:554–559. [PubMed: 23135404]
136. Kerdiles YM, Stone EL, Beisner DR, McGargill MA, Ch'en IL, Stockmann C, Katayama CD, Hedrick SM. Foxo transcription factors control regulatory T cell development and function. Immunity. 2010; 33:890–904. [PubMed: 21167754]
137. Ouyang W, Beckett O, Ma Q, Paik JH, DePinho RA, Li MO. Foxo proteins cooperatively control the differentiation of Foxp3+ regulatory T cells. Nat Immunol. 2010; 11:618–627. [PubMed: 20467422]
138. Song MS, Carracedo A, Salmena L, Song SJ, Egia A, Malumbres M, Pandolfi PP. Nuclear PTEN regulates the APC-CDH1 tumor-suppressive complex in a phosphatase-independent manner. Cell. 2011; 144:187–199. [PubMed: 21241890]
139. Shrestha S, Yang K, Guy C, Vogel P, Neale G, Chi H. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. Nat Immunol. 2015; 16:178–187. [PubMed: 25559258]
140. Zhang P, Tey SK, Koyama M, Kuns RD, Olver SD, Lineburg KE, Lor M, Teal BE, Raffelt NC, Raju J, Leveque L, Markey KA, Varelias A, Clouston AD, Lane SW, MacDonald KP, Hill GR. Induced regulatory T cells promote tolerance when stabilized by rapamycin and IL-2 in vivo. J Immunol. 2013; 191:5291–5303. [PubMed: 24123683]
141. Park Y, Jin HS, Lopez J, Elly C, Kim G, Murai M, Kronenberg M, Liu YC. TSC1 regulates the balance between effector and regulatory T cells. J Clin Invest. 2013; 123:5165–5178. [PubMed: 24270422]

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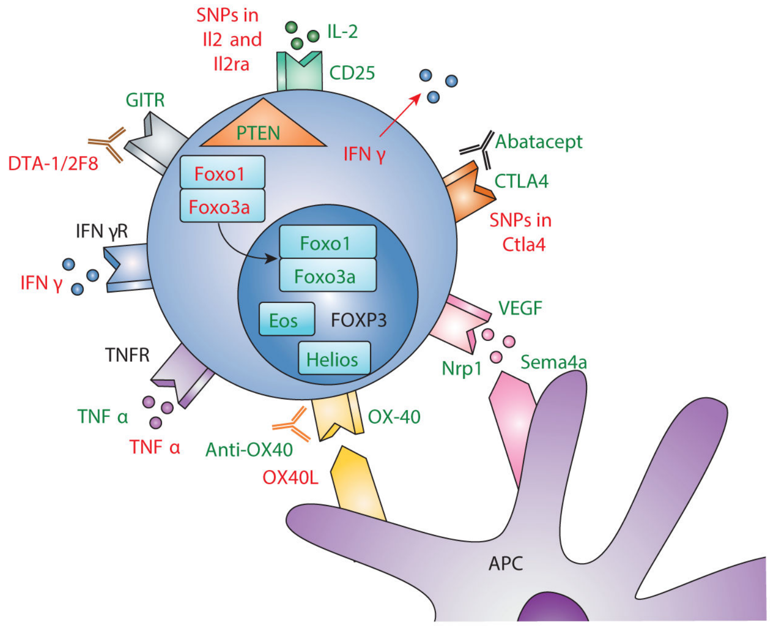

Figure 1. Mechanisms of T_{reg} stability and instability

IL-2 is critical for T_{reg} stability and maintenance where polymorphisms in both *Il2* and *Il2ra* have been seen in diabetes. Pro-inflammatory cytokines including IFNγ and TNFα may alter the T_{reg} phenotype. Many T_{reg}-associated molecules are important for optimal suppressive function including CTLA4, GITR, and OX-40. Interestingly, agonistic antibodies to GITR are detrimental to T_{reg} mediated stability and suppression. Intracellular molecules including Helios, Eos, and PTEN are also key molecules in optimal T_{reg} function. Foxo1/3a localization into the nucleus is necessary to stabilize Foxp3 in T_{regs}. Green: stabilizing signal; Red: destabilizing signal