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Regulatory T Cells in Dominant Immunological Tolerance

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Abstract

Regulatory T (Treg) cells expressing the transcription factor forkhead box P3 (Foxp3) mediate peripheral immune tolerance both to self-antigens and the commensal flora. Their defective function due to inborn errors of immunity or acquired insults is associated with a broad range of autoimmune and immune dysregulatory diseases. While their function in suppressing autoimmunity and enforcing commensalism is established, a broader role for Treg cells in tissue repair and metabolic regulation has emerged, enabled by unique programs of tissue adaptability and specialization. In this review, we focus on the myriad roles played by Treg cells in immune tolerance and host homeostasis and the potential to harness these cells in novel therapeutic approaches to human diseases.

Keywords

regulatory T cells; Foxp3; immunological tolerance; autoimmunity; interleukin-2; regulatory T cell therapy

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Declaration of interests

S.H. has consulted for Merck KGaA. P.G. has consulted for RA Capital and Astro Therapeutics. A.H.S. currently has funding from Quark and AbbVie, unrelated to the submitted work. A.H.S. serves on advisory boards for SQZ Biotechnologies, Selecta, Elpiscience, Monopteros, Bicara, Fibrogen, IOME, Corner Therapeutics and Alixia. She also is on scientific advisory boards for the Massachusetts General Cancer Center, Program in Cellular and Molecular Medicine at Boston Children's Hospital, the Human Oncology and Pathogenesis Program at Memorial Sloan Kettering Cancer Center, Bloomberg-Kimmel Institute for Cancer Immunotherapy, Glaxo Smith Kline, Janssen and Amgen. She is an academic editor for the Journal of Experimental Medicine. A.H.S. has patents/pending royalties on the PD-1 pathway from Roche and Novartis. M.C.H. has patents pending on the PHD3 pathway, is on the scientific advisory board for Alixia, Minovia, and MitoQ, and had research funding from Roche.

Introduction

Immunological tolerance denotes a state of non-responsiveness of the immune system towards self-tissues. Tolerance to self was originally thought to be the default state of an immune system that, as Paul Ehrlich argued, could not be imagined to fall into the trap of self-toxicity (*horror autotoxicus*) [1]. Despite the early demonstration of auto-antibody formation against blood elements, it took the classical experiments of Ray Owen and Peter Medawar showing that immune tolerance is an acquired state learned during the development of the immune system for this concept to be firmly established [2].

The question of immune tolerance arises in the context of an adaptive immune response that is inherently prone to autoreactivity. In principle, adaptive immunity offers the host the incisive advantage of anticipating infections by randomly generating lymphocytes bearing unique antigen receptors that recognize hitherto unencountered pathogens. Lymphocyte clones bearing pathogen-specific antigen receptors expand during an infection and are then maintained in a poised state ready for reactivation, thus providing long-term memory of the initial pathogen encounter. However, the same process may also generate autoreactive antigen receptor-bearing lymphocytes. This conundrum presented a major challenge as to how the immune system minimizes responses to self-antigens while maintaining immune responses to unencountered foreign antigens. To achieve a generalized state of self-tolerance, in which the immune system does not routinely attack self tissues, self-reactive lymphocytes in the generative lymphoid organs are largely removed through a negative selection process that collectively induces a state of central tolerance. During this developmental checkpoint, immature T cells bearing T cell receptors (TCR) with too strong of an affinity for self-peptide major histocompatibility complex (self-peptide-MHC) molecules presented on the surface of thymic epithelial cells are eliminated through induction of death by apoptosis (Figure 1) [3-5]. Meeting a similar fate, T cells whose TCRs fail to bind self-peptide-MHC molecules with low affinity and consequently do not undergo positive selection also undergo apoptosis through a process dubbed “death by neglect” (Figure 1) [6, 7]. Central tolerance, which is leaky by its very nature, is abetted by a process of peripheral tolerance in which regulatory T (Treg) cells actively suppress autoreactivity and restrain overly exuberant immune responses to foreign antigens (Figure 1). The recognition that a host harbors commensal microbial communities, including bacteria, fungi and viruses, that act as part of an “extended self” expands the range of immunological tolerance and in particular the role for Treg cells in enabling commensalism. The scope of Treg cell function has further expanded to encompass a key role in tissue repair and metabolic control, thus linking tolerance mechanisms with organismal homeostasis. In this review we will discuss fundamental concepts in tolerance induction by Treg cells and explore the potential for novel therapies based on manipulation of Treg cells.

Natural and Peripheral Foxp3$^+$ Regulatory T Cells in Immune Tolerance

Reflecting a duality in its developmental ontology, the Treg cell compartment is composed of two distinct populations that act synergistically to maintain peripheral immunological tolerance (Figure 2). Tolerance to self-antigens and tissues is enforced by natural regulatory T (nTreg) cells, which are selected in the thymus on the basis of high-avidity TCR

interactions and thought to arise from two distinct nTreg progenitor subsets that collectively generate a broad self-biased TCR repertoire [8-10]. Selection of nTreg cells in the thymus can also occur through a process of clonal diversion during which thymocytes reactive against peripheral tissue antigens are redirected towards the Treg lineage in a manner that is dependent on the transcriptional autoimmune regulator (Aire)[11-13]. In contrast to nTreg cells, peripheral regulatory T (pTreg) cells play a critical and non-redundant role in the maintenance of tolerance to environmental antigens and components of the "extended-self", including antigens derived from the commensal flora and diet as well as neoantigens arising from developing tumors [14] (Figure 2). Unlike nTregs, pTreg cells are generated extrathymically in mucosal interfaces, where the bioavailability of commensal metabolites such as retinoic acid and specialized antigen presenting cells including $CD101^+$ dendritic cells promote their differentiation [15]. Treg cells can be also induced (iTreg) from conventional naïve $CD4^+$ T cells *in-vitro* upon TCR-stimulation in the presence of TGF-β and interleukin-2 [15, 16] (Figure 2). However, the functional relevance of *in-vitro* derived iTreg cells has been contentious, as these cells exhibit incomplete CpG demethylation at the *Foxp3* locus resulting in unstable expression of Foxp3 and a susceptibility towards destabilization [17, 18]. Although no definitive markers exist to segregate nTreg and pTreg cell populations phenotypically, nTreg cells are characterized by heightened expression of the Ikaros transcription factor family member Helios and additionally in mice the cell surface glycoprotein neuropilin-1 (Nrp-1) [19, 20]. It is now appreciated that the TCR repertoires of nTreg and pTreg cells are largely distinct and share minimal overlap [21 22]. The essentiality of nTreg and pTreg subsets in optimal protection from deleterious autoimmunity is evidenced by the absolute requirement of both populations in the prevention of lethality induced by Foxp3-deficiency [22]. In summary, a division of labor between nTreg and pTreg cells ensures comprehensive coverage over a wide range of antigens expressed by the body's own cells and tissues as well as innocuous antigens originating from the environment, diet and commensal microorganisms that constitute the extended self.

Treg Specialization in Non-Lymphoid Tissues

Unique populations of Treg cells with specialized functions have been identified in non-lymphoid tissues. As a result, a newly found appreciation for the phenotypic heterogeneity and functional diversity of Treg cells has emerged, highlighting the importance of Treg cells not only as sentinels of peripheral tolerance but also as orchestrators of tissue homeostasis. Within the central nervous system (CNS), Treg cells were shown to exert regenerative functions by virtue of promoting the remyelination and differentiation of oligodendrocyte progenitor cells [23]. Treg cells also participate in the resolution of acute lung injury [24],promote wound healing following myocardial infraction and control the development of atherosclerosis [25,26, 27]. A unique population of $Foxp3^+$ Treg cells residing in the visceral adipose tissue (VAT) plays an important role in the regulation of metabolism, including control of insulin sensitivity and VAT inflammation [28]. VAT Treg cells exhibit a unique transcriptome, which is largely driven by the peroxisome proliferator-activated receptor-γ (PPARγ), the master transcriptional regulator of adipocyte differentiation. Importantly, PPARy is necessary and sufficient for the accumulation, phenotypic adaptation and function of VAT Tregs, consistent with the notion that lineage defining-transcription factors license

the specialization of Treg cells in distinct tissue microenvironments. In addition, VAT Treg cells exhibit a unique TCR repertoire when compared to their counterparts in lymphoid tissues and are dependent on IL-33 signaling for their accumulation and proliferation [29, 30].

Another population of Treg cells with dedicated tissue reparative function is found in injured skeletal muscle [31]. These Treg cells rapidly expand following acute muscle injury and play an important role in muscle repair and regeneration [15,32]. Similar to VAT Tregs, muscle Treg cells exhibit a tissue-adapted transcriptome and clonally expanded TCR repertoire with a preferential reliance on IL-33 signaling [31, 33]. Moreover, Treg cells were shown to play an important role in the prevention of infectious lung tissue injury through production of the epidermal growth factor amphiregulin (AREG), which also acts on progenitor muscle cells to enhance muscle regeneration [31, 34]. Interestingly, AREG produced by CNS Treg cells is critical for protection against neurotoxic astrogliosis by suppressing pro-inflammatory IL-6-STAT3 signaling in astrocytes [35]. These results highlight the importance of AREG production by Treg cells as a general mechanism directing the regeneration and repair of various tissues following injury. In contrast, a number of factors secreted by tissue-Treg cells play a privileged and non-redundant role in the regeneration of particular tissues and/or cell types, including the growth regulatory protein CCN3, which accelerates the differentiation and remyelination of oligodendrocytes in the CNS [23]. With the exception of placental and colonic lamina propria Treg cell populations, the majority of tissue-resident Treg cells are thought to be thymically derived and express high levels of nTreg-associated markers Helios and Nrp-1 [36]. Consistent with thymic derivation but not peripheral conversion, VAT and muscle Treg cells are clonally expanded and exhibit a unique TCR repertoire that is not shared with conventional T cells in the same tissue [36]. Collectively, these studies highlight an important functional dualism that extends beyond the classical role of Treg cells in the maintenance of immunological tolerance.

Treg cells at the environmental interfaces

The environmental interfaces in the airways, gut and skin pose special challenges for the immune system because they are heavily colonized by the commensal microbiota. Treg cells play a critical role in shaping and enforcing this commensalism, safeguarding barrier integrity and orchestrating immune responses to inciting agents. The diversity of the tissues involved, their respective metabolic attributes and the different loads of resident microbiota ranging from very high in the gut to very low in the lower airways, are reflected in the heterogeneity of tissue-specific barrier-resident Treg cell populations.

Intestinal Treg cells

Intestinal Treg cells are an important subset of immune cells that play a crucial role in maintaining immune tolerance in the gut. These specialized cells are key regulators of the immune response, helping to prevent immune-mediated damage to the intestines and promoting overall intestinal health. Experimental depletion or functional dysregulation of Treg cells exacerbates intestinal inflammation, which can be alleviated following Treg cell reconstitution [23]. Intestinal Treg cells are enriched in the lamina propria, where they represent more than 25% of $CD4^+$ T cells [37, 38]. This enrichment is lost in germ-free

or antibiotic-treated mice, highlighting the essentiality of the microbiome in intestinal Treg cell differentiation and persistence [39, 40]. Indeed, the intestinal Treg pool is largely composed of pTreg cells, as illustrated by the high abundance of lamina propria Foxp3$^+$ cells lacking expression of the nTreg cell-associated markers Helios and neuropilin-1 [19, 20]. Interestingly, specific deletion of the Foxp3 intronic enhancer CNS1, which leads to a defect in pTreg generation, results in T_H2-type pathologies in the gut in the absence of systemic inflammation, highlighting a critical role for pTreg cells in the maintenance of gut homeostasis [41]. Intestinal Treg cells exhibit a certain degree of plasticity, as they can adapt to the local microenvironment through the expression of effector T (Teff) cell transcription factors, including upregulation of BCL6, GATA3, RORγt and T-bet. A specialized subset of follicular regulatory T (Tfr) cells characterized by the expression of PD-1, CXCR5, and BCL6 play an important role in mucosal IgA production, expansion of follicular helper T (TFH) cell populations, and control of the germinal center reaction [42]. Roughly 40% of intestinal Treg cells express the Teff cell transcription factor RORγt and are induced by microbial products in a STAT3-dependent manner [43-45]. Recent studies have shown the differentiation of this population involves antigen presentation by specific MHCII$^+$ antigen-presenting cells, including type 3 innate lymphoid cells (ILC3) and RORγt -expressing Thetis cells [46-49]. RORγt $^+$ Treg cells are critically involved in the maintenance of gut homeostasis and tolerogenic immune responses to allergens. For instance, susceptibility of mice to food allergy is associated with a preferential decrease in RORγt Tregs; the replenishment of RORγt$^+$ Tregs by treatment with Clostridial and Bacteroidetes species can prevent the development of food allergy [40].

GATA3$^+$ Treg cells represent another Treg cell subset in the intestine and account for 15-20% of the intestinal Treg cell compartment [50]. The majority of these cells are Helios$^+$ and their induction is microbiota-independent, indicative of their nTreg origin [45]. This population expresses interleukin 1 receptor-like 1 (ST2) and can respond to alarmins during intestinal inflammation, including its ligand IL-33 produced by intestinal epithelial cells [51]. IL-33 in combination with antigen recognition and IL-2 promotes GATA3 upregulation and enhances Foxp3 and ST2 expression to support intestinal Treg cell proliferation [51]. Moreover, GATA3 and Foxp3 form a complex following TCR stimulation which promotes the stability and accumulation of Tregs in the intestine [50, 52]. As a result, GATA3-deficient Treg cells fail to accumulate in the inflamed intestine and exhibit defects in Foxp3 expression [50, 53]. In summary, the unique microbial and metabolic attributes of the intestinal mucosal interface is reflected in a diversity of specialized Treg cell populations that serve to enable tissue homeostasis and repair, license microbial commensalism and prevent adverse immune responses to foods and the commensal bacteria.

Lung Treg cells

Treg cells play a pivotal role in the maintenance of tolerance to allergens at environmental interfaces in the airways and prevention of deleterious inflammatory reactions against innocuous airborne antigens. Although both nTreg and pTreg cells are present in the lung, a critical role for pTreg cells in the maintenance of tolerance to airborne antigens is supported by the observation that mice unable to generate pTreg cells develop severe T_H2-type pathologies in the lung, including asthma-like airway allergic inflammation [41]. Consistent

with this finding, adoptive transfer of allergen-specific Treg cells markedly attenuates allergen-induced allergic airway inflammation [54]. Lung antigen-specific pTreg cells can be developed through the consorted action of several myeloid cell populations in the lung, including plasmacytoid dendritic cells, conventional dendritic cells and lung-resident tissue macrophages. Airborne antigens including pollen, dust mites and fungal spores are major targets for tolerogenic Treg cells in the human lung [55]. These results emphasize that during steady state, lung Treg cells are coordinately mobilized to maintain tolerance to potentially harmful allergens as well as innocuous airborne antigens.

In addition to supporting steady state homeostasis in the lung, Treg cells play a central role in the pathogenesis of asthma. $CD4^+CD25^+$ mucosal Treg cells were shown to be critical for reversal of airway hyperresponsiveness following experimental asthma exacerbation [56]. $ST2^+$ lung Treg cells play a major role in actively restraining γδ T cell activation and function through the production of IL-35 during allergic airway inflammation induced by house dust mite exposure [57]. Accordingly, allergic pulmonary inflammation is exacerbated in mice lacking functional $ST2^+$ Treg cells. Interestingly, administration of IL-33 impairs established immunological tolerance to inhaled antigens by promoting the development of dysfunctional T_H2 cell-like mucosal $ST2^+$ Treg cells [58]. Reprogramming of lung Treg cells towards a pathogenic T_H2 cell-like phenotype is also a feature of allergic airway disease induced in OVA-tolerized mice subjected to recurrent respiratory infections with respiratory syncytial virus [59]. These studies suggest that inflammatory triggers may comprise established tolerance and increase susceptibility to allergic disease in later life.

Recent studies support the hypothesis that dysregulated Treg cells play a critical role in the pathogenesis of allergic asthma. Expression of Notch4 on tissue resident lung pTreg cells is involved in the potentiation of type 2 innate lymphoid cell (ILC2) activation via a β-catenin-dependent Treg cell-intrinsic pathway that induces the expression of growth and differentiation factor 15 (GDF15) [60]. GDF15 reinforces allergic airway inflammation by directly acting on ILC2s to promote their expansion and activation.

It is important to note that a failure by Treg cells to initiate lung-tissue reparative programs can in turn lead to unabated inflammation, as demonstrated by aggravated inflammatory immune responses in the lung by Treg cells incapable of producing the tissue repair factor amphiregulin [34, 61 62]. Indeed, lung pTreg cell Notch4 expression was increased as a function of severity in subjects with COVID-19 infections [61]. Mechanistically, Notch4 suppressed the induction of AREG by IL-18, a tissue reparative factor necessary for the prevention of severe lung inflammation [61]. Collectively, these studies demonstrate the importance of Treg cells in the prevention of airway inflammation.

Skin Treg cells

Treg cells are highly abundant in the skin and represent a sizable portion of the $CD4^+$ T cell compartment in humans and mice [63, 64]. Homing of Treg cells to the skin is accomplished through several cutaneous chemokine receptors including CCR4 and CCR6 [65-67]. During neonatal life, epithelial cells within skin hair follicles drive the influx of CCR6-expressing Treg cells via the chemokine CCL20, which supports their accumulation in areas with high hair density [64, 68]. Within the skin, Treg cells exhibit an effector memory-like phenotype and

unique TCR repertoire collectively indicative of tissue residence and specialization [69-71]. Expression of nTreg cell-associated markers Helios and Nrp-1 in skin Treg cells suggests that these cells are largely thymically derived [72 71]. Functional specialization of skin Treg cells is acquired by their heightened expression of the canonical T_H2 transcription factor GATA3, which governs their ability to control type 2 immune responses (Figure 3) [50, 53, 73]. Lineage-specific deletion of GATA3 in Treg cells results in preferential exacerbation of T_H2 cytokine responses in the skin and potentiates fibroblast activation to drive skin fibrosis [74], indicating that GATA3 safeguards the identity and functional specialization of skin Treg cells during inflammatory immune responses.

Another transcription factor enriched in skin Treg cells is the retinoic acid-related orphan receptor α (RORα), whose deletion results in uncontrolled cutaneous inflammation driven by ILC2s (Figure 3) [75]. Similar to GATA3, RORα opposes the destabilization and skewing of skin Treg cells towards pathogenic IL-4 producing Teff-like cells. In an experimental model of psoriasis, Treg cells were shown to be critical for control of cutaneous inflammation by limiting the ability of pathogenic GM-CSF-producing $CD4^+$ T cells to invade and expand within lesional skin and draining lymph nodes [76]. In contrast to their classical “immunosuppressive” role, skin Treg cells can delay barrier repair to facilitate innate inflammation and protection against *Staphylococcus aureus* infection [77]. Interestingly, adhesion of skin Treg cells through the c-type-lectin receptor Laylin impairs their suppressive capacity, suggesting that motility itself acts a cue in the regulation of immune suppression [78]. Skin Treg cells also produce tissue-reparative factors such as AREG which can promote keratinocyte growth and wound healing [79]. Collectively, these studies demonstrate that although skin Treg cells are functionally poised to restrain T_H2-associated skin pathology, they nevertheless exhibit spatiotemporal and contextual plasticity, which enables them to direct both pro-inflammatory and anti-inflammatory responses in the maintenance of skin homeostasis.

The duality of Treg cells as cellular agents directing the response to injury in the skin is now well appreciated [80, 81]. $GATA3^+$ skin Treg cells express receptors for alarmins including IL-33, IL-18 and TSLP that can be released from non-hematopoietic cells following damage (Figure 3). Treg cells have been shown to facilitate cutaneous wound healing and suppress pro-inflammatory macrophage accumulation and IFN-γ secretion through epidermal growth factor receptor (EGFR) signaling [80]. EGFR-deficient-Treg cells in the skin are decreased numerically and this decrease is associated with delayed wound closure and healing (Figure 3) [40]. Epidermal regeneration after injury requires Treg cell mediated attenuation of IL-17A-associated inflammation and suppression of CXCL5 expression from interfollicular epidermal cells [81]. Taken together, these studies highlight the modular nature of skin Treg cells in broadly enabling skin homeostasis.

The Molecular Versatility of Treg Cells

Much like Teff cells, which adopt unique transcriptional programs resulting in the generation of highly specialized anti-pathogen immune responses (i.e T_H1, T_H2 etc.), Treg cells exhibit remarkable molecular versality and can effectively co-opt lineage-defining transcription factors (i.e Tbet, GATA3) required for the specification of particular immune response

types [82]. Molecular co-option represents an important mechanism by which Treg cells assume immune response-specific identities to prevent deleterious immunopathology. For instance, the transcription factors Tbet and STAT3 endow Treg cells with the preferential capacity to suppress T_H1 and T_H17 pro-inflammatory responses respectively, as evidenced by the precipitation of dysregulated T_H1 and T_H17 immune-responses in mice with Treg-cell-specific knockout of Tbet and STAT3 [83, 84]. In Treg cells, immune response type-specific functional adaptation is facilitated by the integration of discrete environmental cues and expression of T_H-type specific molecules by Treg cells. For example, during pathogenic T_H17 responses, expression of the CCR6 chemokine and interleukin-6 receptor and interleukin-1 receptor enable Treg cells to traffic to inflammatory sites where they can compete for cytokines known to promote T_H17 differentiation including IL-6 and IL-1 [83]. During T_H1 immune responses Treg cells upregulate Tbet, the master T_H1 transcriptional regulator, and begin to express the T_H1 cell-associated chemokine receptor CXCR3, which enables them to co-colocalize with $CXCR3^+$ Teff cells [85].

Several studies support the notion of a division of anti-inflammatory labor by Treg cells. Absence of the interferon regulatory factor-4 (IRF4) in Treg cells results in unabated T_H2 inflammation and aberrant antibody production [86]. Moreover, Tregs can co-opt the transcriptional repressor BCL6 to gain entry into germinal centers via expression of CXCR5, where they can support antigen-specific plasma cell differentiation and curtail the outgrowth of non-antigen-specific B cells [87]. Collectively, these studies underscore an important dualism in the functional plasticity of Treg cells, highlighting the essentiality of molecular co-option and Teff cell feature assimilation by Treg cells in the resolution of diverse inflammatory processes. It is now appreciated that in addition to being a cornerstone for the maintenance of peripheral tolerance, Treg cells play disproportionately important roles in enforcing tissue homeostasis broadly, where they adapt unique functional, phenotypic and tissue-reparative properties [88]. This additional layer of Treg cell tissue “specialization” and the consequences of its disruption in peripheral tolerance breakdown will be reviewed in the following section.

Treg Cell Destabilization and Peripheral Tolerance Breakdown

The contentious topic of Foxp3 instability was borne out of the use of *Foxp3-cre* transgenes that were inherently leaky in nature and would readily label non-Treg cells with a transiently active *Foxp3* locus [89-91]. Notwithstanding these pitfalls, it is now appreciated that Treg cells can be phenotypically and functionally destabilized, particularly during persistent inflammatory immune responses. While the capacity of Treg cells to co-opt different transcriptional programs endows them with the ability to control immune response specific outcomes, it also serves as a vulnerability towards their pathological reprogramming and functional degeneration. Pathological reprogramming of Treg cells is largely a feature of the p/iTreg cell compartment and several human polymorphisms associated with inflammatory disorders directly impact the stability and pathogenic potential of allergen-specific pTreg cells. For example, mice carrying a tyrosine (Y) to phenylalanine (F) mutation at position 709 of the murine interleukin-4 receptor alpha chain ($Il4ra^{F709}$), which models human polymorphisms linked to atopy and asthma, exhibit enhanced sensitivity to food allergens and intensified allergen-induced airway hyperreactivity [92, 93]. The formation of allergen-

specific pTreg cells in ovalbumin-allergic *Il4ra*^{F709} mice was shown to be impaired as a result of pathogenic T_H2 cell-like pTreg cell reprogramming (Figure 4) [94]. Mechanistically, the acquisition of a T_H2 cell-like phenotype by pTreg cells resulted from elevated IL-4R-STAT6 signaling, which was also evident in pTreg cells from children with food allergy (Figure 4). Importantly, engraftment of *Il4ra*^{F709} CD4$^+$ T cells into Treg-deficient mice was sufficient to confer susceptibility to food allergy, while Treg cell-specific deletion of Shp1, which mimics the effects of the Il4ra^{F709} mutation, induced the reprogramming of Treg cells into T_H2-like cells and imparted disease susceptibility. These results demonstrate a requisite role for enhanced IL-4R signaling in oral tolerance breakdown and suggest that blockade of the IL-4-IL-4R pathway may enhance mucosal allergen-specific pTreg function in the re-establishment of oral tolerance. It is important to note that nTreg cells can also loose Foxp3 and contribute to immunopathology during inflammatory immune responses. Loss of Foxp3 in antigen-specific nTreg cells was shown to occur during the pathogenesis of experimental autoimmune encephalitis (EAE) [95]. These autoreactive “exTreg” cells produced high levels of IFN-γ and were sufficient to induce EAE following adoptive transfer.

Using mice expressing a coding variant in the interleukin-4 receptor alpha chain (IL-4RR576) that is associated with increased asthma severity, signaling via IL-4RR576 was shown to amplify the expression of Notch4 on lung pTreg cells by recruiting the growth factor receptor-bound protein 2 (GRB2), which in turn mediates super-induction of Notch4 via IL-6 production, ultimately leading to exacerbated lung inflammation [96]. The IL-4RR576 polymorphism was also shown to redirect TGF β-dependent lung pTreg cell differentiation towards the TH17 cell lineage, which occurs via GRB2-MAPK-dependent autocrine IL-6 signaling, highlighting an additional mechanism by which IL-4RR576 reprograms lung pTreg cells towards a pathogenic TH17-like state (Figure 4) [97]. Interestingly, treatment with anti-IL-6 mAb in a severe asthmatic patient homozygous for the IL-4raR576 mutation resulted in immunological and clinical improvement, highlighting the therapeutic potential of intercepting pro-inflammatory signals involved in the destabilization of pTreg cells [98]. In autoimmune arthritis, synovial fibroblast-derived IL-6 was shown to mediate the conversion of Foxp3$^+$ pTreg cells into T_H17 cells with potent arthritogenic and autoreactive properties [99]. Taken together, these studies highlight a critical role for IL-6 dependent T $_H17$ reprogramming of pTreg cells in asthma pathogenesis and point to intervention strategies aimed at re-stabilizing pTreg cell responses relevant to asthma or other inflammatory disorders.

Additional examples of Treg cell destabilization involve the Notch signaling axis (Figure 5) [60, 61, 100-102]. Enforced Notch signaling disrupts peripheral tolerance by promoting T_H1 reprogramming of both nTreg and pTreg cells in a cell-intrinsic and canonical pathway-dependent manner [100]. In contrast, lineage specific disruption of Notch signaling enhances Treg cell fitness and function in the settings of inflammation. Consistent with these results, genetic disruption or pharmacological Notch-1 blockade induces tolerance in murine cardiac and lung transplantation models [101]. Moreover, activation of Notch signaling was shown to negatively impact the immunosuppressive functions of Treg cells during experimental autoimmune uveitis, chronic hepatitis and allergic rhinitis [103 104, 105]. Notch4 expression licenses allergic airway inflammation in asthma by disrupting the capacity of pTreg cells to control ILC2 activation in a Treg cell-intrinsic manner that involves Wnt signaling and

GDF15 (Figure 5) [60]. Interestingly, Notch4 also participates in an alternative mechanism that involves the restraint of AREG production by pTreg cells during severe COVID-19 infection, which results in the exacerbation of lung inflammation (Figure 5) [61]. In addition, Treg cells in multisystem inflammatory syndrome in children (MIS-C) are destabilized through a Notch1-dependent mechanism that promotes the expression of CD22 and leads to aberrant mTORC1 activity (Figure 5) [106]. Collectively these studies illustrate a critical role for Notch signaling in negatively regulating Treg cells and suggest that therapeutic targeting of individual Notch receptors may offer opportunities to restore tolerance in a tissue and disease-specific manner.

A number of molecules with specialized functions play prominent roles in the suppressive capacity of Treg cells. Within the gastrointestinal tract, TGF-β1 was shown to safeguard the functions of colonic Treg by supporting their accumulation and retention through maintenance of integrin αEβ7 (CD103) expression [107]. While Treg cell-specific monoallelic inactivation of *Tgfb1* results in impaired RORγt^{+} Treg cell differentiation, biallelic deficiency results in fatal autoimmunity characterized by dysregulated autoimmune humoral responses [108, 109]. These results demonstrate that Treg-derived TGF-β1 plays a privileged and non-redundant role in the regulation of oral tolerance and autoimmunity. Treg-derived interleukin-10 (IL-10) represents another immunomodulatory cytokine that plays a non-redundant role in restraining autoimmunity at mucosal interfaces, including the colon and lungs, but is dispensable for the control of systemic autoimmunity [89, 110]. Similarly, RORγt expression in Treg cells is important for the maintenance of colonic homeostasis and T_H2-associated pathology is exacerbated in the absence of RORγt^{+} Treg cells [44, 45]. The importance of RORγt^{+} Treg cells is additionally illustrated by the demonstration that protection against food allergy by commensal bacteria is dependent on the induction of RORγt Tin nascent Treg cells [40]. Taken together, these results demonstrate critical roles for TGF-β1, IL-10 and RORγt as factors crucial for the establishment and maintenance of immunological tolerance at particular environmental interfaces and/or inflammatory settings.

In summary, a comprehensive understanding of the inflammatory triggers and molecular mechanisms governing the susceptibility of Treg cells towards functional destabilization may inform conceptually novel approaches aimed at re-invigorating Treg stability in the settings of persistent inflammation.

Resetting Tolerance: Treg Cell Therapies

Since their initial description almost three decades ago, Treg cells have taken center stage as master orchestrators of peripheral immunological tolerance. A growing understanding of the fundamental mechanisms involved in the induction, differentiation, maintenance and suppressive functions of Treg cells has stimulated therapeutic approaches to selectively manipulate these cells to deliver precision immunotherapy for immune dysregulatory diseases. Amongst several approaches, the use of autologous Treg cell products as adoptive cell therapies have already been shown to be safe in a number of clinical trials [111-113]. Currently, Foxp3 gene editing approaches and designer IL-2 muteins engineered to preferentially elicit the activity of Treg cells have been propelled to the forefront of Treg-based cell therapy [114, 115]. Below we summarize recent developments in therapeutic

approaches aimed at the normalization or amplification of Treg cell function, including novel gene editing approaches used to generate and modify Tregs as cellular therapies for inflammatory and autoimmune disorders.

Foxp3 and Treg Gene Editing

In seminal studies demonstrating an essential role for Foxp3 in the development and function of Treg cells, ectopic expression of Foxp3 was shown to confer a regulatory phenotype in conventional murine $CD4^+$ T cells [116-118]. These early observations suggested that conventional $CD4^+$ T cells may be therapeutically repurposed for induction of tolerance in autoimmune disease and transplantation settings through enforced expression of Foxp3 (Figure 6A).

Subsequent studies in human systems demonstrated that retroviral gene transfer of *FOXP3* into naive $CD25^-CD45RO^-CD4^+$ T cells results in the acquisition of several Treg cell features, including *in-vitro* suppressive activity [119, 120]. Lentiviral-mediated expression of FOXP3 promotes the induction of a homogenous and highly suppressive population of Treg-like cells but only when FOXP3 is under the control of an activation-independent promoter [121]. Using a bi-directional lentiviral vector in which expression of FOXP3 is under control of the human elongation factor 1 alpha (EF1α), transduction of naïve or memory $CD4^+$ T cells was shown to result in high and stable expression of FOXP3 (Figure 6A). In a similar approach utilizing homology directed repair (HDR) based gene editing, insertion of strong promoter into the endogenous *FOXP3* locus resulted in the acquisition of a stable regulatory phenotype [122] (Figure 6A). Nevertheless FOXP3-transduced Treg cells exhibited several differences as compared to nTreg cells, including a methylated Treg specific demethylation region (TSDR) and reduced expression of the Ikaros family transcription factor Helios previously shown to be important for Treg cell differentiation and function [123, 124]. A number of functional genetic perturbation approaches, including loss-of-function CRISPR/Cas9 screens, have also been recently employed in the identification of novel regulatory networks and transcriptional regulators of Foxp3 activity and Treg cell identity [125-128].

The utilization of induced pluripotent stem cells (iPSCs) for the generation of Tregs has captured considerable attention as a promising approach to produce readily accessible Tregs. iPSCs offer an appealing and cost-effective solution to address the challenges associated with engineered adoptive Treg cell therapy, encompassing issues related to production, efficacy, immunogenicity, and application. Moreover, IPSCs utilization allows for precise antigenic targeting through genetic modifications [129, 130]. Numerous studies have successfully generated iPSC-derived Tregs that closely resemble natural Tregs and have demonstrated their efficacy in suppressing autoimmune arthritis, Type 1 Diabetes (T1D), and Graft versus host disease [131-133]

Using a catalytically inactive Cas9 variant (dCas9), the feasibility of targeting regulatory regions of FOXP3 was shown by fusing dCas9 with a transcriptional activation domain in order to induce prolonged upregulation of endogenous FOXP3 [134] (Figure 6A). Notably, epigenetic imprinting of Treg cells that precedes FOXP3 expression may represent an important barrier in the generation of functionally stable Treg cells following induction of FOXP3 [135-137]. As previously discussed, selective gene knockout of immune response

specific components (IL-6R, STAT3, STAT6, GATA3 etc.) involved in the functional plasticity of Treg cells may render these cells resistant to phenotypic destabilization and functional dysregulation. Conversely, enforced expression of chemokine receptors (CXCR3, CXCR5, CCR6 etc.) may enhance the trafficking of Treg cells to particular inflammatory sites and reduce the potential of broad off-target immunosuppression. A complete understanding of the elements involved in the specification and maintenance of the Treg lineage beyond Foxp3 will be instrumental for the rational design of gene editing approaches aimed at generating bone-fide Treg cells.

Designer Treg IL-2 Therapy

IL-2 is pleiotropic cytokine that plays a critical role in the induction, homeostatic proliferation, phenotypic stability and suppressor function of Treg cells [138-140]. As a result of a diminished threshold for IL-2R signaling in maintaining Treg cell activation and proliferation, a number of therapeutic approaches leveraging IL-2 have been developed to specifically target Treg cells *in-vivo* ([141]; [142]) (Figure 6B). Previous studies have shown the utility of *in-vivo* administration of immune complexes of IL-2 and specific anti-IL-2 mAbs in stimulating the activation and proliferation of Treg cells [143], and in the treatment of preclinical models of autoimmunity and graft rejection in mice [144]. Treg cell-biased IL-2:anti-IL-2 immune complexes can be achieved by using anti-IL-2 mAbs that sterically block the IL-2:IL-2Rβ interaction while imparting exquisite selectivity towards $CD25^{high}$ cells [145, 146].

Attempts to harness the therapeutic potential of IL-2 in the form of designer muteins engineered to preferentially stimulate the activity of Treg cells have recently gained attention [147] (Figure 6B). The activity of different IL-2 muteins, including IL-2 Fc fusion proteins and pegylated IL-2 variants, are currently being assessed across several autoimmune indications as precision Treg immunotherapies (Figure 6B). Site-specific pegylation of IL-2 can also support IL-2Rα bias and lead to preferential and sustained activation of Treg cells [148], elicit tolerogenic activity in non-human primates and ameliorate disease progression in an experimental model of systemic lupus erythematosus [149]. Several IL-2 Fc fusion proteins with increased dependency towards CD25 and attenuated IL-2Rβ affinity have been shown to exhibit preferential Treg cell activity in-vivo [150-152]. The status of clinical trials assessing the activity of IL-2 Treg-biased muteins in autoimmune indications have been recently reported [153].

Interestingly, a wide dynamic range of IL-2R signaling generates productive Treg cell biological responses *in-vitro,* and distinct minimal thresholds of IL-2R signaling are needed to achieve specific Treg functional outcomes *in-vivo* [154]. The capacity of Treg-biased IL-2 muteins to be utilized in combinatorial approaches is supported by the demonstration that Fc-IL-2 mutein mediated enrichment of Treg cells can promote the *in-vivo* expansion of antigen-specific Treg cells [155]. By using engineered orthogonal IL-2R-IL-2 pairs that specifically bind to one another but not their natural counterparts, it was shown that ortho-IL-2 can effectively and preferentially expand ortho-IL-2Rβ-expressing Treg cells leading to improved hematopoietic stem cell engraftment [156, 157]. The use of messenger RNAs to encode IL-2 muteins with extended half-life and Treg bias has also been recently explored

[158]. Lastly, the application of scRNA-seq analysis has begun to delineate Treg cell subset alterations and distinct gene expression profiles following expansion with IL-2 muteins and holds promise for the identifying tissue-specific patterns of modulation in Treg cells [159]. The constraints of number, function, and stability represent significant limitations in the context of Treg therapies [160, 161]. To circumvent these limitations, combination therapies involving IL-2 and CAR-Tregs have emerged as a potential solution. In fact, the creation of a membrane-bound IL-2 molecule that facilitates autocrine IL-2 signaling in CAR-Tregs has been demonstrated to enhance the expansion, functionality, and durability of CAR-Tregs in a humanized preclinical mouse model [162]. Collectively, these studies have begun to provide a molecular blueprint for the therapeutic engineering of Treg potentiating molecules and hold tremendous promise for a wide range of autoimmune and inflammatory disorders.

Tregs as cellular therapies

The therapeutic utility of Treg cells as adoptive cell therapies (ACT) was first highlighted by the demonstration that transfer of $CD4^+CD25^+$ but not conventional $CD4^+CD25^-$ cells rescues the lymphoproliferative disease of Foxp3 and CTLA-4-deficient mice [116-118]. In 2009, the first use of autologous Treg cells as an ACT in human subjects with GvHD was reported [111]. Although limited to two patients, this case study and a subsequent phase 1 trial in children with recent-onset type 1 diabetes (T1D) from the same group collectively demonstrated the feasibility, safety and potential therapeutic benefit of autologous Treg cell products following their massive expansion *ex-vivo* under good manufacturing practice conditions [112 163]. In 2015, the safety of expanded autologous polyclonal Treg cells in T1D was reported [113]. Expansion of Treg cells *ex-vivo* resulted in the correction of dysfunction associated with Treg cells in T1D subjects, including impaired IL-2-induced STAT5 phosphorylation, and these cells outperformed Treg cells from healthy individuals in *in-vitro* suppression assays. Despite exhibiting superior activity following *ex-vivo* expansion, the majority of the infused Treg cells were absent from circulation 3 months following infusion, with a precipitous drop already apparent 2 weeks following infusion. As high concentrations of IL-2 are required for optimal expansion of Treg cells *ex-vivo,* it was thought that the bioavailability of IL-2 may critically govern longevity of the Treg cell product. Supporting this notion, ex-vivo expanded polyclonal Treg cells become highly dependent on IL-2 and serum IL-2 levels rapidly decline following infusion of Treg cells [161]. To understand whether persistence of Treg cells following infusion depends on the bioavailability of IL-2, Dong et al. examined the effects of low dose IL-2 in combination with Treg ACT in patients with T1D [160]. Although combination therapy led to an increase in the number of exogenous Treg cells in peripheral blood, cytotoxic cells including subsets of activated NK and granzyme/perforin-producing $CD8^+$ T cells were also expanded in the combinatorial arm. These observations provide a strong impetus for the clinical assessment of Treg-biased IL-2 muteins/variants in combination with autologous polyclonal Treg cell therapy and underscore the importance of the pleiotropic effects of IL-2, even with the redirection of specificity of IL-2 towards Treg cells.

The safety and activity of autologous Treg ACT has been investigated in a number of autoimmune indications and transplant settings including liver and kidney transplantation [164; 165; 166; 167 168], multiple sclerosis [169], GvHD [170], and Crohn's disease [171]. Several

clinical trials are currently assessing the safety of autologous polyclonal or antigen-specific Treg cells as ACTs (NCT02711826, NCT03239470, NCT04661254, NCT04817774, and NCT04817774) (Figure 6C). For example, the safety and tolerability of chimeric antigen receptor (CAR) Treg cells targeting HLA-A2, an antigen commonly mismatched in transplantation, is currently underway in the settings of kidney transplantation and end stage renal disease (NCT04817774). As antigen-specific Treg cells have been shown to exhibit superior activity in preclinical models of autoimmunity when compared to polyclonal Treg cells, it will be interesting to understand whether redirection of Treg cells towards autoantigens via TCR and CAR engineering approaches leads to sustained antigen-specific tolerance induction *in-vivo* in humans [172]. In summary, combinatorial modalities leveraging TCR/CAR-Treg engineering approaches coupled with designer cytokine therapy promise to deliver selective and individually disease-tailored Treg cell immunotherapy (Figure 6B-C).

Concluding Remarks and Future Directions

In the past two decades the identification of Foxp3 Treg cells has ushered a revolution in our understanding of fundamental processes governing immunological tolerance; beyond this classical role of Treg cells, a new role for these cells has recently emerged in the maintenance tissue homeostasis and repair. The harnessing of these mechanisms has the potential to deliver innovative therapeutic approaches in autoimmune and inflammatory diseases as well as chronic degenerative diseases.

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Abbreviations used:

Foxp3	forkhead box P3
Treg	regulatory T
self-peptide-MHC	self-peptide major histocompatibility complex
nTreg	natural regulatory T
pTreg	peripheral regulatory T
iTreg	induced regulatory T
CNS	central nervous system
AREG	amphiregulin
Aire	autoimmune regulator
VAT	visceral adipose tissue

PPARγ	peroxisome proliferator-activated receptor-γ
Nrp-1	neuropilin-1
Teff	effector T
Tfr	follicular regulatory T
TFH	follicar helper T
ST2	interleukin 1 receptor-like 1
ILC2	type 2 innate lymphoid cell
ILC3	type 3 innate lymphoid cells
GDF15	growth and differentiation factor 15
GRB2	growth factor-receptor bound protein 2
RORα	retinoic acid-related orphan receptor α
RORγt	RAR-related orphan receptor gamma
EGFR	epidermal growth factor receptor
EAE	experimental autoimmune encephalitis
IRF4	interferon regulatory factor-4
MIS-C	multisystem inflammatory syndrome in children
ACT	adoptive cell therapy
T1D	type 1 diabetes
CAR	chimeric antigen receptor
cTEC	cortical thymic epithelial cells
mTEC	medullary thymic epithelial cells
DR3	death receptor 3
TL1A	TNF ligand-related molecule 1
TSLRPR	thymic stromal lymphopoietin receptor
PENK	proenkephalin
Met-ENK	methionine enkephalin
MAPK	mitogen-activated protein kinase
TSDR	Treg specific demethylation region
HDR	homology directed repair

EF1α	elongation factor 1 alpha

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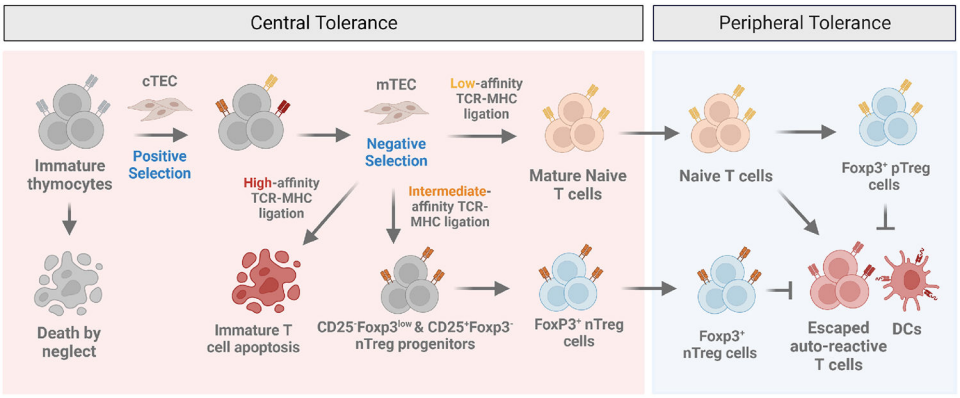

Figure 1. Central and peripheral immunological tolerance

Although T cells originate from the fetal liver and adult bone marrow, the thymus is the major site of T cell maturation. The selection of developing T cells is dependent on T cell receptor (TCR) recognition of self-peptide-major histocompatibility complex (self-peptide-MHC) molecules and results in the elimination of potentially harmful T cell clones. Positive selection is a process mediated by cortical thymic epithelial cells (cTEC) during which immature thymocytes whose TCRs fail to recognize self-peptide-MHC undergo a default pathway of apoptosis; a phenomenon known as “death by neglect”. Thymocytes whose TCRs recognize peptide-MHC presented by medullary thymic epithelial cells (mTEC) with high avidity undergo a process of negative selection resulting in apoptosis. In an alternative process that is incompletely understood, thymocytes bearing TCRs with intermediate avidities towards self-peptide-MHC are selected onto the Treg cell lineage via at least two distinct progenitor subsets including a $CD25^{+}Foxp3^{-}$ and $CD25^{-}$ $Foxp3^{low}$ regulatory T cell population. The elimination of potentially harmful self-reactive T cells in the thymus through negative selection is the basis for0 central tolerance. In contrast, peripheral tolerance is induced in mature lymphocytes by self-antigens, including components of the extended self and innocuous environmental antigens by a pool of regulatory T cells derived from the thymus (nTreg) or generated in the periphery (pTreg) that act in concert to maintain organismal-wide immunological tolerance.

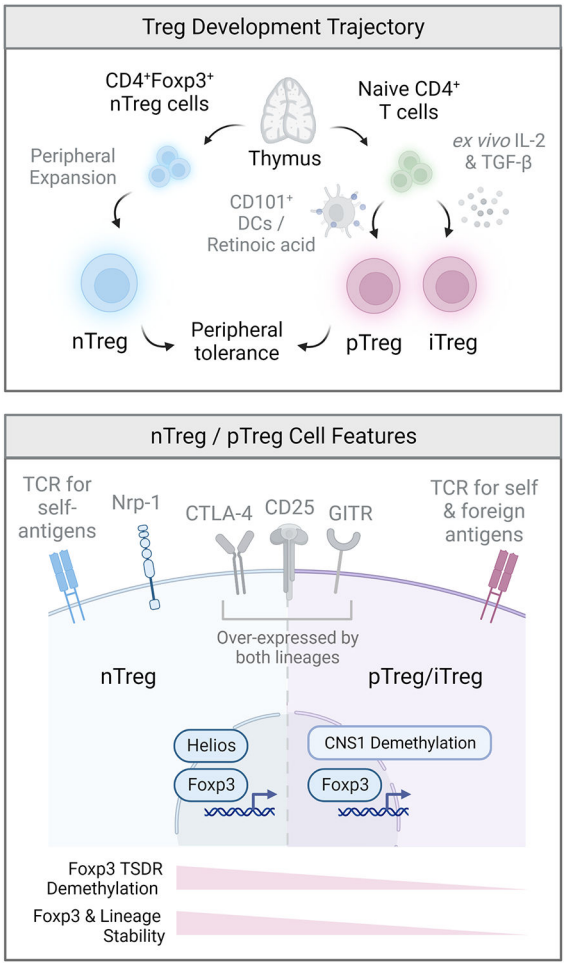

Figure 2. Developmental trajectory and subsets of regulatory T cells

The regulatory T cell pool is composed of two major populations that act in concert to maintain peripheral tolerance. Natural regulatory T (nTreg) cells develop in the thymus and play a critical role in the maintenance of tolerance to self-antigens. Peripheral regulatory T (pTreg) cells are generated from conventional naïve $CD4^+$ T cells at mucosal interfaces and barrier sites and are critically important for the maintenance of tolerance towards dietary, environmental and commensal antigens. Regulatory T cells can also be induced (iTreg) *in-vitro* from conventional naïve $CD4^+$ T cells following TCR ligation in the presence of transforming growth factor beta (TGF-β). Although all three populations express high levels of the transcription factor Foxp3 and share the expression of several canonical Treg cell surface molecules including CTLA-4, CD25 and GITR, the TCR repertoires of nTreg and pTreg/iTreg and are distinct and non-overlapping. Although not uniquely expressed by nTreg cells, the Ikaros family transcription factor Helios and cell surface glycoprotein neuropilin-1 (Nrp-1) are highly expressed in nTreg as opposed to pTreg/iTreg populations. pTreg/iTreg cells exhibit a greater susceptibility towards destabilization and loss of Foxp3 expression, largely due to the absence of an nTreg-like specific demethylation signature at conserved non-coding sequences within the locus of Foxp3 and other Treg signature genes.

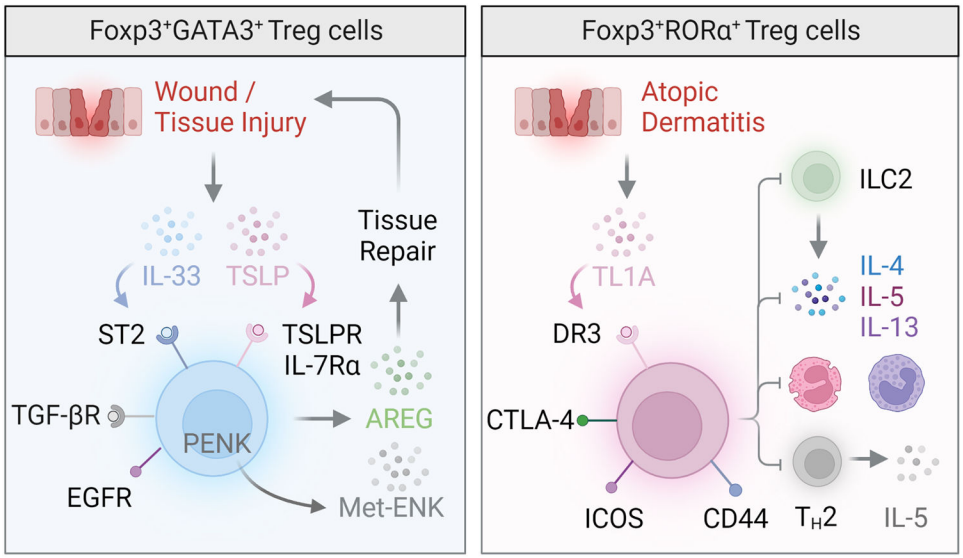

Figure 3. Regulatory T cells in the skin

The skin Treg cell compartment is largely composed of nTreg cells that express the transcription factor GATA-3 (left), although a RORα^{+} population involved in the control of cutaneous inflammation has also been reported (right). These RORα^{+} skin Treg cells express the tumor necrosis factor receptor superfamily member 25, also known as death receptor 3 (DR3), and sequester TNF ligand–related molecule 1 (TL1A) to curtail type 2 innate lymphoid cell activity, eosinophil and basophil accumulation, and type 2 inflammation during atopic dermatitis. GATA-3^{+} skin Treg cells expressing the transcription factor IRF4 play a key role in skin wound repair and healing. Expression of alarmin receptors including the IL-33 receptor (ST2), IL-18 receptor and thymic stromal lymphopoietin receptor (TSLPR) enable skin Treg cells to sense and respond to cues associated with cutaneous damage and stress. Skin Treg cells also express the epidermal growth factor receptor (EGFR), which is thought to contribute to the accumulation of Treg cells in wounded skin and promote wound closure, likely though the production of the tissue reparative factor amphiregulin (AREG). Following UV light exposure, production of the opioid precursor proenkephalin (PENK) by UVB-exposed skin Treg cells contributes to generation of the neuropeptide methionine enkephalin (Met-ENK), which in turn promotes keratinocyte outgrowth and wound healing.

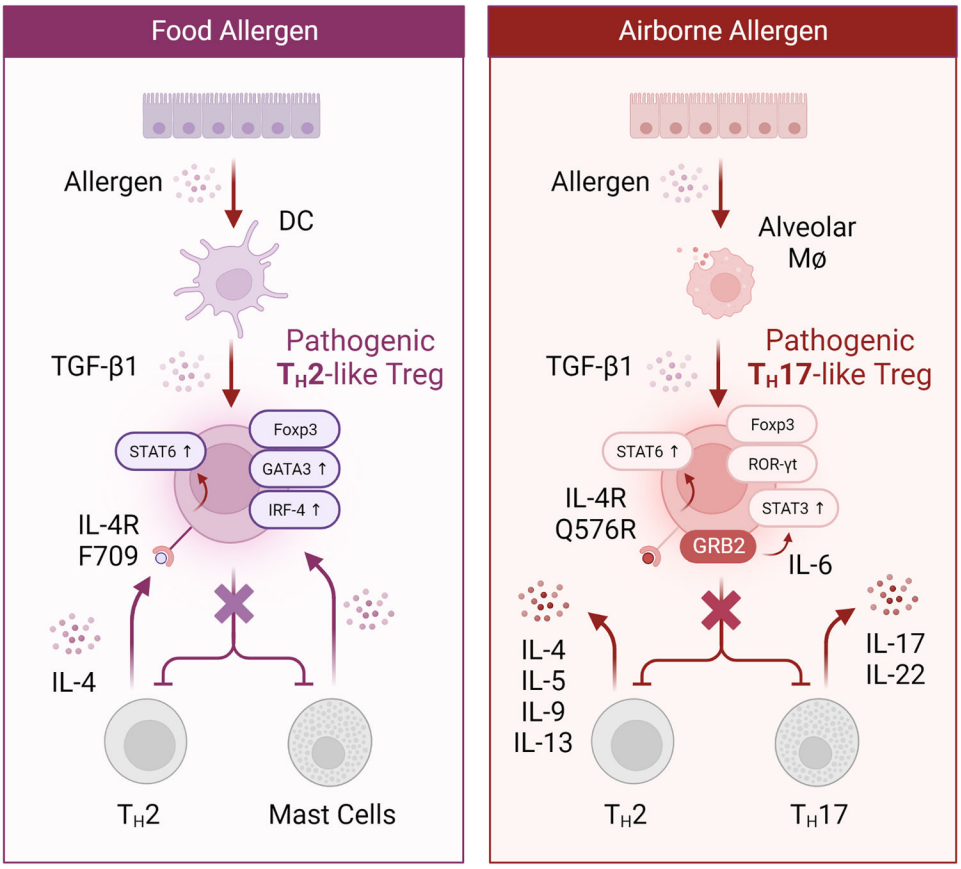

Figure 4. Pathological reprogramming of regulatory T cells in the intestine and lung

Left: Food allergic Il4ra^{F709} mice exhibit deficits in allergen-specific Treg cells. OVA-specific Il4ra^{F709} Treg cells fail to suppress food allergy as a result of undergoing pathogenic T_H2 cell-like reprogramming due to excessive IL-4R-STAT6 signaling. These pathogenic T_H2 cell-like Tregs are characterized by heightened expression of canonical T_H2 program components such as IRF4 and GATA-3. Importantly, allergen-specific Treg cells of human subjects with food allergy harbor similar T_H2 cell-like Tregs, suggesting that blockade of the IL-4R pathway may offer a viable therapeutic strategy for the induction of long-lasting tolerance. Right: Destabilization of IL-4ra^{Q576R} pTreg cells in asthma towards a pathogenic T_H17 cell-like state leads to the exacerbation of pulmonary inflammation. IL-4ra^{Q576}-dependent recruitment of the growth-factor-receptor-bound protein 2 (GRB2) results in mitogen-activated protein kinase (MAPK) activation and heightened autocrine IL-6-STAT3 signaling which collectively derails protective pTreg cell responses in the lung during allergic airway inflammation. Blocking the IL-4R or IL-6R pathways may prevent the destabilization of allergen-specific pTreg cells into T_H2 and T_H17 like cells, respectively, and afford novel Treg intervention strategies for the re-establishment of tolerance.

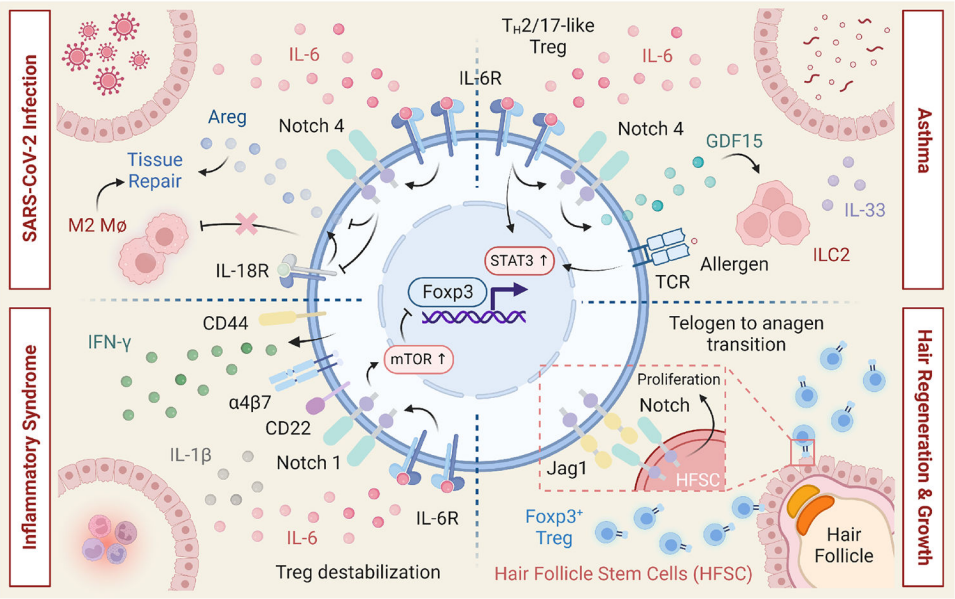

Figure 5. Multifaced roles of Notch signaling in regulatory T cells

Notch signaling plays pleiotropic roles within the Treg cell compartment and is critically involved in the control of Treg cell function in the periphery. Upper Right: In experimental models of asthma, Notch4 expression on lung pTreg cells licenses allergic airway inflammation through a process that involves the destabilization of pTreg cells towards T_H2 and T_H17 cell fates. During allergic airway inflammation, Notch4 expression on lung pTreg cells also potentiates ILC2 activation and expansion, via a growth and differentiation factor 15 (GDF15)dependent mechanism that abrogates pTreg cell-mediated suppression of ILC2s. Upper Left: During severe SARS-CoV-2 infection, pTreg cell Notch4 represses production of the tissue reparative factor amphiregulin (AREG) by inhibiting IL-18 production, which promotes severe lung inflammation. Lower Left: In multisystem inflammatory syndrome in children (MIS-C), subjects harboring mutations in the Notch-related genes, upregulation of Notch1 on Treg cells leads to super-induction of CD22 and augmented TCR signaling, collectively promoting Treg-cell destabilization in an mTOR-dependent manner. Lower Right: Expression of the Notch ligand Jagged-1 by skin Treg cells promotes the proliferation and function of hair follicle stem cells to support hair follicle regeneration and hair growth.

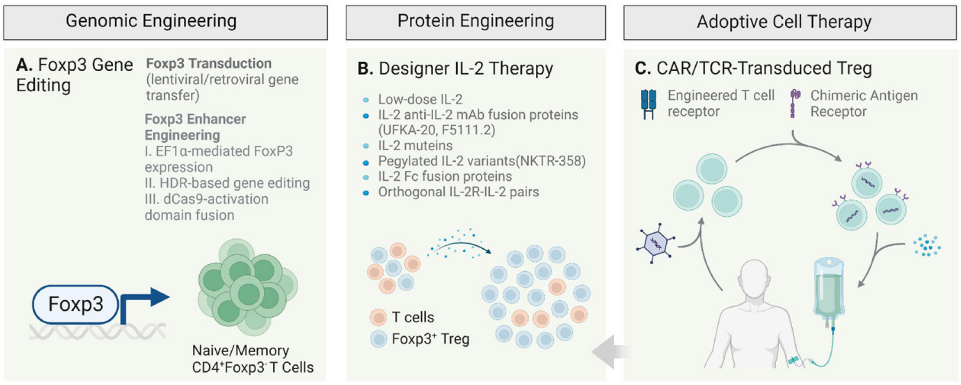

Figure 6. Regulatory T cell therapies

A number of therapeutic approaches are currently attempting to harness the potential of Treg cells as therapeutics for autoimmune and immune dysregulatory disorders. These strategies include: A. genomic engineering strategies to artificially induce long-lasting Foxp3 expression and reprogram conventional $CD4^+$ T cells into Foxp3-expressing Treg-like cells capable of inducing tolerance. B. Modified IL-2 reagents including IL-2 muteins, pegylated-IL-2 variants, IL-2:anti-IL-2 complexes and fusion proteins as well as orthogonal IL-2-IL-2R pairs. C. Adoptive cell therapies (ACTs) utilizing autologous *ex*-vivo expended polyclonal or antigen-specific Treg cells with engineered TCRs including chimeric antigen receptor (CAR) Treg cells.