CellPress

Immunity

Review

Principles of regulatory T cell function

Stanislav Dikiy[1,2,3,*] and Alexander Y. Rudensky[1,*]

[1]Howard Hughes Medical Institute and Immunology Program, Sloan Kettering Institute, Ludwig Center at Memorial Sloan Kettering Cancer Center, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

[2]Immunology and Microbial Pathogenesis Program, Weill Cornell Graduate School of Medical Sciences, New York, NY 10021, USA

[3]Present address: Department of Immunology and Microbiology, Scripps Research, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

*Correspondence: sdikiy@scripps.edu (S.D.), rudenska@mskcc.org (A.Y.R.)

https://doi.org/10.1016/j.immuni.2023.01.004

SUMMARY

Regulatory T (Treg) cells represent a distinct lineage of cells of the adaptive immune system indispensable for forestalling fatal autoimmune and inflammatory pathologies. The role of Treg cells as principal guardians of the immune system can be attributed to their ability to restrain all currently recognized major types of inflammatory responses through modulating the activity of a wide range of cells of the innate and adaptive immune system. This broad purview over immunity and inflammation is afforded by the multiple modes of action Treg cells exert upon their diverse molecular and cellular targets. Beyond the suppression of autoimmunity for which they were originally recognized, Treg cells have been implicated in tissue maintenance, repair, and regeneration under physiologic and pathologic conditions. Herein, we discuss the current and emerging understanding of Treg cell effector mechanisms in the context of the basic properties of Treg cells that endow them with such functional versatility.

INTRODUCTION

The immunosuppressive activity of T cells discovered in the 1960s was ascribed by subsequent studies to a subset of $CD4^+$ T cells characterized by the constitutive expression of the high-affinity binding subunit of interleukin (IL)-2 receptor, CD25, and lower abundance of a high molecular weight isoform of CD45, CD45RB, relative to naive T cells.[1–9] *In vivo* experiments using complementary approaches of adoptive cell transfers and antibody-mediated depletion showed that $CD25^+CD4^+$ or $CD45RB^{lo}CD4^+$ cells effectively suppressed T cell-mediated autoimmunity and colonic inflammation caused by the reactivity of immune cells against the intestinal microbial community.[6–9] Mice lacking genes encoding CD25 (*Il2ra*), CD122 (*Il2rb*), and IL-2 (*Il2*) manifest uncontrolled immune activation and autoimmunity, suggesting a functional role for CD25 and IL-2 signaling in these cells, with a non-redundant cell-intrinsic role of this pathway in regulatory T (Treg) biology demonstrated in cell transfer studies.[10–14] Separately, investigations of a pediatric hereditary monogenic immune disorder termed immunodysregulation, polyendocrinopathy enteropathy X-linked (IPEX), and a spontaneous mouse mutant *scurfy* showed that fatal, early-onset, autoimmune disease in both mice and humans was due to lesions in the *Foxp3* gene.[15–18] The disease is characterized by autoimmune destruction of endocrine organs, enteric inflammation, allergic skin inflammation, dysregulated antibody responses, autoimmune anemia, hyper-IgE syndrome, lympho- and myeloproliferation, and other lesions.[19]

These two lines of inquiry converged with the identification of Foxp3 as a transcription factor required for the differentiation and function of Treg cells.[20–25] Experimentation employing both genetic loss and gain of function, along with classical immunological and cell transfer approaches, showed an exclusive cell-intrinsic role for Foxp3 in Treg cells and demonstrated that the paucity of functional Treg cells accounts for all pathologies resulting from the *Foxp3* deficiency.[20,23] Further studies using $Foxp3^{DTR}$ knockin mice, which express the simian diphtheria toxin receptor exclusively in $Foxp3^+$ cells, enabling the *in vivo* depletion of Treg cells by diphtheria toxin administration, refuted the possibility that the development of these cells guaranteed immune quiescence merely by diverting pathogenic inflammatory self-reactive T cell precursors into a non-inflammatory state. Rather, these studies demonstrated that Treg cells continuously and proactively suppress autoimmunity and inflammation.[26–28]

A PLURALITY OF EFFECTOR MECHANISMS

Considering the singular role of Foxp3 in establishing Treg cell identify and functionality, these studies raised the possibility that their mechanism of suppression was directly controlled by Foxp3 and unique to Treg cells, as opposed to multiple mechanisms of suppression—each individually not unique to Treg cells, but collectively so—in a manner indirectly assisted by Foxp3. Ultimately, the use of genetic tools enabling constitutive or inducible Treg-cell-specific targeting of genes encoding putative effectors of Treg-cell-mediated suppression supported the latter scenario. Numerous studies searching for potential effectors of Treg-cell-suppressive action have revealed an array of molecules and mechanisms, including the immunomodulatory cytokines IL-10 and TGF-β, and a pair of cell surface ectoenzymes CD39 and CD73, which convert extracellular ATP, a potent pro-inflammatory mediator, into its anti-inflammatory product adenosine.[29–32] Single deficiency in the genes encoding some of these effectors in Foxp3-expressing cells does not result in systemic autoimmunity amounting to that observed in *Foxp3* deficiency or upon Treg cell depletion, demonstrating that Treg

cells do not rely on one essential mechanism to enforce systemic immune tolerance.[29,33,34] Indeed, few of these molecular mechanisms appear to be unique to Treg cells but in fact are shared by multiple types of cells of the innate and adaptive immune systems.[35–37]

Despite that, loss of these mechanisms in Treg cells cannot be compensated for by their seemingly redundant deployment by other immune cells. Instead, what has collectively emerged is that the action of a single effector of Treg-cell-mediated suppression can exert nuanced, context-specific, effects in controlling distinct aspects of immune responses in specific inflammatory environments. In this regard, Treg cells deficient in IL-10 production failed to control spontaneous colitis and restrain allergic airway and skin inflammation, in spite of the presence of IL-10 producing macrophages, B lineage cells, and T cells in those tissues, while those deficient in the *Ebi3* gene, expressed by various immune cells in addition to Treg cells, exhibited diminished control of anti-tumor immune responses.[34,38] Conversely, Treg cell suppression has even been linked to an effector molecule ordinarily deployed by innate and adaptive immune lymphocytes mounting a conventional immune response. In this way, Treg-cell-mediated immunosuppression in certain tumors and in allogeneic transplants has been also linked to the expression of granzyme B by Treg cells, even though its mechanisms of action remain unknown.[39,40] Granzyme B produced by Treg cells in these settings might exert its "canonical" cell-extrinsic intracellular proteolytic activity on specific targets, carry out extracellular proteolysis of inflammatory mediators, or might have some undiscovered cell-intrinsic intracellular function in Treg cells. Loss of CTLA-4 or IL2R subunits in Treg cells may appear to be an exception to the above notion, as severe autoimmunity nearly paralleling *Foxp3* deficiency, albeit less aggressive, results from the deletion of these molecules in Treg cells. However, these molecules also perform important cell-intrinsic functions affecting Treg cell development and survival, and T cell receptor (TCR) repertoire selection.[41–43] The bona fide immune suppressive functions of these molecules when deployed by Treg cells appear limited to, in the case of CD25, functioning as a sink for IL-2, thereby controlling the expansion and activation of $CD8^+$ T cells, natural killer (NK) cells, and type 2 innate lymphoid cells, which are all highly sensitive to IL-2 and, in the case of CTLA-4, interfering with dendritic cell (DC)-mediated T cell activation.[43–47] Additionally, although high expression of CTLA-4 and CD25 served as hallmarks of Treg cells prior to the discovery of Foxp3, these mediators of suppression have proven to be hardly unique to Treg cells. Even though Treg cells express arguably the highest levels of CD25 outside of the thymus and are able to reduce IL-2 availability though its consumption, activated effector T cells, innate lymphoid cells, DCs, and even fibroblasts expressing CD25 can sequester IL-2 to achieve immunoregulation.[48,49] Likewise, upregulation of CTLA-4, originally described during conventional T cell activation, has been shown to confer immunoregulatory capacity to effector T cells.[50]

Besides their "archetypal" immune suppressive and modulatory roles, which indirectly support tissue resilience in settings of inflammation, Treg cells have been suggested to contribute directly to the maintenance and repair of a diverse range of tissues. Treg cells in the white adipose tissue have been shown to express the transcription factor Peroxisome proliferator-activated receptor gamma (PPARγ) and regulate the functions of that major metabolic organ, to some degree through the production of IL-10; those in the bone marrow (BM) enforce quiescence in the hematopoietic stem cell (HSC) niche through the CD73/CD39-mediated conversion of extracellular ATP to adenosine; Treg cells in the injured muscle, brain, and lung promote tissue regeneration, at least in part through the elaboration of the growth factor amphiregulin (Areg), and Treg cells in the skin deploy various potential mechanisms, including stimulating Notch or TGF-β receptor signaling in hair follicle stem cells (SCs), through the provision of their corresponding ligands, to support the SC niche and promote hair follicle and epithelial barrier regrowth.[33,51–60] Interestingly, despite the fact that the discovery of the tissue repair function of Treg cells was more recent than that of their canonical immunomodulatory role, it appears as though these two broad functions co-evolved, as zebrafish Treg cells were shown to accumulate at sites of injury and participate in tissue regeneration through the production of organ-specific tissue repair factors rather than immunomodulatory mediators.[61]

A takeaway from the last decade of research of Treg cell function is that no single effector molecule explains all the immunosuppressive or tissue-supportive abilities of Treg cells; rather, Treg cells deploy distinct mechanisms acting upon a range of immune and non-immune target cells and in different contexts. The fact that many effector molecules elaborated by Treg cells, including the ones highlighted above, are also expressed by activated conventional T cells and various innate immune cells, suggest a possibility that the essential, uniquely non-redundant immunosuppressive function of Treg cells has less to do with specific molecules produced and more to do with other features particular to Treg cells. Below, we will discuss the effector mechanisms of Treg cells in the context of these broader features enabling Treg-cell-mediated control of tolerance and inflammation.

PRECISION TARGETING

Treg cells bear a diverse TCR repertoire distinct from that of conventional $CD4^+$ T cells.[26,62–64] This repertoire is highly enriched for receptors recognizing self-antigens, including tissue-restricted antigens expressed in the thymus due to the activity of the autoimmune regulator or AIRE.[26,62,65–68] Furthermore, continuous expression of the TCR itself, and the diversity of the TCR repertoire of Treg cells, is crucial for their ability to suppress untoward immune activation.[69–72]

In addition to diversity and general autoreactivity, it is also clear that the recognition of specific self-antigens guides Treg cell suppression of tissue-restricted autoimmunity. Early studies of the ability of Treg cells to suppress oophoritis or orchitis suggested a requirement for physiological levels of tissue-specific antigens for Treg cells to be able to suppress the corresponding autoimmunity and revealed an enrichment of Treg cell suppressor activity on a per-cell basis in the corresponding draining lymph nodes as opposed to non-draining lymph nodes and spleen, suggesting access to tissue-derived antigens by these potent Treg cells.[73–75] Consistent with these observations, monoclonal Treg cells expressing the chromogranin A-specific BDC2.5 TCR are markedly more efficient at suppressing diabetes in NOD mice than polyclonal Treg cells.[76] Treg-cell-mediated suppression is associated with modulation of the activation

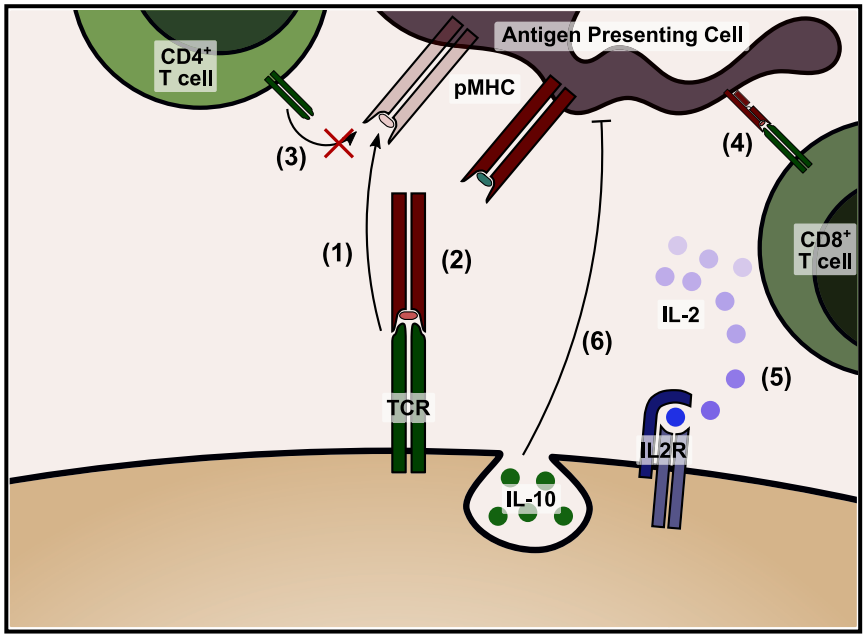

Figure 1. Precision targeting
TCR engagement by cognate ligands presented by DCs allows for co-localization of Treg and conventional T cells (1) during the early stages of T cell activation and enables Treg TCR-assisted removal of shared cognate antigen from DCs (2), restraining the activation of T cells recognizing the same epitopes (3). By co-localizing with DCs (4), Treg cells sequester IL-2 produced by effector T cells through consumption enabled by high CD25 expression (5), allowing them to subvert nascent responses. Additionally, Treg-cell-derived IL-10 can diminish DC stimulatory activity and inflammatory cytokine production (6).

and survival of effector T cells during their interactions with DCs, with recent studies suggesting that the TCRs expressed by Treg cells ensure their positioning within the local niches in the secondary lymphoid organs where the three cell types intermingle (Figure 1).[77,78] Furthermore, antigen-specific Treg cells were able to efficiently suppress conventional CD4$^+$ T cells that recognized a shared peptide epitope and not those recognizing a non-cross-reactive peptide displayed by major histocompatibility complex (MHC) class II molecules on the same DC, through a process mechanistically linked to the direct removal of peptide-MHC complexes from the DC by the TCRs expressed by the Treg cells (Figure 1).[79] In line with this, the CTLA-4 dependent function of Treg cells in disrupting DC activation of T cells in tumors appears to be carried out by Treg cells recognizing tumor-derived antigens, suggesting TCR-guided identification of targets for suppression.[44]

There are other scenarios where TCR-guided "identification" of target cells by Treg cells may be key to their immune suppression function. The induction of IL-10 expression by Treg cells requires the TCR, and mouse genetic models suggest that the major cellular targets of IL-10 are MHC-class-II-expressing mononuclear phagocytes; therefore, it is possible that cognate antigen interactions license Treg cells to target mononuclear phagocytes for IL-10-mediated suppression.[34,69,80,81] Treg-cell-derived IL-10 also plays a role in viral infections by restricting the production of inflammatory cytokines and the expression co-stimulatory molecules by DCs, even though a likely role for specific TCR-peptide-MHC interactions in this process remains untested[82] (Figure 1). Treg cells have been shown to contribute to the regulation of tissue SCs and their niches, and to their mobilization upon challenge, particularly in the skin, intestine, and BM. Although the requirement for cognate antigen recognition through the TCR by Treg cells in these contexts is also untested, it seems likely given the documented expression of MHC class II by SCs and their niche cells. Additional tissue-supportive and -regenerative functions of Treg cells, which may or may not be mediated by interactions with SCs, appear to be dependent on TCR specificity, as the accumulation of Treg cells in challenged adipose tissue and skeletal muscle requires their expression of specific TCRs.[83,84]

However, recognizing cognate antigen and receiving signaling through the TCR cannot be viewed by itself a mechanism of immune suppression. Although Treg cells recognize target cells or their micro-assemblies based on TCR-mediated detection of cognate antigen, they execute their suppressor actions by multiple means beyond antigen removal. Although some mechanisms deployed in this targeting have been well defined, including IL-2 deprivation through CD25 contributing to Treg cell control of CD8$^+$ T cell activation, the means by which Treg cells inhibit CD4$^+$ T cell activation or remove peptide epitopes from APCs, and to what extent the latter mechanism is particular to Treg cells, still remain unclear. Furthermore, TCR-mediated "precision targeting" does not automatically imply that the mechanisms deployed to thereafter achieve suppression depend on TCR signaling in Treg cells. Indeed, elaboration of at least some Treg cell effector molecules, including tissue protective amphiregulin and a suite of immunomodulatory molecules deployed by colonic Treg cells, is uncoupled from, or independent of, TCR signaling—at least after their initial TCR-mediated induction.[52,85] That being said, the profound loss of control of immune responses upon induced TCR ablation in Treg cells, and the pronounced enhancement of immunosuppressive activity upon ablation of PD-1, a major negative regulator of TCR and CD28 signaling, suggest a requirement for continuous TCR signaling for the suppressive functions of Treg cells.[69,72,86,87] Therefore, the deployment of both TCR-dependent and -independent effector functions of Treg cells following cognate antigen stimulation, which activates and guides these cells to the right niches and targets, underpins their far-reaching purview of organismal health.

GETTING THERE

In addition to overlapping TCR specificities enabling Treg cell encounter with and control of their effector T cell targets, the shared expression of chemokine receptors and other cell guidance molecules supports coordinated Treg and effector cell

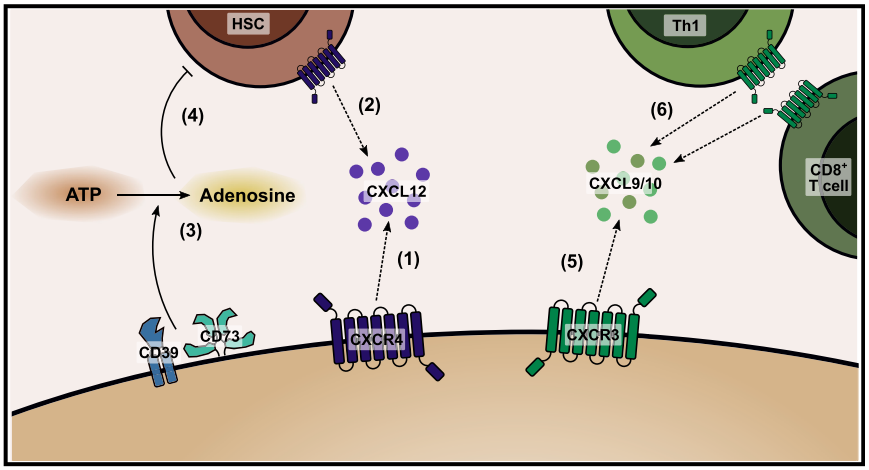

Figure 2. Getting there

Treg cells expressing the chemokine receptor CXCR4 traffic to the bone marrow and access the niches populated by CXCR4-expressing HSCs and B cells (1) guided by the CXCR4 ligand CXCL12 produced by stromal cells (2). The enzymatic activity of CD39 (and CD73 in mice) displayed by Treg cells converts ATP to adenosine (3), which acts on purinergic receptors to support HSC quiescence (4). Treg cells expressing the transcription factor T-bet acquire expression of the chemokine receptor CXCR3 (5), which enables their close spatial opposition to CXCR3-expressing Th1 and activated CD8 T cells, orchestrated by the CXCR3 ligands CXCL9 and CXCL10 (6), and selective repression of type 1 immune responses by T-bet-expressing Treg cells.

trafficking to, and their positioning within, secondary lymphoid organs and non-lymphoid target tissues.[88] This notion stems from findings that the chemokine receptor CCR7 and L-selectin (CD62L), in parallel to their roles in naive CD4 T cells, promote "naive" or "resting" Treg cell recirculation through the secondary lymphoid organs.[89] In addition to these features shared with naive T cells, Treg cells constitutively express high amounts of CCR4, whose ligand CCL22 is displayed by DCs activated upon their engagement by cognate antigen-specific T cells.[90] Such a coordinated process allows for the effective and timely recruitment of Treg cells to niches where they can curtail overexuberant T cell responses in the secondary lymphoid organs. While these and other molecules facilitate Treg cell patrolling of the secondary lymphoid organs and blood vasculature, activated Treg cells express diverse inducible chemokine and other tissue homing receptors. These enable the Treg-cell-mediated restraint of inflammation and the enforcement of tolerance at barrier tissues chronically exposed to microbes and xenobiotics under physiologic conditions and support Treg cell trafficking to sites of induced inflammation in response to infection and injury.[91]

While constitutive CCR4 expression by Treg cells was shown to be a specific prerequisite for preventing inflammation in the skin, differential expression of CCR2, CCR5, CCR6, and CXCR3 by activated Treg cells enables their preferential entry into a variety of inflamed and non-inflamed peripheral tissues.[92–95] Additionally, CCR4 plays a role in Treg cell recruitment to skin and solid organ cancers, where preferential accumulation of these cells subverts anti-tumoral immunity and directly supports tumor progression.[96,97] Notably, a subset of highly activated intratumoral Treg cells displays high amounts of CCR8, whose expression, while dispensable for their migration to and suppressor function within the tumor, offers an opportunity for their therapeutic targeting in human cancers.[92,98–102] The expression of both CCR4 and CCR8 is selectively induced in type 2 helper T cells alongside, and likely in a manner dependent on, GATA3 and IRF4, the lineage specifying transcription factors for Th2 cells.[103–106] Expression of these transcription factors in activated Treg cells identifies those specifically capable of controlling type 2 immunity, and it is reasonable to assume that constitutive expression of those chemokine receptors by these Treg cell subsets grants them preferential access to the site of type 2 inflammation.[107–110]

Likewise, T-bet-expressing Treg cells have been suggested to exhibit a potent ability to selectively restrain type 1 immunity, a capacity linked at least in part to T-bet-dependent expression of CXCR3.[111–113] Indeed, T-bet-expressing Treg cells are found in close opposition to T-bet-expressing $CD8^+$ and $CD4^+$ T effector counterparts in the secondary lymphoid organs[113] (Figure 2). Likewise, in non-lymphoid tissues affected by type 1 inflammation, such as the pancreas in the setting of type 1 diabetes and the kidney in the setting of crescentic glomerulonephritis, the T-bet CXCR3 axis is required for Treg cell localization to and immune suppression within these tissues.[114–116]

Further, CCR6, a chemokine receptor often associated with type 3 immunity, has been also shown to regulate Treg cell trafficking, facilitating Treg cell function in various settings. CCR6 plays a role in recruiting an early wave of Treg cells to the skin, where they promote tolerance to skin commensal microbes by a yet undetermined mechanism.[117,118] Treg cells also require CCR6 for recruitment to the intestine and limiting Th17 responses therein, likely through IL-10 elaboration.[119,120] Besides these canonical chemokine receptors, a recently deorphanized GPCR, GPR15, recognizing a ligand, GPR15L, has also been shown to play an important role in enabling Treg cell trafficking to barrier epithelial tissues, including the colon and skin, in order to suppress colitis and allograft rejection, respectively.[121,122] Similarly, the CXCR4 chemokine receptor expressed by a population of Treg cells guides them to sources of CXCL12 in the BM, where they regulate the numbers and self-reactive antibody production by B-1 B cells and limit the overall amount of serum IgM.[123] Based on the analysis of constitutive Treg-cell-restricted CXCR4 deficiency, CXCR4-guided Treg cells have been also proposed to modulate HSC quiescence[33,124] (Figure 2). There is a strong likelihood, however, that this effect is due to a developmental defect, as the induced loss of CXCR4 in Treg cells and their consequent depletion from the BM in adulthood has not affected the HSC numbers or their differentiation potential.[123]

A Treg cell subset, known as follicular Treg cells (Tfr), is characterized by the expression of CXCR5, a feature shared with follicular helper T (Tfh) cells, whose responses they modulate, along with those of B cells.[125,126] They are suggested to do so

via the production of IL-10, neuritin (previously known as a neuronal-plasticity-associated molecule), TGF-β, and additional mechanisms[127–132] (see Sage and Sharpe[125] for an in-depth review). Intriguingly, as in the case of the conventional follicular helper (Tfh) cells they control, Tfr cells do not require the expression of the B cell follicle homing receptor CXCR5 for their localization or function.[133] However, both require the expression of the transcription factor Bcl6; which Bcl6-regulated localization molecules are necessary, alone or in combination with CXCR5, remains to be examined.[134,135]

Distinct expression of integrins by activated Treg cells also contributes to their trafficking to, positioning in, and function within non-lymphoid tissues. Seemingly specific expression of Raph1, an integrin adaptor molecule, in Treg cells preserves normal Treg cell trafficking in the absence of RIAM (encoded by *Apbb1ip*), a Raph1 paralog, whose deletion has more pronounced effects on conventional T cells.[136] This reinforces the aforementioned notion that Treg cells, more than relying on unique effector molecules, may harbor particular mechanisms by which they traffic to and localize within key non-lymphoid tissues. The αVβ8 and α4β7 integrins play important roles in Treg cell function in controlling intestinal inflammation. While αVβ8 integrin enables Treg cells to activate latent TGF-β, leading to the suppression of enteric inflammation, it could also play a role in recruiting these cells to inflamed gastrointestinal tissue or positioning them within this organ, given the promiscuous binding of this integrin.[137–139] The α4β7 integrin plays a broad role in enabling leukocyte migration to the intestine and associated lymphoid tissues but seems to be of particular importance for Treg cells, as the intestinal inflammation associated with loss of *Itgb7*, which encodes β7, is due in part to the disrupted homing and function of Treg cells.[140–142] Another chemotaxis-related strategy employed by Treg cells for co-localizing with their intended cellular targets is by actively attracting them through the production of chemotactic cues. In this regard, Treg cell production of CCL3 and CCL4 supports the recruitment of pro-inflammatory T cells to Treg cells, allowing them to become targets of suppression.[143]

Populations of Treg cells found at barrier sites, particularly in the intestines, include a large number of peripherally generated Treg (pTreg) cells, that is, cells acquiring Foxp3 expression and Treg cell identity extrathymically.[144–146] The enhanced ability of these cells to persist in—and their specialized function within—these tissues likely depends on their antigenic specificity distinct from self-antigens recognized by their thymically generated (tTreg) counterparts, as it has become increasingly clear that these cells harbor TCRs recognizing antigens derived from the microbiota and, potentially, diet.[147–151] In addition, commensal microbiota-derived metabolites enriched at these sites, including retinoic acid, short-chain fatty acids, and secondary bile acids, contribute to imparting the distinct features of these cells alongside local inflammatory cues.[152–162] It is reasonable to assume that these environmental effects are responsible for the recently demonstrated dispensability of Foxp3 expression by pTreg cells for their stability, fitness, and ability to contain effector T cell expansion.[145] pTreg cells have been shown to perform a number of non-redundant functions, including support of the assembly of beneficial commensal microbial communities and tissue physiology, and curbing local inflammation elicited by commensal microbes and pathobionts.[144,145,148,163,164] While it appears that the effector mechanisms deployed by these cells to these ends might not be unique, their specific localization and ontogeny underpin their distinct functionality.

Upon activation under inflammatory conditions, Treg cells express even higher levels of effector molecules and become markedly more potent suppressors.[165–167] However, this activation is largely transient, with gene expression and immune suppressive ability returning to baseline levels as inflammation subsides.[167] Rather than showing features of persisting memory of heightened suppressor activity, the lasting characteristics of inflammation experienced Treg cells consist largely of stably increased expression of genes whose products promote homing to non-lymphoid tissues.[166,167] This outcome may reinforce the link between the ability of Treg cells to maintain immune tolerance and a heightened capacity for localizing to peripheral tissues.

While most of the studies so far have focused on the functional significance and mechanistic aspects of entry by Treg cells into lymphoid and non-lymphoid tissues and their spatial distribution therein, the regulation and functional significance of their recirculation, entailing emigration from non-lymphoid tissues and re-entry into the draining lymph nodes and eventually circulation, are less well understood. In that "vein," activated Treg cell migration from inflamed skin to draining lymph nodes has been suggested to suppress immune responses to allografts, and the trafficking of Treg cells from large intestine lamina propria to distal locations has been reported.[94,168] Similarly, integrin α2β1 expression marks, and potentially contributes to, the recirculation of a mature, highly suppressive population of Treg cells.[169] Additionally, it has been recently shown that Treg-cell-specific expression of layilin (encoded by *Layn*), a C-type lectin acting as a hyaluronan receptor, may limit Treg-cell-suppressive functions in the skin by restricting Treg cell motility, suggesting the possibility of complementary negative regulation of Treg cell activity through the modulation of their localization.[170]

Although studies over the last two decades revealed a clear relation between the broad localization of Treg cells and their ability to perform effector functions, the finer-grain picture of spatial distribution of Treg cells within lymphoid and non-lymphoid organs and how this enables their function is less well appreciated and understood. Because the effectiveness of a particular effector molecule is a product of its ability to be deployed in the right circumstance and at the proper target cell, identifying the cues that guide them to and within distinct tissues and their different interaction partners therein, alongside the effector molecules deployed by Treg cells, is essential for a better understanding of immune tolerance.

TIMELY ACTION

The developmental timing of Treg cell maturation within and their egress from the thymus has been a major focus of studies since their initial characterization and the first demonstrations of their activity.[3,22,171,172] As it turns out, the timing of thymic egress of Treg cells also enables their proper seeding of and localization within tissues. Early experiments showed that disruption of this process, by thymectomy at three days of age in mice, led to significant organ-specific autoimmunity.[3,171] Recent work from the Mathis and Benoist laboratory has shown that paucity in an early

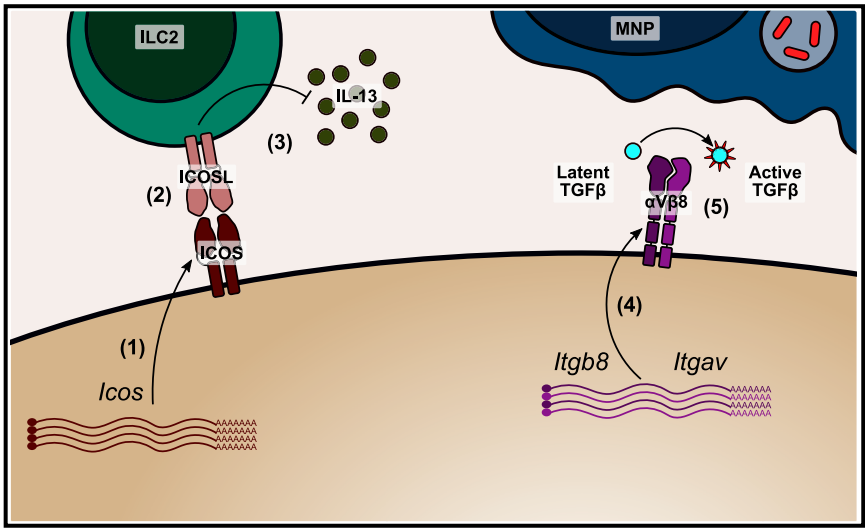

Figure 3. Timely action

Treg cells constitutively express ICOS (1), enabling ongoing interaction with and inhibition of group 2 ILCs (2), leading to reduced production of IL-5 and IL-13 by these cells (3). Treg cells in the mucosa constitutively express the αVβ8 integrin (4), allowing them to efficiently activate latent TGF-β to enforce tolerance at mucosal barriers (5).

wave of thymic Treg cells, enriched for reactivity against AIRE-dependent tissue-restricted antigens, leads to autoimmune lesions in the corresponding tissues.[68] Interestingly, gene expression profiling showed that these Treg cells, though more activated and proliferative than the bulk population, did not uniquely express specific immunosuppressive effectors. Overall, this suggests that proper and timely access to peripheral tissues, more than elaboration of certain effector molecules, is essential for Treg cells to establish and maintain immune homeostasis. More recent work has supported this idea. Treg cells expanding in the liver early in life have been shown to play a role in promoting tolerance in that organ.[173] Further, neonatal Treg cells were suggested to restrain the proliferation of autoreactive neonatal helper T cells, potentially by competing for and limiting IL-33 availability.[174]

Both tTreg and pTreg cells exit their places of birth as pre-activated, antigen-experienced cells, due to their unique developmental trajectory.[145,167,175] This property includes high, constitutive expression of a number of their effector molecules, while for conventional T cells this requires antigen exposure and differentiation. In comparison to conventional T cells, even resting Treg cells in physiologic settings are characterized by markedly higher expression of genes associated with canonical Treg cell suppression mechanisms, e.g., *Il2ra*, *Il10*, *Ctla4*, *Ebi3*, *Gzmb*, and *Entpd1*, among others.[21,165,176,177] It is this attribute that is likely pivotal to the ability of Treg cells to suppress specious immune activation. In this way, Treg cells have a head start on such T cell activation processes. By contrast, effector T cells acquire expression of these same immune suppressive molecules late in their activation and differentiation process, which, while curtailing their activity and contributing to immune response resolution, fails to prevent autoimmunity and inflammation in the absence of Treg cells.

As an illustration of this "background activity" of Treg cells, which stems from their primordial activation and is further tuned in response to the basal inflammatory tone, Treg cells at mucosal barrier sites constitutively express IL-10 and the genes encoding proteins involved in ensuring an efficient display of an active form of TGF-β, which allows them to impart a constitutive immune quiescent state onto macrophages and other tissue resident cells, and thereby prevent colonic inflammation[34,137,139] (Figure 3). Similarly, a large proportion of Treg cells constitutively express high amounts of CD25, allowing them to quench not only nascent $CD8^+$ T cell responses but also pro-inflammatory responses by various innate lymphocytes such as NK cells, which can respond to this cytokine alone.[43,46,47,77] Additionally, Treg cells can control other innate lymphocytes, such as group 2 innate lymphoid cells (ILC2s), because of their heightened, constitutive ICOS expression[178,179] (Figure 3).

Timing is also key for the function of the aforementioned T-bet expressing Treg cells. IL-27, an immunomodulatory cytokine produced by DCs has been shown to play a non-redundant role in inducing T-bet expression in Treg cells, while signaling by IFNγ and IL-12, the cytokines that collectively drive Th1 cell differentiation, are seemingly dispensable or even inhibitory.[180–182] That Treg cells might acquire this program in response to IL-27 alone might enable their function on a different timescale and in different settings than that with which conventional T-bet expressing type 1 polarized T helper cells differentiate, as that process requires sequential IL-12 and IFNγ production by innate and adaptive cells. Presumably acting at later stages, IFNγ can also support T-bet expression in Treg cells, while stimulating IL-27 production by DCs.[180,181] This suggests that Treg cells may act as amplifiers of immunomodulatory cues, i.e., IL-27, elaborated by accessory cells, as a counterpart to the amplification of pro-inflammatory cues by effector $CD4^+$ T cells. Thus, the induction and maintenance of T-bet in Treg cells can be viewed as part of both indirect and direct cell-extrinsic negative loops restraining type 1 immunity.

Interestingly, the ability of Treg cells to mount early responses during pathogen encounter has also been associated with promoting protective immunity in experimental genital herpes, respiratory syncytial virus, and West Nile virus and oral *Candida* infections. Treg cells were shown to contribute to orchestrating migration of innate and adaptive immune cells to infected barrier tissues by limiting production of pro-inflammatory chemokines, and consequently opposing retention of early effectors in the draining lymph nodes, leading to enhanced adaptive immune responses and improved pathogen clearance, and by IL-2 sequestration and CTLA-4 dependent modulation of DC function.[183–187] These unusual vanguard actions of Treg cells are likely intertwined with the well appreciated ability of Treg cells to restrain early, innate inflammation, as in many of these models enhanced adaptive immunity was

also associated with reduced tissue damage and collateral immune pathology, and abundance of various inflammatory mediators. Therefore, the ability to Treg cells to act early in immune responses, ahead of conventional T cell responses, results in a compound benefit: a reduction in inflammation and the associated damage and improved adaptive immune responses and pathogen control. A similar function of Treg cells, resulting in improved virus-specific T cell memory during lymphocytic choriomeningitis virus (LCMV) infection, shown to be dependent on IL-10 production by Treg cells was suggested to function through polarizing DCs away from promoting inflammation and toward CD8 T cell priming.[82] However, the kinetics of IL-10 production by Treg cells throughout LCMV infection leave it unclear whether this results from innate-like or early Treg cell action.

This notion of preparedness for action extends to effector mechanisms of Treg cells that are not directly involved in modulating immune responses, as Treg cells also serve as an important early source of tissue-supportive factors during inflammation and immunity. Treg cells are among the earliest producers of Areg during experimental influenza infection. Thus, they provide essential support for the tissue at a time when the responses of effector T cells capable of Areg production are still ramping up, while mobilized innate immune cells are already inflicting collateral damage.[52] This rapid deployment is likely possible because at least a subset of tissue resident Treg cells constitutively expresses Areg transcript and these cells can rapidly produce Areg in an alarmin responsive, TCR-independent manner.[52,54,188–190] Likewise, Treg cells in the skin constitutively express the Notch ligand Jagged-1, allowing them to support hair follicle SCs and rapidly mobilize them upon tissue damage.[51] While poorly understood, additional mechanisms might explain the ability of Treg cells to outperform conventional T cells in accumulating in peripheral tissues. The capacity of certain Treg cells to expand in response to IL-33, likely stemming from their high expression of the IL-33 receptor ST2, has been suggested to factor in their accumulation in adipose tissue and the injured or aged muscle.[191–193] Even though mature Treg cells have experienced initial TCR stimulation during thymic or peripheral differentiation, timely mobilization of their function in many cases requires subsequent TCR engagement in addition to poorly defined signals, which report on the particularities of the perturbed state, such as the immune tone, inflammation, and tissue stresses. The specifics and timing of these various signals, how they contribute to setting up the pre-activated state of Treg cells as well as driving their full mobilization when required, and finally, which combinations of signals are required for which effector modalities are questions of obvious interest.

SENDING A CLEAR MESSAGE

Treg cells are by far not the only specialized lineage of agonist-selected T cells that emerge from their differentiation process in a pre-activated state. The other non-conventional T cell lineages, including $\gamma\delta$T cells, natural killer T (NKT) and mucosal associated invariant T (MAIT) cells, and intra-epithelial CD8$\alpha\alpha^+$ T cells, likewise constitutively express some, if not all of the aforementioned effector molecules and chemotactic receptors expressed by Treg cells.[194–197] Despite this seeming resemblance, in settings of congenital Treg cell deficiency due to loss-of-function *Foxp3* mutations or experimental depletion of Treg cells, these non-conventional T cell populations fail to effectively carry out essential immunosuppressive functions of Treg cells and prevent autoimmunity. Likewise, the increased expression of IL-10, CTLA-4, CD39, and other immunosuppressive effector molecules by differentiated conventional T cells late in immune responses is inadequate to prevent lethal autoimmunity. Even in narrower contexts, and absent systemic inflammation, the fact that conventional T cells express substantial levels of IL-10 and Areg post-activation, does not allow them to compensate for the lack of these molecules derived from Treg cells.[34,52,82,198] Although this discrepancy is likely explained in part by their localization and timing discussed above, this alone may not fully explain the inability of non-conventional T cells to substitute for Treg cells.

Thus, the activity of Foxp3 within Treg cells coupled with their diverse TCR repertoire is the primary, cell-intrinsic determinant enabling the unique indispensable function of Treg cells for the health of vertebrate animals. Foxp3 has been shown to suppress either directly or indirectly the expression of pro-inflammatory genes at baseline and to maintain this suppressed state particularly when Treg cells are activated in inflammatory environments.[165,199–202] By subtly tuning the expression of relatively few genes, resulting in the modulation of Wnt β-catenin signaling among other changes, Foxp3 ensures a fundamental distinction between Treg cells and all other T cell lineages: Treg cells do not co-deliver pro- and anti-inflammatory signals and are dedicated to the inhibition of immune responses[203–206] (Figure 4). Nevertheless, in human disease settings and in experimental models of inflammation, autoimmunity, and infection Treg cells have been shown to produce cytokines typically considered pro-inflammatory, including IFNγ and IL-13, which may be interpreted as exceptions to this rule.[207–215] Although maladaptive consequences of Treg cell production of IFNγ have been reported, its potential physiological role has not been addressed in sufficient depth, although several reports suggest protective, immunoregulatory functions in settings of allogeneic responses.[208,212,213] In a similar regard, tissue protective, rather than pro-inflammatory, functions for Treg-cell-derived IL-13 have been reported, likely reflecting in part a longstanding misclassification of this tissue remodeling cytokine as pro-inflammatory.[207,209,216] Whether Treg cells have "co-opted" other pro-inflammatory cytokines in particular contexts, and whether this should lead to a re-evaluation of the utility of rigid categorization of cytokines as pro- or anti-inflammatory, deserve further consideration. A recent unexpected observation of an opposite phenomenon of Tfh cells acquiring regulatory capacity and losing immune promoting functions upon upregulation of Foxp3 in late germinal centers offers an interesting wrinkle in the understanding of the importance of Foxp3 in specifying the unique abilities of Treg cells.[217] Additionally, stable Foxp3 expression has also been reported to be induced in CD8 T cells in tumors, conferring some suppressive properties on these cells.[218,219] On the one hand, it appears as though in this case Foxp3 performs a mechanistically consistent function of repression of pro-inflammatory functionalities; on the other hand, this work raises a provocative question about the

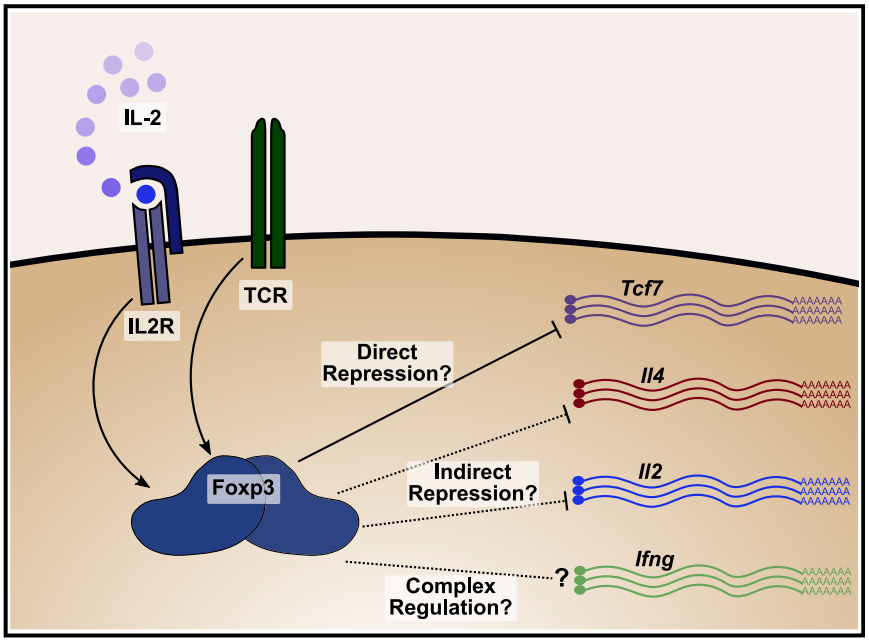

Figure 4. Sending a clear message
Treg cells acquire stable Foxp3 expression in response to TCR and IL-2R signaling during differentiation, which results in the repression of pro-inflammatory gene expression, by direct or indirect means. Repression of some pro-inflammatory cytokines, such as Ifng, is relieved in competent Treg cells in some inflammatory settings, suggesting potential anti-inflammatory or tissue-supportive functions for these molecules.

evolutionary emergence of a dedicated immunosuppressive lymphocyte function and an intriguing possibility of a primordial or parallel function of Foxp3 in T cells.

CONCLUDING REMARKS

To date, efforts to understand the effector functions of Treg cells have been largely focused on identifying and functionally testing the mediators of these mechanisms: immunomodulatory molecules such as IL-10, Ebi3, CD39/CD73, and TGF-β, among others. These collective works have shown that the mediators of Treg cell immunosuppressive and tissue-supportive function are numerous and context dependent. Furthermore, the rather broad expression of many of these effector molecules across Treg cells and effector T cells, not to mention cells of the innate immune system, poses a dilemma that needs further consideration, especially in light of the non-redundancy of many of these molecules revealed by Treg-cell-specific ablation experiments. So far, investigations of the non-redundant functions of various immunosuppressive molecules produced by either Treg cells or conventional T helper cells have been rather biased, with the conspicuous paucity of studies involving side-by-side cell type specific loss-of-function analysis. Beyond the contributions of specific molecular mediators, the principles by which Treg cells carry out their ascribed functions are reasonably well defined and likely broadly applicable. Thus, the special abilities of Treg cells to act at the right place and time provide a framework for future research in the field. While the cues that properly position and marshal Treg cells to deploy known mediators are relatively understudied, investigation of these cues and their interplay with specific functional states of effector Treg cells presents an opportunity for uncovering novel mechanisms of immune regulatory and tissue-supportive functions.

The role of the TCR in this rubric deserves special mention. The majority of Treg cells experience agonist TCR signaling during differentiation followed by tonic TCR stimulation during their maintenance in the periphery, as well as likely heightened TCR engagement in settings of inflammation and other stresses. Further dissection of how these distinct stimulatory modalities result in the constitutive or inducible expression of various effector molecules by Treg cells should help clarify which ones are antigen specific versus TCR dependent, a subtle yet important distinction, or neither. In this regard, the ability of Treg cells to prevent DC-mediated activation of T cells is in large part TCR- and contact-dependent and can be viewed as antigen specific.[69,72,77,220–222] However, TCR stimulation can induce the expression of cell adhesion molecules, such as lymphocyte function-associated antigen (LFA)-1, which could enable subsequent direct, but antigen-independent interactions with DCs.[69,223] As mentioned above, Treg cells are known to interact with and modulate the activity of MHC-class-II-expressing cell types of different origins, including tissue SCs.[33,51,81,224] The contribution of antigen-specific versus TCR-dependent regulation to these cellular circuits, in particular in the context of barrier tissue maintenance, remains to be elucidated. Finally, certain effector mechanisms of Treg cells are deployed in a demonstrably TCR-independent manner, implying that while Treg cells gain competency for certain functions through TCR stimulation, there is not an ongoing obligatory requirement for antigen recognition or TCR signaling for these functions. This heterogeneity in TCR dependence: whether signaling is needed developmentally, chronically, or situationally for the elaboration of distinct mechanisms likely reflects the heterogeneity of the environments in which Treg cells act, as well as of their cellular targets. These properties of Treg cells are also reflected by the breadth of TCR signaling strength, and by extension antigen affinity, which can drive productive Foxp3 expression and Treg cell lineage commitment.[225,226]

Ultimately, the special characteristics of Treg cells—expression of Foxp3 mechanistically coupled with agonist-driven differentiation and further diversification of their functional states—support the manifestation of immune suppression and tissue regeneration modalities at the right place, at the right time, and in a calibrated manner.

ACKNOWLEDGMENTS

We thank A. Mendoza for critical reading of the manuscript. This work was supported by the NCI Cancer Center Support grant P30 CA008748, NIH grant

R37 AI034206 (A.Y.R.), and the Ludwig Center at the Memorial Sloan Kettering Cancer Center (A.Y.R.). A.Y.R. is an investigator with the Howard Hughes Medical Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

DECLARATION OF INTERESTS

A.Y.R. is an SAB member of Amgen, Vedanta Biosciences, Surface Oncology, and Sonoma Biotherapeutics and holds equity in Sonoma Biotherapeutics, Vedanta Biosciences, and Surface Oncology.

REFERENCES

1. Gershon, R.K., and Kondo, K. (1970). Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology *18*, 723–737.

2. Miller, J.F. (1964). The thymus and the development of immunologic responsiveness. Science *144*, 1544–1551. https://doi.org/10.1126/science.144.3626.1544.

3. Nishizuka, Y., and Sakakura, T. (1969). Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. Science *166*, 753–755. https://doi.org/10.1126/science.166.3906.753.

4. Isaković, K., Smith, S.B., and Waksman, B.H. (1965). Immunologic tolerance in thymectomized, irradiated rats grafted with thymus from tolerant donors. Science *148*, 1333–1335. https://doi.org/10.1126/science.148.3675.1333.

5. Isaković, K., Smith, S.B., and Waksman, B.H. (1965). Role of the thymus in tolerance. I. Tolerance to bovine gamma globulin in thymectomized, irradiated rats grafted with thymus from tolerant donors. J. Exp. Med. *122*, 1103–1123. https://doi.org/10.1084/jem.122.6.1103.

6. Morrissey, P.J., Charrier, K., Braddy, S., Liggitt, D., and Watson, J.D. (1993). CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. J. Exp. Med. *178*, 237–244. https://doi.org/10.1084/jem.178.1.237.

7. Powrie, F., Leach, M.W., Mauze, S., Caddle, L.B., and Coffman, R.L. (1993). Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int. Immunol. *5*, 1461–1471. https://doi.org/10.1093/intimm/5.11.1461.

8. Powrie, F., Correa-Oliveira, R., Mauze, S., and Coffman, R.L. (1994). Regulatory interactions between $CD45RB^{high}$ and $CD45RB^{low}$ CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. J. Exp. Med. *179*, 589–600. https://doi.org/10.1084/jem.179.2.589.

9. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). 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. *155*, 1151–1164.

10. Willerford, D.M., Chen, J., Ferry, J.A., Davidson, L., Ma, A., and Alt, F.W. (1995). Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity *3*, 521–530. https://doi.org/10.1016/1074-7613(95)90180-9.

11. Malek, T.R., Yu, A., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. Immunity *17*, 167–178. https://doi.org/10.1016/s1074-7613(02)00367-9.

12. Furtado, G.C., Curotto de Lafaille, M.A., Kutchukhidze, N., and Lafaille, J.J. (2002). Interleukin 2 signaling is required for CD4(+) regulatory T cell function. J. Exp. Med. *196*, 851–857. https://doi.org/10.1084/jem.20020190.

13. Suzuki, H., Kündig, T.M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R., Simard, J.J., Ohashi, P.S., and Griesser, H. (1995). Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. Science *268*, 1472–1476. https://doi.org/10.1126/science.7770771.

14. Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A.C., and Horak, I. (1993). Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell *75*, 253–261. https://doi.org/10.1016/0092-8674(93)80067-o.

15. Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. *27*, 20–21. https://doi.org/10.1038/83713.

16. Brunkow, M.E., Jeffery, E.W., Hjerrild, K.A., Paeper, B., Clark, L.B., Yasayko, S.A., Wilkinson, J.E., Galas, D., Ziegler, S.F., and Ramsdell, F. (2001). Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat. Genet. *27*, 68–73. https://doi.org/10.1038/83784.

17. Chatila, T.A., Blaeser, F., Ho, N., Lederman, H.M., Voulgaropoulos, C., Helms, C., and Bowcock, A.M. (2000). JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J. Clin. Invest. *106*, R75–R81. https://doi.org/10.1172/JCI11679.

18. Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., Perroni, L., et al. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat. Genet. *27*, 18–20. https://doi.org/10.1038/83707.

19. Wildin, R.S., Smyk-Pearson, S., and Filipovich, A.H. (2002). Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J. Med. Genet. *39*, 537–545. https://doi.org/10.1136/jmg.39.8.537.

20. Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. *4*, 330–336. https://doi.org/10.1038/ni904.

21. Fontenot, J.D., Rasmussen, J.P., Gavin, M.A., and Rudensky, A.Y. (2005). A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat. Immunol. *6*, 1142–1151. https://doi.org/10.1038/ni1263.

22. Fontenot, J.D., Dooley, J.L., Farr, A.G., and Rudensky, A.Y. (2005). Developmental regulation of Foxp3 expression during ontogeny. J. Exp. Med. *202*, 901–906. https://doi.org/10.1084/jem.20050784.

23. Fontenot, J.D., Rasmussen, J.P., Williams, L.M., Dooley, J.L., Farr, A.G., and Rudensky, A.Y. (2005). Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity *22*, 329–341. https://doi.org/10.1016/j.immuni.2005.01.016.

24. Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science *299*, 1057–1061. https://doi.org/10.1126/science.1079490.

25. Khattri, R., Cox, T., Yasayko, S.-A., and Ramsdell, F. (2003). An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat. Immunol. *4*, 337–342. https://doi.org/10.1038/ni909.

26. Hsieh, C.S., Zheng, Y., Liang, Y., Fontenot, J.D., and Rudensky, A.Y. (2006). An intersection between the self-reactive regulatory and nonregulatory T cell receptor repertoires. Nat. Immunol. *7*, 401–410. https://doi.org/10.1038/ni1318.

27. Kim, J.M., Rasmussen, J.P., and Rudensky, A.Y. (2007). Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat. Immunol. *8*, 191–197. https://doi.org/10.1038/ni1428.

28. Lahl, K., Loddenkemper, C., Drouin, C., Freyer, J., Arnason, J., Eberl, G., Hamann, A., Wagner, H., Huehn, J., and Sparwasser, T. (2007). Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease. J. Exp. Med. *204*, 57–63. https://doi.org/10.1084/jem.20061852.

29. Gutcher, I., Donkor, M.K., Ma, Q., Rudensky, A.Y., Flavell, R.A., and Li, M.O. (2011). Autocrine transforming growth factor-β1 promotes in vivo Th17 cell differentiation. Immunity *34*, 396–408. https://doi.org/10.1016/j.immuni.2011.03.005.

30. Maynard, C.L., Harrington, L.E., Janowski, K.M., Oliver, J.R., Zindl, C.L., Rudensky, A.Y., and Weaver, C.T. (2007). Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. Nat. Immunol. *8*, 931–941. https://doi.org/10.1038/ni1504.

31. Kamanaka, M., Kim, S.T., Wan, Y.Y., Sutterwala, F.S., Lara-Tejero, M., Galán, J.E., Harhaj, E., and Flavell, R.A. (2006). Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. Immunity *25*, 941–952. https://doi.org/10.1016/j.immuni.2006.09.013.

32. Deaglio, S., Dwyer, K.M., Gao, W., Friedman, D., Usheva, A., Erat, A., Chen, J.F., Enjyoji, K., Linden, J., Oukka, M., et al. (2007). Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J. Exp. Med. *204*, 1257–1265. https://doi.org/10.1084/jem.20062512.

33. Hirata, Y., Furuhashi, K., Ishii, H., Li, H.W., Pinho, S., Ding, L., Robson, S.C., Frenette, P.S., and Fujisaki, J. (2018). CD150high bone marrow Tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine. Cell Stem Cell *22*. 445.e5–453.e5. https://doi.org/10.1016/j.stem.2018.01.017.

34. Rubtsov, Y.P., Rasmussen, J.P., Chi, E.Y., Fontenot, J., Castelli, L., Ye, X., Treuting, P., Siewe, L., Roers, A., Henderson, W.R., et al. (2008). Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity *28*, 546–558. https://doi.org/10.1016/j.immuni.2008.02.017.

35. Li, M.O., and Flavell, R.A. (2008). TGF-beta: a master of all T cell trades. Cell *134*, 392–404. https://doi.org/10.1016/j.cell.2008.07.025.

36. Antonioli, L., Pacher, P., Vizi, E.S., and Haskó, G. (2013). CD39 and CD73 in immunity and inflammation. Trends Mol. Med. *19*, 355–367. https://doi.org/10.1016/j.molmed.2013.03.005.

37. Saraiva, M., and O'Garra, A. (2010). The regulation of IL-10 production by immune cells. Nat. Rev. Immunol. *10*, 170–181. https://doi.org/10.1038/nri2711.

38. Turnis, M.E., Sawant, D.V., Szymczak-Workman, A.L., Andrews, L.P., Delgoffe, G.M., Yano, H., Beres, A.J., Vogel, P., Workman, C.J., and Vignali, D.A.A. (2016). Interleukin-35 limits anti-tumor immunity. Immunity *44*, 316–329. https://doi.org/10.1016/j.immuni.2016.01.013.

39. Cao, X., Cai, S.F., Fehniger, T.A., Song, J., Collins, L.I., Piwnica-Worms, D.R., and Ley, T.J. (2007). Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity *27*, 635–646. https://doi.org/10.1016/j.immuni.2007.08.014.

40. Gondek, D.C., Devries, V., Nowak, E.C., Lu, L.-F., Bennett, K.A., Scott, Z.A., and Noelle, R.J. (2008). Transplantation survival is maintained by granzyme B+ regulatory cells and adaptive regulatory T cells. J. Immunol. *181*, 4752–4760. https://doi.org/10.4049/jimmunol.181.7.4752.

41. Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., Nomura, T., and Sakaguchi, S. (2008). CTLA-4 control over Foxp3+ regulatory T cell function. Science *322*, 271–275. https://doi.org/10.1126/science.1160062.

42. Paterson, A.M., Lovitch, S.B., Sage, P.T., Juneja, V.R., Lee, Y., Trombley, J.D., Arancibia-Cárcamo, C.V., Sobel, R.A., Rudensky, A.Y., Kuchroo, V.K., et al. (2015). Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity. J. Exp. Med. *212*, 1603–1621. https://doi.org/10.1084/jem.20141030.

43. Chinen, T., Kannan, A.K., Levine, A.G., Fan, X., Klein, U., Zheng, Y., Gasteiger, G., Feng, Y., Fontenot, J.D., and Rudensky, A.Y. (2016). An essential role for the IL-2 receptor in Treg cell function. Nat. Immunol. *17*, 1322–1333. https://doi.org/10.1038/ni.3540.

44. Marangoni, F., Zhakyp, A., Corsini, M., Geels, S.N., Carrizosa, E., Thelen, M., Mani, V., Prüßmann, J.N., Warner, R.D., Ozga, A.J., et al. (2021). Expansion of tumor-associated Treg cells upon disruption of a CTLA-4-dependent feedback loop. Cell *184*. 3998.e19–4015.e19. https://doi.org/10.1016/j.cell.2021.05.027.

45. Alissafi, T., Banos, A., Boon, L., Sparwasser, T., Ghigo, A., Wing, K., Vassilopoulos, D., Boumpas, D., Chavakis, T., Cadwell, K., and Verginis, P. (2017). Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. J. Clin. Invest. *127*, 2789–2804. https://doi.org/10.1172/JCI92079.

46. Gasteiger, G., Hemmers, S., Bos, P.D., Sun, J.C., and Rudensky, A.Y. (2013). IL-2-dependent adaptive control of NK cell homeostasis. J. Exp. Med. *210*, 1179–1187. https://doi.org/10.1084/jem.20122571.

47. Gasteiger, G., Hemmers, S., Firth, M.A., Le Floc'h, A., Huse, M., Sun, J.C., and Rudensky, A.Y. (2013). IL-2-dependent tuning of NK cell sensitivity for target cells is controlled by regulatory T cells. J. Exp. Med. *210*, 1167–1178. https://doi.org/10.1084/jem.20122462.

48. Kim, D., Kim, M., Kim, T.W., Choe, Y.H., Noh, H.S., Jeon, H.M., Kim, H., Lee, Y., Hur, G., Lee, K.M., et al. (2022). Lymph node fibroblastic reticular cells regulate differentiation and function of CD4 T cells via CD25. J. Exp. Med. *219*, e20200795. https://doi.org/10.1084/jem.20200795.

49. Li, J., Lu, E., Yi, T., and Cyster, J.G. (2016). EBI2 augments Tfh cell fate by promoting interaction with IL-2-quenching dendritic cells. Nature *533*, 110–114. https://doi.org/10.1038/nature17947.

50. Corse, E., and Allison, J.P. (2012). Cutting edge: CTLA-4 on effector T cells inhibits in trans. J. Immunol. *189*, 1123–1127. https://doi.org/10.4049/jimmunol.1200695.

51. Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.-A., Lai, K., Ahn, R., Corbin, K., Lowe, M.M., Scharschmidt, T.C., et al. (2017). Regulatory T cells in skin facilitate epithelial stem cell differentiation. Cell *169*. 1119.e11–1129.e11. https://doi.org/10.1016/j.cell.2017.05.002.

52. Arpaia, N., Green, J.A., Moltedo, B., Arvey, A., Hemmers, S., Yuan, S., Treuting, P.M., and Rudensky, A.Y. (2015). A distinct function of regulatory T cells in tissue protection. Cell *162*, 1078–1089. https://doi.org/10.1016/j.cell.2015.08.021.

53. Bapat, S.P., Myoung Suh, J., Fang, S., Liu, S., Zhang, Y., Cheng, A., Zhou, C., Liang, Y., LeBlanc, M., Liddle, C., et al. (2015). Depletion of fat-resident Treg cells prevents age-associated insulin resistance. Nature *528*, 137–141. https://doi.org/10.1038/nature16151.

54. Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J.L., Cerletti, M., Jang, Y., Sefik, E., Tan, T.G., Wagers, A.J., Benoist, C., and Mathis, D. (2013). A special population of regulatory T cells potentiates muscle repair. Cell *155*, 1282–1295. https://doi.org/10.1016/j.cell.2013.10.054.

55. Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S.E., Benoist, C., and Mathis, D. (2012). PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature *486*, 549–553. https://doi.org/10.1038/nature11132.

56. Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A.B., Benoist, C., Shoelson, S., and Mathis, D. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat. Med. *15*, 930–939. https://doi.org/10.1038/nm.2002.

57. Ito, M., Komai, K., Mise-Omata, S., Iizuka-Koga, M., Noguchi, Y., Kondo, T., Sakai, R., Matsuo, K., Nakayama, T., Yoshie, O., et al. (2019). Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. Nature *565*, 246–250. https://doi.org/10.1038/s41586-018-0824-5.

58. Kalekar, L.A., Cohen, J.N., Prevel, N., Sandoval, P.M., Mathur, A.N., Moreau, J.M., Lowe, M.M., Nosbaum, A., Wolters, P.J., Haemel, A., et al. (2019). Regulatory T cells in skin are uniquely poised to suppress profibrotic immune responses. Sci. Immunol. *4*, eaaw2910. https://doi.org/10.1126/sciimmunol.aaw2910.

59. Mathur, A.N., Zirak, B., Boothby, I.C., Tan, M., Cohen, J.N., Mauro, T.M., Mehta, P., Lowe, M.M., Abbas, A.K., Ali, N., and Rosenblum, M.D. (2019). Treg-cell control of a CXCL5-IL-17 inflammatory axis promotes hair-follicle-stem-cell differentiation during skin-barrier repair. Immunity *50*. 655.e4–667.e4. https://doi.org/10.1016/j.immuni.2019.02.013.

60. Liu, Z., Hu, X., Liang, Y., Yu, J., Li, H., Shokhirev, M.N., and Zheng, Y. (2022). Glucocorticoid signaling and regulatory T cells cooperate to maintain the hair-follicle stem-cell niche. Nat. Immunol. *23*, 1086–1097. https://doi.org/10.1038/s41590-022-01244-9.

61. Hui, S.P., Sheng, D.Z., Sugimoto, K., Gonzalez-Rajal, A., Nakagawa, S., Hesselson, D., and Kikuchi, K. (2017). Zebrafish regulatory T cells mediate organ-specific regenerative programs. Dev. Cell *43*. 659.e5–672.e5. https://doi.org/10.1016/j.devcel.2017.11.010.

62. Hsieh, C.S., Liang, Y., Tyznik, A.J., Self, S.G., Liggitt, D., and Rudensky, A.Y. (2004). Recognition of the peripheral self by naturally arising CD25+ CD4+ T cell receptors. Immunity *21*, 267–277. https://doi.org/10.1016/j.immuni.2004.07.009.

63. Pacholczyk, R., Ignatowicz, H., Kraj, P., and Ignatowicz, L. (2006). Origin and T cell receptor diversity of Foxp3+CD4+CD25+ T cells. Immunity *25*, 249–259. https://doi.org/10.1016/j.immuni.2006.05.016.

64. Wong, J., Obst, R., Correia-Neves, M., Losyev, G., Mathis, D., and Benoist, C. (2007). Adaptation of TCR repertoires to self-peptides in regulatory and nonregulatory CD4+ T cells. J. Immunol. *178*, 7032–7041. https://doi.org/10.4049/jimmunol.178.11.7032.

65. Aschenbrenner, K., D'Cruz, L.M., Vollmann, E.H., Hinterberger, M., Emmerich, J., Swee, L.K., Rolink, A., and Klein, L. (2007). Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. Nat. Immunol. *8*, 351–358. https://doi.org/10.1038/ni1444.

66. Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat. Immunol. *2*, 301–306. https://doi.org/10.1038/86302.

67. Malchow, S., Leventhal, D.S., Nishi, S., Fischer, B.I., Shen, L., Paner, G.P., Amit, A.S., Kang, C., Geddes, J.E., Allison, J.P., et al. (2013). Aire-dependent thymic development of tumor-associated regulatory T cells. Science *339*, 1219–1224. https://doi.org/10.1126/science.1233913.

68. Yang, S., Fujikado, N., Kolodin, D., Benoist, C., and Mathis, D. (2015). Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. Science *348*, 589–594. https://doi.org/10.1126/science.aaa7017.

69. Levine, A.G., Arvey, A., Jin, W., and Rudensky, A.Y. (2014). Continuous requirement for the TCR in regulatory T cell function. Nat. Immunol. *15*, 1070–1078. https://doi.org/10.1038/ni.3004.

70. Levine, A.G., Hemmers, S., Baptista, A.P., Schizas, M., Faire, M.B., Moltedo, B., Konopacki, C., Schmidt-Supprian, M., Germain, R.N., Treuting, P.M., and Rudensky, A.Y. (2017). Suppression of lethal autoimmunity by regulatory T cells with a single TCR specificity. J. Exp. Med. *214*, 609–622. https://doi.org/10.1084/jem.20161318.

71. Nishio, J., Baba, M., Atarashi, K., Tanoue, T., Negishi, H., Yanai, H., Habu, S., Hori, S., Honda, K., and Taniguchi, T. (2015). Requirement of full TCR repertoire for regulatory T cells to maintain intestinal homeostasis. Proc. Natl. Acad. Sci. USA *112*, 12770–12775. https://doi.org/10.1073/pnas.1516617112.

72. Vahl, J.C., Drees, C., Heger, K., Heink, S., Fischer, J.C., Nedjic, J., Ohkura, N., Morikawa, H., Poeck, H., Schallenberg, S., et al. (2014). Continuous T cell receptor signals maintain a functional regulatory T cell pool. Immunity *41*, 722–736. https://doi.org/10.1016/j.immuni.2014.10.012.

73. Samy, E.T., Parker, L.A., Sharp, C.P., and Tung, K.S.K. (2005). Continuous control of autoimmune disease by antigen-dependent polyclonal CD4+CD25+ regulatory T cells in the regional lymph node. J. Exp. Med. *202*, 771–781. https://doi.org/10.1084/jem.20041033.

74. Samy, E.T., Setiady, Y.Y., Ohno, K., Pramoonjago, P., Sharp, C., and Tung, K.S.K. (2006). The role of physiological self-antigen in the acquisition and maintenance of regulatory T-cell function. Immunol. Rev. *212*, 170–184. https://doi.org/10.1111/j.0105-2896.2006.00404.x.

75. Wheeler, K.M., Samy, E.T., and Tung, K.S.K. (2009). Cutting edge: normal regional lymph node enrichment of antigen-specific regulatory T cells with autoimmune disease-suppressive capacity. J. Immunol. *183*, 7635–7638. https://doi.org/10.4049/jimmunol.0804251.

76. Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J. Exp. Med. *199*, 1455–1465. https://doi.org/10.1084/jem.20040139.

77. Liu, Z., Gerner, M.Y., Van Panhuys, N., Levine, A.G., Rudensky, A.Y., and Germain, R.N. (2015). Immune homeostasis enforced by co-localized effector and regulatory T cells. Nature *528*, 225–230. https://doi.org/10.1038/nature16169.

78. Wong, H.S., Park, K., Gola, A., Baptista, A.P., Miller, C.H., Deep, D., Lou, M., Boyd, L.F., Rudensky, A.Y., Savage, P.A., et al. (2021). A local regulatory T cell feedback circuit maintains immune homeostasis by pruning self-activated T cells. Cell *184*. 3981.e22–3997.e22. https://doi.org/10.1016/j.cell.2021.05.028.

79. Akkaya, B., Oya, Y., Akkaya, M., Al Souz, J., Holstein, A.H., Kamenyeva, O., Kabat, J., Matsumura, R., Dorward, D.W., Glass, D.D., and Shevach, E.M. (2019). Regulatory T cells mediate specific suppression by depleting peptide-MHC class II from dendritic cells. Nat. Immunol. *20*, 218–231. https://doi.org/10.1038/s41590-018-0280-2.

80. Shouval, D.S., Biswas, A., Goettel, J.A., McCann, K., Conaway, E., Redhu, N.S., Mascanfroni, I.D., Al Adham, Z., Lavoie, S., Ibourk, M., et al. (2014). Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. Immunity *40*, 706–719. https://doi.org/10.1016/j.immuni.2014.03.011.

81. Zigmond, E., Bernshtein, B., Friedlander, G., Walker, C.R., Yona, S., Kim, K.-W., Brenner, O., Krauthgamer, R., Varol, C., Müller, W., and Jung, S. (2014). Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity *40*, 720–733. https://doi.org/10.1016/j.immuni.2014.03.012.

82. Laidlaw, B.J., Cui, W., Amezquita, R.A., Gray, S.M., Guan, T., Lu, Y., Kobayashi, Y., Flavell, R.A., Kleinstein, S.H., Craft, J., and Kaech, S.M. (2015). Production of IL-10 by CD4(+) regulatory T cells during the resolution of infection promotes the maturation of memory CD8(+) T cells. Nat. Immunol. *16*, 871–879. https://doi.org/10.1038/ni.3224.

83. Cho, J., Kuswanto, W., Benoist, C., and Mathis, D. (2019). T cell receptor specificity drives accumulation of a reparative population of regulatory T cells within acutely injured skeletal muscle. Proc. Natl. Acad. Sci. USA *116*, 26727–26733. https://doi.org/10.1073/pnas.1914848116.

84. Li, C., DiSpirito, J.R., Zemmour, D., Spallanzani, R.G., Kuswanto, W., Benoist, C., and Mathis, D. (2018). TCR transgenic mice reveal stepwise, multi-site acquisition of the distinctive fat-Treg phenotype. Cell *174*. 285.e12–299.e12. https://doi.org/10.1016/j.cell.2018.05.004.

85. Dikiy, S., Levine, A.G., Pritykin, Y., Krishna, C., Glasner, A., Leslie, C.S., and Rudensky, A.Y. (2022). Terminal differentiation and persistence of effector regulatory T cells essential for the prevention of intestinal inflammation. Preprint at bioRxiv. https://doi.org/10.1101/2022.05.16.492030.

86. Perry, J.A., Shallberg, L., Clark, J.T., Gullicksrud, J.A., DeLong, J.H., Douglas, B.B., Hart, A.P., Lanzar, Z., O'Dea, K., Konradt, C., et al. (2022). PD-L1-PD-1 interactions limit effector regulatory T cell populations at homeostasis and during infection. Nat. Immunol. *23*, 743–756. https://doi.org/10.1038/s41590-022-01170-w.

87. Tan, C.L., Kuchroo, J.R., Sage, P.T., Liang, D., Francisco, L.M., Buck, J., Thaker, Y.R., Zhang, Q., McArdel, S.L., Juneja, V.R., et al. (2021). PD-1 restraint of regulatory T cell suppressive activity is critical for immune tolerance. J. Exp. Med. *218*, e20182232. https://doi.org/10.1084/jem.20182232.

88. Griffith, J.W., Sokol, C.L., and Luster, A.D. (2014). Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu. Rev. Immunol. *32*, 659–702. https://doi.org/10.1146/annurev-immunol-032713-120145.

89. Huehn, J., Siegmund, K., Lehmann, J.C.U., Siewert, C., Haubold, U., Feuerer, M., Debes, G.F., Lauber, J., Frey, O., Przybylski, G.K., et al. (2004). Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like CD4+ regulatory T cells. J. Exp. Med. *199*, 303–313. https://doi.org/10.1084/jem.20031562.

90. Rapp, M., Wintergerst, M.W.M., Kunz, W.G., Vetter, V.K., Knott, M.M.L., Lisowski, D., Haubner, S., Moder, S., Thaler, R., Eiber, S., et al. (2019). CCL22 controls immunity by promoting regulatory T cell communication with dendritic cells in lymph nodes. J. Exp. Med. *216*, 1170–1181. https://doi.org/10.1084/jem.20170277.

91. Mempel, T.R., and Marangoni, F. (2019). Guidance factors orchestrating regulatory T cell positioning in tissues during development, homeostasis, and response. Immunol. Rev. *289*, 129–141. https://doi.org/10.1111/imr.12761.

92. De Simone, M., Arrigoni, A., Rossetti, G., Gruarin, P., Ranzani, V., Politano, C., Bonnal, R.J.P., Provasi, E., Sarnicola, M.L., Panzeri, I., et al. (2016). Transcriptional landscape of human tissue lymphocytes unveils uniqueness of tumor-infiltrating T regulatory cells. Immunity *45*, 1135–1147. https://doi.org/10.1016/j.immuni.2016.10.021.

93. Iellem, A., Mariani, M., Lang, R., Recalde, H., Panina-Bordignon, P., Sinigaglia, F., and D'Ambrosio, D. (2001). Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T cells. J. Exp. Med. *194*, 847–853. https://doi.org/10.1084/jem.194.6.847.

94. Zhang, N., Schröppel, B., Lal, G., Jakubzick, C., Mao, X., Chen, D., Yin, N., Jessberger, R., Ochando, J.C., Ding, Y., and Bromberg, J.S. (2009). Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. Immunity *30*, 458–469. https://doi.org/10.1016/j.immuni.2008.12.022.

95. Sather, B.D., Treuting, P., Perdue, N., Miazgowicz, M., Fontenot, J.D., Rudensky, A.Y., and Campbell, D.J. (2007). Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. J. Exp. Med. *204*, 1335–1347. https://doi.org/10.1084/jem.20070081.

96. Ishida, T., and Ueda, R. (2006). CCR4 as a novel molecular target for immunotherapy of cancer. Cancer Sci. *97*, 1139–1146. https://doi.org/10.1111/j.1349-7006.2006.00307.x.

97. Olkhanud, P.B., Baatar, D., Bodogai, M., Hakim, F., Gress, R., Anderson, R.L., Deng, J., Xu, M., Briest, S., and Biragyn, A. (2009). Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T cells. Cancer Res. *69*, 5996–6004. https://doi.org/10.1158/0008-5472.CAN-08-4619.

98. Plitas, G., Konopacki, C., Wu, K., Bos, P.D., Morrow, M., Putintseva, E.V., Chudakov, D.M., and Rudensky, A.Y. (2016). Regulatory T cells exhibit distinct features in human breast cancer. Immunity *45*, 1122–1134. https://doi.org/10.1016/j.immuni.2016.10.032.

99. Whiteside, S.K., Grant, F.M., Gyori, D.S., Conti, A.G., Imianowski, C.J., Kuo, P., Nasrallah, R., Sadiyah, F., Lira, S.A., Tacke, F., et al. (2021). CCR8 marks highly suppressive Treg cells within tumours but is dispensable for their accumulation and suppressive function. Immunology *163*, 512–520. https://doi.org/10.1111/imm.13337.

100. Villarreal, D.O., L'Huillier, A., Armington, S., Mottershead, C., Filippova, E.V., Coder, B.D., Petit, R.G., and Princiotta, M.F. (2018). Targeting CCR8 induces protective antitumor immunity and enhances vaccine-induced responses in colon cancer. Cancer Res. *78*, 5340–5348. https://doi.org/10.1158/0008-5472.CAN-18-1119.

101. Campbell, J.R., McDonald, B.R., Mesko, P.B., Siemers, N.O., Singh, P.B., Selby, M., Sproul, T.W., Korman, A.J., Vlach, L.M., Houser, J., et al. (2021). Fc-optimized anti-CCR8 antibody depletes regulatory T cells in human tumor models. Cancer Res. *81*, 2983–2994. https://doi.org/10.1158/0008-5472.CAN-20-3585.

102. Kidani, Y., Nogami, W., Yasumizu, Y., Kawashima, A., Tanaka, A., Sonoda, Y., Tona, Y., Nashiki, K., Matsumoto, R., Hagiwara, M., et al. (2022). CCR8-targeted specific depletion of clonally expanded Treg cells in tumor tissues evokes potent tumor immunity with long-lasting memory. Proc. Natl. Acad. Sci. USA. *119*. e2114282119. https://doi.org/10.1073/pnas.2114282119.

103. Mikhak, Z., Fukui, M., Farsidjani, A., Medoff, B.D., Tager, A.M., and Luster, A.D. (2009). Contribution of CCR4 and CCR8 to antigen-specific T(H)2 cell trafficking in allergic pulmonary inflammation. J. Allergy Clin. Immunol. *123*. 67.e3–73.e3. https://doi.org/10.1016/j.jaci.2008.09.049.

104. Panina-Bordignon, P., Papi, A., Mariani, M., Di Lucia, P., Casoni, G., Bellettato, C., Buonsanti, C., Miotto, D., Mapp, C., Villa, A., et al. (2001). The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. J. Clin. Invest. *107*, 1357–1364. https://doi.org/10.1172/JCI12655.

105. Zhu, J. (2015). T helper 2 (Th2) cell differentiation, type 2 innate lymphoid cell (ILC2) development and regulation of interleukin-4 (IL-4) and IL-13 production. Cytokine *75*, 14–24. https://doi.org/10.1016/j.cyto.2015.05.010.

106. Murga-Zamalloa, C., and Wilcox, R.A. (2020). GATA-3 in T-cell lymphoproliferative disorders. IUBMB Life *72*, 170–177. https://doi.org/10.1002/iub.2130.

107. Zheng, Y., Chaudhry, A., Kas, A., deRoos, P., Kim, J.M., Chu, T.-T., Corcoran, L., Treuting, P., Klein, U., and Rudensky, A.Y. (2009). Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. Nature *458*, 351–356. https://doi.org/10.1038/nature07674.

108. Wohlfert, E.A., Grainger, J.R., Bouladoux, N., Konkel, J.E., Oldenhove, G., Ribeiro, C.H., Hall, J.A., Yagi, R., Naik, S., Bhairavabhotla, R., et al. (2011). GATA3 controls Foxp3$^+$ regulatory T cell fate during inflammation in mice. J. Clin. Invest. *121*, 4503–4515. https://doi.org/10.1172/JCI57456.

109. Rudra, D., deRoos, P., Chaudhry, A., Niec, R.E., Arvey, A., Samstein, R.M., Leslie, C., Shaffer, S.A., Goodlett, D.R., and Rudensky, A.Y. (2012). Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat. Immunol. *13*, 1010–1019. https://doi.org/10.1038/ni.2402.

110. Wang, Y., Su, M.A., and Wan, Y.Y. (2011). An essential role of the transcription factor GATA-3 for the function of regulatory T cells. Immunity *35*, 337–348. https://doi.org/10.1016/j.immuni.2011.08.012.

111. Koch, M.A., Tucker-Heard, G., Perdue, N.R., Killebrew, J.R., Urdahl, K.B., and Campbell, D.J. (2009). The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat. Immunol. *10*, 595–602. https://doi.org/10.1038/ni.1731.

112. Yu, F., Sharma, S., Edwards, J., Feigenbaum, L., and Zhu, J. (2015). Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. Nat. Immunol. *16*, 197–206. https://doi.org/10.1038/ni.3053.

113. Levine, A.G., Mendoza, A., Hemmers, S., Moltedo, B., Niec, R.E., Schizas, M., Hoyos, B.E., Putintseva, E.V., Chaudhry, A., Dikiy, S., et al. (2017). Stability and function of regulatory T cells expressing the transcription factor T-bet. Nature *546*, 421–425. https://doi.org/10.1038/nature22360.

114. Tan, T.G., Mathis, D., and Benoist, C. (2016). Singular role for T-BET+CXCR3+ regulatory T cells in protection from autoimmune diabetes. Proc. Natl. Acad. Sci. USA *113*, 14103–14108. https://doi.org/10.1073/pnas.1616710113.

115. Paust, H.-J., Riedel, J.-H., Krebs, C.F., Turner, J.-E., Brix, S.R., Krohn, S., Velden, J., Wiech, T., Kaffke, A., Peters, A., et al. (2016). CXCR3+ regulatory T cells control TH1 responses in crescentic GN. J. Am. Soc. Nephrol. *27*, 1933–1942. https://doi.org/10.1681/ASN.2015020203.

116. Nosko, A., Kluger, M.A., Diefenhardt, P., Melderis, S., Wegscheid, C., Tiegs, G., Stahl, R.A.K., Panzer, U., and Steinmetz, O.M. (2017). T-bet enhances regulatory T cell fitness and directs control of Th1 responses in crescentic GN. J. Am. Soc. Nephrol. *28*, 185–196. https://doi.org/10.1681/ASN.2015070820.

117. Scharschmidt, T.C., Vasquez, K.S., Pauli, M.L., Leitner, E.G., Chu, K., Truong, H.-A., Lowe, M.M., Sanchez Rodriguez, R., Ali, N., Laszik, Z.G., et al. (2017). Commensal microbes and hair follicle morphogenesis coordinately drive Treg migration into neonatal skin. Cell Host Microbe *21*. 467.e5–477.e5. https://doi.org/10.1016/j.chom.2017.03.001.

118. Scharschmidt, T.C., Vasquez, K.S., Truong, H.-A., Gearty, S.V., Pauli, M.L., Nosbaum, A., Gratz, I.K., Otto, M., Moon, J.J., Liese, J., et al. (2015). A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. Immunity *43*, 1011–1021. https://doi.org/10.1016/j.immuni.2015.10.016.

119. Kitamura, K., Farber, J.M., and Kelsall, B.L. (2010). CCR6 marks regulatory T cells as a colon-tropic, IL-10-producing phenotype. J. Immunol. *185*, 3295–3304. https://doi.org/10.4049/jimmunol.1001156.

120. Lochner, M., Peduto, L., Cherrier, M., Sawa, S., Langa, F., Varona, R., Riethmacher, D., Si-Tahar, M., Di Santo, J.P., and Eberl, G. (2008). In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. J. Exp. Med. *205*, 1381–1393. https://doi.org/10.1084/jem.20080034.

121. Kim, S.V., Xiang, W.V., Kwak, C., Yang, Y., Lin, X.W., Ota, M., Sarpel, U., Rifkin, D.B., Xu, R., and Littman, D.R. (2013). GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. Science *340*, 1456–1459. https://doi.org/10.1126/science.1237013.

122. Suply, T., Hannedouche, S., Carte, N., Li, J., Grosshans, B., Schaefer, M., Raad, L., Beck, V., Vidal, S., Hiou-Feige, A., et al. (2017). A natural ligand for the orphan receptor GPR15 modulates lymphocyte recruitment to

epithelia. Sci. Signal. *10*, eaal0180. https://doi.org/10.1126/scisignal.aal0180.

123. Elias, S., Sharma, R., Schizas, M., Valdez, I., Rampersaud, S., Park, S.-M., Gonzalez-Figueroa, P., Li, Q.-Z., Hoyos, B., and Rudensky, A.Y. (2022). CXCR4+ Treg cells control serum IgM levels and natural IgM autoantibody production by B-1 cells in the bone marrow. J. Exp. Med. *219*, e20220047. https://doi.org/10.1084/jem.20220047.

124. Fujisaki, J., Wu, J., Carlson, A.L., Silberstein, L., Putheti, P., Larocca, R., Gao, W., Saito, T.I., Lo Celso, C., Tsuyuzaki, H., et al. (2011). In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. Nature *474*, 216–219. https://doi.org/10.1038/nature10160.

125. Sage, P.T., and Sharpe, A.H. (2020). The multifaceted functions of follicular regulatory T cells. Curr. Opin. Immunol. *67*, 68–74. https://doi.org/10.1016/j.coi.2020.10.009.

126. Crotty, S. (2014). T follicular helper cell differentiation, function, and roles in disease. Immunity *41*, 529–542. https://doi.org/10.1016/j.immuni.2014.10.004.

127. Laidlaw, B.J., Lu, Y., Amezquita, R.A., Weinstein, J.S., Vander Heiden, J.A., Gupta, N.T., Kleinstein, S.H., Kaech, S.M., and Craft, J. (2017). Interleukin-10 from CD4+ follicular regulatory T cells promotes the germinal center response. Sci. Immunol. *2*, eaan4767. https://doi.org/10.1126/sciimmunol.aan4767.

128. Gonzalez-Figueroa, P., Roco, J.A., Papa, I., Núñez Villacís, L., Stanley, M., Linterman, M.A., Dent, A., Canete, P.F., and Vinuesa, C.G. (2021). Follicular regulatory T cells produce neuritin to regulate B cells. Cell *184*. 1775.e19–1789.e19. https://doi.org/10.1016/j.cell.2021.02.027.

129. Turner, J.A., Stephen-Victor, E., Wang, S., Rivas, M.N., Abdel-Gadir, A., Harb, H., Cui, Y., Fanny, M., Charbonnier, L.-M., Fong, J.J.H., et al. (2020). Regulatory T cell-derived TGF-β1 controls multiple checkpoints governing allergy and autoimmunity. Immunity *53*. 1202.e6–1214.e6. https://doi.org/10.1016/j.immuni.2020.10.002.

130. Lu, Y., Jiang, R., Freyn, A.W., Wang, J., Strohmeier, S., Lederer, K., Locci, M., Zhao, H., Angeletti, D., O'Connor, K.C., et al. (2021). CD4+ follicular regulatory T cells optimize the influenza virus-specific B cell response. J. Exp. Med. *218*, e20200547. https://doi.org/10.1084/jem.20200547.

131. Clement, R.L., Daccache, J., Mohammed, M.T., Diallo, A., Blazar, B.R., Kuchroo, V.K., Lovitch, S.B., Sharpe, A.H., and Sage, P.T. (2019). Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses. Nat. Immunol. *20*, 1360–1371. https://doi.org/10.1038/s41590-019-0472-4.

132. Sungnak, W., Wagner, A., Kowalczyk, M.S., Bod, L., Kye, Y.-C., Sage, P.T., Sharpe, A.H., Sobel, R.A., Quintana, F.J., Rozenblatt-Rosen, O., et al. (2020). T follicular regulatory cell-derived fibrinogen-like Protein 2 regulates production of autoantibodies and induction of systemic autoimmunity. J. Immunol. *205*, 3247–3262. https://doi.org/10.4049/jimmunol.2000748.

133. Vanderleyden, I., Fra-Bido, S.C., Innocentin, S., Stebegg, M., Okkenhaug, H., Evans-Bailey, N., Pierson, W., Denton, A.E., and Linterman, M.A. (2020). Follicular regulatory T cells can access the germinal center independently of CXCR5. Cell Rep. *30*. 611.e4–619.e4. https://doi.org/10.1016/j.celrep.2019.12.076.

134. Hollister, K., Kusam, S., Wu, H., Clegg, N., Mondal, A., Sawant, D.V., and Dent, A.L. (2013). Insights into the role of Bcl6 in follicular Th cells using a new conditional mutant mouse model. J. Immunol. *191*, 3705–3711. https://doi.org/10.4049/jimmunol.1300378.

135. Wu, H., Chen, Y., Liu, H., Xu, L.-L., Teuscher, P., Wang, S., Lu, S., and Dent, A.L. (2016). Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. Eur. J. Immunol. *46*, 1152–1161. https://doi.org/10.1002/eji.201546094.

136. Sun, H., Lagarrigue, F., Wang, H., Fan, Z., Lopez-Ramirez, M.A., Chang, J.T., and Ginsberg, M.H. (2021). Distinct integrin activation pathways for effector and regulatory T cell trafficking and function. J. Exp. Med. *218*, e20201524. https://doi.org/10.1084/jem.20201524.

137. Edwards, J.P., Thornton, A.M., and Shevach, E.M. (2014). Release of active TGF-β1 from the latent TGF-β1/GARP complex on T regulatory cells is mediated by integrin β8. J. Immunol. *193*, 2843–2849. https://doi.org/10.4049/jimmunol.1401102.

138. Humphries, J.D., Byron, A., and Humphries, M.J. (2006). Integrin ligands at a glance. J. Cell Sci. *119*, 3901–3903. https://doi.org/10.1242/jcs.03098.

139. Worthington, J.J., Kelly, A., Smedley, C., Bauché, D., Campbell, S., Marie, J.C., and Travis, M.A. (2015). Integrin αvβ8-mediated TGF-β activation by effector regulatory T cells is essential for suppression of T-cell-mediated inflammation. Immunity *42*, 903–915. https://doi.org/10.1016/j.immuni.2015.04.012.

140. Wagner, N., Löhler, J., Kunkel, E.J., Ley, K., Leung, E., Krissansen, G., Rajewsky, K., and Müller, W. (1996). Critical role for beta7 integrins in formation of the gut-associated lymphoid tissue. Nature *382*, 366–370. https://doi.org/10.1038/382366a0.

141. Hadis, U., Wahl, B., Schulz, O., Hardtke-Wolenski, M., Schippers, A., Wagner, N., Müller, W., Sparwasser, T., Förster, R., and Pabst, O. (2011). Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity *34*, 237–246. https://doi.org/10.1016/j.immuni.2011.01.016.

142. Zhang, H.L., Zheng, Y.J., Pan, Y.D., Xie, C., Sun, H., Zhang, Y.H., Yuan, M.Y., Song, B.L., and Chen, J.F. (2016). Regulatory T-cell depletion in the gut caused by integrin β7 deficiency exacerbates DSS colitis by evoking aberrant innate immunity. Mucosal Immunol. *9*, 391–400. https://doi.org/10.1038/mi.2015.68.

143. Patterson, S.J., Pesenacker, A.M., Wang, A.Y., Gillies, J., Mojibian, M., Morishita, K., Tan, R., Kieffer, T.J., Verchere, C.B., Panagiotopoulos, C., and Levings, M.K. (2016). T regulatory cell chemokine production mediates pathogenic T cell attraction and suppression. J. Clin. Invest. *126*, 1039–1051. https://doi.org/10.1172/JCI83987.

144. Josefowicz, S.Z., Niec, R.E., Kim, H.Y., Treuting, P., Chinen, T., Zheng, Y., Umetsu, D.T., and Rudensky, A.Y. (2012). Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature *482*, 395–399. https://doi.org/10.1038/nature10772.

145. van der Veeken, J., Campbell, C., Pritykin, Y., Schizas, M., Verter, J., Hu, W., Wang, Z.-M., Matheis, F., Mucida, D., Charbonnier, L.-M., et al. (2022). Genetic tracing reveals transcription factor Foxp3-dependent and Foxp3-independent functionality of peripherally induced Treg cells. Immunity *55*. 1173.e7–1184.e7. https://doi.org/10.1016/j.immuni.2022.05.010.

146. Kim, K.S., Hong, S.-W., Han, D., Yi, J., Jung, J., Yang, B.-G., Lee, J.Y., Lee, M., and Surh, C.D. (2016). Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. Science *351*, 858–863. https://doi.org/10.1126/science.aac5560.

147. Lathrop, S.K., Bloom, S.M., Rao, S.M., Nutsch, K., Lio, C.-W., Santacruz, N., Peterson, D.A., Stappenbeck, T.S., and Hsieh, C.-S. (2011). Peripheral education of the immune system by colonic commensal microbiota. Nature *478*, 250–254. https://doi.org/10.1038/nature10434.

148. Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., et al. (2015). Mucosal immunology. Individual intestinal symbionts induce a distinct population of RORγ$^+$ regulatory T cells. Science *349*, 993–997. https://doi.org/10.1126/science.aaa9420.

149. Geva-Zatorsky, N., Sefik, E., Kua, L., Pasman, L., Tan, T.G., Ortiz-Lopez, A., Yanortsang, T.B., Yang, L., Jupp, R., Mathis, D., et al. (2017). Mining the human gut microbiota for immunomodulatory organisms. Cell *168*. 928.e11–943.e11. https://doi.org/10.1016/j.cell.2017.01.022.

150. Russler-Germain, E.V., Rengarajan, S., and Hsieh, C.-S. (2017). Antigen-specific regulatory T-cell responses to intestinal microbiota. Mucosal Immunol. *10*, 1375–1386. https://doi.org/10.1038/mi.2017.65.

151. Nutsch, K., Chai, J.N., Ai, T.L., Russler-Germain, E., Feehley, T., Nagler, C.R., and Hsieh, C.-S. (2016). Rapid and efficient generation of regulatory T cells to commensal antigens in the periphery. Cell Rep. *17*, 206–220. https://doi.org/10.1016/j.celrep.2016.08.092.

152. Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., and Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science *317*, 256–260. https://doi.org/10.1126/science.1145697.

153. Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., and Rudensky, A.Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451–455. https://doi.org/10.1038/nature12726.

154. Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature *504*, 446–450. https://doi.org/10.1038/nature12721.

155. Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., et al. (2011). Induction of colonic regulatory T cells by indigenous Clostridium species. Science *331*, 337–341. https://doi.org/10.1126/science.1198469.

156. Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature *500*, 232–236. https://doi.org/10.1038/nature12331.

157. Hang, S., Paik, D., Yao, L., Kim, E., Trinath, J., Lu, J., Ha, S., Nelson, B.N., Kelly, S.P., Wu, L., et al. (2019). Bile acid metabolites control TH17 and Treg cell differentiation. Nature *576*, 143–148. https://doi.org/10.1038/s41586-019-1785-z.

158. Li, W., Hang, S., Fang, Y., Bae, S., Zhang, Y., Zhang, M., Wang, G., McCurry, M.D., Bae, M., Paik, D., et al. (2021). A bacterial bile acid metabolite modulates Treg activity through the nuclear hormone receptor NR4A1. Cell Host Microbe *29*. 1366.e9–1377.e9. https://doi.org/10.1016/j.chom.2021.07.013.

159. Campbell, C., McKenney, P.T., Konstantinovsky, D., Isaeva, O.I., Schizas, M., Verter, J., Mai, C., Jin, W.-B., Guo, C.-J., Violante, S., et al. (2020). Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. Nature *581*, 475–479. https://doi.org/10.1038/s41586-020-2193-0.

160. Song, X., Sun, X., Oh, S.F., Wu, M., Zhang, Y., Zheng, W., Geva-Zatorsky, N., Jupp, R., Mathis, D., Benoist, C., and Kasper, D.L. (2020). Microbial bile acid metabolites modulate gut RORγ+ regulatory T cell homeostasis. Nature *577*, 410–415. https://doi.org/10.1038/s41586-019-1865-0.

161. Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., Glickman, J.N., and Garrett, W.S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science *341*, 569–573. https://doi.org/10.1126/science.1241165.

162. Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., Sparwasser, T., Bérard, M., Cerf-Bensussan, N., and Eberl, G. (2019). A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. Immunity *50*. 1276.e5–1288.e5. https://doi.org/10.1016/j.immuni.2019.02.014.

163. Campbell, C., Dikiy, S., Bhattarai, S.K., Chinen, T., Matheis, F., Calafiore, M., Hoyos, B., Hanash, A., Mucida, D., Bucci, V., and Rudensky, A.Y. (2018). Extrathymically generated regulatory T cells establish a niche for intestinal border-dwelling bacteria and affect physiologic metabolite balance. Immunity *48*. 1245.e9–1257.e9. https://doi.org/10.1016/j.immuni.2018.04.013.

164. Ohnmacht, C., Park, J.-H., Cording, S., Wing, J.B., Atarashi, K., Obata, Y., Gaboriau-Routhiau, V., Marques, R., Dulauroy, S., Fedoseeva, M., et al. (2015). Mucosal immunology. The microbiota regulates type 2 immunity through RORγt^{+} T cells. Science *349*, 989–993. https://doi.org/10.1126/science.aac4263.

165. Arvey, A., van der Veeken, J., Samstein, R.M., Feng, Y., Stamatoyannopoulos, J.A., and Rudensky, A.Y. (2014). Inflammation-induced repression of chromatin bound by the transcription factor Foxp3 in regulatory T cells. Nat. Immunol. *15*, 580–587. https://doi.org/10.1038/ni.2868.

166. Cretney, E., Xin, A., Shi, W., Minnich, M., Masson, F., Miasari, M., Belz, G.T., Smyth, G.K., Busslinger, M., Nutt, S.L., and Kallies, A. (2011). The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nat. Immunol. *12*, 304–311. https://doi.org/10.1038/ni.2006.

167. van der Veeken, J., Gonzalez, A.J., Cho, H., Arvey, A., Hemmers, S., Leslie, C.S., and Rudensky, A.Y. (2016). Memory of inflammation in regulatory T cells. Cell *166*, 977–990. https://doi.org/10.1016/j.cell.2016.07.006.

168. Morton, A.M., Sefik, E., Upadhyay, R., Weissleder, R., Benoist, C., and Mathis, D. (2014). Endoscopic photoconversion reveals unexpectedly broad leukocyte trafficking to and from the gut. Proc. Natl. Acad. Sci. USA *111*, 6696–6701. https://doi.org/10.1073/pnas.1405634111.

169. Fan, X., Moltedo, B., Mendoza, A., Davydov, A.N., Faire, M.B., Mazutis, L., Sharma, R., Pe'er, D., Chudakov, D.M., and Rudensky, A.Y. (2018). CD49b defines functionally mature Treg cells that survey skin and vascular tissues. J. Exp. Med. *215*, 2796–2814. https://doi.org/10.1084/jem.20181442.

170. Mehta, P., Gouirand, V., Boda, D.P., Zhang, J., Gearty, S.V., Zirak, B., Lowe, M.M., Clancy, S., Boothby, I., Mahuron, K.M., et al. (2021). Layilin anchors regulatory T cells in skin. J. Immunol. *207*, 1763–1775. https://doi.org/10.4049/jimmunol.2000970.

171. Miller, J.F. (1961). Immunological function of the thymus. Lancet *278*, 748–749. https://doi.org/10.1016/S0140-6736(61)90693-6.

172. Sakaguchi, S., and Sakaguchi, N. (1990). Thymus and autoimmunity: capacity of the normal thymus to produce pathogenic self-reactive T cells and conditions required for their induction of autoimmune disease. J. Exp. Med. *172*, 537–545. https://doi.org/10.1084/jem.172.2.537.

173. Li, M., Zhao, W., Wang, Y., Jin, L., Jin, G., Sun, X., Wang, W., Wang, K., Xu, X., Hao, J., et al. (2020). A wave of Foxp3+ regulatory T cell accumulation in the neonatal liver plays unique roles in maintaining self-tolerance. Cell. Mol. Immunol. *17*, 507–518. https://doi.org/10.1038/s41423-019-0246-9.

174. Tuncel, J., Benoist, C., and Mathis, D. (2019). T cell anergy in perinatal mice is promoted by T reg cells and prevented by IL-33. J. Exp. Med. *216*, 1328–1344. https://doi.org/10.1084/jem.20182002.

175. Lio, C.W., and Hsieh, C.-S. (2008). A two-step process for thymic regulatory T cell development. Immunity *28*, 100–111. https://doi.org/10.1016/j.immuni.2007.11.021.

176. Hill, J.A., Feuerer, M., Tash, K., Haxhinasto, S., Perez, J., Melamed, R., Mathis, D., and Benoist, C. (2007). Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity *27*, 786–800. https://doi.org/10.1016/j.immuni.2007.09.010.

177. Sugimoto, N., Oida, T., Hirota, K., Nakamura, K., Nomura, T., Uchiyama, T., and Sakaguchi, S. (2006). Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. Int. Immunol. *18*, 1197–1209. https://doi.org/10.1093/intimm/dxl060.

178. Molofsky, A.B., Van Gool, F., Liang, H.-E., Van Dyken, S.J., Nussbaum, J.C., Lee, J., Bluestone, J.A., and Locksley, R.M. (2015). Interleukin-33 and interferon-γ counter-regulate group 2 innate lymphoid cell activation during immune perturbation. Immunity *43*, 161–174. https://doi.org/10.1016/j.immuni.2015.05.019.

179. Rigas, D., Lewis, G., Aron, J.L., Wang, B., Banie, H., Sankaranarayanan, I., Galle-Treger, L., Maazi, H., Lo, R., Freeman, G.J., et al. (2017). Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. J. Allergy Clin. Immunol. *139*. 1468.e2–1477.e2. https://doi.org/10.1016/j.jaci.2016.08.034.

180. Lee, H.-M., Fleige, A., Forman, R., Cho, S., Khan, A.A., Lin, L.-L., Nguyen, D.T., O'Hara-Hall, A., Yin, Z., Hunter, C.A., et al. (2015). IFNγ signaling endows DCs with the capacity to control type I inflammation during parasitic infection through promoting T-bet+ regulatory T cells. PLoS Pathog. *11*, e1004635. https://doi.org/10.1371/journal.ppat.1004635.

181. Koch, M.A., Thomas, K.R., Perdue, N.R., Smigiel, K.S., Srivastava, S., and Campbell, D.J. (2012). T-bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor β2. Immunity *37*, 501–510. https://doi.org/10.1016/j.immuni.2012.05.031.

182. Shafiani, S., Dinh, C., Ertelt, J.M., Moguche, A.O., Siddiqui, I., Smigiel, K.S., Sharma, P., Campbell, D.J., Way, S.S., and Urdahl, K.B. (2013). Pathogen-specific Treg cells expand early during mycobacterium tuberculosis infection but are later eliminated in response to interleukin-12. Immunity *38*, 1261–1270. https://doi.org/10.1016/j.immuni.2013.06.003.

183. Lund, J.M., Hsing, L., Pham, T.T., and Rudensky, A.Y. (2008). Coordination of early protective immunity to viral infection by regulatory T cells. Science *320*, 1220–1224. https://doi.org/10.1126/science.1155209.

184. Soerens, A.G., Da Costa, A., and Lund, J.M. (2016). Regulatory T cells are essential to promote proper CD4 T-cell priming upon mucosal infection. Mucosal Immunol. *9*, 1395–1406. https://doi.org/10.1038/mi.2016.19.

185. Fulton, R.B., Meyerholz, D.K., and Varga, S.M. (2010). Foxp3+ CD4 regulatory T cells limit pulmonary immunopathology by modulating the CD8 T cell response during respiratory syncytial virus infection. J. Immunol. *185*, 2382–2392. https://doi.org/10.4049/jimmunol.1000423.

186. Ruckwardt, T.J., Bonaparte, K.L., Nason, M.C., and Graham, B.S. (2009). Regulatory T cells promote early influx of CD8+ T cells in the lungs of respiratory syncytial virus-infected mice and diminish immunodominance disparities. J. Virol. *83*, 3019–3028. https://doi.org/10.1128/JVI.00036-09.

187. Pandiyan, P., Conti, H.R., Zheng, L., Peterson, A.C., Mathern, D.R., Hernández-Santos, N., Edgerton, M., Gaffen, S.L., and Lenardo, M.J. (2011). CD4(+)CD25(+)Foxp3(+) regulatory T cells promote Th17 cells in vitro and enhance host resistance in mouse Candida albicans Th17 cell infection model. Immunity *34*, 422–434. https://doi.org/10.1016/j.immuni.2011.03.002.

188. Miragaia, R.J., Gomes, T., Chomka, A., Jardine, L., Riedel, A., Hegazy, A.N., Whibley, N., Tucci, A., Chen, X., Lindeman, I., et al. (2019). Single-cell transcriptomics of regulatory T cells reveals trajectories of tissue adaptation. Immunity *50*. 493.e7–504.e7. https://doi.org/10.1016/j.immuni.2019.01.001.

189. Delacher, M., Simon, M., Sanderink, L., Hotz-Wagenblatt, A., Wuttke, M., Schambeck, K., Schmidleithner, L., Bittner, S., Pant, A., Ritter, U., et al. (2021). Single-cell chromatin accessibility landscape identifies tissue repair program in human regulatory T cells. Immunity *54*. 702.e17–720.e17. https://doi.org/10.1016/j.immuni.2021.03.007.

190. Delacher, M., Imbusch, C.D., Hotz-Wagenblatt, A., Mallm, J.-P., Bauer, K., Simon, M., Riegel, D., Rendeiro, A.F., Bittner, S., Sanderink, L., et al. (2020). Precursors for nonlymphoid-tissue Treg cells reside in secondary lymphoid organs and are programmed by the transcription factor BATF. Immunity *52*. 295.e11–312.e11. https://doi.org/10.1016/j.immuni.2019.12.002.

191. Kolodin, D., van Panhuys, N., Li, C., Magnuson, A.M., Cipolletta, D., Miller, C.M., Wagers, A., Germain, R.N., Benoist, C., and Mathis, D. (2015). Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. Cell Metab. *21*, 543–557. https://doi.org/10.1016/j.cmet.2015.03.005.

192. Kuswanto, W., Burzyn, D., Panduro, M., Wang, K.K., Jang, Y.C., Wagers, A.J., Benoist, C., and Mathis, D. (2016). Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. Immunity *44*, 355–367. https://doi.org/10.1016/j.immuni.2016.01.009.

193. Vasanthakumar, A., Chisanga, D., Blume, J., Gloury, R., Britt, K., Henstridge, D.C., Zhan, Y., Torres, S.V., Liene, S., Collins, N., et al. (2020). Sex-specific adipose tissue imprinting of regulatory T cells. Nature *579*, 581–585. https://doi.org/10.1038/s41586-020-2040-3.

194. Chien, Y.H., Meyer, C., and Bonneville, M. (2014). γδ T cells: first line of defense and beyond. Annu. Rev. Immunol. *32*, 121–155. https://doi.org/10.1146/annurev-immunol-032713-120216.

195. Le Bourhis, L., Guerri, L., Dusseaux, M., Martin, E., Soudais, C., and Lantz, O. (2011). Mucosal-associated invariant T cells: unconventional development and function. Trends Immunol. *32*, 212–218. https://doi.org/10.1016/j.it.2011.02.005.

196. McDonald, B.D., Jabri, B., and Bendelac, A. (2018). Diverse developmental pathways of intestinal intraepithelial lymphocytes. Nat. Rev. Immunol. *18*, 514–525. https://doi.org/10.1038/s41577-018-0013-7.

197. Shissler, S.C., and Webb, T.J. (2019). The ins and outs of type I iNKT cell development. Mol. Immunol. *105*, 116–130. https://doi.org/10.1016/j.molimm.2018.09.023.

198. Green, J.A., Arpaia, N., Schizas, M., Dobrin, A., and Rudensky, A.Y. (2017). A nonimmune function of T cells in promoting lung tumor progression. J. Exp. Med. *214*, 3565–3575. https://doi.org/10.1084/jem.20170356.

199. Bettelli, E., Dastrange, M., and Oukka, M. (2005). Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. Proc. Natl. Acad. Sci. USA *102*, 5138–5143. https://doi.org/10.1073/pnas.0501675102.

200. Gavin, M.A., Rasmussen, J.P., Fontenot, J.D., Vasta, V., Manganiello, V.C., Beavo, J.A., and Rudensky, A.Y. (2007). Foxp3-dependent programme of regulatory T-cell differentiation. Nature *445*, 771–775. https://doi.org/10.1038/nature05543.

201. Marson, A., Kretschmer, K., Frampton, G.M., Jacobsen, E.S., Polansky, J.K., MacIsaac, K.D., Levine, S.S., Fraenkel, E., von Boehmer, H., and Young, R.A. (2007). Foxp3 occupancy and regulation of key target genes during T-cell stimulation. Nature *445*, 931–935. https://doi.org/10.1038/nature05478.

202. Schubert, L.A., Jeffery, E., Zhang, Y., Ramsdell, F., and Ziegler, S.F. (2001). Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. J. Biol. Chem. *276*, 37672–37679. https://doi.org/10.1074/jbc.M104521200.

203. Williams, L.M., and Rudensky, A.Y. (2007). Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat. Immunol. *8*, 277–284. https://doi.org/10.1038/ni1437.

204. van der Veeken, J., Glasner, A., Zhong, Y., Hu, W., Wang, Z.-M., Bou-Puerto, R., Charbonnier, L.-M., Chatila, T.A., Leslie, C.S., and Rudensky, A.Y. (2020). The transcription factor Foxp3 shapes regulatory T cell identity by tuning the activity of trans-acting intermediaries. Immunity *53*, 971–984.e5. https://doi.org/10.1016/j.immuni.2020.10.010.

205. Quandt, J., Arnovitz, S., Haghi, L., Woehlk, J., Mohsin, A., Okoreeh, M., Mathur, P.S., Emmanuel, A.O., Osman, A., Krishnan, M., et al. (2021). Wnt-β-catenin activation epigenetically reprograms Treg cells in inflammatory bowel disease and dysplastic progression. Nat. Immunol. *22*, 471–484. https://doi.org/10.1038/s41590-021-00889-2.

206. Sumida, T., Lincoln, M.R., Ukeje, C.M., Rodriguez, D.M., Akazawa, H., Noda, T., Naito, A.T., Komuro, I., Dominguez-Villar, M., and Hafler, D.A. (2018). Activated β-catenin in Foxp3+ regulatory T cells links inflammatory environments to autoimmunity. Nat. Immunol. *19*, 1391–1402. https://doi.org/10.1038/s41590-018-0236-6.

207. Ochoa-Repáraz, J., Rynda, A., Ascón, M.A., Yang, X., Kochetkova, I., Riccardi, C., Callis, G., Trunkle, T., and Pascual, D.W. (2008). IL-13 production by regulatory T cells protects against experimental autoimmune encephalomyelitis independently of autoantigen. J. Immunol. *181*, 954–968. https://doi.org/10.4049/jimmunol.181.2.954.

208. Overacre-Delgoffe, A.E., Chikina, M., Dadey, R.E., Yano, H., Brunazzi, E.A., Shayan, G., Horne, W., Moskovitz, J.M., Kolls, J.K., Sander, C., et al. (2017). Interferon-γ drives Treg fragility to promote anti-tumor immunity. Cell *169*. 1130.e11–1141.e11. https://doi.org/10.1016/j.cell.2017.05.005.

209. Proto, J.D., Doran, A.C., Gusarova, G., Yurdagul, A., Sozen, E., Subramanian, M., Islam, M.N., Rymond, C.C., Du, J., Hook, J., et al. (2018). Regulatory T cells promote macrophage efferocytosis during inflammation resolution. Immunity *49*. 666.e6–677.e6. https://doi.org/10.1016/j.immuni.2018.07.015.

210. Dominguez-Villar, M., Baecher-Allan, C.M., and Hafler, D.A. (2011). Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. Nat. Med. *17*, 673–675. https://doi.org/10.1038/nm.2389.

211. McClymont, S.A., Putnam, A.L., Lee, M.R., Esensten, J.H., Liu, W., Hulme, M.A., Hoffmüller, U., Baron, U., Olek, S., Bluestone, J.A., and Brusko, T.M. (2011). Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. J. Immunol. *186*, 3918–3926. https://doi.org/10.4049/jimmunol.1003099.

212. Koenecke, C., Lee, C.-W., Thamm, K., Föhse, L., Schafferus, M., Mittrücker, H.-W., Floess, S., Huehn, J., Ganser, A., Förster, R., and Prinz, I. (2012). IFN-γ production by allogeneic Foxp3+ regulatory T cells is essential for preventing experimental graft-versus-host disease. J. Immunol. *189*, 2890–2896. https://doi.org/10.4049/jimmunol.1200413.

213. Sawitzki, B., Kingsley, C.I., Oliveira, V., Karim, M., Herber, M., and Wood, K.J. (2005). IFN-gamma production by alloantigen-reactive regulatory

T cells is important for their regulatory function in vivo. J. Exp. Med. *201*, 1925–1935. https://doi.org/10.1084/jem.20050419.

214. Zappasodi, R., Serganova, I., Cohen, I.J., Maeda, M., Shindo, M., Senbabaoglu, Y., Watson, M.J., Leftin, A., Maniyar, R., Verma, S., et al. (2021). CTLA-4 blockade drives loss of Treg stability in glycolysis-low tumours. Nature *591*, 652–658. https://doi.org/10.1038/s41586-021-03326-4.

215. Zhao, J., Zhao, J., Fett, C., Trandem, K., Fleming, E., and Perlman, S. (2011). IFN-γ- and IL-10-expressing virus epitope-specific Foxp3(+) T reg cells in the central nervous system during encephalomyelitis. J. Exp. Med. *208*, 1571–1577. https://doi.org/10.1084/jem.20110236.

216. Liu, Q., Dwyer, G.K., Zhao, Y., Li, H., Mathews, L.R., Chakka, A.B., Chandran, U.R., Demetris, J.A., Alcorn, J.F., Robinson, K.M., et al. (2019). IL-33-mediated IL-13 secretion by ST2+ Tregs controls inflammation after lung injury. JCI Insight *4*, 123919. https://doi.org/10.1172/jci.insight.123919.

217. Jacobsen, J.T., Hu, W., R Castro, T.B., Solem, S., Galante, A., Lin, Z., Allon, S.J., Mesin, L., Bilate, A.M., Schiepers, A., et al. (2021). Expression of Foxp3 by T follicular helper cells in end-stage germinal centers. Science *373*, eabe5146. https://doi.org/10.1126/science.abe5146.

218. Schad, S.E., Chow, A., Mangarin, L., Pan, H., Zhang, J., Ceglia, N., Caushi, J.X., Malandro, N., Zappasodi, R., Gigoux, M., et al. (2022). Tumor-induced double positive T cells display distinct lineage commitment mechanisms and functions. J. Exp. Med. *219*, e20212169. https://doi.org/10.1084/jem.20212169.

219. Chakraborty, S., Panda, A.K., Bose, S., Roy, D., Kajal, K., Guha, D., and Sa, G. (2017). Transcriptional regulation of FOXP3 requires integrated activation of both promoter and CNS regions in tumor-induced CD8+ Treg cells. Sci. Rep. *7*, 1628. https://doi.org/10.1038/s41598-017-01788-z.

220. Kim, J.K., Klinger, M., Benjamin, J., Xiao, Y., Erle, D.J., Littman, D.R., and Killeen, N. (2009). Impact of the TCR signal on regulatory T cell homeostasis, function, and trafficking. PLoS One *4*, e6580. https://doi.org/10.1371/journal.pone.0006580.

221. Thornton, A.M., and Shevach, E.M. (1998). CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J. Exp. Med. *188*, 287–296. https://doi.org/10.1084/jem.188.2.287.

222. Yan, Z., Garg, S.K., and Banerjee, R. (2010). Regulatory T cells interfere with glutathione metabolism in dendritic cells and T cells. J. Biol. Chem. *285*, 41525–41532. https://doi.org/10.1074/jbc.M110.189944.

223. Au-Yeung, B.B., Levin, S.E., Zhang, C., Hsu, L.-Y., Cheng, D.A., Killeen, N., Shokat, K.M., and Weiss, A. (2010). A genetically selective inhibitor demonstrates a function for the kinase Zap70 in regulatory T cells independent of its catalytic activity. Nat. Immunol. *11*, 1085–1092. https://doi.org/10.1038/ni.1955.

224. Biton, M., Haber, A.L., Rogel, N., Burgin, G., Beyaz, S., Schnell, A., Ashenberg, O., Su, C.-W., Smillie, C., Shekhar, K., et al. (2018). T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell *175*. 1307.e22–1320.e22. https://doi.org/10.1016/j.cell.2018.10.008.

225. Feng, Y., van der Veeken, J., Shugay, M., Putintseva, E.V., Osmanbeyoglu, H.U., Dikiy, S., Hoyos, B.E., Moltedo, B., Hemmers, S., Treuting, P., et al. (2015). A mechanism for expansion of regulatory T-cell repertoire and its role in self-tolerance. Nature *528*, 132–136. https://doi.org/10.1038/nature16141.

226. Owen, D.L., Mahmud, S.A., Sjaastad, L.E., Williams, J.B., Spanier, J.A., Simeonov, D.R., Ruscher, R., Huang, W., Proekt, I., Miller, C.N., et al. (2019). Thymic regulatory T cells arise via two distinct developmental programs. Nat. Immunol. *20*, 195–205. https://doi.org/10.1038/s41590-018-0289-6.