

## Review

## Tissue-resident regulatory T cells: modulators of local immunity

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**Regulatory T cells ( $T_{\text{regs}}$ ) have gained renewed attention for their diverse roles beyond immune suppression. This review integrates recent discoveries on how tissue-resident  $T_{\text{regs}}$  integrate immune, metabolic, and neural cues to maintain organ homeostasis and regeneration. Across adipose tissue, intestine, brain, and skin,  $T_{\text{regs}}$  coordinate local networks that couple immune tolerance with metabolic balance and tissue repair. We further discuss therapeutic advances – including antigen-specific chimeric antigen receptor (CAR)/T cell receptor (TCR)  $T_{\text{regs}}$ , interleukin 2 (IL-2) muteins, and metabolic modulation – that aim to harness  $T_{\text{regs}}$  for treating autoimmunity and chronic inflammation. Together, these insights highlight  $T_{\text{regs}}$  as central interpreters of tissue context and as promising targets for next-generation precision immunotherapy.**

 **$T_{\text{reg}}$  cells evolving from ‘immune brakes’ to ‘tissue guardians’**

$T_{\text{reg}}$  cells represent a distinct **Forkhead box P3 (FOXP3)** (see [Glossary](#)) positive lineage within the  $CD4^+$  T cell compartment that maintains immune tolerance and homeostasis by restraining excessive immune responses [1,2]. Traditionally,  $T_{\text{regs}}$  in humans and mice comprise two main subsets based on their origin: thymus-derived  $T_{\text{regs}}$  ( $tT_{\text{regs}}$ ) and peripherally derived  $T_{\text{regs}}$  ( $pT_{\text{regs}}$ ) which develop from conventional T cells ( $T_{\text{conv}}$ ) in peripheral tissues [3]. Helios and neuropilin-1 (Nrp1) serve as markers that help distinguish  $tT_{\text{regs}}$  from  $pT_{\text{regs}}$ .  $tT_{\text{regs}}$  are stable due to complete demethylation of  $T_{\text{reg}}$ -specific demethylated region (TSDR), which secures their long-term suppressive function.  $pT_{\text{regs}}$  display partial demethylation, making them less stable and prone to conversion into effector cells. For many years, studies of  $FOXP3^+ CD4^+$  T cells focused mainly on those within **lymphoid tissues**, but recent findings reveal that  $T_{\text{reg}}$  cells in **non-lymphoid tissues** play essential roles in maintaining local immune balance and coordinating tissue-resident responses [3–5]. Tissue-resident  $T_{\text{regs}}$  not only retain classical immunosuppressive functions but also engage in non-immune functions such as tissue repair, metabolic regulation, and barrier maintenance [6–8]. The phenotypic diversity and functional specialization of tissue-resident  $T_{\text{regs}}$  across organs underscore their importance as key modulators of local immunity [9,10]. Understanding the mechanisms governing their development, maintenance, and function holds significant promise for the development of targeted immunotherapies.

Many studies have shown that tissue  $T_{\text{reg}}$  cells originate from a shared  $FOXP3^+CD4^+$  precursor population residing in lymphoid organs, where basic leucine zipper transcription factor (BATF) drives their tissue-adaptive program. After migrating into non-lymphoid sites, these precursors undergo final specialization through both common and tissue-resident transcriptional modules (Figure 1) [11,12]. Notably, tissue  $T_{\text{regs}}$  across different organs share TCR sequences, supporting transient multi-tissue migration and tissue-agnostic homing capacity [13].  $T_{\text{reg}}$  cells in non-lymphoid tissues display distinct transcriptional and functional profiles from their lymphoid counterparts, contributing not only to immune regulation but also to tissue homeostasis, repair, and regeneration (Figure 1, Table 1). In this review we recapitulate the latest findings on tissue  $T_{\text{reg}}$

## Highlights

Tissue-resident regulatory T cells ( $T_{\text{regs}}$ ) in visceral adipose tissue, skin, intestine, and brain exhibit distinct transcriptional programs and specialized functions that extend beyond immune suppression.

These tissue-resident  $T_{\text{regs}}$  integrate immune tolerance with local tissue remodeling, metabolic regulation, and neural or epithelial repair.

Brain and skin  $T_{\text{regs}}$  integrate immune and regenerative programs, coupling inflammation control with neuroprotection and epithelial renewal to sustain tissue integrity and reveal principles of context-dependent immune adaptation.

Engineered and tissue-resident  $T_{\text{regs}}$  enable precision immune modulation through cytokine, metabolic, and epigenetic reprogramming to achieve durable tolerance and repair.

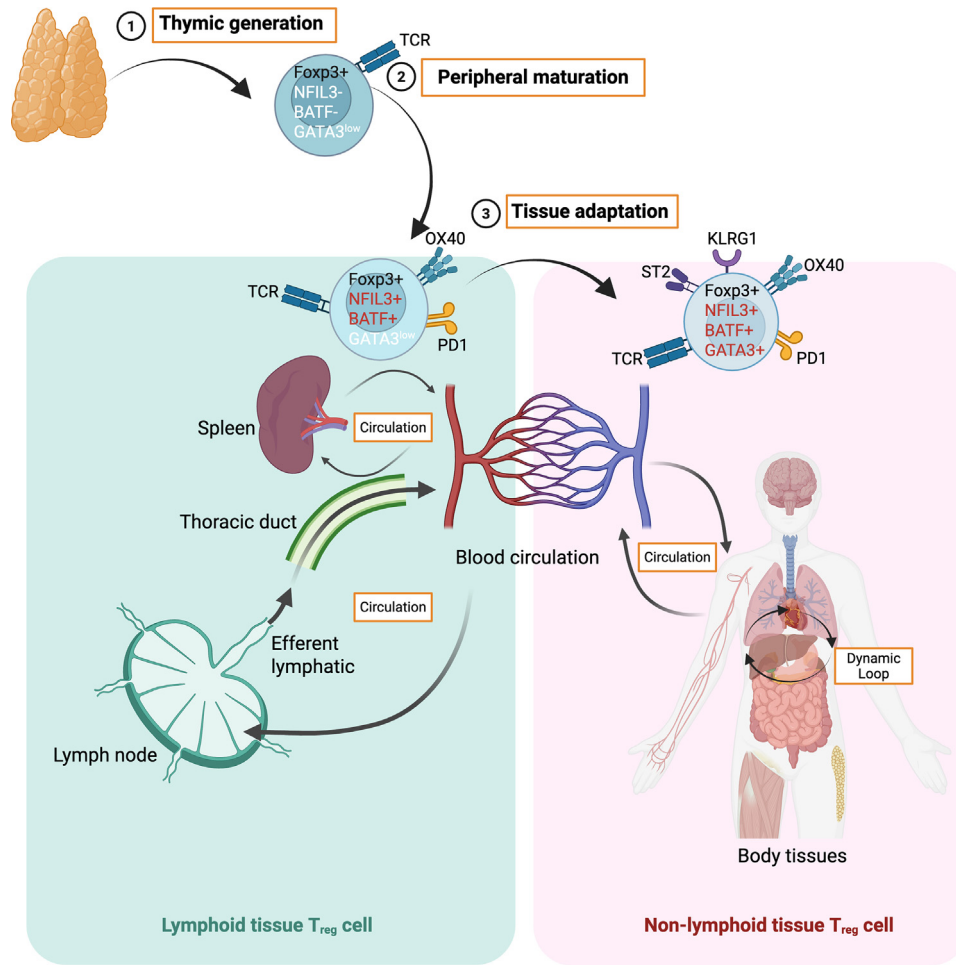
Understanding tissue-specific cues that stabilize or reprogram  $T_{\text{regs}}$  is key to developing next-generation precision  $T_{\text{reg}}$  therapies.

## Significance

Understanding how regulatory T cells ( $T_{\text{regs}}$ ) adapt to distinct tissue environments and how these properties can be harnessed therapeutically is essential for developing next-generation immunotherapies. This review integrates recent discoveries to advance this emerging field and guide future clinical translation.

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Figure 1. Stepwise differentiation and adaptation of regulatory T cells ( $T_{regs}$ ) from thymus to peripheral tissues. ①  $T_{regs}$  originate in the thymus, where FOXP3<sup>+</sup>NFIL3<sup>-</sup>BATF<sup>-</sup>GATA3<sup>°</sup> cells are generated during thymic selection. ② After exiting the thymus, they undergo peripheral maturation within secondary lymphoid organs such as the lymph nodes and spleen, expressing the transcription factors NFIL3 and BATF, gaining access to circulation through the thoracic duct and efferent lymphatic vessels. ③ Once recruited to non-lymphoid sites,  $T_{regs}$  undergo tissue adaptation, acquiring context-specific phenotypes characterized by the expression of receptors such as ST2, PD-1, OX40, and KLRG1. These signals integrate environmental cues to sustain tissue homeostasis and repair. Circulating and tissue-resident  $T_{regs}$  form a dynamic loop that maintains immune tolerance and coordinates organ-specific functions. Abbreviations: BATF, basic leucine zipper transcription factor; FOXP3, Forkhead box P3; GATA3, GATA-binding protein 3; KLRG1, killer cell lectin-like receptor subfamily G, member 1; NFIL3, nuclear factor interleukin 3; OX40, tumor necrosis factor receptor superfamily member 4; PD-1, programmed cell death protein 1; ST2, suppression of tumorigenicity 2; TCR, T cell receptor. Figure created with BioRender.

cells, focusing on their development and functions in adipose tissue, intestine, brain, and skin. We also discuss emerging therapeutic strategies that target  $T_{regs}$  or harness  $T_{regs}$  for immune regulation. Our aim is to connect tissue-resident features of  $T_{regs}$  with their therapeutic potential, offering conceptual and translational insights into immune tolerance across different organs.

Visceral adipose tissue  $T_{regs}$

Visceral adipose tissue (VAT)  $T_{regs}$  were first identified in **lean mice** as a distinct Foxp3<sup>+</sup>CD4<sup>+</sup> population [29]. In obesity models, VAT  $T_{reg}$  number decreases and accumulates in ‘crown-

Table 1. Tissue-resident T<sub>regs</sub> in different tissues<sup>a</sup>

Tissue	Important molecules	Function	Regulatory mechanisms	Refs
VAT	PPAR $\gamma$ , ST2, KLRG1	Regulation of metabolic homeostasis; control of adipose inflammation	IL-33 signaling; tissue-derived metabolic cues; secreting oncostatin M	[14,15]
Intestine	ROR $\gamma$ t, GATA3, MAF	Maintenance of mucosal tolerance; control of microbiota-induced inflammation	Limit IgA production; TGF- $\beta$ , IL-27; inhibit Th2 inflammation	[16–18]
Skeletal muscle	ST2, amphiregulin (AREG)	Promotion of tissue repair; regulation of local inflammation	IL-33–ST2 axis; interaction with ILC2s and eosinophils	[19]
Skin	GATA3, Blimp1, BATF, ST2	Immune homeostasis; hair follicle regeneration; wound healing	Jagged 1, PENK and AREG from T <sub>reg</sub> cells	[20,21]
Lung	PPAR $\gamma$ , AREG, IL-10, ST2	Maintain immune tolerance; regulate allergic inflammation; tissue protection	PPAR $\gamma$ signaling; IL-33–ST2 axis; AREG- production	[22]
CNS	ST2, AREG, IL-10, TGF- $\beta$	Control neuroinflammation; promote remyelination; support tissue repair after injury	IL-33–ST2 axis; AREG-mediated tissue repair pathways	[23,24]
Liver	IL-10, CTLA-4, TGF- $\beta$ , IL-2	Control hepatic immune tolerance; suppress autoimmune inflammation; regulate fibrosis	IL-2, TGF- $\beta$ , and local hepatic immune cues	[25]
Heart	IL-10, AREG, TGF- $\beta$	Limit post-infarct inflammation; promote cardiac repair and remodeling	IL-10, AREG; TGF- $\beta$ mediated repair pathways	[26]
Kidney	ST2, AREG, PPAR $\gamma$ , GATA	Limit acute kidney injury; promote renal repair; amelioration of renal injury	IL-33–ST2 signaling; AREG-mediated repair	[27]
Bone marrow	FOXP3, IL-10, TGF- $\beta$	Support hematopoietic stem cell niche; regulate bone marrow inflammation	IL-10, TGF- $\beta$ ; stromal niche-derived signals	[28]

<sup>a</sup>Abbreviations: BATF, basic leucine zipper transcription factor; Blimp 1, B-lymphocyte-induced maturation protein-1; CNS, central nervous system; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MAF, musculoaponeurotic fibrosarcoma oncogene homolog; PENK, proenkephalin; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; ROR $\gamma$ t, RAR-related orphan receptor gamma t; ST2, suppression of tumorigenicity 2, also called IL-33 receptor (IL1RL1); TGF, transforming growth factor; VAT, visceral adipose tissue.

like' structures at adipocyte junctions [29,30]. By contrast with non-tissue-resident T<sub>regs</sub>, VAT T<sub>regs</sub> rely on the transcription factor peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) and the cytokine IL-33 for their maintenance and differentiation. Genetic ablation of either PPAR $\gamma$  or IL-33 signaling leads to a selective loss of VAT T<sub>regs</sub>, accompanied by impaired systemic glucose metabolism [14,15,31]. By contrast, exogenous administration of IL-33 promotes the expansion of VAT T<sub>regs</sub> and ameliorates metabolic dysfunction in obese mice [15], indicating their critical function in glucose metabolism. It was recently found that suppression of tumorigenicity 2 (ST2<sup>+</sup>) VAT T<sub>regs</sub> are highly enriched in the VAT of male mice [14], whereas a newly identified population of C–X–C motif chemokine receptor 3-positive (CXCR3<sup>+</sup>) T<sub>regs</sub> are preferentially enriched in females [32]. These two subsets play opposing roles in metabolic homeostasis: ST2<sup>+</sup> T<sub>regs</sub> promote anti-inflammatory responses and improve insulin sensitivity, while CXCR3<sup>+</sup> T<sub>regs</sub> display proinflammatory properties and contribute to glucose intolerance under high-fat diet conditions [32]. ST2<sup>+</sup> T<sub>regs</sub> increase with age and adiposity, whereas CXCR3<sup>+</sup> T<sub>regs</sub> remain stable and correlate inversely with body and VAT weight [32] (Figure 2). These findings indicate that sex- and age-dependent shifts between CXCR3<sup>+</sup> and ST2<sup>+</sup> T<sub>regs</sub> subsets help regulate VAT homeostasis, reflecting their evolutionary adaptation to changing metabolic demands. Nevertheless, these

## Glossary

**Anagen:** the active growth phase of the hair cycle, when hair follicles produce new hair.

**Chimeric antigen receptor T<sub>regs</sub> (CAR-T<sub>regs</sub>):** a type of cell therapy that involves genetically engineering T<sub>reg</sub> cells to express CARs, enabling antigen-specific recognition and suppression of immune responses.

**Clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated**

**protein 9 (Cas9):** a powerful genome-editing tool derived from a bacterial immune system. CRISPR RNA guides the Cas9 endonuclease to a specific genomic sequence, enabling precise DNA cuts and allowing targeted modifications.

**Epigenetic modulation:** regulation of gene expression through DNA methylation, histone modification, and chromatin remodeling.

**Forkhead box P3 (FOXP3):** a transcription factor that defines the T<sub>reg</sub> lineage and controls its transcriptional and epigenetic programs. Stable FOXP3 expression is essential for maintaining T<sub>reg</sub> identity and suppressive function under inflammatory conditions.

**Graft-versus-host disease (GVHD):** a severe complication of allogeneic transplantation in which donor immune cells attack host tissues.

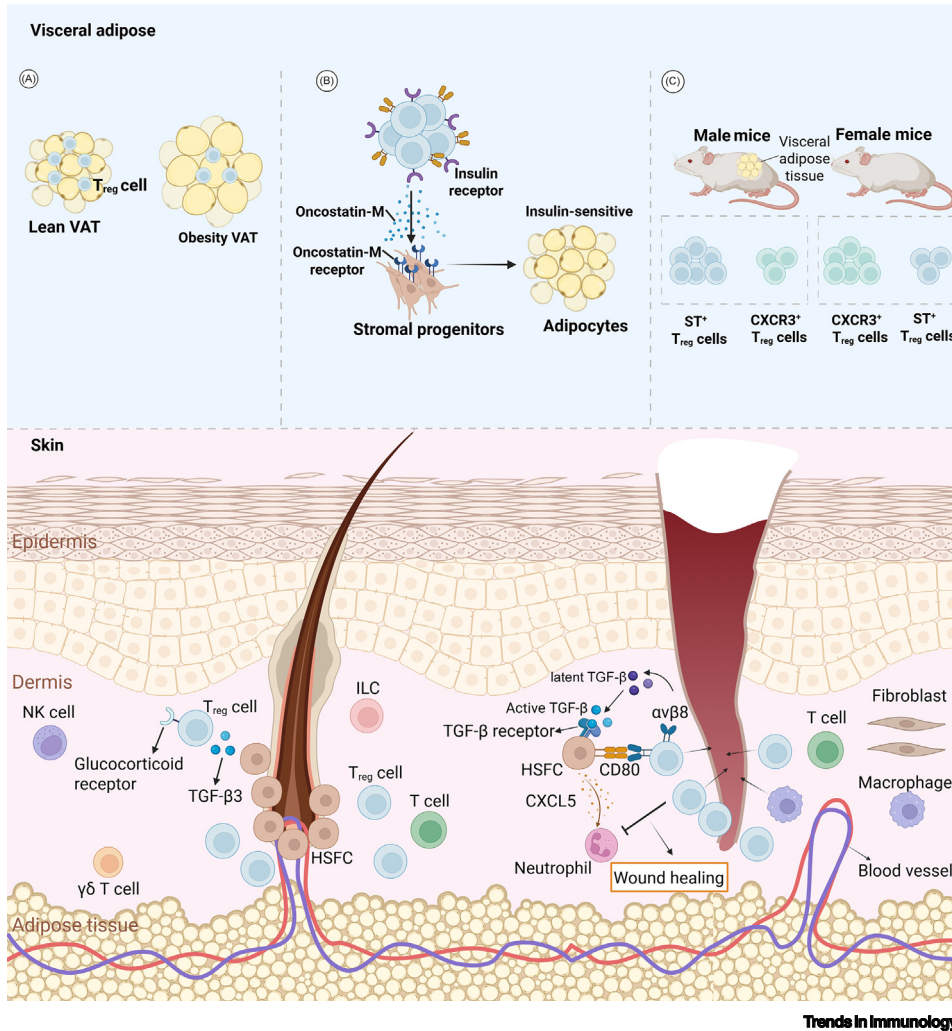
**IL-33–ST2 axis:** a signaling pathway in which the alarmin IL-33 activates ST2-expressing T<sub>regs</sub> to promote tissue repair and immune tolerance, partly through induction of AREG and other effector molecules.

**Inflammatory gliosis:** a reactive response of glial cells (mainly astrocytes and microglia) to injury or inflammation in the CNS, characterized by glial activation, proliferation, and increased expression of inflammatory markers.

**Lean mice:** mice that exhibit a low body mass and reduced adiposity, typically maintained on a regular chow diet and used as a physiological control group in metabolic studies.

**Lymphoid tissues:** tissues that support the proliferation, maturation, and activation-induced differentiation of lymphocytes: namely, the spleen, lymph nodes, and mucosa-associated lymphoid tissues.

**Metabolic reprogramming:** the adaptive remodeling of cellular metabolism to support lineage-specific function. T<sub>regs</sub> rely on oxidative



**Figure 2.** Tissue-resident regulatory T cells ( $T_{reg}$ s) integrate local cues to maintain homeostasis and repair in visceral adipose tissue (VAT) and skin.  $T_{reg}$  cells are abundant in lean VAT but diminished in obese VAT. VAT  $T_{reg}$ s maintain insulin sensitivity and metabolic balance through oncostatin-M-mediated crosstalk with stromal progenitors. Distinct subsets, such as  $ST2^+$  and  $CXCR3^+$   $T_{reg}$ s, exhibit sex-dependent differences in frequency and function. Skin:  $T_{reg}$ s accumulate around hair follicles, glucocorticoid receptor (GR) signaling in skin  $T_{reg}$ s drives transforming growth factor (TGF)- $\beta$ 3–Smad2/3 activation in hair follicle stem cells (HFSCs) to promote regeneration.  $T_{reg}$ s accumulate around site of injury, where they coordinate wound healing by interacting with HFSCs via TGF- $\beta$  and CD80–CD80L pathways, promoting epithelial regeneration and immune restraint. Abbreviations: CXCR3, C–X–C motif chemokine receptor 3; ILC, innate lymphoid cell; NK cell, natural killer cell;  $ST2$ , suppression of tumorigenicity 2. Figure created with BioRender.

findings raise several key questions for future investigation. What are the distinct roles and mechanisms of these populations? How are  $CXCR3^+$  and  $ST2^+$   $T_{reg}$ s developmentally programmed? What roles do sex hormones and aging play in shaping their balance and function?

VAT  $T_{reg}$ s help maintain tissue and metabolic homeostasis by attenuating local and systemic inflammation, thereby mitigating the risk of type 2 diabetes and metabolic syndrome [33,34]. They preserve metabolic homeostasis by secreting oncostatin M (OSM), which engages OSM receptors on preadipocytes to inhibit their differentiation, thereby restraining aberrant adipogenesis and sustaining insulin sensitivity [35] (Figure 2). Hyperinsulinemia-induced intrinsic insulin

phosphorylation and fatty acid oxidation, while metabolites such as lactate and short-chain fatty acids stabilize their suppressive phenotype.

**Microbial coating:** a process by which secretory immunoglobulins (mainly IgA) bind to the surface of commensal or pathogenic bacteria in the intestinal lumen.

**Mutins:** protein variants created by mutation, usually engineered to change or improve their biological activity, stability, or specificity.

**Nanoparticles:** engineered or naturally occurring particles ranging in size from 1 to 100 nm. In immunology, nanoparticles serve as carriers for targeted drug delivery, vaccine formulation, and adjuvant development, aiming to enhance immunogenicity or selectively deliver therapeutics to tumors.

**Non-lymphoid tissues:** peripheral tissues outside the thymus, spleen, and lymph nodes, including barrier and metabolic organs where immune cells support local homeostasis and repair.

**Umbilical-cord-blood-derived  $T_{reg}$ s (UCB- $T_{reg}$ s):**  $T_{reg}$ s isolated and expanded from umbilical cord blood, characterized by broad TCR diversity, strong lineage stability, and low alloreactivity. UCB- $T_{reg}$ s show potent suppressive and reparative functions with minimal risk of GVHD, representing a scalable and clinically promising source for cell therapy in transplantation and autoimmune disorders.

signaling in  $T_{\text{regs}}$  contributes to their dysfunction, aggravating VAT inflammation and metabolic syndrome: an effect reversed by  $T_{\text{reg}}$ -specific insulin receptor deletion [36]. In lean mice, VAT  $T_{\text{regs}}$  enhance insulin sensitivity by limiting local inflammation, whereas their loss in obesity amplifies tissue inflammation and impairs metabolic control [37] (Figure 2). Under metabolic stress, however, VAT  $T_{\text{regs}}$  can shift from protective to pathogenic roles, as BLIMP1-driven IL-10 from  $T_{\text{regs}}$  suppresses adipocyte thermogenesis and lowers energy expenditure, promoting weight gain and insulin resistance [38]. Despite growing recognition of their metabolic relevance, the precise functions of VAT  $T_{\text{regs}}$  remain poorly defined and are still a subject of ongoing research.

### Skin $T_{\text{regs}}$

#### Crosstalk between $T_{\text{regs}}$ and hair follicle stem cells

Skin  $T_{\text{regs}}$  have been recognized for over two decades, yet their distinctive properties and functions have only recently emerged. Accumulating evidence indicates that  $T_{\text{regs}}$  play active roles in hair follicle (HF) regeneration and wound repair [7,20,39]. A landmark study showed that highly activated  $T_{\text{regs}}$  preferentially accumulate around HFs during late telogen phase. As follicles transition into **anagen**, these  $T_{\text{regs}}$  become activated and produce Jagged1, which engages Notch receptors expressed on HF stem cells (HFSCs) to promote their proliferation and differentiation. However, the upstream signals that activate  $T_{\text{regs}}$  in this context remain unclear [20]. Recent work has provided a mechanistic clue, showing that steroid hormone glucocorticoids act as key initiators of the interaction between skin  $T_{\text{regs}}$  and stem cells. Glucocorticoid receptor (GR) signaling in skin  $T_{\text{regs}}$  induces transforming growth factor (TGF)- $\beta$ 3 production, which activates Smad2/3 in HFSCs and promotes their regeneration [39] (Figure 2). In addition, UVB-expanded  $T_{\text{regs}}$  express proenkephalin (PENK) and amphiregulin (AREG), both of which enhance keratinocyte growth and wound healing [21]. Moreover, PENK-producing  $T_{\text{regs}}$  were recently shown to modulate nociceptor activity, thereby restraining local inflammation and highlighting an unanticipated layer of tissue–neural crosstalk [40]. Thus, skin  $T_{\text{regs}}$  orchestrate tissue renewal by coupling stem cell activation with immune regulation and neuroimmune communication.

#### $T_{\text{regs}}$ protect HFSCs in harsh inflammatory environment

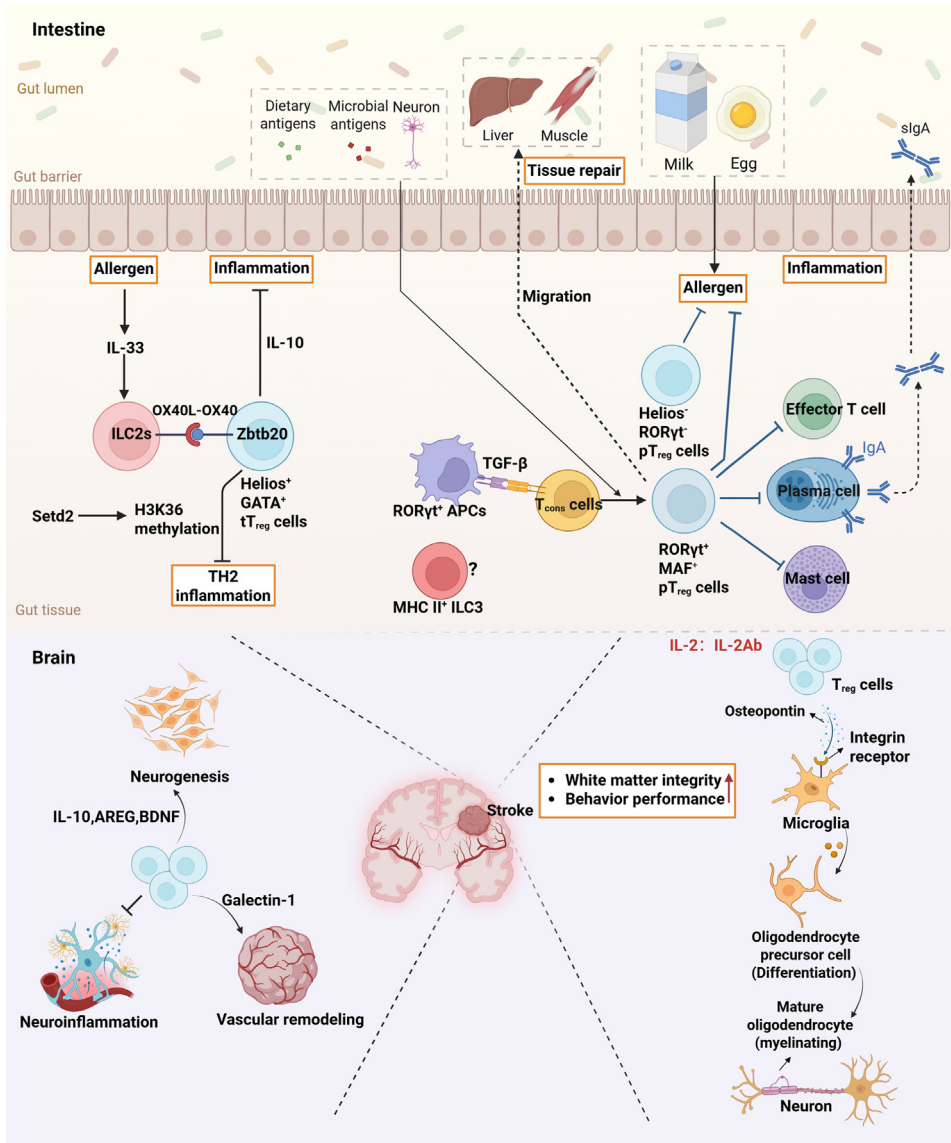
Following injury, HFSCs migrate and convert into epidermal stem cells to restore the damaged tissue, yet they encounter a hostile inflammatory milieu that can compromise their reparative capacity. To cope with this challenge, HFSCs actively coordinate local immune responses. In a mouse skin injury model, stem cells orchestrate immune responses by expanding  $pT_{\text{regs}}$  to provide protection while transiently recruiting neutrophils to generate the inflammatory milieu needed to prevent infection [41]. Mechanistically, HFSCs express CD80 to promote local  $pT_{\text{regs}}$  expansion, which restrains CXCL5–IL-17-mediated neutrophilic inflammation, thereby facilitating HFSC differentiation into epithelial cells and accelerating skin barrier repair [41,42]. Interestingly, skin  $T_{\text{regs}}$  can also play a paradoxical role when the skin barrier is disrupted. Following mechanical injury in a mouse model, skin  $T_{\text{regs}}$  expressing the integrin  $\alpha\text{v}\beta$ 8 activate latent TGF- $\beta$ , which in turn prompts epithelial cells to produce CXCL5 and recruit neutrophils, thereby promoting innate inflammation [43] (Figure 2). Although this delays re-epithelialization, it enhances protection against *Staphylococcus aureus* infection [43], indicating the important function of skin  $T_{\text{regs}}$  in balancing the critical immune responses for skin repair and clearance of infection. Under chronic inflammatory conditions, however, the stability of skin  $T_{\text{regs}}$  becomes compromised. Single-cell RNA sequencing (scRNA-seq) of chronically inflamed skin identified the polyamine-regulating enzyme spermidine/spermine  $N^1$ -acetyltransferase 1 (SSAT1) as a key determinant of  $T_{\text{reg}}$  stability, wherein 4-1BBL<sup>+</sup> keratinocytes convert  $T_{\text{regs}}$  into Th17-like, non-suppressive cells: a phenotype reversible by SSAT1 inhibition [44]. Collectively, these findings highlight the remarkable plasticity of skin  $T_{\text{regs}}$ , which can vary between regenerative, inflammatory, and dysfunctional states depending on the surrounding microenvironment.

### Intestinal T<sub>regs</sub>

Intestinal T<sub>regs</sub> comprise three main subsets: **Helios<sup>+</sup>GATA3<sup>+</sup> tT<sub>regs</sub>**, retinoic acid-related orphan receptor  $\gamma$  (ROR $\gamma$ )**t<sup>-</sup>NRP1<sup>-</sup> dietary antigen-induced pT<sub>regs</sub>**, and **ROR $\gamma$ t<sup>+</sup>MAF<sup>+</sup> microbiota-induced pT<sub>regs</sub>** [16]. Helios<sup>+</sup>GATA3<sup>+</sup> tT<sub>regs</sub> primarily restrain autoimmunity, whereas the two pT<sub>reg</sub> subsets maintain tolerance to dietary antigens and commensal microbiota [45]. The aryl hydrocarbon receptor (AHR) acts as an environmental sensor for microbial and dietary metabolites and enhances intestinal T<sub>reg</sub> generation and function through a cell-intrinsic mechanism [46]. Studies showed that AHR is preferentially expressed by intestinal pT<sub>regs</sub> and is essential for their suppressive function and gut-homing capacity [47]. Mechanistically, AHR integrates FOXP3 and enhances its binding to open chromatin sites in the *Gpr15* locus and upregulate the gut-homing receptor GPR15, thereby enabling CD4<sup>+</sup> T cells, including T<sub>regs</sub>, to migrate to the large intestine [48]. By contrast, ROR $\gamma$ t can compete with AHR binding and inhibit *GPR15* expression in T<sub>regs</sub> and Th17 cells [48]. Furthermore, recent work has demonstrated that dietary L-tryptophan is metabolized by host indoleamine 2,3-dioxygenase 1/2 (IDO1/2) into AHR-activating metabolites that selectively promote *GPR15* expression in T<sub>regs</sub>, independently of the gut microbiota [49]. By comparison, T<sub>regs</sub> in the small intestine rely on a retinoic-acid-dependent imprinting program in CD103<sup>+</sup> dendritic cells that induces the canonical CCR9–CCL25 and  $\alpha$ 4 $\beta$ 7–MAdCAM-1 homing modules, thereby guiding their selective entry into the small intestinal mucosa and supporting local tolerance [50,51]. Together, these findings highlight the AHR as a key metabolite-sensing hub that links dietary and microbial cues to intestinal T<sub>reg</sub> generation, suppressive function, and gut-homing capacity. Consequently, dietary interventions or strategies that increase the availability of AHR ligands may represent promising therapeutic approaches for restoring immune homeostasis in inflammatory bowel disease (IBD).

Tolerance to intestinal microbes depends on the conversion of naïve T cells into pT<sub>regs</sub> upon recognition of commensal antigens. However, the antigen-presenting cells (APCs) that drive this conversion remain incompletely defined. Recent studies have identified a novel **ROR $\gamma$ t<sup>+</sup> APCs** subset that can promote pT<sub>reg</sub> differentiation, but they disagree on its precise identity [52–54] (Figure 2). Conversion requires major histocompatibility complex II (MHCII), CCR7, and  $\alpha$ v $\beta$ 8 integrin-mediated TGF- $\beta$  activation [52,53]. One study reported that MHCII-expressing type 3 innate lymphocytes (ILC3s) act as APCs to generate microbiota-specific ROR $\gamma$ t<sup>+</sup> T<sub>regs</sub> [54]. By contrast, another study found that MHCII on ROR $\gamma$ t<sup>+</sup> ILC3s or classical dendritic cells (DCs) was not essential for pT<sub>reg</sub> conversion [52] (Figure 3). Thus, while the involvement of ROR $\gamma$ t<sup>+</sup> APCs is supported, their exact identity and mechanisms of action remain unresolved and require further investigation.

T<sub>regs</sub> in the intestine safeguard mucosal balance by suppressing immune reactivity to nutrients and commensals [55]. The intestinal T<sub>regs</sub> restrain T cell activation via producing anti-inflammatory cytokines such as IL-27, TGF- $\beta$ , and IL-10 [17,56]. Their deficiency exacerbates intestinal inflammation, promotes mast cell activation, and increases susceptibility to food allergy [57]. ROR $\gamma$ t<sup>+</sup> T<sub>regs</sub> also limit IgA production – loss of this subset elevates IgA<sup>+</sup> plasma cells and enhances **microbial coating** – indicating a reciprocal feedback loop between T<sub>regs</sub> and mucosal IgA responses [18] (Figure 3). Most T<sub>regs</sub> reside in the lamina propria, with a subset infiltrating the epithelium and sharing transcriptional programs with extraintestinal ROR $\gamma$ t<sup>+</sup> T<sub>regs</sub> found in the spleen and lymph nodes. Beyond gut tolerance, microbiota-dependent T<sub>regs</sub> can migrate to distant tissues, including muscle and liver, to facilitate repair after injury [58,59]. The Helios<sup>+</sup>GATA3<sup>+</sup> tT<sub>regs</sub> remain understudied. Recently, a distinct tT<sub>reg</sub> subset, characterized by *Zbtb20* expression, was found to be enriched in the gut, produce IL-10, and protect against colitis [56]. These T<sub>regs</sub> inhibit Th2 inflammation by interacting with ILC2s via OX40L–OX40 and CCL1–CCR8 signaling, while Setd2-dependent epigenetic H3K36 methylation further sustains their capacity to restrain



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Figure 3. Tissue-resident regulatory T cells ( $T_{reg}$ s) integrate local cues to maintain homeostasis and repair in intestine and brain. Intestine: Helios<sup>+</sup>GATA<sup>+</sup> t $T_{reg}$ s and gut-enriched Zbtb20<sup>+</sup> t $T_{reg}$ s produce IL-10 to suppress Th2 inflammation via OX40L–OX40 and CCL1–CCR8 signaling, supported by Setd2-dependent H3K36 methylation; Helios<sup>-</sup> RORyt<sup>+</sup>  $T_{reg}$ s in the gut prevent food allergy by maintaining oral tolerance; RORyt<sup>+</sup>MAF<sup>+</sup>  $T_{reg}$ s preserve tolerance to commensals, restrain inflammation, and regulate mast cells, IgA<sup>+</sup> plasma cells, and mucosal responses to food and vaccines. Brain: CNS-resident  $T_{reg}$ s modulate neuroinflammation, vascular remodeling, and oligodendrocyte differentiation through IL-10, AREG, and BDNF signaling;  $T_{reg}$ -derived osteopontin reprograms microglia through integrins to drive oligodendrocyte and white matter repair. Abbreviations: AREG, amphiregulin; BDNF, brain-derived neurotrophic factor; IL, interleukin; ILC2, group 2 innate lymphoid cells; ILC3, group 3 innate lymphoid cells; MAF, musculoaponeurotic fibrosarcoma oncogene homolog; MHCII, major histocompatibility complex class II; OX40, tumor necrosis factor receptor superfamily member 4; p $T_{reg}$ , peripherally derived  $T_{reg}$ ; RORy, retinoic acid-related orphan receptor  $\gamma$ ; TH2, T helper 2; t $T_{reg}$ , thymus-derived  $T_{reg}$ . Figure created with BioRender.

Th2 responses and suppress intestinal inflammation [60,61] (Figure 3). These observations underscore the functional diversity of intestinal  $T_{\text{regs}}$ , which integrate microbial, cytokine, and epigenetic signals to coordinate immune tolerance and tissue repair. Defining how distinct subsets such as  $\text{ROR}\gamma\text{t}^+\text{MAF}^+$ ,  $\text{Helios}^+\text{GATA3}^+$ , and  $\text{Zbtb20}^+ T_{\text{regs}}$  cooperate within the mucosal niche will be key to understanding how tolerance and regeneration are balanced in the gut.

#### Neurons talk, $T_{\text{regs}}$ listen: neural modulation of gut immune tolerance

The critical role of neuro-immune communication within the gut is being increasingly recognized. Researchers have applied the designer receptors exclusively activated by designer drugs (DREADDs) system to probe how intestinal neurons regulate mucosal immunity. By selective activation of defined neuronal subsets [62], it was found that  $\text{Nos1}^+$  nitrergic neurons controlled Th17-like cells,  $\text{ChAT}^+$  cholinergic neurons shaped neutrophils, and  $\text{Trpv1}^+$  nociceptors broadly reduced colonic myeloid cells and intestinal  $\text{ROR}\gamma\text{t}^+ T_{\text{regs}}$  through calcitonin gene-related protein (CGRP)–receptor activity-modifying protein 1 (RAMP1) signaling [62]. In addition, disruption of vagal signaling lowers colonic  $T_{\text{regs}}$ , particularly  $\text{ROR}\gamma\text{t}^+$  cells [63], while enteric neuron-derived IL-6 in co-cultures reduces total  $pT_{\text{regs}}$  but enriches the  $\text{ROR}\gamma\text{t}^+$  fraction [64]. These findings highlight that neuronal control of immune cells is a key and targetable intestinal immune regulation, linking sensory circuits to  $T_{\text{reg}}$  homeostasis and inflammatory susceptibility. Such insights may enable therapeutic modulation of neuronal inputs to restore mucosal tolerance in inflammatory disorders.

#### Brain $T_{\text{regs}}$

The brain, once considered immune privileged, is now known to engage in dynamic crosstalk with peripheral immune cells through a complex neuroimmune network [23,65]. Recently, parabiosis studies reveal that most  $T_{\text{regs}}$  enter the brain in an activated state, with a small fraction rapidly converting to  $\text{CD69}^+$  resident cells that persist for weeks, whereas non-resident  $T_{\text{regs}}$  remain only transiently [24]. After acquiring tissue residency, brain  $T_{\text{regs}}$  shift from primarily immunomodulatory roles to promoting neuronal survival and regeneration.

#### Immune and neuroprotective functions of brain $T_{\text{regs}}$

Beyond suppressing adaptive immune responses, brain  $T_{\text{regs}}$  contribute to neuroprotection by limiting **inflammatory gliosis** [66,67]. Recent studies have demonstrated that  $T_{\text{regs}}$  infiltrate the brain within 1–5 weeks following experimental stroke in mice. Their depletion leads to impaired oligodendrogenesis, reduced white matter regeneration, and delayed functional recovery [67]. Mechanistically,  $T_{\text{reg}}$ -derived osteopontin engages integrin receptors on microglia, enhancing their reparative activity. This reprogramming of microglia ultimately promotes oligodendrocyte differentiation and white matter repair [67]. Moreover, post-stroke expansion of  $T_{\text{regs}}$  by an IL-2–anti-IL-2 antibody complex or astrocyte-targeted IL-2 delivery has been shown to improve white matter integrity and neurological outcomes, without affecting peripheral immunity [66,67] (Figure 3). Similarly, in a Parkinson's disease mouse model, CD28 superagonist-induced  $T_{\text{reg}}$  expansion mitigates neuroinflammation and protects dopaminergic neurons [68]. In the context of aging, brain-specific IL-2 delivery expands brain  $T_{\text{regs}}$ , suppresses low-grade neuroinflammation that disrupts the neurogenic niche, and prevents neurological decline while partially restoring cognitive function in aged mice [69].

#### Neuroregenerative roles of brain $T_{\text{regs}}$

In addition to their immunoregulatory functions, emerging evidence suggests that brain  $T_{\text{regs}}$  also play a role in tissue repair. Brain  $T_{\text{regs}}$  have been shown to enhance neural stem cell proliferation and support neurogenesis, primarily through the actions of IL-10 [70], amphiregulin (AREG) [71,72], and brain-derived neurotrophic factor (BDNF) [73] (Figure 3). In addition,  $T_{\text{reg}}$ -derived

galectin-1 is implicated in promoting vascular remodeling, further supporting regenerative processes in the injured brain [74]. Myelin regeneration represents one of the most critical regenerative functions of  $T_{\text{regs}}$  in the central nervous system (CNS). Brain  $T_{\text{regs}}$  promote remyelination by directly inducing oligodendrocyte progenitor cell differentiation into myelin-producing oligodendrocytes, partly through the secretion of CCN3 [75]. However, recent findings suggest that CCN3 is not essential for remyelination *in vivo* [76]. Additional candidates such as melanoma cell adhesion molecule 1 (MCAM1) and integrin  $\alpha 2$  (ITGA2) may mediate  $T_{\text{reg}}$ -driven oligodendrocyte differentiation [77].  $T_{\text{regs}}$  also mitigate myelin damage by suppressing neuroinflammation and microglial pyroptosis through the TLR4–MyD88–nuclear factor  $\kappa B$  (NF- $\kappa B$ ) pathway [78]. These findings highlight a non-immunomodulatory, regenerative role for  $T_{\text{regs}}$  in the CNS.

Brain  $T_{\text{regs}}$  provide neuroprotection through multiple mechanisms, including suppressing adaptive immune activation, exerting direct neuroprotective effects, and promoting regeneration. Yet, the relative contributions of these pathways and their cellular interactions within the brain remain poorly understood. Most studies have focused on the acute injury phase, when increased blood–brain barrier permeability facilitates  $T_{\text{reg}}$  recruitment. Far less is known about their role in later stages, when barrier restoration limits  $T_{\text{reg}}$  entry and activity. It is unclear whether  $T_{\text{regs}}$  can still promote regeneration under these conditions or rather act remotely via secreted factors.

### Therapeutic implications of targeting tissue-resident $T_{\text{regs}}$ : strategies and challenges

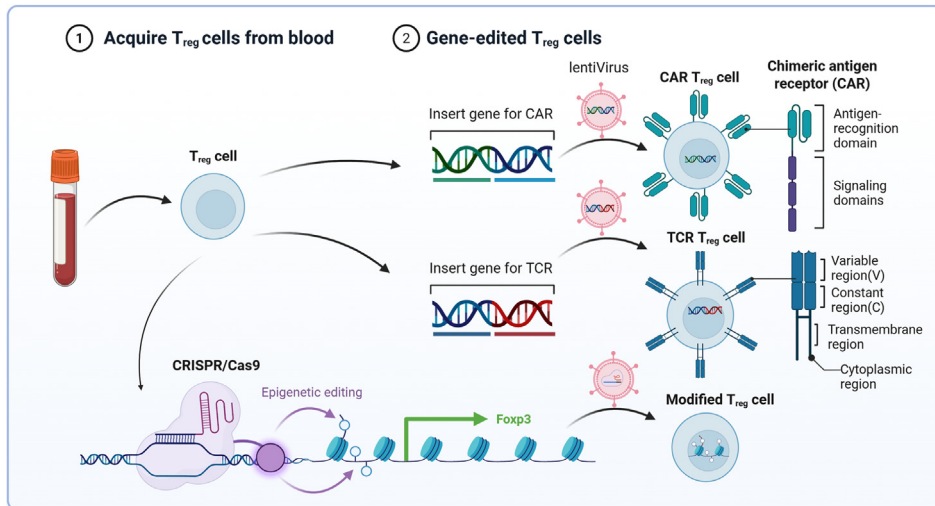
Once regarded primarily as mediators of tolerance,  $T_{\text{regs}}$  are now emerging as therapeutic targets that extend beyond classical immunosuppression. Tissue-resident  $T_{\text{regs}}$  integrate immune regulation with repair and other functions, placing them at the center of precision immunotherapy. Therapeutic strategies are shifting from nonspecific approaches such as low-dose IL-2 toward engineered cells, selective molecular modulators, and metabolic or epigenetic reprogramming (Figure 4, Table 2). These advances promise not only to treat autoimmunity and inflammation but also to enhance regeneration and recalibrate antitumor immunity.

#### Engineered $T_{\text{reg}}$ -based therapy

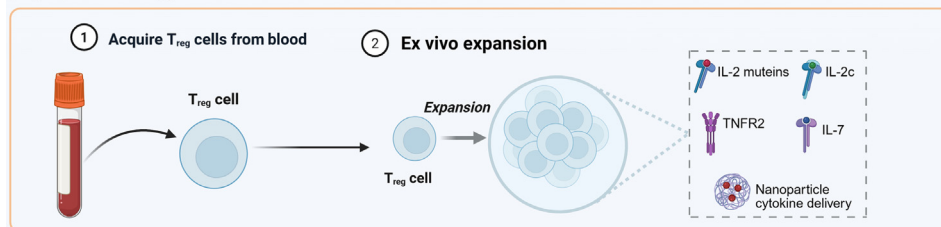
$T_{\text{regs}}$  constitute only 2–5% of peripheral  $CD4^+$  T cells, making their large-scale expansion essential for therapeutic use. Conventional bead-based enrichment yields limited purity and requires rapamycin to suppress contaminating effector T cells [79]. **Umbilical-cord-blood-derived  $T_{\text{regs}}$  (UCB- $T_{\text{regs}}$ )** provide a promising alternative, offering broader TCR diversity, greater lineage stability, and reduced risk of **graft-versus-host disease (GVHD)**; glycoengineering strategies further enhance their homing capacity. Several Phase 2 trials are now testing UCB- $T_{\text{regs}}$ , underscoring ongoing efforts to overcome the barriers of yield, purity, and sources in  $T_{\text{reg}}$ -based therapies [80,81]. Limitations in yield and purity, along with efforts to find alternative sources such as UCB, have driven clinical studies of  $T_{\text{reg}}$ -based therapies, which so far have focused mainly on autologous polyclonal  $T_{\text{reg}}$  infusions.

Over 150 clinical trials – both completed and ongoing – have established the safety and feasibility of  $T_{\text{reg}}$  therapy across autoimmune disease, transplantation, metabolic disorders, and cancer. Clinical proof-of-concept studies further underscore their potential. For example, adoptive transfer of *ex vivo* expanded donor-derived  $T_{\text{regs}}$  in children with therapy-refractory chronic GVHD resulted in clinical improvement, enhanced immune reconstitution, and long-term persistence of infused cells, with an excellent safety profile [82]. Similarly, in children with recent-onset type 1 diabetes, the combination of autologous expanded  $T_{\text{regs}}$  with the B cell-depleting antibody rituximab was superior to  $T_{\text{reg}}$  monotherapy or standard care, delaying the decline in  $\beta$ -cell function, prolonging remission, and demonstrating sustained safety over 2 years [83]. Nonetheless,

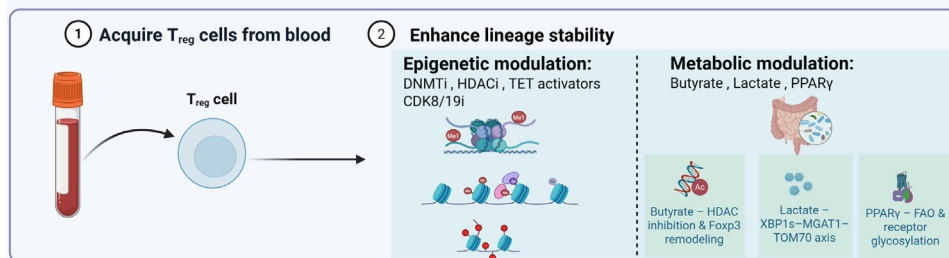
(A) T<sub>reg</sub>-cell based therapy



(B) Expansion of T<sub>reg</sub> cells



(C) Improve stability of T<sub>reg</sub> cells



Trends in Immunology

**Figure 4. Strategies for regulatory T cell (T<sub>reg</sub>)-based therapy.** (A) Engineered T<sub>reg</sub> cells. Peripheral blood T<sub>reg</sub>s are isolated and genetically modified using lentiviral or CRISPR–Cas9-based approaches. CAR- or TCR-engineered T<sub>reg</sub>s acquire antigen-specific recognition and enhanced suppressive function, while epigenetic editing reinforces stable FOXP3 expression. (B) *Ex vivo* expansion of T<sub>reg</sub> cells. Circulating T<sub>reg</sub>s are expanded *ex vivo* under optimized cytokine conditions, including IL-2 muteins, TNFR2, and IL-7 signaling, or with nanoparticle-based cytokine delivery, to increase yield and maintain suppressive phenotype before reinfusion. (C) Improving T<sub>reg</sub> stability. Epigenetic modulators (e.g., DNMT1, HDAC inhibitors, TET activators, CDK8/19 inhibitors) and metabolic regulators (e.g., butyrate, lactate, PPAR $\gamma$  agonists) enhance lineage stability by promoting FOXP3 maintenance, mitochondrial function, and receptor glycosylation. Together, these approaches aim to achieve durable, antigen-specific immune tolerance for clinical application. Abbreviations: CAR, chimeric antigen receptor; CRISPR–Cas9, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9); DNMT1, DNA (Cytosine-5)-methyltransferase 1; FOXP3, Forkhead box P3; HDAC, histone deacetylase; IL, interleukin; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; TCR, T cell receptor; TET, ten-eleven translocation methylcytosine dioxygenase; TNFR2, tumor necrosis factor receptor 2. Figure created with BioRender.

Table 2. Selected clinical trials of T<sub>reg</sub> cell-based therapies<sup>a</sup>

Cell product type	Indication	Clinical phase/trial status	Study design	Primary endpoint	Statistical methods	Patient population	SAEs	NCT number
T <sub>reg</sub>	ESRD kidney transplant recipients on everolimus	Early Phase 1 (completed)	Single-arm, open-label pilot study	Safety, feasibility	Descriptive stats	Adults 18–65 renal transplant recipients with subclinical graft inflammation	NR	NCT03284242 <sup>i</sup>
UCB-T <sub>reg</sub>	Diabetes mellitus, type 1	Phase 1/2 (recruiting)	Randomized, open-label, parallel-group trial	Safety, beta-cell function	Descriptive + exploratory	Children and adults 6–60 with type 1 diabetes	NR	NCT02932826 <sup>ii</sup>
Donor T <sub>reg</sub> cells	GVHD	Phase 1 (completed)	Single-arm, open-label prophylaxis study	Safety, acute GVHD incidence	Descriptive stats	Adults 18–70 undergoing allogeneic HSCT at risk for acute GVHD	NR	NCT01795573 <sup>iii</sup>
Purified T <sub>reg</sub> DLI	Chronic GVHD	Phase 1/2 (completed)	Single-arm, open-label dose-escalation and expansion study	Safety, preliminary efficacy	Descriptive stats	Adults ≥18 with severe refractory chronic GVHD after allogeneic HSCT	NR	NCT02749084 <sup>iv</sup>
Polyclonal T <sub>reg</sub> infusion	Post-transplant complication	Phase 1 (completed)	Single-arm, open-label study	Safety, graft inflammation	Descriptive stats	Adults ≥18 renal transplant recipients with subclinical graft inflammation	NR	NCT02088931 <sup>v</sup>
Autologous polyclonal T <sub>regs</sub> (NP001)	Amyotrophic lateral sclerosis (ALS)	Phase 1 (recruiting)	Open-label, multicenter dose-escalation study	Safety/tolerability	Descriptive stats	Adults 18–70 with neurodegenerative disorders including ALS	NR	NCT06671236 <sup>vi</sup>
Allogeneic T <sub>regs</sub>	Hematologic malignancies	Phase 1/2 (terminated)	Single-arm, open-label study	Safety, immune reconstitution	Descriptive stats	Children and adults ≤60 with hematologic malignancies undergoing haploidentical HSCT	NR	NCT01050764 <sup>vii</sup>
CD6-CAR T <sub>reg</sub>	Chronic GVHD hematologic and lymphocytic disorder	Phase 1 (suspended)	Single-arm, open-label, dose-escalation trial (3+3-style)	DLTs, RP2D	Descriptive stats	Adults ≥18 with chronic GVHD or lymphoid malignancies after allogeneic HCT	NR	NCT05993611 <sup>viii</sup>
TX200-TR101	Kidney transplant rejection/end-stage renal disease	Phase 1/2 (active, not recruiting)	Open-label, dose-escalation and expansion study	Safety, PK/PD	Descriptive stats	Adults 18–70 HLA-A*02+ living-donor kidney transplant recipients	NR	NCT04817774 <sup>ix</sup>
PolyT <sub>regs</sub> +IL2	Type 1 diabetes mellitus	Phase 1 (completed)	Open-label, single-arm dose-finding study	Safety, immune response	Descriptive stats	Adults 18–45 with type 1 diabetes	NR	NCT02772679 <sup>x</sup>
Interleukin 2	Rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, and 14 other autoimmune diseases	Phase 2 (completed)	Multi-cohort, open-label Phase 2 study	Safety, efficacy	Descriptive + exploratory	Adults ≥18 with autoimmune or autoinflammatory diseases	NR	NCT01988506 <sup>xi</sup>

(continued on next page)

Table 2. (continued)

Cell product type	Indication	Clinical phase/trial status	Study design	Primary endpoint	Statistical methods	Patient population	SAEs	NCT number
Orca-T (HSPC + T <sub>reg</sub> + T <sub>con</sub> )	Allogeneic HSCT for hematologic malignancies	Phase 3 (active, not recruiting)	Randomized, open-label, parallel-arm	GVHD-free survival, safety	Descriptive + comparative	Adults 18–65 undergoing myeloablative allogeneic HCT for hematologic malignancies	NR	NCT05316701 <sup>xii</sup>
CD-CAR T <sub>reg</sub> (SBT77101)	Rheumatoid arthritis	Phase 1 (recruiting)	Single-dose, open-label dose-escalation interventional study	Safety, tolerability, pharmacokinetics	Descriptive + exploratory	Adults 18–70 with refractory rheumatoid arthritis	NR	NCT06201416 <sup>xiii</sup>

<sup>a</sup>Abbreviations: DLI, donor lymphocyte infusion; DLTs, dose-limiting toxicities; RP2D, recommended Phase 2 dose; PK/PD, pharmacokinetics/pharmacodynamics; SAEs, serious adverse events.

polyclonal T<sub>reg</sub> products are limited by poor antigen specificity, heterogeneity, and exhaustion. Development of engineered T<sub>regs</sub> with greater stability, precision, and durability is thus critical for advancing immunotherapy [84].

Antigen-specific T<sub>regs</sub> provide greater precision by homing to diseased tissues and limiting systemic toxicity. Cellular engineering has enabled next-generation approaches, with **chimeric antigen receptor T<sub>reg</sub> cells (CAR-T<sub>regs</sub>)** showing superior efficacy over polyclonal T<sub>regs</sub> in animal models of autoimmunity, allergy, hemophilia, transplantation, and GVHD. Human leukocyte antigen (HLA)-A2–CAR-T<sub>regs</sub> have advanced into clinical trials [85], and CD19-CAR-T<sub>regs</sub> suppress autoreactive B cells in antibody-mediated disease [86]. Emerging platforms such as TCR fusion construct (TRuC)-T<sub>regs</sub>, universal CAR-T<sub>regs</sub> (UniCAR-T<sub>regs</sub>), and B cell antigen receptor (BAR)-T<sub>regs</sub> expand versatility, though challenges in tuning activation thresholds and ensuring long-term stability remain. Future iterations, including advanced CARs and TCR-like designs, promise to refine efficacy and accelerate translation. As these architectures proliferate, it has become increasingly clear that improving receptor design alone is insufficient, prompting a shift from optimizing formats to rethinking how engineered T<sub>regs</sub> acquire specificity. The UniCAR system demonstrated that antigen recognition can be modularized and reassigned through adaptor molecules rather than fixed single-chain variable fragment (scFv) domains, enabling a single CAR-T<sub>reg</sub> product to be rapidly retargeted [87]. More recently, artificial immune receptors (AIRs) employ TNF receptor-derived ligand-binding domains to sense inflammatory cues and convert them into activation signals, divorcing T<sub>reg</sub> triggering from predefined antigens [88]. These approaches shift CAR-T<sub>reg</sub> design from static antigen targeting toward programmable, microenvironment-responsive immune control.

TCR-engineered T<sub>regs</sub> harness physiological, antigen-dependent activation to accumulate inflamed or transplanted tissues and exert localized suppression. In transplantation models, TCR-T<sub>regs</sub> targeting donor MHC-II or minor histocompatibility antigens such as H-Y have effectively prevented allograft rejection and GVHD, while preserving graft-versus-leukemia activity in the H-Y setting [89]. Preclinical studies in type 1 diabetes have further demonstrated that islet-specific TCR-T<sub>regs</sub> can stably persist in pancreatic tissue and prevent disease progression, and this approach is now advancing clinically for the treatment of multiple sclerosis (ABA-101). More sophisticated multi-editing strategies – such as combining TCR transfer with *FOXP3* editing and synthetic IL-2 signaling modules (GNTI-122) – highlight the translational potential of

engineered  $T_{\text{regs}}$  to establish durable and disease-specific immune tolerance in autoimmune disorders [90]. Genome editing, particularly **clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)**, have expanded the therapeutic landscape by enabling precise manipulation of  $T_{\text{reg}}$  stability, function, and lineage integrity. Depending on disease context, CRISPR can be used to either attenuate  $T_{\text{reg}}$  activity to enhance antitumor immunity [91] or augment  $T_{\text{reg}}$  function to reinforce immune tolerance in autoimmunity and transplantation [92]. Honaker *et al.* devised a gene-editing strategy using CRISPR–Cas9 combined with homology-directed repair (CRISPR–HDR) to insert regulatory elements at the *FOXP3* locus in human  $CD4^+$  T cells, thereby generating cells that closely resemble natural  $T_{\text{regs}}$  in phenotype, transcriptome, and function [93]. These edited  $T_{\text{regs}}$  effectively suppressed effector T cell proliferation *in vitro*, alleviated inflammation in *in vivo* models, and exhibited stable, durable FOXP3 expression. Despite advances in high-fidelity Cas9 variants, nanoparticle delivery, and CRISPR screening technologies that promise safer and more efficient next-generation  $T_{\text{reg}}$  therapies, engineered  $T_{\text{regs}}$  remain prone to phenotypic drift or effector conversion under stress. Key challenges remain: how can we ensure the long-term stability and lineage fidelity of engineered  $T_{\text{regs}}$  within inflammatory or tissue-resident microenvironments? Moreover, what strategies can best enhance their persistence *in vivo*, tissue homing, and functional endurance across diverse disease contexts?

#### Expansion of endogenous $T_{\text{regs}}$

Clinical trials in systemic lupus erythematosus, rheumatoid arthritis, other autoimmune diseases, GVHD, and transplantation confirm IL-2-induced  $T_{\text{reg}}$  growth and symptomatic benefit, though efficacy is variable and limited by off-target activation of natural killer (NK) and  $CD8^+$  T cells [94,95]. To improve selectivity, IL-2 **muteins**, IL-2–anti-IL-2 immune complexes (IL-2c), and nanocarrier-based delivery platforms have been developed to preferentially expand tissue-resident  $T_{\text{regs}}$  to enhance immune tolerance [94,96]. **Nanoparticles** such as poly(lactic-co-glycolic) acid (PLGA), nanoparticles-encapsulating rapamycin (ImmTOR), and gold nanorods (AuNRs) enable targeted delivery and sustained release of IL-2, reducing off-target effector T cell activation, promoting antigen-specific  $T_{\text{reg}}$  expansion, and prolonging functional stability while lowering the dose and toxicity [97,98]. Recent work has shown that engineering  $T_{\text{regs}}$  to express an IL-2 partial agonist (IL-2pa) sustains their expansion and long-term persistence while limiting toxicity and off-target activation. Compared with wild-type IL-2 or unmodified  $T_{\text{regs}}$ , IL-2pa- $T_{\text{regs}}$  display superior durability and functional stability, effectively restrain autoimmune pathology in murine models, and control GVHD in humanized settings, highlighting a promising strategy to overcome key bottlenecks in  $T_{\text{reg}}$ -based therapies for autoimmune disease [99].

Beyond IL-2, tissue-resident  $T_{\text{regs}}$  display distinct cytokine responsiveness in context-dependent ways. IL-33 potently induces  $ST2^+$   $T_{\text{regs}}$  across adipose tissue, muscle, lung, skin, and kidney, where it activates repair programs and drives AREG and other mediators [100]. AREG, an IL-33-induced effector molecule, supports regeneration but also contributes to fibrosis, depending on context [101]. The IL-33– $T_{\text{reg}}$  axis thus emerges as a critical circuit for maintaining epithelial integrity while restraining fibrotic remodeling [102].  $T_{\text{reg}}$  cell-specific deletion of the IL-33 receptor *ST2* exacerbates defects in muscle repair, whereas exogenous supplementation of IL-33 restores  $T_{\text{reg}}$  accumulation and significantly promotes muscle regeneration in aged mice (**IL-33–*ST2* axis**) [103]. IL-7 sustains naïve and tissue  $T_{\text{regs}}$  during lymphopenia or regeneration, promotes persistence via **metabolic reprogramming**, and is essential for adipose tissue  $T_{\text{reg}}$  maintenance; it has also been used to optimize autologous  $T_{\text{reg}}$  products [104]. A tumor necrosis factor receptor 2 (TNFR2)-selective TNF- $\alpha$  mutein Fc-fusion protein robustly expanded murine and human  $T_{\text{regs}}$ , enhancing suppressive function and mitigating inflammation in hypersensitivity and arthritis models [105]. Similarly, agonists of TNFR2, glucocorticoid-Induced TNFR-related protein

(GITR), and death receptor 3 (DR3) each promoted  $T_{reg}$  expansion and protection in GVHD, though with distinct kinetics: DR3 agonists induced rapid but transient effects, TNFR2 agonists induced slower but sustained expansion, and GITR agonists provide early survival benefits but with late inflammatory rebound [106]. Collectively, cytokine pathways act as a rheostat for tissue  $T_{regs}$  and their rational manipulation – via systemic cytokines, engineered complexes, or local delivery – offers promising strategies for next-generation immunotherapies.

### Ensuring $T_{reg}$ stability

The success of  $T_{reg}$  therapy hinges on maintaining lineage stability and durable suppressive function *in vivo*. Both recent inducible FOXP3-degradation studies highlight that the requirement for FOXP3 is temporal and context-dependent: mature  $T_{regs}$  can transiently function without FOXP3 in the steady state but rely heavily on continuous FOXP3 expression during inflammation or in the tumor microenvironment [107,108]. However,  $iT_{regs}$  often lack FOXP3 locus hypomethylation and are prone to conversion into pathogenic effectors under inflammation [109]. Different strategies are used to enforce FOXP3 expression and stability, including targeted **epigenetic modulation** of FOXP3 promoters and super-enhancers through inhibition of epigenetic enzyme DNA methyltransferase (DNMT) or histone deacetylase (HDAC), dCas9-fused transcriptional activators, and vitamin C-mediated activation of ten-eleven translocation (TET) enzymes [110–112]. Additional approaches aim to protect FOXP3 from degradation by inhibiting its negative regulators – such as DBC1, Stub1, or Janus kinase 2 (JAK2) – or by enhancing the activity of stabilizing deubiquitinases such as USP7 [113]. Moreover, epigenetic reprogramming induced by TGF- $\beta$  stimulation or pharmacological inhibition of CDK8/19 has been demonstrated to convert conventional T cells into functional FOXP3<sup>+</sup>  $T_{regs}$  with therapeutic benefit across multiple autoimmune settings [114]. Together, these multilayered strategies provide a framework for ensuring durable  $T_{reg}$  stability in immunotherapy.

Metabolites critically shape  $T_{reg}$  stability. Butyrate suppresses Th17 differentiation and promotes  $T_{reg}$  induction by inhibiting HDACs and remodeling FOXP3/RORC loci, an effect enhanced and stabilized by butyrate-loaded liposomes [115]. Lactate augments human  $T_{reg}$  oxidative phosphorylation, proliferation, and suppressive activity via an XBP1s–MGAT1–TOM70 axis that drives mitochondrial N-glycosylation; MGAT1 knockdown abrogates these benefits, underscoring this pathway as a therapeutic target [116]. PPAR $\gamma$  activation promotes fatty acid oxidation while enhancing N-linked glycosylation of TGF- $\beta$ RII and IL-2R $\alpha$ , thereby sustaining FOXP3 expression and reinforcing TGF- $\beta$ –Smad and IL-2–STAT5 signaling [117]. Together, these pathways sustain suppressive programs (FOXP3, IL-10, CTLA4, TIGIT) and preserve  $T_{reg}$  identity, highlighting metabolism–glycosylation crosstalk as a therapeutic axis in autoimmunity and inflammation.

### Concluding remarks

The 2025 Nobel Prize in Physiology or Medicine, awarded for the discovery of  $T_{regs}$ , marks a milestone in modern immunology and underscores their central role in maintaining immune balance and tissue health. As highlighted throughout this review, tissue-resident  $T_{regs}$  serve as pivotal mediators linking immune regulation with tissue integrity and repair. Despite recent progress, several unresolved issues require further investigation (see [Outstanding questions](#)). A key priority for the field is to understand how  $T_{regs}$  interpret signals from their tissue environment – including immune mediators, metabolic factors, and neural inputs – and translate them into context-dependent programs that sustain homeostasis and drive repair. Meeting this challenge requires uncovering the molecular mechanisms that confer organ-adapted identities and regenerative potential. Defining how tissues instruct  $T_{regs}$ , whether through epigenetic reprogramming, metabolic regulation, or signal pathways such as AHR and IL-33–ST2, and determining whether these processes can be engineered will inform the design of next-generation  $T_{regs}$  capable of navigating distinct niches

### Outstanding questions

Although the origin of tissue-resident  $T_{regs}$  has been well characterized in mice under steady-state conditions, do human tissue-resident  $T_{regs}$  also arise from lymphoid precursors and undergo similar phenotypic transitions? How does inflammation reshape their biological properties and functional identity within tissues?

What drives the differentiation and maintenance of tissue-resident  $T_{regs}$ ? How do their functions vary across tissue and disease states? What are the prospects for targeting these cells in immunology?

What mechanisms underlie the selective loss of VAT  $T_{regs}$  during obesity, while  $T_{reg}$  abundance in lean mice remains stable? Which adipose-tissue-derived signals (e.g., cytokines, lipids, or metabolites) drive the reduction of  $T_{regs}$  under conditions of obesity? What are the phenotypic and functional characteristics of  $T_{regs}$  residing in brown and beige adipose tissues (BATs)? Do  $T_{regs}$  in BAT and beige fat share regulatory pathways with VAT  $T_{regs}$ , or do they adopt unique transcriptional and metabolic profiles?

What strategies will enhance  $T_{reg}$  *in vivo* persistence, tissue homing, and metabolic fitness across disease contexts? How can scalable manufacturing, built-in safety switches, and integration within endogenous immune networks be optimized to enable durable and clinically translatable tolerance therapies?

How do brain and skin  $T_{regs}$  sense and integrate neural, stromal, and immune signals to couple inflammation control with regeneration across distinct barrier environments? What local antigenic or metabolic cues endow them with neuroprotective or pro-regenerative functions, and do  $T_{reg}$ -derived mediators – such as osteopontin, amphiregulin, and IL-10 – act directly on stem cells or indirectly via microglia, fibroblasts, and keratinocytes?

What mechanisms drive  $T_{reg}$  instability and proinflammatory conversion in chronic neuroinflammation or barrier disruption, and how can these processes be restrained? Can  $T_{regs}$  be selectively engineered or delivered

and executing reparative functions with precision. Emerging technologies, including multi-omics analysis, CRISPR screens, and single-cell profiling, provide promising avenues to advance  $T_{reg}$  research.

to the brain and skin to promote lasting tissue regeneration without compromising immune defense?

Therapeutically, recent advances in the clinical translation of  $T_{regs}$  – including cytokine engineering (e.g., IL-2 muteins), antigen-specific cellular therapies (CAR- or TCR- $T_{regs}$ ), and metabolic reprogramming – hold great promise for restoring tolerance in autoimmunity and tissue injury. Yet key challenges remain in sustaining  $T_{reg}$  stability and functional precision in inflammatory microenvironments. Future studies combining epigenetic stabilization, metabolic rewiring, and clinical translation will further delineate how  $T_{regs}$  integrate systemic and local signals, paving the way for next-generation precision  $T_{reg}$  therapies.

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### Declaration of interests

The authors have no potential conflicts of interest to declare.

### Resources

<sup>i</sup><https://clinicaltrials.gov/study/NCT03284242>

<sup>ii</sup><https://clinicaltrials.gov/study/NCT02932826>

<sup>iii</sup><https://clinicaltrials.gov/study/NCT01795573>

<sup>iv</sup><https://clinicaltrials.gov/study/NCT02749084>

<sup>v</sup><https://clinicaltrials.gov/study/NCT02088931>

<sup>vi</sup><https://clinicaltrials.gov/study/NCT06671236>

<sup>vii</sup><https://clinicaltrials.gov/study/NCT01050764>

<sup>viii</sup><https://clinicaltrials.gov/study/NCT05993611>

<sup>ix</sup><https://clinicaltrials.gov/study/NCT04817774>

<sup>x</sup><https://clinicaltrials.gov/study/NCT02772679>

<sup>xi</sup><https://clinicaltrials.gov/study/NCT01988506>

<sup>xii</sup><https://clinicaltrials.gov/study/NCT05316701>

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