REVIEW

Treg–tissue cell interactions in repair and regeneration

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Regulatory T (Treg) cells are classically known for their critical immunosuppressive functions that support peripheral tolerance. More recent work has demonstrated that Treg cells produce pro-repair mediators independent of their immunosuppressive function, a process that is critical to repair and regeneration in response to numerous tissue insults. These factors act on resident parenchymal and structural cells to initiate repair in a tissue-specific context. This review examines interactions between Treg cells and tissue-resident non-immune cells—in the context of tissue repair, fibrosis, and cancer—and discusses areas for future exploration.

Introduction

Regulatory T (Treg) cells are a specialized CD4+ T cell subset first identified for their critical immunosuppressive functions (Josefowicz et al., 2012; Sakaguchi et al., 2020). The absence of Treg cells, most definitively seen in Scurfy mice, results in uncontrolled inflammation, tissue damage, and death—a phenotype that can be rescued by the transfer of CD4+ T cells overexpressing Foxp3 (Fontenot et al., 2003; Hori et al., 2003). The demonstration of Foxp3 as the lineage-defining transcription factor of Treg cells resulted in a close study of the critical roles that Treg cells play in maintaining immunological tolerance to self-antigens and to innocuous environmental antigens such as food and commensal bacteria (Josefowicz et al., 2012). Extensive study has identified the immunosuppressive mediators—and the mechanisms through which they act—that enable Treg cell function, including cytokines such as IL-10 and TGF-β, cell surface proteins such as CTLA-4, sequestration of the critical T cell cytokine IL-2 through constitutive CD25 expression, and microenvironmental metabolic changes, such as conversion of proinflammatory ATP to anti-inflammatory AMP via expression of CD73 and CD39, among other processes (Dikiy and Rudensky, 2023; Josefowicz et al., 2012). The effect of these mediators on other immune cells, including macrophages, dendritic cells, and T cells, represents the canonical function of Treg cells and remains under intense investigation (Dikiy and Rudensky, 2023).

Beyond their effect on modulating immune responses, more recent work has shown that Treg cells can play critical roles in tissue repair and regeneration. This can occur via protection of tissue stem cell niches from inflammatory activity (Fujisaki et al., 2011) or by direct signaling to non-immune tissue cells and/or stem cells, which is the subject of this review (Fig. 1 A). Critically, the production of non-immunomodulatory mediators is distinct from Treg cell immunosuppressive function (Arpaia et al., 2015). This suggests a model in which Treg cells promote tissue repair and regeneration through distinct modalities; namely, indirectly by limiting collateral tissue damage through immunosuppressive molecules that act on immune cells, and directly through the production of tissue factors that are sensed by mesenchymal, epithelial, and endothelial cells (Fig. 1 A). In most damage resolution scenarios, both of these mechanisms of action are likely at play; however, many of the reports we review herein effectively isolate these individual processes through elegant experimentation. Crucially, the sensing of Treg cell–derived mediators by specialized tissue-resident non-immune cells allows a common input to generate a context- and tissue-specific program that is unique and critical for regeneration and repair (Fig. 1 B). In this review, we will discuss the interplay of Treg cells with tissue-specific parenchymal and structural cells and their emerging role in tissue protection.

Treg cell–tissue cell intercommunication in tissue regeneration

Several recent reviews discuss the roles of Treg cells during homeostasis and disease in various non-lymphoid tissue sites (Astarita et al., 2023; Boothby et al., 2020; Estrada Brull et al., 2022; Jovisic et al., 2023; Panduro et al., 2016; Zhang et al., 2017); however, none have focused specifically on how Treg cells communicate with other tissue cell types. Thus, herein we review existing literature reporting interactions between Treg cells and epithelial, mesenchymal, or endothelial cells (hereafter referred to as "tissue cells") that mediate regeneration (Fig. 2 A),

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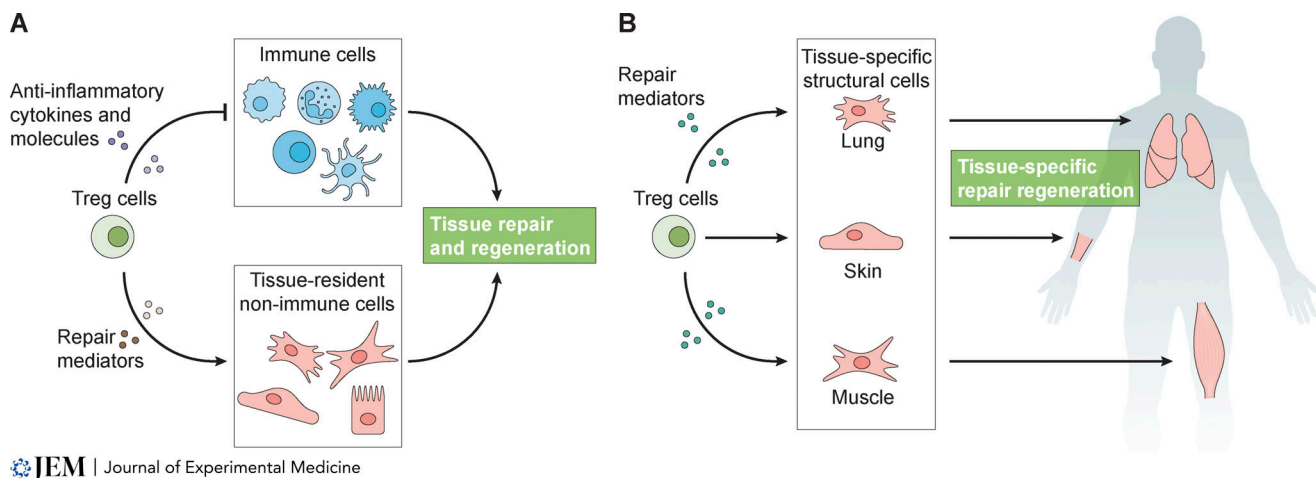
Figure 1. **Function of Treg cells in tissue repair. (A)** Treg cells mediate tissue repair and regeneration via the production of anti-inflammatory molecules that reduce inflammation (top) and through the production of mediators that influence tissue-resident non-immune cells (bottom)—a function that is distinct from immunosuppression. **(B)** Treg cell–derived mediators are sensed by unique tissue-specific parenchymal and structural cells, which generate a tissue- and context-specific repair program.

as well as the tissue cell–derived signals that modulate Treg cell repair activity (Fig. 2 B).

Treg cell–epithelial cell interactions

There have been several reports investigating Treg cell interactions with epithelial cells across multiple organ systems and in several disease states. We first discuss these examples in the context of experimental lung disease.

A seminal report by the King group (D'Alessio et al., 2009) is the first to our knowledge indicating that Treg cells may promote tissue reparative processes. The authors observed deficient resolution of tissue damage following acute lung injury (ALI) in the absence of lymphocytes, a phenotype that could be rescued by adoptive transfer of CD4+CD25+ splenic T cells, but not other

T cell subsets; however, this was attributed to Treg cell–mediated effects on alveolar macrophages, not to their direct action on tissue cells. In a follow-up study, the authors imply that lung Treg cells reduce the production of CXCL12 by lung epithelial cells (LECs) (Garibaldi et al., 2013).

Additional reports by this group demonstrated direct Treg cell–LEC communication in this context. One such report explored the role of CD103 (Itgae, an integrin receptor for E-cadherin) expression by lung Treg cells (Mock et al., 2014). In this study, the proliferation of E-cadherin+ LECs was shown to correlate with an increase in CD103+ Treg cells in the lung following ALI, and LEC proliferation was decreased upon Treg cell depletion. This was also tested in a Treg cell–replete environment by adoptively transferring wild type or Itgae−/− Treg cells into
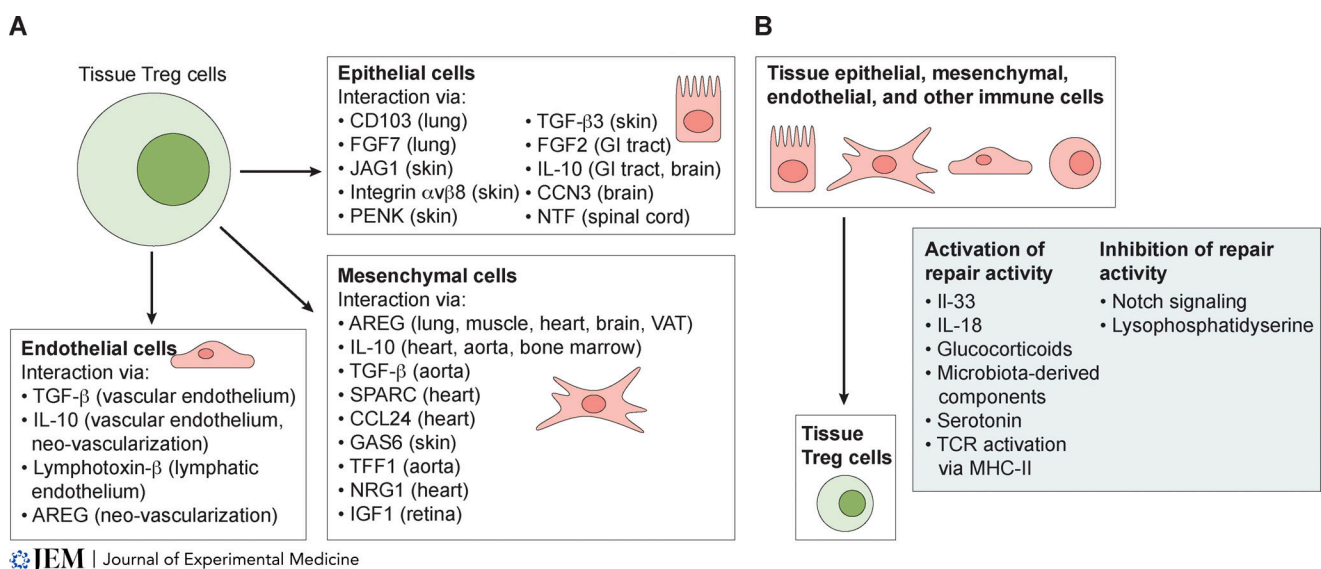

Figure 2. **Mediators of Treg cell interactions with tissue cells. (A)** Treg cell–derived factors shown to mediate communication with each type of tissue cell, as well as the organ(s) where these effects were studied. **(B)** Tissue-derived mediators shown to influence Treg cell reparative programs.

$Rag1^{-/-}$ mice, showing decreased LEC proliferation in the latter. In a later report, it was found that Treg cell–derived FGF7 (KGF) promotes LEC proliferation in both the LPS ALI and pneumectomy contexts (Dial et al., 2017). These reports also showed that $Itgae^{-/-}$ and $Fgf7^{-/-}$ Treg cells are deficient in directly augmenting LEC proliferation in vitro. Additional studies demonstrated that this function of Treg cells in the setting of lung pathology can be altered by the inhibition of DNA methyltransferases or by using Treg cells from aged mice; however, the authors interpreted these findings as being due to intrinsic alterations in Treg cell function rather than effects on direct Treg cell–tissue cell interaction (Morales-Nebreda et al., 2021; Singer et al., 2015).

The study of Treg cell–epithelial cell interactions in the skin has been particularly extensive. One such study investigated the role of Treg cells in wound healing using a skin punch biopsy mouse model (Nosbaum et al., 2016). In this setting, activated Treg cells accumulate in wounded skin and Treg cell depletion prevented proper wound healing, which the authors attributed to the lack of Treg cell–mediated immunosuppression rather than direct interaction with tissue cells. Another report by this group utilized models of hair regrowth to investigate Treg cell interactions with hair follicle epithelial stem cells (HFSCs) (Ali et al., 2017). In this study, tissue-resident Treg cells of an activated phenotype were enriched during the anagen (follicle regrowth) phase of hair growth compared with the telogen (follicle quiescence) phase. Furthermore, Treg cell depletion impaired hair regrowth in a depilation model, with reduced HFSC proliferation in Treg cell–depleted mice likely being the culprit for this failure to regrow hair. Importantly, immunosuppression was not altered in this context, and similar to observations in the lung, Treg cells appear to spatially colocalize with HFSCs. The authors identified Notch ligand JAG1 as a critical Treg cell–derived mediator of HFSC proliferation, which they confirmed through the generation and experimental use of Treg cell–specific $Jag1$ knockout mice.

Another set of reports by this group focused on the Treg cell–keratinocyte signaling axis. Mathur et al. (2019) used a subacute skin injury model to investigate hair follicle regeneration. In this model, Treg cells repressed CXCL5 production by keratinocytes, limiting the recruitment of inflammatory Th17 cells and neutrophils to promote healing. A later report from the same group (Moreau et al., 2021) found that Treg cell expression of αvβ8 integrin activates latent TGF-β in the surrounding tissue microenvironment, which subsequently signals to keratinocytes to induce CXCL5 production. This in turn promotes the recruitment of Th17 cells and neutrophils that inhibit regeneration. Despite the seemingly conflicting nature of these reports, the authors argue that both mechanisms are likely involved in the proper adjustment of the immune response in various contexts.

Evidence from other groups substantiates the role of Treg cell interactions with tissue cells in the skin. UVB irradiation of skin results in an increase in activated, clonally expanded Treg cells, and UVB-mediated acceleration of wound healing (using the punch biopsy model) is dependent on the presence of Treg cells (Shime et al., 2020). The authors experimentally attribute these effects to UVB-induced Treg cell expression of opioid precursor proenkephalin (Penk), a novel molecule in the tissue repair

context. In another report, the authors found that skin Treg cells require glucocorticoid signaling to mediate HFSC proliferation and direct hair growth (Liu et al., 2022b). In addition, Treg cell–derived TGF-β3, induced by glucocorticoid signaling, was shown to directly engage TGF-β receptors on HFSCs to promote their activation and proliferation both in vitro and in vivo.

Although the complexity of the gastrointestinal tract's immune system and microbiota makes studies of specific cellular interactions difficult, some recent reports have successfully elucidated Treg cell–epithelial cell relationships in this context. In one such report (Song et al., 2015), the authors explored the role of FGF2 and IL-17 in colitis models, finding that knockout of either worsens reparative intestinal epithelial cell (IEC) proliferation. Both mediators were shown to be required in tandem for proper repair, and Treg cells and Th17 cells were reported to be the likely source for each. They also found that FGF2 induction by Treg cells is microbiota and TGF-β dependent.

Despite not being considered traditional antigen-presenting cells, intestinal epithelial stem cells (ISCs) express MHC-II, making them a particularly interesting cell type to study in the context of T cell interactions. A report studying this interaction (Biton et al., 2018) demonstrated that ISCs can utilize MHC-II to stimulate antigen-specific T cell responses and that ISC-specific MHC-II deletion leads to a reduction of CD4$^+$ T cells in crypts, which correlated with a profound decrease in ISC differentiation (Biton et al., 2018). The authors showed that the addition of Treg cells or provision of recombinant IL-10 could increase the number of ISCs but not differentiated cell types in vitro. These findings suggest that Treg cell–derived IL-10 may be acting directly on epithelial cells to promote stemness in a role separate from its long-appreciated immunosuppression functions.

While the brain has historically been viewed as an immune-privileged organ, it is now clear that immune cells—including Treg cells—frequently access this site, particularly during damage. In the brain, the role of Treg cells has been most extensively investigated in the model of ischemic stroke using middle cerebral artery occlusion (MCAO). There are numerous reports querying how Treg cell depletion or expansion can affect outcomes in this model, with most studies attributing the protective effects of Treg cells in ischemic stroke to their immunosuppressive capacity (Liesz et al., 2015). However, one report using the MCAO model highlighted a potential direct role of Treg cell–derived IL-10 on neuronal outgrowth (Wang et al., 2015). The authors found that activated Treg cells promoted the proliferation of a Mash1$^+$ population of neural stem cells, precursors to neurons, at homeostasis and after MCAO injury in vivo, and neuron proliferation in vitro—an effect that was blocked by anti-IL-10 antibody treatment.

Addressing another neurological disease state, Dombrowski et al. (2017) investigated the role of Treg cells in remyelination of neurons using demyelination models involving lysolecithin (spinal cord) or cuprizone (corpus callosum) injury. The authors showed that oligodendrocyte progenitor cell (OPC) differentiation and subsequent remyelination are impaired upon Treg cell depletion in both models and that adoptive transfer of Treg cells can rescue proper remyelination. Furthermore, they identified matricellular growth regulatory protein CCN3 as a potential

Treg cell–derived factor that stimulates OPC differentiation in several coculture models. However, a follow-up report (Dittmer et al., 2018) utilizing similar methods indicated that Treg cell–derived CCN3 may not signal directly to OPCs. Studies by a different group investigating Treg cell–induced reparative remyelination (Shi et al., 2021) found that Treg cell–derived matricellular ECM protein osteopontin signals to microglia to induce OPC differentiation, highlighting a microglia-mediated role for Treg cells in this context. However, given the multifunctional nature of ECM components, osteopontin could also impact other tissue cells in this or related disease contexts.

Treg cell–mesenchymal cell interactions

While these earlier reports regarding Treg cell–epithelial cell interactions prompted many to view this as the primary mode of Treg cell–mediated tissue affectation, subsequent work has found that the target of Treg cell–derived signals in other tissue contexts is mesenchymal cells. Utilizing a model of lung influenza A virus (IAV) infection, an initial study investigated a potential role for Treg cell–derived production of the epidermal growth factor receptor (EGFR)–ligand amphiregulin (Areg) (Arpaia et al., 2015). Using an *Areg* conditional knockout mouse, this report showed that animals specifically lacking Areg production by Treg cells exhibited reduced blood oxygen saturation and increased lung edema during the course of infection. Notably, these conditional knockout mice displayed equivalent IAV-specific CD4+ and CD8+ T cell responses with no alterations in lung viral load, pointing to a tissue cell–driven effect on lung recovery—mediated by Treg cell–derived Areg that is separable from their canonical immunosuppressive function.

Given that epithelial cells express EGFR in many tissues/cell lines and the aforementioned findings of Treg cell–LEC interactions by other groups, it was expected that the target tissue cells of Treg cell–derived Areg were likely LECs. However, in a recent report by our group (Kaiser et al., 2023), it was found that type II alveolar epithelial cells (AT2s)—the LEC type for which activation/differentiation is necessary for lung recovery after IAV infection—express little to no *Egfr* and thus are unlikely to be direct sensors of Areg. Instead, we found that mesenchymal cells expressed the highest levels of *Egfr* in the lung and identified a particular *Egfr*-high subset of *Col14a1*-expressing lung mesenchymal cells (LMCs), which we termed Col14+ LMCs (also known as adventitial fibroblasts), that is uniquely responsive to Areg. Notably, Treg cells are spatially positioned nearby these Col14+ LMCs, and Treg cell–derived Areg was shown to specifically prevent the death of this LMC subset in IAV-infected lungs. Genetically targeting *Egfr* for deletion in mesenchymal cells further underscored that Treg cell–derived Areg signaling to LMCs mediates alveolar repair in this context. A subsequent signaling relay from LMCs to LECs likely provides support for regeneration of the latter; this is supported by studies using a Treg cell/LMC coculture model in which lung Treg cells, but not spleen Treg cells, were able to induce pronounced tissue reparative gene expression in LMCs.

Treg cell–mesenchymal cell interactions have also been highlighted in muscle tissue. In an influential early report by the Mathis/Benoist group (Burzyn et al., 2013), the authors found

that Treg cells accumulate at the site of tissue damage in several models of muscle injury, and Treg cell depletion inhibited muscle repair. Finding that Treg cells from injured muscles produce large amounts of Areg, the authors determined that Treg cell–derived Areg promotes satellite cell (muscle-associated mesenchymal stem cells) proliferation and differentiation to facilitate muscle recovery. Similarly, another report (Castiglioni et al., 2015) demonstrated that Treg cells are the primary T cell type recruited to injured muscle and showed reduced muscle recovery and satellite cell mobilization after injury in *Rag2*−/−*Il2rg*−/− mice. This report also showed that specifically Treg cells, but not other T cell populations, increase satellite cell proliferation in vitro. Other reports (Panduro et al., 2018; Villalta et al., 2014) also found detrimental effects of Treg cell depletion on repair in muscle injury models but attributed these results to increased immune activation in this context. Interestingly, in the setting of parasitic muscle infection, the presence of Treg cells may inhibit proper muscle repair due to their influence on macrophage polarization (Jin et al., 2017).

There have also been multiple reports highlighting the roles of Treg cell–mesenchymal cell interactions in cardiovascular damage. Several of these reports (Saxena et al., 2014; Sharir et al., 2014b; Tang et al., 2012; Weirather et al., 2014; Xia et al., 2020) investigate the role of heart Treg cells in myocardial infarction (MI) models, while others (Ait-Oufella et al., 2013; Li et al., 2019a; Meng et al., 2014) explore this in experimental induction of abdominal aortic aneurysm (AAA). These studies found that Treg cells accumulate in the heart and aorta during MI and AAA respectively, have a tissue-adapted phenotype disparate from Treg cells in secondary lymphoid organs, and Treg cell depletion leads to worse disease outcomes. Although the authors in these reports primarily attribute the observed pathology to heightened inflammation, some of these reports also demonstrate that beyond their immunosuppressive role, Treg cells signal to cardiomyocytes (CM) or aortic smooth muscle cells (SMCs) via IL-10 (Meng et al., 2014; Tang et al., 2012), TGF-β (Meng et al., 2014), or deposition of matricellular ECM protein SPARC (Xia et al., 2020) to mediate protection from cardiovascular disease.

Treg cell accumulation has also been observed in thrombi, with one study demonstrating that Treg cell–depletion inhibits thrombus resolution (Shahneh et al., 2021). This group also identified SPARC as a novel Treg cell–derived mediator in this context; administration of SPARC-knockout Treg cells to *Rag1*−/− mice resulted in impaired thrombus resolution. While the authors attribute this effect to the ability of SPARC to induce monocyte MMP activity, ECM proteins are known to be pleotropic and interact with several types of tissue cells, so the effect of SPARC in this context could be multifarious.

In the setting of neonatal cryoinjury, one report investigated the role of Treg cells in heart tissue regeneration (Li et al., 2019b). Mice lacking adaptive immune cells failed to undergo proper heart regeneration, which could be rescued by adoptive transfer of Treg cells, and Treg cell depletion resulted in worsened regeneration characteristics. The authors identified three candidate Treg cell–derived mediators that promoted CM proliferation in vitro: chemokine CCL24, growth-stimulating secreted factor GAS6, and Areg. In a mouse AAA model (Li et al.,

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2022), another study identified a novel Treg cell–derived mediator of tissue-cell intercommunication in the context of vascular damage. This study found that Treg cells, which accumulate in injured aorta, produce trefoil factor 1 (TFF1), a lectin-like peptide component of mucous in barrier tissues. Treg cell–specific *Tff1* deletion resulted in increased damage, as measured by aortic diameter and SMC apoptosis. Correspondingly, *Tff1* overexpression improved recovery, and treatment of in vitro primary vascular SMCs with recombinant TFF1 demonstrated antiapoptotic effects.

Treg cells have also been shown to affect mesenchymal cells in the brain, where an accumulation of activated Treg cells is observed in the later stages of the MCAO model (Ito et al., 2019). In this setting, Treg cell depletion resulted in increased pathologic activation of astrocytes—brain mesenchymal cells—and impaired neurological recovery. Adoptive transfer of Treg cells improved recovery of MCAO-injured mice lacking lymphocytes. As the authors found that brain Treg cells express *Areg,* they used in vitro coculture experiments and adoptive transfer of *Areg*-knockout Treg cells to show that Treg cell–derived Areg signals to astrocytes and microglia—brain-resident macrophages—to promote the production of IL-6, which in turn ameliorates pathologic astrocyte outgrowth and improves neurologic recovery following MCAO.

Potential Treg cell–mesenchymal cell interactions have additionally been investigated in non-traditional tissue environments. One report (Camacho et al., 2020) found an enrichment of Treg cells in the mouse bone marrow (BM), demonstrating proficiency for homing to the BM following intravenous injection. The authors found that Treg cells signal to BM mesenchymal cell populations to restrict their improper growth and to support healthy hematopoietic stem cell (HSC) generation—a feature that they attributed to Treg cell–derived IL-10. Notably, disruption of this Treg cell–mesenchymal cell IL-10 axis led to deficiencies in the long-term engraftment capability of HSCs.

Further highlighting the ability of Treg cells to signal toward either epithelial cells or mesenchymal cells in different tissue contexts, a report demonstrating interspecies conservation of Treg cell–tissue cell interaction effects leveraged the high regenerative potential of zebrafish to study Treg cells in organ damage/regeneration models (Hui et al., 2017). This group identified zTreg cells, a Treg cell–like population of cells that express an ortholog of Foxp3, and showed their enrichment in regenerating the spinal cord, heart, and retina; regeneration in these tissues was profoundly dysregulated upon zTreg cell depletion. This report identified various mediators that are critical for tissue cell activation in different tissues: neuronal growth factor neurotrophin-3 (ntf3) from zTreg cells induces neural cell progenitor proliferation in damaged spinal cord, EGFR ligand neuregulin-1 (nrg1) induces CM proliferation in damaged heart, and insulin-like growth factor 1 (igf1) induces glial cell proliferation in the damaged retina. Whether these mediators are important for Treg cell–tissue cell communication in other vertebrates remains to be seen.

Treg cell–endothelial cell interactions
As the entryway for immune cells into tissue, endothelial cells constitute an unconventional cell type often considered a subset of "tissue cells," with several reports indicating direct interactions with Treg cells. In one such report (Maganto-García et al., 2011), the authors found that endothelial cells, via MHC-II–based antigen presentation, stimulate Treg cells to produce TGF-β, which in turn leads to changes in vascular endothelial cells that result in decreased transmigration of other T cell subsets. Fu et al. (2014) further suggested this was due to Treg cell T cell receptor (TCR)-endothelial MHC-II interactions that promote Treg cell transmigration in an allospecific manner, inhibiting the recruitment of other T cell subsets from the blood. A series of reports from the Bromberg group (Brinkman et al., 2016; Piao et al., 2020; Saxena et al., 2022) explore interactions between Treg cells and the lymphatic endothelium, showing that binding of Treg cell–derived lymphotoxin αβ to endothelial lymphotoxin receptors promotes the recruitment of Treg cells from lymphatic vessels; however, this interaction increases the recruitment of other T cell subsets.

A number of reports (Leung et al., 2018; Liu et al., 2022a; Sharir et al., 2014a; Zouggari et al., 2009) have shown a role for Treg cells in improving neovascularization in ischemic tissue models, though one report found a detrimental effect (Hellingman et al., 2012). In one such report (Leung et al., 2018), the authors found that mice with diet- or genetically induced diabetes-like states exhibited reduced reperfusion in a model of muscle ischemia. In this context, improved neovascularization was dependent on heightened Treg cell reparative activity toward endothelial cells. Further, the authors found that Treg cells, or Treg cell–conditioned media, IL-10, or Areg can directly promote endothelial cell tube formation in vitro. In a separate report, Treg cell–derived Areg was also shown to directly promote endothelial cell activity in vitro (Liu et al., 2022a).

Treg cell "anchoring" in tissue
To potentiate their interactions with tissue cells, Treg cells have also been suggested to "anchor" themselves in tissues, in a manner that could promote optimal interaction with tissue cells. In Mock et al. (2014), the authors find that in ALI, adoptively transferred *Itgae*$^{-/-}$ Treg cells are less able to engraft lung tissue than wild type Treg cells, suggesting that CD103 on Tregs may poise Treg cells for enhanced tissue cell interaction. In Kaiser et al. (2023), we made the observation that Tregs in IAV-infected lungs localize in areas deficient in certain types of extracellular matrix (ECM); Treg cell–ECM interaction has not been extensively explored, but this may be an important factor in confining Tregs to tissue regions with cognate mesenchymal cells. In the skin (Mehta et al., 2021), ECM-binding C-type lectin receptor layilin (*Layn*) was highly expressed in both mouse and human skin Treg cells. Through the generation of Treg cell–specific *Layn* knockout mice, the authors found that *Layn* is critical for Treg cell localization, with mice with Treg cell–specific *Layn* deletion having impaired wound healing.

Activating tissue repair function in Treg cells
IL-18 and IL-33, two factors released upon tissue damage, have been shown to promote Areg production by Treg cells (Arpaia et al., 2015). IL-33 has also been demonstrated to be a critical mediator of tissue Treg cell reparative activity in the muscle

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(Kuswanto et al., 2016), cardiovascular system (Li et al., 2019a, 2022), and brain (Ito et al., 2019). Additional mediators reported to induce reparative Treg function include glucocorticoids in the skin (Liu et al., 2022b), serotonin in the brain (Ito et al., 2019), and commensal microbiota–derived components in the gut (Song et al., 2015). These observations suggest that damage-associated signals drive the activation of Treg cell tissue repair programs. While identifying the cell types that produce these mediators remains an active area of investigation, some reports have identified specific cellular sources in various tissue damage contexts.

Though traditionally viewed as being derived primarily from epithelial cells (Drake and Kita, 2017), a key role for IL-33 production by other tissue cell types has begun to be appreciated as important for initiating repair and regeneration. Adventitial fibroblasts are a key source of IL-33 in the lung that mediate repair following helminth infection (Dahlgren et al., 2019) and could influence Treg cell repair programs given their colocalization (Kaiser et al., 2023). Several additional examples suggest that tissue-specific fibroblast populations direct Treg cell tissue repair function. Mesenchymal cell–derived IL-33 promotes Treg cell repair programs in the damaged muscle (Kuswanto et al., 2016).

The first indication that tissue-resident Treg cells were distinct from circulating Treg cells came from studies in visceral adipose tissue (VAT) (Muñoz-Rojas and Mathis, 2021). VAT Treg cells express high levels of Areg (Burzyn et al., 2013) and have a close spatial interaction with IL-33–producing mesenchymal cells, with mesenchymal cell–derived IL-33 leading to the expansion of VAT Treg cells (Spallanzani et al., 2019). Further, Treg cell activity negatively regulates IL-33 expression by VAT mesenchymal cells and thus prevents aberrant VAT inflammation (Spallanzani et al., 2019). Notably, VAT Treg cells are significantly reduced in mice fed a high-fat diet as compared with mice fed normal chow (Feuerer et al., 2009; Winer et al., 2009). Adoptive transfer of Treg cells or Treg cell gain of function reduces insulin resistance in high-fat diet–fed mice, demonstrating that VAT Treg cells are critical for the maintenance of insulin sensitivity—a feature attributed to their role in immunosuppression (Feuerer et al., 2009). Together, these studies suggest that loss of VAT Treg cells in high-fat diet feeding leads to increased IL-33 levels in VAT due to unrestrained expansion of IL-33–producing mesenchymal cells, resulting in aberrant inflammation that promotes insulin resistance. However, the effect of VAT Treg cell–derived tissue repair mediators, such as Areg, on resident mesenchymal cells or adipose progenitor cells and the potential role of mesenchymal cell–derived IL-33 in driving that activity are yet to be described.

Interestingly, other tissue mediators appear to inhibit reparative Treg cell activity. For example, Harb et al. (2021) showed that Notch4 signaling in Treg cells reduces Areg expression and negatively impacts tissue recovery in viral infection models, indicating that inhibition of this axis could potentiate Treg cell reparative phenotypes. Global knockout of GPR174—a G-protein-coupled receptor for lysophosphatidylserine (LysoPS)—resulted in increased Treg cell generation and tissue residency (Barnes et al., 2015), while GPR174-knockout

Treg cells have heightened production of Areg and increased ability to induce neovascularization in a muscle ischemia injury model (Liu et al., 2022a), suggesting LysoPS-mediated GPR174 activation inhibits Treg cell tissue reparative activity. Given the pronounced levels of LysoPS present across tissue sites (Barnes et al., 2015), further exploration of this axis with regard to Treg cell reparative phenotypes is warranted.

In the lung, Treg cells do not require expression or activation of the TCR to produce Areg (Arpaia et al., 2015); however, in the brain, TCR signaling was shown to be required (Ito et al., 2019). Based on previous work that found enrichment for a particular Treg cell TCR in the injured muscle (Burzyn et al., 2013), a follow-up study generated transgenic mice expressing this specific TCR and demonstrated that Treg cells from these animals preferentially migrate to and are retained in muscle, have a muscle repair-oriented transcriptional signature, and enhance muscle regeneration (Cho et al., 2019). However, the self-antigens that may induce this potential in muscle Tregs are yet to be identified. VAT Treg cells have also been shown to have a unique TCR repertoire (Feuerer et al., 2009; Winer et al., 2009), and transgenic TCR approaches have demonstrated a role for TCR signaling in VAT Treg cell accumulation and differentiation (Li et al., 2018); however, the role of antigen-specificity in VAT Treg cell activation or Areg production is unclear. These reports collectively suggest that the role of antigen-driven tissue repair function may be context and/or model dependent—potentially a function of the type, severity, or duration of damage in a given tissue. Although epithelial, mesenchymal, and endothelial cells are known to present antigens to Treg cells via MHC-II expression (Biton et al., 2018; Cruickshank et al., 2004; Fu et al., 2014; Maganto-García et al., 2011; Westendorf et al., 2009), whether this contributes to activation of the Treg cell tissue repair program or mediates the retention of Treg cells in specific tissues is unclear.

Overall, the studies discussed above suggest that the reparative activity of Treg cells is activated by early tissue damage–associated mediators and other niche-specific factors, with a possible additional contribution for antigen stimulation.

Treg cell–structural cell interactions in chronic disease and cancer

Treg cells in fibrosis and chronic tissue injury

The role of Treg cell interactions with fibroblasts and epithelial cells as pro- or anti-fibrotic in chronic tissue injury has been largely unexplored. Suggestive of their potential role in the development of tissue fibrosis, Treg cells are enriched in the livers of mice and humans with chronic liver disease (Claassen et al., 2010; Ikeno et al., 2020; Langhans et al., 2013; Savage et al., 2024). Further, in human liver cirrhosis, Treg cells are believed to interact closely with hepatic stellate cells (Langhans et al., 2013), a liver resident fibroblast population key to liver fibrosis (Mederacke et al., 2013). Treg cell depletion has also been shown to worsen liver and skin fibrosis (Ikeno et al., 2020; Kalekar et al., 2019; Katz et al., 2011), with Gata3+ Treg cells thought to be anti-fibrotic in mouse skin fibrosis models. However, recent work from our group demonstrated that Treg cells interact with hepatic stellate cells in multiple models of
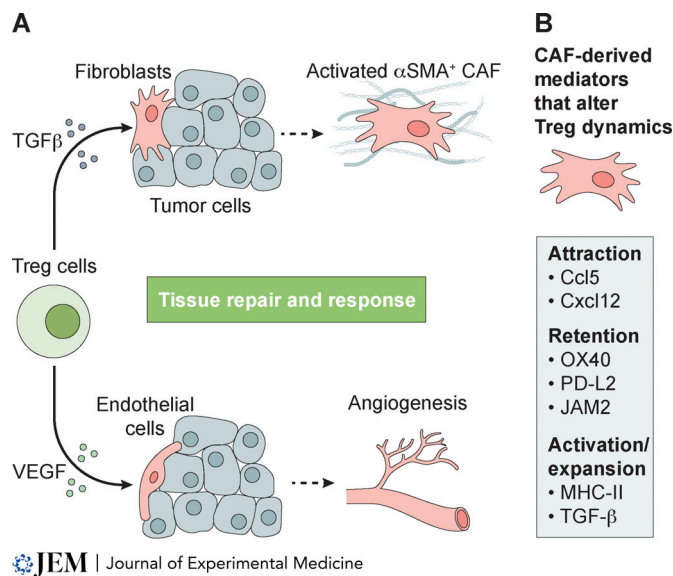
A

Fibroblasts

Activated αSMA⁺ CAF

TGFβ

Tumor cells

Treg cells

Tissue repair and response

Endothelial cells

Angiogenesis

VEGF

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B

CAF-derived mediators that alter Treg dynamics

Attraction
• Ccl5
• Cxcl12

Retention
• OX40
• PD-L2
• JAM2

Activation/ expansion
• MHC-II
• TGF-β

Figure 3. Treg cell repair function in cancer. (A) Treg cell–derived mediators are sensed by cancer-associated stromal cells and influence their function. **(B)** Specific CAF–Treg cell interactions can influence Treg cell phenotype and function in the TME.

chronic liver injury, with Treg cell–derived Areg activating EGFR signaling pathways in hepatic stellate cells to promote the development of liver fibrosis (Savage et al., 2024). Further, hepatic stellate cells activated by Treg cell–derived Areg produce IL-6 that stimulates hepatocyte gluconeogenesis and leads to glucose intolerance (Savage et al., 2024).

In the lung, there is conflicting evidence suggesting Treg cells can both promote (Birjandi et al., 2016; Chakraborty et al., 2018; Xiong et al., 2015) and ameliorate lung fibrosis (Kamio et al., 2018). Using in vitro cultures and in vivo Treg cell depletion, a prior study suggested that PDGF-β from Treg cells may promote lung fibrosis by directly stimulating lung fibroblasts (Lo Re et al., 2011).

As in vivo Treg cell depletion results in increased inflammation and tissue damage that could worsen fibrosis—thereby confounding attempts to isolate the tissue repair function of Treg cells—more definitive studies of Treg cell interactions with fibroblasts in the setting of chronic disease will be required to identify critical tissue cell– and Treg cell–derived mediators that regulate fibrosis development.

Treg cell interactions with stromal cells in cancer

Cancer is a unique chronic disease in which tumor cells often hijack normal tissue repair processes to drive growth. Across various malignancies, Treg cell–mediated immunosuppression of anti-tumor T cell responses has been extensively studied and reviewed elsewhere (Plitas and Rudensky, 2020; Togashi et al., 2019). Mechanistic studies of Treg cell–stromal interactions are a newer area of study with less extensive literature, but their importance to our understanding of cancer progression is clear and growing.

In highly desmoplastic malignancies, cancer-associated stromal cells make up a significant proportion of the tumor

microenvironment (TME) and modify the immune cell landscape. Treg cells have been shown to primarily associate with stromal cells in the TME of solid tumors (Kinoshita et al., 2013), suggesting a level of crosstalk may occur between the two cell types. This is most notably studied in pancreatic ductal adenocarcinoma (PDAC)—a cancer type with a fibroblast-rich TME. In a mouse model of PDAC, depletion of Treg cells results in the functional reprogramming of cancer-associated fibroblast (CAF) populations, resulting in the loss of tumor-restraining α-SMA⁺ CAFs and tumor outgrowth (Zhang et al., 2020); the loss of Treg cell–derived TGF-β may drive α-SMA⁺ CAFs to lose their myofibroblastic phenotype, resulting in increased carcinogenesis, the generation of aberrant immune responses, and unrestricted tumor growth. Notably, this result is in contrast to what would be expected with the loss of immunosuppressive functions of Treg cells and further emphasizes the importance of studying Treg cell–CAF interactions. Furthermore, depletion of α-SMA⁺ CAFs alone stimulates tumor growth (Özdemir et al., 2014), with an increase in tumor-infiltrating Treg cells and decreased overall survival. Though Treg cells are prominent sources of TGF-β, more targeted genetic approaches are necessary to determine how Treg cell–derived TGF-β (or other mediators) specifically affect CAF function. Interestingly, in this model, Treg cells express increased levels of CTLA-4, and administration of anti-CTLA-4 antibody slowed PDAC progression and increased overall survival.

Beyond TGF-β, Treg cell–derived Areg drives greater lung tumor burden in a model of metastatic breast cancer (Green et al., 2017), although the target cells through which Areg mediates this effect are unknown. It is possible that Treg cell–derived Areg signals through stromal cells in lung cancer, as it does in other disease contexts discussed above (Kaiser et al., 2023), to promote tumor growth. To understand how Treg cells modulate CAF phenotypes to potentially regulate carcinogenesis, further investigation of Treg cell–CAF interactions, including the identification of other mediators, will be required.

Crosstalk between CAFs and Treg cells is bidirectional, with CAF populations influencing Treg cell functional dynamics in the TME. Interestingly, fibroblast populations with the capacity to present antigen (apCAFs) have been shown to promote the induction of Foxp3⁺ Treg cells from naïve CD4⁺ T cells (Huang et al., 2022). Moreover, these Treg cells suppress antigen-specific T cell responses. Though apCAF populations have been shown to impact antigen-specific CD4⁺ T cell responses in several models of cancer (Kerdidani et al., 2022), the overall impact of apCAF-induced Treg cells on cancer outcomes remains largely understudied. More broadly, studies have demonstrated that fibroblast-derived chemokines such as CCL5 and CXCL12 lead to the specific migration and retention of Treg cells to tumors (Cheng et al., 2018; Costa et al., 2018; Givel et al., 2018; Karnoub et al., 2007), which can influence the level of immunosuppression in the TME and overall survival.

Treg cell–derived factors have also been implicated in promoting endothelial sprouting in cancer. Facciabene et al. (2011) demonstrated that tumor hypoxia leads to the recruitment of CCR10⁺ Treg cells, which secrete high levels of VEGF, and ablation of these Treg cells suppressed angiogenesis. Moreover,

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increased angiogenic sprouting was observed in in vitro assays utilizing hypoxic Treg cell–conditioned medium.

The activation of tissue repair functionality in tumor-associated Treg cells may drive a tissue-specific, wound-healing modality of cancer-associated stromal cells (Fig. 3). The effect of this interaction is that highly context-dependent—tissue-specific programs likely guide the function and phenotype of tumor-associated stromal cells—thus, continued mechanistic studies of Treg cell–stromal interactions in cancer will be needed in order fill this knowledge gap.

Concluding remarks

In this review, we have discussed the roles of Treg cells in promoting tissue repair/regeneration through interactions with tissue-resident parenchymal and structural cells, a non-canonical function that is distinct from their immunosuppressive properties. We have highlighted numerous studies examining Treg cell–derived mediators that underpin these interactions and their importance for tissue repair and regeneration—recognizing the critical role tissue cells play in translating common Treg cell–derived inputs into tissue-specific outputs. These processes suggest potential targets for the treatment of human disease; however, there remains much to learn in terms of identifying additional factors and their associated mechanisms of action, especially in the setting of chronic disease. Overall, the interplay of Treg cells with non-immune cells offers an exciting new area of study in the field of Treg cell biology that could further our understanding of mechanisms that maintain tissue integrity.

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