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The Phenotypic and Functional Diversity of Tissue Tregs, and Their Role in Chronic Viral Infections

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Abstract

Regulatory T cells (Tregs) are a subset of CD4+ T cells characterized by the expression of the transcription factor FoxP3. While Tregs are known to suppress immune cells to mediate immune responses, they exhibit location-specific functions across lymphoid and non-lymphoid tissues. This review examines the factors that influence location-specific functions of Treg phenotypes. While Tregs share some common characteristics, their roles are dependent on a range of tissue-specific factors. Exposure to varied stimuli across tissue environments, such as cytokines that act through distinct signaling pathways, as well as diverse cell-to-cell interactions, influence gene expression and drive differential behavior of Tregs. Additionally, Tregs play crucial roles in chronic viral infections, where they balance the facilitation of protective immune activity against foreign antigens with the need to prevent immunopathology, thereby allowing viral persistence. Through analysis of whole genome sequences, the role of Tregs in chronic norovirus infections was investigated. There was an accumulation of mutations in viral T cell epitopes over time in chronic infections, indicating that the virus better evades recognition by immune cells. This suggests the role of Tregs in suppressing antiviral immunity, while dampening pathogen-induced immunopathology. An understanding of the tissue-specific adaptations of Treg populations and their behavior in viral infections can inform novel therapies targeting Tregs for diseases and chronic infections in specific tissues.

Keywords: Immunity, Tregs, Tissue-specific, Chronic

Introduction

Humans are constantly surrounded by viruses and microorganisms that can infiltrate or inhabit the body; some are harmful and cause disease. Infectious diseases are one of the leading causes of death. In 2021, while COVID-19 was ranked second, lower respiratory infections were ranked as the fifth leading cause of death, making them the world's most deadly communicable diseases. In low-income countries, lower respiratory infections were the leading cause of death, with malaria, COVID-19, tuberculosis and HIV/AIDS ranked fourth, sixth, eighth and tenth, respectively (World Health Organization, 2024). Thus, there is an urgent need to combat the devastating impact of and to develop treatments against infectious diseases. One of the major challenges to preventing the spread of infectious diseases is an incomplete understanding of immunity.

The physical and chemical barriers of the epidermis and mucous membranes prevent the entry of unwanted bacteria or viruses. The cutaneous production of antimicrobial peptides by various cell types acts as a protective chemical shield against microbes on the surface of the skin (Schauber & Gallo, 2009). If these pathogens penetrate the body, a complex immune response unfolds. The innate immune response is immediate and nonspecific, involving these antimicrobial proteins that initiate a host response, innate immune cells like macrophages or natural killer cells, and inflammation.

In contrast to this generalized response, the adaptive immune system tailors its responses to target particular pathogens. Antigens (derived from 'antibody generators') may be self- antigens on our cells but are often foreign molecules derived from pathogens. These antigens are often large, complex protein molecules but may also include large polysaccharides, nucleic acids, lipoproteins, or glycoproteins. Antigen molecules generally contain many small, recurring molecular groups, called epitopes, specific to a pathogen that provoke immune responses. Acquired immunity develops only after exposure to an antigen and results in dormant memory cells that can effectively eradicate the pathogen in the case of a subsequent infection. Adaptive immunity relies on 2 types of lymphocytes: B lymphocytes and T lymphocytes. Each lymphocyte expresses antigen receptors (T-cell receptors, TCRs, or B-cell receptors, BCRs) of a single specificity (Daëron, 2022). Antibody-mediated immunity, or humoral immunity, involves the production of antibodies by B lymphocytes that differentiate into active plasma cells. Cell- mediated immunity entails the development and proliferation of activated T lymphocytes that directly destroy infected cells. T lymphocytes can be broadly categorized into 3 types: cytotoxic or killer CD8+ T cells that destroy cells displaying foreign antigens or intracellular pathogens; helper CD4+ T cells that activate other immune cells; and regulatory T cells (Tregs).

Regulatory T cells are a diverse subset of CD4+ cells, debatably 'the most versatile immunosuppressive cells' (Sharma & Rudra, 2018), with a range of functions. Tregs are characterized by expression of the transcription factor forkhead box protein 3 (FoxP3), which is required both for initial differentiation and Treg suppressor functions (Plitas & Rudensky, 2017).

Tregs are indispensable in the mediation of the immune response. Constituting roughly 5-10% of CD4+ T cells (Georgiev et al., 2019), Tregs play an essential role in maintaining immune homeostasis by controlling both innate and adaptive immune responses. Tregs contribute to the suppression of autoimmune responses, including those of CD4+ T cells that avoid negative selection when maturing in the thymus, as well as in tissue repair. They play an important role in enforcing tolerance to self-antigens (Georgiev et al., 2019). Thus, abnormal Treg function can cause disease. During infection, Tregs are critical to minimize overstimulation of the immune response (Liu et al., 2024) and immune pathology. They regulate host inflammation while balancing pathogen control (Traxinger et al., 2022). However, during chronic viral infections immunosuppression of antiviral immunity by Tregs may in some cases promote disease progression by delaying viral clearance, allowing low levels of viral replication, virus persistence and latency (Ciurkiewicz et al., 2020).

Tregs can be generated through multiple processes. The typical generation of Tregs involves early development in the thymus. Regulatory T cells that develop in the thymus are known as thymic Treg (tTreg) or natural Treg (nTreg) cells (Sharma & Rudra, 2018). Initial stages of thymic development involve TCR and co-stimulatory signaling, followed by subsequent cytokine signaling that induces FoxP3 expression. The differentiation of tTreg cells involves affinity interactions with self-peptide MHC complexes (Josefowicz et al., 2012). Cell interactions between thymocytes and thymic antigen-presenting cells is also involved in Treg cell generation (Hsieh et al., 2012). Alternatively, a subset of Tregs differentiates de novo from conventional CD4+ T cells in the periphery, mediated by interleukin (IL)-2 and transforming growth factor (TGF)-β signaling (Ciurkiewicz et al., 2020); this Treg subset is referred to as induced Tregs (iTreg). This differentiation occurs as a result of antigen (non-self) stimulation (Liu et al., 2024).

Several subsets of Tregs of differing origin (peripheral or thymic) and location (lymphoid or non-lymphoid) exist, showcasing considerable phenotypic heterogeneity. Some Tregs become tissue specific. Tissue Tregs in non-lymphoid tissues demonstrate differing characteristics and may have different functions. Local tissue-specific factors and external stimuli in the body are thought to drive these differing phenotypes. Their respective functions have important consequences for the regulation of immune responses to infection and disease.

While extensively studied in the context of autoimmunity, the role of Tregs in infections is variable and less defined (Traxinger et al., 2022). Much is not understood about how local phenotypes of Tregs contribute to or inhibit pathogen-specific immunity in infectious disorders. This review outlines how the phenotypic and functional characteristics of Tregs vary across different tissue environments, and how this distinction can be harnessed in the fight against infectious diseases. Norovirus genome sequences collected longitudinally from individual chronic infections are employed to determine whether there is an increase in viral T cell epitope mutations over time, driven by Tregs. There was an accumulation in T cell epitope mutations observed through time across patients, suggesting a role of Tregs in allowing for viral persistence by suppressing immunity. Overall, a deeper understanding of the highly adaptable characteristics of Tregs in different locations (Sharma & Rudra, 2018) could help inform therapeutic strategies targeting regulatory T cells, particularly for tissue-specific viral infections or diseases where the role of Tregs is pivotal to understand.

External Factors and Stimuli Influencing Treg Phenotypes

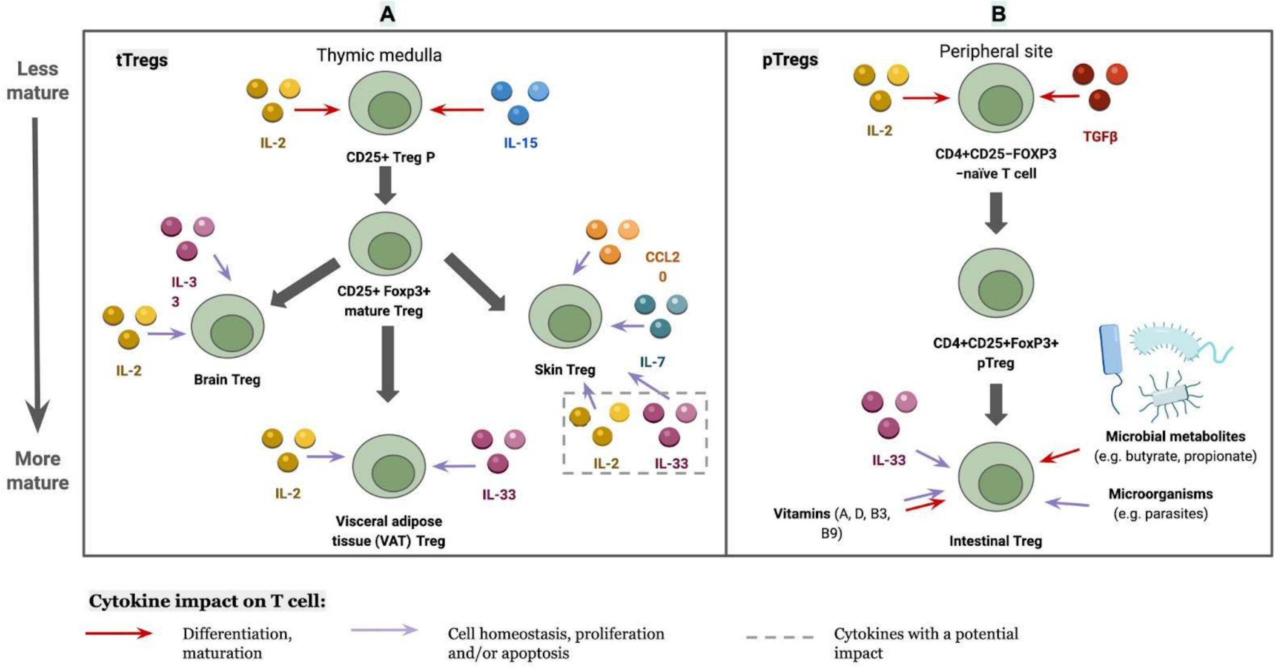
To distinguish between differing Treg phenotypes, the triggers and external stimuli that affect the phenotypic and functional properties of Tregs first need to be understood. Two key external factors driving Treg phenotypes are cytokines (signaling molecules that orchestrate different functions of immune cells, including promoting lymphocyte proliferation and differentiation) and direct contact with other immune cell types. These factors act throughout Treg development. The impact of cytokines on Treg development will first be reviewed.

As outlined above, most Tregs share their initial stages of development in the thymus. Although mature Tregs can develop via a CD25-Foxp3lo intermediate (Savage et al., 2020), tTregs typically undergo a two-stage development process, as seen in Figure 1.

The first stage is driven by the stimulation of the TCR and CD28 of developing CD4+ single-positive (SP) thymocytes. This results in CD25+Foxp3− precursors (CD25+ TregP). The second stage involves γ-chain cytokines that lead to the generation of CD25+ Foxp3+ mature Tregs. In particular, Interleukin-2 (IL2) provided by the autoreactive CD4+ SP thymocytes and IL-15 produced by medullary thymic epithelial cells (mTEC) help in maturation (Borelli et al., 2024). Thus, the indispensable role of IL-2 and IL-15 has been established in intrathymic Treg-development, with IL-2 playing a more substantial role than IL-15. After development in the thymus, mature Tregs migrate to non-lymphoid tissues (Liston et al., 2022). They egress into the periphery, where IL-2 is required for peripheral survival and function (Apert et al., 2022).

Figure 1

Signaling molecules drive Treg development



Key signaling molecules regulating the stages of development of (A) tTregs and (B) pTregs are shown. Signaling molecules are depicted in different colors; arrow colors depict their impact on Tregs. Tregs that instead differentiate extrathymically at peripheral sites are referred to as pTregs (Shevach & Thornton, 2014). pTregs differentiate from conventional T cells (Tconv) lacking FoxP3, usually naïve CD4+ T cells, in the periphery (Apert et al., 2022). In mice, TGF-β and IL- 2 are necessary to convert CD4+CD25−FoxP3−naïve T cells into CD4+CD25+FoxP3+ pTregs, with anti-inflammatory conditions further augmenting the effects of TGF-β (Hoeppli et al., 2015). Little is known about whether dedicated mechanisms exist to coordinate pTreg differentiation, and what those mechanisms comprise, including the environmental constituents involved. However, it is suggested that pTregs may undergo microbiota-dependent cell differentiation in tissues like the colon, potentially driven by foreign, exogenous antigens, including peptides from commensal microbiota, dietary constituents, and fetal antigens (Savage et al., 2020). Further development of Tregs can be influenced both by factors that are shared across tissues and tissue-specific factors. Tissue Tregs in several non-lymphoid tissues, including the skin, intestine, lung, fat, heart and brain, share some common features (Watanabe et al., 2024). In general, Treg cell homeostasis is controlled by common cytokines. IL-2 is a key cytokine necessary for robust Treg development and function (Sjaastad et al., 2021) in the periphery, controlling proliferation and apoptosis (Liston et al., 2023). The development of several tissue- specific Treg phenotypes, such as Visceral adipose tissue (VAT) Treg cells, is IL-2 dependent (Muñoz-Rojas & Mathis, 2021). Interleukin-33 (IL-33) is also an important factor for the expansion and homeostasis of a range of Treg phenotypes which express ST2 (the IL-33 receptor), including accumulation after tissue injury (Spath et al., 2022). For example, IL-33 secreted by intestinal epithelial cells and enteroendocrine cells drives the expansion of GATA3+ intestinal Treg cells (Ramanan et al., 2023). CNS Tregs are also maintained by IL-33 signaling, while VAT Treg cells and skeletal muscle Tregs are much more responsive to IL-33 than to IL-2 (Muñoz-Rojas & Mathis, 2021). It has been found that the local environment is the primary determinant of tissue-specific characteristics of Tregs (Watanabe et al., 2024). This is likely driven by multiple factors.

Tissue- specific factors include exposure to local cytokines, many of which are tissue-specific. The local microenvironment includes different concentrations of various cytokines within the immediate vicinity of the cell. Additionally, exposure to microbiota can influence Treg development; the presence and diversity of microbiota will vary between tissues. The skin and intestine provide representative examples of tissue-specific factors influencing Treg development.

Skin Tregs

The phenotypes of skin Tregs, which are believed to originate from the thymus (Liston et al., 2022), are influenced by local levels of several cytokines. This includes the chemokine CCL20 which is produced by follicles in response to skin microbiota, and drives Treg proliferation. Local keratinocytes produce relatively high levels of IL-7 which regulates Treg homeostasis through the IL-7 receptor. Conversely, constitutive IL-2 production is low in the skin; therefore, skin Tregs do not heavily depend on IL-2 for maintenance. IL-33 dependence is also low. However, these cytokines may influence antigen-specific responses of skin Tregs (Muñoz-Rojas & Mathis, 2021)

Intestinal Tregs

Similar to skin Tregs, intestinal T cell homeostasis is controlled by local cytokines. This is observed in the colon and small intestine in the gastrointestinal tract, a major site of pTreg induction. The generation and accumulation of pTregs in the gut is largely affected by transforming growth factor beta (TGFβ). Intestinal epithelial cells also secrete cytokines, including IL-18, promoting intestinal Treg cell function (Ramanan et al., 2023).

Gut Treg differentiation and function are further known to be modulated by dietary metabolites in the local tissue environment (Hoeppli et al., 2015). Several factors like dietary vitamin A, vitamin D, Niacin (Vitamin B3), folic acid (Vitamin B9), and tryptophan regulate intestinal Tregs (Sharma & Rudra, 2018). The primary bioactive metabolite of vitamin A, all trans retinoic acid (ATRA), plays an important role in the differentiation of pTregs in the gut (Hoeppli et al., 2015). Specifically, the treatment of naive CD4+ T cells in vitro with ATRA and TGF-β allows Treg promotion by inducing FoxP3 expression. The TGF-β-mediated generation of pTregs in the tissue promotes their homing to mesenteric lymph nodes and the small intestine, crucially establishing mucosa ‘oral tolerance’ (Zeng & Chi, 2015). This refers to the suppression of immune responses to a specific antigen due to prior oral administration of the antigen (Kurihara et al., 2024). Vitamin D3 induces Foxp3 expression and enhances the suppressive activity of Tregs, while vitamin B3 also induces Treg differentiation. Tetrahydrofolate derived from folic acid maintains Treg cell proliferation and survival by inhibiting apoptosis (Zeng & Chi, 2015). Finally, tryptophan helps maintain the function of Tregs. Aryl hydrocarbon receptor (aHr) ligands produced by the microbiota-catabolism of tryptophan, or kynurenine pathway (KP) metabolites involved in tryptophan metabolism, induce the generation and differentiation of Tregs (Ding et al., 2020).

Furthermore, microbiota and bacterial components from the local tissue can significantly influence Treg differentiation. It has been demonstrated that secondary bile acids produced by bacterial metabolism potentiate Treg cell differentiation. Microbial metabolites or by-products, such as butyrate or propionate, can boost intestinal Treg differentiation in the colon (Lee & Lee, 2018). Apart from cytokines, dietary metabolites and microbiota, other microorganisms exposing various molecular structures can activate innate immune sensors and/or adaptive immune receptors of intestinal Tregs. For example, the parasite Heligmosomoides polygyrus can cause Treg expansion by secreting a molecule that mimics TGFβ (Ramanan et al., 2023). Thus, external stimuli in the local tissue environment are crucial to modulate Treg phenotype and development.

Cell-to-Cell Interactions of Tregs in Tissues

In addition to signaling molecules, the phenotypes of Tregs are influenced by direct cell-to-cell interactions. Interactions with antigen presenting cells (APCs) are especially important. The initial thymic development and further peripheral regulation of organ-specific Treg cells are dependent on antigen presentation by dendritic cells (DCs). DCs can provide antigenic and accessory signaling that control Treg cell activation, their distribution and positioning within organs, and access to distinct environmental cues (Leventhal et al., 2016). Furthermore, environmental antigens including microbial or food antigens presented by mucosal tissue- resident DCs can drive pTreg cell development (Lee & Lee, 2018). For example, crosstalk between intestinal epithelial cells and DCs influences Treg cell abundance (Ramanan et al., 2023). Additionally, lung Tregs induced from CD4+ T cells in the periphery depend on a lung microenvironment-adapted tolerogenic dendritic cell population, termed the IFNAR1hiTNFR2+ conventional DC2 (iR2D2) population (Ni & Chen, 2023). While DCs are the major APCs required for Treg differentiation, other subsets of APCs also play a role.

In the context of intestinal Tregs, it has been demonstrated that antigen presentation by a subset of

RORγt + APCs, called Thetis cells IV (TC IV), induced by eosinophils (Kurihara et al., 2024), is necessary for food-induced pTreg cell differentiation (Parisotto et al., 2024). Oral tolerance to oral or food-derived antigens, due to prior exposure to the antigens, may thus differ from person- to-person based on the dietary antigens intestinal Tregs have been exposed to. DCs are not required for Treg cell control by Thetis cells. Homeostasis of intestinal Treg cell subsets can be influenced by several other different cell types including macrophages, RORγ+ Janus cells, and neurons (Ramanan et al., 2023).

It is possible that other immune or non-immune cell types directly interact with Tregs to influence their phenotype and development. However, there is limited data available regarding the nature of these interactions. This could be owing to challenges in studying cell-cell interactions in vitro. With more advanced in vitro systems and methods, including ex vivo tissue slices or organoids incorporating Tregs (Muñoz-Rojas & Mathis, 2021), further insights may be more easily gained in the future.

Gene Expression of Treg Phenotypes

Cytokines and cell-cell interactions drive different Treg phenotypes by influencing and altering gene expression. These alterations, and the regulation of different stages of Treg development in general, are orchestrated by several transcription factors (TFs). Key TFs driving early stages of development include BACH2, IRF4 and BATF, which allow for entry into the tissue-Treg-cell precursor pool in lymphoid organs. IRF4 and/or BATF then further induce the expression of other TFs, such as

BLIMP1, MAF, GATA3 and ID2, in tissue Treg-cell precursors. These TFs play crucial roles in Treg development. For example, BLIMP1 is essential to induce tissue-Treg cell effector molecules like IL-10, and inhibits DNA methyltransferase DNMT3a. This drives the hypermethylation of the Foxp3 locus and a high level of FOXP3 expression (Muñoz-Rojas & Mathis, 2021). The development and suppressive function of Tregs requires the master regulator transcription factor Foxp3 (Zhao et al., 2017). Further Treg differentiation in tissues is determined by other transcription factors. There are some common TFs that regulate the differentiation pathway of tissue Tregs. For example, GATA-binding protein 3 (GATA3) is important in tissue Treg differentiation at multiple non- lymphoid sites, with GATA3-defined populations present in the skin, VAT, lung, liver and colon (Sjaastad et al., 2021). Additionally, BATF has been identified as a programmer in non-lymphoid tissue Treg precursors (Delacher et al., 2020) and a critical regulator of tissue Tregs (Hayatsu et al., 2017). For example, BATF regulates the expression of KLRG1 and IL-33R in mouse lung tissue Tregs. The expression of other TFs is also regulated by BATF in VAT tissue Treg cells. Tregs from various non-lymphoid tissues have common characteristics, such as elevated expression of IL-33R, ST2 and tissue-regenerative factor amphiregulin (AREG) (Ni & Chen, 2023), with AREG highly expressed in nearly all tissue Tregs. However, there are other unique transcription factors that drive the differentiation of tissue- specific Tregs, exerting tissue-preferential effects on Treg cell pools. A few examples will be explored below.

VAT Tregs

One example is the VAT Treg phenotype, which has a distinct gene expression profile, T- cell receptor repertoire and chemokine receptor expression from its Treg counterparts in the spleen and lymph nodes (Cipolletta et al., 2012). The VAT Treg signature (Muñoz-Rojas & Mathis, 2021) and phenotype (Cipolletta et al., 2012) are driven by peroxisome proliferator- activated receptor-γ, PPARγ, which induces the elevated expression of CCR1, CCR2, CCR9, CXCL10 and low CXCR3 expression (Zhao et al., 2017). Furthermore, this transcription factor is also essential for accumulation and function of VAT Tregs, while it does not affect the accumulation of other tissue-specific phenotypes such as skeletal muscle Tregs. Meanwhile, RORα and RARα are also associated with the transcriptional regulation of VAT Treg cells (Muñoz-Rojas & Mathis, 2021).

Lung Tregs

Transcription factors contributing specifically to the lung tissue Treg signature include Nfil3, Maf and the Delta-Notch signaling pathway. These transcription factors regulate the increased expression of CD103, PD-1, GITR, CTLA-4 and KLRG1, which are unique to lung Tregs. Thus, while the core signature of lung tissue Tregs overlaps with other nonlymphoid tissues, its overall phenotype is influenced by tissue-specific cues and locally acting transcription factors (Traxinger et al., 2021).

Differing Functions in Tissue Environments

Canonical Functions

Tregs fulfill their conventional function of suppressing immune cells and modulating the inflammatory responses across tissue environments. Mechanisms of immunosuppression may vary between tissues depending on the local tissue-specific environment and unique Treg cell activity.

Tregs in the respiratory tract work to restrain immune responses and allergic reactions to innocuous antigens, with loss-of-function mutations in Foxp3 resulting in immune dysregulation, autoimmune manifestations, and severe allergic inflammatory conditions in the respiratory tract (Traxinger et al., 2021). Inflammatory responses are dampened by the upregulation of Mmp12 which inhibits neutrophil recruitment. Lung Tregs also inhibit the activation of type 2 innate lymphocytes (ILC2s) through direct inducible costimulator (ICOS) induction, which suppresses the production of IL-5 and IL-13 (Nirmala et al., 2023). VAT Tregs act as immunoregulators by suppressing other cells. Adipose-resident Tregs express hydroxyprostaglandin dehydrogenase (HPGD) which can inhibit VAT conventional T cells (Nirmala et al., 2023). Their ablation leads to macrophage and monocyte accumulation, as well as the induction of proinflammatory cytokines and mediators. In mice, they express high levels of IL-10, an anti-inflammatory cytokine that allows them to inhibit inflammation (Lui et al., 2020). Brain Tregs also play roles in modulating immune responses to neuroinflammation, with an impairment in the suppressive function of Tregs correlated with the severity of neurodegenerative diseases, such as Alzheimer's Disease. In the context of stroke, high levels of AREG expressed serve to suppress IL6 production from microglia and astrocytes, limiting inflammation (Liston et al., 2022).

Regulatory T cells play crucial roles in developing immune tolerance at various sites, particularly in mucosal surfaces which are frequently exposed to antigens and pathogens. In the gastrointestinal tract, pTregs that are neuropilin-1 (NRP1) and Helios double-negative, as well as RORγt negative, are critical for tolerance to dietary antigens. They play a significant role in preventing food allergies. Microbiota-induced RORγt+c-Maf+ pTregs are also crucial to maintain tolerance to both food and commensal bacteria (Traxinger et al., 2021).

Functions of Regeneration and Wound Healing

Furthermore, it has been discovered that Tregs play important roles in tissue repair. Amphiregulin (AREG) mediated tissue repair may be a generalized mechanism employed (Sharma & Rudra, 2018). For example, when muscle is damaged, muscle Tregs in mice produce the growth factor AREG that acts directly on satellite cells (Traxinger et al., 2021), a population of muscle stem cells that maintain, grow, and regenerate adult muscle. This promotes muscle cell differentiation in vitro and enhances tissue repair in vivo (Nirmala et al., 2023). Brain Tregs promote neurological recovery and prevent neurotoxic astrogliosis, depending on the high expression of AREG (Ni & Chen, 2023). Similarly, the secretion of amphiregulin is a non- redundant requirement for respiratory tract tissue Tregs, enhancing the proliferation and differentiation of alveolar epithelial cells in order to repair damaged lung tissue (Nirmala et al., 2023). However, other location-specific mechanisms are employed to resolve tissue injury. In the respiratory tract, the keratinocyte growth factor (KFG) is expressed for tissue repair. Lung Tregs downregulate Sik1 leading to an increase in the expression of CD103, which benefits Treg adhesion and retention in the injured lung (Ni & Chen, 2023). CD103 plays a similar role in muscle tissue (Nirmala et al., 2023). In the intestine, the knockout of FGF2 and IL-17 produced by Tregs worsens reparative intestinal epithelial cell (IEC) proliferation, suggesting functions of tissue repair. Brain Tregs can accumulate in the brain at the chronic phase of ischemic brain injury, expressing high levels of Osteopontin to enhance microglia-mediated repair. Thus, they can play a protective role during stroke, limiting the extent of pathology (Liston et al., 2022).

Finally, skin Tregs accumulate in large quantities following injury. They highly express “jagged1” which encodes a ligand for the Notch signaling pathway, responsible for hair follicle stem cell (HFSC) proliferation. This modulation aids in the acceleration of cutaneous wound healing (Sharma & Rudra, 2018). However, Tregs also showcase functional heterogeneity by performing location-dependent, sometimes paradoxical roles in various non-lymphoid organs and mucosal tissues. Some examples exploring these highly specialized roles are outlined below.

Functions of Tregs in Health and Disease

VAT Tregs upregulate LDLR and DGAT which have important effects on lipid metabolism in the tissue. They also regulate insulin sensitivity. The induction of VAT Tregs could ameliorate obese-related insulin resistance, while VAT Tregs with upregulated B lymphocyte–induced maturation protein 1(Blimp-1), which modulates IL-10 expression, could worsen insulin resistance.

The paradoxical effects of these Tregs and their mechanisms on insulin sensitivity require further research (Ni & Chen, 2023). GI Tregs contribute to humoral mucosal functions such as control of germinal center reactions to drive secretion of immunoglobulin A (IgA), which blocks invading pathogens from attaching to the mucosal epithelium. A loss of c-Maf + RORγt+ Tregs, however, leads to excessive Th17 and IgA responses. This implies a unique role to balance protection from external pathogens with regulating the mucosal immune response as described earlier (Traxinger et al., 2021).

Uterine Tregs play a role in pregnancy, promoting pregnancy in early stages of conception by suppressing immune responses to the foreign antigen of the sperm while mediating protection against STIs. They also play unique roles in the prevention of gestational hypertension and preeclampsia (Traxinger et al., 2021).

This pattern is observed in other tissues. Skin Treg cell expression of αvβ8 integrin activates latent TGF-β in the surrounding microenvironment, which then signals to keratinocytes to induce CXCL5 production. This promotes the recruitment of Th17 cells and neutrophils that inhibit tissue regeneration. The role of brain Tregs in Alzheimer's Disease is controversial, with different studies suggesting either a protective or deleterious role of Tregs. While their immunosuppressive functions are reduced in patients, low doses of systemic IL2 increased the number of Tregs and improved cognitive ability in mice (Liston et al., 2022).

Treg Cell Memory in Different Tissues

In general, immunological memory is an important feature of the adaptive immune system. The consensus is that cells must fulfill three criteria to be considered memory cells: they must show evidence of prior activation and/or expansion, they must be preserved without continual exposure to the cognate antigen, and they must display enhanced activity when re-exposed to the antigen compared to initial exposure (Rosenblum et al., 2015). Memory T regulatory cells also display these characteristics and are essential to regulate memory effector responses and mitigate aberrant immune reactions to evade collateral damage to tissues. They also perform functions in autoimmunity, antimicrobial host defense, and maternal-fetal tolerance. They may rely on specific cytokine signals, transcription factors and metabolic pathways for generation and maintenance (Rosenblum et al., 2015), with data supporting multiple pathways of homeostatic maintenance that exist specifically for distinct populations of tissue Tregs (Gratz et al., 2014).

The lack of memory-specific markers on memory Treg cells has posed challenges to their phenotypic and functional characterization in many tissues (Rosenblum et al., 2015). However, tissue resident regulatory memory has been studied particularly in the context of skin Tregs, with upregulated transcripts encoding memory markers observed in the skin-Treg transcriptome (Muñoz-Rojas & Mathis, 2021). In the skin, 70% to 85% of the total pool of tissue-resident memory T cells consists of CD4+ T cells, and about 10% of these cells express Foxp3 (defining them as memory Tregs, mTregs). Early evidence of antigen-specific memory FOXP3+ Tregs was showcased through the controlled introduction of an antigen in mice skin. Antigen expression resulted in the activation and proliferation of antigen-specific Tregs, with the persistence of a population of CTLA4hi Tregs in the subsequent absence of the antigen, and a quicker resolution of inflammation when the antigen was reintroduced. Skin mTreg cells proliferate in an antigen-independent manner upon contact with dermal fibroblasts in the presence of IL-15. mTreg cells in human skin express memory markers (CD27 and BCL-2) and elevated levels of activation markers (CTLA4, CD25, and ICOS) (Khantakova et al., 2022).

The existence of resident memory Treg cells in barrier tissues like mucous membranes, in the brain and other sites of the body is becoming apparent (Khantakova et al., 2022), and further research can significantly contribute to therapeutic treatments.

Results

Norovirus Accumulates Mutations in T Cell Epitopes During Chronic Infections

To investigate the possible role of interactions of Tregs with chronic viral infections, a dataset of genetic sequences of norovirus from 6 infections was obtained. The norovirus is an RNA virus with a genome size of 7.5 to 7.7 kb (Cotten et al., 2014). It is the leading cause of acute gastroenteritis globally, causing over 200,000 deaths yearly in low and middle-income countries (Sarmento et al., 2023). While most infections occurring in healthy hosts are short and quickly shed for a median of seven days (Ludwig-Begall et al., 2021), some chronic infections occur in immunocompromised individuals (Green et al., 2020). Previous studies have sampled these chronic infections longitudinally and sequenced multiple viruses from those individual infected patients. Examining norovirus chronic infections can help test whether Tregs are influencing evolution in chronic viral infections. The aim was to determine whether there were mutations in T cell epitopes for individual patients, and whether these accumulate through time. A paper (Beek et al., 2017) was used to source the accession numbers of the norovirus sequences, which were subsequently submitted to the NCBI nucleotide database (NCBI, 2023). The sequences were copied and submitted to a norovirus genotyping tool, in order to download the results in FASTA format.

Initially, it was ensured that the 3 open reading frames in the norovirus genome were all in frame, and aligned at the amino acid level using muscle in SeaView version 5.0.5 (PRABI-Doua: SeaView, n.d.). A phylogenetic tree was reconstructed with IQ-TREE v3.0.1 (Wong et al., 2025), using the HKY nucleotide substitution model, viewing it with FigTree.v1.4.4 (*BioWeb*, 2025).Clusters of highly related sequences in the tree were used to identify individual patient samples, which were validated using the NCBI nucleotide database, as seen in Table 1. Patient sequences were extracted into separate alignments in SeaView.

Table 1

Sample accession numbers, patients, and days of sampling

Sampling days represent the number of days that have passed since the first sample was collected from the patient on day 0.

Accession number	Patient	Day of sampling
MF140669	A	0
MF140670	A	402
MF140671	B	0
MF140672	B	72
MF140673	B	122
MF140674	C	0
MF140675	C	355
MF140681	D	0
MF140682	D	18
MF140683	D	204
MF140684	E	0
MF140685	E	287
MF140686	E	365
MF140687	E	434
MF140688	E	553
MF140689	E	637
MF140690	F	0
MF140691	F	173
MF140692	F	269
MF140693	F	340

To identify amino acid substitutions within the alignments, variable sites were extracted from the protein sequences of all patients. The T cell epitopes for all sequences of all patients were predicted using IEDB (Vita et al., 2025) MHC-II binding positions, with a threshold rank of 0.1 applied for the results. At this threshold, there was a median of 32.5 T cell epitopes in each sample, as seen in Table 2. The amino acid positions that mutated during the course of the chronic infection were identified and checked for whether they were in predicted T cell epitopes.

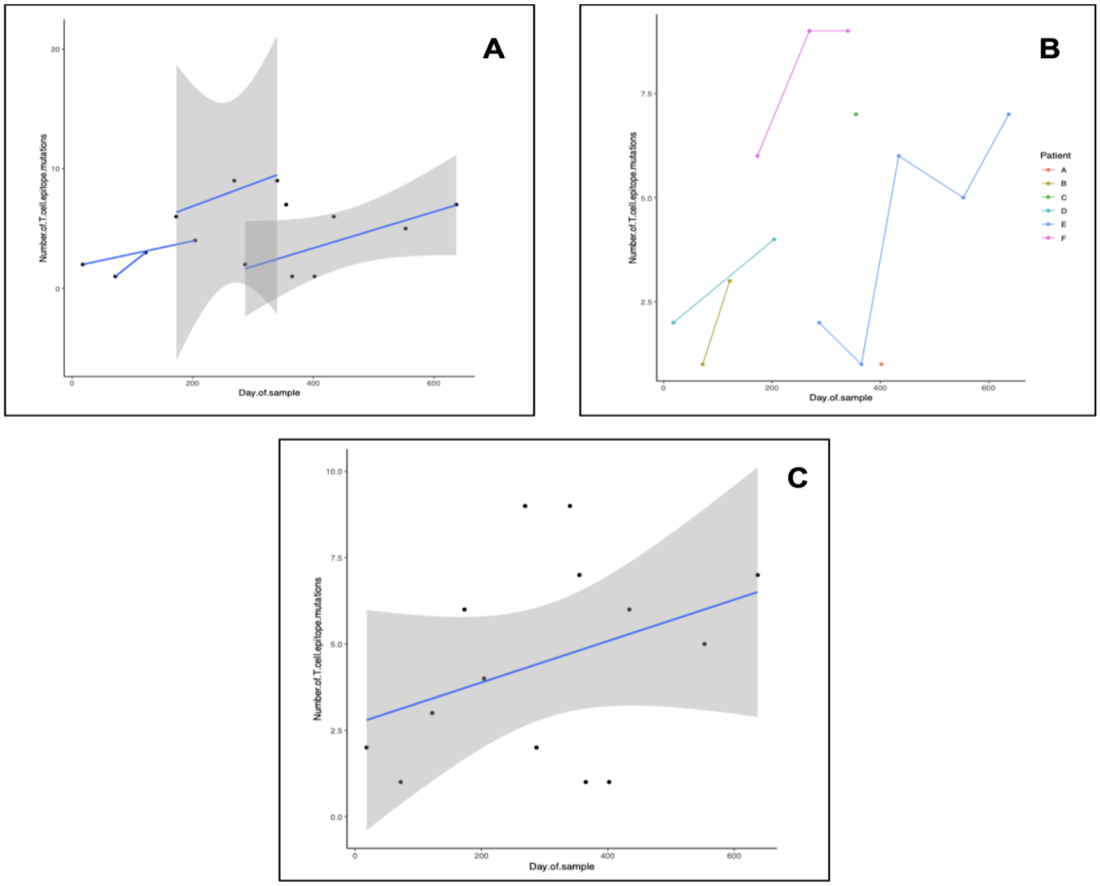
Table 2

Proportion of predicted T cell epitopes that mutate for each sample

Patient	Day of sample	Number of epitopes	Number of mutations in epitopes	Proportion of predicted T cell epitopes that mutate
A	402	34	1	2.94%
B	72	36	1	2.78%
B	122	39	3	7.69%
C	355	37	7	18.92%
D	18	33	2	6.06%
D	204	31	4	12.90%
E	287	31	2	6.45%
E	365	31	1	3.23%
E	434	29	6	20.69%
E	553	32	5	15.63%
E	637	30	7	23.33%
F	173	31	6	19.35%
F	269	33	9	27.27%
F	340	33	9	27.27%
		Median number of epitopes: 32.5		

The data demonstrated an accumulation of T cell epitope mutations over time, as seen in Figure 2. This is consistently observed across multiple patients, with 4 patients demonstrating an increase in the number of T cell epitope mutations through time. The pattern is observed in the short-term, such as across a span of 122 days (Patient B), as well as in the long-term, such as across a span of 637 days (Patient E). An accumulation of these mutations leads to T cells that recognise the virus less efficiently than they did before mutation, indicating that the norovirus gets better at evading recognition by T cells as the duration of the chronic mutation extends. This may hinder the activation of T cells like Tregs, which require the binding of an MHC-peptide complex with the TCR which is mutated.

Figure 2



Graph A displays the correlation between the day on which a sample was taken and the number of T cell epitope mutations accumulated in it, relative to Day 0, the first sample from the patient. The lines indicate the best fit line for each patient. Graph B displays a line graph of the correlation between time and the number of T cell epitope mutations for each patient. Graph C displays a best fit line for the correlation between time and the number of accumulated T cell epitope mutations across all patients.

The results have various implications. The accumulation of mutations in T cell epitopes could be deleterious, signifying that Tregs are allowing for viral persistence and latency, suppressing antiviral immunity. However, there could be benefits to this function, as Tregs may play a role in dampening pathogen-induced immunopathology. Both of these were observed with Tregs' role during neurotropic viruses infecting the CNS (Ciurkiewicz et al., 2020).

It is important to consider if the virus is simply accumulating mutations that happen to fall in T cell epitopes, with the background non-T cell epitope mutation rate similar to that of T cell epitope mutation rate. Evolutionary analysis has concluded that the GII.4 VP1 capsid gene evolves at a rate of 4.3 x 10-3 nucleotide substitutions/site/year (Bok et al., 2009). Taking into account the approximate genome size, the norovirus accumulates roughly 32 nucleotide substitutions per year. Thus, the accumulation of T cell epitope mutations could be due to T cell selection, or it could be random chance as previous literature has outlined independent mutation rates.

Supplementary Data Tables

Norovirus Cumulated Patient Data: Mutations in T Cell Epitopes Through Time
Table 3. Patient data displaying with each relevant variable site and its overlapping T cell epitope. Details including the HLA molecule, the start and end location of the epitope's codons, whether the variable site falls in the core peptide of the epitope, its position in the epitope and whether the variable site was mutated following day 0 (considered the consensus sequence) are tabulated.

Patient A raw data and table
Patient B raw data and table
Patient C raw data and table
Patient D raw data and table
Patient E raw data
Patient E table
Patient F raw data and table

Conclusion

Overall, it is clear that tissue Tregs showcase considerable phenotypic and functional heterogeneity, though the timing of these location-specific adaptations and the factors impacting them still remain unclear for many subsets. Much is still not known about Tregs and their roles compared to other immune cell types. There is a critical need to develop better methods to determine the functions and cell-cell interactions of Tregs within tissues. Systems that incorporate immune cells, such as organoids, are emerging, but are often complicated and expensive to run. Immortal cell lines also face challenges, unable to effectively replicate the behaviour of primary cells. Meanwhile, research regarding viruses is predominantly focused around antibodies, rather than T cells, much less Tregs. More studies must be conducted regarding how the phenotypes of Tregs evolve during immune responses to infection, tissue injury or during disease. A more comprehensive understanding of these knowledge gaps can help develop novel therapies for diseases, including more effective genetically engineered Tregs targeted at patients suffering from disease or infection in specific tissues.

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