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Review

T cell trafficking in human chronic inflammatory diseases

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SUMMARY

Circulating T cells, which migrate from the periphery to sites of tissue inflammation, play a crucial role in the development of various chronic inflammatory conditions. Recent research has highlighted subsets of tissue-resident T cells that acquire migratory capabilities and re-enter circulation, referred to here as "re-circulating T cells." In this review, we examine recent advancements in understanding the biology of T cell trafficking in diseases where T cell infiltration is pivotal, such as multiple sclerosis and inflammatory bowel diseases, as well as in metabolic disorders where the role of T cell migration is less understood. Additionally, we discuss current insights into therapeutic strategies aimed at modulating T cell circulation across tissues and the application of state-of-the-art technologies for studying recirculation in humans. This review underscores the significance of investigating T trafficking as a novel potential target for therapeutic interventions across a spectrum of human chronic inflammatory diseases.

INTRODUCTION

In this review, we aim to discuss two aspects of T cell recirculation: the transition from circulation to tissue residency, and the reverse process where tissue-resident memory T (Trm) cells re-enter the circulation. For clarity, we will refer to "recirculating T cells" as Trm cells that rejoin the circulation, and "circulating T cells" to identify classical circulating T cell subsets, such as central and effector memory T cells. We will address these aspects within the pathological contexts of various human chronic inflammatory diseases. Furthermore, we will present the latest data on drugs recently introduced into clinical trials and discuss novel compounds targeting T cell circulation. Finally, we will provide an overview of techniques used to identify recirculating T cells in human samples.

BIOLOGY OF T CELL RECIRCULATION

From the circulation to tissue residency

Tissue infection triggers the activation of naive T cells in secondary lymphoid organs through dendritic-cell mediated antigen-presentation. Following activation, naive T cells differentiate into different subtypes of T cells. Effector T cells infiltrate the tissue to eradicate the infection through cell lysis and secretion of TNF, IFN-γ, IL-2, IL-12, IL-27, and IL-33.[1] After pathogen clearance, most effector T cells undergo apoptosis, but a fraction remains in the tissue as tissue-resident memory T cells (Trm).[2] These Trm cells differ from central memory (Tcm) and effector memory (Tem) T cells in their functional properties and migratory patterns. While Tcm recirculate through lymphatic vessels, blood, and secondary lymphoid organs,[3] Tem mainly traffic between tissues and blood. Trm cells, on the other hand, activate an epigenetic program that enables tissue residency,[4] which is reflected in their transcriptional profile shared across tissues and distinct from circulating memory T cells.[5]

The RUNX3-TGF-β axis plays a pivotal role in establishing tissue residency for T cells.[6] This axis promotes tissue entry, upregulates adhesion molecules, and contributes to the downregulation of T-bet and Eomes transcription factors.[7–10] T-bet and Eomes are necessary for the differentiation into effector and circulating memory T cells, respectively.

Trm express low level of lymphoid-homing molecules CCR7, S1PR1, CD62L, and upregulate tissue adhesion molecules such as CD103, CD101, CRTAM, and retention marker CD69 which blocks S1PR1 and is required for preventing egress from tissues.[5,11] However, there is no consensus on tissue residency markers. Variable expression of adhesion molecules is observed in Trm population within a single tissue[4,12]; moreover CD69 expression is not restricted to Trm, being upregulated by activated T cells as well.[13] On the other hand, other markers such as CD103 are expressed only by a fraction of Trm and are not able to capture the whole realm of resident populations.[5,12] In addition to a core transcriptional signature specific to tissue localization, Trm cells possess the capacity to express tissue-specific markers, such as CCR9 required for gut homing.[14,15] This indicates that the regulation of tissue migration is not solely governed by the functional subset of T cells, but also influenced by the microenvironment of the tissue itself. For instance, heightened expression of CXCR5 characterizes Tfh-like T cell clusters in the tonsils, while skin-specific Trm clusters prominently display cutaneous lymphocyte antigen (CLA).[14]

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Furthermore, metabolism can alter the migratory abilities of T cells.[16,17] For instance, glycolysis is required for migration in CD4 T cells,[18] which can be inhibited by accumulation of sodium lactate in extracellular space. Also, CD8 T cell motility is negatively affected by lactate metabolites, but independently of glycolysis.[18] However, the net effect of lactate can be context-dependent, i.e., in obese mice, the deficiency of monocarboxylate transporter 1, a lactate transporter, results in a reduced infiltration of CD8 T cells in adipose tissue, likely due to the inhibition of cell proliferation.[19]

From tissue residency to recirculation

Trm cells play a surveillance role in tissues to ensure a rapid response upon reinfection. Although they have been considered a stable population permanently residing within the tissue,[20,21] mounting evidence recently indicate that Trm cells are heterogeneous and plastic, and for a fraction of Trms, tissue residency is not the ultimate fate.

Experiments utilizing lineage tracing and parabiotic mouse models have revealed intriguing dynamics of CD8 Trms. Upon antigen stimulation, CD8 Trms are dislodged from tissues and re-enter circulation between 8- and 30-day post-reinfection.[22–24] Furthermore, investigations involving the separation of cojoined mice post-LCMV infection have shown the presence of circulating ex-Trms in the blood even without secondary infection. Notably, Wijeyesinghe's work[25] indicates that ex-Trms comprise approximately 15–30% of all LCMV-specific memory T cells in peripheral blood 350 days post-infection. These findings suggest that under physiological conditions, Trms slowly exit tissues through a process termed retrograde migration. However, reinfection alters migration dynamics, prompting a more rapid recall response in circulation.

In line with this, secondary infection has been correlated with the downregulation of Hobit, a key regulator of tissue residency, in Trms. This observation is substantiated by the diminished expression of Hobit in ovoalbumin-specific T cells upon antigen exposure. Upon transition into circulation, ex-Trms manifest a distinct molecular profile compared to circulatory memory T cells. They exhibit reduced expression of Trm signature genes such as *Xcl1*, *Rgs1*, *Osgin1*, and *P2rx7*, while pathways associated with lymphocyte migration, including *S1pr1*, *S1pr5*, and *Klf2*, are upregulated. Notably, ex-Trms retain a degree of tissue-specificity, as evidenced by the persistence of CD103, CD49a, CD43, and gut residency marker CCR9, particularly in those originating from the small intestine.[22,23,25] Furthermore, ex-Trms in the bloodstream display heterogeneous expression of CD62L, indicative of potential plasticity in generating diverse memory T cell subsets. These insights underscore the dynamic nature of Trms and their responses to secondary infection.

The profile of circulating ex-Trms appears to undergo changes over time following secondary infection. In Behr et al.'s study,[23] conducted 60 days post-secondary infection, the majority of ex-Trms exhibited an effector phenotype, characterized by positivity for CX3CR1 and KLRG1 markers. Conversely, in Wijeyesinghe's work,[25] observed a different scenario at a later time point (350 days post-primary infection), where ex-Trms lacked expression of CD62L and KLRG1 markers, suggesting an enrichment in the Tem subset. However, the transition of ex-Trms from an effector profile to an effector memory one over time remains unclear. Fonseca et al. noted that 10 days post-infection, circulating Trms resemble an effector memory phenotype, lacking KLRG1 and CD62L expression. The observed variability in dynamics may stem from differences in animal models, tissue of origin, and type of infection. Nonetheless, coherence across various studies indicates that ex-Trms can give rise to a small fraction of circulating Tcm, as reported by Behr and Fonseca.[22,23]

The migration of Trm cells extends beyond circulation; they also accumulate in draining lymph nodes post-infection, while remaining absent in distant lymph nodes.[22,26] Resident T cells within lymph nodes express the resident marker CD69 and exhibit phenotypic similarity to Trm cells.

As discussed, Trm cells exhibit considerable heterogeneity both across and within tissues, with no consensus on definitive markers for their identification. Variable expression of resident markers CD103 and CD69 is observed among Trm populations, ranging from predominantly $CD103^{+}CD69^{+}$ Trms in the skin to a diverse mixture in the liver, reflecting their heterogeneous nature.[24] This heterogeneity extends to Trm functionality, plasticity, and migratory behavior. Skin-derived Trms, for instance, exhibit lower cytokine production, reduced Ki67 expression, and resistance to migration compared to their counterparts in the liver. Moreover, skin-derived Trm show reduced differentiation potential, being unable to populate spleen and liver and expand upon antigen stimulation.[24] In a parabiosis model, liver-derived Trms from partner mice were found in the host's liver, while skin-derived Trms remained exclusively in the donor mice, underscoring tissue-specific differences in migratory capacity.[24] The non-migratory nature of skin-derived Trms is further corroborated by findings from Jiang et al.[27] However, ex-Trms possess the ability to reseed tissues, as demonstrated by the localization of Trms in the spleen following the transplantation of liver sections containing antigen-specific Trms into naive mice.[23,24] Trm cells demonstrate a remarkable propensity to repopulate their tissue of origin, as evidenced by tracking ex-Trms, which exhibit a higher frequency in the small intestine compared to other organs.[22] In contrast to the skin Trms, characterized by marked stability and residency, lung Trms exhibit a different post-infection behavior. Instead of persisting in the tissue, lung Trms decline and migrate to draining mediastinal lymph nodes.[28] These diverse behaviors highlight the multifaceted nature of Trm cells and underscore the importance of considering tissue-specific factors in understanding their dynamics.

Data on retrograde migration in humans remain limited. A study on patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) suggests that Trms constitute a stable population, with approximately 50% of human cutaneous T cells being comprised of host cells even 10 years post-transplant.[29] However, in another study, a T cell population expressing the skin-resident marker LGALS3 was found circulating in a patient receiving allo-HSCT, though the data are insufficient to definitively conclude if Trms exit the tissue.[30] Notably, in a liver transplant study, no donor cells were detected in the blood years after the transplant, suggesting a potential lack of Trm recirculation from the liver.[31]

T cell trafficking and residency in chronic inflammatory diseases

T cell migration plays a pivotal role in various chronic inflammatory conditions, where the infiltration of T cells into tissues often initiates autoimmune damage, as observed in multiple sclerosis (MS), and contributes to the relapsing-remitting nature of inflammatory bowel diseases (IBD) and autoimmune arthritis. However, in other chronic inflammatory diseases, such as obesity and type 2 diabetes (T2D), the involvement of T cells and their migration patterns remains inadequately investigated and therefore poorly understood. Additionally, retrograde migration of Trms in these conditions has been largely overlooked.

In this section, we present the latest research findings concerning both circulating and recirculating T cells in chronic inflammatory diseases, with a focus on human data when available.

Multiple sclerosis

In MS, the infiltration of autoreactive T cells into the brain represents a crucial step in the pathogenesis of autoimmune damage, as extensively demonstrated in rodent models of experimental autoimmune encephalomyelitis (EAE).[32–34] This pathogenetic model find confirmation in human disease as evidenced by (i) increased T cell infiltration in acute and RR phases,[35] (ii) efficacy of natalizumab and fingolimod, targeting crucial molecules for T cell migration, in reducing the risk of progression in RRMS patients,[36,37] (iii) but limited benefits in patients with secondary progressive multiple sclerosis (SPMS).[38,39]

Recent genomic studies have identified key proteins involved in the entry of autoreactive T cells into the brain, organized into three functional modules: adhesion, chemotaxis, and egress. Within the adhesion module, genes such as *Itgae* (coding for α4-integrin, the target of natalizumab), *Fermt3* (intracellular binding protein of α4-integrin), and *Hsp90b1* (chaperone α4-integrin) play critical roles in T cell accumulation within the central nervous system (CNS), as demonstrated by genetic deletion studies in EAE models.[40] Analysis of the chemotaxis module CXCR3 revealed that TBX21 and GNAI2 modulate CXCR3 function, with TBX21 regulating its expression and GNAI2 mediating it's signaling in T cell migration.

S1PR1, a known regulator of egress, and GRK2, which affects diapedesis, were demonstrated to be relevant in the MS pathology, with GRK2 deletion impairing S1PR1 internalization. This is confirmed by the impaired internalization of S1PR1 when GRK2 is deleted in circulating CD4 T cells isolated from healthy donors. Moreover, the transcription factor ETS1 was identified as a negative regulator of T cell migration, as its deletion enhances CNS migration of autoreactive T cells by increasing responsiveness to cytokine signals and cytotoxic molecule production. In line with these findings, a positive correlation between the expression of *HSP90B1*, *GNAI2*, and *S1PR1* and the propensity to migration—defined as the frequency of overlapping clones between periphery and cerebrospinal liquid—was found in CD4 T cell clusters from MS patients, while *ETS1* expression negatively correlated.[40]

Once entered in CNS, T cells likely acquire a resident phenotype as the majority of T cells in MS lesions are positive for CD103. CD103 expression is highest in patients with acute or RRMS. Notably, patients entered progressive phase, show a reduced number of CD3 T cells and CD103 expression,[35] suggesting that Trms may exit the CNS. However, these data are insufficient to sustain that retrograde migration takes place in MS, this aspect should be investigated in mice with lineage tracing experiments and with a deeper profiling of T residency markers in human samples spanning different MS stages.

Inflammatory Bowel Diseases

IBD, including ulcerative colitis (UC), and Crohn's disease, are strongly associated with T cell recruitment to the gut, as demonstrated by successful clinical trials targeting molecules involved in gut entry by T cells such as CCR9 and α4 integrin.[41,42]

A single-cell study integrating RNA and T cell receptor (TCR) sequencing has revealed heightened T cell trafficking between peripheral blood and intestinal tissue in patients with UC. Specifically, a distinct cluster expressing both tissue residency markers (CD69, ITGAE, CD101, CCR6, and ITGA1) and circulating T cell markers (KLF2 and S1PR1), predominantly enriched in gut samples compared to peripheral blood, exhibited clonal expansion in UC patients. Furthermore, this cluster diverged from other Trms due to elevated expression of genes encoding inflammatory molecules and cytolytic granules.[43] These findings suggest not only the migration of T cells from peripheral blood to the inflamed gut but also the presence of T cell subsets with intermediate characteristics between Trm and circulating Tem cells. These subsets may exist in both physiological and pathological contexts, recirculate back to the circulation, and potentially exert detrimental effects in IBD patients due to their pro-inflammatory phenotype.

Furthermore, it is plausible to hypothesize that extra-intestinal manifestations frequently observed in IBD patients could result from retrograde migration of autoreactive T cells primed in the gut. However, it remains possible that other mechanisms, such as antigen dissemination or migration of activated dendritic cells, contribute to these manifestations.[44]

Autoimmune arthritis

In murine models of rheumatoid arthritis (RA), Trm cells within inflamed joints are characterized as a strictly resident population, unresponsive to CCL21-stimulated egress. These Trms mediate arthritis flare in murine models of antigen-mediated joint inflammation by recruiting lymphocytes from the circulation.[45] Retention of infiltrating lymphocytes in the synovia is sustained by expression of the lactate transporter, whose metabolism reduces the migratory potential of T cells.[18] Evidence suggests that preventing joint inflammation recurrence with S1P inhibitor treatment, which blocks T cell circulation, underscores the role of Trms in recruiting circulating T cells, likely facilitated by the high production of the CCL5 chemokine.[45] Although preclinical models suggest that Trm cells primarily act locally and do not migrate

into other joints, studies in patients with juvenile idiopathic arthritis (JIA) have revealed intriguing insights. Clonally expanded TCR-β clones from both CD4 and CD8 T cell subsets, sharing identical amino acid sequences, are present in both left and right joints within the same patient, suggesting the possibility of T cell recirculation in joint inflammation.[46,47]

In ankylosing spondylitis (AS), a subpopulation of T cells with a Trm phenotype and expression of gut-associated markers is found in the synovial fluid from AS patients.[48] AS patients frequently suffer from IBD,[49] suggesting that gut-originating Trms may migrate to joints, contributing to tissue damage.

Psoriatic arthritis (PsA), often occurring as an evolution of psoriasis,[50] exhibits increased Th17 cell frequency in the blood and synovial fluid.[51,52] Considering the established role of autoreactive Trm CD8 T cells in psoriasis,[53] it is hypothesized that joint manifestations in PsA may result from retrograde migration of ex-Trms exiting the skin and infiltrating the joints. This hypothesis is supported by the finding of a circulating T cell subset, enriched in PsA patients compared to those affected only by psoriasis, featuring residency molecules and the CCR10 skin marker.[54]

Metabolic disorders: Obesity, type 2 diabetes, non-alcoholic steatohepatitis, and cardiovascular disease

Recent evidence suggests that T cell recirculation may play a role in non-autoimmune-mediated metabolic inflammatory conditions, including obesity, T2D, and non-alcoholic steatohepatitis (NASH). T cells isolated from human visceral fat tissue (VAT) exhibit phenotypic alterations in obese individuals,[55–57] with dysglycemia correlating with a partially exhausted phenotype.[57] Furthermore, T cell abnormalities in T2D extend to migratory properties. In dysglycemic obese patients, a subset of PD-1$^+$ CD4 Tconv cells demonstrated an increased rate of recirculation between peripheral blood and VAT compared to BMI-matched normoglycemic individuals, with a preferential expansion of recirculating clones in peripheral blood. Moreover, peripheral blood-derived PD-1$^+$ CD4 Tconv cells exhibited heightened expression of *ITGAX* and *ITGAV* genes, both associated with motility functions, and downregulation of *ITGA1*, a tissue residency marker. Notably, PD-1$^+$ CD4 T cells were found to be specifically enriched in the liver of dysglycemic patients with NASH compared to normoglycemic NASH patients.[57] Another study reported increased TCR clone recirculation in a T2D patient, showing a higher number of shared TCR sequences among CD8 and CD4 Tconv cells derived from peripheral blood, spleen, pancreas, and mesenteric/inguinal lymph nodes.[58] These findings collectively suggest that T cells in T2D possess migratory potential and tend to traffic between various tissues, with implications for metabolic dysregulation that require further elucidation.

In the context of NASH, characterized by elevated frequency of Th1 cells in both the bloodstream and liver,[59–61] T cell migration from gut to liver has been demonstrated in a preclinical model. Indeed, a subset T cells expressing gut-associated markers was found in the liver of NASH patients.[54] Furthermore, this gut-to-liver migration was found to be induced by local interactions with B cells.[62] Additionally, patients with NASH displayed T cell clusters with a more circulating phenotype than those with metabolic dysfunction-associated steatotic liver disease (MASLD), indicating that increased recirculation of T cells is associated with disease severity.[63]

A study conducted by Chowdhury RR et al.[64] on human samples revealed that aortic plaques are infiltrated by antigen-experienced CD8 T cells lacking SELL and CCR7 expression. Furthermore, aortic plaques showed an enrichment of clones specific for EBV, CMV, and Influenza epitopes compared to blood. The authors conclude that the accumulation of virus-specific T cells may be driven by antigen-specific stimuli likely due to molecular mimicry of viral antigens and human proteins highly expressed in the heart. In contrast to findings in T2D diabetes, where PD-1$^+$ CD4 T cells are expanded in the peripheral blood,[57] the clonality of clonotypes shared between blood and aortic plaques is comparable. Notably, in two patients, a small fraction of shared clones was found highly expanded in both sites. This suggests that CD8 T cells may circulate between tissues in response to antigens, whether this is triggered by viral infections or self-antigens in an inflammatory milieu, is yet to be explored. Figure 1 illustrates T cell development, localization, and recirculation in health and chronic inflammatory conditions.

Pharmacological targeting of T cell trafficking in chronic inflammatory diseases

The crucial role of T cell migration in different inflammatory diseases has spurred the development of multiple drugs targeting distinct mechanisms involved in T cell trafficking, providing valuable therapeutics tools. Circulating and recirculating T cells express the same surface markers that enable them to move through the bloodstream. Similarly, Trm cells and recirculating ex-Trm cells express the same tissue-homing markers. As described in the previous chapter, there are no unique markers or migration patterns of cells involved in inflammatory diseases; therefore, most pharmaceutical agents lack specificity, being effective on both circulating and recirculating T cells. Although several compounds that have proven efficacy and safety in chronic inflammatory diseases are currently available on the market or under experimentation, little is known about their specificity for T cell subsets. Below, we collect the most recent literature on clinical trials (Table 1) targeting key molecules in T cell migration and discuss the potential of these drugs in the context of recirculation.

S1PRs

Spingosine-1-phosphate (S1P) is a key molecule that regulates the egress of T lymphocytes from secondary lymphoid organs (SLOs) and guides their migration through the bloodstream into inflamed tissue.[65] Several molecules have been developed in the attempt to suppress the recruitment of T lymphocyte to inflammatory sites by inhibiting the S1P/S1PR axis.[66] S1PR modulators constitute a class of drugs that act as agonists binding to the S1P receptor (S1PR), thereby triggering its internalization and subsequent degradation,[67] consequently affecting T cells trafficking. The first S1PR modulator approved by FDA and EMA, in 2010 and 2011, respectively, was fingolimod (FTY720). Fingolimod is a non-selective S1PR inhibitor, acting on S1PR1, S1PR3, S1PR4, and S1PR5, and is currently indicated for the treatment of relapsing-remitting

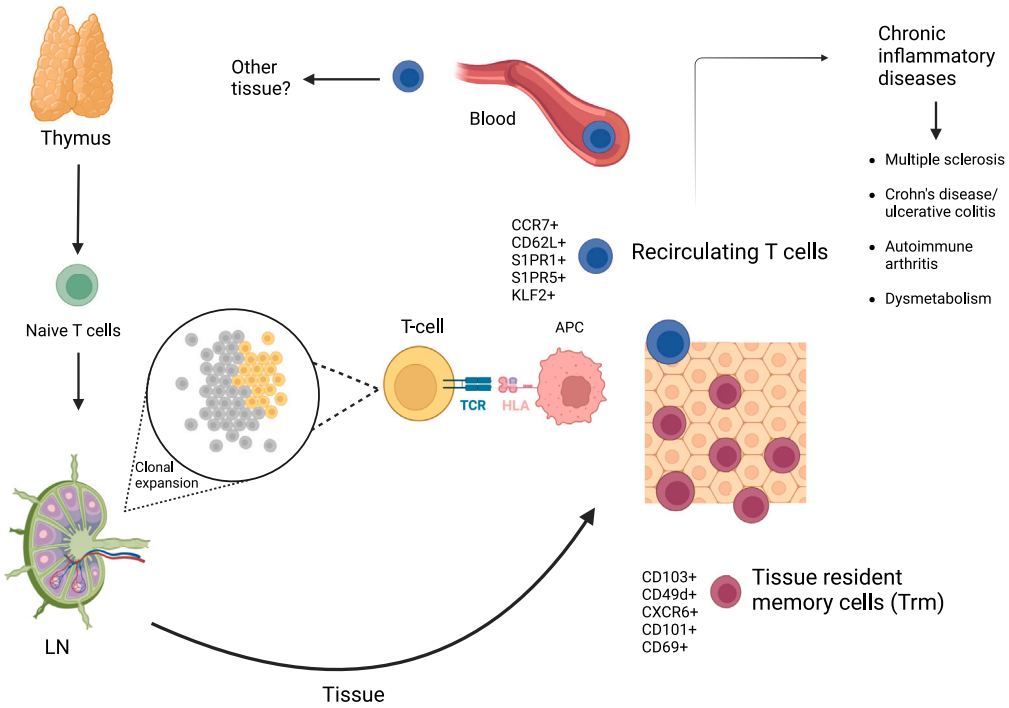

Figure 1. Human T cell development, localization and recirculation in health, and chronic inflammatory conditions

The process initiates in the thymus, where naive T cells are produced. Subsequently, in the lymph nodes, clonal expansion occurs alongside the interaction between T cell receptors (TCR) and antigen-presenting cells (APCs). Upon exiting the lymph nodes, T cells migrate to various tissues, where tissue resident memory T cells (Trm) are formed, distinguished by the expression of specific markers (CD103+, CD101+, CD49d+, CXCR6+, CD69^{+}). Some of the cells decrease the expression of resident markers and acquire a circulating phenotype featured by the expression of CCR7, CD62L, S1PR1, S1PR5, and KLF2, facilitating the re-entry into the bloodstream. Once re-entering the bloodstream, ex-Trms have the ability to differentiate into effector memory and central memory cells, and can migrate to various tissues, displaying distinct homing markers and capacities based on their tissue of origin. Enhanced T cell trafficking between the bloodstream and tissues is observed in chronic inflammatory conditions such as MS, IBD, autoimmune arthritis, and metabolic disorders.

MS. However, the clinical use of fingolimod have been associated with a number of adverse events, including infections,[68] nervous system disorders,[69] and heart diseases.[70] The lack of selectivity of fingolimod has driven the search for safer alternatives through the development of selective S1RP1/S1PR5 modulators. This led to the introduction of siponimod, ozanimod, and ponesimod, all of which have received the approval for the treatment of MS.[71–73] Siponimod and ozanimod are inhibitors of S1PR1 and S1PR5, with ozanimod more recently approved for the treatment of UC.[74] The latest S1PR inhibitor obtaining the approval for the treatment of MS is ponesimod, a selective inhibitor for S1PR1.[72] Administration of ponesimod has demonstrated beneficial effects also in patients with moderate to severe chronic plaque psoriasis, leading to reduction in psoriasis area and severity index scores (PASI).[75] Cenerimod, a novel selective S1PR1 receptor modulator, has shown an acceptable safety profile and potential efficacy in treating patients with systemic lupus erythematosus in a phase II study,[76] while the phase III trial is still ongoing (ClinicalTrials.gov ID NCT05648500). In two phase III clinical trials, etrasimod, an inhibitor of S1PR1, S1PR4, and S1PR5, demonstrated effectiveness and safety in treating moderate-to-severe UC through daily administration of a single oral dose. Patients achieved clinical remission in both induction and maintenance therapy, leading to approval for the treatment of UC by FDA in 2023 and by EMA in 2024.[77]

The efficacy of all S1PR modulators is likely due to the blockade of circulating T cells, which can no longer infiltrate target tissues and trigger disease. This nonspecific mechanism of action is supported by evidence of reversible lymphopenia observed in patients treated with all S1PR modulators.[78] Furthermore, the use of fingolimod has been associated with a predominant reduction in circulating naive and central memory T cells[79,80], as well as significant changes in T cell composition, including a reduction in the frequency of circulating IFN-γ-producing T cells and an increase in Treg.[81]

Expression of S1PRs is a key process involved in Trm retrograde migration, and S1PR modulators represent a potential therapeutic strategy for the inhibition of this process. However, the side effects related to the impairment of all circulating lymphocytes need to be further investigated.

Integrins

Integrins are transmembrane heterodimeric adhesion receptors that, upon activation, regulate various T cell migration processes, including adhesion, migration speed, and tissue retention. They represent crucial targets for the treatment of autoimmune diseases such as psoriasis,

Table 1. Pharmacological compounds targeting T cell recirculation in chronic inflammatory diseases

Molecules	Target	Disease	Trial phase	NCT	Status
Fingolimod	S1PR1, S1PR3, S1PR4, S1PR5	Multiple sclerosis	On the market		
		Type 2 diabetes	Phase IV	NCT05307731	Recruiting
Siponimod	S1PR1, S1PR5	Multiple sclerosis	On the market		
		Dermatomyositis	Phase II	NCT02029274	Terminated[a]
		Polymyositis and Dermatomyositis	Phase II	NCT01148810	Terminated[b]
Ozanimod	S1PR1, S1PR5	Multiple sclerosis and ulcerative colitis	On the market		
		Crohn's disease	Phase II/III	NCT05470985	Recruiting
			Phase III	NCT03467958	Recruiting
			Phase III	NCT03464097	Recruiting
			Phase III	NCT03440385	Completed
			Phase III	NCT03440372	Recruiting
Ponesimod	S1PR1	Multiple sclerosis	On the market		
		Psoriasis	Phase II	NCT01208090	Completed
		Plaque psoriasis	Phase II	NCT00852670	Completed
		Chronic Graft-Versus-Host Disease	Phase II	NCT02461134	Terminated[b]
Etrasimod	S1PR1, S1PR4, S1PR5	Ulcerative colitis	On the market		
		Atopic dermatitis and eczema	Phase II/III	NCT05732454	Active, not recruiting
		Crohn's disease	Phase II/III	NCT04173273	Recruiting
		Alopecia areata	Phase II	NCT04556734	Completed
		Eosinophilic esophagitis	Phase II	NCT04682639	Completed
		Primary biliary cholangitis	Phase II	NCT03155932	Terminated[c]
Cenerimod	S1PR1, S1PR5	Systemic lupus erythematosus	Phase III	NCT05672576	Recruiting
			Phase III	NCT05648500	Recruiting
Natalizumab	α4	Multiple sclerosis and Crohn's disease	On the market		
		Rheumatoid arthritis	Phase II	NCT00831649	Terminated[a]
			Phase II	NCT00083759	Terminated[a]
		Acute graft-Versus-Host Disease	Phase II	NCT02133924	Completed
Vedolizumab	α4β7	Ulcerative colitis and Crohn's Disease	On the market		
		Type 1 diabetes	Phase I	NCT05281614	Recruiting
		Graft Versus Host Disease	Phase II	NCT05132166	Active, not recruiting
Abrilumab	α4β7	Ulcerative colitis	Phase II	NCT01694485	Completed
		Crohn's disease	Phase II	NCT01696396	Completed
AJM300	α4	Ulcerative colitis	Phase III	NCT03531892	Completed
Etrolizumab	β7	Crohn's disease	Phase III	NCT02403323	Terminated[d]
			Phase III	NCT02394028	Completed
		Ulcerative colitis	Phase III	NCT02165215	Completed
			Phase III	NCT02171429	Completed
			Phase III	NCT02163759	Completed
			Phase III	NCT02136069	Completed
			Phase III	NCT02100696	Completed

Compounds targeting S1PRs, integrins and CCL20 are reported, including their latest advancement phase studies for each chronic inflammatory disease. References to clinical trial are indicated by the NCT, only for molecules that are not yet on the market. Completed indicates that the study is concluded; terminated indicates that the study has been stopped prematurely.

[a]Terminated as the study did not provide sufficient efficacy.

[b]Terminated due to enrollment challenges.

[c]Terminated due to sponsor's decision.

[d]Terminated due to program discontinuation, based on mixed efficacy results in the parent studies.

MS, IBD, RA, and lupus erythematous.[82] Only two molecules are approved as anti-integrin therapies, but this number is expected to increase with the molecules currently being tested in clinical trials. Natalizumab was the first approved anti-integrin agent in 2004 for the treatment of MS and Crohn's disease. It is a recombinant humanized IgG monoclonal antibody that binds the α4 subunit, thus blocking integrin α4β1 implicated in MS and α4β7 involved in IBD.[83] Despite reducing T cell trafficking, natalizumab induces a proinflammatory profile in recirculating T cells,[84] implying that synergistic strategies are needed in addition to blocking recirculation for chronic inflammatory diseases. Furthermore, its use has been restricted due to the risk of developing progressive multifocal leukoencephalopathy,[85] a risk non observed with vedolizumab.[86] Vedolizumab is a monoclonal antibody against α4β7 that selectively inhibits lymphocytes recruitment in the intestine, without affecting the central nervous system. It has proven effective in inducing and maintaining remission in UC and Crohn's disease.[87,88] Similarly, abrilumab, an antibody targeting α4β7, induced remission in patients with moderate to severe UC based on data from a phase IIb trial.[89] In a phase III clinical trial, the α4 integrin antagonist AJM300 emerged as an alternative for patients with UC with inadequate response to mesalazine.[90] Etrolizumab, an anti-β7 subunit antibody, was initially considered a candidate for IBD treatment due to its ability to bind both α4β7 and αEβ7. However, clinical data regarding its efficacy in UC are contradictory; while the HICKORY and HIBISCUS I studies showed that etrolizumab is effective in inducing remission, this was not confirmed by the HIBISCUS II trial.[91,92] Furthermore, etrolizumab did not improve the remission of patients during the maintenance phase.[91,93] Controversially, in Crohn's disease, etrolizumab seems to induce clinical remission during maintenance but not during induction phase.[77]

Overall, strategies aimed at blocking integrins have shown efficacy in the treatment of MS and IBD by inhibiting the homing of pathogenic circulating T cells. However, recent evidence suggests that T cells expressing gut-homing molecules could reach the CNS and play a pathogenic role in MS.[94,95] Supporting this hypothesis, treatment of relapsing-remitting MS patients with natalizumab increased the proinflammatory phenotype of memory CD4 T cells expressing the β7 integrin. These cells are likely recirculating cells primed in the gut and may be responsible for the rebound effect observed after discontinuation of natalizumab.[84]

CCL20

Chemokines are small polypeptides that bind to chemokine receptors on leukocytes and induce their directional movement. Elevated expression of the chemokine CCL20 has been observed in chronic inflammatory skin disorders such as psoriasis and atopic dermatitis, as well as in the colon of patients with IBD.[82,96,97] CCL20 may also play a role in MS, RA, and AS, suggesting that strategies aimed at blocking this chemokine or its receptor could hold therapeutic promise across various diseases.[98–101] Only a few compounds have been identified with the ability to block CCL20. One such compound is 18β-glycyrrhetinic acid, derived from the glycyrrhetinic acid of licorice. In a mouse model of psoriasis-like skin lesions, 18β-glycyrrhetinic acid was found to be effective in improving skin lesions by downregulating the transcription factor ATF2, resulting in reduced CLL20 levels in lesioned epidermis.[102]

Another molecule developed to inhibit CCL20 is GSK3050002, an anti-CCL20 antibody. While it was well tolerated in a phase I clinical trial, a subsequent toxicology study in cynomolgus monkey revealed vascular and organ inflammation.[103,104] An alternative approach to blocking CCL20 involves the inhibition of its receptor CCR6. Through in silico and *in vitro* functional assays a promising molecule acting as CCR6 antagonist has been identified. Study in a mouse model of colitis showed an improvement in general conditions and attenuation of macroscopic injuries, while its dual administration in a model of peritonitis exhibited an anti-inflammatory effect.[105]

The identification of new agents that can inhibit the interaction between CCL20 and its receptor CCR6 may represent a strategy to block the recirculation of T cells with intermediate properties between Trm and circulating Tem, as observed in UC patients.

New therapeutic approaches

Given the pivotal role of chemokine receptors and their ligands in T cell trafficking, targeting CCR9 and CCR4 holds promise in the management of IBD. CCR9 serves as a gut-specific homing molecule, binding to CCL25 expressed in the small intestine.[106] Although a CCR9 antagonist, vercirnon, was tested for Crohn's disease treatment, it did not meet efficacy expectations in a phase III trial.[107] Currently, a new humanized monoclonal antibody, AZD7798, targeting CCR9, is undergoing phase I testing (ClinicalTrials.gov ID NCT05452304, NCT06053424).

CCR4 is another chemokine receptor that has the potential to be a therapeutic target for skin diseases, including atopic dermatitis and psoriasis.[108] Selectively expressed by memory T cells, CCR4 contributes to T cell recruitment in inflamed skin. However, clinical investigation of zelnecirnon, a CCR4 antagonist, has been halted by the FDA in two phase II trials due to suspected liver failure, currently under investigation.[109]

Novel strategies targeting T cell recirculation may involve GTPase mitochondrial dynamin-related protein 1 (DRP1). DRP1 plays a role in various cellular processes, including T cell migration and survival.[110] The first developed DRP1 inhibitor, the mitochondrial division inhibitor mdivi-1, has shown beneficial effects on the vascular system and neuroprotection.[111,112] However, due to off-target side effects, research is ongoing to identify new DRP1 inhibitors.[113] Recently, Rosdah et al. identified a small molecule called DRP1i27, which selectively binds to human DRP1 isoform 3.[114] Nevertheless, given the broad spectrum of DRP1's actions, precise clinical evaluation of potential adverse effects is imperative.

Pharmaceutical agents targeting molecules involved in T cell migration represent a promising approach to treating various inflammatory diseases centered on T cell trafficking. For many of these agents, extensive research in preclinical models and human patients has led to significant advancements in clinical practice. However, the role of retrograde migration of Trm cells remains largely unexplored in the context of inflammatory diseases, as it has been recently discovered in mice and studied primarily in the resolution of acute infections. Additionally,

ex-Trms acquire intermediate features between resident and circulating T cells, making it challenging to clearly differentiate these subsets. Nevertheless, in certain diseases it is possible to identify recirculating T cells in the blood, as seen with gut-homing markers like CCR9. Ancillary studies of clinical trials aiming to assess the role of recirculation in inflammatory diseases should be designed using the techniques presented in the next section.

Techniques for identifying recirculating T cells in human blood and tissue

Labeled antibodies techniques for multiplexed single-cell analysis

The identification of recirculating T cells represents a challenge, as ex-Trm express genes and proteins associated to circulation, while retaining the expression of tissue residency markers. To overcome this obstacle, techniques relying on advanced single-cell phenotyping, such as mass cytometry and flow cytometry for the simultaneous detection of multiple markers can be employed.

Mass cytometry, or cytometry by time-of-flight (CyTOF), is an immunological technique enabling the simultaneous analysis of more than 40 cellular markers per single cell. Unlike flow cytometry, which relies on fluorophore-labeled antibodies, mass cytometry utilizes heavy-metal labels, thus reducing spectral overlap issues and improving the accuracy of markers quantification. This distinction allows mass cytometry to circumvent the limitations of flow cytometry associated with color overlap.[115] However, flow cytometry boasts faster acquisition rates and better preservation of cell integrity for subsequent analyses. Its drawbacks include constraints in multiplexing capabilities and the stability of fluorophores.

Both mass cytometry and flow cytometry techniques utilize antibodies targeting cell markers to identify circulating, resident or recirculating T cells including CD69, CD62L, S1PR1, CD103, CD49d, CD101, CXCR3, CXCR6 (described in Chapter 1).[116] These markers enable the determination of the frequency and phenotype of these cells. For instance, a recent study employing mass cytometry investigated the turnover of CD4 T cells in HIV-1-infected patients who initiated antiretroviral therapy during the acute or chronic phase of infection. Through the study of T cell subgroups, the heterogeneity of immune cells in the blood was assessed, revealing clusters of CD4 T cells with low expression of CD45RA but high expression of CD27, CD28, CD127, and CD44, indicative of memory cells migrating to inflamed tissues. Additionally, cell clusters highly expressing the T cell trafficking molecule CXCR3, which directs T cells to inflamed tissue, were also identified (10.1172/jci.insight.125442).[116]

Both technologies enable single-cell detection of T cell dynamics in human circulation and tissues. The choice between mass cytometry and flow cytometry depends on factors such as the number of specific markers needed for detection, user-friendliness, instrument availability across institutes, and experimental workflow preferences.

Single-cell sequencing of transcriptome and T cell receptor

Single-cell transcriptome profiling (scRNA-seq) has emerged as a revolutionary technique, particularly impactful in immunology, enabling unbiased exploration of T cell diversity in health, disease, and response to therapies at an unprecedented resolution. This technique allows for the gene expression analysis of thousands of individual cells from a large heterogeneous population, including cells isolated from tissue biopsies. It reveals individual cell functions within their microenvironment, providing a novel perspective that enables clear distinction between recirculating and non-recirculating cells. Through scRNA-seq, signature genes of recirculating T cells can be enriched in different cell clusters, facilitating the identification and detailed profiling of unique cell subsets. Sets of genes highly expressed in tissue-resident vs. non-resident memory T cells, including elements associated with microtubules (genes coding for tubulin TUBA1A, TUBA1B, TUBB, TUBB4B; S100A4), cytoskeleton, cell matrix, membrane scaffold, and adhesion molecules (VIM or vimentin, galectins LGALS1/LGALS3, AMICA1, ITM2C, EZR, annexins ANXA1/ANXA2), have been identified in the context of cancer.[117] Such findings suggest that the localization of T cells in tissues likely involves structural changes in the cell that facilitate interactions with the tissue matrix—a feature less pronounced in blood-resident cells that express these genes only in trace amounts. Beyond characterization, scRNA-seq has enabled high-resolution mapping of cellular heterogeneity, development, and activation states across different systems, hinting at its potential application in distinguishing resident from (re)circulating T cells. This method has been instrumental in discerning clusters of circulating and resident T cells within skin lesions of patients with systemic sclerosis (SSc), shedding light on the direct involvement of T cells in vascular damage. In contrast to skin samples from healthy controls, which predominantly consist of Trm clusters characterized by broad expression of CD69 and variable expression of ITGAE, biopsies from SSc patients exhibit an enrichment in memory subsets expressing circulation-associated genes such as CCR7, SELL, KLF2, S1PR1, and the skin-homing receptor SELPLG (CLA).[118] A computational framework called ProjecTILs[119] was devised for projecting new scRNA-seq data onto existing reference atlases. This method enables the characterization of previously unknown cell states that "deviate" from the reference, thus facilitating the identification and characterization of new cell states. The validity of ProjecTILs to date has only been assessed on mice implanted with cancer cells, but the presence of well-founded data lays the groundwork for extending this framework to humans, creating subsets to differentiate resident T cells from recirculating ones, and promises to advance our understanding of T cell dynamics in different biological contexts and environments.

Cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) serves to further enhance and fortify the results obtained from scRNA-seq. In addition to transcriptomic data, CITE-seq can capture protein information at the single-cell level through the use of specific antibodies, thus furnishing more robust insights for categorizing T cell subsets. This approach has proven particularly valuable in discerning memory T cell subsets, as scRNA-seq struggles to profile CD45RA effectively due to the short reads generated by next-generation sequencing.[120] Another promising approach for distinguishing resident and non-resident T cells involves integrating single-cell RNA

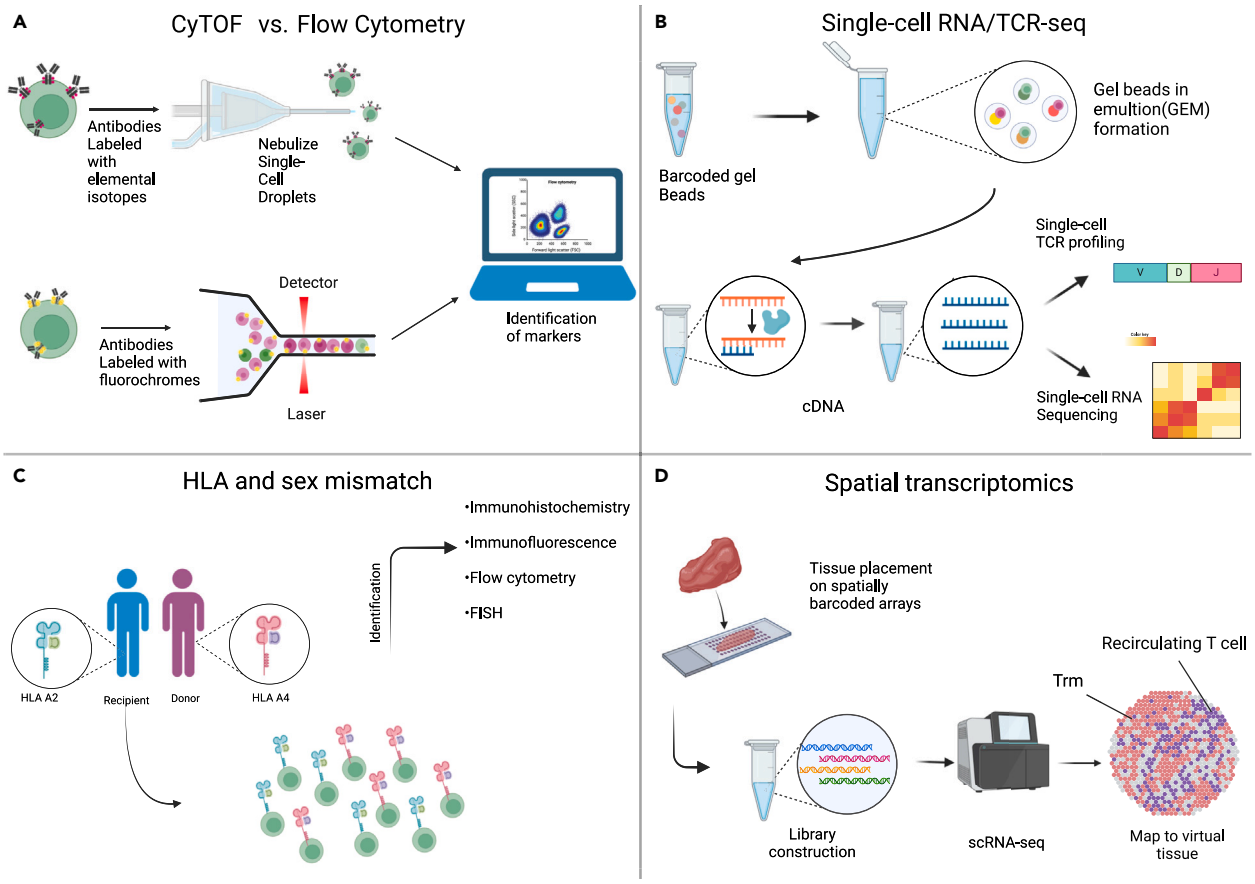

Figure 2. State-of-the-art technologies for identifying recirculating T cells in human blood and tissue

(A) Labeled antibodies techniques for multiplexed single-cell analysis include mass cytometry, utilizing heavy metal labels, and flow cytometry, which employs fluorophore-labelled antibodies. Both technologies enable the identification of cell clusters, each characterized by a homogeneous profile of markers.

(B) Single-cell transcriptome and TCR profiling (scRNA-seq) involves labeling cells with cytometric beads, followed by the conversion of RNA populations into a library of cDNA fragments. These fragments are then sequenced using high-throughput next-generation sequencing techniques, and the reads are mapped to the reference genome. The identification of shared TCRs between different tissues enables the tracking of recirculating T cell clones.

(C) HLA and sex chromosomes mismatch analysis allows for the investigation and tracking of T cells in patients undergoing organ or allogeneic hematopoietic stem cell transplantation, facilitating the study of host- and donor-derived immune cell populations separately. Techniques such as immunohistochemistry, immunofluorescence, flow cytometry, and FISH are employed for this purpose.

(D) In spatial transcriptomics, the tissue is placed on spatially barcoded arrays, after which libraries are created, sequenced, and a virtual map of the starting tissue is obtained.

sequencing with TCR sequencing. This method allows to identify T cells trafficking across tissues based on the homology of CDR3beta sequences found in T cells from different districts within the same individuals. The directionality of the movement can be inferred by analyzing the preferential expansion of a specific between various tissues. In a recent study utilizing bulk RNA-seq and TCR-seq, we demonstrated an increased recirculation of PD-1-expressing CD4 T cells from adipose tissue to blood in obese patients with dysglycemia.[57] However, to delineate the specific phenotype of recirculating vs. non-recirculating cells, scRNA/TCR-seq analysis would have been necessary. Exploring this technique in the investigation of T cell recirculation between target organs affected by human chronic inflammatory diseases holds significant promise.

Donor-recipient HLA and sex mismatch in transplantation

HLA mismatch analysis in organ or hematopoietic stem cell transplantation serves as a valuable tool for studying T cells, enabling the identification of different subsets of memory T cells, such as recirculating and resident T cells. By investigating the fate of memory T cells in patients undergoing allo-HSCT, researchers can effectively study host- and donor-derived immune cell populations separately. This methodology allows for the differentiation between recirculating and resident cells based on phenotypic markers. Immunohistochemistry techniques can detect HLA mismatch by using antibodies against mismatched HLA antigens, thus discriminating between donor and recipient T cells. For instance, in a study tracking T cells from HLA-mismatched donors in patients undergoing complete

facial skin transplants, the anti-Bw4 antibody identified the HLA-B Bw4 allele antigen expressed by donors, while the anti-B7 antibody detected the HLA-B B7 allele antigen expressed by recipients. Subsequently, double-labelling immunofluorescence was employed to complement immunohistochemistry, enabling the dual-channel identification of epitopes co-expressed at similar or overlapping subcellular locations.[121]

Flow cytometry can also differentiate recipient and donor T cells. In a study of patients with type 1 diabetes following pancreatic-duodenal transplantation, HLA mismatch was assessed using HLA type I allo-type-specific antibodies targeted to donor- and/or recipient-derived cells.[122]

Additionally, fluorescence *in situ* hybridization (FISH) offers insight into distinguishing memory T cells. Data from skin and blood samples of individuals with cutaneous graft-versus-host disease (GVHD) demonstrated the determination of donor- and host-derived T cells using automated quantification of X- and Y chromosomes. This involved X/Y chimerism analysis on skin cryosections combined with surface marker labeling, followed by incubation with a FISH probe.[29]

To conclude, by employing HLA mismatch analysis in the context of organ or cell transplantation, researchers can obtain a comprehensive understanding of T cell subsets, thereby discriminating resident T cells from circulating ones more effectively.

Spatial transcriptomics

Understanding the spatial distributions of T cell clonotypes within tissues is essential for comprehending their behavior, fate, and dynamics in health and disease. Spatial VDJ sequencing represents a cutting-edge methodology capable of mapping TCR sequences within human tissue, addressing the critical need to preserve spatial information often lost during tissue dissociation into single-cell suspensions.[123] Even more groundbreaking is the emerging slide-TCR-seq technique, which facilitates the sequencing of transcriptomes and TCRs with a spatial resolution of 10 μm. This approach not only allows for the exploration of complex spatial relationships among T cell clonotypes but also among other cell types within healthy and diseased tissues. Consequently, it sheds light on gene expression dynamics in contexts related to residency and recirculation.[124] These advancements in spatial genomics provide a promising avenue for gaining deeper insights into the intricate interplay between immune cells and their microenvironment. Figure 2 describes the state-of-the-art techniques for the identification of recirculating and resident T cells.

Conclusions

This review provides a comprehensive exploration of T cell (re)circulation in chronic inflammatory diseases in humans, shedding light on its crucial role in disease progression and relapse. Insights from clinical trials underscore the therapeutic potential of targeting molecules like S1PRs, integrins, and CCL20 to modulate T cell recruitment. The recent demonstration of retrograde migration of Trms, though minimally explored in chronic inflammatory diseases, merits further investigation due to its potential role in propagating pathology across tissues. Of particular interest is the role of T cell recirculation in metabolic disorders, where understanding T cell-mediated crosstalk between insulin-sensitive tissues could lead to significant advancements in treating prevalent conditions like obesity and T2D. Advanced techniques such as single-cell analysis and spatial transcriptomics offer promise in deepening our understanding of T cell dynamics. However, further exploration of innovative and accessible methodologies is needed to advance T cell tracking during clinical trial monitoring phases.

AUTHOR CONTRIBUTIONS

A.G., V.C., and M.P. performed literature search, drafted and edited the manuscript. A.P. conceptualized the manuscript, reviewed its progressive versions, and provided constructive feedback. All the authors gave their final approval to the version submitted for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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