Synovial tissue macrophages in joint homeostasis, rheumatoid arthritis and disease remission

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Abstract | Synovial tissue macrophages (STMs) were principally recognized as having a pro-inflammatory role in rheumatoid arthritis (RA), serving as the main producers of pathogenic tumour necrosis factor (TNF). Recent advances in single-cell omics have facilitated the discovery of distinct STM populations, providing an atlas of discrete phenotypic clusters in the context of healthy and inflamed joints. Interrogation of the functions of distinct STM populations, via ex vivo and experimental mouse models, has re-defined our understanding of STM biology, opening up new opportunities to better understand the pathology of the arthritic joint. These works have identified STM subpopulations that form a protective lining barrier within the synovial membrane and actively participate in the remission of RA. We discuss how distinct functions of STM clusters shape the synovial tissue environment in health, during inflammation and in disease remission, as well as how an increased understanding of STM heterogeneity might aid the prediction of clinical outcomes and inform novel treatments for RA.

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Tissue macrophages are ancient innate immune cells of embryonic or adult bone-marrow origin. Macrophages have various tissue-specific roles that facilitate tissue homeostasis, including engulfment and destruction of pathogens, such as those present in the lung airways during gas exchange. Macrophages also have important roles in the inflammatory response, serving to initiate and then contain sterile injury through the promotion and subsequent resolution of inflammation to re-instate tissue immune-homeostasis[1]. In response to instructive signals, such as tissue damage, resident macrophages are assisted by tissue-infiltrating monocyte-derived macrophages that mediate inflammation. The interplay between tissue-resident and tissue-infiltrating macrophages determines the chronicity of tissue inflammation and the quality of tissue repair[2].

Rheumatoid arthritis (RA) is a common, chronic, inflammatory autoimmune disease that is characterized by articular inflammation, often with subsequent progressive joint damage and disability[3]. Several seminal clinical observations provided the first evidence for a key contribution of macrophages to RA pathogenesis. These included the positive association between the abundance of synovial tissue macrophages (STMs) and the extent of joint damage[4], and the positive association between a high disease activity score in local joints and the abundance of macrophages in the synovial sublining layer[5]. The expression of TNF and IL-6 is also associated with the severity of joint pain[5], and until recently, STMs were principally recognized as the main producers of these pro-inflammatory molecules[6]. However, recent advances in single-cell multi-omics have uncovered unexpected heterogeneity in STM function, revealing a rich atlas of discrete phenotypic clusters among tissue-resident and tissue-infiltrating STMs[7,8]. Together with ex vivo and in vivo studies of STM populations, these data have refined our knowledge of STM biology in healthy and arthritic joints, revealing that while certain STM clusters contribute more towards RA pathogenesis, others phenotypes have a key role in RA remission[8,9].

In this Review, we discuss how distinct functions of different STM clusters shape the synovial tissue environment in health, and how they control RA disease progression and remission. We also discuss the potential origin of different STM clusters and the role of the synovial tissue niche in their local differentiation and spatial distribution. Finally, we discuss how understanding STM heterogeneity might aid in the management of RA and inform novel treatments for RA and other rheumatic diseases.

A functional taxonomy of STMs

Over the past 5 years, human and mouse studies from several groups have advanced our understanding of STMs and their role in joint homeostasis and the inflammatory response[7–10]. Here, we attempt to integrate these data into a single taxonomy (FIG. 1). All human

Key points

- New technologies have identified macrophage populations in both the human and mouse joint synovium, with distinct homeostatic, protective and inflammatory functions.
- Tissue-resident synovial tissue macrophages (STMs) form an immune-protective lining barrier, control the development of experimental arthritis, and actively participate in maintaining RA in remission.
- The progression of RA is associated with phenotypic changes in resident STMs and the influx of monocytes that differentiate into STMs with pro-inflammatory functions, driving chronic pathology.
- Capitalizing on the joint-protective and inflammation-resolving biology of newly identified STM clusters might assist the development of novel therapeutics that are aimed at treating arthritis and maintaining disease remission.
- The relative proportions of STM clusters can predict a flare of arthritis following treatment tapering or cessation and might be a useful component of flare prediction algorithms.

and mouse STMs express the pan macrophage markers CD64, CD11b and CD68 (human) or F4/80 (mouse)[8–10]. Within the human synovium, two main populations of macrophages can be distinguished based on cell membrane expression of MerTK[11], a tyrosine protein kinase receptor involved in efferocytosis and resolution of inflammation (BOX 1), and CD206, a C-type lectin mannose receptor that acts as a pattern recognition receptor for pathogens[12]. Specifically, these populations are MerTK^{pos}CD206^{pos} tissue-resident macrophages and MerTK^{neg}CD206^{neg} tissue-infiltrating macrophages[8]. During arthritis MerTK^{pos}CD206^{pos} STMs acquire expression of CD163. Single-cell transcriptomics (scRNAseq) and multiparameter flow cytometry experiments have revealed that these two STM populations contain multiple transcriptionally distinct phenotypic clusters that differ in function and distribution between different stages of arthritis progression.

Tissue-resident (MerTK^{pos}CD206^{pos}) STMs are the predominant macrophage subtype in healthy human and mouse synovium. Two distinct subpopulations of tissue-resident STMs have been identified (FIG. 1). The first is a TREM2^{pos}CX3CR1^{pos}FOLR2^{pos} cluster[8,9] (referred to as TREM2^{pos} from here on) that forms a protective lining layer in the synovial membrane[9] and is likely to be the equivalent of the CD64^{pos}MHCII^{neg} cluster described in healthy mouse synovium[10]. The second subpopulation is a LYVE1^{pos}FOLR2^{high} cluster (RELMα^{pos} in mouse[9]; referred to as LYVE1^{pos} or RELMα^{pos} from here on) that mostly resides in the synovial sublining layer[8,9] and is ostensibly the equivalent of the mouse CD64^{pos}MHCII^{high} cluster[10]. In turn, hierarchical clustering analysis has revealed that both of these resident STM subpopulations (TREM2^{pos} and LYVE1^{pos}) contain distinct phenotypic clusters, which might reflect diverse activation states[8]. For example, a TREM2^{low} cluster is a discrete activation state of TREM2^{pos} STMs, whereas FOLR2^{pos}ICAM1^{pos} and FOLR2^{pos}ID2^{pos} clusters are an activation state and a lineage precursor of LYVE1^{pos} STMs[8], respectively. A third large subpopulation of myeloid cells in the healthy synovium are cells with dendritic cell phenotype, which are CLEC10A^{pos} in humans, and AQP1^{pos} in the mouse[8,9]. Thus, heterogeneity exists within the tissue-resident STMs.

During joint inflammation, such as in patients with RA and in experimental models of arthritis, a shift in the composition of STM clusters is observed. A key feature of this shift is that the synovial protective lining layer becomes distorted and the tissue-infiltrating population (MerTK^{neg}CD206^{neg}) of STMs becomes predominant, increasing in abundance with disease progression. Like resident macrophages, this tissue-infiltrating STM population contains distinct phenotypic clusters, including a CD48^{pos}S100A12^{pos} cluster[8] (previously described as an M1 population[7]), a CD48^{pos}SPP1^{pos} cluster (equivalent to mouse CCR2^{pos}IL-1β^{pos} STMs[9,10]) and a CD48^{pos}ISG15^{pos} cluster with an interferon signature[8] (previously described as an M4 population[7] and equivalent to the mouse CCR2^{pos}ARG1^{pos} STMs[9,10]). By contrast, during sustained remission in patients with RA, tissue-infiltrating (MerTK^{neg}CD206^{neg}) STM clusters are reduced in number, with restoration of the normal, healthy composition of tissue-resident (MerTK^{pos}CD206^{pos}) TREM2^{pos} and LYVE1^{pos} STM subpopulations[8].

The abundance of distinct STM populations seems to be dependent on the type of joint disease. Unlike in RA, non-inflammatory, mechanical stress-induced osteoarthritis (OA) is characterized by a high abundance of tissue-resident (MerTK^{pos}CD206^{pos}) STMs (previously identified as FOLR2^{pos}[13] and an M2 population[7]), compared with the healthy synovium. However, whether the transcriptomic signature of this STM population differs from that in healthy tissue, and whether it contributes to hallmark OA pathological conditions such as cartilage degradation, remains unknown. STM clusters are also poorly characterized in other chronic inflammatory joint diseases, such as psoriatic arthritis (PsA). Mass cytometry analysis of synovial fluid from patients with PsA has identified the robust presence of a myeloid cluster, characterized by high expression of the bone-remodelling mediator osteopontin (SPP1)[14], that resembles the tissue-infiltrating CD48^{pos}SPP1^{pos} cluster observed in active RA[8]. Further analyses, including single-cell transcriptomic and proteomic studies, are needed to elucidate the shared pathogenic roles of different STM phenotypes in RA, OA and PsA, as well as those that contribute to pathological conditions that are unique to these diseases, such as new bone formation and tendinopathy in patients with PsA.

Distinct STM clusters in health and disease

Healthy homeostasis. The synovial membrane is a highly specialized, multifunctional structure consisting of an inner lining and a sublining layer (FIG. 2). The inner lining is thin (<2 mm in radiocarpal joints[15]) but highly cellular, composed of tissue-resident TREM2^{pos} STMs[8,9,16]. This STM population interacts closely with lining-layer synovial fibroblasts (PRG4^{pos}PDPN^{pos}CD90^{neg})[17] that produce components of synovial fluid, such as lubricin and hyaluronic acid, to lubricate the joint during movement. In line with a protective role of the lining layer of the synovial membrane, mouse and human TREM2^{pos} STMs express tight-junction proteins that enable the formation of a physical epithelial-like barrier, protecting against pathogens and controlling the apparent influx

Disease activity score
A composite index to quantify RA activity, calculated with a formula including painful and swollen joints (over 44 or 28 joints), inflammatory lab tests (erythrocyte sedimentation rate or C-reactive protein) and patients' Global Health evaluation.

Synovial membrane
Specialized connective tissue that lines the inner surface of capsules of synovial joints, tendon sheath and bursae.

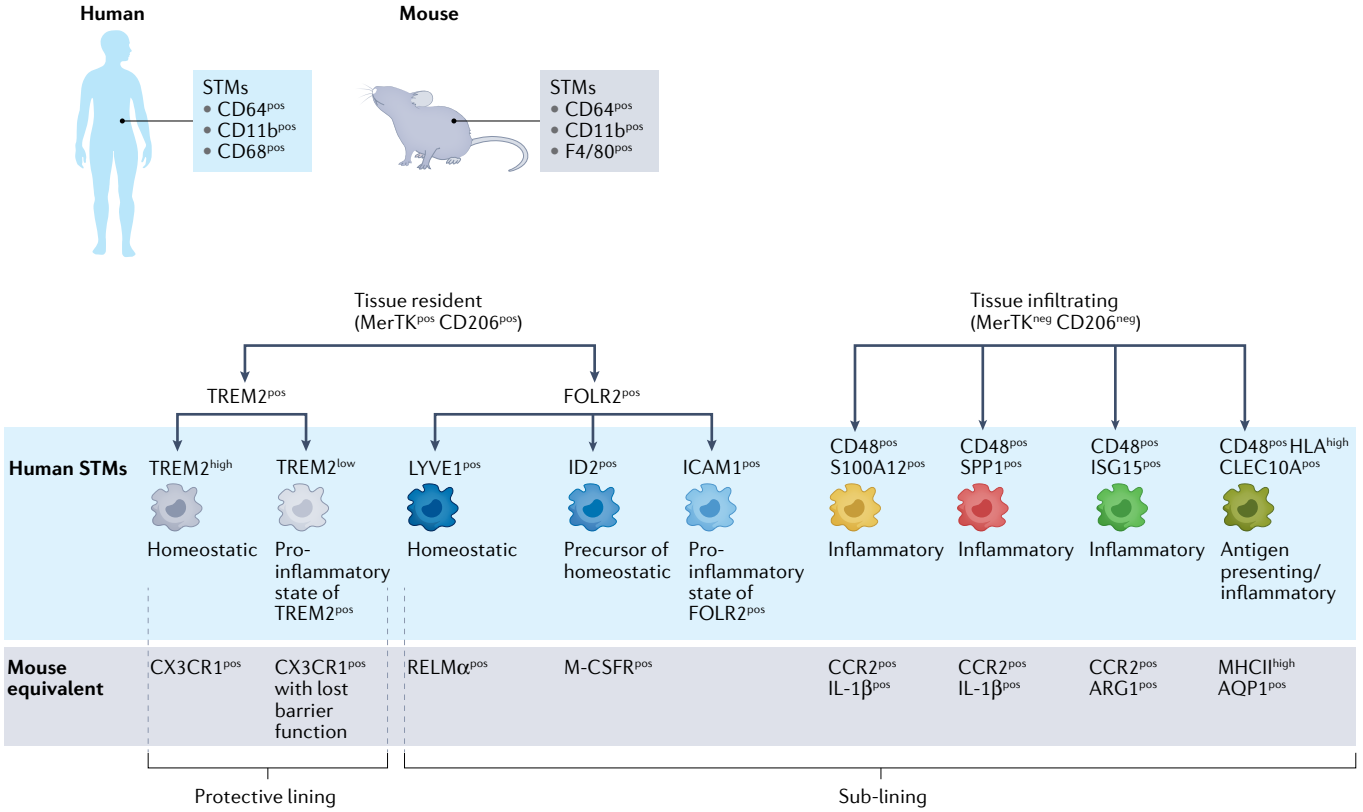


Fig. 1 | **A novel taxonomy of human and mouse STMs defined by single cell omics.** Schematic showing human and mouse synovial tissue macrophage (STM) heterogeneity, revealed by single-cell transcriptomics in Alivernini et al.[8] and Culemann et al.[9]. Human and mouse STMs are clustered based on their transcriptomics, functions and spatial localization within the synovial membrane, in health, rheumatoid arthritis and experimental models of arthritis.

of inflammatory cells[8,9]. In addition, of all known STM clusters, they constitutively express the highest levels of defensins, such as defensin B1, as well as complement proteins such as C1Q[8], which likely reflects their local role in preventing infections and in clearing damaged tissue to prevent rapid cartilage degradation, such as that described in patients with septic arthritis[18]. Consistent with such a function is the finding that both human and mouse TREM2^{pos} STMs express a wide range of phagocytic receptors, including AXL, MARCO and TIMD4 (REFS[8,9]), and constitutively express enzymes involved in phagocytosis (such as lysosomal cathepsins)[8]. Together, these findings suggest that the inner lining STMs have an important role in engulfing apoptotic cells during homeostatic tissue remodelling and removal of dying short-lived inflammatory cells to prevent sterile inflammation[8,9,19].

In addition to their phagocytic function, the regulatory transcriptome of TREM2^{pos} STMs suggests a role in actively restraining inflammation. Pathways enriched in this macrophage population are related to MerTK and AXL, tyrosine kinase receptors that inhibit cytokine- and Toll-like receptor (TLR)-induced pro-inflammatory responses. Additional upregulated pathways include retinoic acid and VSIG4 (a PDL1-like molecule) pathways that inhibit effector T cell activation while facilitating the expansion of regulatory T cells[8,9]. Moreover, of all known STM clusters, TREM2^{pos} STMs have the highest expression levels of receptors and enzymes involved in attenuation of inflammation, such as those related to lipid binding and metabolism (e.g. APOE-binding receptor TREM2 (REF.[8])) and the production of lipid mediators (e.g. resolvin D1)[8]. In this respect, the TREM2^{pos} STM population might share the same tissue function as that of TREM2^{pos} adipose tissue macrophages, the disruption of which leads to adipose tissue inflammation and the development of insulin resistance[20]. Accordingly, data from experimental mouse models suggest that a reduced abundance of lining-layer STMs precedes the development of spontaneous arthritis[21]. In summary, TREM2^{pos} STMs form a protective barrier that maintains synovial lining-layer integrity and limits the development of sterile and pathogen-induced inflammation.

The sublining layer (interstitium) of the synovium contains loose, adipocyte-rich connective tissue and sublining fibroblasts (CD90^{pos}), which provides support to the lining layer[7,17,22]. Sympathetic and sensory nerves, as well as blood and lymphatic vasculature, are also present in the sublining layer, providing a link between the synovium and systemic responses to changes within the tissue environment[16]. Across several tissues, the healthy interstitium contains at least two distinct macrophage clusters: nerve-associated macrophages (NAMs) and perivascular macrophages. NAMs, localized adjacent to the sympathetic nerves, are tissue-resident macrophages characterized by low LYVE1 and high MHCII expression. Studies in mice have identified an important functional interaction

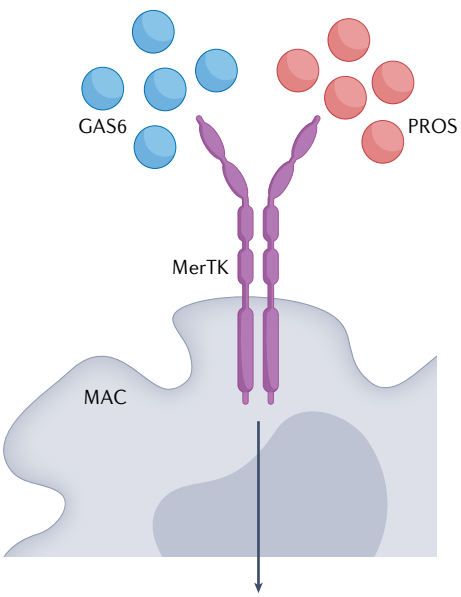
between nerves and NAMs that prevents excessive inflammation in tissues, such as lung, dermis and heart[23,24]. Indeed, NAMs have an immunoregulatory phenotype, in that they express high levels of IL-10 (REFS[23,24]), are potent inducers of regulatory T cells[23] and their depletion in mouse lung leads to an excessive inflammatory response during viral infections[24]. In addition, NAMs can provide essential cues for nerve homeostasis, suggesting two-way communication between macrophages and the nerve. NAMs in the gut produce bone morphogenic protein 2 (BMP2) that binds to its receptor (BMP2R) on enteric neurons and promotes gut neuron-mediated gastrointestinal motility[25]; depletion of NAMs in the gut disrupts normal gut peristatic activity[25]. To date, NAMs have not been formally identified in the human or mouse synovium, although their transcriptomic profile suggests that they might be embedded within the recently described tissue-resident LYVE1^{low}ID2^{pos} STM cluster[8]. Refined characterization of the synovium NAM population is critical to improve our understanding of NAM function and might inform therapeutic strategies targeting the aetiology of joint pain that characterizes inflammatory and non-inflammatory conditions.

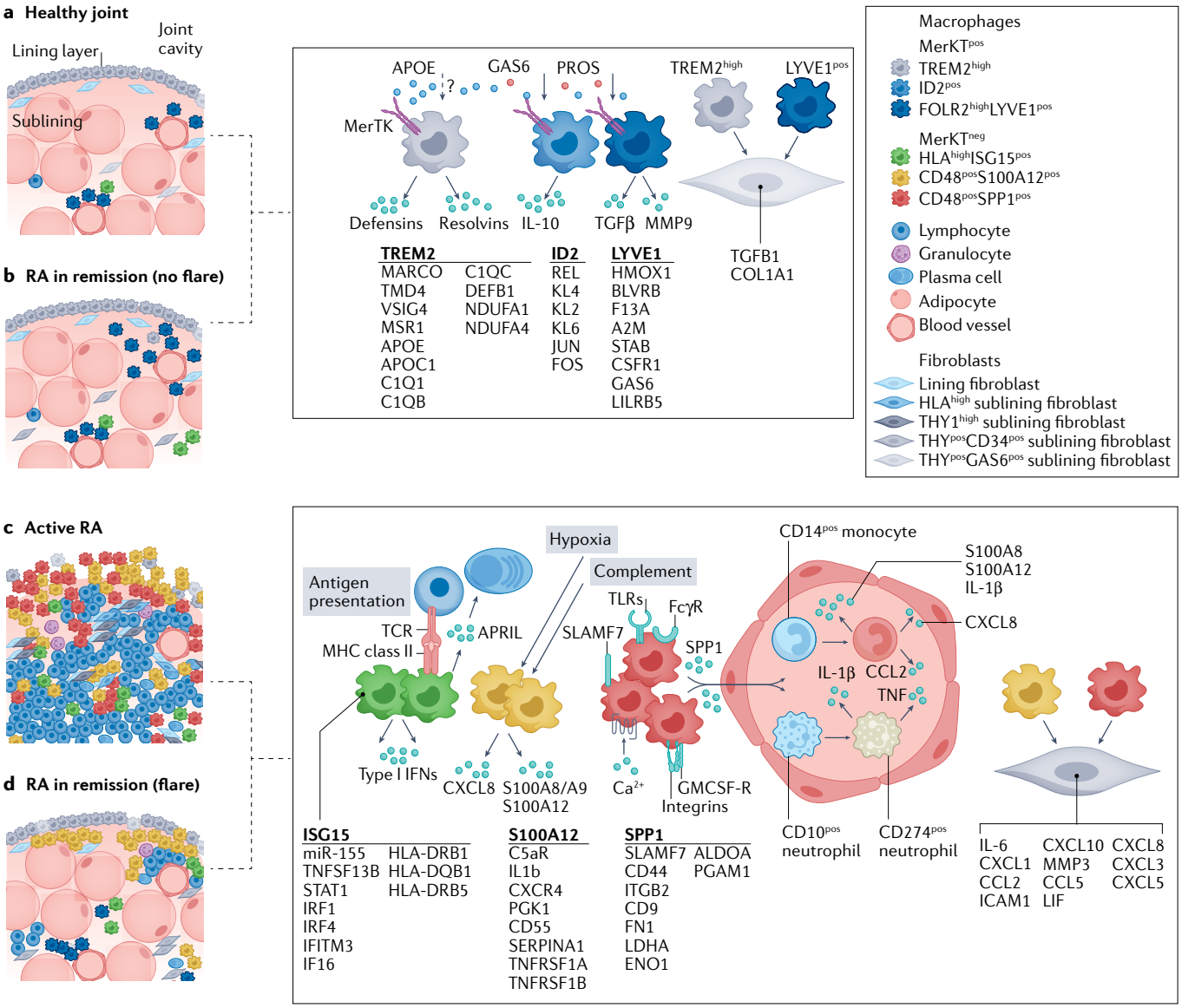
Alongside the NAM population, the healthy synovial sublining layer contains a robust LYVE1^{pos} perivascular macrophage cluster (termed RELM1a^{pos} in the mouse) with a transcriptional profile that indicates various regulatory functions. Among all identified STM clusters, the LYVE1^{pos} cluster expresses the highest levels of enzymes involved in haem metabolism (such as HMOX1 and BLVRB)[8], which suggests it has a role in the phagocytosis of erythrocytes, iron recirculation and the prevention of haem-triggered inflammation after blood vessel disruption or injury[26]. Computational[27] and experimental studies[23] have shown that a similar LYVE1^{pos} macrophage cluster is present in many tissues, with a conserved role in regulating blood vessel stiffness and permeability[23,28]. Mechanistically, the LYVE1^{pos} cluster can produce IL-10, transforming growth factor-β (TGFβ) family members and matrix metalloproteinase 9 (MMP-9), which together control inflammation and regulate the dynamics of collagen deposition during resolution of inflammation[23,28]. The experimental deletion of LYVE1^{pos} macrophages in the lung leads to increased leukocyte infiltration and excessive fibrosis[23], whereas a lack of these cells around the arterial wall leads to increased arterial stiffness owing to greater deposition of collagen by smooth muscle cells[28]. In the synovium, LYVE1^{pos} macrophages are not only located in close proximity to blood vessels but also interact directly with CD90^{pos} (also known as THY1^{pos}) sublining synovial fibroblasts[8] that provide the extracellular matrix that supports the structure of the synovium[16], suggesting a similar role for LYVE1^{pos} macrophages in regulating interstitial extracellular matrix deposition. Many regulatory functions of this cluster, such as extracellular matrix homeostasis and vessel mechanics, are mediated by the interaction of LYVE1 with its ligand hyaluronic acid, which is expressed on recipient stromal cells (such as smooth muscle cells)[28,29]. In summary, LYVE1^{pos} STMs likely control synovial blood vessel permeability and blood cell infiltration within the tissue and regulate tissue remodelling.

Initiation of inflammation in the synovium. Studies in patients, as well as experimental animal models, have revealed dynamic STM behaviour during the very early stages of arthritis (that is, undifferentiated arthritis)[8,9,21]. These works indicate that a phenotypic switch in the tissue-resident lining-layer (TREM2^{pos}) STM population precedes the influx of both neutrophils and monocyte precursors of tissue-infiltrating, inflammatory macrophage clusters (CD206^{neg}MerTK^{neg}), which are the main producers of TNF in chronic stages of arthritis[8,9,21]. Initial mouse studies showed that MHCII^{neg} tissue-resident STMs (close equivalents of human TREM2^{pos}) are crucial in limiting the inflammatory response in the joint[10]. Subsequent studies confirmed the key role of an intact TREM2^{pos} STM lining layer barrier in controlling inflammation. Indeed, diphtheria toxin-induced depletion of TREM2^{pos} lining-layer macrophages (that is, TREM2^{pos} STM expressing diphtheria toxin receptor under the CX3CR1 promoter) or pharmacological disintegration of lining layer tight junctions resulted in early and accelerated neutrophil influx that exacerbated the onset and severity of arthritis in experimental models of

Box 1 | **The function of MerTK in tissue resident macrophages**

MerTK is a protein tyrosine kinase receptor that belongs to the TAM family receptors (Tyro3, AXL, MerTK). MerTK is mainly expressed by tissue-resident macrophages (MAC) and mediates the immuno-homeostatic functions of these cells. Activation of MerTK by one of the two ligands: growth-arrest-specific 6 (GAS6) or protein S (PROS), promotes apoptotic cell removal[103,104], resolution of tissue inflammation[105,106], tissue remodelling[104] and tissue spatial organization[107].




a Healthy joint
Lining layer
Joint cavity
Sublining
b RA in remission (no flare)
c Active RA
d RA in remission (flare)
APOE
GAS6
PROS
TREM2high
LYVE1pos
MerTK
Defensins
Resolvins
IL-10
TGFβ
MMP9
TGFB1
COL1A1
TREM2
MARCO
TMD4
VSIG4
MSR1
APOE
APOC1
C1Q1
C1QB
C1QC
DEFB1
NDUFA1
NDUFA4
ID2
REL
KL4
KL2
KL6
JUN
FOS
LYVE1
HMOX1
BLVRB
F13A
A2M
STAB
CSFR1
GAS6
LILRB5
Macrophages
MerKTpos
TREM2high
ID2pos
FOLR2highLYVE1pos
MerKTneg
HLAhighISG15pos
CD48posS100A12pos
CD48posSPP1pos
Lymphocyte
Granulocyte
Plasma cell
Adipocyte
Blood vessel
Fibroblasts
Lining fibroblast
HLAhigh sublining fibroblast
THY1high sublining fibroblast
THYposCD34pos sublining fibroblast
THYposGAS6pos sublining fibroblast
Hypoxia
Complement
Antigen presentation
TCR
MHC class II
APRIL
SLAMF7
TLRs
FcγR
SPP1
Type I IFNs
CXCL8
S100A8/A9
S100A12
Ca2+
GMCSF-R
Integrins
CD14pos monocyte
S100A8
S100A12
IL-1β
CXCL8
IL-1β
CCL2
TNF
CD10pos neutrophil
CD274pos neutrophil
IL-6
CXCL10
CXCL8
CXCL1
MMP3
CXCL3
CCL2
CCL5
CXCL5
ICAM1
LIF
ISG15
miR-155
TNFSF13B
STAT1
IRF1
IRF4
IFITM3
IF16
HLA-DRB1
HLA-DQB1
HLA-DRB5
S100A12
C5aR
IL1b
CXCR4
PGK1
CD55
SERPINA1
TNFRSF1A
TNFRSF1B
SPP1
SLAMF7
CD44
ITGB2
CD9
FN1
LDHA
ENO1
ALDOA
PGAM1

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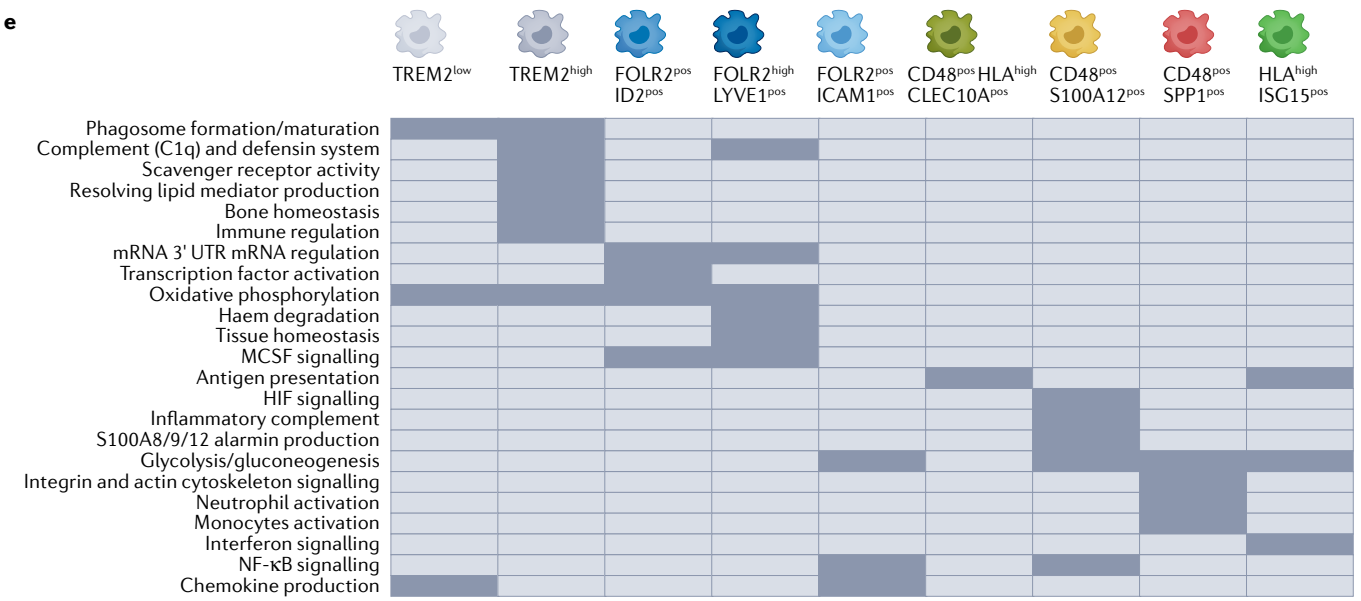

TREM2low
TREM2high
FOLR2pos ID2pos
FOLR2high LYVE1pos
FOLR2pos ICAM1pos
CD48pos HLAhigh CLEC10Apos
CD48pos S100A12pos
CD48pos SPP1pos
HLAhigh ISG15pos
Phagosome formation/maturation
Complement (C1q) and defensin system
Scavenger receptor activity
Resolving lipid mediator production
Bone homeostasis
Immune regulation
mRNA 3' UTR mRNA regulation
Transcription factor activation
Oxidative phosphorylation
Haem degradation
Tissue homeostasis
MCSF signalling
Antigen presentation
HIF signalling
Inflammatory complement
S100A8/9/12 alarmin production
Glycolysis/gluconeogenesis
Integrin and actin cytoskeleton signalling
Neutrophil activation
Monocytes activation
Interferon signalling
NF-κB signalling
Chemokine production

Fig. 2 | **Diverse functions of STM clusters in health, inflammation and remission in RA. a** | The healthy synovial membrane is a pauci-cellular tissue that covers the inner surface of the joint cavity. It contains tissue-resident MerTK^{pos}CD206^{pos} synovial tissue macrophages (STMs) with two main clusters: TREM2^{pos}, which forms a barrier layer, and LYVE1^{pos}, which is mostly localized in the sublining layer. **b** | In patients with rheumatoid arthritis (RA) in sustained clinical and ultrasound remission without flare, the STM composition is similar to that of the healthy joint with a restoration of MerTK^{pos}CD206^{pos} clusters, whereby TREM2^{pos} and LYVE1^{pos} clusters actively promote the resolution of inflammation by releasing resolvins and anti-inflammatory mediators (such as IL-10, defensins and TGFβ). These tissue-resident STMs induce an anti-inflammatory and a repair programme (characterized by TGFB1, COL1A1, COL1A1.1 and CD24) in stromal cells, contributing to maintaining an inflammation-free joint environment. GAS6, PROS and APOE represent potential drivers of the phenotypes of MerTK^{pos}CD206^{pos} clusters. **c** | The synovial membrane in active RA is characterized by progressive changes in the synovial lining and sublining layers, as well as infiltration of immune cells that leads to synovial hyperplasia. These tissue alterations are associated with changes in the composition and phenotype of STMs. Synovial inflammation leads to breakdown of the epithelial-like barrier and change in the phenotype of lining-layer STMs from TREM2^{high} to TREM2^{low} with reduced regulatory pathway function and increased expression of chemokines. These events contribute to the development of a permissive synovial tissue niche that fosters the influx and differentiation of monocyte-derived tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs (CD48^{pos}S100A12^{pos}, CD48^{pos}SPP1^{pos} and CD48^{pos}HLA^{pos}ISG15^{pos}) that amplify synovitis through the production of pro-inflammatory mediators, activation of stromal cells and promotion of T and B lymphocyte activation. For example, these tissue-infiltrating MerTK^{neg}CD206^{neg} STMs induce chemokines, cytokines and MMP production by synovial fibroblasts. **d** | The synovium of remission patients with RA who flare is characterized by the persistence of the CD48^{pos}S100A12^{pos} STM cluster, which produces alarmins to rekindle inflammation. **e** | Clusters constituting the main human MerTK^{neg}CD206^{neg} and MerTK^{pos}CD206^{pos} STM populations differ in metabolic and immune pathways, suggesting cluster-specific functions (generated from information in Alivernini et al.[8] and Clayton et al.[102]).

antibody-transfer RA. Human studies on early arthritis showed that disruption to this lining-layer barrier can be preceded by an increase in the numbers of lining layer macrophages[30] and their differentiation into a TREM2^{low} cluster[8]. The TREM2^{low} cluster has decreased expression of many regulatory pathways, such as VSIG4 and retinoic acid pathways and increased expression of inflammatory mediators such as S100A9, IL-8 and SPP1, compared with the mother TREM2^{pos} cluster[8]. The switch from a TREM2^{pos} into a TREM2^{low} cluster was most prominent in very early arthritis[8] and mouse studies suggest that immune complexes containing autoantibodies might be responsible for this phenotypic change[9]. In humans, such differentiation likely permits the development of inflammation in response to anti-citrullinated protein autoantibodies (ACPA) or endogenous TLR ligands. In summary, TREM2^{pos} STMs control the onset of inflammation, and changes in their phenotype towards TREM2^{low} might be the first step in the initiation of synovitis, such as by producing mediators attracting neutrophils (for example, IL-8) and monocytes (for example, SPP1).

Perpetuated joint inflammation. Clinical arthritis is associated with hypertrophy of the synovial membrane, which reflects a number of alterations to the synovium, including hyperplasia of the inner lining and sublining layers, fibroblast expansion, increased blood and lymphatic vasculature and an influx of immune cells[31]. In at least two of the most common types of synovitis — myeloid (macrophage rich) and lymphoid (T and B cell follicle rich) — inflammatory macrophages (CD206^{neg}MerTK^{neg}) are the key cellular component[31,32]. Notably, this population is different from tissue-resident (CD206^{pos}MerTK^{pos}) STMs that are present in healthy synovium and likely differentiates locally from circulating monocytes that are attracted to the synovium by chemokines[33]. Indeed, recent studies have provided evidence of accelerated monocytopoiesis in bone marrow, and the egress of immature CD14^{pos} precursors from bone marrow and their migration into inflamed joints[34]. In vivo tracking of labelled blood CD14^{pos} cells from patients with RA confirmed that CD14^{pos} cells migrate to the joints[35,36]. Whether other monocyte subpopulations, such as CD16^{pos} and CD14^{pos}CD16^{pos} cells, are also trafficked into and within human synovial tissue is unclear. However, in line with this possibility, mouse studies have revealed that Ly6C^{pos} classical monocytes (equivalent of human CD14^{pos} monocytes) and Ly6C^{neg} patrolling monocytes (equivalent of human CD16^{pos} and CD14^{pos}CD16^{pos} monocytes) differentiate into pro-inflammatory macrophages that mediated joint pathology[10,37].

Tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs are abundant macrophage population in the synovium of patients with active RA, including in patients who are naive to treatment or resistant to conventional DMARDs. Consistent with their pro-inflammatory function, an ex vivo study of synovial biopsy-derived, FACS-sorted tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs showed that these cells are the main producers of pro-inflammatory cytokines (such as TNF, IL-6 and IL-1β) and chemokines (such as CCL2)[8]. This fact is in sharp contrast to tissue-resident (CD206^{pos}MerTK^{pos}) STMs, which show limited production of these inflammatory mediators in accordance with their more homeostatic and regulatory role[8].

In addition to the production of pro-inflammatory cytokines and chemokines, each of the three identified clusters that comprise the tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM population seem to have their own unique pathogenic mediator signature, suggesting discrete mechanistic contributions to synovitis. First, the CD48^{pos}S100A12^{pos} cluster shows the highest expression levels of inflammation-triggering alarmins (such as S100A8, S100A9 and S100A12) and CXCL8 (REF.[8]), which are potent inducers of monocyte and fibroblast activation and chemo-attractants for neutrophils, respectively. This cluster also seems to be the major source of IL-1β in the inflamed synovium[7,8]. Second, the CD48^{pos}SPP1^{pos} cluster is characterized by high levels of glycolytic enzymes, cytoskeletal proteins and integrins, suggesting an activated migratory phenotype. Moreover, its marker SPP1 (also known as osteopontin) has bone-resorbing properties, indicating a specific contribution of this cluster to bone damage[38]. SPP1 also drives differentiation of the pro-inflammatory neutrophil cluster (PDL1^{pos})[39], suggesting that this STM cluster might also contribute to the activation of neutrophils attracted to the synovial fluid. It was shown recently that their strong pro-inflammatory activation, including TNF production, is driven by the engagement of signalling lymphocytic activation molecule family member 7 (SLAMF7)[40]. Finally, the transcriptome of the third tissue-infiltrating cluster (CD48^{pos}ISG15^{pos}) suggests that this population is a source of type I IFNs in the synovium and therefore

Monocytopoiesis
A process that leads to the differentiation of monocytes from hematopoietic precursors in the bone marrow.

might contribute to type I interferon-driven pathological conditions observed in some patients with a poor response to therapies[41]. Of the three clusters, this one also expresses the highest level of the plasma cell survival factor APRIL, suggesting that it has a role in ectopic germinal centre formation. A recent study showed that their pro-inflammatory activation is likely driven by the post-transcriptional regulator, microRNA-155 (REF.[42]) that limits the cellular pool of inhibitors of inflammatory responses, such as SOCS-1 and SHIP1 proteins[43]. While all three clusters are present in patients with active RA, SPP1^{pos} and ISG15^{pos} clusters are further increased in patients that are resistant to conventional DMARDs treatment[8,42]. Together, these findings highlight that each of the three tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM subpopulations might make unique contributions towards synovitis. Nonetheless, although these STM clusters are all localized in the sublining layer, their specific local pathogenic micro-niches (such as perivascular, ectopic germinal centre or enrichment in adipocytes) have yet to be identified. Understanding the precise niche in which these STM populations exert biological effects is important to better understand the contribution of macrophage diversity to different pathogenic features of synovitis.

The mechanism of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM-mediated inflammation involves activation of synovial fibroblasts. Micro-co-cultures of synovial biopsy-derived tissue-infiltrating STMs with synovial tissue stromal cells demonstrated that this population, but not tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters, triggered MMP production from lining layer fibroblasts, and pro-inflammatory cytokines and chemokines from sublining layer synovial fibroblasts[8,44]. In addition, in vitro modelling of the CD48^{pos}S100A12^{pos} cluster of tissue-infiltrating STMs (the approximate equivalent of the M1 population[7]) showed that this cluster produces heparin-binding EGF-like growth factor (HBEGF) that, in turn, promotes epidermal growth factor receptor (EGFR)-dependent invasiveness of synovial fibroblasts[44]. Together, these data suggest a key role of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs in driving the pathogenic response of synovial fibroblasts. Interestingly, an elegant study in mice showed that inflammation-imprinted lining (PDPN^{pos}CD90^{neg}) and sublining (PDPN^{pos}CD90^{pos}) fibroblasts could perpetuate their respective pathogenic functions (joint damage or inflammation) after adoptive transfer to healthy joints[17]. Thus, reciprocal interactions between tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM clusters and synovial fibroblasts might perpetuate chronic synovitis. The role of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STM clusters in regulating synovial tissue effector T cell responses (such as the recently identified PD1^{high} CXCR5^{neg} helper T cells[45]) is unclear; however, their high expression of MHCII and their production of a wide range of mediators suggests that they direct the adaptive immune response. Several studies show that monocytes from the synovial fluid of patients with RA can drive the differentiation of T helper 1 and T helper 17 cells, supporting this hypothesis[46]. In summary, tissue-infiltrating STM clusters are a major source of a wide range of inflammatory mediators and trigger and imprint chronic activation of the synovial stromal compartment in RA.

Disease remission. Remission of RA is defined by the resolution of systemic and joint inflammation[47]. At a minimum, this includes restoration of the disease activity score 28 (DAS28) to less than 2.6 (REF.[47]) and resolution of synovial inflammation as confirmed by normal synovial blood flow on Power Doppler ultrasound[15,48]. Note that patients who meet these criteria for remission could still have active synovitis[49] and that more stringent criteria, such as the Boolean-based definition, have been suggested. Clinically, remission is a key part of the recovery process from RA, leading to improved joint function via restoration of homeostatic turnover of bone and reduced loss of cartilage[48,50,51]. Although sustained drug-free remission is rare, it is not impossible; thus, understanding the homeostatic mechanisms of sustained remission might encourage new therapeutic strategies.

Recent studies in patients with arthritis, as well as experimental arthritic models, suggest that STMs, particularly the tissue-resident (CD206^{pos}MerTK^{pos}) population, have a pivotal role in RA disease remission[8–10]. The synovium of patients with RA in remission shows a substantially reduced number of pathogenic tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs that are hugely abundant in active RA, and restoration of the healthy tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters TREM2^{pos} and LYVE1^{pos} in the lining and sublining layers, respectively[6,8]. Investigations into the biology of remission-related tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters have demonstrated their role in limiting inflammation and re-instating synovial homeostasis. This STM population produces inflammation-resolving mediators (such as Resolvin D1), rather than pro-inflammatory mediators, and induces a repair programme in synovial fibroblasts, such as increased expression of collagens and the TGFβ pathway[8]. It follows, therefore, that global deletion of tissue-resident STMs in mice during the peak of inflammation delays the resolution of experimental arthritis[10]. In summary, tissue-resident (CD206^{pos}MerTK^{pos}) STM clusters might be actively involved in the resolution of synovial inflammation and contribute to maintenance of remission.

Flare of arthritis. Of those patients with RA who respond to treatment and achieve remission, approximately half will relapse within months of treatment cessation[48,52]. A recent study revealed that the presence of tissue-infiltrating (CD206^{neg}MerTK^{neg}) STMs prior to treatment cessation, and specifically the CD48^{pos}S100A12^{pos}cluster, as identified by scRNAseq, is associated with an increased risk of subsequent flare[8]. Isolation of these STMs from biopsy material of patients with RA in sustained clinical and ultrasound remission, revealed high expression levels of inflammation-triggering alarmins, such as S100A8, S100A9 and S100A12, similar to levels expressed by equivalent CD48^{pos}S100A12^{pos} clusters isolated from patients with active RA. These findings suggest that the inflammatory activities of the CD48^{pos}S100A12^{pos}

Boolean
The ACR/EULAR definition of remission by which, at any time point, a patient must satisfy all of the following: painful joint ≤1, swollen joint ≤1, C-reactive protein ≤1 mg/dl and Patient Global Assessment ≤1 (on a 0–10 scale).

Epigenetic imprinting
Changes in the chromatin structure around a specific gene that is induced by the environment and that makes a gene primed for either higher or lower expression levels. These changes can be passed from mother to daughter cell.

STM clusters might be responsible for triggering flares of arthritis if not controlled by the treatment and/or the regulatory functions of CD206^{pos}MerTK^{pos} STMs. Other cell types likely also contribute to disease flares. An elegant study recently identified circulating stromal cells in the blood of patients with RA prior to flare, with a CD45^{neg}CD31^{neg}PDPN^{pos} phenotype that resembled synovial sublining-layer fibroblasts[53]. Uncovering whether there is a functional and temporal link between the breakdown of the synovial lining layer barrier, emergence of the CD48^{pos}S100A12^{pos} STM cluster and the appearance of CD45^{neg}CD31^{neg}PDPN^{pos} fibroblasts in the circulation prior to flare, would help to establish a cellular interactome that might be prognostic or targeted therapeutically to improve patient management.

Sustained remission versus healthy homeostasis. The structure and histology of the RA synovium in sustained remission can resemble the healthy synovium, with both containing similar inflammation-controlling STM clusters. However, even prolonged remission eventually flares[54,55], demonstrating that remission is fragile and far from a complete return to health. Thus, STM phenotypes in the context of remission require closer scrutiny to better understand the pathological mechanisms. Detailed molecular studies of patients with RA in remission have shown that, compared with healthy controls, inner lining TREM2^{pos} and sublining LYVE1^{pos} STM clusters fail to restore the expression of certain regulatory pathways that are downregulated in active RA[8]. The results suggest that these 'super-repressed' genes might contribute to the tendency for flare-ups in drug-free RA remission. For example, STMs from patients with RA in remission remain deficient in *VSIG4*, which encodes a B7-related co-inhibitory molecule[1], in *LILRB* genes, which encode inhibitors of Fc gamma receptor-driven activation[56], and in genes encoding enzymes involved in retinoic acid production (such as *ALDH1A1* and *RBP4*)[8]. The role of these molecules in STM-driven joint homeostasis is unknown, although their known functions in other contexts suggest a role in restricting adaptive immunity[57]. For example, the expression of VSIG4 in myeloid cells inhibits effector T cell function and activates FOXP3^{pos} regulatory T cells[32]. Moreover, global *Vsig4*$^{-/-}$ mice spontaneously develop inflammatory and autoimmune pathological conditions[58], whereas a VSIG4-Fc fusion-protein protects against experimental arthritis[59].

The mechanisms that sustain the super-repressed gene expression pattern in remission are unknown. Possible candidates include the action of autoantibodies (that is, the breach of self-tolerance) and/or epigenetic changes in STM precursors. Serological evidence of remission, defined as the disappearance of a previously positive test for anti-citrullinated protein antibody (ACPA) and/or rheumatoid factor after successful therapy, is a rare event in patients with RA. It is noteworthy, though, that specific B cell targeted therapies, such as rituximab and CTLA4-Ig, can decrease autoantibody titres[60–62]. In support of the notion that autoantibodies might be responsible for the different signatures of STMs from healthy individuals and from patients with RA in remission, recent studies have demonstrated that ACPA positivity is associated with a higher probability of disease flare after treatment modification[63,64]. An additional, but not mutually exclusive, possibility is that tissue-resident (MerTK^{pos}CD206^{pos}) STMs and their local precursors undergo epigenetic imprinting mediated by prior inflammation. Indeed, fate-mapping studies of murine counterparts of both TREM2^{pos} and LYVE1^{pos} populations suggest that tissue-resident STMs can be long-lived[9,10], rendering them susceptible to epigenetic imprinting by the local environment. A better understanding of the difference in myeloid pathways between remission and healthy synovium will help to develop new treatment strategies to extend remission towards restoration of self-sustained, normal joint homeostasis.

Specification of distinct STM clusters

Tissue macrophages undergo dramatic and diverse responses to danger signals, including infection, aberrant immunity and tissue injury. This development of distinct immune-homeostatic functions in response to such signals might be determined by at least two factors: their ontogeny and adaptation to tissue-specific cues.

Ontogeny

Murine models have established that adult healthy tissues contain resident macrophages that can originate from three different sources: embryonic yolk sac precursors, such as microglia; fetal liver precursors, such as alveolar macrophages; and adult bone marrow-derived monocytes, such as the majority of macrophages in the gut and dermis[65]. Yolk sac and liver prenatal precursors are established in tissues during early embryonic development and macrophage numbers are maintained in adulthood by in situ proliferation. In certain tissues, such as the lung, macrophage numbers can be supplemented postnatally by recruiting blood monocytes. In other tissues, such as the gut, the tissue-resident macrophages are constantly replenished by recruited blood monocytes[66,67].

In addition to the initial source of macrophages, the tissue-resident macrophage population is enriched by monocyte-derived macrophages during infection or injury, which respond to locally generated cytokine danger signals[66]. Recent studies in mice suggest that TREM2^{pos} synovial lining-layer tissue-resident macrophages are of prenatal origin and maintained by precursors proliferating in situ[9,10]. First, bone marrow transplant studies showed that synovial tissue-resident MHCII^{neg} (equivalent of TREM2^{pos} STMs) were long-lived, with their tissue pool not requiring a contribution from bone marrow for at least 2 months post-transplant[10]. This finding was supported by data showing that TREM2^{pos} macrophages are present in the synovium by embryonic day 15.5, suggesting that TREM2^{pos} STMs are derived from embryonic precursors[9]. Using a cell fate-mapping approach, it was demonstrated that the half-life of mouse TREM2^{pos} STMs is approximately 5 weeks, and that these cells are maintained in adulthood by M-CSFR^{pos} precursors proliferating in situ and independent of precursors from the circulation[9]. These mouse studies indicate a similar

embryonic origin for the synovial tissue RELMa^{pos} population (human LYVE1^{pos})[9]. It is still unclear whether human TREM2^{pos} and LYVE1^{pos} STMs clusters similarly differentiate from prenatal precursors that proliferate in situ, or whether these clusters are maintained by recruited circulating monocytes. A small population of LYVE1^{pos}ID2^{pos} STMs, closely resembling mouse M-CSFR^{pos} precursors, have been identified in healthy and arthritic human synovium[8], providing indirect evidence to support the prenatal precursor hypothesis. Human tissue-resident TREM2^{pos}, LYVE1^{pos} and LYVE1^{low}ID2^{pos} STM clusters express high levels of FOLR2, a protein that mediates the delivery of folic acid derivatives into the cell. Given that derivatives of folic acid are key for DNA synthesis, DNA repair, maintaining epigenetic marks and adhesion to collagen, the folic acid–folate receptor 2 (FOLR2) pathway might contribute to the fitness and longevity of these tissue-resident STM clusters[68–71].

Uncovering the origin of human tissue-resident STMs might improve our understanding of the mechanisms that underpin the deficiency in several regulatory pathways that persists in patients with RA, and in disease remission, such as that seen in TREM2^{pos} and LYVE1^{pos} STM clusters. Mouse studies suggest that monocyte-derived macrophages that adopt a tissue-resident phenotype might have different, perhaps impaired functions compared with the original resident macrophage population. For example, experimental repopulation of the lung alveolar niche with monocyte-derived macrophages impairs lung immune homeostasis[67,72]. This scenario resembles the changes in alveolar macrophages that occur with advanced age or lung injury, in which fetal liver-derived homeostatic alveolar macrophages are replaced by inflammatory monocyte-derived alveolar macrophages. Although the monocyte-derived alveolar macrophages can efficiently recycle pulmonary surfactant to enable efficient gas exchange[72], they have a more pro-fibrotic[73] and pro-inflammatory (as potent producers of IL-6)[74] phenotype than the original resident alveolar macrophages. Thus, a similar replacement of initial TREM2^{pos} macrophages of prenatal origin with inflammation-directed monocyte-derived macrophages in the synovium of patients with RA might contribute to the suboptimal joint immune homoeostasis in disease remission. Although emerging evidence suggests a prenatal origin of tissue-resident (MerTK^{pos}CD206^{pos}) STM clusters, experimental data suggest that pro-inflammatory tissue-infiltrating (MerTK^{neg}CD206^{neg}) STM clusters differentiate from infiltrating blood precursors. Human studies with radiolabelled CD14^{pos} monocytes[36], as well as mouse bone-marrow transplant and cell fate-tracking studies[9,10], strongly suggest that during arthritis both the CD14^{pos}CD16^{neg} and CD14^{pos}CD16^{pos} populations of blood monocytes give rise to the inflammatory macrophages, although it is unclear which monocyte population gives rise to which MerTK^{neg}CD206^{neg} STM clusters. In mice, depletion of blood monocytes using an anti-CCR2 antibody, or replacement of CCR2 competent (wild type) bone marrow with *Ccr2*$^{-/-}$ bone-marrow transplant, prevented the development of the mouse equivalent of human tissue-infiltrating (MerTK^{neg}) CD48^{pos}S100A12^{pos} and CD48^{pos}ISG15^{pos} STM clusters in the synovium that was associated with attenuated joint swelling in a mouse model of arthritis[10]. In summary, to better understand the origin of different human STMs, a close tracking of STM populations in patients administered autologous radiolabelled precursors, or monitoring of patients with RA after bone-marrow transplant, is required.

Tissue-specific cues

All tissue macrophage precursors acquire their identity via the action of the lineage-determining transcription factor PU.1, which itself is transcriptionally controlled by stromal compartment-derived M-CSF and IL-34 (REF.[1]). Subsequently, tissue-specific identity is provided by tissue-specific cues that induce differentiation of macrophages towards tissue-specific functions, such as bone remodelling osteoclasts, surfactant-recycling alveolar macrophages or iron-recycling red zone macrophages in the spleen[67]. The identity of 'tissue-identity' signals for many types of tissue macrophages have been uncovered, although the signals determining the identity of the lining-layer TREM2^{pos} STMs and sublining layer LYVE1^{pos} STMs, remain elusive.

In the lung, epithelial cell-derived granulocyte–macrophage colony-stimulating factor (GM–CSF) and surfactants act through transcription factors PPARγ[75] and BACH2 (REF.[76]) to induce the unique identity of alveolar macrophages, generating a cell capable of recycling surfactants to maintain patency and facilitate gas exchange[77]. Mice and humans lacking functional alveolar macrophages develop severe lung proteinosis owing to uncontrolled accumulation of surfactants, demonstrating the importance of this macrophage population for tissue homeostasis. Intriguingly, human TREM2^{pos} lining-layer STMs share transcriptomic similarities with human alveolar macrophages that also express TREM2 (REF.[39]), potentially reflecting similar biological functions. One possibility is that TREM2^{pos} STMs recycle components of synovial fluid, such as lubricin, which is produced by PDPN^{pos}PRG4^{pos} lining-layer fibroblasts to facilitate joint movement[78]. Thus, by analogy with the lung, lining-layer fibroblast-derived lubricin might drive the protective, inflammation-resolving barrier functions of the lining-layer.

Transcription factors that are specific to the lining (TREM2^{pos}) STM cluster have not yet been identified, but FLIP (FLICE-like inhibitory protein) might, at least in part, be responsible for their differentiation and inflammation-resolving phenotypes, including their expression of VSIG4, a receptor with immunosuppressive function[21]. Similarly, the drivers and transcription factors of the sub-lining (LYVE1^{pos}) cluster are unknown. Making some progress in this area, a recent study demonstrated that endothelial cell-derived JAG1 acts via its receptor NOTCH3 to determine the transcriptomic identity of the sublining synovial fibroblast clusters (CD90^{pos})[22]. A similar pathway might be involved in the function of the LYVE1^{pos} STM cluster that localizes around blood vessels in the joint and might regulate influx of inflammatory cells. Studies of STMs

that have transcriptional similarities to those in other tissues in homeostasis will likely provide insight into additional regulators of their functions. These might include the haem-driven transcription factor NRF2 (REF.[26]) or endothelial cell-derived R-spondin 3 (an activator of the canonical WNT signalling pathway)[79], both of which regulate the function of blood vessel-associated macrophages in liver and lung. Identification of STM-identity cues could help to design therapeutic strategies to boost their numbers or homeostatic functions in patients with RA.

Response to emerging danger signals

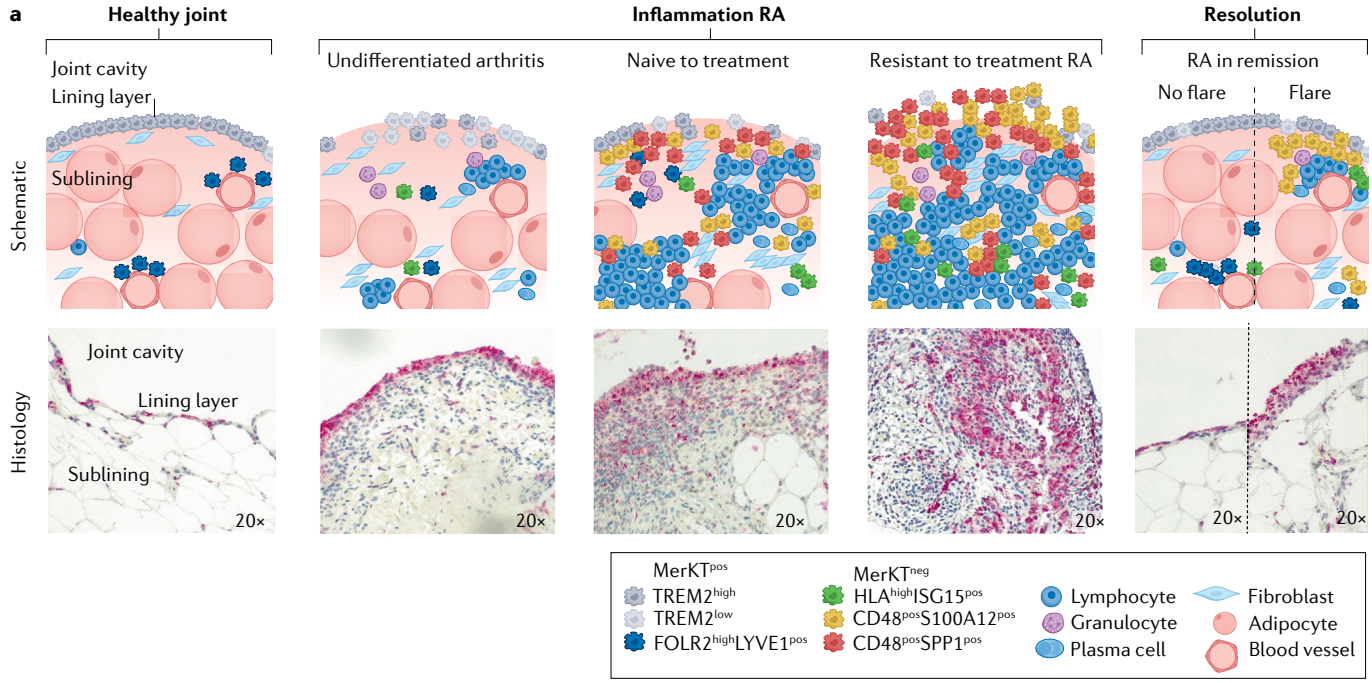
Tissue-resident and infiltrating monocyte-derived macrophages can shape the tissue environment in response to emerging 'immediate demand' signals, such as during infection or injury. During the development of synovitis, a variety of signals are thought to drive the pro-inflammatory activation of tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs, including pro-inflammatory cytokines, endogenous TLR ligands, oxidized lipids, interactions with epigenetically imprinted synovial fibroblasts and T cells (reviewed in REF.[80]). However, the precise upstream regulators of individual tissue-infiltrating STM clusters remain unknown. A comparison of scRNAseq datasets from human macrophage clusters in a range of inflamed tissues, including synovium, identified two inflammatory clusters that are shared between tissues: CXCL10- and CCL2-expressing macrophages and FCN1^{pos} macrophages[81], which are the equivalents of CD48^{pos}SPP1^{pos} and CD48^{pos}S100A12^{pos}[8,39] STM clusters, respectively. Emergence of these clusters in multiple tissues during inflammation suggests that they share a common upstream regulator. A preliminary computational prediction, supported by in vitro data, suggests that a combination of TNF plus IFNγ might be responsible for the differentiation of, at least, the CXCL10 and CCL2 cluster[81] (CD48^{pos}SPP1^{pos}[8,39]), but in vivo confirmation is needed. Recent studies suggest that high extracellular calcium levels can also drive differentiation of the CD48^{pos}SPP1^{pos} macrophage cluster from monocyte precursors, and that monocytes from patients with RA more easily respond to the calcium signal[82]. High levels of calcium are present in synovial fluid of patients with RA[83] and may be responsible for the differentiation of joint infiltrating monocytes into CD48^{pos}SPP1^{pos} pro-inflammatory macrophages in the RA synovium.

Compared with tissue-infiltrating (MerTK^{neg}CD206^{neg}) STMs, tissue-resident (MerTK^{pos}CD206^{pos}) clusters seem to respond differently to inflammatory signals, involving engagement of the GAS6–MerTK pathway to resolve inflammation. GAS6 is expressed by sublining synovial fibroblast clusters[7,8] and this expression is increased in synovial fibroblasts of patients in RA disease remission[8]. GAS6–MerTK interactions drive the expression of transcription factors (including KLF4 and NR4A2) that govern the active inflammation-resolving phenotypes of both MerTK^{pos}TREM2^{pos} and MerTK^{pos}LYVE1^{pos} STM clusters[84,85]. More detailed studies are required to confirm how the molecular interplay between the tissue-resident STMs and the tissue-infiltrating STMs determine chronicity or resolution of tissue inflammation.

STMs as treatment response targets in RA

Sustained disease remission is currently the target clinical outcome in RA management[86]. Although progress has been made in predicting the response to different therapies, the goal of personalized medicine, enabling identification of the most effective drug to induce early remission for a particular patient, is still elusive. Many of the therapeutics approved for the treatment of RA inhibit the inflammatory function of macrophages. In addition, early studies showed that macrophages can be biomarkers of a good therapeutic response, and therefore useful predictors of the response to some RA treatments. For example, an effective treatment response to gold, sulphasalazine, methotrexate or leflunomide was associated with a reduced macrophage infiltration into synovial sublining tissue following treatment[87]. STMs isolated from patients with RA undergoing synovectomy showed that leflunomide and methotrexate inhibit the release of pro-inflammatory cytokines and NF-κB expression[88]. Similarly, treatments targeting TNF (such as etanercept and infliximab) are associated with a reduction in the number of, and upregulated apoptotic signal in, STMs[89]. However, most mechanistic data investigating the therapeutic effect of various recent biological treatments on macrophage functions are derived from in vitro studies on monocyte-derived macrophages from the blood of patients with RA or from the assessment of mouse models of arthritis. Although these studies are informative[90], they provide limited insight into the changes in the composition of STM clusters and their resolving functions. For example, although in RA the main anti-inflammatory mechanism of methotrexate is the modulation of adenosine metabolism[91], methotrexate is also a folate antagonist with low binding affinity to FOLR2 (REF.[92]), a receptor that is highly expressed on tissue-resident TREM2^{pos} and LYVE1^{pos} STMs. Even though patients with RA are given supplementary folic acid with methotrexate, the role of methotrexate in modulating tissue-resident lining layer and perivascular STM functions are unknown. Thus, understanding the impact of therapeutics on various STM clusters, and the consequence on the synovial environment, requires synovial biopsy-driven investigations.

The development of minimally invasive biopsy techniques has enabled synovial tissue collection for high-throughput analysis and markedly improved our understanding of RA heterogeneity and response to therapies. The synovial biopsy-based study of the Pathobiology Early Arthritis Cohort (PEAC) established three different synovial pathotypes: 'diffuse myeloid', characterized by monocyte or macrophage enrichment; 'lympho-myeloid', characterized by aggregates of B and T lymphocytes with a variable degree of inflammatory cell infiltrate; and 'pauci-immune fibroid', characterized by a lack of an inflammatory cell infiltrate. RA consists predominantly (~85–90%) of the 'diffuse myeloid' and 'lympho-myeloid' pathotypes, and macrophages are the commonest histopathological feature regardless of lymphocyte enrichment[93]. The degree of enrichment of STMs can predict an effective response to TNF inhibition, compared with the poor response in patients with RA with a pauci-immune synovial pathotype[94].



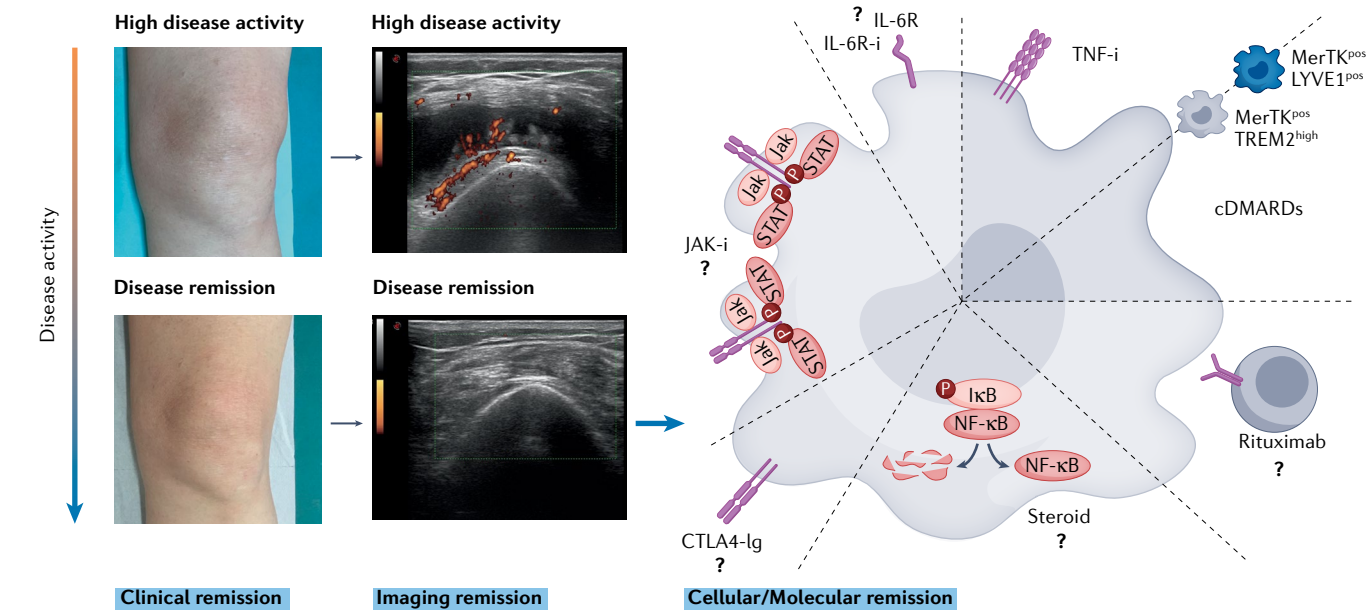


Fig. 3 | **STM clusters as novel biomarkers of treatment-response in RA.** **a** | Top: schematic showing changes in synovial tissue macrophage (STM) composition during RA progression. In RA, barrier-forming TREM2^{pos} switch to an inflammation-permitting TREM2^{low} phenotype in very early undifferentiated arthritis. In RA that is naive to treatment, an increase in the number of sublining MerTK^{neg}CD206^{neg} clusters S100A12^{pos}, SPP1^{pos} and ISG15^{pos} is observed. A further increase in SPP1^{pos} and ISG15^{pos} clusters is seen in patients with RA that are resistant to conventional DMARDs. Restoration of healthy STM composition, including the lining layer TREM2^{pos} barrier cluster and sublining LYVE1^{pos} cluster, occurs in patients in remission RA who do not flare after treatment tapering/cessation, whereas patients who flare exhibit the emergence of the pro-inflammatory MerTK^{neg}CD206^{neg} S100A12^{pos} cluster in the sublining layer. Bottom: representative histology images of staining for pan macrophage marker CD68 (red) on ultrasound-guided minimally invasive synovial tissue biopsies are shown at different RA stages. **b** | Levels of RA remission. The current definition of remission includes clinically based (such as DAS28- or Boolean-based) and imaging-based (such as ultrasonography) parameters. Representative images of the knee joint from a patient with active RA and from a patient with RA in sustained clinical remission (left) and their ultrasound Power Doppler evaluation (right) are shown. These illustrate an increased blood flow associated with inflammation (positive Power Doppler signal) in the knee of a patient with active RA and a lack of Power Doppler signal in the knee of a patient in remission, confirming a lack of inflammation. Patient knee images, ultrasonography and histology images come from the SYNGem Biopsy Unit (Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy). Emerging studies indicate that inclusion of a cellular and molecular description of STMs can enhance the power of algorithms designed to predict sustained disease remission in patients with RA. For example, patients who achieved disease remission with methotrexate and TNF inhibitors (TNF-i) have a reduced risk of disease flare after treatment cessation if their synovium is enriched in the MerTK^{pos}CD206^{pos} STM clusters, TREM2^{pos} and LYVE1^{pos}. The phenotype and molecular signature of STMs during sustained clinical and imaging remission achieved with other treatments (such as IL-6R inhibition (IL-6Ri), JAK inhibition (JAKi), CTLA4-Ig, steroids and rituximab) are unknown.

Omics
Genomics, proteomics, metabolomics and transcriptomics aimed at the collective characterization and quantification of pools of biological molecules revealing the biology of the cells.

In addition, synovial tissue expression of gene modules characteristic of pro-inflammatory macrophages correlated with clinical parameters such as disease activity measured with DAS28-CRP[95]. This finding is in line with those of earlier studies showing that synovial tissue enriched with macrophages expressing CD163, MRP14 (now called S100A9) and MRP8 (now called S100A8) that produced TNF in situ predicted a good response to TNF inhibition[96]. Similarly, comparison of anti-IL-6R with TNF-inhibitor therapy (the ADACTA trial) defined that a baseline myeloid synovitis was associated with a good clinical response at 24 weeks of TNF inhibitor treatment[97]. By contrast, patients with RA who responded inadequately to TNF inhibition, and who were classified as having a B cell poor synovitis (defined histologically and by RNA sequencing) had more successful treatment responses to IL-6R inhibition compared with TNF or B cell-targeted therapy[98].

Preliminary studies suggest that the macrophage cytokine profile released by synovial explant in vitro can predict response to RA therapies. For example, the concentration of inflammatory mediators, such as IL-6, MCP-1 and TNF, were associated with disease activity and structural characteristics of the source joint[99]; high production of IL-6 and MCP-1 correlated with the subsequent response to TNF inhibitiors[100]. Thus, pro-inflammatory macrophage-rich synovitis is a useful biomarker for predicting therapeutic responses, at least to TNF inhibitors.

Recent analysis of prospective longitudinal synovial samples from patients with RA suggests a continuum in synovial pathotypes from a pauci-immune, fibroid pathotype to a lympho-myeloid pathotype, with progressive involvement of innate and adaptive immune cells leading to the formation of ectopic lymphoid structures with plasma cells[95,101]. In this inflammatory spectrum, infiltrating macrophages that function as a source of pro-inflammatory cytokines and antigen-presenting cells to T follicular helper cells might be fundamental for local B cell activation and maturation of plasma cells. Therefore, in this context, despite pathotypes representing different types of synovitis characterized by different pathogenetic and inflammatory milieu, the heterogeneous functions of STMs could represent evolving states of activation in the disease process[95]. Thus, identifying specific STM clusters associated with progression between pathotypes will help us to understand the cellular and molecular mechanisms that underlie this transition and provide potential biomarkers to predict the evolution of the disease in terms of response to specific treatments.

Patients with RA who achieve sustained disease remission might experience disease flare immediately after tapering or cessation of treatment, with approximately half of patients developing a flare within a year[15]. Typically, the duration of drug-free remission is limited[54], with a progressive increase in the risk of disease flare over time[55]. Thus, identifying patients at risk is the first step in preventing the occurrence of flare, although prediction methods are lacking. A recent study showed that in patients who achieved sustained remission (defined by clinical and ultrasound criteria) with anti-TNF plus methotrexate treatment, the relative proportions of the main STM populations (that is, tissue-resident regulatory MerTK^{pos}CD206^{pos} versus tissue-infiltrating, pro-inflammatory MerTK^{neg}CD206^{neg}) at the time of treatment tapering or cessation can predict whether the patient remains in remission for at least a year[8]. This finding suggests that identifying the phenotypes of STMs might provide insight into the underlying mechanisms of remission and serve as a useful predictor of sustained disease remission versus disease flare. Similar studies are required to investigate the composition and phenotypes of STMs in patients with RA in remission following different treatments (FIG. 3). Recently, an increasing number of algorithms have been developed to predict the response to treatments, such as by quantifying the nature and extent of synovial tissue inflammation, tissue pathotypes and transcriptomics, duration of symptoms, autoantibody positivity and the response to first-line treatment[30,95]. Emerging evidence presented in this Review suggests that recognition of the phenotype and function of the STM clusters, as well as their relative proportions, could strengthen the predictive power of these models.

Conclusions

Recent advances in single-cell omics have uncovered a rich heterogeneity in STMs. The discovery of distinct STM clusters that are vital for joint homeostasis and maintaining disease remission provides a paradigm shift in our understanding of the role of macrophages in arthritis. Capitalizing on the joint protective and inflammation-resolving biology of newly identified STM clusters might aid the development of novel therapeutics that combat arthritis and maintain disease remission. In addition, emerging data suggest that profiling STM clusters could clarify the mechanisms of disease progression and help to predict response to treatment in patients with RA and other chronic inflammatory joint disorders.

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