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Sphingosine-1-Phosphate and Rheumatoid Arthritis: Pathological Implications and Potential Therapeutic Targets

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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects approximately 1% of the population worldwide. RA mainly targets the synovial tissues of the small joints of the hands and feet, although larger joints are also affected. The disease is characterized by 1) proliferation of synovial fibroblasts, leading to synovial hyperplasia; 2) recruitment of inflammatory cells into joint tissue, resulting in tissue destruction; and 3) excessive secretion of pro-inflammatory cytokines/chemokines, contributing directly to synovium inflammation. While the etiology of RA remains unknown, inflammatory mediators appear to drive the evolution of the disease. In particular, TNF- α together with proinflammatory cytokines, including IL-1 β and IL-6, have been shown to be pivotal in promoting cytokine, chemokine and matrix metalloproteinase production within the RA synovium, along with cellular activation and joint erosion [1,2]. Given the complexity of the inflammatory cascade in the RA synovium, it is of great importance to identify novel biochemical signalling moieties that have the potential to constitute intracellular molecular checkpoints within the cell.

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite which is formed from sphingosine by sphingosine kinases (SphKs) and degraded by S1P phosphatases (SPPs) and S1P lyase (SPL). S1P is critically involved in both physiological and pathological processes. The lipid is implicated in many cellular processes including proliferation, apoptosis and migration via binding to and activation of its G protein-coupled cell surface receptors. Alterations in S1P signalling as well as in the enzymes involved in its synthesis and metabolism have been observed in many types of pathological situations such as angiogenesis, metastasis, and autoimmunity. Accumulating evidence now suggests a role for S1P in various as-

pects of RA biology. This can involve, for example, activation of SphKs [3] and an elevated level of S1P [4] in the synovium and synovial fluids of patients with RA, as well as alterations in S1P signalling that lead to synovial fibroblast migration, proliferation, survival and production of proinflammatory cytokines/chemokines [4,5]. This review will highlight how S1P is involved in RA pathology and the mechanisms of its action. In addition, the therapeutic potential of drugs that alter S1P actions will be examined with reference to RA.

2. S1P biology

Sphingolipids are a class of complex, structurally-related compounds derived from sphingoid bases, with hundreds of known class members; they represent a major class of lipids that are ubiquitously expressed in eukaryotic cell membranes. Apart from their structural functions, sphingolipids have emerged as the source of important signalling molecules; these sphingolipid metabolites have important roles in stimulus/agonist-mediated signalling which regulate many cellular processes including inflammation, cell proliferation, apoptosis, angiogenesis, and transformation [6]. Sphingolipid-mediated signalling also influences the pathophysiology of many diseases including cancer, and autoimmune and inflammatory diseases.

S1P is one of the most important sphingolipid metabolites. It was first identified as a potent second messenger in the early 1990s [7,8]. Since then, S1P has been shown to be involved in many important cell signalling pathways and physiological processes such as angiogenesis, cell migration and movement, cell survival and proliferation, cellular architecture, cellular contacts and adhesions, heart development, vascular development, atherogenesis, acute lung injury and acute respiratory distress, tumorigenicity and metastasis, and inflammation and immunity (reviewed in [9,10]). New tools, such as specific agonists and antagonists and the generation of targeted knockouts, have led to a surge of interest in the role of S1P in numerous diseases. Recent studies have shown, for example, that it modulates the pathophysiological consequences of various autoimmune diseases, such as Sjogren's syndrome [11] and systemic sclerosis [12].

2.1. S1P metabolism

S1P is present at submicromolar concentrations in various biological fluids and tissues [13]. It is predominantly present in the platelets and erythrocytes in the blood, at concentrations of 100 nM to 4 μ M [14], because the platelets lack the S1P degradation enzyme SPL [15]. Human serum is also a rich source of S1P with concentrations ranging from 340 nM to 1 μ M [16,17]. Moreover, the erythrocytes appear to be the cells mostly responsible for the storage and constant supply of plasma S1P [14].

S1P is produced intracellularly by a series of enzymatic reactions (Figure 1); all cells are able to generate it during the normal physiologic metabolism of sphingolipids. Sphingomyelin hydrolysis is considered to be the first step in the pathway generating S1P. The reaction is catalyzed by sphingomyelinases yielding ceramide. Ceramide is the central step

in sphingolipid metabolism and can be also synthesized *de novo* from serine and palmitate by the action of ceramide synthase [18]. Ceramide is, in turn, deacylated by ceramidase to release sphingosine, which is then phosphorylated either by SphK1 or SphK2, to yield S1P. While both SphK1 and SphK2 can phosphorylate sphingosine, SphK1 produces most of the S1P [19]. SphKs can be activated by a large variety of agonists, such as TNF- α (reviewed in [20-22]). Activation of SphK1 leads to its translocation to the plasma membrane where its substrate sphingosine is located, resulting in the production of S1P [23,24]. S1P, in turn, activates specific S1P receptors present on the surface of the same cell or on nearby cells in autocrine and/or paracrine manners [20]. This “inside-out” signalling of intracellularly generated S1P is crucial for many of its functions [25]. S1P is degraded through two distinct mechanisms, the reversible dephosphorylation into sphingosine by SPPs, and the irreversible degradation by SPL to hexadecenal and ethanolamine phosphate [26,27]. Consequently, the cellular levels of S1P are tightly regulated by its formation from sphingosine through the activity of SphKs and its degradation through the activity of SPPs and SPL. In the basal state, the balance between S1P generation and degradation results in low cellular levels of S1P [28,29].

The mechanism by which S1P is exported to the outside of cells after synthesis is not fully understood. Several studies suggested the involvement of the ATP-binding cassette (ABC) family of transporters in S1P secretion [30-32]. It has been shown that its release from mast cells is regulated by ABCC1 [31] while the ABCA1 transporter is critical for release of S1P from astrocytes [32]. Altogether, these studies suggested that members of the family of ABC transporters may be important for the transport of S1P out of cells.

2.2. S1P receptors and S1P receptor-mediated signalling

S1P exerts diverse biological activities under physiological and pathological conditions via both intracellular and extracellular signalling pathways, but mostly the latter. To date, five cell surface G protein-coupled S1P receptors (S1P1-5), belonging to the endothelial differentiating gene (EDG) family, have been identified [13]. S1P receptors exhibit variable tissue distribution: S1P1, S1P2, and S1P3 are widely expressed in various tissues, whereas the expression of S1P4 and S1P5 is more restricted to cells of the immune system and nervous system, respectively (reviewed in [33]). Each S1P receptor couples to a specific heterotrimeric G protein: $G_{i/o}$, G_q and $G_{12/13}$. When activated, the G protein dissociates into its α and $\beta\gamma$ subunits and transduces signals toward the downstream pathways. In particular, S1P1 is coupled predominantly to $G_{i/o}$ through which it activates signalling known to be essential for embryonic blood vessel development as the murine S1P1 knockout is lethal at the embryonic stage, as a result of hemorrhage [34]. In addition, plasma S1P has been shown to elicit egress of lymphocytes into the blood in an S1P1-dependent manner [35] and to regulate basal and inflammation-induced vascular leak *in vivo* [36]. S1P2 and S1P3 are linked predominantly to G_q and $G_{12/13}$; knockout of both receptors in mice decreases litter size and survival rates [37]. S1P4 and S1P5 are the least studied receptors, although it is known that S1P4 is involved in T-cell proliferation [38] and S1P5 is required in natural killer cell trafficking [39]. More detailed information on the various signalling pathways triggered by S1P receptor activation

can be found in previous reviews [40,41]. The five known S1P receptors can lead to activation of different downstream targets, such as Rac, ERK, PI3K, adenylyl cyclase, phospholipase-C, Rho or JNK, resulting in the abovementioned cellular responses [42]. The extracellular pathways mediated by each S1P-specific receptor are common; however, given the existence of agonists and antagonists that exhibit receptor specificity, it is probable that the S1P receptors are not totally redundant [43]. Upon binding to one of the five known cell surface receptors, S1P initiates signal transduction leading to various cellular responses.

S1P also exerts its action as a second messenger via intracellular pathways. For example, it intracellularly targets the histone deacetylases HDACs, regulating specific and contextual chromatin states that impact gene transcription [44]. S1P has been shown to promote growth and survival, independently from its G protein-coupled receptors, in mouse embryonic fibroblasts devoid of S1P receptors [35]. There is also evidence supporting a role for intracellular S1P in calcium mobilization [45].

3. Alteration of S1P in rheumatoid arthritis

TNF- α is the predominant proinflammatory cytokine in RA. TNF- α can activate SphK, which leads to the production of S1P [46]. Indeed, SphK1, SphK2 [3,47] and S1P levels [48] are elevated in the synovium of patients with RA. Moreover, administration of S1P to RA synovial fibroblasts causes their proliferation, survival, and migration, as well as cytokine/chemokine and other proinflammatory mediator production [4,5]. The findings suggest that S1P may play a role in RA pathology (Figure 2).

3.1. SphK activity and S1P levels in RA synovium

Activated SphKs and elevated S1P levels are associated to RA (Table 1). For instance, increased SphK1 expression and activity was found in RA B lymphoblastoid cell lines, and identified as the underlying mechanism of impaired Fas-mediated death signalling in RA [47]. More recently, SphK2 has been shown to be strongly expressed in RA synovial fibroblasts *in vivo* and *in vitro*, which is associated with upregulation of S1P [3]. Suppression of SphK2 by siRNA results in a more aggressive disease and greater secretion of proinflammatory cytokines, such as IL-6, TNF- α and IFN- γ in a murine collagen-induced arthritis (CIA) model [49]. Of more interest, in a murine CIA model, administration of a pharmacological SphK inhibitor, *N,N*-dimethylsphingosine (DMS), and an siRNA approach to knockdown SphK1 isoform markedly suppressed joint pathologies such as adjacent cartilage and bone erosion, synovial hyperplasia, and inflammatory infiltration into the joint compartment [49].

At present, evidence for roles in RA of other S1P metabolic enzymes, such as SPPs and SPL, is limited. However, up-regulation of SPP2 was detected in samples of skin lesions from patients with psoriasis, a chronic inflammatory skin disease [50]. Interestingly, an elevated mRNA expression of SPP1 and SPL was observed in RA synovial fibroblasts, as compared to non-arthritic synovial fibroblasts (Zhao et al., unpublished data).

S1P is widely expressed in RA synovium. Elevated levels were detected in both synovial tissue and synovial fluids from patients with RA [4,48]. Moreover, the S1P content in synovial fluids from patients with RA was compared to that from patients with osteoarthritis (OA), a degenerative joint disease [4]. S1P levels were shown to be much higher in synovial fluids of RA patients than in those of OA patients. In that study, the S1P level in RA synovial fluids was even higher than those in serum or plasma from normal donors. A similar experiment was performed with a different experimental strategy, in which the S1P level in RA synovial fluids was five fold higher than that in OA synovial fluids [48]. Peripheral blood B lymphoblastoid cell lines from patients with RA exhibited a high level of S1P level as well [47]. This increase in S1P level could be responsible for the recruitment and retention of the immune infiltrates in the synovium.

	RA	OA	Normal	Experimental Strategy	Reference
S1P content in synovial fluids	17.51±4.23 µM	3.45±0.85 µM	N/A	Competitive ELISA	[48]
	1,078.92 pM/ml	765.01 pM/ml	N/A	HPLC	[4]
S1P intracellular level in synovial cells	Increased level in LDLs compared to normal control cells	N/A		Chromatography	[47]
SphK activity in synovial cells	Markedly increased enzymatic activity in LDLs compared to normal control cells	N/A		Sphk enzymatic activity assay	[47]
SphK expression in synovial fibroblasts	More SphK2 positive cells than those in OA	Weakly positive	N/A	Immunofluorescence	[3]
S1P1 receptor expression in synovial tissue	Markedly enhanced expression than that in OA	Weak expression	Weak expression	immunostaining	[4]
N/A: not applicable or not available					

Table 1. SphK expression/activity, S1P levels, and S1P receptor expression in synovium of RA, OA, and normal donors.

3.2. S1P receptor expression in synovial fibroblasts

Synovial fibroblasts or fibroblast-like synovial synoviocytes (FLS) are key contributors to RA chronic inflammation due to their abnormal growth and erosive activity. During RA disease progression, these cells become hyperplastic, closely interact with infiltrated immune cells to form the aggressive pannus tissue that invades and degrades the cartilage and bone and eventually promotes joint destruction (reviewed in [51]). Synovial fibroblasts also contribute to the local production of cytokines/chemokines, small molecule mediators of inflammation, and proteolytic enzymes that degrade the extracellular matrix [52].

RA synovial fibroblasts have been reported to express three of five known S1P receptors, S1P1-3 [4,5,53]. Expression of S1P1 in RA inflamed synovial tissue is significantly higher than that in OA synovial tissue [4]. Of more interest, pretreatment of RA synovial fibroblasts with TNF- α , the cytokine well-recognized to be critical in RA, results in up-regulation of S1P3 receptor expression in synovial fibroblasts, which likely contributes to the synergistic production of inflammatory cytokines/chemokines, migration (or invasion) and survival of these cells upon subsequent exposure to S1P [5]. Thus, it seems that the elevated TNF- α levels observed in the synovial fluid of RA patients could make synovial fibroblasts more responsive to increases of S1P in RA synovium; in turn, the enhanced responsiveness to S1P through the S1P3 receptor could increase synovial fibroblast survival, migration and production of cytokines/chemokines, processes that all likely contribute to the pathology of RA.

3.3. Influence of S1P on the secretion of proinflammatory cytokines/chemokines and other proinflammatory mediators by synovial fibroblasts

One key feature of RA is the large amounts of pro-inflammatory cytokines and chemokines produced by activated synovial cells. These cytokines/chemokines may directly contribute to cartilage and bone erosion by promoting matrix metalloproteinase (MMP) production and chondrocyte/osteoclast destruction function [51]. S1P can stimulate synovial fibroblasts to release various inflammatory mediators, including cytokines, chemokines, and prostaglandin E2 (PGE2) [4,5]. S1P administration notably stimulates the synovial fibroblast secretion of IL-8, IL-6, MCP-1 and RANTES via S1P2 and S1P3 receptors and through modulation of p38, ERK, and Rho kinase activities [5]. The S1P-induced cytokine/chemokine secretion and S1P receptor-mediated signalling pathways were further suggested as the driving force for synovial fibroblast invasion into the surrounding tissue [5]. Moreover, inhibition of S1P production by a potent SphK1 inhibitor, DMS, significantly suppresses production of these cytokines [48]. The effect of S1P on cytokine secretion is further amplified by TNF- α [5], suggesting that the cytokine-rich environment of the inflamed synovium may synergize with S1P signalling to exacerbate the disease process [5].

In addition, S1P has been found to indirectly stimulate IL-17 secretion by activating T-cell receptor-activated CD4 T cells [54]. IL-17 is produced by cultured peripheral blood mononuclear cells (PBMC) and synovial membrane cells, and elevated levels of IL-17 are detected in the synovial fluid of RA patients [54,55]. It was reported that IL-17-deficient mice showed resistance to CIA [56]. Furthermore, IL-17 was demonstrated to contribute to the severity of synovial inflammation and bone destruction in RA by stimulating the production of proin-

flammatory cytokines and chemokines such as TNF- α , IL-1 β , IL-6, MMPs, and receptor activator of NF- κ B ligand (RANKL) [57,58].

S1P is able to stimulate the production of other inflammatory mediators, such as PGE2, and of its metabolic enzymes cyclooxygenases (COXs) in RA synovial fibroblasts [4,53]. PGE2 is an autocrine lipid mediator derived from arachidonic acid metabolism by COX-1 or COX-2 [59]. The inflammation characteristic of RA is actually closely associated to the production of PGE2 by synovial fibroblasts, as PGE2 stimulates angiogenesis in rheumatoid synovium [60] and triggers bone resorption by osteoclasts [61]. Thus, S1P may aggravate synovial hyperplasia, inflammation and angiogenesis through the induction of COX-2 and PGE2 in RA synovial tissues.

3.4. Upregulation of immune cell recruitment and retention by S1P in the RA synovium

Recruitment and retention of inflammatory cells, such as neutrophils, monocytes and T lymphocytes, are other fundamental features of RA synovitis. This process is coordinated by the presence of chemo-attractant proteins at the site of inflammation, assisted by the expression of adhesion molecules. Chemokines and other small chemo-attractant molecules are abundant in the RA synovium and can be produced by synovial fibroblasts in the intimal lining. S1P, as a chemo-attractant, plays a pivotal role in the immune cell egression. S1P1, for example, is essential for lymphocyte recirculation since it regulates lymphocyte egress from both thymus and peripheral lymphoid organs [62,63]. As S1P exists at lower levels in tissues and higher levels in the blood and lymph, it is suggested that the S1P gradient between tissues and the blood/lymph drives such a migration. Likewise, the elevated S1P levels in the synovial fluid of RA patients [48] could be responsible for the recruitment and retention of the immune infiltrates in the RA synovium. Indeed, inhibition of S1P1 down-regulated inflammatory cell accumulation in an adjuvant-induced arthritis (AIA) animal model [64].

S1P may also contribute indirectly to the recruitment and retention of inflammatory cells into RA synovium by stimulating the secretion of other chemo-attractants. In RA, the recruitment of immune cells into the synovium may be due to the large amount of CC and CXC chemokines produced by activated cells of the synovial lining. In particular, IL-8 exhibits selective chemotactic activity for neutrophils, whereas MCP-1, MIP-1 α , -1 β and RANTES primarily attract monocytes. RA synovial fibroblasts do not secrete detectable levels of cytokines or chemokines, except for low amounts of MCP-1 in the resting state; upon treatment with S1P, however, the secretion of IL-8, IL-6, MCP-1 and RANTES is strongly induced, indicating S1P contribution to and/or amplification of the secretion of chemokines by cells of the inflamed synovium [5]. Since immune cells express a wide repertoire of chemokine receptors, including those of IL-8, MCP-1, SDF-1 α , IP-10 and RANTES [65], S1P may indirectly drive the recruitment and retention of inflammatory cells in RA by this chemokine secretion. Indeed, a bioactive lipid structurally-related to S1P, lysophosphatidic acid (LPA), is able to recruit leukocytes into an *in vivo* inflammatory site by stimulating inflammatory cytokine/chemokine secretion [66].

3.5. Promotion of proliferation and/or survival of synovial fibroblasts, B lymphocytes, and chondrocytes by S1P in the RA synovium

Abnormal growth of synovial fibroblasts and chondrocytes has been suggested to contribute directly to hyperplasia of the rheumatoid synovium [67]. The growth in RA of the synovial fibroblast population is likely attributable to an imbalance between cell proliferation, survival, and death. In fact, synovial fibroblast proliferation is difficult to demonstrate in RA. Nonetheless, limited numbers of mitotic figures or cells expressing cell cycle markers suggest that synovial fibroblast DNA synthesis is not a major influence [68,69]. Instead, the RA synovial environment promotes survival of these cells and discourages their depletion through apoptosis. S1P, through S1P₁, protects synovial fibroblasts from apoptosis [5]. Thus, the ability of S1P to promote synovial fibroblasts survival, whether or not this agent can also increase cell proliferation, could contribute to RA pannus hyperplasia [70].

B lymphocytes play an important role in the pathogenesis of RA. B-cell accumulation and maturation in the inflamed synovium can form ectopic germinal centers [71-73] and activate T cells [74]. Moreover, mature plasma cells secrete autoantibodies, such as the rheumatoid factor, which are key features of RA [75]. The importance of B cells in RA is illustrated by the success obtained when targeting CD-20-positive B cells with the chimeric monoclonal anti-CD20 antibody, rituximab [76]. In that study, a single short course of rituximab, either alone or in combination with cyclophosphamide or continuing methotrexate, provided patients with significant improvements in disease symptoms, a reduction in B-cell numbers, rheumatoid factors, and total immunoglobulin levels. S1P appears capable of increasing cell survival and inhibiting apoptosis of B lymphocytes derived from RA patients, as these cells are uniquely resistant to Fas-mediated apoptosis [47]. This effect is due to an increased activity of SphK1 and an overproduction of S1P. In a murine experimental arthritis model, administration of SphK inhibitor, DMS, and of SphK1 siRNA significantly decreased the production of anticollagen IgG2a in the mouse serum [48,49].

S1P was also reported to induce chondrocyte proliferation through stimulation of COX-2 and PGE₂ production and via activation of ERK [77,78], and was thus suggested to be able to modulate cartilage homeostasis.

3.6. Contribution of S1P to osteoclastogenesis

Pathologic bone loss is a common feature of RA in which progressive destruction of bone is associated with joint inflammation. Focal bone erosion occurs at the pannus-bone interface and in the immediate subchondral bone early in RA, and is associated over time with significant morbidity for patients [79]. Bone-resorbing osteoclasts have been identified as important effector cells in inflammation-induced bone loss in both experimental animal models and human RA. Osteoclasts are derived from hematopoietic precursor cells of the myeloid lineage [80,81]. In normal skeletal remodelling, the balance between bone resorption by osteoclasts and bone formation by osteoblasts is critically regulated [82-84] and osteoclast differentiation is dependent on the presence of two key factors, RANKL and colony-stimulating factor-1(CSF-1), provided by cells of the osteoblast lineage [81,85,86]. In experimental animal models of arthritis, osteoclasts have been observed at sites of focal bone

erosion [87,88]. In addition to the RANK/RANKL pathway, many cytokines (such as TNF- α , IL-6, and IL-17) and growth factors elaborated by inflamed synovial tissues may contribute to osteoclast differentiation and activation in RA [89].

S1P has been found to induce chemotaxis of osteoclast precursors and osteoclastogenesis *in vitro* [90] and *in vivo* [91]. In a bone marrow-derived macrophage and osteoblast coculture system, for instance, S1P addition greatly increased osteoclastogenesis by increasing RANKL in osteoblasts via cyclooxygenase-2 and PGE2 regulation [90]. S1P also chemoattracted osteoblasts and enhanced their survival [90]. Moreover, S1P controls the migratory behaviour of osteoclast precursors between bone tissues and the blood stream, dynamically regulating bone mineral homeostasis via S1P receptors. Cells with the properties of osteoclast precursors indeed express functional S1P1 receptors and exhibit positive chemotaxis along an S1P gradient *in vitro* [92]. On the other hand, S1P2 requires a higher concentration of S1P for activation and induces negative chemotactic responses to S1P gradients [91]. S1P2 activation causes cells to move from the bloodstream into bone marrow cavities [91].

3.7. SphKs and S1P in experimental arthritis

Both CIA and AIA are well-established models for studying RA. Administration of the S1P receptor agonist FYT720, which down-regulates S1P receptors, in rat CIA and AIA models inhibits rat hind paw oedema and joint destruction and decreases lymphocyte invasion into the joints [93,94]. In addition to receptor modulation, non-specific inhibition of SphK with DMS in a murine CIA model has been shown to significantly reduce adjacent cartilage and bone erosion, synovial hyperplasia, and inflammatory infiltration into the joint compartment [48,49]. Moreover, suppression of SphK1 via siRNA knockdown results in similar reduction in joint pathology, serum levels of IL-6, TNF- α , IFN- γ and S1P, and the *in vitro* production of these proinflammatory mediators in response to collagen [48,49]. In another murine arthritis model, the transgenic human TNF- α model that develops spontaneous erosive arthritis, Sphk1-deficient mice exhibit significantly less synovial inflammation and joint pathology than the wild-type mice [95].

Interestingly, SphK isoforms may play different roles in RA. In a murine CIA model, down-regulating SphK1 via specific small interfering RNA (siRNA) significantly reduced the incidence, disease severity, and articular inflammation. Treatment with SphK1 siRNA also down-regulated serum levels of S1P, IL-6, TNF- α , IFN- γ , and IgG2a anticollagen antibody [49]. On the other hand, mice receiving SphK2 siRNA developed a more aggressive disease, and higher serum levels of IL-6, TNF- α , and IFN- γ , when compared with control siRNA recipients. These results suggest distinct immunomodulatory roles for SphK1 and SphK2 in the development of inflammatory arthritis via the regulation of the release of proinflammatory cytokines and T cell responses.

S1P metabolism and SphK/S1P/S1P receptor axis-mediated signalling pathways in rheumatoid arthritis synovium and the potential role of S1P in RA pathogenesis are illustrated in Figure 1 and Figure 2, respectively.

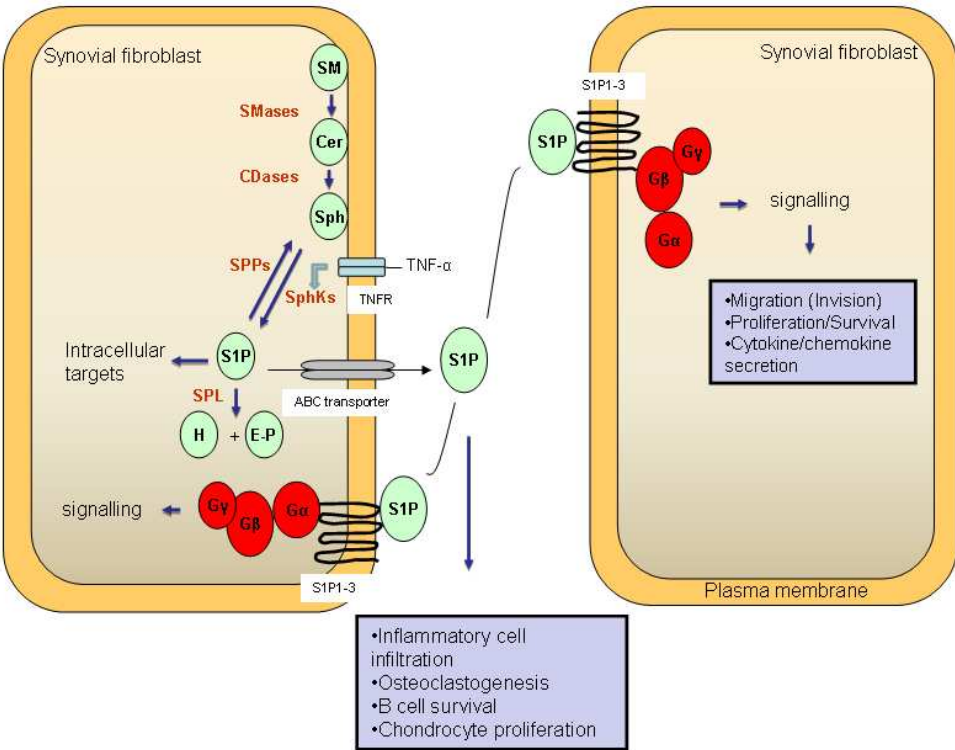


Figure 1. S1P metabolism and SphK/S1P/S1P receptor axis-mediated signalling pathways in RA synovium. S1P homeostasis is tightly regulated by the balance between its synthesis and degradation via three enzyme families: (1) Sphks (SphK1 and SphK2), which generate S1P through phosphorylation of its precursor, sphingosine (Sph), (2) SPPs (SPP1 and SPP2), which reversibly convert S1P back to Sph, and (3) SPL, which irreversibly degrades S1P to generate ethanolamine phosphate and hexadecenal. In the inflamed RA synovium, the specific binding of agonists, such as TNF- α , to their receptors (TNFR) induces the expression of SphKs, which in turn converts the membrane-bound Sph into S1P in synovial fibroblasts. The generated S1P then exits the cell through the ATP binding cassette (ABC) transporter and exerts its action through the G-protein coupled S1P receptors in an autocrine and/or paracrine fashion, activating specific S1P receptors presenting on the surface of the same cell or on nearby cells. Each S1P receptor couples to a specific heterotrimeric G protein. When activated, the G protein dissociates into its α and $\beta\gamma$ subunits and transduces signals toward the downstream pathways to regulate cell proliferation, growth, migration, apoptosis, etc. Extracellular S1P can stimulate the infiltration of immune cells, the proliferation and/or survival of synovial fibroblasts, B lymphoblastoid cells, and chondrocytes, as well as the osteoclastogenesis in the synovium. S1P also exerts its action as a second messenger via intracellular pathways, regulating chromatin states that impact gene transcription, cell growth and survival, and calcium mobilization independent of its G protein-coupled receptors. SM, sphingomyelin; Cer, ceramide; H, hexadecenal; E-P, ethanolamine phosphate; SMases, sphingomyelinases; CDases, ceramidases.

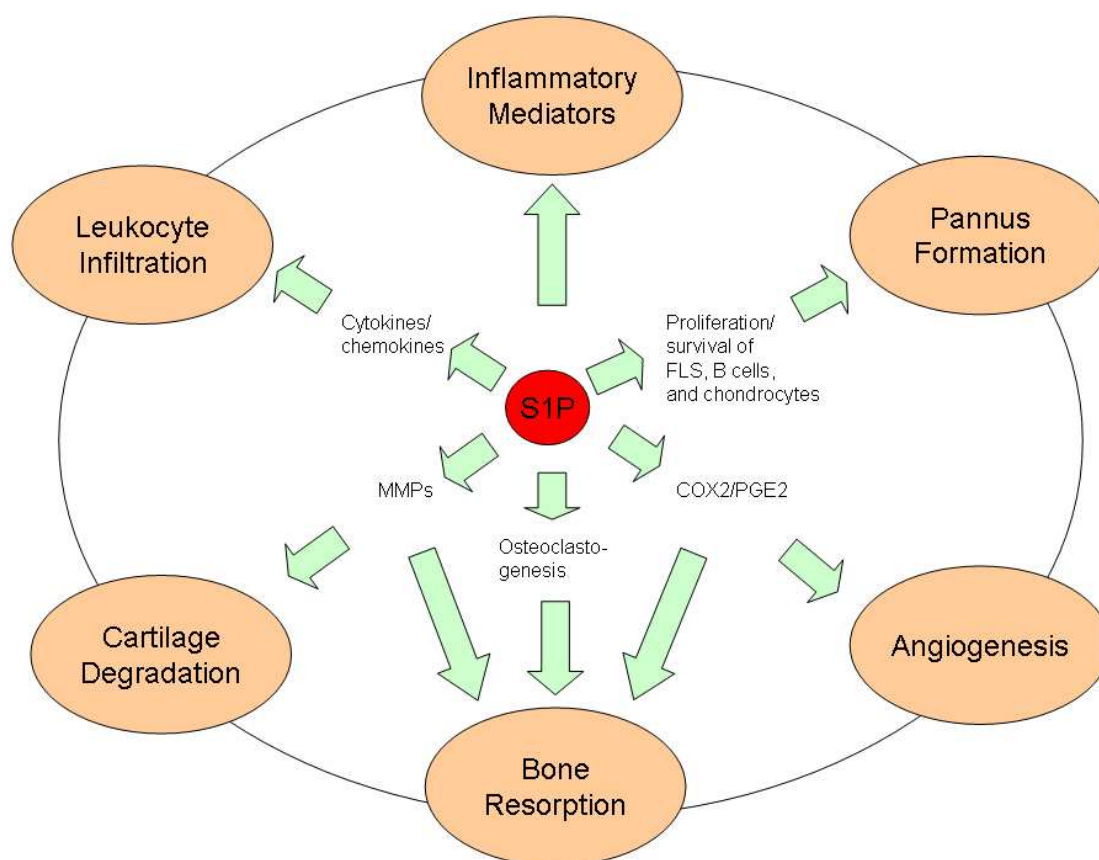


Figure 2. Potential role of S1P in the pathogenesis of RA.

4. Therapeutic POTENTIAL of S1P in rheumatoid arthritis

The introduction of novel biological therapies in the mid-1990s markedly improved clinical outcomes in RA. Cytokine antagonists, such as biologic agents that inhibit $\text{TNF-}\alpha$, IL-6, or IL- 1β , decrease inflammation and joint destruction [96]. The impressive efficacy of these biologic agents, however, is only seen in about half of the patients. Similarly, B cell depletion and T cell co-stimulation blockers [96] are beneficial only in non- or partially-overlapping subsets of patients. There is undoubtedly a necessity to develop therapies that target other pathways. As S1P modulates RA pathogenesis in many aspects, manipulation of endogenous amounts of bioactive S1P and/or its receptor activation may be beneficial for joint inflammation and destruction.

4.1. Targeting S1P levels

Decreasing S1P level by inhibiting SphK1 activity may represent a therapeutic approach against RA. Blockage of SphK1 activity in an animal arthritis model indeed significantly suppressed articular inflammation and joint destruction, reduced disease severity, and

down-regulated proinflammatory cytokine production and inflammatory cell infiltration into the synovium [49]. In fact, inhibition of S1P synthesis by blocking SphK activity has proven useful as an anti-inflammation strategy in cancer therapy [97-101].

Depleting S1P level by utilizing S1P-blocking agents, such as specific antibodies, may also have therapeutic implications for RA. Indeed, anti-S1P antibodies have been developed and are currently tested in clinical studies for treatment of cancer, fibrosis, inflammation, macular degeneration, diabetic retinopathy, glaucoma, and other diseases or symptoms (reviewed in [102]). These antibodies bind to and inactivate S1P, reducing the extracellular pool of bioactive S1P and inhibiting its stimulating activity [103]. A preclinical study using blocking S1P antibodies to prevent tumour progression, for example, was recently reported [104]. In that study, a specific anti-S1P monoclonal antibody reduced, and in some cases completely eliminated, tumour formation and accompanying tumour angiogenesis. These results suggest that antibody-mediated inhibition of S1P signalling may be developed as a strategy for inhibiting pannus formation and angiogenesis in RA.

SPL, the major S1P-degrading enzyme, catalyzes the irreversible degradation of intracellular S1P. Inhibition of SPL leads to the accumulation of S1P in tissues, including lymphoid tissues [105], and induces premature internalization of the exit-signal-sensing S1P1 receptor on lymphocytes, rendering them unresponsive to S1P and preventing their egress from thymus and lymph nodes [106]. One physiological outcome of this systemic redistribution of lymphocytes is potent immunosuppression, which offers new opportunities for developing immunoregulatory agents to treat autoimmune and inflammatory diseases [107-111]. In fact, SPL-deficient mice showed resistance to various inflammatory and autoimmune challenges [112-114]. Early studies are undergoing on the application of SPL inhibitors to RA treatment. The evaluation of a synthetic SPL inhibitor, LX2931, is currently in phase-II clinical trials in patients with active RA [112]. There are also studies on the SPL inhibitor, LX 3305, which inhibits lymphocyte migration, concerning its potential for RA clinical treatment [115].

4.2. Targeting S1P receptors: S1P receptor agonist FTY720

FTY720 (generic name fingolimod) is a synthetic sphingosine analog (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol) that can be phosphorylated by SphKs. The designation FTY-P is used for the phosphorylated compound. FTY720 acts as an agonist with high affinity for all S1P receptors except S1P2 [109], and very effectively down-regulates S1P receptor expression [63,102]. It very actively induces the internalization, ubiquitylation and subsequent degradation of S1P receptors, consequently rendering cells unresponsive to S1P [63]. Compared to FTY720, S1P is much less effective at inducing receptor degradation [116,117].

FTY720 has emerged as a clinically promising novel immunosuppressive drug that presumably acts by limiting effector lymphocyte egress from lymph nodes [118,119]. It rapidly induces lymphopenia through the sequestration of lymphocytes in lymph nodes and by blocking the emigration of mature thymocytes from the thymus through receptor down-

modulation [116,117,120]. It differentially affects the sequestration of regulatory T-cells and increases their suppressive activity [121]. It is also reported to display anti-angiogenic activity and to potently diminish angiogenesis and tumor vascularization *in vivo* in growth factor implants and corneal models [122] and via the S1P1 receptor [123].

FTY720 has been proven effective in the treatment of multiple sclerosis (MS). MS and RA are both autoimmune diseases with similar clinical inflammatory characterization, such as the infiltration of immune cells into the inflammatory sites, with RA affecting the joints, while MS affecting the brain and spinal cord. In MS, the most relevant effect of FTY720-P relates to its interaction with S1P1 receptor. After binding with the drug, S1P1 is internalized for several days. This receptor internalization, reduces the number of available S1P1 receptors and subsequently renders T lymphocytes unresponsive to S1P signals, which would otherwise mediate their migration from the thymus and lymph nodes to the peripheral blood and from there to the brain. This way, FTY720 prevents lymphocytes from recirculating to peripheral sites of inflammation [124]. Treatment of human patients with relapsing-remitting MS with considerably low doses of FTY720 has proven to be beneficial [125,126]. The results of a phase-II clinical trial, evaluating the efficacy and safety of FTY-720 for treating relapsing MS, showed that the annualized relapse rate of the FTY-720 group was significantly lower [126]. More recently, two large-scale, phase-III clinical trials conducted on relapsing-remitting MS patients demonstrated that FTY720 reduces relapse rates by more than 50%, as compared with the control groups [127,128]. FTY720, which can be taken orally [129], is therefore a highly promising immunomodulatory drug for MS. In fact, FTY720 has been approved by the US Food and Drug Administration for the treatment of relapsing forms of MS.

The therapeutic effect of FTY720 on RA was recently examined in animal models. FTY720 administration suppresses the progression of laminarin-induced arthritis in the SKG mice [130]. FTY720 treatment decreases IL-6 and TNF- α expression in synovial fibroblasts and inflammatory cells, as well as bone destruction. The numbers of CD4+ and CD8+ T cells were significantly increased in the thymus and decreased in the spleen in FTY720-treated SKG mice. FTY720 enhanced IL-4 production by CD4+ T cells stimulated by allogeneic spleen cells and inhibited PGE2 production by a TNF- α -stimulated synovial fibroblast cell line. The anti-arthritis effect of FTY720 was also evaluated in AIA rats [131]. In that study, FTY720 treatment inhibited the incidence of arthritis, hind paw oedema and bone destruction. In addition, it markedly decreased the number of peripheral blood lymphocytes. In a separate study, ovariectomized mice were injected with an arthrogenic anti-collagen II antibodies cocktail and then with lipopolysaccharide (LPS), so that they developed arthritis in their paws [132]. These mice thus exhibit both arthritis and osteoporosis and can be regarded as a model of elderly female RA patients. Results from this study showed that FTY720 was as potent as corticosteroid for suppressing arthritis. In addition, it induced recovery of the ovariectomy-induced bone density loss. These results clearly suggest that S1P-targeted therapy, such as S1P receptor agonists, would be beneficial for treating RA patients with both immunological and bone resorptive disorders.

5. Conclusion

In summary, S1P, via SphK1/S1P/S1P-receptor signalling, appears to play an essential role in modulating RA pathogenesis since activated SphK1 and elevated level of S1P are detected in RA synovium. Moreover, S1P receptor expression is upregulated in inflamed synovium. S1P can stimulate proinflammatory cytokines/chemokines secretion, drive immune cell recruitment into inflammatory sites, and promote the proliferation and/or survival of synovial fibroblasts, B lymphoblastoid cells, and chondrocytes. Although the mechanism of its effect in RA remains unclear, S1P represents an exploitable target for the development of a novel therapeutic approach for RA.

6. Key Points

- S1P plays an important role in rheumatoid arthritis pathogenesis. Proofs are:
 - Levels of S1P and its synthetic enzymes SphKs are elevated in RA synovium;
 - RA synovial fibroblasts are more responsive to S1P;
 - S1P stimulates the production of proinflammatory cytokines/chemokines by RA synovial fibroblasts;
 - S1P drives recruitment and retention of immune cells in RA synovium;
 - S1P promotes RA proliferation and/or survival of synovial fibroblasts, B cells, and chondrocytes;
 - S1P induces osteoclastogenesis in RA synovium; etc.
- S1P represents a potential therapeutic target for rheumatoid arthritis. Evidences are:
 - Decreasing S1P level by inhibiting SphK1 activity reduces RA disease severity in animal models of arthritis;
 - Depleting S1P level by utilizing S1P-blocking agents inhibits the growth of tumours, which is relevant to pannus formation in RA;
 - The strategy of inhibiting SPL activity – with the aim of rendering immune cells unresponsive to S1P and thus decreasing immune cells infiltration– is undergoing in phase-III clinical trials in patients with active RA;
 - Blocking S1P receptor activity by utilizing S1P receptor agonist FTY720 suppresses arthritis in animal models of RA; FTY720 has been approved by the US Food and Drug Administration for the treatment of relapsing forms of another chronic inflammatory disease multiple sclerosis.

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