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TOPIC HIGHLIGHT

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Role of sphingosine kinase and sphingosine-1-phosphate in inflammatory arthritis

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Abstract

The importance of sphingosine kinase (SphK) and sphingosine-1-phosphate (S1P) in inflammation has been extensively demonstrated. As an intracellular second messenger, S1P plays an important role in calcium signaling and mobilization, and cell proliferation and survival. Activation of various plasma membrane receptors, such as the formyl methionyl leucyl phenylalanine receptor, C5a receptor, and tumor necrosis factor α receptor, leads to a rapid increase in intracellular S1P level *via* SphK stimulation. SphK and S1P are implicated in various chronic autoimmune conditions such as rheumatoid arthritis, primary Sjögren's syndrome, and inflammatory bowel disease. Recent studies have demonstrated the important role of SphK and S1P in the development of arthritis by regulating the pro-inflammatory responses. These novel pathways represent exciting potential therapeutic targets.

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Key words: Cytokines; Inflammation; Rheumatoid arthritis; Sphingosine kinase; Sphingosine-1-phosphate

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SPHINGOLIPIDS

Sphingolipids represent a major class of lipids that are ubiquitously expressed in eukaryotic cell membranes. They were first discovered by Thudichum JLW in 1876, who named the chemical backbone of sphingolipids after the mythological beast, the Sphinx, for their enigmatic 'Sphinx-like' properties. Sphingolipids are characterized by their sphingoid backbone, and primary structural roles in membrane formation. Apart from their structural functions, they have emerged as the source of important signaling molecules[1], and are potentially involved in pathophysiological processes[2,3]. Sphingosine kinase (SphK) is a key enzyme in the sphingolipid metabolic pathway because it provides an essential checkpoint that regulates the relative levels of ceramide, sphingosine, and S1P[4]. Following stimulation of various plasma membrane receptors, an enzymatic cascade is activated and sphingosine is rapidly metabolized into sphingosine-1-phosphate (S1P) by SphK.

S1P

Bioactive lysophospholipid, S1P, is a unique signaling molecule that has the ability to act as an intracellular second messenger, as well as an extracellular stimulus through specific G-protein coupled receptors[5-7]. To date, five S1P receptors, S1P$_{1\text{-}5}$[8-11], which belong to the endothelial-differentiating gene family, have been discovered. Binding of S1P to these receptors triggers a wide range of cellular responses including proliferation, enhanced extracellular matrix assembly, stimulation of adherent junctions, formation of actin stress fibers, and inhibition of apoptosis[11-15]. These receptors mediate their diverse cellular functions through differential coupling to various heterotrimeric G-proteins and through heterogeneity in their expression patterns[16]. S1P has also been proposed to play an intracellular role as a second messenger after observations that stimulation of various plasma membrane receptors, such as the platelet-derived growth factor receptor[17,18], FcγR I and FcεR I antigen receptors[19-21], the formyl methionyl leucyl phenylalanine receptor[22], the C5a receptor[23,24], and tumor necrosis factor (TNF)-α receptor[25] trigger rapid production of S1P through SphK activation. Moreover, inhibition of SphK strongly reduced cellular events triggered by these receptors, such as receptor-triggered DNA synthesis, calcium mobilization and vesicular trafficking[17-22].

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic and symmetric polyarthritis with a prevalence of 1% in the industrialized world. RA is characterized by chronic inflammatory infiltration of the synovial membrane, which is associated with the destruction of cartilage and underlying bone. In particular, within inflamed RA synovial membrane, the levels of pro-inflammatory cytokines [TNF-α, interleukin (IL)-1β, IL-6, IL-15 and IL-17) exceed those of anti-inflammatory agents, and this probably contributes directly to cartilage and bone erosion through promoting matrix metalloproteinase (MMP) production and dysregulated chondrocyte/osteoclast function[26-28].

SphK/S1P SIGNALING AND CHEMOTAXIS IN RA

S1P plays a crucial role in homing of immune cells to lymphoid organs, and in controlling their egress into the blood and lymph. An important factor that drives such egression is the S1P gradient that exists between the tissues (which have low S1P levels) and the blood/lymph (which have high S1P levels). One of the five S1P receptors, S1P$_1$, has been shown to be involved in the egression of B and T cells from the peripheral lymphoid organs[29], and for the exit of mature T cells from the thymus[30]. Furthermore, it has been shown that S1P plays a central role in leukocyte chemotaxis in purified human peripheral blood neutrophils, eosinophils, monocytes and macrophages[22-24]. We have found that the concentration of S1P in synovial fluids from patients with RA is significantly higher than that from those with osteoarthritis, a degenerative joint disease. This increase in S1P level due to the chronic inflammation in RA could be responsible for the recruitment and retention of the immune infiltrates in the synovium[31]. Indeed, in a murine collagen-induced arthritis (CIA) model, we demonstrated that, by inhibiting SphK activity using a pharmacological inhibitor, *N*, *N*-dimethylsphingosine (DMS), adjacent cartilage and bone erosion, synovial hyperplasia, and inflammatory infiltration into the joint compartment, were clearly markedly suppressed as compared to the control group that received PBS. A similar result was observed using the siRNA approach to knockdown SphK1 isoform, whereby serum S1P level is lowered and joint pathologies reduced[31].

SphK/S1P AND AUTOIMMUNE B CELLS

Mature plasma cells that secrete autoantibodies[32] are a prominent cellular component of rheumatoid synovium. The classical autoantibody associated with RA is rheumatoid factor (RF), which is an antibody directed against the Fc portion of IgG. However, RF can be detected in other rheumatic diseases, infectious diseases, and even in 3%-5% of apparently healthy individuals. The current diagnostic marker used in the clinics now is the anti-cyclic citrullinated peptide (CCP) antibody. Anti-CCP antibody can be detected years before onset[33], and it has been shown that anti-CCP antibody correlates with a more erosive disease[34]. In addition to the classical role of autoantibody production, it is now clear that B cells play a pivotal role in activation of synovial T cells in the synovial tissue[35]. The importance of B cells in RA is supported by the moderate success of targeting CD20+ B cells with the chimeric monoclonal anti-CD20 antibody, rituximab[36]. It is of interest that B-cell lines derived from RA patients are uniquely resistant to Fas-mediated apoptosis, in part, due to overactivity of SphK1, and overproduction of S1P, which can inhibit apoptosis and regulate lymphoid migratory pathways[37]. A follow-up study by the same group has shown that the Fas death signaling aberration in RA lymphoblastoid B-cell lines is caused by extracellular S1P, which triggers phosphoinositide-3-kinase-dependent SphK overactivity through a Gi protein-coupled receptor-mediated signaling cascade[38]. In addition, FTY720-phosphate, which binds to S1P receptors, causes rapid disappearance of peritoneal B cells by inhibiting their emigration from parathymic lymph nodes, and reduced peritoneal B-cell-derived intestinal secretory IgA production[39]. In our study using the CIA model, we also have found that the production of anti-collagen IgG2a is lower in the serum of mice administered DMS and SphK1 siRNA[31,40]. Together, this suggests that S1P plays an important role in regulating B-cell survival, trafficking and antibody production.

SphK/S1P AND SYNOVIAL FIBROBLASTS

The high proliferative rate and erosive activity of RA fibroblast-like synoviocytes (FLSs) implicate FLSs as im-

portant contributors to chronic RA inflammation. In inflammatory arthritis, the FLSs become hyperplastic[41], and they closely interact with invading immune cells to form the aggressive pannus tissue that invades and degrades the cartilage and bone. Activated T lymphocytes in the synovium drive FLS activation *via* production of soluble mediators or direct cell-cell contact[42,43]. These activated FLSs then become potent producers of various effector molecules that act on a variety of cells (lymphocytes, monocytes, mesenchymal cells) to modulate joint inflammation and promote matrix degradation[44].

Elevated SphK1, S1P and $S1P_1$ levels have been detected in RA synovium, and S1P signaling *via* $S1P_1$ has been found to promote synoviocyte proliferation, inflammatory cytokine-induced cyclooxygenase-2 expression and prostaglandin E2 production[37,45]. In human RA, FLSs have been shown to express $S1P_1$, $S1P_2$ and $S1P_3$ receptors[46]. Moreover, exogenously applied S1P induces FLS migration, secretion of inflammatory cytokines/chemokines, and protection from apoptosis. Signaling *via* $S1P_1$ has been shown to be essential for survival, whereas signaling *via* $S1P_1$/$S1P_3$ stimulates FLS migration, and activation of $S1P_2$/$S1P_3$ enhances IL-6 and IL-8 secretion. The effects of S1P on FLSs are further amplified by addition of TNF-α, which suggests that the cytokine-rich environment of the inflamed synovium synergizes with S1P signaling to exacerbate the clinical manifestations of RA. More recently, SphK2 has been shown to be strongly expressed in rheumatoid synovial fibroblasts[47]. In contrast to SphK1, which is normally found in the cytosol, SphK2 expression is found in and around the nuclei. Furthermore, SphK2 is responsible for FTY720-mediated apoptosis in the synovial fibroblasts, which suggests that it regulates autonomous proliferation of synovial fibroblasts.

SphK/S1P AND OSTEOCLASTS

S1P has also been shown to induce chemotaxis and regulate migration of osteoclast precursors in culture and *in vivo*[48]. Cells with the properties of osteoclast precursors express functional $S1P_1$ receptors and exhibit positive chemotaxis along an S1P gradient *in vitro*. Osteoclast/monocyte lineage-specific conditional $S1P_1$ knockout mice show osteoporotic changes due to increased osteoclast attachment to the bone surface, because these osteoclast precursors are unable to recirculate from bone tissues to systemic blood flow. Treatment with FTY720 ameliorates bone loss in a murine model of postmenopausal osteoporosis by promoting the recirculation of osteoclast precursor monocytes from the bone surface. Furthermore, SphK activity and S1P signaling can drive the production pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, which stimulate osteoclastogenesis and drive bone resorption.

INTERACTIONS OF S1P AND TH17 CELLS

Th17 recently has been designated as a unique subset of CD4 T cells that are characterized by production of IL-17[49,50]. IL-17 has been suggested to be an important cytokine in the pathogenesis of inflammatory and autoimmune disease in animals and humans[51]. It has been shown that the key to Th17 differentiation in mice is the combination of transforming growth factor (TGF)-β and IL-6[52-54], and expands up to full potential in the presence of IL-23[55,56]. In contrast, IL-1β is the most effective inducer of IL-17 expression in human naive T cells. IL-6 and IL-23 induce a small amount of IL-17 alone and greatly enhance Th17 differentiation in the presence of IL-1β[57,58].

It has been reported recently in the murine model that S1P has the same potential as IL-23 *in vitro* to increase proliferation and IL-17-secreting activity of T-cell-receptor-activated $CD4^+$ T cells grown in the presence of IL-1β, IL-6 and TGF-β1[59,60]. The differentiation into Th17 cells that is induced by S1P occurs with corresponding suppression of Th1 and Th2 cytokine production, interferon (IFN)-γ or IL-4, respectively[60]. Furthermore, the introduction of FTY720 into cultures of Th17 cells that develop under the influence of S1P substantially suppresses generation of IL-17[60].

ROLE SphK/S1P IN CELL-CONTACT-MEDIATED PRO-INFLAMMATORY CYTOKINE PRODUCTION

Studies pioneered by Dayer and colleagues, as well as work from several other laboratories, have demonstrated that direct contact with stimulated T lymphocytes is a potent pro-inflammatory mechanism that triggers massive up-regulation of cytokines such as TNF-α, IL-1β, IL-6, as well as, metalloproteinases from human monocytes and macrophages[61-65]. In chronic inflammatory diseases such as RA, the synovium is very cellular and several different cell types, including T lymphocytes and macrophages lie in close proximity to one another, which allows reciprocal cellular crosstalk. McInnes *et al*[66] have demonstrated that freshly isolated, paraformaldehyde-fixed T lymphocytes from the synovial fluid might induce TNF-α production directly by blood or synovial macrophages, *via* direct cell-to-cell contact without additional exogenous stimulation. This effect is enhanced when the T cells are activated with cytokines such as IL-15. In another study by Brennan *et al*[67], cytokine-stimulated T-cells or T cells isolated from RA synovial tissue displayed the ability to induced TNF-α production in normal blood monocytes *via* the nuclear factor-κB pathways.

In our *in vitro* study, we found that cell-contact induced production of inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and proteinase MMP-9, is dependent on SphK activity. Jurkat T cells activated with phytohemagglutinin/phorbol myristate acetate induce substantial production of TNF-α, IL-1β, IL-6, and MCP-1 by U937 monocytic cells, and such cytokine synthesis is markedly reduced when the cells are treated with DMS. To validate the results obtained using human cell lines, peripheral blood mononuclear cells from RA patients have been used in identical cell contact experiments. Likewise, activated peripheral T lymphocytes from RA patients induced sub-

stantial production of TNF-α, IL-1β, IL-6, and monocyte chemotactic protein-1 by autologous peripheral monocytes, and DMS treatment significantly suppressed production of these cytokines[31]. Overproduction of MMP-9 has been observed in the synovial fluid of RA patients. MMP-9 from macrophages and neutrophils is thought to play a key role in migration of these cells during inflammation in RA[68] and erosion of the cartilage matrix. We found that MMP-9 production by monocytes is also induced by T cell contact, and that this production is significantly suppressed when SphK activity is inhibited[31]. These results demonstrate the importance of SphK/S1P signaling in cell-contact-induced inflammatory mediators, which is relevant to RA pathogenesis.

THE DISTINCT ROLES OF SphK1 *VS* SphK2 IN INFLAMMATORY ARTHRITIS

To date, two mammalian SphKs have been cloned, sequenced and characterized. These kinases are encoded by two genes, SphK1[4,69,70], and SphK2[71]. Human SphK1 localizes to chromosome 17 (17q25.2), whereas SphK2 maps to chromosome 19 (19q13.2). Although the two mammalian isoforms possess five evolutionarily conserved domains that are found in all SphKs and are highly similar in amino acid sequence, they differ in kinetic properties and in temporal and spatial distribution, which implies that they have distinct physiological functions.

Using the CIA model, we found that knocking down SphK1 *via* the siRNA approach resulted in significantly reduced joint pathology and subsequent pro-inflammatory responses[31,40]. This suggests that SphK modulation possesses inhibitory action on inflammatory cell infiltration into the joints, and subsequent synovium hyperplasia and erosion. Moreover, mice that receive SphK1 siRNA have significantly reduced serum levels of pro-inflammatory IL-6, TNF-α, and IFN-γ, as well as *in vitro* production of these pro-inflammatory cytokines in response to collagen. In contrast, downregulation of SphK2 results in increased disease activity and pro-inflammatory responses. SphK2 siRNA-treated mice exhibit increased joint pathology as compared to control and SphK1 siRNA treatment groups. These mice are also associated with higher serum levels of pro-inflammatory IL-6, TNF-α and IFN-γ, and higher production of such cytokines in response to collagen *in vitro*. In addition, we have found that at day 27 prior to development of arthritis, SphK2-siRNA treatment can potentially polarize *in vitro* T-cell anti-collagen responses in a pro-inflammatory manner, and is unlikely to be associated with changes in the concentration of serum S1P[40]. Furthermore, a recent study has shown that SphK2 is highly expressed in rheumatoid synovial fibroblasts and is responsible for FTY720-mediated apoptosis in these cells[47].

THERAPEUTIC IMPLICATIONS

Despite many years of intensive research, neither the initiating events nor the perpetuating factors in the pathogenesis of RA are clearly understood. Most of the current knowledge about the inflammatory process and cellular infiltration in the rheumatoid joint comes from the study of synovium in established, rather than early, disease. To date, the gold standard for treating RA is the disease-modifying anti-arthritic drugs, with methotrexate being the most widely used[72], although the clinical success of anti-TNF-α therapy suggests that targeting the cytokine network can be of immense benefit. The cytokine network is vast and complicated, with each cytokine linked to another in distinct and/or overlapping manners[26-28]. Hence, targeting one specific cytokine might not be enough to efficiently control, much less ameliorate the disease in the long term.

SphKs and S1P signaling appears to play an important role in modulating RA pathogenesis. The SphK1 pathway is activated and appears to play a similar pro-inflammatory role in a mouse model of inflammatory arthritis. More fascinating is the fact that blockade of SphK1 activity simultaneously reduces several antigen-specific inflammatory responses such as pro-inflammatory cytokines and anti-collagen antibody production, and inflammatory infiltration into the synovium, yet does not compromise global immunity. On the contrary, SphK2 plays a protective role in inflammatory arthritis. Therefore, selective targeting and/or dual-targeting of the two SphK isoforms might be required to modulate effectively the sphingolipid metabolic pathway in such a way that it becomes therapeutic in chronic inflammatory arthritis.

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