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Anti-Inflammatory Effects of Sphingosine Kinase Modulation in Inflammatory Arthritis

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Sphingolipids are sources of important signaling molecules in addition to their role as structural components of the eukaryotic cell membranes. In particular, sphingolipid metabolites such as ceramide and sphingosine-1-phosphate (SIP) have emerged as a new class of potent bioactive messengers involved in an array of cellular processes, including angiogenesis, proliferation, and apoptosis (1, 2). Recently, interest in SIP has focused on two distinct cellular roles, namely its function as an intracellular second messenger, or extracellularly as a specific and high-affinity ligand for a family of G protein-coupled receptors previously known as the endothelial differentiation gene (EDG) family. To date, five SIP receptors in the endothelial differentiation gene family have been identified, including EDG-1, EDG-3, EDG-5, EDG-6, and EDG-8, now collectively known as SIP1–5 (2–4). Sphingosine kinase (SphK) is a key enzyme in the sphingolipid metabolic pathway, responsible for phosphorylating sphingosine into sphingosine-1-phosphate (S1P). SphK/S1P play a critical role in angiogenesis, inflammation, and various pathologic conditions. Recently, SIP, receptor was found to be expressed in rheumatoid arthritis (RA) synovium, and SIP signaling via S1P, enhances synoviocyte proliferation, COX-2 expression, and prostaglandin E2 production. Here, we examined the role of SphK/S1P in RA using a potent SphK inhibitor, N,N-dimethylsphingosine (DMS), and a molecular approach against one of its isoenzymes, SphK1. We observed that levels of S1P in the synovial fluid of RA patients were significantly higher than those of osteoarthritis patients. Additionally, DMS significantly reduced the levels of TNF-α, IL-6, IL-1β, MCP-1, and MMP-9 in cell-contact assays using both Jurkat-U937 cells and RA PBMCs. In a murine collagen-induced arthritis model, i.p. administration of DMS significantly inhibited disease severity and reduced articular inflammation and joint destruction. Treatment of DMS also down-regulated serum levels IL-6, TNF-α, IFN-γ, S1P, and IgG1 and IgG2a anti-collagen Ab. Furthermore, DMS-treated mice also displayed suppressed proinflammatory cytokine production in response to type II collagen in vitro. Moreover, similar reduction in incidence and disease activity was observed in mice treated with SphK1 knock-down via small interfering RNA approach. Together, these results demonstrate SphK modulation may provide a novel approach in treating chronic autoimmune conditions such as RA by inhibiting the release of pro-inflammatory cytokines. The Journal of Immunology, 2008, 181: 8010–8017.
significant contribution from B cells, macrophages, mast cells, and fibroblasts (16, 17). T cell effector function may be via cytokine secretion (e.g., IL-17) or through cell contact-dependent cognate interactions with macrophages via ligand pairs such as LFA-1/ICAM-1 and CD40/CD154 (20–24). Recently, elevated SphK1, S1P, and S1P, levels have been detected in RA synovium, and S1P signaling via S1P1 was found to promote synoviocyte proliferation, inflammatory cytokine-induced COX-2 expression, and prostaglandin E2 production (25, 26). In the present study, we investigated whether targeting SphK activity, either by DMS or specific blockade of SphK1 through a molecular approach, may possess any immunomodulatory, antiarthritic properties. We show that synovial fluid of RA patients exhibited higher levels of S1P than did those of osteoarthritic (OA) patients and in RA-derived human cells in which DMS suppressed cytokine and MMP-9 release by PBMCs and by monocytes following cell contact-dependent interaction with activated T lymphocytes. The potential clinical relevance of these observations is illustrated by the ability of DMS and SphK1 small interfering RNA (siRNA) to effectively suppress murine collagen-induced arthritis (CIA) both in vivo and in vitro via specific suppression of the pathologic proinflammatory and Th1 responses.

Materials and Methods

Human studies

Peripheral blood (PB) was collected from RA patients who fulfilled the American College of Rheumatology 1987 diagnostic criteria (27), follow-
ing approval from the hospital’s institutional review board (Tan Tock Seng Hospital, Singapore), and written informed consent was obtained from all patients. PB T cell and monocyte subpopulations were prepared as described previously (22). Briefly, human Jurkat or RA patient-derived PB T cells were stimulated for 72 h with PHA (5 μg/ml)/PMA (10 nM, both Sigma-Aldrich) in RPMI 1640 with 2 mM t-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (all Invitrogen), then fixed in 4% paraformaldehyde. Control nonactivated Jurkat and PB T cells and were also fixed for comparative purposes. Fixed Jurkat or PB T cells were then cocultured with the myelomonocytic U937 cells or autologous RA PB T cells monocytes in the presence or absence of DMS (Cayman Chemical). DMS was prepared as a 10 mg/ml stock in ethanol and diluted to the appropriate concentration in RPMI 1640 before use as described previously (8, 9, 11–13). The antisense down-regulation of SphK1 was conducted as previously described (8, 12–14). The sequences of the oligonucleotides were: 5'-CC CGCAGGATCCATAACCTC-3', 5'-GGCGAAGGCUCCUGAUCUCUUGCTC-3' as scrambled control (Qiagen). Supernatants were harvested after 48 h of coculture for cytokine estimation by ELISA.

Synovial fluid was obtained from RA and OA patients and stored at −70°C until estimation of S1P by ELISA (Rheumatology Division, University of California, Los Angeles). Clinical details are as follows: for RA, mean age 49.1 ± 6.0 years (mean ± SEM), mean disease duration 72.6 ± 22.4 mo, 4 male/10 female; for OA, mean age 52.1 ± 4.9 years, mean disease duration 74.1 ± 25.2 mo, 3 male/5 female. The degree of inflammation, synovial hyperplasia, and erosion. Each parameter was analyzed separately against a set of predefined sections graded 0 (normal), 1 (mild), 2 (moderate), and 3 (severe).

Treatment protocols

To investigate the effect of SphK modulation in murine CIA, DBA/1 mice received daily i.p. injections of DMS at the doses of 200 or 400 mg/kg/mouse (n = 16 mice/group) from days 21–23 and then once every 2 days until day 45. The doses of DMS chosen are consistent with previous in vivo murine studies (15, 28). Control mice received PBS supplement with eth-

Collagen-specific in vitro culture

Draining lymph nodes (popliteal and inguinal; four per mouse) were aseptically removed from the mice and passed through cell striainers (BD Biosciences) to prepare a single-cell suspension. Cells were cul-
tured at 2 × 10^6 cells/ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 2 mM glutathione, and 10% heat-inactivated FCS (all Invitrogen). Cells were stimulated with graded concentrations of CIA (5 μg/ml, equivalent to 200 μg/kg, in 0.2 ml final volume of PBS) i.p. on days 20–22, and then once every 2 days until day 36. Control mice received scrambled siRNA at the same time points. The efficiency of SphK1 siRNA transfection was consistently measured with a S1P competitive ELISA kit (Echelon Biosciences) according to the manufacturer’s instructions. Cytokine concentrations were analyzed with the nonparametric Mann-Whitney U test. Differences between cumulative incidences at a given time point were analyzed by the χ² contingency analysis. Cytokine- and collagen-specific IgG levels were compared using Student’s t test.
Results

Detection of S1P in RA synovial fluid

We first compared the levels of S1P in synovial fluids collected from 14 RA patients and 8 patients suffering from OA, a degenerative joint disease. S1P levels were measured by a competitive ELISA, and up to 17.5 ± 4.2 ng/ml of S1P was detected in RA synovial fluids, significantly higher than those observed in OA fluids (3.45 ± 0.85 ng/ml, p < 0.05, Mann-Whitney U test). As the levels of S1P detected in synovial fluid of RA patients could be complicated by nonspecific binding of rheumatoid factor, RA synovial fluids (n = 10) were diluted 1/10 with delipidized pooled human serum, then spiked with 1 μM of external S1P and assayed by ELISA. The average recovery rate was 96% (range 89–107%), thus ruling out any potential interference by rheumatoid factor in our ELISA.

DMS inhibits cell contact-induced cytokine production via cognate interactions

T cells from RA synovium or PB, particularly those activated by PHA/PMA, or cytokines like IL-15 or IL-18 are capable of driving macrophages to produce TNF-α in a cell contact-dependent manner (29, 30). We therefore investigated the ability of DMS, a potent SphK inhibitor, to influence the production of proinflammatory cytokines such as TNF-α by monocytes in response to activated T cells in a cell contact coculture system (22–24). Jurkat T cells were cultured with PHA and PMA, fixed with paraformaldehyde, and then cocultured with human U937 monocytic cells in the presence or absence of DMS. As expected, activated T cells induced substantial production of TNF-α, IL-6, IL-1β, and MCP-1 by U937 cells (Fig. 1A–D). Such cytokine synthesis was markedly reduced when the U937

FIGURE 1. DMS inhibits cytokine production from human Jurkat T cells and U937 monocytes in vitro. Paraformaldehyde-fixed PMA/PHA-stimulated (Ts) or medium control (Tc) Jurkat T cells were cocultured with U937 cells either in medium alone or with increasing concentrations of DMS. Activated Jurkat T cells induced TNF-α (A), IL-1β (B), IL-6 (C), and MCP-1 (D) production in a cell contact-dependent fashion and were significantly inhibited by DMS in a dose-dependent manner. Similarly, SphK1 antisense oligonucleotide-treated U937 cells exhibited significant reduction of IL-6 (E) and TNF-α (F) release upon coculture with PMA/PHA-activated Jurkat T cells. Data are means ± SEM of triplicate cultures and are representative of five similar experiments. *, p < 0.05 by Student’s t test.

FIGURE 2. DMS suppresses cell-mediated monocyte cytokine release induced via cognate interactions. Cytokine production was measured in a cell contact assay in which RA patient-derived PB T cells were stimulated for 72 h with PMA/PHA (Ts) or in medium control (Tc) and then fixed in paraformaldehyde before coculturing with autologous PB monocytes with or without DMS (10 μM) for 48 h. Significant suppression of TNF-α (A), IL-1β (B), IL-6 (C), and MCP-1 (D) production in coculture was observed with DMS. Data are means ± SEM from five RA patients, with each experiment performed in triplicate. *, p < 0.05 by Student’s t test.
cells were treated with DMS in a dose-dependent manner (Fig. 1A–D). Similar data were obtained when identical experiments were performed using PB T cells derived from RA patients and cocultured with autologous PB monocytes (Fig. 2).

It was important to determine whether similar effects could be achieved through direct inhibition of SphK. To this end, we performed parallel studies in which the ability of SphK1 antisense oligonucleotide to modify IL-6 and TNF-α production by U937 monocytes was evaluated. We observed that coculture-induced IL-6 and TNF-α release was significantly suppressed by SphK1 antisense oligonucleotide when compared with scrambled control (Fig. 1A and D). No evidence of increased apoptosis was observed at the concentrations of DMS employed using annexin V/7-aminoactinomycin D staining by FACS analysis (annexin V+/U937: 3.9 ± 1.7% vs 10 μM DMS: 3.8 ± 1.7%, 5 μM DMS: 3.5 ± 1.3%, 1 μM DMS: 3.9 ± 1.8%; annexin V+ RA PB monocytes: 6.8 ± 1.8% vs 10 μM DMS: 7.6 ± 2.1%), indicating that reduced cell survival is unlikely to explain the observed inhibition. These results therefore demonstrate that SphK modulation may regulate proinflammatory cytokine synthesis in a system directly relevant to clinical arthritis.

**DMS inhibits cell contact-induced MMP-9 production**

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 the migration of these cells during inflammation in RA (31). To determine the role of SphK in cell contact-induced MMP-9 synthesis by monocytes, Jurkat T cells or RA PB T cells were stimulated, fixed, and then cocultured with U937 monocytic cells or autologous PB monocytes in the absence or presence of DMS as described above. Such coculture-induced monocyte MMP-9 release was significantly suppressed by DMS in a dose-dependent manner (Fig. 3A). Moreover, similar reduction was observed in experiments using PB T cells and monocytes derived from RA patients (Fig. 3B).

**FIGURE 3.** Effect of DMS on cell contact-induced MMP-9 production by monocytes. Jurkat T cells or PB T cells purified from RA patients (n = 5) were stimulated with PMA/PHA for 72 h, fixed in paraformaldehyde, and cocultured with U937 monocytic cells or autologous PB monocytes in the absence or presence of DMS for 48 h. DMS significantly suppressed MMP-9 released by U937 cells in a dose-dependent manner (A) or RA patients at 10 μM DMS (B). Data are means ± SEM of triplicate cultures and are representative of five similar experiments (A) or five RA patients (B). Ts indicates PMA/PHA activated T cells; Tc, medium control. *, p < 0.05 by Student’s t test.

**FIGURE 4.** DMS dose-dependently attenuated the progression of murine CIA. Collagen-primed DBA/1 mice were injected i.p. with 200 μg/kg DMS (n = 16 mice/group), 400 μg/kg DMS (n = 16 mice/group), or with PBS carrier control (n = 15 mice/group) from days 21 to 23 and then once every 2 days until day 45. Mice were monitored for disease progression as indicated by (A) incidence, (B) mean articular index, and (C) mean paw thickness (mm). DMS-treated mice developed significantly less severe disease in a dose-dependent manner compared with PBS carrier controls. Data are mean ± SEM. *, p < 0.05 by Mann-Whitney U test.

**Treatment with DMS inhibits the development of murine CIA**

We next investigated the effect of DMS on the development of CIA in DBA/1 mice, a surrogate model of human RA. DBA/1 mice were immunized with CII/CFA as described in Materials and Methods. Mice began to show clinical signs of arthritis on day 27 after immunization. Mice were injected i.p. daily with 200 μg/kg DMS, 400 μg/kg DMS, or with PBS carrier control from days 21 to 23 and then once every 2 days until day 45. DMS dose-dependently suppressed the incident, mean articular index, and mean paw thickness of developing CIA (Fig. 4). To determine whether DMS administration modified articular destruction, we evaluated cartilage and bone integrity histologically. Adjacent cartilage and bone erosion, synovial hyperplasia, and inflammatory infiltration into the joint compartment were clearly evident in the PBS carrier controls (Fig. 5). Each of these parameters was markedly suppressed in mice that received 400 μg/kg DMS (Fig. 5). Taken together, these data clearly indicate that DMS potently suppressed the development of CIA and that such activity can prevent progression of articular damage.

**Effect of DMS on serum cytokines, S1P, and anti-collagen Ab production in vivo**

A potential mechanism by which SphK modulation via DMS could suppress CIA pathology is by blocking proinflammatory cytokine
release in vivo. To compare the extent of such modulation, serum cytokine concentrations from arthritic mice were measured by ELISA at the end of the treatment period (day 45). High concentrations of IL-6, TNF-α, and IFN-γ were detected in PBS carrier controls. These were present at significantly (p < 0.05) reduced levels in mice treated with 400 μg/kg of DMS (Fig. 6, A–C). Moreover, reduction in serum IL-6 levels (Fig. 6A), a surrogate marker for suppression of the acute phase response, suggested that systemic inflammatory responses were modified. It was important to determine the level of S1P modulation achieved by DMS at either 200 or 400 μg/kg, and serum S1P levels were assessed by ELISA. A significant reduction in serum S1P levels was observed in a dose-dependent manner in DMS recipients (Fig. 6D), suggesting that S1P responses were indeed modified by the presence of DMS. Finally, we sought evidence for anti-collagen Ab production. CII-specific IgG1 and IgG2a levels were also analyzed by ELISA and found to be reduced in mice treated with 400 μg/kg DMS (Fig. 6, E and F).

**DMS reduced in vitro collagen-specific proinflammatory immune responses**

We next investigated the immunological mechanisms by which SphK modulation suppresses articular inflammation. CIA is associated with a proinflammatory immune response, rendering it an excellent model to explore the effect of DMS upon functional T cell response in vivo. CII-specific immune responses were examined in vitro in pooled draining lymph node cells obtained at day 45. Cells from DMS-treated mice produced significantly less CII-induced proliferation, IFN-γ, TNF-α, and IL-6 compared with cells from control animals (Fig. 7A–D), whereas antiinflammatory cytokine IL-10 synthesis was enhanced (Fig. 7E). Immune modulation by DMS in vivo was Ag-specific since Con A-induced production of IFN-γ, TNF-α, IL-6, and IL-10 in parallel cultures was not affected (data not shown).

**SphK1 siRNA attenuated the development of murine CIA**

To verify that the inhibitory effects of DMS from the above observations were mediated by direct SphK inhibition rather than
SphK1, one of the two SphK isoenzymes, attenuated the development of CIA in vivo.

The pathways that drive cytokine release in RA synovium remain unclear, but T cells play a significant role by direct release of IL-17 or via cell contact with synovium macrophages (17–19, 21, 29, 30). DMS was effective in suppressing T cell-monocyte interactions, an important pathway driving proinflammatory cytokine production in the synovium (29, 30). We have demonstrated in a series of cell contact experiments employing either Jurkat/U937 cells or PB T cells and autologous monocytes derived from RA patients that DMS significantly reduced cell contact-induced proinflammatory TNF-α, IL-1β and IL-6, and chemokine MCP-1 synthesis in a dose-dependent manner. More importantly, MMP-9, a collagenase that plays a direct role in cartilage degradation and subsequent bone erosion in RA (31), was significantly inhibited by the presence of DMS, which may potently block MMP-9 synthesis either by directly interfering with monocyte-T cell interaction (39) or indirectly by suppressing TNF-α and IL-1β (40).

CIA represents an ideal opportunity to explore the diverse inflammatory effects on SphK modulation in an inducible, autoimmune model. CIA has served as a translational model in which the role of inflammatory cytokines in RA, particularly TNF-α, IL-1β, and IL-17, were defined (16, 20). Our data indicate that DMS administration into CIA mice resulted in significantly reduced joint pathology and subsequent proinflammatory responses. Activation of various plasma membrane receptors, such as the fMLP receptor (11), the C5a receptor (12, 13), and TNF-α receptor (14) (receptors that are of direct relevance in RA), leads to rapid increase in intracellular S1P levels via SphK stimulation. S1P can promote neutrophils, monocytes, and lymphocyte activation and migration (12–15, 41–44). Our data suggest that SphK modulation via DMS may possess inhibitory action on inflammatory cell infiltration into the joint, subsequent synovial hyperplasia, and erosion as revealed by histological examination. Such suppressive action of DMS on leukocyte migration is consistent with previous studies showing that SphK plays a role in chemotaxis of human peripheral blood neutrophils and macrophages (12, 13, 15). Inhibition of SphK may have a direct effect on cellular migratory machinery such as calcium mobilization and expression of adhesion molecules, including VCAM-1 and E-selectin (45). Finally, reduced S1P production after DMS treatment may also be directly responsible for the reduced cell infiltration, as S1P has been shown to act as a chemoattract signal (35, 36).

We show that blockade of SphK activity with DMS significantly reduced the levels of CII-specific IgGα and IgG2a Abs in the serum of arthritic mice. Our data indicate that DMS has an inhibitory action on the levels of circulating S1P in serum of treated mice in a dose-dependent manner as compared with carrier controls. Interestingly, 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 (26). Additionally, FTY720-phosphate, which binds to S1P receptors, caused the rapid disappearance of peritoneal B cells by inhibiting their emigration from parathymic lymph nodes, as well as reducing peritoneal B cell-derived intestinal secretory IgA production (46). This suggests that S1P may play an important role in regulating B cell survival, trafficking, and Ab production.

It is important to establish that the inhibitory effects of DMS in the above observations were mediated by direct SphK inhibition rather than by nonspecific off-target side effects (32), and to determine the efficacy of SphK1 in murine arthritis using a

### Table I. SphK1 siRNA attenuated the progression of murine CIA

<table>
<thead>
<tr>
<th>Time (Day 36)</th>
<th>Scrambled siRNA (n = 12)</th>
<th>SphK1 siRNA (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (%)</td>
<td>75%</td>
<td>33.3%*</td>
</tr>
<tr>
<td>Mean articular index</td>
<td>5.86 ± 1.20</td>
<td>2.50 ± 0.64*</td>
</tr>
<tr>
<td>Arthritic paws</td>
<td>2.87 ± 0.34</td>
<td>0.50 ± 0.25*</td>
</tr>
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*S Collagen-primed DBA/1 mice were injected i.p. with scrambled or SphK1 siRNA (both at 5 μg/animal; equivalent to 200 μg/kg, n = 12 mice/group) from day 20 to 22 and then once every 2 days until day 36. Mice were monitored for disease progression as indicated by incidence (%), mean articular index, and mean number of arthritic paws. SphK1 siRNA-treated mice developed significantly less incidence of arthritis and severe disease compared with mice that received scrambled siRNA. Data are expressed as means ± SEM. *p < 0.05, determined by Mann-Whitney U test for clinical scores, and incidence rate was analyzed by χ² contingency analysis.

nonspecific off-target side effects (32), and to determine whether SphK1 inhibition could influence the progression of murine CIA, DBA/1 mice were given i.p. 200 μg/kg of SphK1 siRNA or scrambled siRNA as control on days 20–22 and then once every 2 days until day 36 as described in Materials and Methods. The siRNA dose chosen is consistent with our previous in vivo murine studies (typically 4–8 μg/animal) where we observed that SphK1 protein expression in lysates of PBMC, spleen, lung, and liver was effectively suppressed by administration of SphK1 siRNA (28, 49). To determine the levels of SphK1 protein inhibition, spleen cells were isolated from mice (n = 5) 24 h after the last treatment of SphK1 siRNA as described above, and they were found to be significant reduced when compared with mice that received scrambled control siRNA, while its isoenzyme, SphK2, remains unaffected (data not shown). The severity of arthritis was measured by sequential estimation of the articular index and was compared for involved animals within each group. In agreement with the previous DMS results, SphK1 siRNA significantly suppressed the incidence and severity of development of CIA when compared with control mice that received scrambled siRNA (p < 0.05, Table I), suggesting that such inhibition is dependent of SphK1 rather than SphK2. Therefore, these results clearly demonstrate that SphK modulation, either in the form of DMS administration or targeting SphK1 via siRNA, can directly inhibit proinflammatory cytokine synthesis and progression of arthritis.

**Discussion**

There is currently considerable interest in the potential of immunomodulatory therapies in the treatment of inflammatory diseases, particularly those targeting cytokine expression. Recently, SphK and S1P were implicated in various autoimmune conditions such as RA (25, 26), primary Sjögren’s syndrome (33), and multiple sclerosis (34). The pivotal role of SphK and S1P in inflammation has been widely established and was summarized in a recent review (2). SphK/S1P have pleiotropic effects on a variety of leukocytes such as T and B cells (28, 35, 36), macrophages and monocytes (16, 37), and neutrophils (13, 15, 38). Moreover, studies have shown that SphK/S1P participate in several inflammatory responses such as leukocyte chemotaxis (12, 13), and cytokine production and blockade of SphK activity suppresses such responses (12–15, 28). Our data indicate that levels of S1P in the synovial fluid of RA patients were significantly higher than those of osteoarthritis patients. Moreover, we have shown SphK modulation either through DMS or SphK1 antisense oligonucleotidite significantly reduced the levels of proinflammatory mediator synthesis in our cell contact assays. In a murine CIA model, i.p. administration of DMS significantly inhibited disease severity and reduced articular inflammation and joint destruction, as well as proinflammatory responses both in vivo and in vitro. Similarly, inhibition of...
highly specific siRNA (28). Our data showed that i.p. administration of SphK1 siRNA significantly reduced both incidence and disease severity in the development of murine CIA. Recently, it was suggested that SphK1 knockout mice developed CIA with normal incidence and severity (47). It is possible that mice lacking the SphK1 gene during embryonic development may be adapted to not rely on this pathway for inflammatory responses postnatally. There are a number of precedents including the initial Src kinases, Syk, and Zap-70 knockout mice (48). Thus, blockade of SphK in normal animals may lead to modulation of inflammatory responses. To this end we have recently validated that SphK1 plays a critical role in vivo in a model of acute inflammation (49).

In conclusion, we demonstrate in this study that synovial fluid of RA patients exhibited higher levels of S1P than did their non-inflammatory OA counterparts and in RA-derived human cells in which DMS suppressed cytokine and MMP-9 release by PBMCs following cell contact-dependent interaction with activated T lymphocytes. While testing such hypotheses reliably in preclinical studies remains challenging, the potential clinical relevance of the above observations is illustrated by the ability of SphK modulation either through DMS or SphK1 siRNA to effectively suppress murine CIA both in vivo and in vitro via specific down-regulation of the pathologic proinflammatory and Th1 responses. Therefore, it is conceivable that SphK modulation may play a beneficial role in the treatment of inflammatory diseases such as RA.

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Disclosures

The authors have no financial conflicts of interest.

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