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Mini Review

Role of sphingosine 1-phosphate signaling for the pathogenesis of autoimmune diseases

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Sphingosine 1-phosphate (S1P) acts as an extracellular mediator by binding to G protein-coupled receptors, regulating cell proliferation, angiogenesis and inflammation. FTY720 (FTY) is a high-affinity agonist for S1P receptors, inducing internalization of receptors, rendering the cells unresponsive to S1P. Here, we review the role of S1P signaling for the pathogenesis of autoimmune diseases and the therapeutic effects of FTY on autoimmune diseases.

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Introduction

Sphingosine 1-phosphate (S1P) is one of the cell-derived lysophospholipid growth factors that signal diverse cellular functions [1]. S1P is generated by metabolism of sphingomyelin with S1P levels being tightly regulated by series of enzymes including sphingosine kinase (SK) and S1P phosphatase. S1P acts as an extracellular mediator by binding to G protein-coupled receptors (GPCRs). To date, five closely related GPCRs, namely $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$, have been identified as high-affinity S1P receptors [2] (Fig. 1). S1P was defined as a novel regulator of angiogenesis and seems to be a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells through $S1P_1$ [3]. In the immune system, S1P interacts with naive and memory T cells through $S1P_1$, regulating T cell development and tissue-homing patterns [4, 5]. FTY720 (FTY) is a high-affinity agonist for S1P receptors, and after phosphorylation by SK, FTY720P (FTY-P) activates $S1P_1$, $S1P_4$ and $S1P_5$ receptors with an EC50 of approximately 1 nM, is a partial agonist on $S1P_3$ receptors and is inactive on $S1P_2$ [6]. FTY induces internalization of the receptor, rendering the cells unresponsive to S1P. Its immunomodulatory effects are primarily exerted by sequestration of lymphocytes within the thymus and secondary lymphoid organs, thereby denying them the ability to recirculate to peripheral sites of inflammation [7-9]. Here, we review the role of S1P signaling for the pathogenesis of autoimmune diseases and the therapeutic effects of FTY on autoimmune diseases including our own experimental results.

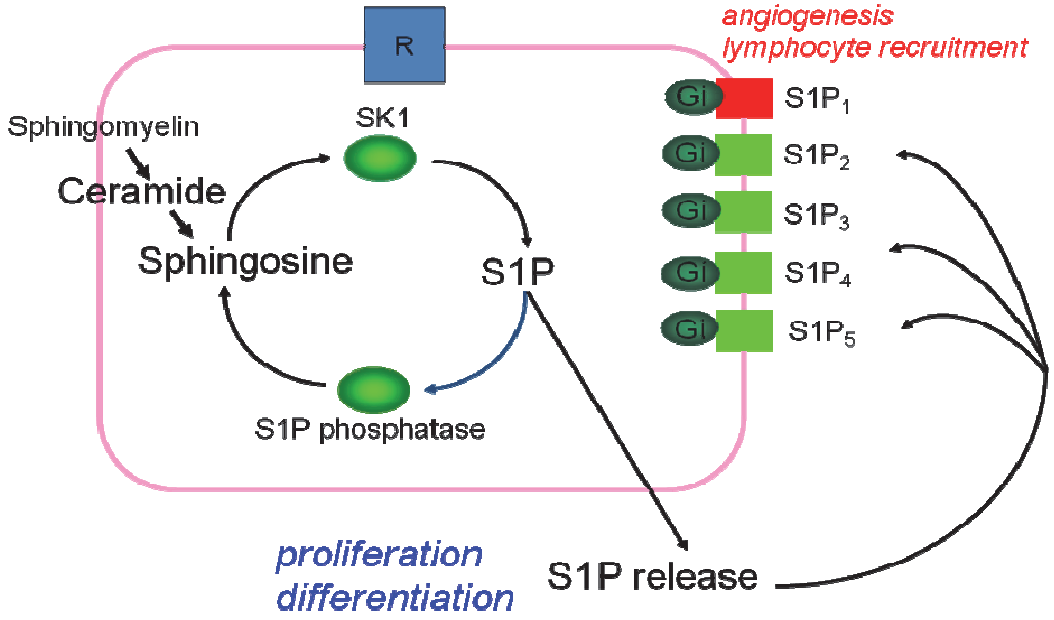

Fig. 1.

Regulatory role of S1P for cell proliferation and differentiation. S1P is generated by metabolism of sphingomyelin with S1P levels being tightly regulated by series of enzymes including sphingosine kinase (SK) and S1P phosphatase. S1P acts as an extracellular mediator by binding to G protein-coupled receptors (GPCRs) such as $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$.

Role of S1P signaling for the pathogenesis of autoimmune diseases

The sphingolipid metabolites ceramide, sphingosine, and S1P have recently emerged as a new class of lipid messengers that regulate cell proliferation, differentiation, and survival in opposite directions [10-12]. The balance of these three lipid-signaling molecules is critically regulated by SK, which converts sphingosine to S1P by phosphorylating sphingosine. Recent studies have demonstrated that the agonist-inducible SK, SK1, is up-regulated in azoxymethane-induced colon cancer cells and in B cells resistant to Fas-mediated apoptosis from patients with

rheumatoid arthritis (RA) [13, 14]. The mechanisms by which SK1 promotes carcinogenesis and resistance to Fas-mediated apoptosis probably depend on its ability to phosphorylate sphingosine to produce S1P. We have demonstrated that S1P signaling is up-regulated in patients with RA and primary Sjogren's syndrome (pSS) [15, 16].

Role of S1P signaling for the pathogenesis of RA

We examined $S1P_1$ and SK1 protein expressions in RA synoviocytes. Both $S1P_1$ and SK1 were more strongly expressed in synovial lining cells, vascular endothelial cells, and inflammatory mononuclear cells of RA synovium compared with osteoarthritis synovium (Fig. 2). S1P increased the proliferation, COX-2 expression and PGE2 production of RA synoviocytes. These findings suggest that S1P signaling via S1P receptors plays an important role in cell proliferation and inflammatory cytokine-induced COX-2 expression and PGE2 production by RA synoviocytes [15].

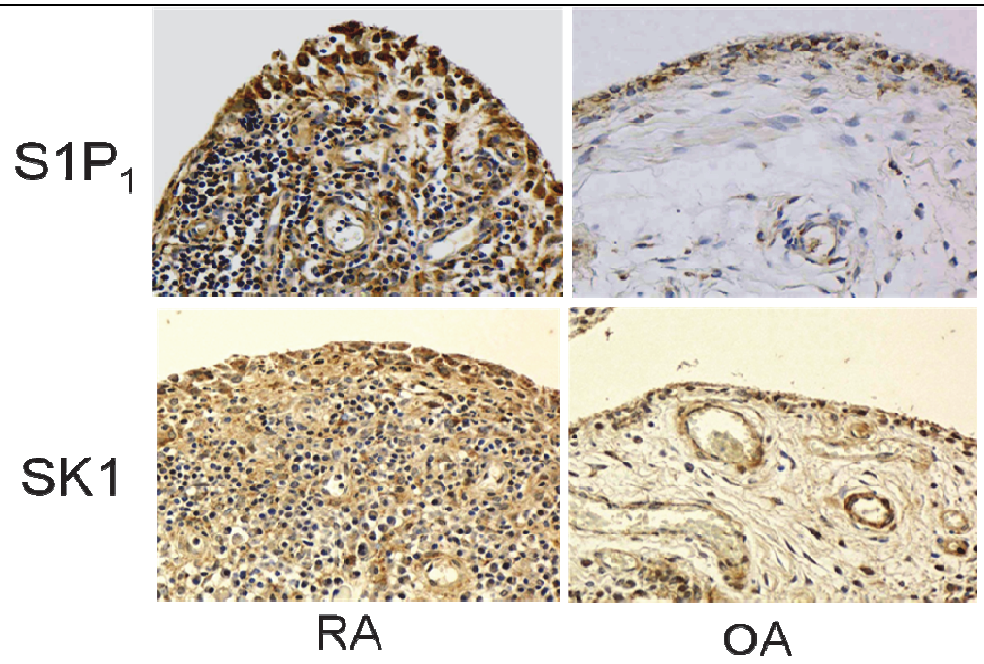

Fig. 2.

Immunohistochemistry for $S1P_1$ and SK1 of synovium in RA or OA patients. Both $S1P_1$ and SK1 were more strongly expressed in synovial lining cells, vascular endothelial cells, and inflammatory mononuclear cells of RA synovium compared with OA synovium. Original magnification, x400.

Role of S1P signaling for the pathogenesis of pSS

To study the role of S1P-$S1P_1$ interactions in the pathogenesis of pSS, we examined the expression and localization of SK1 and $S1P_1$ in labial salivary glands (LSG) from patients with pSS by immunohistochemistry. The immunoreactivity of both SK1 and $S1P_1$ exhibited a similar cellular distribution, as both SK1 and $S1P_1$ were expressed within cytoplasm of inflammatory mononuclear cells, vascular endothelial cells, and salivary gland epithelial cells in LSG biopsy specimens. We next extended this approach to various stages of sialoadenitis and examined the extent and intensity of both SK1 and $S1P_1$ immunostaining. Although SK1 staining intensity was not different between grade 1 and grade 4 LSG biopsy specimens, $S1P_1$ staining in inflammatory mononuclear cells was significantly more extensive in the grade 4 LSG biopsy specimens than in the grade 1 LSG biopsy specimens (Fig. 3). These results indicate that S1P-$S1P_1$ interactions occur in salivary glands from patients with pSS. We observed that IFN-γ significantly increased Fas mRNA expression in a salivary gland ductal epithelial cell line, NS-SV-DC. Fas mRNA expression was also significantly increased by S1P (0.1–0.5 μM). Furthermore, S1P enhanced IFN-γ-induced Fas mRNA expression in NS-SV-DC cells (Fig. 4). These results indicate that both S1P and IFN-γ secreted by infiltrating $CD4^+$ T cells increase

Fas expression on salivary gland epithelial cells. We also examined caspase-3 expression in NS-SV-DC cultured with IFN-γ or S1P. Both IFN-γ and S1P (0.1–0.5 μM) significantly increased caspase-3 expression in NS-SV-DC in the presence of anti-Fas mAb (Fig. 4) [16].

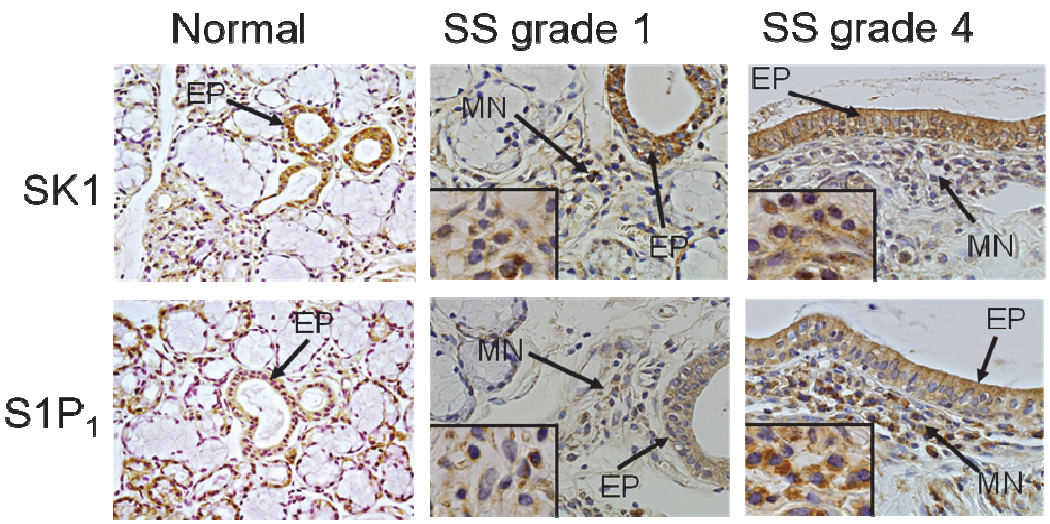

Fig. 3.

$S1P_1$ and SK1 expression in salivary glands by pSS patients. SK1 staining intensity was not different between grade 1 and grade 4 LSG biopsy specimens, but $S1P_1$ staining in inflammatory mononuclear cells was significantly more extensive in the grade 4 LSG biopsy specimens than in the grade 1 LSG biopsy specimens. Original magnification, x400, with insets, x1000. EP, Epithelial cell; MN, mononuclear cell.

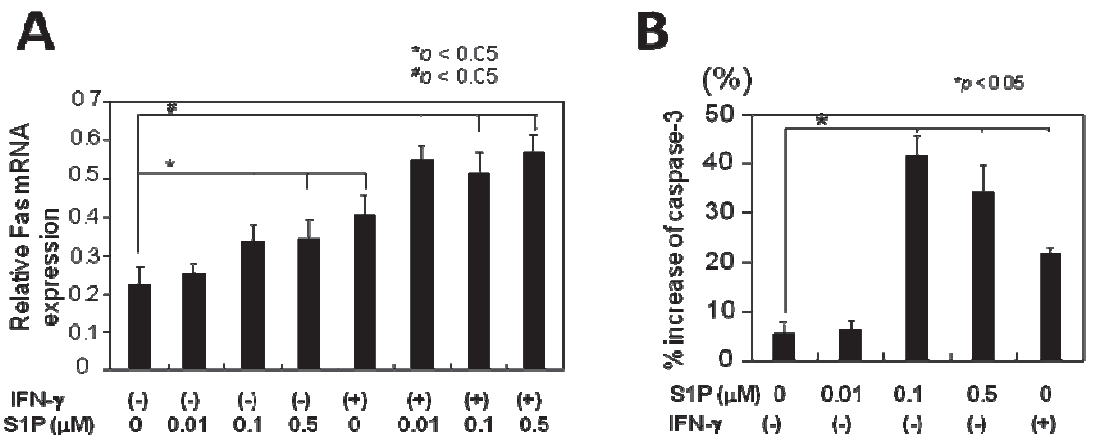

Fig. 4.

S1P induces apoptosis of salivary gland epithelial cells. A. NS-SV-DC cells (1 x 10^6) were treated with 0.01–0.5 μM S1P without (–) or with (+) IFN-γ (0.2 μg/ml) for 6 h, and semiquantitative RT-PCR for the expression of Fas mRNA in NS-SV-DC cells was performed. Fas mRNA expression levels were determined by normalizing expression with respect to GAPDH mRNA expression levels. B. NS-SV-DC cells (1 x 10^6) were cultured in the presence (+) or absence (–) of S1P (0.01–0.5 μM) or IFN-γ (0.2 μg/ml). After 72 h of culture, anti-Fas mAb (100 ng/ml) was added and caspase-3 activity of the cell lysates was analyzed. Percentage increase of anti-Fas mAb-induced caspase-3 activity was calculated.

Experimental trial of FTY for the treatment of autoimmune diseases

FTY is a synthetic compound produced by modification of a metabolite from Isaria sinclairii, a kind of vegetative wasp. FTY is phosphorylated in vivo by SK2 to FTY-P, which acts as potent S1P receptors (S1PRs) agonist. These receptors are critically involved in cell survival, cytoskeletal rearrangements, cell motility, and cell migration [17, 18]. FTY-P binds to four types of S1PRs ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$) except for $S1P_2$ and acts as a high affinity agonist at these receptors. FTY-P acts as a potent agonist at $S1P_1$, internalizes $S1P_1$ on lymphocytes, and inhibits the migration of lymphocytes toward S1P. This immunomodulatory effects are primarily exerted by sequestration of lymphocytes within the thymus and secondary lymphoid organs, thereby denying them the ability to recirculate to peripheral sites of inflammation [7-9]. FTY has been shown to be a useful agent for the prevention of transplant rejection and autoimmune diseases such as multiple sclerosis (MS) using animal models [19-22]. There are several reports demonstrating that FTY is effective for the treatment of RA using collagen or adjuvant-induced arthritis models [23-25]. We have observed that FTY is effective for the treatment of systemic lupus erythematosus (SLE) or RA using lupus model of chronic graft-versus-host disases (GVHD) mice or RA model of SKG mice, respectively. [26].

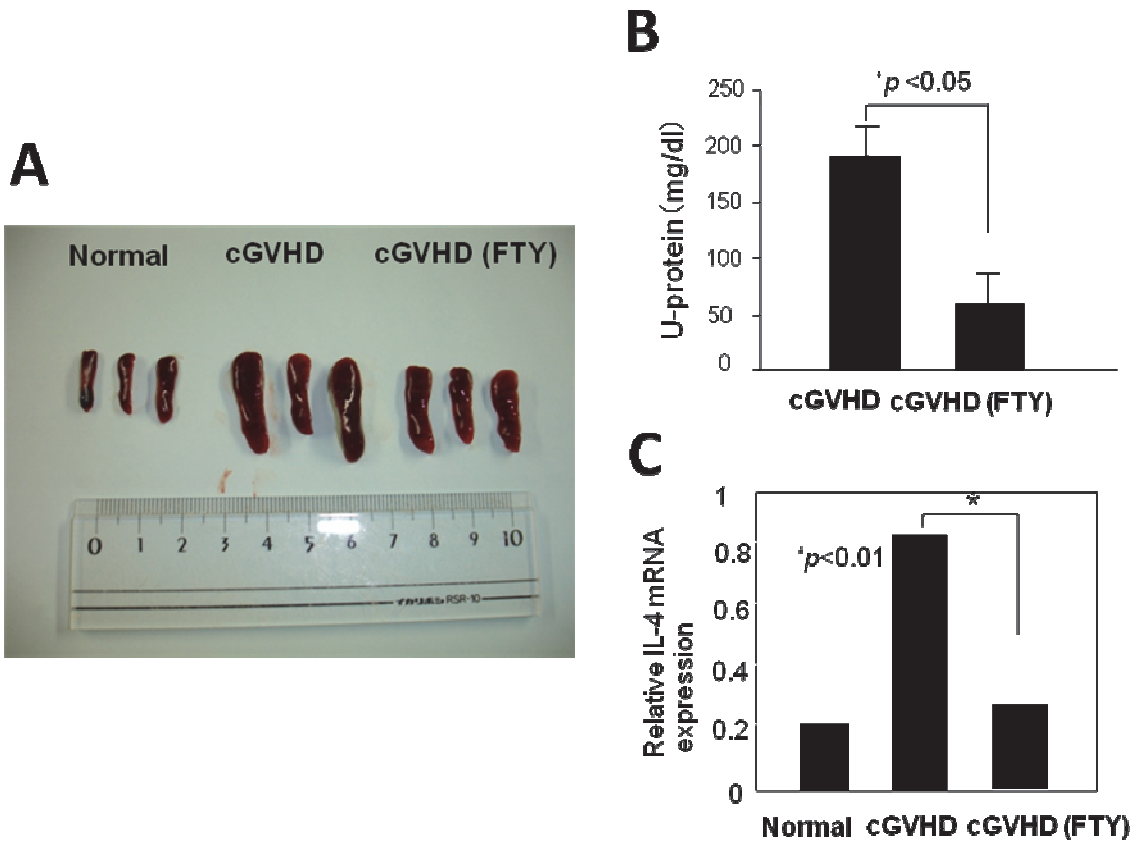

Fig. 5.

Effect of FTY on lupus model of chronic GVHD mice. Chronic GVHD was induced by injection of DBA/2 spleen cells (5×10^7) into (B6 x DBA/2) F1 mice. FTY treatment of chronic GVHD mice reduced the spleen size at 2 weeks (A) and significantly inhibited proteinuria at 8 weeks after GVHD induction (B). FTY treatment also inhibited IL-4 mRNA expressions in the spleen at 2 weeks after GVHD induction (C).

Therapeutic effect of FTY on SLE model mice

Therapeutic effects of FTY has been studied using SLE model of lpr mice. FTY-treated lpr mice had significantly prolonged live and the increased proportion of $CD3^+$ $B220^+$ and $CD4^-$ $CD8^-$ cells in the thymus. Apoptotic cells were detected in all the lymphoid organs [27, 28]. Pathogenic T cells that recognize self-antigens and drive B cell hyperactivity play a central role in the pathogenesis of both human and murine lupus [29-31]. Chronic GVHD, which is induced in (C57BL/6 × DBA/2) F1 (BDF1) mice by injection of DBA/2 spleen cells, is associated with the activation of donor $CD4^+$ T cells that recognize host major histocompatibility complex antigens and drive host B cell hyperactivity [32, 33]. Mice of this parent-into-F1 chronic GVHD model show increased T helper (Th) 2 immune responses, and exhibit autoimmune disorders that resemble human SLE, primary biliary chirrhosis, and pSS, which are characterized by lymphocyte infiltration into organs such as the kidneys, liver and salivary glands [34]. We observed that FTY treatment of chronic GVHD mice significantly inhibited proteinuria and histopathological changed in the kidneys, liver, and salivary glands. FTY treatment also inhibited Th2 cytokine mRNA expressions in the spleen and kidneys (Fig. 5).

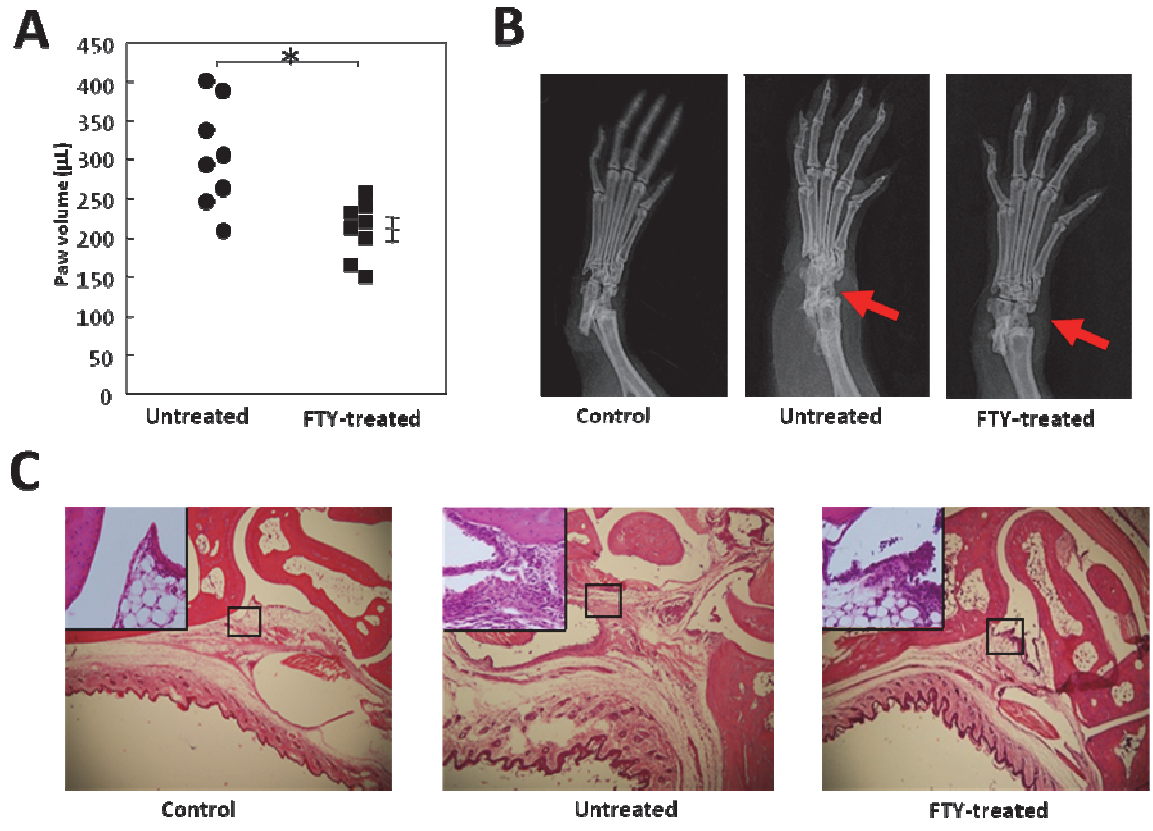

Fig. 6.

FTY administration inhibited joint swelling (A). X-ray examination of the ankle joints of untreated SKG mice at 60 days following β-glucan injection revealed erosion of the cartilage and subchondral bone while these changes were inhibited in FTY-treated SKG mice (B). Histopathology of swollen joints in the untreated SKG mice revealed vigorous proliferation of synovial cells and infiltration by mononuclear cells and neutrophils of the synovial tissues. In contrast, these pathological changes were significantly inhibited in FTY-treated SKG mice. Original magnification x40. Inserts show higher magnifications of the indicated part of the synovium (C).

Therapeutic effect of FTY on RA model of SKG mice

We observed that FTY administration inhibited joint swelling. Histopathology of swollen joints in the untreated SKG mice revealed vigorous proliferation of synovial cells and infiltration by mononuclear cells and neutrophils of the synovial tissues as has been observed in human RA. In contrast, these pathological changes were significantly inhibited in FTY-treated SKG mice. X-ray examination of the ankle joints of untreated SKG mice at 60 days following β-glucan injection revealed erosion of the cartilage and subchondral bone while these changes were inhibited in FTY-treated SKG mice (Fig. 6) [26].

Clinical trial of FTY treatment

The first Phase II, multicenter, open-label, dose-finding study compared FTY (0.25, 0.5, 1.0, or 2.5 mg) with mycophenolate mofetil (MMF), in combination with cyclosporine and corticosteroids has been reported. FTY at 2.5 mg was found to be as effective as MMF in combination with cyclosporine for the prevention of acute rejection after renal transplantation [35]. Next multicenter, double-blind, Phase II, randomized study evaluated the safety and efficacy of 5 mg FTY vs. 2.5 mg FTY vs. MMF in de novo renal transplant patients receiving full dose cyclophosphamide and prednisone. Although FTY provided adequate protection from acute rejection the safety profile was less favorable for adverse events such as bradycardia and respiratory disorders than current standard immunosuppression in de novo renal transplant patients [36].

FTY was highly effective in Phase II clinical trials with relapsing MS [37, 38]. FTY at an oral dose of 1.25 mg or 5.0 mg, or placebo is administered daily for 6 months to 281 patients and total of 255 patients has completed the clinical study. The median total number of gadolinium-enhanced lesions on magnetic resonance imaging (MRI) was lower in patients receiving 1.25 mg (1 lesion, $P<0.001$) and 5.0 mg (3 lesions, $P=0.006$) of FTY than those receiving placebo (5 lesions). Recently, one year Phase III TRANSFORMS study of FTY in relapsing remitting MS has been reported [39]. In this study, FTY at oral dose of 0.5mg and 1.25mg showed a superior efficacy compared with a standard treatment of interferon-β. Another 2 years placebo-controlled Phase III studies (FREEDOMS and FREEDOMS II) to assess the impact of FTY in reducing the frequency of relapses and slowing the progression of disability have been reported [40]. Furthermore, Phase III study (INFORMS) of FTY is also ongoing for the treatment of patients with primary progressive MS.

Conclusions

In this paper, we briefly reviewed clinical and experimental reports of S1P for the pathogenesis of autoimmune diseases including our experimental results. We also reviewed clinical and experimental reports of FTY for the treatment of autoimmune diseases. Many preclinical and clinical data supported that FTY has efficacy to many diseases such as acute rejection after renal transplantation and MS. The regulation of S1P/S1P1 receptors signaling using FTY may become a new approach for the therapy not only of autoimmune diseases but also of many other diseases.

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