Review Article

Jak and Syk: Emerging their relevance to the treatment of inflammatory diseases

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The multiple soluble ligands such as cytokines play a pivotal role in the pathogenesis of inflammatory diseases. The appropriate intracellular signaling pathways must be activated via cytokine receptors on the cell surface and the tyrosine kinase transduce the first "outside to in" signals to be phosphorylated following receptor binding to its ligand. Among them, members of Janus kinase (Jak) family and Spleen tyrosine kinase (Syk) family are essential for the signaling pathways of various cytokines and are implicated in the pathogenesis of rheumatoid arthritis (RA), a representative autoimmune inflammatory disease.

Selective inhibition of Jak3 was considered as a potential target in the treatment of RA and an orally available Jak3 inhibitor CP-690,550 (tofacitinib), is currently in clinical trials for RA with satisfactory effects and acceptable safety. Our in vitro experiments have indicated that the inhibition could be mediated through the suppression of IL-17 and IFN-γ production and proliferation of $CD4^+$ T cells without directly affecting synovial fibroblasts and monocytes. Multiple global clinical examinations indicate that JAK inhibition with CP-690,550 in patients with RA results in rapid and remarkable clinical effects without severe adverse events.

A selective Syk inhibitor R788 (fostamatinib) has been shown to be effective for the treatment of not only RA but also bronchial asthma, B-cell lymphoma and idiopathic thrombocytopenic purpura. We have found that Syk-mediated B cell receptor (BCR)-signaling is prerequisite for optimal induction of toll-like receptor (TLR)-9, thereby allowing efficient propagation of CD40 and TLR9-signaling in human B cells. These results indicate that inhibition of Syk have a potential to regulate B-cell mediated inflammatory diseases such as systemic lupus etythematosus (SLE). We here document the in vitro and in vivo effects of a Jak inhibitor and a Syk inhibitor for the treatment of autoimmune inflammatory diseases, mainly RA and SLE.

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"Outside to in signals" in inflammation

Inflammatory processes are mediated by a sequence of signaling pathway consisting of intercellular signals, outside to in signals and intracellular signals, whose hyper-activation and/or dysregulation result in the onset as well as persistency of inflammatory diseases. Various intercellular signaling plays a pivotal role during the process, some of which are mediated by soluble ligands such as cytokines and growth factors and others are done by cognate interaction through costimulatory molecules and adhesion molecules.

The importance of cytokines in the pathological processes of inflammatory diseases has become apparent from the clinical efficacies of biological agents targeting TNF and IL-6 on rheumatoid arthritis (RA), a representative autoimmune disease[1-3]. The regulation of these proinflammatory cytokines leads to inhibition of not only the disease activity but also destructive changes of the inflamed joints. For such cytokines to exert their biological activities, the appropriate intracellular signaling pathways must be activated by the engagement of their specific receptors on the cell surface, that is "outside to in signaling".

The intracellular signals are represented by the following pathway; i) phosphorylation of protein kinase such as serine/threonine kinase, tyrosine kinase and MAP kinase kinase; ii) GTP-binding proteins including small G-protein such as Rho and Ras and heterotrimeric G-protein consisting of Gα, Gβ, Gγ; iii) second messengers such as cyclic AMP and GMP; iv) protease activating apoptotic-related proteins such as caspase; v) ubiquitination. The transduction of these intracellular signals leads to various cellular functions through directly, sequentially activating or regulating one another.

Phosphorylation of kinase proteins plays an important role in immune system and more than 99% of them are serine/threonine kinases in physiological and ordinary condition. On the other hand, the tyrosine kinase is the first intracellular signaling molecules to be phosphorylated following receptor binding in a cytokine response and is involved in fundamental function such as cell proliferation, differentiation and adhesion in various pathological processes including inflammation and cancer. Therefore, many investigators have shed light on tyrosine kinases as the target of the treatment of various diseases. More than 90 genes encoding tyrosine kinases have been identified from human genome-wide studies and 14 tyrosine kinases are known to be involved in RA[4].

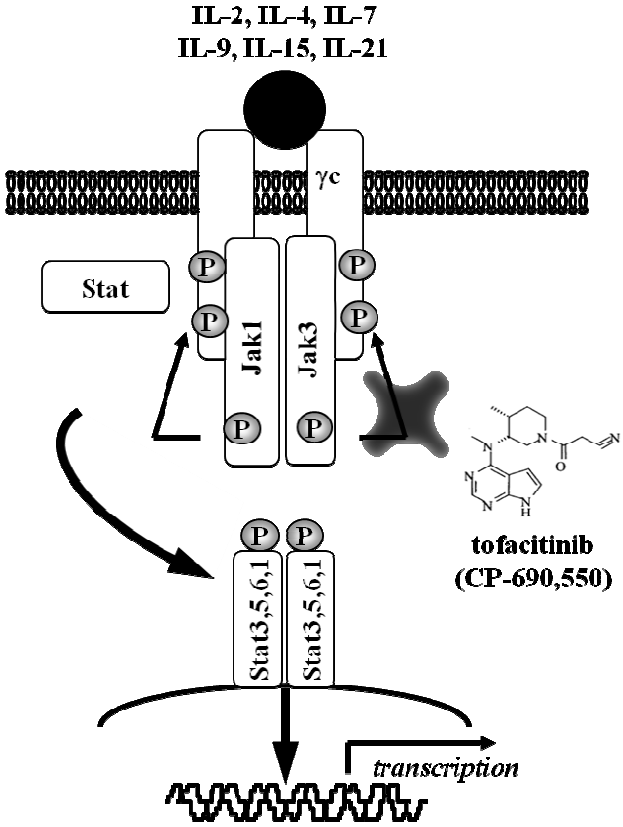

Figure 1. The JAK-STAT signaling pathway
JAK3 expression is essentially limited to hematopoietic cells and constitutively binds to the γc chain which is a common receptor subunit for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.

Jak-Stat pathway in inflammation

Of tyrosine kinases, Janus kinase (Jak) family, consisting of Jak1, Jak2, Jak3 and Tyk2, has gathered particular attention since Jaks are essential for the signaling pathways of various cytokines. Jaks are phosphorylated just after cytokines bind to their receptors and consecutively activate transcription factor signal transducers and activators of transcription (Stat) (Figure 1)[5-9]. After the engagement of homodimeric or heterodimeric receptors for cytokines and growth factors, which are constitutively bound to Jaks. Jaks are activated by a conformational change in the receptor that allows trans- and/or auto-phosphorylation of the two bound Jaks. These in

turn phosphorylate the cytokine receptors. Stat proteins bind the phosphorylated receptor chains, which allow the Jaks to phosphorylate the Stats. Phosphorylated Stats form dimers and translocate into the nucleus, where they regulate gene expression. Thus, Jak-Stat pathway regulates multiple immune functions. For instance, different Stat is involved in differential cytokine production from $CD4^+$ T cell subsets: Stat1 and Stat4 mainly induce IFN-γ from Th1, Stat6 does IL-4 from Th2, Stat5 does TGF-β from regulatory T cells (Treg) and Stat3 dose IL-17 from Th17.

Jak3 expression is essentially limited to hematopoietic cells and constitutively binds to the common γ-chain which is a common receptor subunit for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, the deficiency or dysfunction of Jak3 leads to severe combined immunodeficiency both in human and mice. Thus, numbers of tyrosine kinase inhibitors have recently been evaluated in clinical trials, selective inhibition of Jak3 was considered as a potential target in the treatment of RA without affecting other organ systems.

Jak inhibition for the treatment of RA

RA is a representative autoimmune disease characterized by chronic and destructive inflammatory synovitis and multiple organ manifestations that causes severe disability and mortality. A new concept of 'treat-to-target' is emerging in treatments of RA, whereby patients are treated according to pre-specified goals, such as remission. Traditional disease-modifying anti-rheumatic drugs (DMARDs), most commonly methotrexate (MTX), remain the cornerstone of RA treatment. Patients who have an inadequate response to the conventional MTX are often recommended to be treated with a growing number of biological DMARDs targeting TNF and IL-6, either as monotherapy or in combination with MTX. The combined use of a TNF-inhibitor and MTX has produced significant improvements in clinical, structural and functional outcomes that were not previously seen and has revolutionized the treatment goal of RA to clinical remission. However, since approximately 30% of patients treated with the emerging therapy attained clinical remission, treatments in the next generation are prerequisite to patients with refractory RA[1-3].

Based on these backgrounds, an orally available Jak3 inhibitor CP-690,550, which is now designated tofacitinib, was developed with expectations to be a new immunosuppressant with few side effects[10,11]. Tofacitinib improved endpoints of both murine collagen-induced arthritis and rat adjuvant-induced arthritis. Tofacitinib was also reported to highly suppress Jak3 with low concentration with few side effects in a graft versus host disease experiment[10-12]. Furthermore, tofacitinib is currently in clinical trials for RA with satisfactory effects and acceptable safety.

Kremer, et al reported a Phase 2 dose-ranging trial which was carried out to investigate the efficacy, safety, and tolerability of oral tofacitinib in 264 patients with active RA in whom MTX, etanercept, infliximab, or adalimumab caused an inadequate or toxic response[13,14]. Patients were randomized to placebo, 5 mg, 15 mg or 30 mg of tofacitinib twice daily for 6 weeks, and were followed up for an additional 6 weeks after treatment. The American College of Rheumatology 20% improvement criteria (ACR20) response rate was 26.9%. 70.5%, 81.2%, and 76.8% in the placebo, 5 mg, 15 mg, and 30 mg twice daily groups, respectively, at 6 weeks. Thus, patients treated with tofacitinib in all treatment groups were satisfied with the primary efficacy end point, ACR20 response rate at 6 weeks, significantly compared with the placebo group. Rapid improvements in disease activity were observed in patients treated with tofacitinib and ACR50 and ACR70 response rates significantly improved in all treatment groups by week 4. The most common adverse events reported were headache and nausea. The infection rate in the 15 mg twice daily group and the 30 mg twice daily group was 30.4% (26.2% in placebo) and opportunistic infections or deaths were not observed.

A phase 2 double blinded study was also carried out to investigate the efficacy and safety of an orally available tofacitinib in Japanese patients with active RA and inadequate response to MTX (MTX-IR). A total of 140 patients were randomized to tofacitinib[15] 1, 3, 5, 10 mg, or placebo twice daily in this 12-week and remained on background MTX. ACR20 response rates at week 12, a primary endpoint, were significant for all tofacitinib treatment groups. The ACR20 response rate was 14.3%, 64.3 %, 77.8%, 96.3%, 80.8 % in the placebo, 1 mg, 3 mg, 5 mg and 10 mg twice daily groups, respectively, at 12 weeks. Furthermore, in patients with high disease activity at baseline (DAS28 >5.1), the greatest percentage of patients achieving disease activity-score (DAS) remission at week 12 was observed in the tofacitinib 10 mg twice daily group

(45.5%). In patients with low to moderate disease activity at baseline (DAS28 ≤5.1), the tofacitinib 5 mg twice daily group contained the greatest percentage of patients achieving DAS remission at Week 12 (80.0%) at week 12 (Figure 2).

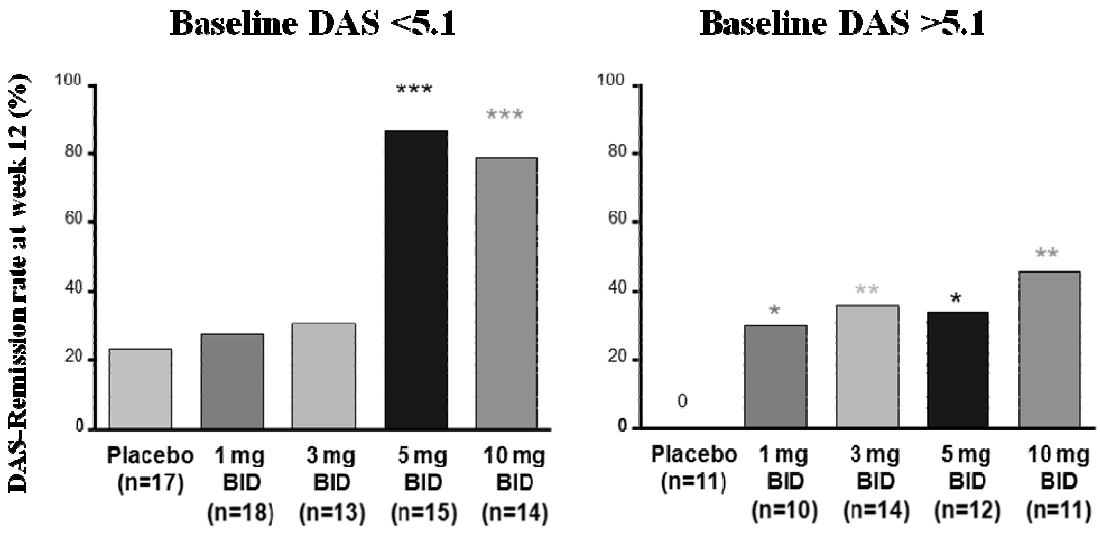

Figure 2. DAS28-3 (CRP) remission (<2.6) and low disease activity state (≤3.2) rate (%) in patients with baseline DAS28-3 (CRP) ≤5.1 and >5.1 at Week 12 (LOCF) and treated with placebo or different dose of tofacitinib

The most commonly reported adverse events were nasopharyngitis, and increased hepatic transaminase. These adverse events were mild or moderate in severity. Serious adverse events were reported by 5 patients, but no deaths occurred. Taken together, in Japanese patients with active RA and MTX-IR, an orally available Jak inhibitor tofacitinib, in combination with MTX over 12 weeks was efficacious and had a manageable safety profile. Accordingly, longer-duration and dose-ranging studies of this novel Jak inhibitor tofacitinib in the treatment of RA are on-going by multiple global clinical examinations and efficacy for the regulation of progress in structural damages and functional disabilities is another important outcome.

In vitro effects of a Jak inhibitor in RA

However, mode of action of tofacitinib in patients with RA remains unclear. Walker, et al reported that Jak3, Stat1, Stat4 and Stat6 were highly expressed in synovium in patients with RA, whereas the expression was scarce in synovium in normal volunteers and patients with osteoarthritis and spondyloarthropathy. According to the important role of Jak3 in lymphocyte development, differentiation and proliferation, we assessed the effects of tofacitinib on $CD4^+$ T cells at the local inflammatory sites in patients with RA. The proliferation of $CD4^+$ synovial T cells in RA patients stimulated with anti-CD3 and anti-CD28 antibodies was inhibited by tofacitinib in a dose-dependent manner. Furthermore, treatment of synovial $CD4^+$ T cells with tofacitinib inhibited production of IL-17 and IFN-γ, but had no effect on IL-6 and IL-8 production. However, $CD14^+$ monocytes and synovial fibroblasts isolated from synovium in patients with RA were not affected by tofacitinib. Our results suggested that the effects of tofacitinib in RA are mediated through the suppression of IL-17 and IFN-γ production and proliferation of $CD4^+$ T cells without affecting synovial fibroblasts and monocytes. Since IFN-γ and IL-17 are produced by Th1 and Th17 cells respectively and are important drivers of destructive arthritis in mice and humans, Jak3 in $CD4^+$ T cells, presumably Th1 and Th17 cells, plays a crucial role in rheumatoid synovitis (Figure 3).

Syk in inflammation

Spleen tyrosine kinase (Syk) is a 72 kDa another protein tyrosine kinase expressed by B cells, T cells, mast cells, macrophages, neutrophils and synovial fibroblasts. Syk is involved in intracellular signaling through multi-chain immune receptors, including Igα (B-cell receptor; BCR), ζ chain of T-cell receptor

(TCR), FcγR, FcεR and integrins, which contain the immune-receptor tyrosine-based activation motif (ITAM)[16-18]. The engagement of the immune receptor phosphorylates ITAMs, which in turn phosphorylates Syk and leads to multiple downstream signaling, resulting in proliferation and cytokine production (Figure 4). Thus, Syk is also involved in T-cell and B-cell receptor-mediated signaling, potentially making Syk targets for the treatment of autoimmune diseases.

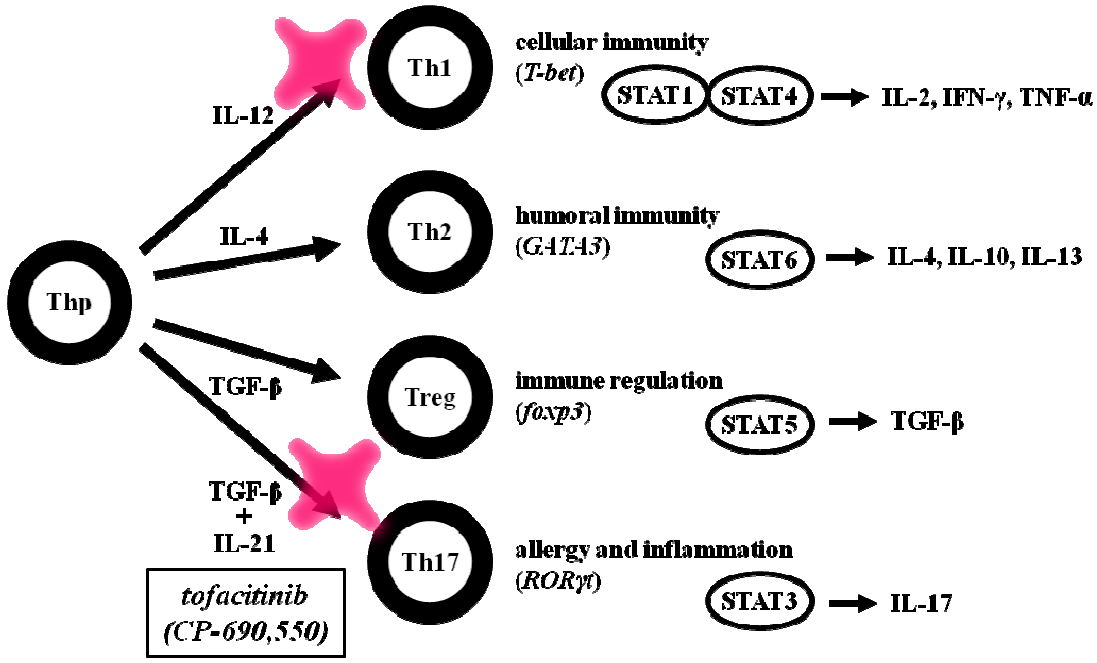

Figure 3. The JAK-STAT signaling pathway in T cell subsets
Effects of tofacitinib in RA are mediated through the suppression of IL-17 and IFN-γ production from CD4-positive T cells.

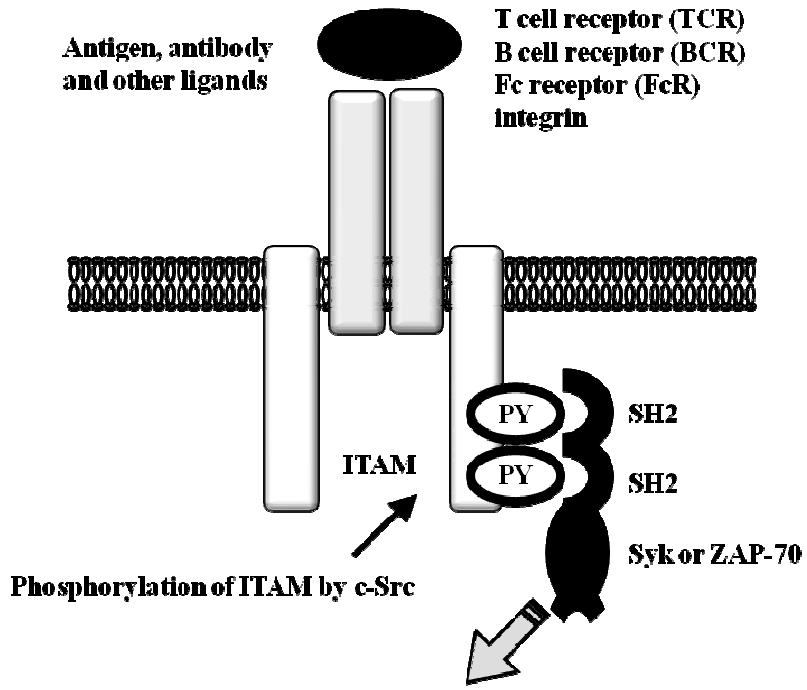

Figure 4. Spleen tyrosine kinase (Syk) in intracellular signaling

B cell aberrations are a hallmark of the pathogenesis in SLE, a representative autoimmune disease. Syk is well known to function as a key molecule in BCR-mediated signaling, but a role of Syk in human B cell aberrations remains elusive. We have demonstrated that BCR-crosslinking, in conjunction with CD40 and toll-like receptor (TLR)-9 stimulations, efficiently induced various functions of human B cells such as remarkable proliferation, expression of costimulatory molecules, cytokine production and Ig production in memory B cells, compared with naïve B cells. The highly specific Syk inhibitor significantly abrogated these functions of B cell subsets. It is noteworthy that BCR-crosslinking alone markedly induced expression of TLR9 and TRAF-6. Additional CD40 and TLR9 stimulations further induced expression of TRAF-6 and phosphorylation of NF-κB, whereas a Syk inhibitor significantly inhibited them. These results indicate that inhibition of Syk have a potential to regulate B-cell mediated inflammatory diseases (Figure 5).

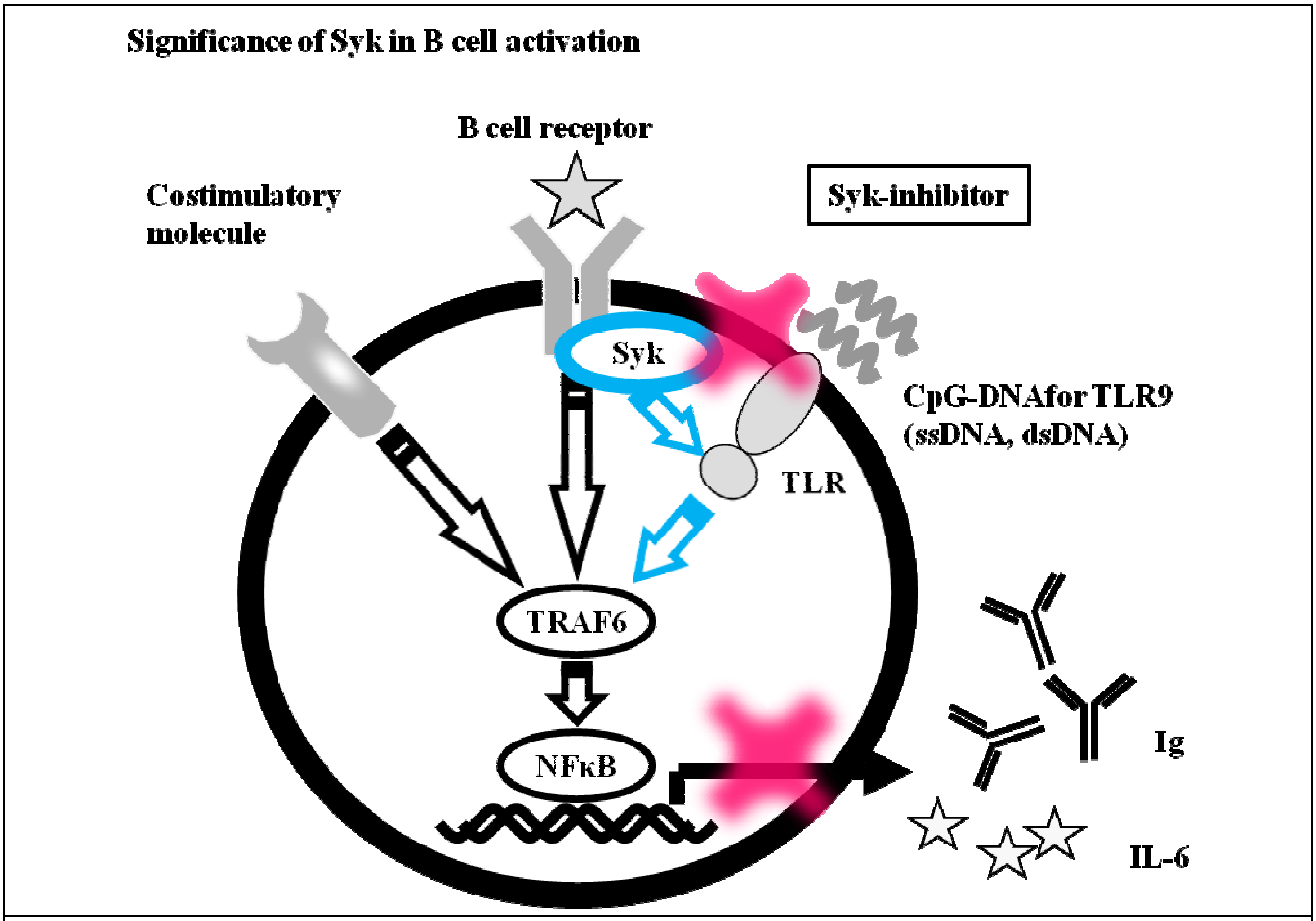

Figure 5. A schematic regarding a role of Syk during B cell activation

Syk and a Syk inhibitor for RA and SLE

Pine et al. showed that a Syk inhibitor R788 (fostamatinib disodium), a pro-drug of R406, suppressed the severity of arthritis, bone erosions, pannus development and synovitis in murine collagen-induced arthritis (CIA)[19]. The reduced expression of SyK in the fostamatinib-treated mice correlated with an amelioration of arthritis, a reduction in cytokine and metalloproteinase production, including IL-1. IL-6 and MCP-1. A selective Syk inhibitor fostamatinib has thus far been shown to be effective for the treatment of not only RA but also bronchial asthma, B-cell lymphoma and idiopathic thrombocytopenic purpura[20-23]. Moreover, Syk inhibition prevents the development of skin and kidney lesions in lupus-prone mice[24,25].

A phase 2 double blinded study was undertaken to evaluate an orally available Syk inhibitor fostamatinib among patients with active RA and MTX-IR[23]. In this randomized trial, a significant effect was seen with fostamatinib at a dose of 100 mg twice a day in

addition to MTX in the ACR 20, 50, and 70 response rates and in the rates of DAS28 remission at 6 months, with a higher response observed in the group that received. A clinically significant effect was noted by the end of the first week of treatment and majority of the patients in whom there was a response at month 6 already had a response by month 2. Major adverse effects included diarrhea (in 19% of subjects taking the 100-mg dose of fostamatinib vs. 3% of those taking placebo), upper respiratory infections (14% vs. 7%) and neutropenia (6% vs. 1%). Fostamatinib was associated with an increase in systolic blood pressure of approximately 3 mm Hg between baseline and month 1. Thus, in this phase 2 study a Syk inhibitor fostamatinib was satisfied with the primary objective to determine the efficacy and safety, as compared with placebo, in patients with active RA and MTX-IR at 6 months. Thus, inhibition of the Syk pathway offers a new drug target for the treatment of RA and additional studies are needed to further assess the safety and efficacy of the therapy in RA patients.

We have recently found that Syk inhibitors have another potential to regulate B-cell-mediated inflammatory diseases such as SLE, from clinical and experimental studies to assess the molecular mechanisms of Syk in activation of human B cell subsets and address its relevance to pathogenesis of SLE. As described above, Syk-mediated BCR-signaling is prerequisite for optimal induction of TLR-9, thereby allowing efficient propagation of CD40 and TLR9-signaling in human B cells. It is noteworthy that high levels of Syk phosphorylation and TRAF6 expression were noted in B cells in patients with active SLE, compared with those from inactive patients or healthy donors. Furthermore, treatment of B cells from patients with a Syk inhibitor prominently abrogated Syk phosphorylation as well as TRAF6 expression. All these results indicate a vital role of Syk in B-cell-mediated pathological processes in SLE and a therapeutic potential of Syk inhibition in patients with SLE.

We here document the in vitro and in vivo effects of a Jak inhibitor and a Syk inhibitor for the treatment of autoimmune inflammatory diseases, mainly RA and SLE. Biological DMARDs such as TNF inhibitors have changed the paradigm of the treatment strategy of RA. Clinical remission is, however, obtained in 1/3 of patients treated even with biologics and anti-biological product antibodies sometimes experienced. Also, intravenous or subcutaneous administration of the drug is required and the economic issues and long-term safety remain unsolved. Accordingly, orally available low molecular weight products such as tofacitinib and fostamatinib, targeting intracellular signaling molecules, would provide enormous power and flexibility in the treatment of inflammatory autoimmune diseases including RA and SLE. Moreover, since it is possible to design low molecular weight products recognizing particular conformation of target molecules in the signaling cascade, the success in tofacitinib and fostamatinib will accelerate new development of multiple products not only for RA but also for many inflammatory diseases and malignancies.

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