Mini Review

The possible mode of action of Tofacitinib, a JAK inhibitor

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Janus kinase (JAK) family members constitutively bound to cytokine receptors and play important roles in the cytokine signal transduction through the activation of the signal transducer and activator of transcription (STAT). Tofacitinib, which is selective for JAK1 and JAK3, is highly effective on rheumatoid arthritis (RA) indicating its wide spread as a common treatment tool for RA. Although the precise action of tofacitinib on the JAK/STAT pathway has been investigated in mouse, the exact mechanism of action under inflammatory conditions in humans remains unclear. We conducted two studies with human synovium and dendritic cells (DCs) and revealed that tofacitinib act on multilineage cells thereby inhibiting lymphocytes both directly and indirectly. Tofacitinib inhibited IL-17 and IFN-γ production by $CD4^+$ T cells subsequently suppressing IL-6 and IL-8 production by RA synovial fibroblasts and $CD14^+$ monocytes, with decreased cartilage destruction in a *ex vivo* human arthritis model (SCID-HuRAg). On the other hand, tofacitinib directly acted on DCs and suppressed inflammatory cytokine production and expression of co-stimulatory molecules by inhibiting the positive feedback loop consisted of type-I IFN. Consequently, tofacitinib suppressed the T-cell stimulatory capability of DCs. These results indicate the wide range of biological effect of tofacitinib that directly and indirectly affect acquired immunity.

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Introduction

Janus kinase (JAK) is a tyrosine kinase known to play important roles in cytokine receptor binding-triggered signal transduction through the translocation of signal transducers and activator of transcription (STAT) to the cell nuclei. The JAK family consists of four members: JAK1, JAK2, JAK3, and Tyk2 and more than 40 different cytokines and growth factors have been shown to activate specific combinations of JAKs and STATs. Since JAKs selectively associate with various cytokine receptors, they are highly associated with functions that can be attributed to cytokines. For example, JAK1$^{-/-}$ cells are unresponsive to three distinct families of cytokine receptors; all class II cytokine receptors (interferon and IL-10 related cytokines), common gamma (γc)-cytokines, as well as IL-6 and other gp130-using cytokines. On the other hand, JAK3 associate with only one cytokine receptor, the γc chain, a shared receptor subunit that pairs with other ligand-specific subunits to form the receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Due to the important role of JAKs in multiple cytokine signaling pathways, they are essential for not only immune system development but also inflammatory immune response[1] and in pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA)[2, 3]. A number of kinase inhibitors targeting JAKs have been developed and tofacitinib, a novel selective JAK inhibitor was reported to exhibit high efficacy in clinical trials for RA and is known to be highly selective against JAK1 and JAK3[4]. Tofacitinib was approved as an anti-rheumatic drug by the U.S. Food and Drug Administration in 2012, and Japan in 2013. Its efficacy shown in clinical trials on RA was numerically similar to TNF inhibitors[5]. Hence, tofacitinib could provide an effective treatment option in patients with an inadequate response to TNF inhibitors[6]. These striking clinical benefits indicated that JAK1 and JAK3 play an important role in the pathology of RA. Here we described our two reports revealing the mode of action of tofacitinib, a novel selective JAK inhibitor.

Effect of tofacitinib on human lymphocyte

JAKs are essential for lymphocyte function and we have previously shown the effect of tofacitinib on T lymphocytes[7]. In this study, we used CD4$^+$ T cells, RA synovial fibroblasts (RASFs), and CD14$^+$ monocytes purified from the synovium and peripheral blood of patients with RA. We observed that tofacitinib did not directly affect IL-6 and IL-8 production by RASFs, CD14$^+$ monocytes, whereas IL-17 and IFN-γ production by CD4$^+$ T cells *in vitro* was markedly decreased. However, when RASFs and CD14$^+$ monocytes were cultured with supernatant from tofacitinib treated CD4$^+$ T cells, the production of IL-6 by RASFs and IL-8 by CD14$^+$ monocytes was significantly reduced in a concentration-dependent manner of tofacitinib. This suggested that tofacitinib acted on RASFs and CD14$^+$ monocytes in an indirect manner with CD4$^+$ T cells. Lack of direct effects of tofacitinib on RASFs is assumed to depend on the followings: i) Because RASFs express JAK1 and JAK2 but not JAK3, we expect off-target effects; ii) Because LPS and IL-1β do not activate the JAK/STAT signaling pathway. In order to clarify the mode and mechanism of action of tofacitinib *in vivo*, we utilized SCID-HuRAg mice a *ex vivo* human arthritis model mouse. Synovium and cartilage from RA were transplanted into the back of SCID mice and tofacitinib was administered continuously via osmotic minipumps. This experiment was the first to show that tofacitinib inhibited both human IL-6 and IL-8 derived from RA synovium implanted in SCID mice. Moreover, histological evaluation of the implanted specimen revealed that number of IL-6, IL-8 and IL-17 positive cells were significantly reduced, with decreased cartilage destruction in mice treated with tofacitinib (Fig.1). These results suggested that tofacitinib-induced specific inhibition of IL-17 and IFN-γ production by CD4$^+$ T cells (presumably Th1 and Th17 cells) resulting in the suppression of IL-6 and IL-8 production by RASFs and CD14$^+$ monocytes, with decreased cartilage destruction.

The mechanism of tofacitinib-induced inhibition of IL-17 and IFN-γ production by CD4$^+$ T cells remains unknown. We and others observed that the production of IL-2 by CD4$^+$ T cells stimulated with anti-CD3/anti-CD28 antibodies was apparently increased when tofacitinib was added to the culture[7, 8]. This suggests that tofacitinib might inhibit the consumption of IL-2, which is produced by CD4$^+$ T cells stimulated with anti-CD3/anti-CD28 antibodies. Alternatively, IL-2 production by CD4$^+$ T cells could be enhanced by the inhibition of IL-2—mediated negative feedback activated through JAK3-STAT5[9]. Meanwhile, proliferation induced by IL-2 could also be suppressed by tofacitinib leading to decreased IL-17 and IFN-γ production. Furthermore, immunohistochemical analysis demonstrated that IL-6, IL-8 and IL-17 were highly expressed in the RA synovium grafts in SCIDHuRAg mice treated with vehicle,

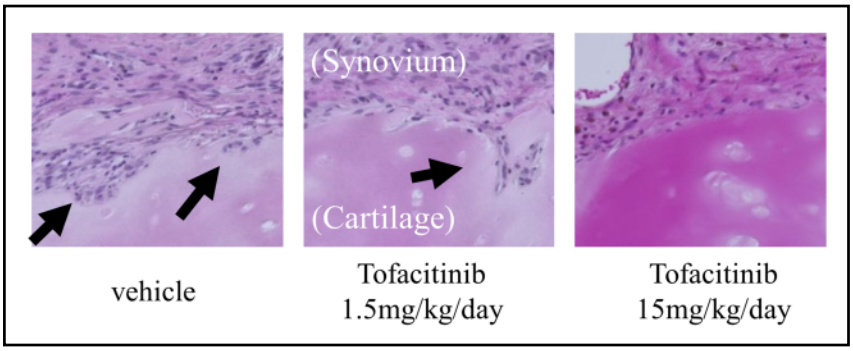

Fig.1 Tofacitinib suppressed RA cartilage destruction in SCID-HuRAg mice
Rheumatoid arthritis synovium with articular cartilage were co-implanted onto the back of SCID mice. Treatment with vehicle or tofacitinib (1.5 mg and 15 mg/kg/day) was initiated and the co-implants were taken out on day 35 and stained for histological evaluation. Arrows show the invasive front of synovial tissue.

however treatment with tofacitinib significantly reduced the number of IL-6, IL-8 or IL-17 — positive cells[7]. Taken together, our observations suggest that tofacitinib could inhibit the IL-2 — mediated JAK/STAT signaling pathway, which might lead to the reduction of production of IL-17 and IFN-γ by $CD4^+$ T cells.

Effect of tofacitinib on human dendritic cells

As described previously, tofacitinib selectively suppresses the production of cytokines and the proliferation of lymphocyte. These functions can be predicted to some extent by the important role of JAK family members in the differentiation and proliferation of lymphocytes. However, considering the dramatic effect of tofacitinib in RA, the direct suppressive effect of tofacitinib on T cells alone does not thoroughly explain the mechanism. Dendritic cells (DCs) play a key role in bridging natural immunity and acquired immunity. An increase in the number of invasive DCs has been observed in the synovitis tissues of rheumatoid arthritis (RA). In addition, an increase in the number of DCs that express high levels of TLR4 ligands in synovial fluid has been reported[10, 11], suggesting the activation of DCs and the disruption of immunotolerance. We have reported that DCs also express JAK1, JAK2, and JAK3; however, DCs that were derived from a JAK3-deficient mouse have been shown to overproduce IL-10 and exhibit anti-inflammatory activity[12].

We next investigated the effects of the inhibition of JAK1 and JAK3 by tofacitinib in human monocyte-derived DCs (MoDCs) on maturation and T-cell stimulatory function. In this report, we have shown that tofacitinib promoted a tolerogenic phenotype in human DCs[13]. We observed that lipopolysaccharide (LPS) induced clustering and expression of co-stimulatory molecules on human monocyte-derived DCs *in vitro*. However, the addition of tofacitinib to MoDC inhibited clustering of cells and reduced expression of CD80 and CD86 but not HLA-DR in a dose-dependent manner. The production of TNFα, IL-1β, and IL-6 was induced by the stimulation with LPS, while the production of these cytokines was suppressed by tofacitinib in a concentration-dependent manner. Although overproduction of IL-10 was observed in JAK3 deficient mice[12], tofacitinib did not affect TGF-β and IL-10 production from human monocyte-derived dendritic cells. The precise mechanism to explain the discrepancy remains unclear, it may be caused by the difference in species or selectivity of JAK1 inhibition by tofacitinib. On the other hand, Lck/Fyn inhibitor (PP1), p56lck/p59fynT/Hck inhibitor (PP2) and JAK2 inhibitor (G6) did not demonstrate these effects. Expression of CD80/CD86 mRNA after LPS stimulation in MoDCs was also suppressed by cyclohexamide treatment. Activation of TLR4 signaling by LPS does not involve JAK-STAT pathway; therefore, an indirect mechanism was considered in which the suppression of CD80/CD86 expression by tofacitinib occurred through a proteinogenic mechanism. The expression of CD80/CD86 was induced by type-I IFN stimulation alone and was completely inhibited by tofacitinib. Correspondingly, phosphorylation of STAT1 and STAT2 induced by type-I IFN was complete inhibited by tofacitinib, and the expression of CD80/CD86 induced by LPS was suppressed by an anti-type-I IFN receptor antibody. These results suggested that the inhibition of JAK1/3 in MoDCs partially suppressed the expression of CD80/CD86 through the inhibition of type-I IFN signaling (Fig.2). Furthermore, among the transcription factors required for CD80/CD86 expression, IRF7 was the only one suppressed by tofacitinib in LPS-stimulated DCs and the production of type-I IFN was also reduced by tofacitinib. Finally, co-culture of tofacitinib-treated DCs with naïve $CD4^+$ T cells re-

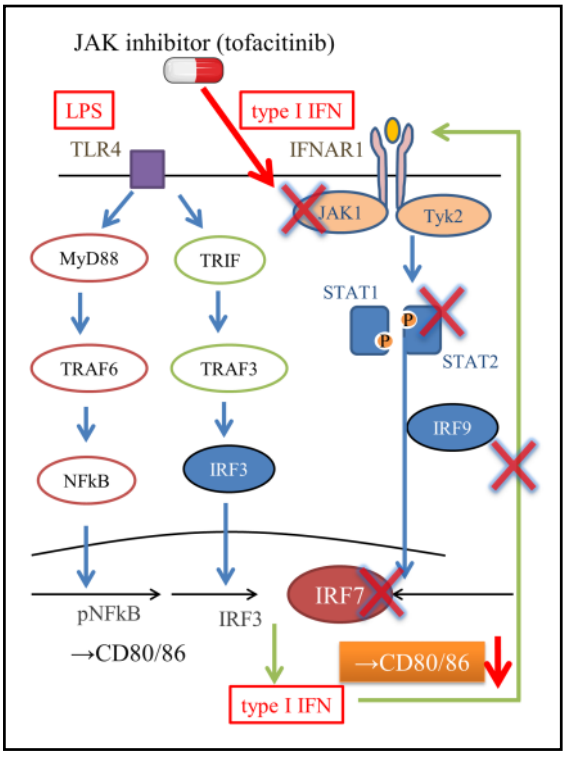

Fig.2 Tofacitinib suppressed a production and stimulation loop of type-I IFN

Tofacitinib suppressed the phosphorylation of STAT1/STAT2 induced by autocrine stimulation with type-I IFN, continuously suppressing IRF7 expression and the production of type-I IFN, which decreased CD80/86 expression in MoDCs.

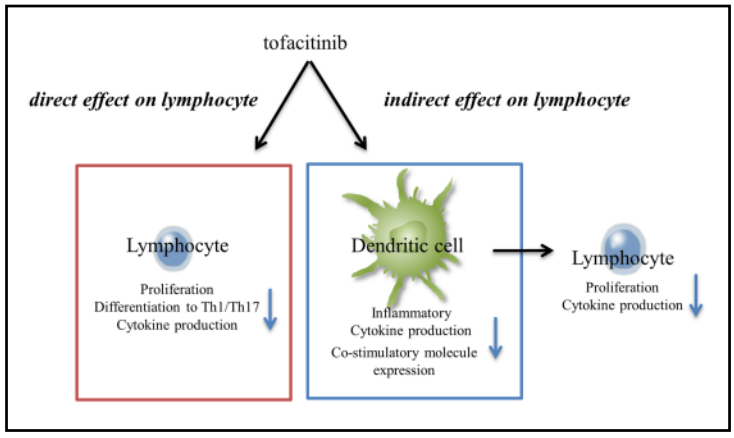

Fig.3 Direct and indirect effect of tofacitinib in human lymphocyte

Tofacitinib directly affected lymphocyte and decreased proliferation and cytokine production. Treatment of dendritic cells with tofacitinib decreased inflammatory cytokine production and co-stimulatory molecule expression resulting in reduced allo-T cell stimulation.

duced T-cell proliferation and IFN-γ production. Additionally, tofacitinib markedly increased the expression levels of indoleamine 2, 3-dioxygenase in DCs. We concluded that tofacitinib suppressed production and stimulation loop of a type-I IFN through JAK1/JAK3, decreased CD80/CD86 expression, and suppressed T-cell stimulatory capabilities that were accompanied by the induction of IDO expression. Thus, tofacitinib not only suppressed cytokine production but also suppressed the expression of costimulatory molecules by inhibiting the positive loop of type-I IFN in DCs, which leads to immunomodulatory effects.

Conclusions

Tofacitinib, which is selective for JAK1 and JAK3, is highly effective for patients with rheumatoid arthritis. This finding supports the notion that JAK1 and JAK3 plays an important role in autoimmune diseases. Furthermore, it is thought that the elucidation of the mode of action of JAK family members *in vivo* will lead to a better understanding and treatment of autoimmune diseases. Here, we have focused on the two our studies about tofacitinib. Tofacitinib acts on multilineage cells resulting in direct and indirect effect on lymphocytes (Fig.3). On the basis of these results, a novel therapeutic strategy for autoimmune diseases by tofacitinib should be considered.

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Conflicts of Interest

Y Tanaka received consulting fees, lecture fees, and/or honoraria from Mitsubishi-Tanabe Pharma, Eisai, Chugai Pharma, Abbott Japan, Astellas Pharma, Daiichi-Sankyo, Abbvie, Janssen Pharma, Pfizer, Takeda Pharma, Astra-Zeneca, Eli Lilly Japan, Glaxo SmithKline, Quintiles, MSD, Asahi-Kasei Pharma, and received research grants from Bristol-Myers, Mitsubishi-Tanabe Pharma, Abbvie, MSD, Chugai Pharma, Astellas Pharma, Daiichi-Sankyo. K Yamaoka received consulting fees from Pfizer.

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