Role of JAKs in myeloid cells and autoimmune diseases

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In the treatment of autoimmune diseases, therapy targeting inflammatory cytokines such as tumor necrosis factor and interleukin-6 has shown dramatic effect. In addition to these biological agents, several small molecules are currently in clinical development for the treatment of autoimmune diseases. Due to the important role of Janus kinase (JAK) in multiple cytokine signaling pathways, JAKs are essential not only for the development of the immune system but also in inflammatory responses and in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA). Tofacitinib, a novel selective JAK inhibitor, has shown high efficacy in clinical trials for RA and is known to be selective against JAK1 and JAK3. In addition to its therapeutic potential, tofacitinib is a favorable reagent to study the roles of JAKs in vivo. Dendritic cells are antigen-presenting cells that regulate T-cell responses and have been implicated in RA pathogenesis. Meanwhile, macrophages are resident phagocytic cells in lymphoid and non-lymphoid tissues and are believed to be involved in steady-state tissue homeostasis via the clearance of apoptotic cells and the production of growth factors. Macrophages also play an important role in synovial inflammation and tissue destruction in RA. Although the biological roles of JAKs in lymphocytes are well known, its function in monocyte-lineage cells remains elusive. This review describes the role of JAKs in myeloid cells and autoimmune disease.

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Introduction

In the treatment of autoimmune diseases, therapy targeting inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-6 has shown dramatic effect, leading to a paradigm shift of treatment strategy. However, parenteral administration and cost might limit their availability to vast numbers of patients. In addition to these biological agents, several small molecules are currently
in clinical development for the treatment of autoimmune diseases. Due to the important role of Janus kinase (JAK) in multiple cytokine signaling pathways, JAKs are essential not only for the development of the immune system but also in inflammatory responses and in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA). Indeed, a number of kinase inhibitors targeting JAKs have been developed, although most of the previous compounds were low in potency and selectivity. Among the compounds currently under development, tofacitinib, a novel selective JAK inhibitor, has shown high efficacy in clinical trials for RA and is known to be selective against JAK1 and JAK3. In addition to its therapeutic potential, tofacitinib is a favorable reagent to study the roles of JAKs in vivo. Although previous studies confirmed the specific action of tofacitinib on CD4+ T cells, other immune cells expressing JAKs, such as monocyte-lineage cells could be targeted. Here, we describe the role of JAKs on antigen-presenting cells (APCs) in autoimmune disease.

**Immunologic function of JAK**

JAK is a tyrosine kinase that plays crucial roles in cytokine receptor binding-triggered signal transduction activating the transcription factor signal transducers and activator of transcription (STAT). The JAK family consists of four members: JAK1, JAK2, JAK3, and Tyk2. More than 40 different cytokines and growth factors have been shown to activate specific combinations of JAKs and STATs. Genetic knockout studies have shown that JAKs and STATs have highly specific functions in the control of various immune responses (Table 1). Since JAKs selectively associate with different cytokine receptors, they are highly associated with functions that can be attributed to these various cytokines. JAK1- and JAK2- cells are unresponsive to three distinct families of cytokine receptors; all class II cytokine receptors (interferon and IL-10 related cytokines), common gamma chain (γc)-cytokines, as well as IL-6 and other gp130-cytokines. Jak2−/− cells fail to respond to erythropoietin, thrombopoietin, IFN-γ, IL-3, or granulocyte/macrophage colony-stimulating factor (GM-CSF) and deficiency of JAK1 or JAK2 results in a lethal phenotype. On the other hand, JAK3 associates with only one cytokine receptor the γc, a shared receptor subunit that pairs with other ligand-specific subunits to form the receptors for 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, JAKs are essential not only for development of the immune system but also in inflammatory immune response and in pathogenesis of autoimmune diseases such as RA. As a matter of fact, JAKs and STATs have been reported to have expression in the synovium of RA patients. At the present day, there are multiple JAK inhibitors in clinical trials at different phases with promising outcomes, proving its important role in RA.

**Antigen-presenting cells in autoimmune disease**

Three major types of APCs are known; dendritic cells (DCs), macrophages and B cells. APCs detect pathogens via pattern recognition receptors that interact with different types of pathogen-derived molecules or pathogen-associated molecular patterns and play a key role in the immune response, linking innate and adaptive responses. DCs are APCs that regulate T-cell responses and have been implicated in RA pathogenesis. Myeloid DCs with high

### Table 1. In vivo immunological functions of JAK-STAT signaling components

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype of null mice</th>
</tr>
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<tbody>
<tr>
<td><strong>Jak</strong></td>
<td></td>
</tr>
<tr>
<td>Jak1</td>
<td>Perinatal lethality, defects in lymphoid development</td>
</tr>
<tr>
<td>Jak2</td>
<td>Embryonic lethality, failure of erythropoiesis</td>
</tr>
<tr>
<td>Jak3</td>
<td>SCID caused by cytokine-signaling defects from γc containing receptors</td>
</tr>
<tr>
<td>Tyk2</td>
<td>Hypersensitivity to pathogens due to interferon- and IL-12-signaling defects</td>
</tr>
<tr>
<td><strong>Stat</strong></td>
<td></td>
</tr>
<tr>
<td>Stat1</td>
<td>Impaired interferon signaling, susceptibility to viral infections</td>
</tr>
<tr>
<td>Stat2</td>
<td>Impaired interferon signaling</td>
</tr>
<tr>
<td>Stat3</td>
<td>Embryonic lethality, impaired responses to pathogens, cell-survival defects</td>
</tr>
<tr>
<td>Stat4</td>
<td>Defects in Th1 cell differentiation, impaired IL-12 pathway</td>
</tr>
<tr>
<td>Stat5a</td>
<td>Defects in mammary-gland development, impaired prolactin signaling</td>
</tr>
<tr>
<td>Stat5b</td>
<td>Growth-hormone pathway defects, defective NK-cell-mediated proliferation and cytolytic activity</td>
</tr>
<tr>
<td>Stat5a/b</td>
<td>No NK cells, impaired IL-2-induced T-cell proliferation</td>
</tr>
<tr>
<td>Stat6</td>
<td>Defects in Th2 cell differentiation, impaired IL-4/IL-13 pathway</td>
</tr>
</tbody>
</table>
T-cell stimulatory capacity are enriched in the synovial tissue of RA patients\(^1\). These DCs are considered to present arthritogenic antigens to T cells and perpetuate the inflammatory autoimmune responses. Meanwhile, DCs are important for the balance between immunity and tolerance. Immature DCs induce T-cell tolerance whereas mature DCs activate T cells and induce immunity. Accordingly, enhancement of DC survival can break immune tolerance, resulting in autoimmune disease\(^2\) whereas the ablation of DCs abrogates T-cell priming to antigens and inhibits autoimmunity in mice\(^3\).

Macrophages are resident phagocytic cells in lymphoid and non-lymphoid tissues and are believed to be involved in steady-state tissue homeostasis via the clearance of apoptotic cells and the production of growth factors. Macrophages are equipped with a broad range of pathogen recognition receptors that make them efficient at phagocytosis and induce production of inflammatory cytokines including TNF-\(\alpha\), IL-1, IL-6 and IL-12, and chemokine such as IL-8 and CXCL10. Macrophages also play an important role in synovial inflammation and tissue destruction in RA\(^4\).

**Regulation of APC by JAK inhibitors**

Tofacitinib, a novel selective JAK inhibitor has shown high efficacy in clinical trials for RA and is known to be selective against JAK1 and JAK3. The safety and effectiveness of tofacitinib have been established in multiple clinical studies\(^5,6\). Phase III studies demonstrated that, in patients with RA receiving background methotrexate, tofacitinib was significantly superior to placebo and was numerically similar to adalimumab (TNF inhibitor) in efficacy\(^6\). Although the biological roles of JAKs in lymphocytes are well known, knowledge of its function in APCs is limited.

We have recently reported that tofacitinib inhibited both human IL-6 and IL-8 derived from implanted synovium tissue from RA patients in SCID (SCID-HuRAg) mice\(^7\). However, we observed that tofacitinib did not directly affect IL-6 and IL-8 production from synovial fibroblasts (RASF) and CD14\(^+\) monocytes. Instead, tofacitinib directly affected CD4\(^+\) T cells and reduced IL-17 and IFN-\(\gamma\) production in vitro. When RASFs and CD14\(^+\) monocytes were cultured with supernatant from tofacitinib treated CD4\(^+\) T cells, production of IL-6 from RASFs and IL-8 from monocytes were reduced in a tofacitinib concentration-dependent manner, suggesting an indirect mechanism. Accordingly, IL-6 and IL-8 positive cells were significantly reduced with decreased cartilage destruction of the implanted specimen in SCID-HuRAg mice treated with tofacitinib. Therefore, our results suggest that inhibition of IL-17 and IFN-\(\gamma\)-production from CD4\(^+\) T cells (presumably Th1 and Th17 cells) by tofacitinib leads to suppression of IL-6 and IL-8 production and decreases cartilage-destruction in SCID-HuRAg mice.

Recent reports have shown additional effects of tofacitinib on monocyte lineage cells. Pattison M.J. et al. showed that the inhibition of JAKs using tofacitinib results in pro-inflammatory effects in isolated LPS-stimulated macrophages due to the inhibition of IL-10 signaling, resulting in an increase in proinflammatory cytokine production\(^8\). However, tofacitinib was also found to inhibit IFN-\(\gamma\) signaling in macrophages more potently than IL-10 signaling. This is presumably why its final biological effect results in anti-inflammation. Although IFN-\(\gamma\) and IL-10 both signal via JAK1 and Tyk2, the underlying mechanism potently inhibiting IFN-\(\gamma\) rather than IL-10 is unknown.

Yarlina A. et al. also demonstrated that tofacitinib effectively suppressed activation of blood-derived and RA synovial macrophages, including a subset of inflammatory re-
sponses induced by the pathogenic cytokine TNF\(^{17}\). Stimulation with TNF induced autocrine IFN-mediated STAT1 activation that promoted inflammatory chemokine production. This was efficiently inhibited by tofacitinib. In addition, tofacitinib suppressed the late phases NF-\(\kappa\)B activation and of inflammatory cytokine production, while augmenting TNF-mediated induction of c-Jun and NFATc1. Correspondingly, tofacitinib effectively suppressed K/BxN serum transfer arthritis, which is an arthritis model entirely dependent on innate immune cells\(^{17}\).

Among the monocyte lineage cells, the role of JAKs in DCs is still unknown. Previously, we have reported that DCs from JAK3\(^{-}\) mice produce increased IL-10, but not IL-6 or TNF-\(\alpha\), compared to wild-type DCs, in response to Toll-like receptor ligands\(^{18}\). In order to evaluate the direct effect of tofacitinib on human DCs, monocyte derived DCs were matured with lipopolysaccharide in the presence of tofacitinib. Treatment with tofacitinib decreased inflammatory cytokine production and co-stimulatory molecule expression resulting in reduced alloergenic T cell stimulation (submitted). These results suggest that tofacitinib directly affects not only the adaptive immunity, but also innate immunity, which possibly results in greater immunosuppression than expected (Fig.1).

Conclusions
We have focused on the role of APCs in autoimmune disease and tofacitinib is likely to regulate the disease activity of RA by acting on DCs. Affecting the innate immunity is an important strategy in aiming for cure in autoimmune disease, although, at the same time, it can result in over suppression of the immune system. Improved knowledge of the underlying mechanisms of tofacitinib would contribute to better understanding of the pathogenesis of RA and also to further application of the drug to other diseases.

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Conflicts of interest

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Mini Review  Role of JAKs in APC  
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