Abstract

The Janus kinase/Signal Transducer and Activator of Transcription (Jak/STAT) signaling pathway mediates the signal transduction of multiple pro-inflammatory cytokines, downstream of Type I and Type II cytokine receptors. Jak inhibitors suppress cytokine-mediated inflammatory responses and are used to treat inflammatory diseases such as rheumatoid arthritis (RA), psoriasis (PsO) and inflammatory bowel disease (IBD). Although Jak inhibitors block inflammatory cytokine singling cascades, patients with Jak inhibitor treatments show relatively low risk for infectious complications. The underlying molecular mechanisms for this unexpected clinical observation are poorly understood. A better knowledge about the regulation of immune responses, especially during Jak inhibitor treatments are crucial for a better understanding and optimizing therapeutic approaches.

Macrophages play important roles in immune defense against invading pathogens. The pathogen-recognizing Toll-like receptors (TLRs) not only induce the expression of pro-inflammatory cytokines, but also trigger the expression of the anti-inflammatory interleukin (IL)-10 in immune cells, which is known to play a crucial role in host evasion. The fact that IL-10/IL-10 receptor (IL-10R) signaling mediates signal transduction via Jak1 and tyrosine kinase 2 (Tyk2), prompted us to investigate the effect of the Jak inhibitor Tofacitinib (Tofa) on IL-10/IL-10R signaling in human, TLR4-activated, monocyte-derived macrophages (MDMs). Furthermore, we explored in more detail the role of IL-10/IL-10R signaling in two important macrophage host defense mechanisms. On the one hand, the vitamin D-dependent induction of the antimicrobial peptide cathelicidin (CAMP). On the other hand, the hepcidin (HAMP)-regulatory iron sequestration, which albeit helping to fight extracellular microbes, has beneficial consequences for intracellular pathogens. At last, we investigated the effect of Tofa on these two host defense pathways, as well as antigen-presentation makers in TLR4-activated human macrophages. As a model, we used MDMs stimulated with *Neisseria gonorrhoeae* lysate (GC) and the membrane-containing lipopolysaccharide (LPS), which both bind and activate TLR4.

First, we confirmed that TLR4 activation results in IL-10 induction and subsequent autocrine phosphorylation of STAT3 (pSTAT3) by ELISA analyses of IL-10 secretion as well as by FACS analyses of pSTAT3. Moreover, we found that Tofa efficiently inhibits IL-10-mediated pSTAT3 in TLR4-activated human MDMs. Specifically, the blockage of the IL-10R by the monoclonal antibody (mAb) as well as the usage of Tofa inhibited pSTAT3 in GC-activated MDMs. Second, we found that TLR4-induced IL-10/IL-10R signaling modulates human macrophage responses. In this regard, TLR4 activation results in vitamin D-induced CAMP repression and the blockage of IL-10R by the use of mAb, enhanced the expression of CAMP in TLR4-activated human MDMs, measured by qPCR and FACS analyses. Moreover, the blockage of IL-10R promoted the TLR4-mediated expression of the antigen-presenting molecules cluster of differentiation

(CD) 86 and HLA-DR. Furthermore, the mAb against IL-10R reduced the TLR4-induced HAMP expression. Third, we found that Tofa has diverging effects on these host defense pathways. The treatment with Tofa abolished CAMP expression as well as the expression of CAMP-associated host defense gene in TLR4-activated human MDMs, but restored CD86 and HLA-DR expression. Moreover, Tofa prevented the TLR4-mediated intracellular HAMP upregulation in human MDMs.

Altogether, our project supports the concept of a complex immune evasion strategy of intracellular pathogens by inducing IL-10/IL-10R signaling to modulate human macrophage response. Moreover, we demonstrated diverging effects by Tofa on macrophage functions that provide insights of the effects of Jak inhibitors. Such insights are needed for the molecular explanations of the clinical safety profile in Jak inhibitor therapies with respect to infectious complications. In this instance, we offer one possible explanation for the low risk of infectious adverse events in Jak inhibitor treatments. Concerning the *anti-microbial, pro-host* effect of Tofa on HAMP expression, we propose the TLR4-IL-10-STAT3-HAMP axis as a potentially therapeutic target to interrupt pathogen-induced immune evasion.