Small Molecule Inhibitors Targeting the Th17 Cell Transcription Factor RORγt for the Treatment of Autoimmune Diseases

Jianfei Yang*, Jill Skepner, Mark Trocha and Shomir Ghosh
Tempero Pharmaceuticals, a GSK company, Cambridge, MA, USA

Naïve CD4+ T helper cells can differentiate into Th1, Th2, Th17, T regulatory, Th9 and Th22 cells. Th1 cells are characterized by their production of IFNγ but not IL-4 and IL-5 while Th2 cells are characterized by their expression of IL-4 and IL-5 but not IFNγ. T-bet and GATA3 are the master transcription factors for Th1 and Th2 cells, respectively [1]. Th1 cells mediate cellular immunity against intracellular pathogens while Th2 cells induce humoral immunity to parasitic helminthes. Th1/Th2 cells are believed to play an important role in several types of autoimmune diseases. Th9 cells produce IL-9 that has been associated with a variety of inflammatory diseases. Th22 cells, which express IL-22 only, may mediate both protective immunity and inflammation. T regulatory cells, which are characterized by CD25 and the master transcription factor Foxp3, play critical role in regulation of overactive immune systems. Th17 cells, which are characterized by the expression of IL-17A, IL-17F, IL-22, IL-23R and CCR6, have been shown to play a critical role in a variety of autoimmune diseases including rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease (IBD), and asthma as well as in tumor immunity [2,3].

Th17 cells express two IL-17 family members, namely IL-17A and IL-17F. Both cytokines signal through the same receptors, IL-17RA and IL-17RC. IL-17A can be detected in sera of asthmatic patients and synovial fluids of arthritic patients. Suppression of allergic cellular and humoral responses was observed in IL-17A-deficient mice [4]. In addition, IL-17A-deficient mice are protected from collagen-induced arthritis (CIA) [5] and treatment with neutralizing anti-mouse IL-17A antibody after the onset of CIA significantly reduced the severity of CIA [6]. Fully human monoclonal antibodies targeting IL-17A and the IL-17A/IL-17R have shown clinical efficacy in psoriasis, rheumatoid arthritis, and uveitis [7-9].

Naïve CD4+ T cells, upon TCR signaling and cytokine costimulation (IL-12, IL-6, TGFB), can differentiate into Th17 cells in vitro. In vivo, IL-23 has been shown to play a critical role in the generation of Th17 cells. Using IL-23ra-deficient mice, Cua et al. demonstrated that the terminal differentiation of Th17 cells in vivo requires IL-23 signaling [10]. Furthermore, IL-23 is required for the generation of pathogenic Th17 cells. Both IL-23 and IL-6/TGFβ can independently induce the generation of IL-17A-producing Th17 cells ex vivo while only IL-23-induced Th17 cells can adoptively transfer disease in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis [11,12]. In humans, genome-wide association studies in autoimmune patient populations have demonstrated that IL23r polymorphisms are associated with several autoimmune diseases including IBD, ankylosing spondylitis, and psoriasis [13-16]. In addition, the IL-23R coding SNP Arg381Gln was found to be protective against psoriatic arthritis [16]. Further, monoclonal antibodies against IL-12/IL-23p40 or IL-23p19 have shown spectacular efficacy in psoriasis patients [17].

While targeting single cytokines such as IL-17A or IL-23 using neutralizing monoclonal antibodies has shown good efficacy in psoriasis, they appear to be less efficacious in the treatment of arthritis. Since pathogenic Th17 cells express not only IL-17A but also other proinflammatory cytokines such as IL-17F and IL-22, targeting the Th17 lineage could result in better efficacy for the treatment of autoimmune diseases including arthritis and IBD. The retinoic acid receptor-related orphan nuclear receptor RORγt has been identified as the master transcription factor for the generation of Th17 cells and the induction of IL-17A. RORγt-deficiency abolishes Th17 cell generation while forced expression of RORγt induces the differentiation of Th17 cells [18]. Thus, targeting RORγt with small molecule inhibitors may be an attractive approach to blocking the pathogenic effects of the Th17 lineage. Several other transcriptional factors such as STAT3, BATF and IRF4 have also been found to be important for the generation of Th17 cells. These transcription factors function through complex protein-protein interactions which are challenging to target with small molecule inhibitors.

The nuclear receptor RORγt contains a ligand binding domain (LBD) and a DNA binding domain (DBD). When an agonist or inverse agonist binds to the RORγt LBD, it induces a conformational change in the RORγt DBD, resulting in a change of promoter activity. A single A325F mutation in the putative ligand-binding pocket abolishes RORγt activity [19]. One method for screening small molecule libraries to identify RORγt inhibitors would be to use a RORγt-LBD/GAL4-DBD fusion protein which can activate a luciferase reporter gene by binding to upstream GAL4 binding sites. Indeed, Littman and colleagues have discovered a small molecule inhibitor of RORγt, digoxin, after screening several thousand small molecule compounds using this method [19]. Several other groups have further discovered a variety of small molecule inhibitors of RORγt such as SR221, SR1001 and Ursolic acid [19-21]. These compounds inhibit Th17 cell differentiation in vitro and suppress IL-17A production by memory CD4+ T cells, and are believed to be important for the maintenance of Th17 cell functions. More importantly these small molecule inhibitors reduce the severity of EAE [19-22].

As part of our research program at Tempero, we have discovered a novel series of RORγt inverse agonists. These compounds block in vitro Th17 cell differentiation. Furthermore, we demonstrated that these RORγt inverse agonists block in vivo Th17 cell differentiation. Naïve mouse draining lymph node cells do not express IL-17A upon stimulation with MOG peptide. However, draining lymph node cells from mice immunized with MOG35-55, in Complete Freund’s Adjuvant (CFA) do produce IL-17A after ex vivo restimulation with MOG35-55.

*Corresponding author: Dr. Jianfei Yang, Tempero Pharmaceuticals, a GSK company, 200 Technology Square, Suite 602, Cambridge, MA, USA, Tel: 617-374-8830; Fax: 617-374-8839; E-mail: JianfeiYang2012@Gmail.com

Received May 22, 2013; Accepted May 23, 2013; Published May 29, 2013


Copyright: © 2013 Yang J, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
indicating the cells have differentiated into Th17 cells in vivo. In our compound studies, mice were immunized with MOG_35-55 in CFA and dosed with Tempebro RORγt compound B or vehicle for 6 days. The draining lymph node cells were then isolated and restimulated with MOG_35-55. The expression of pathogenic Th17 signature genes such as IL-17a, IL-17f, and IL-22 were significantly reduced in the cells from mice treated with Tempebro RORγt compounds.

We and several other groups have demonstrated that RORγt small molecule inhibitors block Th17 cell differentiation in vitro and in vivo. The inhibitors also block IL-17A expression by RORγt-positive Th17 cells. Thus, RORγt small molecule inhibitors may block the pathogenic effects of the Th17 lineage and may find potential use in treating a variety of autoimmune diseases. A summary of RORγt as a therapeutic target for the Th17/IL-17 pathway is illustrated in Figure 1.

Figure 1: RORγt as a therapeutic target for the Th17/IL-17 pathway.

References