TYK2 promotes IL-23 induced type 3 immunity and disease progression in SpA
Eric Gracey1, Dominika Gogova2, Melissa Lim1, Zoya Qaiyum1, Yuriy Baglaenko1, Craig Masse3, William Westlin4, Birgit Strobl2, Mathias Muller2, Wenyen Miao3 & Robert Inman3

1Department of Immunology, University of Toronto & Krembil Research Institute, Toronto, Canada
2University of Veterinary Medicine, Vienna, Austria
3Nimbus Therapeutics, Cambridge, Massachusetts, USA

Introduction

Th17 cells play an important role in the pathogenesis of ankylosing spondylitis (SpA), a debilitating arthritis of the axial skeleton.1 TYK2, a member of the Janus Kinase (JAK) family of signaling molecules, associates with a number of receptors, including the type 1 interferon, IL-10/IL-22, and IL-12/IL-23 families of cytokine receptors. TYK2 plays a crucial role in Th17 cell function through mediating IL-23 intracellular signaling, making TYK2 an attractive target for the treatment of AS. TYK2 was the first JAK to be associated with AS in genome-wide association studies2 and is associated with psoriatic arthritis (PsA). The majority of AS/PsA-associated SNPs impart non-synonymous mutations, suggesting these variants impact a change in TYK2 function and not expression. Recent work suggests altered function of TYK2 is the most likely cause of TYK2's association across multiple autoimmune diseases.3

Nimbus Therapeutics have developed a selective and potent catalytic inhibitor of TYK2, NDI-031407. Here we examine the role of TYK2 in murine models of local and systemic IL-23 inflammation, type 3 immune cells in vitro and correlate immune cell phenotype and AS disease progression with TYK2 risk SNPs.

TYK2 plays a pro-inflammatory role in IL-23 induced inflammation in vivo. The IL-23 minicircle was used to induce systemic IL-23 overexpression in male B10.BR mice. A) Schematic of IL-23 minicircle experiment. Briefly, 6µg minicircle administered by hydrodynamic delivery and NDI-031407 treatment begun one week post minicircle treatment. B) Composite SpA score (blepharitis, enthesitis, iritis) with NDI-031407 treatment begun one week post minicircle treatment. B) Cohort 1 examined for TYK2 gene expression 5 days with anti-CD3/CD28 stimulation with the indicated cytokines. TYK2 inhibition using mice with kinase-dead TYK2 (TYK2KD). F) Representative flow cytometry gating showing dermal γδ T cell identification and cytokine production. G) Dermal γδ T cell IL-17/IL-22 with NDI-031407 treatment or in TYK2KD mice. Data assessed by Mann-Whitney test, one-way ANOVA or Wilcoxon test where appropriate.

TYK2 does not play a universal role in IL-23 activation of murine γδ T cells in vitro. γδ T cells identified by flow cytometry in whole lymph node extract. A) Representational γδ T cell pSTAT3 staining in WT or TYK2KD mice with the indicated stimuli. Lymphocytes treated under the indicated conditions from B) WT or TYK2KD γδ T cells or C) NDI-031407 treated WT T cells. To examine down-stream effectors of IL-23 stimulation, whole lymph node extracts stimulated for 4.5 hours with 20ng IL-18 and 20ng IL-23. D) Representative gating strategy of IL-23 induced cytokines in γδ T cells. IL-17A/IL-22 production in E) WT cells pretreated with NDI-031407 or F) WT, TYK2 kinase dead (923E) or knock-out (KO) cells. G) WT and TYK2KD cells pretreated with the JAK inhibitor, ruxolitinib, prior to stimulation with IL-12/IL-23. All graphs from single experiment representative of 2 independent repeats per experiment.

AS-associated TYK2 LoF SNP correlates with AS progression

AS-Associated TYK2 SNPs do not alter TYK2 expression, but associate with altered Th1 frequency and AS disease progression. A) Clinical features of two AS patient cohorts from the Toronto Western Hospital SpA clinic. All patients fulfilled mNY criteria for AS and the imaging arm for axSpA. B) Cohort 1 examined for TYK2 expression across all autoimmune diseases. TYK2 SNPs associated with AS (and PsA), likely exert their effect through altering TYK2 function. It has been reported that TYK2 loss of function (LoF) SNPs are the primary variants associated with autoimmune disease. Consistent with this, we find the LoF SNP rs1272056 is associated with reduced frequency of IL-12 dependent cells (Th1 and NK) and to associate with protection against arthritic progression. Loss of TYK2 in humans1 and mice4 has no effect on Th17 frequencies, consistent with the lack of effect of rs1272056 on Th17 frequency. We show that TYK2 inhibition in SpA models has a protective effect associated with inhibition of type 3 immunity. The protective effect may be mediated through blocking IL-22 via TYK2. Anti-IL-23 is beneficial for PsA and the SpA-linked diseases, psoriasis and IBD, but not AS. While this work does not explain why blocking IL-23 may be ineffective in AS, it does shed light on the dissociation between IL-23 and IL-17A. As has previously been reported, we have demonstrated that IL-23 alone cannot drive an IL-17A response. Co-stimulation, especially with IL-18, is essential for IL-23 to induce effector cytokines. We show for the first time that IL-17A and IL-22 are regulated by distinct IL-23 intracellular signaling pathways, involving JAK2 independent of STAT3 and JAK1/JAK2/TYK2/TSTAT3 respectively.

These data provide strong pharmacological support that TYK2 inhibition is a valid therapeutic target for SpA through inhibition of type 3 immunity.

Conclusion

Our results in AS patients support the notion that TYK2 SNPs associated with AS (and PsA), likely exert their effect through altering TYK2 function. It has been reported that TYK2 loss of function (LoF) SNPs are the primary variants associated with autoimmune disease. Consistent with this, we find the LoF SNP rs1272056 to be associated with reduced frequency of IL-12 dependent cells (Th1 and NK) and to associate with protection against arthritic progression. Loss of TYK2 in humans1 and mice4 has no effect on Th17 frequencies, consistent with the lack of effect of rs1272056 on Th17 frequency. We show that TYK2 inhibition in SpA models has a protective effect associated with inhibition of type 3 immunity. The protective effect may be mediated through blocking IL-22 via TYK2. Anti-IL-23 is beneficial for PsA and the SpA-linked diseases, psoriasis and IBD, but not AS. While this work does not explain why blocking IL-23 may be ineffective in AS, it does shed light on the dissociation between IL-23 and IL-17A. As has previously been reported, we have demonstrated that IL-23 alone cannot drive an IL-17A response. Co-stimulation, especially with IL-18, is essential for IL-23 to induce effector cytokines. We show for the first time that IL-17A and IL-22 are regulated by distinct IL-23 intracellular signaling pathways, involving JAK2 independent of STAT3 and JAK1/JAK2/TYK2/TSTAT3 respectively.

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References

6. A) Represenational flow cytometry showing dermal γδ T cell identification and cytokine production. G) Dermal γδ T cell IL-17/IL-22 with NDI-031407 treatment or in TYK2KD mice. Data assessed by Mann-Whitney test, one-way ANOVA or Wilcoxon test where appropriate.