Talk

Validation of STAT1 variants found in patients with primary immunodeficiencies and evaluation of the effect of JAK inhibition using an in vitro model

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ABSTRACT

Regulation of cellular responses to interferons, cytokines, growth factors and hormones is mediated by signal transducer and activator of transcription (STAT) proteins. In the immune system binding of a cytokine (e.g. IFN) to the corresponding surface receptor, Janus kinase (JAK) molecules are phosphorylated, resulting in the docking and phosphorylation of the associated STAT proteins. The STATs will form homo or heterodimers and translocate to regulate transcription of pro-inflammatory target genes. Mutations in STAT1 are known to result in immunodeficiency and/or immune dysregulation syndromes.

In this project, the functional impact of variants in the STAT1 gain-of-function gene (STAT1 GOF) will be analyzed on a protein level. The effect of a directed treatment approach targeting the JAK-STAT pathway (JAK inhibitors) will be evaluated in an in vitro model. Freshly isolated PBMCs or whole blood samples from patients and healthy controls were obtained. The cells were stimulated with IFNg and the treated with the JAK inhibitor Ruxolitinib. Extra and intracellular staining with anti-human fluorochrome conjugated antibodies was performed in order to determine the expression of STAT1 and pSTAT1 on monocytes by means of flow cytometry.

Two pediatric patients and one related adult patient were studied and the pathogenicity of the variants was confirmed as STAT1 and pSTAT1 levels in the patients at baseline as well as after IFNg stimulation were markedly increased, when compared with healthy controls. The in vitro administration of different concentrations of the JAK inhibitor Ruxolitinib resulted in the normalization of pSTAT1 levels in the cells obtained from patients with STAT1 GOF mutations. Patients with STAT1 GOF mutations show a severe clinical phenotype with recurrent bacterial and fungal infections. Currently no specific treatment options are available. However recent case reports suggested the benefit of JAK inhibition. Therefore we studied the effect of this drug using primary cells in an in vitro model on a molecular level. Importantly two patients have been started on this medication and have achieved a significant clinical response. We are currently evaluating the capacity of our protocol to identify patients with alterations of the JAK-STAT pathway eligible for this targeted treatment approach.

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