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Understanding clinical and immunological features of patients suffering from dominant negative STAT3 mutations (Job Syndrome)– exploring new therapeutic options and improving quality of life

The signal transducer and activator of transcription (STAT) proteins are critical transcription factors for the appropriate regulation of cellular responses to interferons, cytokines, growth factors, and hormones. Binding of these molecules to their corresponding receptor leads to the activation of Janus Kinase (JAK) resulting in the tyrosine phosphorylation of cytoplasmic STAT proteins that translocate into the nucleus to initiate the corresponding transcription process.

Our research activity focuses on STAT3 mutations that lead to the clinical phenotype found in autosomal-dominant Hyper IgE syndromes (AD-HIES) or dominant negative STAT3 mutations. Although the real incidence of this syndrome is still unknown, registries resulted in estimations of 1:100.000. The main purposes of our research are to obtain novel information about the pathophysiologic mechanism, improve the understanding of their clinical features and identify potential directed treatments for the AD-HIES. It is important to mention that patients with STAT1 gain-of-function (GOF) mutations and AD-HIES show a surprisingly overlap in terms of clinical manifestations and cellular phenotypes, suggesting similar pathophysiologic mechanisms. While several reports have shown the clinical benefit of JAK inhibition for patients with STAT1 GOF mutations, AD-HIES therapeutic intervention is limited to antibiotic or antifungal prophylaxis or immunoglobulin substitution.

Our main hypothesis is that STAT1/STAT3 equilibrium is disturbed in AD-HIES similar as in STAT1 GOF patients with deleterious STAT1 excess. In fact, we and other authors have shown that many patients with AD-HIES showed levels of STAT1 and phosphorylated pSTAT1 after stimulation with cytokines similar to patients with STAT1 GOF mutations. Considering this scenario, we are working on the evaluation of a JAK 1/2 inhibitor, named Ruxolitinib, as a first directed treatment option for AD-HIES patients. We have shown that incubation of patient's cells with different doses of Ruxolitinib significantly reduced the activation of pSTAT1 in those patients with higher STAT1 levels. By flow cytometry, we have shown that STAT1 and pSTAT1 levels of AD-HIES patients are normalized. Using quantitative PCR, we have observed a reduction in the transcription (mRNA) of STAT1-dependent genes (*STAT1, CXCL10, SOCS1, SOCS3, PD-L1*). Similarly, the secretion of the chemokine CXCL10, widely known to be activated by STAT1, was also reduced and normalized to levels of healthy volunteers.

On the other hand, lung infections and secondary pulmonary complications are common in AD-HIES. Therefore, we also focus on the study of the impact of lung infections in STAT3 LOF mutant

mice with relevant pathogens such as *Staphylococcus aureus*. To date, clinical experience has shown that patients are prone to develop lung tissue damage after recurrent lung infections and that colonization with *Pseudomonas* and *Aspergillus* are often secondary consequences. It is our purpose to evaluate this clinically relevant aspect using transgenic STAT3 mouse models. In this regard our hypothesis is that the STAT1 excess creates a deleterious pro-inflammatory environment in the lungs that will promote higher susceptibility to infections followed by increased lung tissue damage. Following our determination to evaluate the effect of Ruxolitinib in the AD-HIES context, we aim also to assess the effect of this molecule in the severity of the infection and secondary consequences.

In summary, our goal is to contribute to the knowledge of this rare but potentially devastating disease, to improve its diagnosis and management thereby potentially improving quality of life of affected patients. The identification of new therapeutic alternatives is crucial to reduce immunological complications including auto-immunity and inflammation phenomena