

## Study of the switch-over from the profile Th17 lymphocytes to Th1-like during the evolution of Experimental Autoimmune Encephalomyelitis

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### Abstract

It has been demonstrated the cytotoxic activity of TCD4+ encephalitogenic lymphocytes and its crucial role in the development of Experimental Autoimmune Encephalomyelitis (EAE). Furthermore, our laboratory has seen a switch from Th17 profile to Th1-like during the evolution of EAE. Thus, these lymphocytes decreased IL-17A production while begin to produce IFN $\gamma$  when infiltrate the central nervous system. Therefore, our goal was to verify the mechanisms that governs the conversion of Th17 cells into IFN $\gamma$ -producing cells.

### Key words:

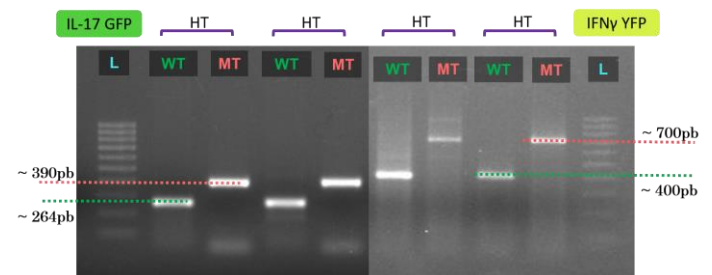
Experimental Autoimmune Encephalomyelitis, T CD4+ cells, JAK/STAT/SOCS.

### Introduction

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative autoimmune disease of the central nervous system (CNS), in which encephalitogenic lymphocytes attack and destroy myelin. In the Experimental autoimmune encephalomyelitis (EAE), experimental model of MS, it has been shown that two subtypes of lymphocytes (Th1 and Th17) act in a synergistic and coordinated manner during the development of the disease. Particularly during clinical stage of the disease, clones "Th17" begin to express IFN $\gamma$  after entering the CNS, assuming a "Th1" profile. Thus, it is possible for the same encephalitogenic clones to assume distinct profiles during the progression of the disease. The resulting microenvironment of cytokines released seems to be the main factor to influence the differentiation and function of T lymphocytes. Cytokines transmit its signal by binding to receptors of cytokine associated with Janus kinases (JAKs), culminating in the phosphorylation of signal transducers and activators of transcription (STATs). STATs induce transcription of suppressor of signals of cytokines (SOCS), SOCS proteins control cytokines responses. Therefore, it is possible that these CD4+ T encephalitogenic lymphocytes that change their expression of cytokines have a regulation of the JAK/STAT/SOCS pathway which have not been described in the literature yet. The aim of this study is to evaluate the regulation of this pathway in CD4+ encephalitogenic lymphocytes during the clinical development of EAE. STATs phosphorylation was measured through PhosFlow cytometry in the different lymphocytes subtypes T CD4+ (IFN $\gamma$ +IL-17-, IFN $\gamma$ +IL-17+ e IFN $\gamma$ -IL-17+). These subtypes will be sorted to perform qPCR and analysis of SOCS family molecules expression.

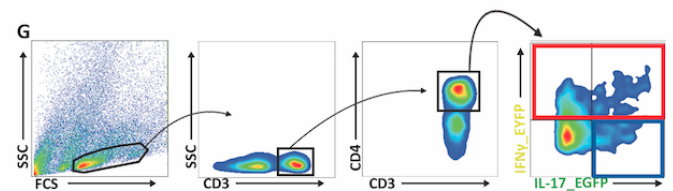
### Results and Discussion

We obtained double-reporter mice for IL17 and IFN $\gamma$  that were bred from single-reporters for each gene. To guarantee that the animals are reporter for both genes, we genotyped the offspring (Figure 1). When the result was either heterozygous or mutant for IL17 and IFN $\gamma$ , we immunized them with MOG and at the peak of the disease (13 days after immunization), the animals were sacrificed, the spinal cord and lymph nodes were collected and the cells processed and analyzed by flow cytometry (Figure 2).



**Figure 1:** electrophoresis of the genotyping of mice heterozygous for IL-17 and IFN $\gamma$ . (L – ladder, WT – wild type, HT – heterozygous, MT – mutant, BP – base pairs).

Further, total CD4+, IL-17+ or IFN $\gamma$ + T cells from CNS and lymph nodes were sorted for qPCR expression analysis. Our results demonstrated a significant increase of SOCS2, SOCS6 and CIS after CD4+ T cells reach the CNS. Interesting, when we subdivided the total CD4+ T cells in CD4+IL-17+ or CD4+IFN $\gamma$ +, we verified a downregulation of SOCS1, SOCS3 and CIS in IFN $\gamma$ -producing cells. Moreover, we have observed an increased expression of SOCS4 in IFN $\gamma$ -producing cells. Of note, to date, there is not a single description of SOCS4 function in hematopoietic cells.



**Figure 2.** Analysis strategy of the protein expression of IL-17\_GFP and IFN $\gamma$ \_YFP in the spinal cord and lymph nodes of double-reporter animals

### Conclusions

Up to now, our results indicated a non-canonical regulation of SOCS molecules during the conversion of Th17 cells into IFN $\gamma$ -producing cells. This mechanism is crucial for the development of the autoaggressive response in EAE model. Further experiments may provide a better understanding of the mechanisms governing the conversion of Th17 cells into IFN $\gamma$ -producing cells.

### Acknowledgement

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