ImmunoTools *special* Award 2014

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New therapeutic approaches for Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease that consists of an immune-mediated destruction of the hepatocytes. It is characterized by a T-cell rich interface hepatitis with an up-regulation of all pathways associated with Th1 immune responses.

Most of patients respond complete or partially to standard immunosuppressive therapies with corticosteroids but, however, there is still a 20% of the patients that fails to respond and their AIH can either rapidly progress to liver failure or develop a chronic process that will finally lead to cirrhosis and liver failure.

The aetiology of the breakdown of liver immunotolerance remains still unknown and the understanding of its underlying molecular mechanism is critical for the development of effective therapies for difficult-to-treat. New animal models able to resemble human AIH clinical features are essential for this purpose.

We recently developed a new non-transgenic mouse model for AIH, through the hepatic expression of low levels of pro-inflammatory cytokines, that recapitulates AIH clinical features.

We first performed a preliminary analysis of the immune populations present in the livers of these mice and our next aims are summarized in the following points:

- Deep analysis of circulating, splenic or intrahepatic immune populations by flow cytometry.
- *In vitro* analysis of the effect of the exposure of these populations to different recombinant cytokines (previously reported as anti-inflammatory) that could potentially be able to reverse the Th1 phenotype and restore the immune balance.
- *In vivo* evaluation of the effect of the injection of the recombinant cytokines identified as potential candidates for AIH therapy.

Our first goal is to determine if AIH mice show significant differences, when compared to naïve controls, in the different immune cell populations (neutrophils, eosinophils, inflammatory and resident monocytes, B cells, NK cells and T cells). For this purpose we will perform flow cytometry on whole blood, total splenocytes and intrahepatic lymphocytic populations.

As there are strong evidences that AIH is driven by antigen-specific T cells, we also want to go more in depth for this cell type and see how are naïve, memory and effector compartments distributed, determine if there are changes in the percentages of α/β and γ/δ T cells, etc.

This would constitute also an exploratory study that will allow us to find any significant difference with healthy controls, not yet described for AIH patients, that could be latter studied in humans.

In addition to this characterization it is also important for us to look for new therapeutic strategies which can be potentially applied to the clinic.

In this regard, we plan to perform an initial *in vitro* approach to see if the treatment with different cytokines (previously reported as anti-inflammatory) is able to reverse the Th1 profile of the intrahepatic T cells isolated from mice suffering AIH.

For this purpose, we will isolate intrahepatic lymphocytic populations and co-culture them with the different cytokines. After that, we will analyze by different techniques (flow cytometry, ELISA, quantitative PCR, etc.) if we were able to significantly reduce the level of activation of these cells.

Once we identified some candidates *in vitro*, we next will move to an *in vivo* approach to determine if the injection of the recombinant selected cytokines is able to improve AIH progression on these animals.

Finally, the successful candidates would be included in a viral vector to see if their inducible expression in the liver is able to improve AIH onset and may have clinical benefits in patients with difficult AIH.