ImmunoTools *special* Award 2013

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Identification of Autoimmunity as an etiology of Chronic Pelvic Pain Syndrome: study of the pathogenic immune mechanisms involved and its consequences on male fertility

In our project, we aim to identify the immune mechanisms underlying Autoimmune Prostatitis development and the inflammatory detrimental consequences on the male reproductive tract. We search for the immune cell populations and cytokines involved in the disease induction and progression and also possible regulatory T cell dysfunctions.

We perform experiments using samples from patients bearing Chronic Non Bacterial Prostatitis (a disease in which autoimmunity has been suspected to be a cause) and, in parallel, studying animal models of Experimental Autoimmune Prostatitis developed in NOD and B6 mice.

In experiments using patient samples, perform in vitro cultures of peripheral blood mononuclear cells in the presence of different prostate antigens and then we measure the content of IL-6, IFN-γ, IL-17, IL-4, IL-10, TGF-β and TNF-α in culture supernatants. Also, we perform intracellular staining of different cytokines using these in vitro cultured cells ($CD45^+CD3^+CD4^+$, $CD45^+CD3^+CD8^+$, $CD45^+CD11b^+$, or $CD45^+CD11c^+$ cells secreting different cytokines such as IFN-γ, IL-17, IL-10, IL-4, IL-6, TNF-α, TGF-β) activated in the presence of prostate antigens. Besides, we quantify the total amounts of T regulatory cells ($CD3^+CD4^+CD25^+FoxP3^+TGFβ^+$ cells) in patient samples.

On the other hand, we analyze the content of total leukocytes ($CD45^+$ cells) in semen samples from patients. Moreover, we characterize the subpopulations present: macrophages ($CD11b^+$), dendritic cells ($CD11c^+$), T lymphocytes ($CD3^+CD4^+$ or $CD3^+CD8^+$), B lymphocytes ($CD19^+$), NK cells ($CD16^+$, $CD56^+$) and granulocytes ($Gr1^+$, $Ly6GC^+$, $Ly6C^+$). Also, we test the state of activation of T cells (CD25, CD44, CD62L) present in semen samples and in in vitro cultures after antigen stimulation; and the levels of sperm apoptosis in semen samples (Annexin V/Propidium Iodide) by flow cytometry.

We also routinely perform experiments using our animal model of Experimental Autoimmune Prostatitis in NOD and C57BL6 mice. We induce the disease by

immunizing animals with prostate antigens plus adjuvants and then analyze the induced prostate-specific immune response. We perform in vitro cultures of spleen and lymph node cells in the presence of prostate antigens and then quantify the levels of cytokines (IL-6, IFN-γ, IL-17, IL-4, IL-10, TNF-α and TGF-β) in culture supernatants by ELISA and ELISPOT, and also by intracellular staining followed by flow cytometry analysis ($CD45^+CD3^+CD4^+$, $CD45^+CD3^+CD8^+$, $CD45^+CD11b^+$, or $CD45^+CD11c^+$ cells secreting different cytokines such as IFN-γ, IL-17, IL-10, IL-4, IL-6, TNF-α, TGF-β).

In parallel, we disrupt and digest prostate tissue samples and quantify total leukocyte infiltrating cells and the different leukocytic subpopulations (as explained above for human samples). We also perform intracellular staining of different cytokines in infiltrating cells and the levels of T regulatory cells.

Also, we polarize naive specific T cells in vitro to Th1, Th2, Th17 or T reg cells and then transfer them to recipient SCID mice and evaluate the induction of the disease when certain cell subsets are transferred. For those in vitro polarization experiments we use recombinant cytokines (rh IL-2, rh IL-4, IL-12, IFN-γ, rh IL-6, rh TGF-β) and neutralizing antibodies (anti-IL-4, anti-IFN-γ, anti-IL-12).

Also, we are interested in investigating the role of chronic prostate inflammation as a trigger of Prostate Cancer. We are involved in a project studying models of chronic infectious and autoimmune prostatitis and the incidence and initiation of prostate cancer, specially focused in the appearance of cancer stem cells ($CD133^+$, $CD44^{hi}$, integrin $\alpha2\beta1^+$, $CD177^+$, $Sca1^+$, Androgen Receptor α^-) within areas of inflammation in tissue samples from animals under study.

In our projects, we usually perform flow cytometry (our main used methodology) detecting organ infiltrating cell populations, staining of intracellular cytokines and cell surface specific markers. Also, we usually perform adoptive transfer of specific T cell populations into recipient mice and then we track those cells by flow cytometry. Besides, we routinely measure the content of secreted cytokines by ELISA and ELISPOT techniques.