ImmunoTools *special* Award 2015

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Role of TGF-beta induced targets in the protection against T cell cytotoxicity in type 1 diabetes

Diabetes is characterized by high levels of glucose in the blood as a consequence of insufficient insulin for the body's needs. Two major types of diabetes have been described: type 1 diabetes (T1D) which is a polygenic autoimmune disease characterized by smoldering inflammatory response directed against the insulin-producing β islet cells of the pancreas and type 2 diabetes mellitus (T2DM), the most common form of the disease, influenced by lifestyle factors, such as age, pregnancy, and obesity, for which the key problem of the disease is the reduced β cell function (Ashcroft et al., 2012).TGF-βi/Bigh3 is a secreted protein and is capable of binding to both extracellular matrix (ECM) and cells. It thus acts as a bifunctional molecule enhancing ECM and cell interactions, a lack of which results in dysfunction of many cell types. TGF-βi is also found in the cytoplasm and nucleus of cells. It was recently demonstrated that TGF-βi is a diabetes risk gene based on mouse and human genetic studies (Han et al., 2014). Previous studies demonstrated that recombinant TGFβi could preserve the integrity and enhance the function of cultured pancreatic islet cells (Han et al., 2011). Islets from TGFβi transgenic mice with actin-promoter driven TGFβi overexpression showed better integrity and insulin release after *in vitro* culture (Han et al., 2011). Conversely, TGFβi KO islets function was compromised *in vivo* after transplantation in diabetic recipients (Han et al., 2014). These studies point out that TGFβi might play an active role in the protection of β-cell against T cell cytotoxic insult in T1D.

In order to understand the role of TGF-βi in the protection in T1D, we will recover T cells from mouse diabetic pancreata, treat them for 24h with TGFβi and assess them for the production of IFNγ, Granzyme B, as well as CD107a, as a read-out of the cytotoxic capacity.

Alternatively we will inject those T cells in WT animal in order to monitor the impact *in vivo*. We will monitor the activation level of the transferred T cell in the lymph nodes and in the spleen by flow cytometry looking for the expression of CD44, Ly6C, CD62-L as well as the production of inflammatory cytokines as IFN-γ, IL-17 and TNF-α.

As a proof of concept of the data we have found in mouse, we also propose to evaluate the modifications of T cell activation upon TGFβi stimulation in the peripheral blood of T1D patients and evaluate the correlation (if any) with the activity/stability of the disease. Peripheral blood monocyte cells (PBMC) will be purified by ficoll gradient and stained for a panel of immunological markers (CD3, CD8, CD4, CD45RO, CD45RA). We will study by flow cytometry the activation level of the Ag specific T looking for the expression of CD44, Ly6C, CD62-L as well as the production of cytokines as IFN-γ, IL-17 and IL-4.

Thus, flow cytometry is the main technology we use to perform our study; this is why ImmunoTools antibodies would be very helpful to carry out our project.

A major goal of T1D research is to restore beta cell function while eliminating diabetogenic T cells by immunotherapy in the hope of achieving insulin independence. To develop an effective immunotherapy, there must be a continued effort on defining the molecular basis that underlies T cell response to pancreatic islet antigens in T1D. Therefore, identifying new targets involved in the protection against cytotoxic T lymphocyte attack might be important for defining new immunotherapy hints. Our work is expected to dissect an emerging concept of the intrinsic role of TGF-βi in the protection of β-cell against the islet autoreactive T cells.

ImmunoTools *special* AWARD for **Ana Hennino** includes 21 reagents

FITC - conjugated anti-human CD3,

PE - conjugated anti-human CD44, CD45RB, CD69, IFN-gamma,

PerCP - conjugated anti-human CD4,

APC - conjugated anti-human CD8,

recombinant human cytokines: rh IFNb1b, rh IFNgamma, rh TGF-beta3,

FITC - conjugated anti-mouse CD3e, CD45,

PE - conjugated anti-mouse CD4, CD44, CD62L,

APC - conjugated anti-mouse CD8a,

recombinant mouse cytokines: rm GM-CSF, rm IFNgamma, rm IL-1beta, rm IL-2, rm VEGF

DETAILS more AWARDS