ImmunoTools *special* Award 2014

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Study of Autoimmune diabetes in the NOD mouse model.

Autoimmune diabetes (Type 1 Diabetes mellitus or T1D) is a T-cell mediated condition characterized by the selective destruction of insulin-producing β cells**.** The mechanisms underlying β cell death in T1D have been thoroughly studied and three major death effectors have been proposed: first, the Fas/FasL pro-apoptotic pathway**),** second, the perforin pro-apoptotic pathway (CD8 T cell-dependent) and third, cytokine-induced β cell death via iNOS. Among these mechanisms, the most extensively pursued has been the Fas(CD95)/FasL(CD95L) pathway, which in turn, seems to be one of the main pathways or targets involved in cytokine-induced β cell death. Fas is a death receptor located on the cell surface that belongs to the TNFR family, and trimerizes once engaged by its trimeric ligand, 3FasL, a member of the TNF family. Fas trimerization triggers the death cascade: FADD (Fas-associated death domain) is recruited to the trimerized Fas-receptor and in turn recruits pro-caspase-8 (a pro-cysteine-protease), leading to its trans-activation and therefore to activation of caspase-3 and other caspases (cysteine-proteases). One of the final steps in Fas-induced apoptosis is the activation of Caspase-Activatable-DNAses (CADs) which cleave DNA into 200bp fragments. Fas expression on β cells is upregulated by IL-1β in conjunction with IFN-γ, in mice and induces their apoptosis**.**

NOD mice deficient in either Fas (NOD/lpr) or FasL (NOD/gld) do not develop spontaneous diabetes and NOD/lpr mice are not susceptible to adoptively transferred diabetes . Interestingly, beta cell specific Fas or FADD deficiency respectively impairs spontaneous diabetes onset. Moreover, transgenic expression of FasL on beta cells exacerbates the diabetic phenotype in NOD mice, suggesting that there may be a gradual up-regulation of Fas on beta cells during the course of islet infiltration prior to diabetes onset, and the early presence of FasL on neighboring beta cells would accelerate fratricidal beta cell death. Several groups have demonstrated that CD4 T cells are required to promote insulitis and diabetes in NOD mice and they are able to transfer diabetes into immunodeficient NOD/SCID recipients, without CD8 T cells. These data suggest a scenario in which the reciprocal activation of macrophages and CD4 T cells, upon an inflammatory signal in the local pancreatic environment, triggers IL-1beta and IFN-gamma production by macrophages and Th1 CD4 T cells respectively. Both cytokines, in turn, up-regulate Fas on beta cells causing their death as soon as the Fas receptor is engaged by its ligand, FasL.

The overall aim of our study was to understand the role of Fas in the induction of autoimmune diabetes. Therefore, discrimination between the role of Fas expression on beta cells as a consequence of the infiltration and Fas expression on activated lymphocytes infiltrating the islets themselves is essential. Moreover, it is difficult to demonstrate Fas up-regulation on beta cells in spontaneous diabetes, since Fas-bearing beta cells are more likely to undergo apoptosis.

We aimed to elucidate whether CD4 T cells from NOD mice induce beta cell death in a Fas-dependent fashion. Finally, we have tested the role of IL-1beta in the induction of spontaneous and adoptively transferred diabetes in NOD mice. The antibodies selected below will be used to characterize the haematopoietic cell subsets responsible for the autoimmune attack to pancreatic beta cells.