ImmunoTools IT-Box-Cy55M-Award 2013

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The Effects of a Single Nucleotide Polymorphism in the CTLA-4 Gene on Alternative Splicing and Type 1 Diabetes Development in the NOD Mouse

Type 1 diabetes (T1D) is a multifactorial disease both in humans and in the non-obese diabetic (NOD) mouse model. In addition to environmental influences, the disease is linked to a number of genetic susceptibility regions. In the NOD mouse these regions are called insulin dependent diabetes (Idd). My PhD focuses on the Idd5.1 region which contains the important immune genes ICOS and CTLA-4 (cytotoxic T lymphocyte antigen 4).

Congenic studies have revealed that NOD mice are partially protected from T1D development if they carry the Idd5.1 region of the non-diabetic B10 mouse. Mice carrying the B10 version of Idd5.1 also produce 4 times more liCTLA-4 mRNA compared to mice carrying NOD Idd5.1. This difference in pre-mRNA splicing can be attributed to the numerous single nucleotide polymorphisms (SNPs) between the Idd5.1 regions in the NOD and B10 mouse.

The mutation I focus on is a single base pair change at position 77 on exon 2 of the CTLA-4 gene, from a guanine residue in the NOD mouse to an adenine residue in the B10 mouse. In order to investigate whether this mutation causes the difference in CTLA-4 splicing, I have generated a transgenic NOD mouse (77A-NOD). This mouse contains the B10 genotype (guanine) at position 77 but is otherwise identical to the wild type NOD mouse.

The CTLA-4 gene has important immune regulatory functions. Complete CTLA-4 knockout mice die of uncontrolled immune proliferation at 4 weeks of age. Foxp3 conditional knockout mice (no CTLA-4 in Tregs) die at 7 weeks of age. The CTLA-4 gene is expressed in activated T cells and regulatory T cells. In mice the gene is spliced into three different isoforms: A full length isoform (flCTLA-4), a soluble isoform which excludes the transmembrane domain (solCTLA-4), and a ligand independent isoform (liCTLA-4). flCTLA-4 and solCTLA-4 bind to co-stimulatory molecules, CD80 and CD86, on the surface of antigen presenting cells (APCs). liCTLA-4 is predicted to exist intracellularly. Several different mechanisms of action have been proposed for CTLA-4 some of which focus on the interaction between T cells and APCs.

Since the generation of my transgenic mouse I have confirmed by qPCR analysis that the single base pair change at position 77 on exon 2 alters the splice pattern of CTLA-4 to produce 4-fold more liCTLA-4 mRNA. I am also running a mouse cohort to investigate the effect of the introduced mutation on the development of T1D.

I would now like to investigate how this mutation and the resulting increased levels of liCTLA-4 affect the interaction between dendritic cells (DCs) and T cells. To this end I have crossed my transgenic NOD mouse onto the BDC2.5 NOD background. BDC2.5 mice are T cell receptor (TCR) transgenic. Their T cells specifically recognise a peptide present in pancreatic β cell islets.

I will compare BDC2.5 CD4 T cell polarisation into T helper cell subsets by dendritic cells *in vitro*. Different DC subsets will be derived from NOD bone marrow by culture with different cytokines:

GM-CSF + IL-4 + IL-13 → alternatively activated dendritic cells
GM-CSF + IL-12 + IFNgamma → classically activated dendritic cells

Once DC cell subsets have been achieved, I will pulse the DCs with BDC2.5 mimotope (the peptide which is recognised by the BDC2.5 TCR). The antigen competent DCs will then be co-cultured with naïve BDC2.5 T cells. These $CD4^{+}CD25^{low}$ T cells will be isolated from **BDC2.5 77A-NOD** mice or wild type **BDC2.5 NOD** mice.
Following co-culture, the efficiency of T cell polarisation towards the different T cell subsets will be analysed using flow cytometry (intracellular staining) and ELISA.

A difference in T cell polarisation by the DC subsets would provide evidence that the single base pair change in the CTLA-4 gene influences the function of the immune system. It would then be possible to further investigate the role liCTLA-4 in co-stimulation.

Following this experiment I would like to investigate whether higher liCTLA-4 levels alter T cell intrinsic properties. I would initially investigate the capacity of naive CD4+ T cells from 77A or 77G NOD mice to be polarised towards different helper T cell subsets. To this end I have crossed the NOD 77A mutation onto the NOD Foxp3-GFP background. This enables the cell sorting of naive T cells without regulatory T cell interference. The subsets of interest would be Th1, Th2, iTreg and Th17 cells. (Cytokines required for this polarisation would include IL12, IL-4, IL-2, TGFβ, IL-6, IL-21, IL-1β)

In a successive experiment I would adoptively co-transfer the *in vitro* derived Th1 or Th17 cells with natural Treg cells into cyclophosphamide treated NOD mice. A difference in the ability of Th1 or Th17 cells derived from 77A NOD mice, to be regulated by nTregs, would result in differential diabetes development. In short, this experiment would determine the influence of liCTLA-4 levels on effector T cell function in the context of Type 1 Diabetes.

For the described experiments as well as for further investigations into T cell dependent CTLA-4 interactions with immune cells, I would be very grateful for the chance to use the ImmunoTools *IT-Box-Cy55M* cytokines.

ImmunoTools *IT-Box-Cy55M* for **Fabian Jakubczik**

includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS.