Laboratory of Immunobiology

Immune tolerance, TLRs in embryonic haematopoiesis, TCR signalling

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The main goal of our research is to elucidate the mechanism(s) guiding the process of central and peripheral tolerance. In the last couple of years, our research refocused on the contribution of cellular and molecular factors controlling the process of central and chiefly peripheral tolerance. We have shown that the physiological role of enteric α-defensin production in the thymus is critical for the maintenance of central tolerance in the small intestine. These molecules, expressed by Paneth cells in the crypts of small intestine, are also expressed by a sizable fraction of medullary thymic epithelial cells (mTECs), where their expression is dependent on the AIRE transcription regulator. The immunological consequences of defective enteric α-defensin expression in the thymus were confirmed by the presence of anti-HD5 autoantibodies in the sera of APECED patients who are deficient in AIRE function. Moreover, our new mouse model of APECED demonstrated that self-reactive enteric α-defensin-recognizing T cells alone are sufficient to drive the process of initiation of Paneth cell destruction (Fig. 1), leading to intestinal microbiome dysregulation and enhanced Th17 responses, which further amplify inflammatory autoimmunity in the intestine. In addition, we have characterized a functionally distinct lymph node cell population with the capacity to delete self-reactive CD8+ and CD4+ T cells or mediate conversion of the latter into Tregs (manuscript in preparation).

We are also very interested in the expression pattern and function of Toll-like receptors (TLRs) and other TIR domain-containing immune-related proteins during the early mammalian embryogenesis (Fig. 2). We have shown that TLRs expressed on embryonic macrophages couple inflammatory signals to iron metabolism during early ontogenesis. In addition, Toll-like receptor 2 (TLR2) seems to be a suitable surface marker that allows tracking the earliest haematopoietic progenitors in a precirculation embryo. Our newly generated transgenic mice, which enable performing genetic lineage tracing experiments, provided evidence that these early TLR2 expressing progenitors contribute not only to primitive but also to definitive haematopoiesis (manuscript submitted).

We also continue in our effort to understand the earliest biochemical events leading to the activation of T cells. This mainly concerns the processes associated with the regulation of the proximal T-cell signalling where two Src-family tyrosine kinases (SFK), Lck and Fyn, provide critical functions. Towards this end we have identified several candidate proteins involved in the regulation of translocation of Lck to lipid rafts via linking this process to microtubular cytoskeletal network (manuscript submitted).

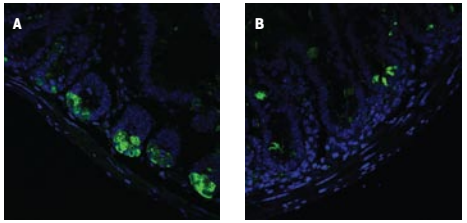

Fig. 1. The loss of Paneth cells visualized by lysozyme staining (green) on small intestinal sections in the absence (A) and presence (B) of enteric defensin-specific self-reactive T cells.

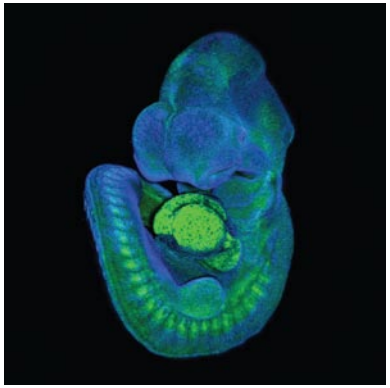

Fig. 2. A macro confocal macroscopy image of a mouse E10.5 embryo stained with Hoechst 33342 (blue), and paternally inherited EGFP fluorescence (green) driven from the β-actin promoter which enables distinguishing haematopoietic cells of maternal and embryonic origin .

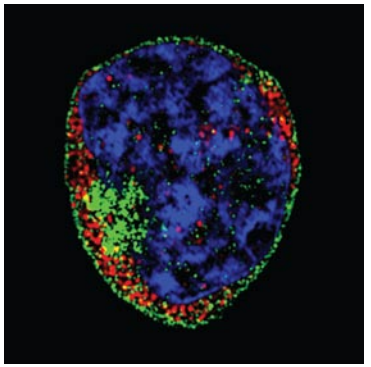

Fig. 3. Jurkat T-cell stained with anti-Lck antibody (green) and antibody against the candidate Lck-interacting protein (red) and visualized by N-SIM super-resolution microscope.

- TACR, GAMA TG01010066 – Applied molecular genetics and biology – IMG, 2014-2016, D. Filipp
- GACR, GBP302/12/G101 – Molecular mechanisms of signalling through leukocyte receptors, their role in health and disease, 2012-2018, V. Hořejší, Pe. Dráber, D. Filipp
- GACR, 14-17194P – Synthetic oligofuranosides, their enzymatic preparation and the mode of cellular signalling, 2014-2016, I. Chlubnová
- GACR, GA310/09/2084 – Characterization of the molecular machinery regulating the recruitment of signalling molecules to lipid rafts, 2009-2013, D. Filipp

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From the left: Adéla Fellnerová / Diploma Student (since October 2014, Dominik Filipp, PhD / Head of Laboratory, Jana Balounová, MSc / PhD Student, Research Assistant (since July 2014), Tomáš Brabec, BSc (since October 2014), Martina Benešová, MSc / Research Assistant, Matouš Vobořil / Diploma Student, PhD Student (since September 2014), Jan Dobeš, MSc / PhD Student, Ondřej Ballek, MSc / PhD Student, Lenka Súkeníková, BSc (since October 2014), Iva Šplíchalová / PhD Student (since September 2014)

Not in the picture: Aleš Neuwirth, MSc / PhD Student (until January 2013), Vijay Bharathi Arumugham, MD / PhD Student (until March 2013), Ilona Chlubnová / Postdoc (since January 2014)