Laboratory of Immunobiology

Immune tolerance, TLRs in embryonic haematopoiesis, TCR signalling

Dominik Filipp

dominik.filipp@img.cas.cz

www.img.cas.cz/research-groups/dominik-filipp

The main theme of our research is the understanding of the mechanisms guiding the process of central and peripheral tolerance. Specifically, our research is focused on the contribution of cellular and molecular factors to the onset and maintenance of diabetes [1,2]. In collaboration with clinical laboratories we were able to demonstrate increased levels of myeloid α-defensin expression in the capillary blood of recently diagnosed patients with diabetes [3]. We have extended these analyses to the question related to the physiological role of enteric α-defensin production in the thymus and the maintenance of central tolerance in the small intestine. We showed that these molecules expressed by Paneth cells in the crypts of the small intestine are also expressed by a sizable fraction of medullary thymic epithelial cells (mTECs) [Fig. 1], 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 deficient in AIRE function. This seropositivity highly correlated with the clinical manifestation of idiopathic diarrhea. In addition, we demonstrated a strong correlation between the severity of intestinal damage and the multiplicity of intestinal immunoreactivities in the serum of APECED patients [Fig. 2]. As GI symptoms and their underlying pathogenesis in APECED patients are still poorly understood, these findings provide new insight into the process of maintenance of central tolerance in the small intestine [manuscript submitted]. In addition, we have started to characterize functionally distinct stromal cell populations present in lymph nodes [Fig.3].

We are also very interested in the expression pattern of Toll-like receptors and other TIR domain-containing immune-related proteins during the early mammalian embryogenesis. Data accumulated so far point to the spatially and temporarily strictly regulated expression of these receptors. Surprisingly, Toll-like receptor 2 seems to be a suitable surface marker which allows tracking the earliest haematopoietic progenitors in a precirculation embryo [manuscript submitted].

We also continue in our effort to understand the very early biochemical events leading to the activation of T cells. The main goal of this line of research is the characterization of the molecular mechanism and its structural elements underpinning the recruitment of Lck and other signalling molecules to lipid rafts. Interestingly, we showed that a small pool of kinase active Lck in primary naive CD4+ T cell is maintained in a special type of lipid microdomains, called 'heavy lipid rafts' [4,5][Fig. 4].

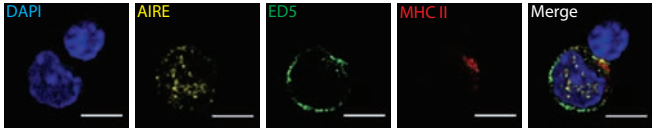

Fig. 1. Confocal immunofluorescence images of enteric defensin-positive thymic stromal cell [ED5, green] interacting with a thymocyte. The cells were co-stained with AIRE [yellow] and MHCII [red]. Nuclei are stained by DAPI [blue]. White bar represents 5-μm scale.

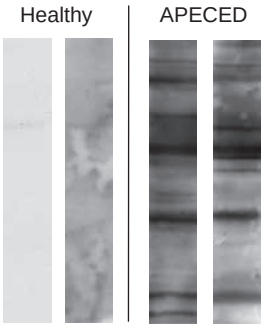

Fig. 2. Western blot analysis of sera derived from APECED patients and controls showed increased numbers of autoantibodies against intestinal antigens in the former. Representative images from two patients from each group are shown.

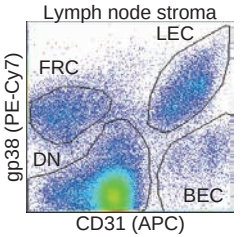

Fig. 3. Distinct immunophenotypes of stromal cell populations present in lymph nodes revealed by staining with gp38 and CD31 surface antigens.

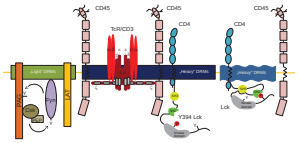

Fig. 4. A new model of TCR triggering incorporates 'heavy' DRMs as a structurally and functionally important component of cellular membranes. For detail see [4,5].

- GA AS CR, IAA500520707 - The role of innate immune molecules in embryonic homeostasis and sterile inflammation, 2007-2011, D. Filipp
- Ministry of Education, Youth and Sports of the Czech Republic, 2B08066 - Novel treatment of genetically determined metabolic disease, type 1 diabetes, using an immunotherapeutical approach, 2008-2011, D. Filipp [co-applicant]
- Ministry of Defence of the Czech Republic, 0801 8 8110 R - Study of novel prophylactic agents against Francisella tularensis infection, 2008-2011, D. Filipp [co-applicant]
- GA CR, GA310/09/2084 - Characterization of the molecular machinery regulating the recruitment of signaling molecules to lipid rafts, 2009-2013, D. Filipp
- GA CR, P302/12/6101 - Molecular mechanisms of signaling through leukocyte receptors – their role in health and disease, 2012-2017, D. Filipp [co-applicant]

1. Stechova K, Halbhuber Z, Hubackova M, Kayserova J, Petruzelkova L, Vcelakova J, Kolouskova S, Ulmannova T, Faresjö M, Neuwirth A, Spisek R, Sediva A, Filipp D, Sumnik Z: Case report: type 1 diabetes in monozygotic quadruplets. **Eur J Hum Genet 2012** 20(4): 457-462.
2. Stechova K, Kolar M, Blatny R, Halbhuber Z, Vcelakova J, Hubackova M, Petruzelkova L, Sumnik Z, Obermannova B, Pithova P, Stavikova V, Krivjanska M, Neuwirth A, Kolouskova S, Filipp D. Healthy first degree relatives of patients with type 1 diabetes exhibit significant differences in basal gene expression pattern of immunocompetent cells compared to controls: expression pattern as predeterminant of autoimmune diabetes. **Scand J Immunol 2012** 75(2): 210-219.
3. Neuwirth A, Dobeš J, Oujezdská J, Ballek O, Benešová M, Sumník Z, Včeláková J, Koloušková S, Obermannová B, Kolář M, Stechová K, Filipp D: Eosinophils from patients with type 1 diabetes mellitus express high level of myeloid alpha-defensins and myeloperoxidase. **Cell Immunol 2012** 273(2): 158-163.
4. Ballek O, Broučková A, Manning J, Filipp D. A specific type of membrane microdomains is involved in the maintenance and translocation of kinase active Lck to lipid rafts. **Immunol Lett 2012** 142(1-2): 64-74.
5. Filipp D, Ballek O, Manning J. Lck, membrane microdomains, and TCR triggering machinery: defining the new rules of engagement. **Front Immunol 2012** 3(155): 1-14.

From the left:
Aleš Neuwirth, MSc / PhD Student (until 2012) · Jana Balounová, MSc / PhD Student · Jan Valečka BSc / Diploma Student · Vijay Bharathi Arumugham, MD / PhD Student (since 2012) · Dominik Filipp, PhD / Head of Laboratory · Jan Dobeš, MSc / PhD Student
Matuš Vobořil, BSc / Diploma Student (since 2012) · Martina Benešová, MSc / Research Assistant · Ondřej Ballek, MSc / PhD Student

Not in the picture:
Zdeňka Hanusová, MSc / Diploma Student (until 2012)