



LABORATORY OF
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Central and peripheral immune tolerance, T cell signalling, embryonic haematopoiesis, toll-like receptors

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An overarching theme of our research is the cellular, molecular and signalling processes underpinning immune homeostasis.

Central and peripheral tolerance. To eliminate self-reactive T-cells, transcription regulator Aire promotes expression of tissue-restricted antigens in the medullary thymic epithelial cells [mTECs]. In this context, we have shown that if not removed in the thymus, self-reactive enteric α -defensin-recognizing T cells in the periphery can destroy Paneth cells, leading to intestinal microbiome dysregulation and enhanced inflammatory Th17 responses.¹ Using single-cell RNA-sequencing, we have also studied the process of cooperative antigen transfer and its importance for the generation of T-regulatory cells [Voboril *et al*, *Nature Communications*, *in revision*].

Interestingly, AIRE is not exclusively expressed in mTECs, but also in extrathymic cells present in the lymph nodes, spleen and testes. To enable cell type-specific ablation of the *Aire* gene, we generated transgenic mice with a *LoxP*-flanked *Aire* locus.² We have also identified Aire-expressing cells in lymph nodes with typical group 3 innate lymphoid cell [ILC3] characteristics.

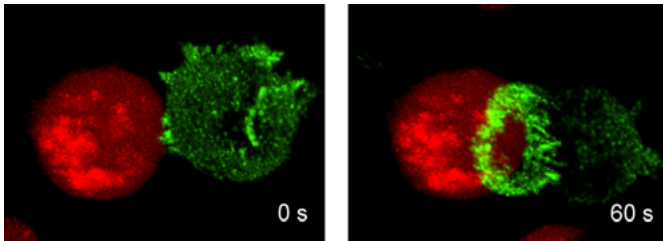


Figure 1. Expression of TLR2 in the embryo
Immunofluorescence of E7.5 embryo. TLR2⁺ cells are in green. Nuclei were stained by DAPI (blue). YS, yolk sac, EP, embryo proper, PPS, posterior primitive streak. E, day of embryonic development.

They express MHCII, costimulatory molecules, and present antigens to CD4⁺ T cells. These findings define a novel type of ILC3-like cells with potent APC features.³

Toll-like receptors and embryonic haematopoiesis. We have shown that TLRs are expressed during early embryogenesis [Fig. 1]. The expression of TLR2 on E7.5 c-kit⁺ cells mark the emergence of precursors of erythro-myeloid progenitors [EMPs]. Using *in vivo* fate mapping, we demonstrated that at E8.5, the *Tlr2* locus is already active in emerging EMPs and in progenitors of adult haematopoietic stem cells [HSC]. Together, we showed that the activation of the *Tlr2* locus tracks the earliest events in the process of EMP and HSC specification.⁴

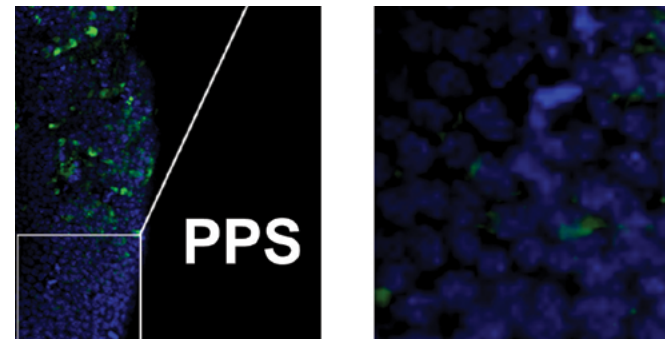


Figure 2. Early T cell activation
Live-cell imaging of immunological synapse formation between RAJI B cell (red) and Jurkat T cell expressing α -actinin-1 (green).

TCR proximal signalling.

We continue in our effort to understand the earliest events leading to the activation of T cells [Fig. 2]. Toward this end, we have contributed to studies on membrane heterogeneities in T cells.⁵

Selected publications:

1. Filipp D*, Brabec T, Vobořil M, Dobeš J (2019) Enteric α -defensins on the verge of intestinal immune tolerance and inflammation. *Semin Cell Dev Biol*, **88**:138-146.
2. Dobeš J, Edenhofer F, Vobořil M, Brabec T, Dobešová M, Čepková A, Klein L, Rajewsky K, Filipp D* (2018) A novel conditional Aire allele enables cell-specific ablation of the immune tolerance regulator Aire. *Eur J Immunol*, **48**:546-548.
3. Yamano T¹, Dobeš J¹, Vobořil M, Steinert M, Brabec T, Zietara N, Dobešová M, Ohnmacht C, Laan M, Peterson P, Benes V, Sedláček R, Hanayama R, Kolar M, Klein L^{1*}, Filipp D* (2019) Aire-expressing ILC3-like cells in the lymph node display potent APC features. *J Exp Med*, **216**:1027-1037. ¹ equal contribution
4. Balounová J, Šplichalová J, Dobešová M, Kolář M, Fišer K, Procházková J, Sedláček R, Jurisicová A, Sung, Alberich-Jorda M, Filipp D* (2019) Toll-like receptor 2 expression on c-kit⁺ cells tracks the emergence of definitive hematopoietic progenitors in a pre-circulation embryo. *Nat Commun*, **10**:5176.
5. Angelisova P, Ballek O, Sykora J, Benadfa O, Cajka T, Pokorna J, Pinkas D, Horejsi V* (2019) The use of styrene-maleic acid copolymer (SMA) for studies on T cell membrane rafts. *Biochim Biophys Acta Biomembr*, **186**:130-141.



In the picture: 1. Ballek Ondřej | 2. Vobořil Matouš | 3. Brabec Tomáš | 4. Filipp Dominik | 5. Manning Jasper Eugene | 6. Březina Jiří | 7. Vičíková Kristína | 8. Šplíchalová Iva | 9. Petrusová Jana