Laboratory of Signal Transduction

Plasma membrane signalosomes, immunoreceptor signalling, KIT and tetraspanin activation, chemotaxis

Petr Dráber

petr.draber@img.cas.cz

www.img.cas.cz/research-groups/petr-draber

Our long-term research goal is to understand the molecular mechanisms governing signal transduction from the plasma membrane receptors to the cytoplasm. We focus on the high-affinity IgE receptor, KIT, and tetraspanins in mast cell degranulation and chemotaxis. We also analyse the role of transmembrane adaptor proteins (NTAL, LAT, and PAG), galectins, and endoplasmic reticulum-associated proteins (STIM1, ORMDL3) and cross-talk of all these proteins during cell activation. To reach our goal, techniques of molecular biology, immunology, immunochemistry and immunohistochemistry are used. The techniques include use of mice with genetically enhanced or reduced expression of the proteins studied (through lentiviral transduction of proper probes or CRISPR/Cas techniques), expression profiling, and high-throughput screenings. Our research also involves development of new unique antibodies and DNA aptamer probes, as well as a variety of microscopic techniques (live cell imaging, TIRF microscopy, and super-resolution microscopy). An integral part of our studies is to verify performance of the signalling pathways under in vivo conditions. To this end we established several systems for analysis of mast cell activation, including passive cutaneous anaphylaxis and passive systemic anaphylaxis measured by computerized telemetry. Using these and other techniques we found and described new functions of the transmembrane adaptor proteins, tetraspanins, ORMDL3 protein, and galectin 3. Our studies deepen knowledge of the cellular and molecular mechanisms of the cells involved in allergic and inflammatory diseases, a prerequisite for development of anti-allergic and anti-inflammatory drugs.

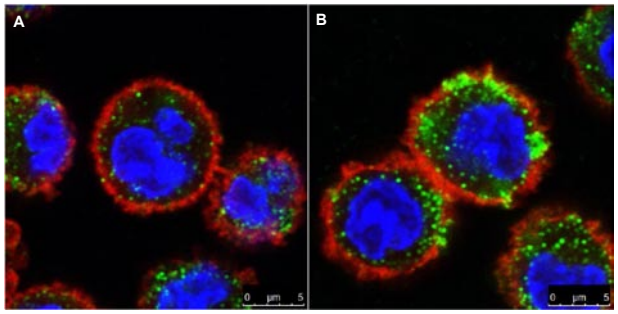

Fig. 1. Confocal microscopy images of IgE-sensitized bone marrow-derived mast cells before activation (A) and 5 min after activation with antigen (B). In activated cells, elevated amount of plasma membrane-bound signalosomes with tyrosine phosphorylated proteins is observed. Red – high-affinity IgE receptor with bound IgE detected with anti-IgE conjugated to Alexa Fluor 568. Green – tyrosine phosphorylated proteins detected with phosphotyrosine-specific antibody, followed by anti-IgG-Alexa Fluor 488 conjugate. Blue – nucleus stained with Hoechst 33258 Stain.

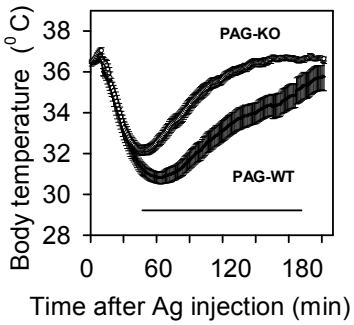

Fig. 2. The figure shows changes in body temperature in wild-type (WT) and PAG-deficient (PAG-KO) mice during passive systemic anaphylaxis. The mice were passively sensitized with antigen-specific IgE and 24 h later challenged with antigen to induce systemic anaphylaxis. Body temperature responses at various time intervals after antigen administration were measured with an accuracy of ± 0.1°C using the VitalView data acquisition system with ER-4000 energizer receivers, G2 E-mitter transponders implanted intra-abdominally, and VitalView software (Mini Mitter). Means ± SE are shown. Statistically significant differences ($P<0.05$) between PAG-WT and PAG-KO mice are indicated by black line below the curves.

- TACR, TG01010066 – Applied molecular genetics and biology – IMG, sub-project: Hybrid DNA polymerase Twa with enhanced enzymatic activity, 2014-2016, Pe. Dráber
- GACR, 14-00703S – ORMDL family proteins in mast cell signalling, 2014-2016, P. Dráber
- GACR, 14-09807S – Signalling pathways involved in mast cell chemotaxis, 2014-2016, L. Dráberová
- 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
- MEYS, LD12073 - COST CZ – Membrane signalosomes of mast cells and basophils – targets for innovative therapies, 2012-2014, P. Dráber
- MIT, FR-TI3/067 – Genetically modified polymerases and their use for amplification of unpurified DNA, 2011-2014, Pe. Dráber
- GACR, GA301/09/1826 – Topography and function of Csk-binding proteins of the plasma membrane in mast cells, 2009-2013, Pe. Dráber
- GACR, GAP302/10/1759 – Function-structure relationships between transmembrane adaptor-based signalosomes in mast cells, 2010-2013, L. Dráberová
- TACR, TA01010436 – New generations of DNA aptamers, 2011-2013, Pe. Dráber, Pa. Dráber

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From the left: Pavol Utekal, MSc / Research Assistant, Petr Dráber, DSc /Head of Laboratory, Viktor Bugajev, PhD / Research Fellow, Ivana Hálová, PhD / Research Fellow, Tomáš Paulenda, MSc / PhD Student, Romana Budovičová, MD / Research Assistant, Monika Bambousková, MSc / PhD Student, Magda Tůmová, PhD / Postdoctoral Fellow (maternity leave until IX/2014), Lucie Stegurová, MSc / PhD Student, Sergi Junyent Espinosa, Oleksii Redchenko, PhD / Research Fellow, Iva Polakovičová, MSc / PhD Student, Hana Mrázová / Technician, Lubica Dráberová, PhD / Research Fellow, MSc / PhD Student (currently abroad)

Not in the picture: Helena Dráberová