Theodor Kocher Institute

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Prof. Britta Engelhardt
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PD Dr. Charaf Benarafa
Research Group Leader and Co-Head*

PD Dr. Ruth Lyck
Research Group Leader and Coordinator of the Microscopy Imaging Center

Prof. Jens V. Stein
Research Group Leader

Dr. Giuseppe Locatelli
Research Group Leader (since November 2017)

Dr. Urban Deutsch
Research Group Leader and Head**

PD Dr. Marlene Wolf
Research Group Leader and Coordinator of the Graduate Schools

* Research Group Leader and Co-Head of the transgenic mouse and cryoconservation unit (until June 2017). Since July 2017 at the Institute of Virology and Immunology, Vetsuisse Faculty, University of Bern
**Research Group Leader and Head of the Transgenic Mouse and Cryoconservation Unit

Selected Research Partners

- Interfaculty Bioinformatics Unit, University of Bern, Bern, Switzerland
- Clinic for Neurology, University Hospital Zurich, Zurich, Switzerland
- Institute for Research in Biomedicine, Bellinzona, Switzerland
- Department of Pathology and Immunology, University Hospital Geneva, Geneva, Switzerland
- Centre de Physiopathologie de Toulouse-Purpan, INSERM UMR1043, Toulouse, France
- Center for Genome Research, Barcelona, Spain
- Kennedy Institute of Rheumatology, University of Oxford, UK
- Integrated Cardio Metabolic Centre, Karolinska Institutet, Huddinge, Sweden
- Centre de Recherche, Laboratoire de la barrière hémato-encéphalique, Université d'Artois, Lens, France
- Department of Biomedical Engineering, University of Rochester Medical Center, NY, USA

Research Profile

Founding of the TKI in 1950 was made possible by a donation of the Bernese Nobel laureate Theodor Kocher in 1912. Current research at the TKI is dedicated to investigate cellular and molecular mechanisms involved in inflammation. A special focus hereby lies on immune cell migration during immune surveillance and inflammation employing cutting-edge 3D *in vitro* and *in vivo* live cell imaging methodologies and targeted transgenic models. Ongoing research projects address for example how and where activated immune cells enter the immune privileged central nervous system (CNS) during neuroinflammatory diseases such as multiple sclerosis and stroke, or how immune cell subsets interact within the lymph node to mount an immune response or how tumor cells cross the blood-brain barrier forming brain metastases. Additional projects on the role of innate immunity in inflammation complement this portfolio. Knowledge on the migration strategies employed by different immune cell subsets will allow to improve immunomodulatory therapies for numerous diseases. Research competence at the TKI allows for the coordination of the Microscopy Imaging Center (MIC, www.mic.unibe.ch) and the heading of the Mouse Cryoconservation and Mouse Transgenic and Genetic Engineering Facility, a member of the transgenesis platform of the Swiss Animal Facilities Network (SAFN).

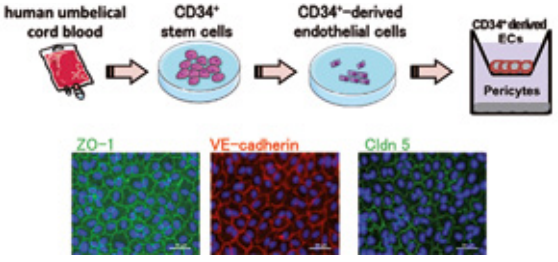
Teaching Profile

The TKI is involved in local, national and international teaching efforts. The institute offers a large portfolio of lectures and practical classes in immunology, microscopy, vascular cell biology, transgenic mouse technologies, inflammation and high end in vitro and in vivo live cell imaging for bachelor, master and graduate students in the Medical, Science and Vetsuisse Faculties. The institute is furthermore involved in teaching of students of medicine and biomedicine of the University of Fribourg. The TKI hosts the two interfaculty Graduate Schools (Graduate School for Cellular and Biomedical Sciences (www.gcb.unibe.ch) & Graduate School for Health Sciences (www.ghs.unibe.ch). In addition, coordination of the Swissuniversities supported PhD programs "Cell Migration" and "Cutting Edge Microscopy" are localized at the TKI. Britta Engelhardt is coordinator of the Horizon2020 funded international PhD student training program BtRAIN (www.btrain-2020.eu.

Highlights 2017

Modelling the multi-step migration of human T-cell subsets across in vitro models of the human blood-brain barrier and blood-cerebrospinal fluid barrier

Molecular mechanisms mediating T-cell migration into the CNS in multiple sclerosis (MS) have largely been studied in animal models, which do not mimic the full picture of

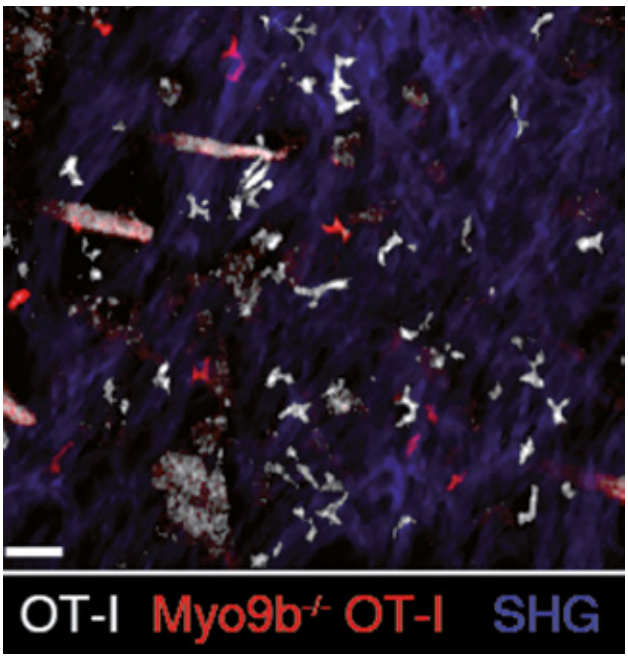


Human in vitro models of the BBB and BCSFB. CD34+ stem cells are sorted from umbilical cord blood and *in vitro* differentiated into endothelial cells (ECs). CD34+ derived ECs are then co-cultured with bovine pericytes to acquire BBB characteristics. Immunofluorescence stainings show junctional localization of ZO-1, claudin-3 and VE-cadherin by the brain like ECs.

MS neuropathology. In a collaborative approach, we have established novel in vitro models of the human blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), allowing us to side by side compare the cellular and molecular mechanisms involved in the migration of human $CD4^+$ T cell-subsets across these two brain barriers. We have furthermore established a novel nanomembrane based microfluidic device with unique optical characteristics and a very small scale, now allowing to study the extravasation of rare patient derived T-cell subsets across the BBB under physiological flow *in vitro*.

The Rho regulator Myo9b enables non-lymphoid tissue seeding of protective $CD8^+$ T cells

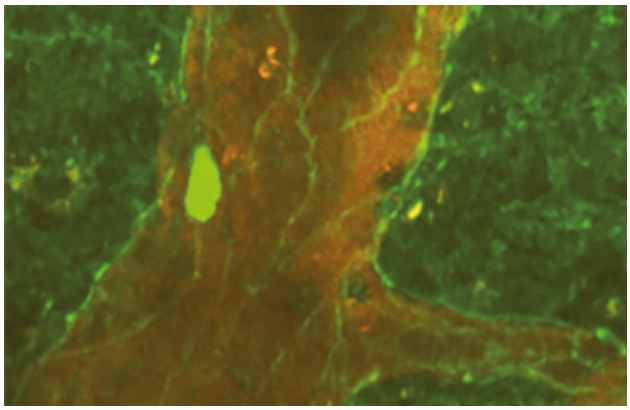
Tissue-resident memory T cells (T_{RM}) populate barrier organs such as skin, but their behavior in these exposed organs is not well understood. Using intravital imaging of cellular motility and interactions in LNs and skin, we noted that TCR-transgenic OT-I $CD8^+$ T cells deficient in the actomyosin regulator Myo9b were defective in their ability to cross the basement membrane separating dermis from epidermis. This results in blunted capacity of Myo9b-deficient T_{RM} to protect the host from a local viral infection.



Intravital imaging image of dsRed-expressing OT-I (white) and GFP-expressing Myo9-deficient OT-I T cells (red) in skin on day 30 post viral infection. SHG, second harmonic generation indicating collagen. Scale bar, 30 µm.

Two-Photon imaging of T-cell interactions with the cervical spinal cord microvasculature during neuroinflammation in vivo.

Two-photon intravital microscopy (2P-IVM) has been established as a powerful tool to study cell-cell interactions in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). We established a novel spinal cord window preparation allowing to use 2P-IVM to image the post-arrest multistep T-cell extravasation across the cervical spinal cord microvessels. The technology will allow to study the cellular pathway of T-cell diapedesis across the BBB by establishing visualization of endothelial junctions in this vascular bed.



Visualizing junctions of the blood–brain barrier endothelium in VE-cadherin-GFP knock-in mice with experimental autoimmune encephalomyelitis (EAE). An autoagressive $CD4^+$ T cell (green) can be seen crawling along the junction of the spinal cord microvessel. Endothelial junctions are visible as green lines in VE-cadherin-GFP knock-in mice. Contrast enhancement of the blood vessels was achieved by injection of Texas Red-dextran (MW = 70,000).

Selected Competitive Grants

- Swiss National Science Foundation (31003A_17013, CRSII3_154483, 31003A_172994, 16CRSII5_170969, CR23I3_156234, 310030_173137)
- European Union: (FP7 MCA-ITN 607962nEUROinflammation; H2020-MSCA-ITN-2015 675619)
- State Secretary for Education, Research and Innovation (Eurostars Project E!9059 SIAGEN-MS)
- Swiss Multiple Sclerosis Society
- Swiss Cancer League (KFS-3524-08-2014)

Selected Publications

(*corresponding author if not senior author)

- Ackerknecht, M, Gollmer K, Germann P, Ficht X, Abe J, Fukui Y, Swoger J, Ripoll J, Sharpe J, Stein JV. 2017. Antigen availability and DOCK2-driven motility govern CD4+ T cell interactions with dendritic cells in vivo. J Immunol. Jul 15;199(2):520-530
- Haghayegh Jahromi N, Tardent H, Enzmann G, Deutsch U, Kawakami N, Bittner S, Vestweber D, Zipp F, Stein JV, Engelhardt B. 2017. A Novel Cervical Spinal Cord Window Preparation Allows for Two-Photon Imaging of T-Cell Interactions with the Cervical Spinal Cord Microvasculature during Experimental Autoimmune Encephalomyelitis. Front Immunol. 2017 Apr 11; 8:406. doi: 10.3389/fimmu.2017.00406. eCollection 2017
- Moore TL, Hauser D, Gruber T, Rothen-Rutishauser B, Lattuada M, Petri-Fink A, Lyck R. Cellular Shuttles: Monocytes/Macrophages Exhibit Transendothelial Transport of Nanoparticles under Physiological Flow. ACS Appl Mater Interfaces 2017, 9 (22): 18501-18511
- Lyck R*, Lécuyer MA, Abadier M, Wyss C, Matti C, Rosito M, Enzmann G, Zeis T, Michel L, Garcia A, Sallusto F, Gosselet F, Deutsch U, Weiner JA, Schaeren-Wiemers N, Prat A, Engelhardt B. 2017. ALCAM (CD166) is involved in extravasation of monocytes rather than T cells across the blood-brain barrier. J Cereb Blood Flow Metab. 37(8):2894-2909
- Uster S, Matos Coelho F, Aeberli D, V Stein J, Hofstetter W, Engelhardt B*, Seitz M. 2017. TNF-a blockade mediates bone protection in antigen - induced arthritis by reducing osteoclast precursor supply. Bone 107:56-65