

Probing microglia microenvironment with adaptive-optics imaging and selective optical microsurgery

Clemens Alt, PhD

Summary of research project

Microglia are multifunctional agents of neural physiology and pathology. These resident phagocytes are suspected of playing a major role in inflammatory and degenerative diseases of the central nervous system (CNS), such as Parkinson's and Alzheimer's diseases, Amyotrophic Lateral Sclerosis (ALS), and macular degeneration in the retina. While it is known that microglia constantly probe their immediate vicinity by reshaping their processes, their exact role in these pathogenesises remains controversial. An answer of whether activated microglia are cause or effect of a more-widespread immune response and neurodegeneration remains elusive. Some studies have studied microglial activation after photic injury. They have shown that microglia assume an activated state after laser injury and that their motility becomes directed towards the injury site. However, by placing photocoagulation laser spots in the retina or the brain, adjacent tissue structures likely were damaged. Due to this lack of specific laser damage, observed microglial activation can be interpreted as resulting from neural injury, barrier disruption or both.

We propose to study microglial activation *in vivo* in response to selective laser targeting of specific retinal microenvironments using a novel laser microsurgical platform integrated into a high-resolution scanning laser ophthalmoscope (SLO). Our custom-built adaptive optics SLO (AO-SLO) was developed specifically for *in vivo* retinal imaging in the mouse eye. Confocal reflectance and fluorescence images can be acquired at three colors simultaneously at video-rate. The AO-SLO aims to optimize image resolution by minimizing optical aberrations introduced by the mouse eye. A deformable mirror imposes the inverse of the mouse eye aberration on the incident imaging laser beam, canceling the distortions of the mouse cornea. The instrument resolves retinal microstructure, such as capillaries, microglia processes and ganglion cell axons, a unique capability not found in commercial SLOs. For retinal microsurgery, a laser scanner that steers the beams of high power lasers into the retina will be adapted to the AO-SLO. The inner and outer blood retinal barriers will be targeted by means of their intrinsic melanin and hemoglobin absorption, respectively, using microsecond-short exposures generated from a green laser. Tissue structures without specific absorption signatures will be targeted with a laser scalpel, using optical breakdown caused by a femtosecond regenerative amplifier.

With this high-resolution imaging and laser surgical platform, we focus on microglia activation and immune recruitment in response to injury of particular microenvironments. We will investigate microglial activation and their interaction with adoptively transferred lymphocytes after causing either neuronal injury or disruption of the BRB in CX3CR1^{+GFP} mice that express the green fluorescent protein (GFP). In the short term, our results can help explain the efficacy of retinal laser treatments. However, the mouse retina, as an 'extension of the brain,' can serve as a model system of CNS adaptation to injury. It allows *in vivo* longitudinal assessment of glial interaction on a cellular level at close to physiological condition without the need for retinal flat mounts or skull thinning surgery. Long-term implications of this research imply impact on the understanding of mechanism underlying secondary neurodegeneration and aging processes.