SL07 **Intravital Imaging of Autoimmunity in the Central Nerve System: Exploring the Mechanisms and Therapeutic Targets**

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Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination and axonal loss caused by immune cell infiltration, including T cells, B cells, and macrophages, in the central nerve system (CNS). Accordingly, immune suppressive drugs are the first choice for the treatment of MS. Although these show beneficial effect, up to date MS remains incurable disease. Therefore, understanding the mechanisms of MS pathogenesis is of great importance. Accumulating evidence suggested that autoreactive T cells, which exist in healthy immune repertoire, have critical role in initiating MS. Current working hypothesis suggest that these T cells can be activated by endogenous autoantigen presented by local antigen presenting cells (APCs) in the CNS which induces production of inflammatory cytokines and opening of the blood-brain barrier (BBB). Once BBB is open other immune cells including B cells and macrophages are recruited to the inflammation site contributing to MS pathology.

We have been exploring the mechanisms of autoreactive T cell infiltration into the CNS by using animal model of MS, experimental autoimmune encephalomyelitis (EAE). Among many ways to induce EAE we are using adoptive transfer of autoantigen specific T cells. Recently, we have successfully for the first time, visualized the infiltration and activation of T cells in the CNS by using intravital two-photon imaging. The autoreactive T cells appeared at the spinal cord leptomeninges and crawled on the intraluminal surface of the vessels, before extravasating into the perivascular space. There T cells recognized autoantigen presented by local APCs and became activated, which was visualized by newly developed in vivo activation sensors.

This approach deepens the understanding of MS pathogenesis and is, in addition, suitable for evaluation of therapeutic targets. For example, intravenous injection of blocking antibody against integrin alpha4, the molecule known to be important for T cell migration, diminished intraluminal crawling within a few minutes after infusion, preventing following infiltration into the CNS, and development of clinical symptoms. Indeed, humanized anti integrin alpha4 antibody was approved and used for MS treatment. In addition, other treatments, for example, one preventing T cell activation and the other trapping autoreactive T cells in peripheral organs, also prevented the clinical EAE, indicating these are potentially interesting for therapeutic treatment.

In summary, the intravital imaging enables visualization of cellular motility and function at the single cell resolution When it is combined with traditional analysis, such as histology, flowcytometry, and RNA expression, it can contribute to the development of new therapeutic treatments.