



Assessing Neuroinflammation-related Neural Damage by Monitoring the Retinotectal System

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Questions & Answers from the Presentation

Does neuroinflammation cause fronto-parietal dementia?

We did not investigate models of frontotemporal dementia. However, there is literature by others demonstrating that neuroinflammation (comprising CD8⁺ T cells) seems to play a major role in different models of dementia.

Do you have any data/idea how this immune response in the CNS is initiated? What causes inflammation and accumulation of T cells?

We know from our disease models with gene defects affecting specific cells (e.g. oligodendrocytes) that the mutations result in cell stress, impaired proteostasis, and activation of signaling pathways that promote immune cell recruitment and activation (e.g. cytokine expression/antigen presentation). Similar reactions seem to occur in long-lived glial cells during aging. We are presently studying how these alterations cause T cell accumulation.

Is there any tissue/cell-type specificity requirement for the suppressor mutation?

Rag1 is mostly expressed in the immune system and Rag1^{-/-} mice are unable to generate mature adaptive immune cells. There are reports about the expression of Rag1 in the central nervous system. However, our bone marrow transplantation experiments (that specifically restore adaptive immune cells in Rag1^{-/-} mice) show that the observed effects are due to immunodeficiency and not direct effects of Rag1 deficiency in the CNS.

What are the CD8⁺ T cells targeting? Do they recognize a specific antigen?

Our data and that from others indicate that they drive neural damage in an antigen-specific manner and that they undergo clonal expansion. However, we presently do not know which specific antigens are recognized by the CD8⁺ T cells. In myelin disease models and aging, they infiltrate the white matter, and oligodendrocytes show cell stress and increased MHC-I expression. We therefore speculate that they might target myelin components, indirectly resulting in axonal damage. In the models of CLN disease, they might target neuronal/axonal structures directly.

You showed images of immune cells at the nodes of Ranvier forming spheroids. Were you able to identify their cell type? Were resident immune cells present?

We observed that both activated resident immune cells (microglia) and infiltrating CD8⁺ T cells preferentially localize near nodes of Ranvier. At these axonal domains (the juxtaparanodal regions), early signs of damage and spheroid formation are often detected. There is a good spatial and temporal correlation.

Which come first, T cell appearance or microglial cells becoming reactive?

Microglial activation seems to occur a little bit earlier. Soon afterwards, there is an accumulation of CD8⁺ T cells detectable.

Is ERG also affected in these different models? Can ERG be used as a surrogate for neuroinflammation?

We did not investigate ERG. It is likely that there would be alterations in PLPmut mice due to pronounced RGC degeneration. There is literature using ERG in the CLN disease models and aging, indeed showing alterations especially in b-wave amplitudes. However, we presently do not know how well ERG changes reflect neuroinflammation-related damage.

You found decline in the visual acuity measured by the OptoDrum between 24- and 28-month-old WT aged mice, but not between 12 and 24-month-old mice. What biological features underlie this change in visual acuity at 28 months?

We do not know this exactly. There could be different compensatory mechanisms at 24 months, e.g. functional/structural changes in different compartments of the retinotectal system to preserve visual acuity despite ongoing damage. For behavioral readouts it is typical that a certain threshold of neural damage must be reached to detect a significant functional deficit. When we performed systemic challenge (by i.p. LPS injection at 23 months) to increase inflammation-related damage, visual acuity was decreased at 24 months already, likely because we passed that threshold.

You did not see visual decline at 24 months of age. Does that mean the OptoDrum is not very sensitive?

We think it is sensitive for a behavioral readout, and it accurately reflects the actual biological property of the animal at that age (visual acuity, in this case). Whether or not a certain readout shows deficits at a specific time point strongly depends on disease model/condition and the affected neural circuits. This must be tested for the specific model/condition.

Does neuroinflammation have a role in Alzheimer's disease?

We did not look at AD models, but there is extensive literature indicating that neuroinflammation seems to play a major role in AD.

Is the blood brain barrier degraded in aged compared to young mice?

There are contradictory reports in the literature with most studies indicating that there is at least some impairment of BBB integrity with age. We did not focus on this in aging mice. From our other disease models, we know that an accumulation of T cells can occur without strong impairment of BBB integrity. There are different possible routes of T cell trafficking to the CNS.

Would you repeat some of these experiments in vitro with cultured neurons of humans?

This strongly depends on the question you want to answer. We are interested in complex interactions of different cell types of the immune system and the nervous system in disease and aging. We think that many of these interactions are difficult to properly model in vitro.

What modulation of the immune system would you suggest to ameliorate the effects of aging?

It remains to be determined if/which type of immunomodulation might have beneficial effects regarding aging-related neurological decline. The risks also must be carefully considered. We cannot give any recommendations now.

Your aged mice show a decreased motor function (rotarod). Do your PLP mice show this as well? Can the effect that you see in the optomotor test also be caused by this motor deficit? In general: can you perform an optomotor test on mice that have motor deficits?

PLPmut mice indeed show a deficit in motor coordination (rotarod). However, in contrast to aging, the impaired motor performance (15 months) occurs much later than the decline in visual acuity (8 months) in PLPmut mice. Overall, there is no good correlation between deficits in optomotor reflex and motor coordination in our models. The optomotor reflex is also dependent on modification of the visual stimulus (stripe pattern), which is difficult to explain with a pure motor phenotype. We therefore consider it very unlikely that the observed decline in visual acuity is related to motor deficits.

What is the role B cells play in this neuroinflammation in your models?

We did not do experiments to study the specific role of B cells in detail. However, based on our bone marrow transplantation experiments with CD8^{-/-} donors into Rag1^{-/-} recipients (which restores CD4⁺ T cells and B cells, but not CD8⁺ T cells), B cells (and CD4⁺ T cells) are unable to damage axons in the absence of CD8⁺ T cells.

Debris laden microglia can leave the CNS via the vascular system. Could the microglial movement facilitate immune cells entering the CNS?

We presently do not have any evidence that microglia leave the CNS in our models. Based on recent insights by others, the peripheral adaptive immune system can survey the CNS also via meningeal neuroimmune interfaces.

For the microglia depletion experiment, when do you think is a good time window and how long did you treat the animals? Is the depletion or the repopulation important for the beneficial effects?

We think this depends on the specific model/condition. For our genetic disease models, immune-targeted treatment approaches usually work best when we start them early, ideally before disease onset. We therefore performed long-term (several months), low-dose, and constant microglia depletion (reduction) without repopulation to attenuate neuroinflammation. Microglia also have beneficial functions related to homeostasis, phagocytosis and repair and therefore compromising their function can also have detrimental consequences in some conditions.

What other systemic stimuli to induce inflammation could be used instead of intraperitoneal injections?

Other stimuli/conditions that are known to induce systemic inflammation and might affect the diseased or aged CNS include bacterial/viral infection, dysbiosis, obesity, chronic stress, or xenobiotics. Since CD8⁺ CNS-associated T cells can cause neural perturbation, their expression of checkpoint molecules (e.g. LAG3, PD-1) might also explain why immune checkpoint inhibition for cancer therapy is often associated with cognitive and behavioral changes. These stimuli/conditions could be modeled to confirm susceptibility of the diseased/aged CNS and the role of neuroinflammation.