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Microglia Get a Little Help from "Th"-eir Friends

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The presence of CD4+ T cells in the healthy brain parenchyma remains controversial due to the barrier function of the glia limitans. Pasciuto, Burton, Roca et al. in *Cell* describe the dynamic recruitment of CD4$^+$ T cells within the brain parenchyma, their unexpected contribution to microglial maturation, and, ultimately, their influence on behavior.

While central nervous system (CNS) invasion and disease-shaping functions of T cells in neurological disorders have been a major research subject for decades, the presence and role of adaptive immune cells in the mammalian immune privileged CNS parenchyma at steady state remain a matter of debate. Making use of interdisciplinary methodology from neuroimmunology, bioinformatics, and applied mathematics, a recent study by Pasciuto et al. (2020) published in *Cell* provides convincing evidence for the presence of rare and distinct subsets of brain resident CD4$^+$ T cells in the healthy mouse and human brain parenchyma, offering novel insights on density and localization of these lymphocytes and altogether proposing a model for their immigration dynamics. Furthermore, the study unveils an unexpected function of rare brain resident CD4$^+$ T cells in supporting the final steps of microglial development, thus influencing synaptic pruning and ultimately behavior.

In a detailed analysis that combines confocal microscopy of brain tissue sections, multidimensional flow cytometry and single-cell RNA sequencing, Pasciuto, Burton, Roca et al. report the presence of about 2,000 CD4$^+$ T cells in the mouse brain at steady state. Harboring 25% of these brain resident CD4$^+$ T cells, the meninges are confirmed as a key brain compartment ensuring T cell mediated CNS immune surveillance. Nevertheless, the remaining 75% CD4$^+$ T cells were found located within the brain parenchyma beyond the glia limitans. Notably, the number of brain CD4$^+$ T cells was maximal around birth, declined in the post-natal period and slowly increased with age, thus suggesting a potential developmental function of the recruited T cells besides a role during aging. In accordance to previous observations that identified T cells in mouse and human brains as mature CD69$^+$ CD62L^{lo} CD44$^+$ brain resident T cells and thus distinct from their blood counterparts (Korin et al. 2017), the present study by Pasciuto, Burton, Roca et al. confirms a scarcity of naive T cell clusters and rather identifies activated CD69$^+$ IFN-γ^{hi} conventional and FoxP3$^+$ regulatory CD4$^+$ T cells in the mouse brain parenchyma.

To shed light on the temporal dynamics of brain invasion by CD4$^+$ T cells, the authors analyzed parabiotic animals and found that most newly invading CD4$^+$ T cells were antigen-experienced CD44^{hi}CD62L^{lo} cells acquiring expression of CD69 2 weeks after entry into the mouse brain and displaying a median brain dwelling time of ~7 weeks. Mathematical data modeling suggested that naive CD4$^+$ T cells have instead a markedly lower chance of accessing the brain parenchyma and rather quickly outflowed or died by apoptosis. Among the immigrated activated T cells, infiltrating Foxp3$^+$ Treg cells appeared to transition to CD69$^+$ dwellers 100 times more efficiently than conventional T cells.

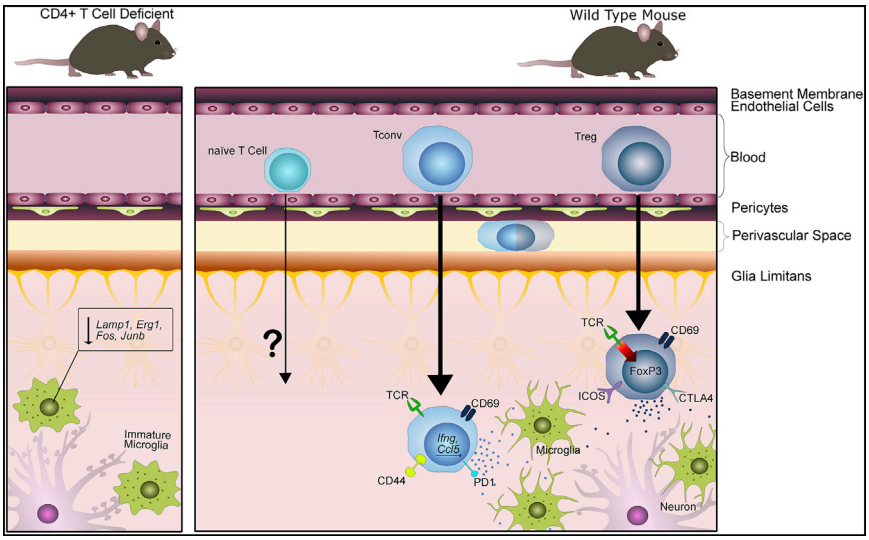

Figure 1. Brain CD4 T Cells as Drivers of Microglial Cell Maturation
In the left panel, the absence of CD4$^+$ T cells results in impaired maturation of microglia and, in turn, synaptic pruning. The right panel shows CD4$^+$ T cell trafficking toward the brain parenchyma by crossing endothelial cells of the blood-brain barrier and glia limitans at steady state. Compared with naive T cells (T naive), activated conventional CD4$^+$ T cell (T conv) have a ~10-fold higher chance of entering the brain parenchyma. Conventional T cells and Treg cells can then locally convert to CD69$^+$ resident T cells up-regulating specific molecules as depicted in the figure. Unknown soluble factors released from CD69$^+$ CD4$^+$ T cells aid microglia maturation ultimately regulating correct synaptic pruning. This figure was designed by Jasmin Steudler.

In a series of complementary experiments utilizing different reporter and transgenic mouse models, the authors suggest that T cell receptor (TCR) engagement in the periphery is necessary for the brain recruitment of conventional CD4$^+$ T cells, while stimulation of the TCR by self-antigens within the brain specifically allows residency of Foxp3$^+$ Treg cells. Accordingly, cross comparison of animals with different gut flora showed that mice with an enriched and diversified microflora possessed a higher amount of conventional brain CD4$^+$ T cells. These observations led the authors to propose that CD4$^+$ T cells might function as a "conveyor belt" of peripheral information to the mammalian brain, constituting an additional player in systemic gut-brain symbiosis.

Intriguingly, the authors show that major histocompatibility complex (MHC) class II-deficient mice that lack most CD4$^+$ T cells have a substantial block in microglial maturation, as determined both by gene expression and morphology (Figure 1). While the use of MHC class II-deficient mice poses obvious technical issues, similar results were obtained by *in vivo* injections of depleting anti-CD4 antibodies 5 days after birth, but not at 3 weeks of age. These data suggest the existence of a precise temporal window for the signaling functions of brain T cells toward microglia during post-natal development. Additionally, by modeling the interaction of microglia and CD4$^+$ T cells with brain slices and utilizing *in vitro* microglial cultures, the authors corroborated their *in vivo* findings and implicated soluble factors rather than direct contact as mediators of the observed function.

Exploring the functional impact of the reported block in microglia maturation the authors found in MHC class II-deficient mouse brains a higher density of immature long thin spines in cortical pyramidal neurons. This abnormality correlated with behavioral abnormalities in these mice, similar but not identical to previous reports suggesting that brain-antigen-specific T cells support learning behavior in mice (Radjavi et al. 2014).

The questions raised by this seminal study will initiate several future research directions. Most importantly, the anatomical routes and molecular mechanisms employed by CD4$^+$ T cells to reach the meninges versus the brain parenchyma during development will be exciting to explore. In principle, T cells can accumulate in the cerebrospinal fluid or in the brain perivascular spaces by crossing distinct brain barriers and, from there, during neuroinflammation migrate across the glia limitans to finally access the brain parenchyma (Engelhardt et al. 2016). This multistep process across brain checkpoints seems to significantly differ in development and might contribute to the shaping of CD4$^+$ T cell functions, as suggested by the observation that CNS antigens are accessible to naive auto-aggressive T cells only during the first 10 days after birth (Na et al. 2008). In this context, it will be crucial to understand if CD4$^+$ T cell trafficking to the CNS in neonatal mice is relevant for establishing self-tolerance to CNS-specific antigens, as previously shown for other tissues (Alferink et al. 1998).

The potential distinct functions of brain resident Treg cells (recognizing CNS antigens) versus conventional T cells (recognizing peripheral antigens) remains a future area of inquiry. Do both CD4$^+$ T cell subsets affect the maturation of microglia? And is the described effect modulated or amplified by other glial cells within the parenchymal syncytium? Moreover, besides microglia, other resident macrophage subsets populate the steady state brain at its interfaces, regulating CNS immune surveillance and brain function. Whether these barrier-associated myeloid cells are also affected by CD4$^+$ T cell recruitment will be important to clarify. Furthermore, while the proposed function of CD4$^+$ T cells as key "messengers" along the gut-brain axis is indeed intriguing, this notion appears as a small piece in a puzzle of higher complexity. The influence of gut flora on the nervous system is a blooming area of research, with previous reports indicating a direct influence of the microbiome on microglia as well as on brain endothelial cells (Braniste et al. 2014). Further evidence is needed to consolidate the notion of CD4$^+$ T cells as important players in this essential systemic communication. Pasciuto, Burton, Roca et al. note an increase in parenchymal CD4+ T cells with age. However, the underlying mechanisms of this increased density are yet to be explored (Pasciuto et al., 2020). Are the increased numbers of CD4$^+$ T cells in the parenchyma due to local proliferation or enhanced CD4$^+$ T cell trafficking to the aging brain? Supposedly brain resident CD4$^+$ T cells in the aged brain could

protect neurons from degeneration as proposed in the concept of "protective autoimmunity" coined by Michal Schwartz that assigns CNS-specific effector and regulatory T cells a role in CNS maintenance and repair.

Lastly, the actual action radius of a $CD4^+$ T cell within the brain parenchyma remains to be unraveled. The $CD4^+$ T cell density reported by Pasciuto, Burton, Roca et al. in the mouse cortex is ~4 cells/mm^3, an extremely rare population considering the local density of microglia (~26,231 cells/mm^3) and neurons (~102,320 cells/mm^3) (Erö et al. 2018). While the authors propose that $CD4^+$ T cells can reach the proximity of this vast number of microglia by fast Lévy walk behavior, such kinetic pattern may rather apply for T cells scanning the leptomeninges, a location likely governed by different physical restrictions when compared to the brain parenchyma harboring extremely narrow extracellular spaces.

Taken together, this significant study from Pasciuto, Burton, Roca et al. lays the groundwork for a novel understanding of adaptive immune cell actions within the brain, underscoring unexpected roles of brain $CD4^+$ T cells that go beyond maintaining CNS immunity. More in-depth understanding these functions will be of fundamental importance for considering potential adverse effects of T cell targeting therapies as used for the treatment of multiple sclerosis.

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