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INSIGHTS

Dural mural cells paint an anti-inflammatory picture

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Mural cells directly contact macrophages in the dural layer of the meninges to suppress pro-inflammatory phenotypes, including antigen presentation and lymphocyte differentiation. These mechanisms represent new targets for modulating CNS immune surveillance and pathological inflammation (Min et al. 2024. *J. Exp. Med.* https://doi.org/10.1084/jem.20230326).

A recent study by Min et al. (2024) discovered that mural cells suppress macrophage antigen presentation in the dural layer of the meninges, influencing immune surveillance in the brain. The meninges are a key interface between the immune system and the central nervous system (CNS) (Derk et al., 2021). The meninges surround the CNS in several distinct layers: the outer dura mater lies outside the CNS and contains fenestrated or "leaky" blood vessels, while the arachnoid, the subarachnoid space containing cerebrospinal fluid (CSF), and underlying pia mater—together called the "leptomeninges"—are within the CNS. Blood vessels in the leptomeninges and the brain tissue itself have blood–brain barrier (BBB) properties that selectively permit only necessary molecules into the CNS (see figure, panel A). The arachnoid forms a blood–CSF barrier that isolates the dura from the CSF-containing subarachnoid space. Together, the BBB and blood–CSF barriers tightly regulate peripheral immune cell access to the CNS.

Mural cells are specialized cells that wrap around the endothelial cells that form blood vessels (Siekmann, 2023). Pericytes are mural cells that line capillaries, while vascular smooth muscle cells typically encircle larger vessels. In the CNS, pericytes regulate BBB formation, contribute to vascular maintenance and vascular tone, and limit immune cell trafficking across the BBB (Armulik et al., 2010; Daneman, 2012; Török et al., 2021). Vascular smooth muscle cells can control vessel diameter and regulate local blood flow in response to neuronal activity (Ando et al., 2022). Although the dura is outside the CNS, recent evidence suggests that CNS antigens access this space, where they can be sampled by the adaptive immune system (Rustenhoven et al., 2021). However, how mural cells may regulate this process has not been thoroughly investigated.

During infection or disease, T cells can become activated by antigen-presenting cells. After activation, T cells will migrate and divide to target foreign antigens such as virus-infected cells or tumors. In the autoimmune disease multiple sclerosis, this pathway works against the body by causing T cells to target myelin in the brain and spinal cord (Almolda et al., 2011). Myelin surrounds neuronal axons, and loss of myelin causes neuron degeneration as well as physical symptoms including muscle weakness and paralysis.

Within the CNS, macrophages and dendritic cells are antigen-presenting cells that are known to activate T cells. This interaction was thought to predominantly occur in the leptomeninges, where border-associated macrophages display tissue-specific transcriptional signatures that regulate their function (Van Hove et al., 2019). However, there is greater appreciation for how these events may occur near the CNS while remaining outside of CNS barriers, such as antigens being presented in the dura. One recent study shows that T cells can be activated in the dura (Rustenhoven et al., 2021), and that this process is facilitated by mural and endothelial cells.

Insights from Nicole C. Lummis, Benjamin D. Gastfriend, and Richard Daneman.

In their paper titled "Mural cells interact with macrophages in the dura mater to regulate CNS immune surveillance," Min et al. (2024) describe a new mechanism in which mural cells limit CNS immune surveillance by directly contacting macrophages in the dura. The authors first observed that mural cell abundance in the dura was reduced during the presymptomatic phase of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. To test for a functional role of mural cells in regulating CNS autoimmunity, the authors used a mouse model in which diptheria toxin receptor expression is induced under the control of the *Pdgfrb* (encoding the platelet derived growth factor receptor-β, PDGFRβ) promoter. Under the authors' diptheria toxin administration paradigm, dural mural cell abundance was reduced, but leptomeningeal and parenchymal mural cell abundance was unaffected, offering the opportunity to specifically examine dural mural cell function.

The authors transferred unstimulated 2D2 T cells into experimental mice; these

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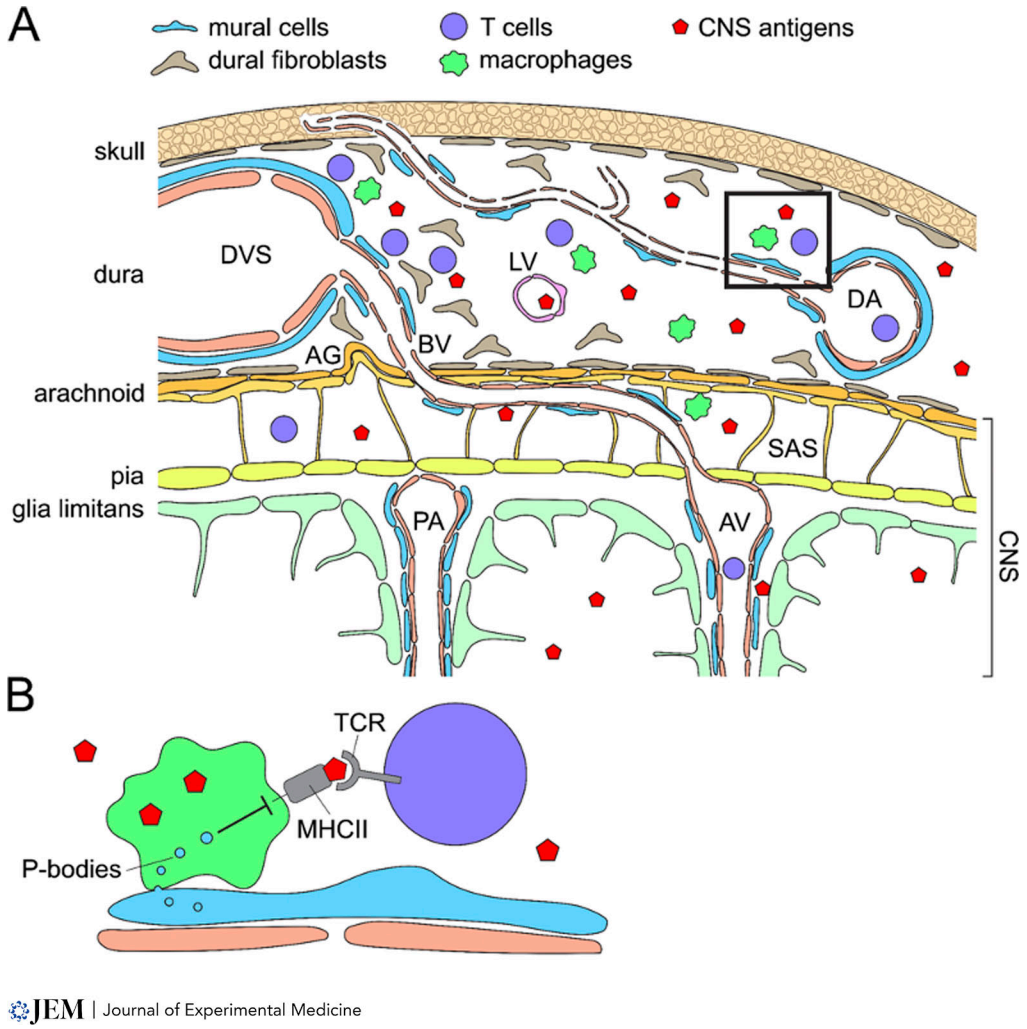

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Meninges as a site of CNS immune surveillance. (A) Anatomy of the CNS border. The dura mater lies directly below the skull but outside the CNS and contains fenestrated blood vasculature—including dural arteries (DA) and dural venous sinuses (DVS)—as well as lymphatic vasculature (LV). The arachnoid forms a barrier between the dura and the CSF-containing subarachnoid space (SAS). CSF that contains CNS antigens reaches the dura, potentially via arachnoid granulations (AG) or similar structures, or via the perivascular space along bridging veins (BV). Blood vessels in the leptomeninges are non-fenestrated and exhibit BBB properties. The glia limitans is formed by astrocyte endfeet and forms a contiguous border at the tissue surface and around the perivascular spaces of penetrating arterioles (PA) and ascending venules (AV). (B) Model for mural–macrophage–T cell interactions in the dura. Mural cell (blue) signaling to macrophages (green) inhibits macrophage antigen presentation, and concomitantly reduces antigen-dependent retention of T cells (purple) in the dura via T cell receptor (TCR) signaling. This interaction may be mediated through the transfer of processing bodies (P-bodies).

T cells have transgenic receptors that specifically recognize the CNS antigen myelin oligodendrocyte glycoprotein. Mural cell–depleted mice had increased numbers of 2D2 T cells in the dura compared to controls, suggesting that loss of mural cells leads to greater T cell recruitment at the CNS borders. Notably, no such increase was observed for adoptively transferred control T cells or endogenous T cells that had not been activated, suggesting that dural mural cells regulate T cell trafficking in an antigen-dependent manner. Indeed, dural macrophages had significantly elevated expression of antigen presentation and costimulatory machinery in mural cell–depleted mice. Furthermore, dural macrophages that contained mural cell–derived cytoplasmic components (processing bodies [P-bodies]) had lower levels of MHCII, which physically presents an antigen to a receiving T cell. In vitro experiments demonstrated that macrophages co-cultured with mural cells suppressed T cell proliferation, but this effect was not observed when processing body formation was inhibited in mural cells (see figure, panel B). Together, these results support a model in which dural mural cells contribute to a homeostatic level of CNS immune surveillance by secreting contents in their processing bodies that suppress presentation of CNS antigens by dural macrophages.

The results reported here contribute to a growing body of evidence that the dura may serve as a site where the adaptive immune system can sample CNS antigens, although the routes by which CNS antigens access the dura (see figure, panel A) remain incompletely understood. Rustenhoven et al. (2021) recently demonstrated that within the dura, macrophages and T cells are highly enriched around the venous sinuses. Thus, it will be important to further clarify the specific anatomical sites important for interactions between dural mural cells, antigen-presenting

cells, and T cells. Is the dura surrounding the brain the only location that such interactions take place, or do similar mural–macrophage interactions occur at other border sites including the choroid plexus, spinal cord meninges, or parenchymal perivascular spaces? Additionally, the contribution of each meningeal layer in regulating T cell activation and trafficking is the subject of debate. For example, Merlini et al. (2022) demonstrated that the leptomeninges, and not the dura, were the predominant site of T cell extravasation and activation in the context of CNS autoimmunity. Therefore, when T cells undergo activation in the dura, it is important to identify whether they enter the CNS and how they carry out effector functions.

While antigen presentation by macrophages and dendritic cells is a well-studied phenomenon, there is a growing body of work suggesting that lymphocyte recruitment and activation is regulated by other cell types. For example, it has recently been proposed that vascular endothelial cells can themselves process and present antigens to lymphocytes during inflammatory conditions, including EAE and malaria (Aydin et al., 2023; Fain et al., 2023). However, it is not known whether this also occurs in the dura. Additionally, production of CXCL12 by dural PDGFRβ-expressing cells may directly influence recruitment of T cells (Rustenhoven et al., 2021). Together, these observations motivate additional investigation of the cellular and molecular interactions regulating CNS immune surveillance.

Despite the potential impact of these findings, one complication lies in possible off-target effects of the PDGFRβ-based depletion strategy. While PDGFRβ is commonly used as a mural cell marker due to its high expression in pericytes and vascular smooth muscle cells, PDGFRβ is also abundant in fibroblasts and potentially expressed by other dural cells that have not yet been completely characterized (Muhl et al., 2020). Although the authors demonstrated that dural mural cells, and not fibroblasts, were the primary target of in vivo depletion, it remains possible that depletion of other cell types contributed to the observed phenotypes. Additionally, diptheria toxin acts by inhibiting protein synthesis, and it is possible that a sublethal dose could affect the function of PDGFRβ-expessing cells in the dura, leptomeninges, brain, or other tissues (Cherubin et al., 2018).

Min et al. (2024) have described a new mural cell–macrophage interaction that may be especially relevant in inflammatory disease states. If mural cells can suppress antigen presentation from dural macrophages, strengthening this process may limit the number of immune cells that are allowed to enter the CNS in disease. This is especially exciting regarding the observation that reduced mural cell coverage coincides with inflammation in EAE, a mouse model of multiple sclerosis. Further study is necessary to understand the exact mechanisms that drive these changes. What are the upstream signals that lead to mural cell loss in the presymptomatic phase of EAE? How do mural cells transmit cytoplasmic components to macrophages? What are the specific mRNAs and proteins contained in mural cell–derived P-bodies, and how do these regulate macrophage function? And what are the precise signaling pathways that suppress macrophage antigen presentation and T cell activation? If mural cell–macrophage interactions can be enhanced, perhaps pathological inflammation can be suppressed further limiting autoimmune neuroinflammation as occurs in multiple sclerosis. Conversely, encouraging CNS antigen presentation could help combat cancer or infection. If therapeutics could be developed to exogenously target these signaling pathways, we might be able to slow or prevent disease progression in patients with multiple sclerosis, encephalitis, or other neuroinflammatory conditions.

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