and antibody act in concert, or if this occurs independently of antibody (Figure 1).

Mamedov et al.'s study identifies a new subset of γδ T cells, polarized toward M-CSF production, that can directly influence the myeloid compartment in the chronic stage of *Plasmodium* infection. This might have important implications on the crosstalk between the γδ T cell and myeloid compartments but at the very least represents an important new installment in the malaria immunology canon. M-CSF-producing γδ T cells, newly coined as TγδM, might hold the key to myeloid cell recruitment in the control of chronic malaria infection and might serve as a biomarker correlating with immunity. Myeloid cells and macrophages that have undergone epigenetic remodeling, such as in response to M-CSF-producing γδ T cells, might represent a previously unexplored "trained innate" component in the acquisition and maintenance of immunity to malaria. This engaging hypothesis warrants further investigation.

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Microglia: You'll Never Walk Alone!

Nàdia Villacampa[1] and Michael T. Heneka[1,2,*]
[1]Department of Neurodegenerative Diseases and Gerontopsychiatry, University of Bonn, Sigmund-Freud Straße 25, 53127 Bonn, Germany
[2]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), 53127 Bonn, Germany
*Correspondence: michael.heneka@ukbonn.de
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In this issue of *Immunity*, Mrdjen et al. (2018) use high-dimensional single-cell proteomics and high parametric mass cytometry to provide insight into the long-lasting issue of how to identify and characterize both resident and recruited leukocyte populations in healthy, aged, and diseased CNS.

Moving from the old-fashioned view of the brain as an immune-privileged organ to our current understanding of a pivotal role of myeloid cells for both normal brain function and disease pathogenesis has been a major challenge for the neuroscience field. One of the biggest conundrums still to address is the identification of myeloid subsets in the brain and their association to physiological and pathological conditions. Until now, in the diseased brain, resident immune cells have been almost indistinguishable from their blood-derived relatives due to pathology-induced phenotypic changes (Prinz et al., 2011). In this issue of *Immunity*, Mrdjen et al. (2018) shed new light on the question of how many different myeloid subsets exist in the brain. By creating a high-dimensional single-cell proteome atlas of immune populations using high parametric mass cytometry (Bandura et al., 2009) coupled with 22-color fluorescence cytometry, genetic

fate-mapping systems, and confocal microscopy, the authors identify various subsets of myeloid cells that together account for physiological and pathophysiological immune functions. Importantly, these subsets are being distinguished in the healthy and aging brain and, moreover, in two models of the most prevalent neurodegenerative conditions: Alzheimer's disease and multiple sclerosis.

The central nervous system (CNS) parenchyma is populated by microglia derived from the yolk sac around embryonic day 8.5–9 (Ginhoux et al., 2010). As the brain's resident immune cells, they persist there for the remaining life of the organism due to their capacity for self-renewal. In the steady state, adult ramified microglia are far from being considered "dormant soldiers," simply awaiting a CNS challenge, but rather represent "sentinels" with very motile branches that constantly survey their nearby microenvironment, clear debris, and provide neurotrophic factors. During recent years, microglia emerged as a key factor for the maintenance of CNS homeostasis, synapse remodeling, and immune surveillance. Outside the CNS parenchyma, i.e., in meninges, the choroid plexus, and perivascular spaces, immune tasks are performed by bone-marrow-derived dendritic cells (DCs) (Greter et al., 2005) and CNS border-associated macrophages (BAMs; Bechmann et al., 2001). Until now, a clear distinction of microglia and BAMs was achieved solely on the basis of their localization and morphology by immunohistochemistry. Moreover, overlapping CNS compartments have prevented thus far any precise discrimination between DCs and BAMs. In their paper, Mrdjen et al. (2018) describe a 43-heavy metal isotope-tagged surface antibody for mass cytometry that was key to unraveling the whole complexity of myeloid subpopulations in the healthy, aged, and diseased CNS: macrophage-like cells, several types of monocytes (Ly6C^{hi} and Ly6C^{lo} monocytes and monocyte-derived cells [MdCs]), as well as classical DCs (cDCs), plasmacytoid DCs (pDCs), B cells (CD24^{+} and CD24^{-}), T cells, natural killer (NK) cells, NKT cells, innate lymphoid cells (ILCs), eosinophils, and mast cells. Furthermore, the authors hypothesized that non-microglia macrophage-like cells carrying a specific surface receptor pattern (CD45^{lo}CD11b^{lo}F4/80^{hi}CD64^{hi}MeTK^{+}Cx3CR1^{+}CD88^{hi}Siglec^{-}CD206^{+}CD38^{+}) were BAMs and later confirmed such affirmation using an antagonistic colony-stimulating factor 1 receptor (CSF1R) antibody treatment, by which they depleted BAMs but not microglia. They also used the recombinant growth factor Flt3L, which specifically expands DCs, to increase the frequency of DCs in the CNS and confirm their algorithm-guided identification. Furthermore, myeloid cells were examined from the CNS of *Sall1*^{GFP} reporter mice, where GFP expression was found to be restricted to microglia, confirming Sall1 as a key transcription factor to distinguish the parenchyma-associated microglia from BAMs and DCs. Using *Cx3cr1*^{CreER} *Rosa26*-RFP inducible fate-mapping mice, which show irreversible expression of red fluorescent protein (RFP) in CX3CR1^{+} cells upon tamoxifen treatment, long-lived cells (RFP^{+}) and short-lived cells (RFP^{-}) were easily distinguished. In fact, most microglia and BAMs retained the RFP label, while RFP-DCs were rapidly replaced by bone marrow progenitors. The authors move then to an in-depth BAM phenotype analysis showing that BAMs in fact represent a heterogeneous class of macrophages. In total, four different BAM subsets were identified based on differential expression of CD38, MHCII, and CCR2, with one of the four subsets (CD38^{+}MHCII^{-}CCR2^{-}) comprising around 76% of the total BAMs. In addition, histology revealed that the identified BAM subpopulations were specifically enriched in different CNS compartments: subset 1 was present in all locations, subset 2 (CD38^{+}MHCII^{+}CCR2^{-}) was enriched in the pia mater and the perivascular space, and subset 3 (CD38^{-}MHCII^{+}CCR2^{-}) and 4 (CD38^{-}MHCII^{+}CCR2^{+}) in the dura mater.

DCs are considered the main antigen-presenting cells (APCs) and represent the intersection of the innate and adaptive immune systems (Sie and Korn, 2017); however, little is known of their role within the CNS. Using their mass cytometry approach, Mrdjen et al. (2018) identified three main DC subsets corresponding to cDC1s, cDC2s, and pDCs, differentiated by their expression of CD11b and CD24. In addition, cDC2s were further separated into four subsets and, as in peripheral organs, were found to be more abundant than cDC1s. Also, after Flt3L treatment, cDC1s were IRF8^{hi}IRF4^{hi} and cDC2s were IRF8^{-}IRF4^{hi}, but both expanded similarly. By confocal microscopy, CD11c^{+}MHCII^{+} DCs were found exclusively in the choroid plexus, pia mater, and dura mater, identifying these locations as putative entry sites for MHC-dependent T cells.

During brain aging, substantial changes affect the CNS immune cell populations; for example, senescent microglia acquire a pro-inflammatory phenotype and play a major role in neurodegenerative diseases (Heneka et al., 2014). Mrdjen et al. (2018) show that aged microglia express high levels of CD11c and CD14. In addition, aged mice had a substantial increase in T cells and CD135^{+} cDC2s, accompanied by a decrease in NKT cells, pDCs, and CD24^{+} cDC2s and changes in the proportions of BAM subsets. Were similar changes present in the context of neurodegenerative disease? To answer this question, the authors used 4-month-old APP/PS1 mice and a mouse model of Alzheimer's disease displaying cerebral amyloidosis and compared the amyloid-β plaque-containing cortex to plaque-free cerebellar tissue. Interestingly, young APP/PS1 mice displayed increased T cells but unaltered BAM distribution, suggesting BAM's change requires brain aging. Microglia in young APP/PS1 mice express the phagocytosis-related markers CD11c and CD14 similarly to microglia from aged mice. In addition, they are characterized by an increase in CD86, CD44, PDL1, and MHCII, while CX3CR1, MerTK, and Siglec-H decreased. This observation confirms that microglia in young APP/PS1 animals switch from a homeostatic to an activated phenotype displaying a phagocytic and pro-inflammatory profile (Sarlus and Heneka, 2017). These findings were then compared to myeloid population changes in response to experimental allergic encephalitis (EAE), an animal model of multiple sclerosis (Kipp et al., 2017). During myelin oligodendrocyte glycoprotein (MOG)-induced EAE, highly reactive microglia display a phenotype similar to those of aged wild-type and young APP/PS1 mice, characterized by an increase of CD86, CD44, PDL1, and CD11c and a concomitant decrease of CX3CR1, MerTK, and Siglec-H. In contrast to aging and APP/PS1 expression, EAE caused a decrease in CD14 and an increase of MHCII and Sca-1 in microglia. In-depth analysis of further myeloid subpopulations in EAE

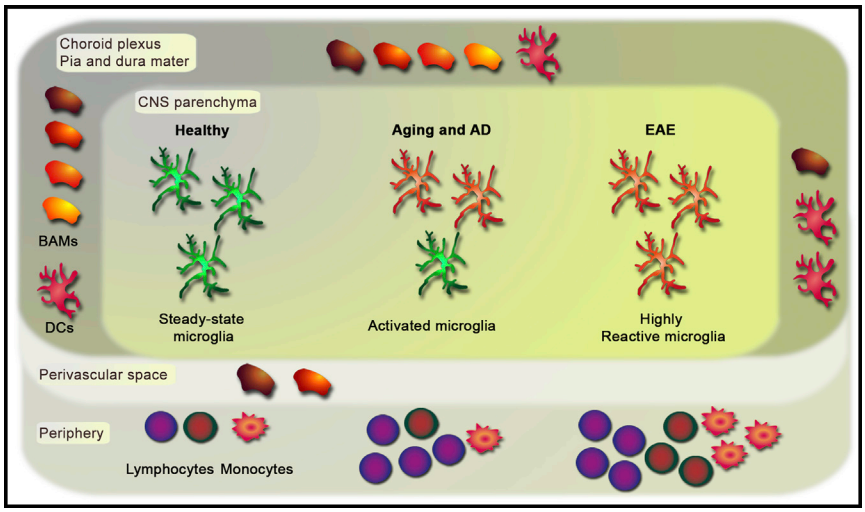

Figure 1. Schematic Representation of CNS Myeloid Cells in Health, Aging, and Disease
In health, steady-state microglia (green) are the sole myeloid cells in the central nervous system (CNS) parenchyma, while four different subsets of border-associated macrophages (BAMs) and dendritic cells (DCs) are found in the choroid plexus, the pia mater, and dura mater. Some subsets of BAMs reside also in the perivascular spaces. Cells from the peripheral immune system, such as lymphocytes and monocytes, are also present. During both aging and Alzheimer's disease (AD), microglia adopt an activated phenotype (red), BAMs and DCs are present in the CNS borders, and lymphocyte recruitment is increased. In experimental allergic encephalitis (EAE), microglia become highly activated and DCs are more abundant in the CNS borders, whereas subsets of BAMs decrease. Significant recruitment of several subsets of lymphocytes (violet) and monocytes (red/orange) is observed.

showed that MdCs, monocytes, and cDCs are distinctly separated from pDCS and BAMs. Also, similar to microglia, the latter express MHCII, CD44, Sca-1, PDL1, CD117, and CD11c. Invading Ly6C^{hi} monocytes during EAE differentiate into highly activated inflammatory MdCs expressing Sca-1, MHCII, PDL1, CD11a, CD86, CD38, CD14, and CD16/32. For years, the classical approach of gating CD11b^{+}CD45^{high} and CD11b^{+}CD45^{mid} cells prevented the detection of distinct phenotypic differences of resident microglia, BAMs, and recruited MdCs. Using their novel approach, Mrdjen et al. (2018) now mastered this problem and were able to successfully distinguish not only those populations but also the different subsets of cDCs and pDCs and their lineage EAE during peak. Future studies will now have to link function to the different phenotypes and locations that have been identified in this manuscript. Moreover, they need to delineate if and how these different myeloid cells interact with astrocytes, oligodendrocytes, and neurons. It seems possible that those myeloid subsets have different effects on their neighboring brain cells, in particular under pathological conditions.

In summary, as pictured in Figure 1, the study by Mrdjen et al. (2018) indicates a major step forward to identifying and characterizing the phenotypic signature of CNS myeloid cells, i.e., microglia, BAMs, and DCs in health, aging, and disease conditions. Moreover, the authors succeed in assigning locations and roles, putting some on the pitch and others in the stands. They demonstrate that using mass cytometry allows them to distinguish highly activated CNS-resident myeloid cells from recruited leukocytes and their respective subsets. From these studies on aging, APP/PS1 expression, and EAE, we now learn that microglia, like Liverpool F.C.'s players on those nights on Anfield Road, will never walk alone. When the heat is on and the game becomes tight, their stands filled with their supporters, relatives, and friends—the different types of myeloid subsets—will support them through an invisible net of emotions (immune mediators) they have spun for that occasion only and that unifies them for this one moment, for better or worse, until the match is decided.

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