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REVIEW

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Perivascular tissue resident memory T cells as therapeutic target in multiple sclerosis

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ABSTRACT

Introduction: Multiple sclerosis (MS) is characterized by inflammatory attacks of infiltrating leukocytes at onset but evolves into a smoldering, progressive disease within the central nervous system at its later stages. The authors discuss the contribution of white matter lesions to the pathology of advanced MS, thereby paying particular attention to the role of T cells.

Areas covered: Diagnostic biopsy and autopsy studies of white matter lesions in early MS show different pathological patterns of demyelination and leukocyte infiltration. Brain autopsies from advanced MS display substantial inflammation without distinct patterns and suggest a role for perivascular $CD8^+$ tissue-resident memory T (T_{RM}) cells in active and mixed active/inactive MS white matter lesions. When compared to control and normal-appearing white matter, these lesions are enriched for parenchymal $CD8^+$ T cells. In the perivascular space, cuffs containing $CD8^+$ T_{RM} cells are observed also in progressive MS, and could be sites of local reactivation.

Expert opinion: Recent findings point toward the perivascular space as an immunological hotspot, which could be targeted in order to suppress a contribution of T_{RM} cells to ongoing white matter lesion activity in advanced progressive MS. The authors discuss approaches, which may be explored to suppress T_{RM}-cell reactivation in the perivascular space.

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1. Introduction

Multiple sclerosis (MS) in an inflammatory, immune-mediated disease of the central nervous system (CNS). This view is supported by a vast body of genetic evidence, pointing toward T- and B-cell interactions as drivers of the disease [1,2], and has led to the development of disease-modifying treatments (DMTs) that successfully silence relapses and magnetic resonance imaging (MRI) lesions in the early phases of MS[3]. DMTs may be responsible for the, in general, milder disease course noted in contemporary patient cohorts[4], since relapse rate and MRI lesions in the early phases predict the accumulation of disability in the later phases of MS[5]. Oligoclonal immunoglobulin (Ig)G and high levels of soluble CD27 in the cerebrospinal fluid (CSF) indicate intrathecal adaptive immune activation and predict a more severe disease course [5–7]. Of note, the currently available DMTs fail to have a meaningful impact at late, progressive stages of the disease. Despite brain atrophy and cortical demyelination are the most prominent features of progressive MS pathology[8], a contribution of ongoing focal white matter inflammation to disability progression in advanced disease has also been suggested.

In this review, we discuss postmortem studies of the natural disease course of MS that point toward the perivascular space (PVS) as a relevant hotspot of immune (re)activation in the context of white matter lesion development in progressive MS. Moreover, we elaborate on potential approaches to target T cells in the PVS for the benefit of people suffering from progressive MS.

2. Distinct white matter lesion profiles in early MS

In the majority of people with MS, the onset of the disease is characterized by sub-acute, temporary exacerbations of clinical symptoms, reflecting focal dysfunction of the CNS[4]. These attacks are caused by inflammatory cells, which invade the CNS and cause focal inflammatory, demyelinated lesions. In the currently leading immunological concept of MS, exacerbations are initiated by presentation of unknown molecular structures by antigen-presenting cells (APCs) to T cells in the lymph nodes[9]. This process leads to T-cell activation and clonal expansion, and to the recruitment of T cells, B cells, and bone marrow-derived circulating monocytes toward the CNS. Waves of circulating, inflammatory cells migrate to the focally inflamed endothelium, cross this specialized endothelium of the blood brain barrier (BBB) at the level of post-capillary venules, and enter the PVS in close association with lesion formation. Here, the T cells are reactivated by resident APCs and move into the parenchyma to contribute to inflammatory, demyelinated lesions. Early in their development, these lesions are characterized by cellular infiltrates consisting of T cells, B cells,

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Article highlights

- The phenotype of MS evolves in the majority of patients from a relapsing–remitting onset, characterized by inflammatory attacks of leukocytes infiltrating the CNS, into a smoldering, slowly progressing inflammatory disease.
- Diagnostic biopsy and autopsy studies of white matter lesions in early MS show distinct profiles of demyelination and leukocyte infiltration, while autopsy studies in advanced MS lack clear separate pathological patterns.
- In advanced MS, the presence of active and mixed active/inactive white matter lesions correlates with a faster accumulation of disability, disease progression, and an unfavorable genetic risk profile.
- Active and mixed active/inactive lesions are enriched for $CD8^+$ and to a lesser extent $CD4^+$ T cells, proportionally infiltrating the parenchyma and having a T_{RM}-cell phenotype, the latter suggests mobilization from the PVS rather than recruitment from the circulation.
- In progressive MS, perivascular cuffs containing T_{RM} cells are observed and may be sites of antigen presentation and T_{RM}-cell reactivation.
- Targeting the reactivation and mobilization of perivascular brain T_{RM} cells could be effective in controlling a T cell-mediated contribution to white matter lesions and related disability progression in advanced MS.

activated HLA-positive microglia, and (possibly) infiltrating monocyte-derived macrophages [10,11]. At a later stages, these lesions are characterized by a demyelinated, hypocellular sclerotic core with an active rim of myeloid cells (Figure 1)[10]. This lesion type is known by many names (smoldering, slowly-expanding, chronic active) but currently mostly referred to as mixed active/inactive.

Focal disruption of BBB integrity can be visualized in MS patients on MRI scans as gadolinium-enhancing T1 lesions. Current disease-modifying treatments in MS generally or more specifically inhibit critical components of this model, as take place in lymph nodes and circulation (i.e. outside the CNS)[3]. For instance, teriflunomide disables clonal expansion of lymphocytes[12], fingolimod prevents immune cells from leaving the lymph nodes[13], and natalizumab inhibits attachment of immune cells to the endothelium[14]. Circulating T and B cells are depleted by drugs as cladribine[15], alemtuzumab[16], and ocrelizumab[17]. MS treatment with autologous hematopoietic stem cell transplantation also depends on the depletion of circulating lymphocytes and their precursors in the host[18]. With these treatments, relapses and MRI lesions can be prevented.

The pathological characteristics at the earliest phases of MS have been investigated using diagnostic brain biopsies of patients with MS and autopsy brain material of MS patients who died shortly after disease onset. A point of caution in the interpretation of these studies lies in the fact that only a minor proportion of MS patients receives a diagnostic biopsy, and the representativeness of this sub-group for the pathological profile at onset in the entire MS population is uncertain. Luchinetti *et al*. characterized white matter lesions in a combined autopsy and biopsy cohort, consisting of patients with a short disease duration[19]. White matter lesions were in all donors characterized by T-cell infiltrates and myelin-containing microglia/macrophages, coinciding with distinct patters of demyelination, IgG accumulation, and activated complement deposition. In a longitudinal study, these different patterns were consistent within donors with multiple subsequent biopsies[20]. This observation led to the proposition of four distinct pathological patterns in early MS[21]. Type I lesions are defined as perivenous, radially expanding lesions, which contain T cells and macrophages, and display degeneration of myelin. Type II lesions are type I lesions with IgG and complement deposition at site of demyelination. In type III lesions, T-cell and macrophage activation coincides with small vessel vasculitis and degeneration of distal oligodendrocytes. Type IV lesions are similar to type III lesions but characterized by oligodendrocyte loss and less by inflammation. Notably, the four pathological patterns in early biopsy samples did not result in differences in clinical disease course. It is at present unclear whether these pathological patterns are also associated with a distinct phenotypic profile of infiltrating T cells. Furthermore, Breij *et al*. could not distinguish these different patterns in active MS lesions at later disease stages in an autopsy study but rather observed a homogenous pattern of demyelination with complement and IgG deposition in all donors. This suggests that in later phases of MS, ongoing demyelination is mediated by complement and IgG[22].

3. Ongoing inflammation in white matter lesions in end-stage MS

In the later phases of MS, exacerbations of the disease are often lacking, and patients may experience a gradual deterioration of neurological symptoms[4]. In patients with long-standing relapsing–remitting MS, gadolinium-enhanced lesions become less prevalent when compared to people early in their disease[23]. In primary progressive MS, gadolinium-enhancing lesions were only found in early phases and markedly declined during 5-years follow-up[24]. These observations indicate that focal BBB leakiness, associated with local trafficking of leukocytes into the white matter and gadolinium-enhancing MRI lesions, is less prevalent at the later, progressive stages of MS. Nevertheless, postmortem pathological studies showed in progressive MS altered immunostaining profiles, associated with a reduced BBB integrity, which was supported by the observation of fibrinogen-depositions in the adjacent white matter [25,26]. This leakiness apparently differs from the local disruption of the BBB associated with lymphocyte trafficking toward acute white matter lesions in early, active MS[27], since gadolinium-enhancing MRI lesions are sparse in advanced MS.

The pathology of white matter lesions in the most advanced end stages of MS has been the focus of extensive autopsy studies performed on post mortem human MS brain samples. Several groups characterized the presence of inflammatory white matter lesions in MS. We reported that 78% of n = 182 MS brain donors of the Netherlands Brainbank (NBB) displayed active and/or mixed active/inactive white matter lesions at the time of death[28]. Of all white matter lesions studied, mixed active/inactive lesion were most prevalent (33%), followed by inactive lesions (27%) and active lesions (24%). Earlier work by Prineas *et al*. and Michailidou *et al*. showed that chronic mixed active/inactive lesions, but not early acute lesions, are associated with complement C3

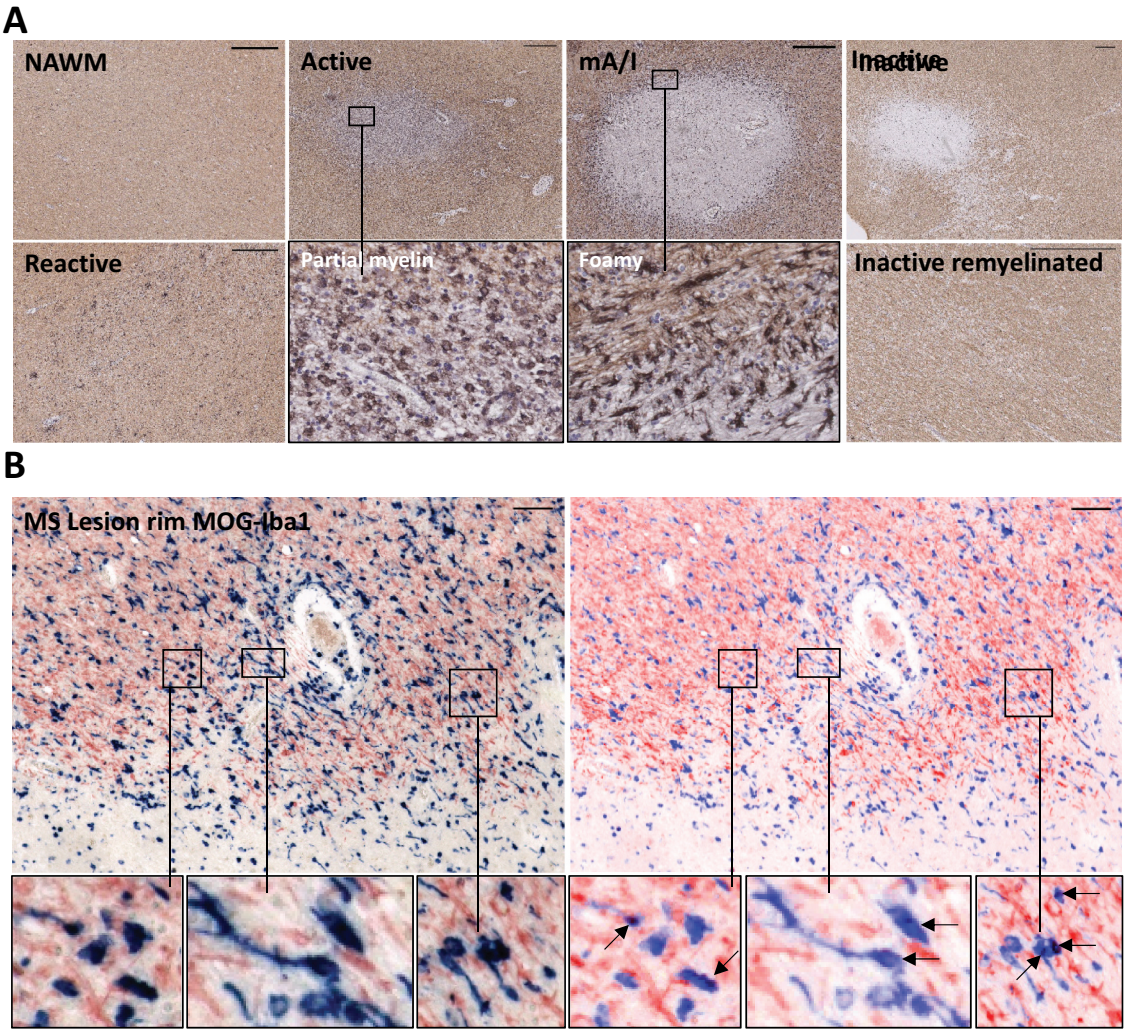

Figure 1. Characterization of postmortem MS white and gray matter lesions. (a) Immunohistochemical staining of formalin-fixed paraffin-embedded postmortem white matter MS tissue for HLA-DR (black) and PLP (brown). Panels show normal-appearing white matter (NAWM; no demyelination, no infiltration of HLA-DR$^+$ cells), reactive site (no demyelination, infiltration of HLA-DR$^+$ cells), active lesion (demyelination, infiltration of HLA-DR$^+$ cells throughout the lesion), mixed active/inactive lesion (mA/I; demyelination, infiltration of HLA-DR$^+$ cells at the lesion rim), inactive lesion (demyelination, no infiltration of HLA-DR$^+$ cells), and inactive remyelinated/shadow plaque (partial demyelination/loose myelin, no infiltration of HLA-DR$^+$ cells). Bar = 500 μM. (b) Double staining for MOG (red) and Iba-1 (blue), showing the original (left) and optimized (right) figures. The arrows show internalized MOG-positive fragments by Iba-1 positive cells, indicating active myelin uptake. Bar = 50 μM.

deposition on partial axon demyelination [29,30]. These findings contrast with observations in biopsy material. In the NBB collection, shadow plaques, suggestive of remyelinated lesions, were encountered least prevalent (16%)[28]. These lesions were significantly enriched in brain donors with a preserved relapsing–remitting disease course at autopsy. This finding is in line with the positive correlation of remyelinated area proportion with disease duration reported by Patrikios *et al.* in an autopsy cohort of n = 51 MS brain donors[31]. A longer disease duration between diagnosis and autopsy in postmortem studies is a marker of a less severe disease course[32]. Frisher *et al.* analyzed samples of n = 102 postmortem MS brain donors of the Vienna and Mayo MS autopsy collections, consisting of both, acute (those died within 1 year after diagnosis) and chronic MS cases, which showed a slightly different distribution of lesion types as compared to the NBB collection. Of all white matter lesions studied, active plaques were most prevalent (35%), followed by inactive lesions (35%), mixed active/inactive lesions (15%), and shadow plaques (14%)[33]. Where active lesions dominated the pathology in donors with a short MS duration, mixed active/inactive lesions were most prevalent in donors with a longer disease duration and a progressive disease course. Mixed active/inactive lesions can be considered as ongoing demyelinating or post-demyelinated lesions, based on the presence of myelin degradation products inside the microglia/macrophages [10,28]. The inverse correlation between remyelinating and mixed active/inactive lesions observed in NBB donors suggests that these lesion types may reflect two fundamentally distinct fates of active MS white matter lesion progression[28]. It remains to be consolidated whether ongoing active demyelination in the rim hampers remyelination or processes underlying remyelination suppress lesion activity.

It can be concluded that inflammatory disease activity in white matter lesions is still prevalent in advanced MS. The

relative contribution of these active and mixed active/inactive lesions to clinical disability progression in MS can be debated. Many studies point toward cortical demyelination as a critical pathological process in progressive MS. Although cortical demyelination is already present early in MS[34], it is far more extensive in progressive MS[35]. In primary progressive MS, Choi *et al*. reported a proportionally larger cortical area to be demyelinated, when compared to white matter [36]. Active cortical demyelination has been associated with the formation of follicle-like inflammatory structures in the overlying meninges [36–38]. These structures contain T-cell, B-cell, and plasma cell zones, which resembles tertiary lymphoid structures [38,39]. The presence of these follicle-like structures correlated with a more severe disease course, characterized by earlier onset of disease, faster accumulation of disability, and earlier death [36,38]. Progressive MS is also characterized by more diffuse instead of focal changes in the normal-appearing white matter[35]. However, the persisting relevance of focal white matter lesions in advances progressive MS is supported by the association of pathological findings with clinical characteristics. In the NBB tissue collection, donors with a high percentage of mixed active/inactive lesions showed a shorter time between first symptoms and walking with a stick or being wheelchair-bound and also displayed a shorter total disease duration[28]. Additionally, several prognostic factors associated with a faster accrual of disability during life were also associated with a higher proportion of active and mixed active/inactive lesions. Male MS brain donors showed a higher percentage of mixed active/inactive lesions in both the NBB and Vienna/Mayo cohorts [28,33]. MS brain donors with a progressive disease course showed a higher lesion load and a higher percentage of mixed active/inactive lesions when compared to donors without progressive disease. A similar association of mixed active/inactive lesions with progressive disease was observed in the Vienna/Mayo cohort[33]. Genetic polymorphisms, which have been associated with a more detrimental disease course of MS during life, also correlated with a higher proportion of either active or mixed active/inactive lesions. These include single nucleotide polymorphisms (SNPs) within genes, such as Fas, Kv channel-interacting protein-1 (KCN1P1), and C-type lectin domain-containing 16A (CLEC16A)[40].

The association of the inflammatory lesion activity in the mixed active/inactive lesion rim with disability progression and prognostic markers of disability progression supports its relevance for the disease process of MS. These observations corroborate the idea that mixed active/inactive white matter lesions are a relevant contributor to progressive MS [30,33]. Therefore, targeting this inflammatory response could be of therapeutic benefit for people with advanced MS. Acknowledging the clinical and pathological differences between early and end-stage MS can provide insight into the fundamentally different efficacy of current DMTs in modulating meaningful clinical endpoints. With the absence of gadolinium-enhancing lesions on MRI scan, suggesting absence of extensive local trafficking of infiltrating leukocytes into the PVS at sites of lesion formation in advanced MS, the role of lymphocytes also likely changes with the course of disease. We will focus on the role of T cells in advanced MS, as investigated recently in postmortem human autopsy studies.

4. T-cell presence in non-inflamed brain white matter

In the absence of inflammatory conditions, low numbers of T cells can be observed in postmortem human white matter (Figure 2) [41–44]. Although substantial variation exists, $CD8^+$ T cells in general outnumber $CD4^+$ T cells [41–44]. Approximately three T cells/mm^2 could be encountered in white matter of donors without brain diseases[42]. Under non-inflammatory conditions, most T cells in white matter are found in close association with the extra-luminal side of blood vessels[41]. Laminin staining revealed that the majority of T cells is located in the PVS [42], and that T cells only occasionally exist in the parenchyma (Figure 2).

Brain white matter T cells show a phenotypic profile consistent with tissue resident-memory T (T_{RM}) cells. In contrast to central memory and effector memory T cells (T_{CM} and T_{EM} cells, respectively), T_{RM} cells arise locally in a multitude of tissues after a primary infection and have the cardinal hallmark that they do not recirculate[45]. In skin, lung, gut, and vagina, among other barrier tissues, T_{RM} cells are believed to serve as sentinels to mount a swift immune response after re-exposure to their antigen [46,47]. They are characterized by a core transcriptional and phenotypic profile, of which expression of CD69 and CD103 are important markers, among many others [48]. We optimized our approach to isolate viable primary human microglia from postmortem rapid autopsy-acquired brain tissue for the isolation of viable brain T cells[49]. The clear phenotypic differences between T cells isolated from postmortem rapid autopsy-acquired blood samples and brain tissue supported the applicability of this approach to study brain T-cell phenotypes (Figure 3) [41,42]. Viable brain white matter T cells displayed a profile of surface markers and transcription factors resembling T_{RM} cells [41,42]. They express markers of memory cells (CD44, CD45RO, CD127), lack receptors for lymph node homing (CCR7), and expose molecules associated with tissue residency (CD49A, CD69, CD103, CTLA-4, PD-1). Functionally, postmortem human brain T_{RM} cells produced low levels of granzyme B but detectable amounts of granzyme K directly *ex situ* and made lots of interferon (IFN) γ and tumor necrosis factor (TNF) upon reactivation *in vitro*. Since T_{RM}-cell populations in other tissues have been described to arise after exposure to a wide range of viral or bacterial antigens[45], we reasoned the common human brain T_{RM} cells to be most likely directed against common neurotropic viruses. In experimental models of neurotropic virus infections, populations of specific T_{RM} cells are generated [50–54]. The dominant localization of human brain T_{RM} cells inside the PVS is a marked difference compared to the distribution of $CD8^+$ T_{RM} cells in other tissues, where these cells are scattered through the tissue. This tissue organization of T_{RM} cells in the human brain outside the context of acute neurotropic virus infection may be attributable to the unique characteristics of the PVS. Notably, although the PVS is

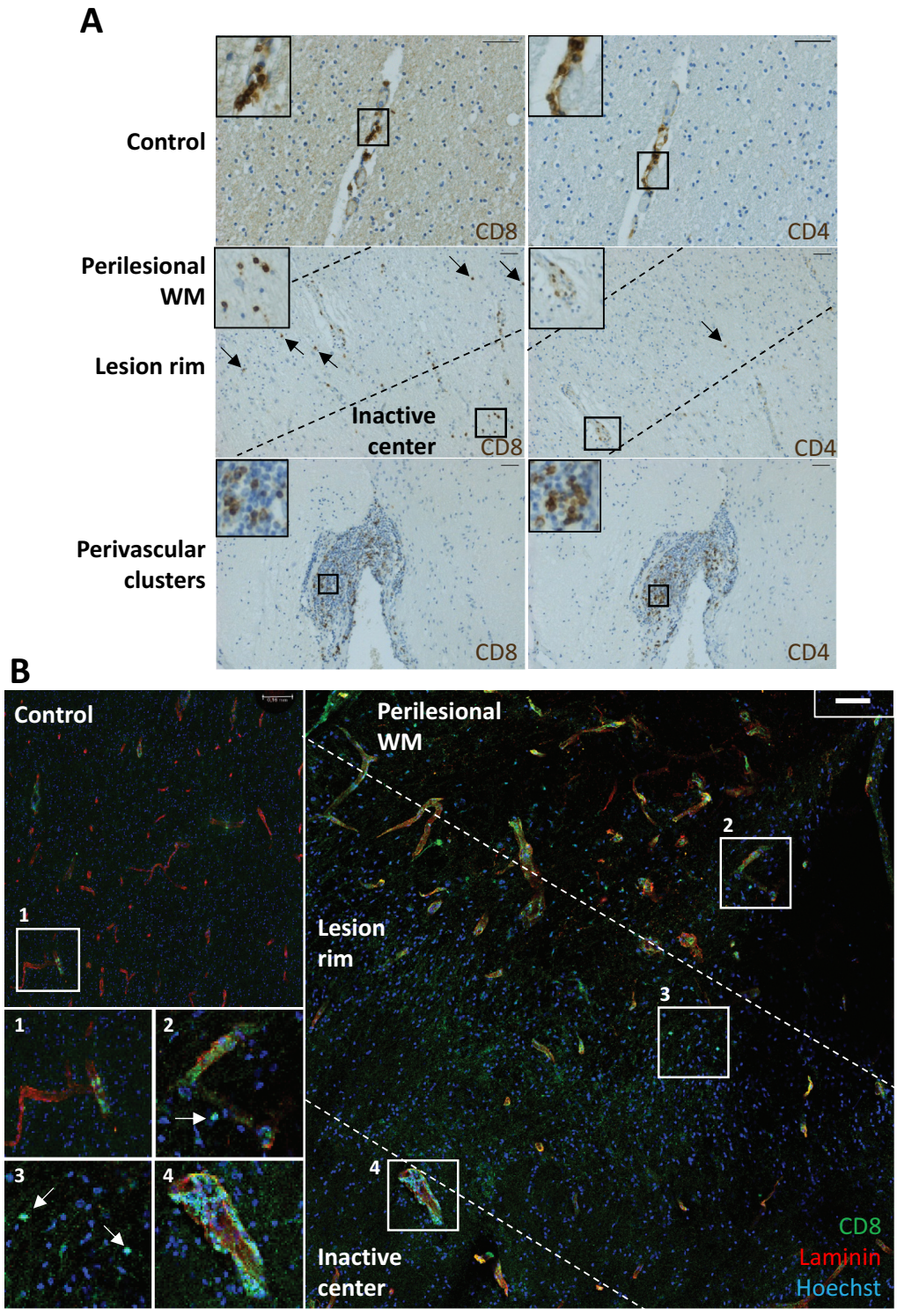

Figure 2. T-cell distribution in postmortem white matter control and MS tissue. (a) Staining for CD8 and CD4, showing perivascular distribution in control white matter, parenchymal localization of some $CD8^+$ and $CD4^+$ T cells in the rim of active lesions, and presence of both $CD8^+$ and $CD4^+$ T cells in perivascular MS clusters. (b) Double staining for CD8 (green) and laminin (red), confirming predominant localization of $CD8^+$ T cells in the PVS of a control donor. In a mixed active/inactive white matter lesion, $CD8^+$ T cells are observed in the parenchyma of the active rim and surrounding perilesional white matter. Bar = 50 μM.

a continuum with the subarachnoid space, the phenotype of T cells isolated from these compartment show substantial differences. In CSF acquired by lumbar puncture, $CD4^+$ T cells are more prevalent than $CD8^+$ T cells and show a contrasting T_{CM}-cell phenotype, including expression of CCR7 [55,56]. The small population of CSF $CD8^+$ T cells was also reported to display a T_{CM}-cell phenotype[56]. These CSF T-cell populations have been argued to enter the CSF via the choroid plexus and meningeal vessels[27]. How and whether these CSF T-cell population relate to the development and maintenance of white matter PVS T_{RM}-cell populations is not known. Additionally, whether T cells in meninges and choroid plexus also display a T_{RM}-cell profile has to our knowledge not been extensively studied.

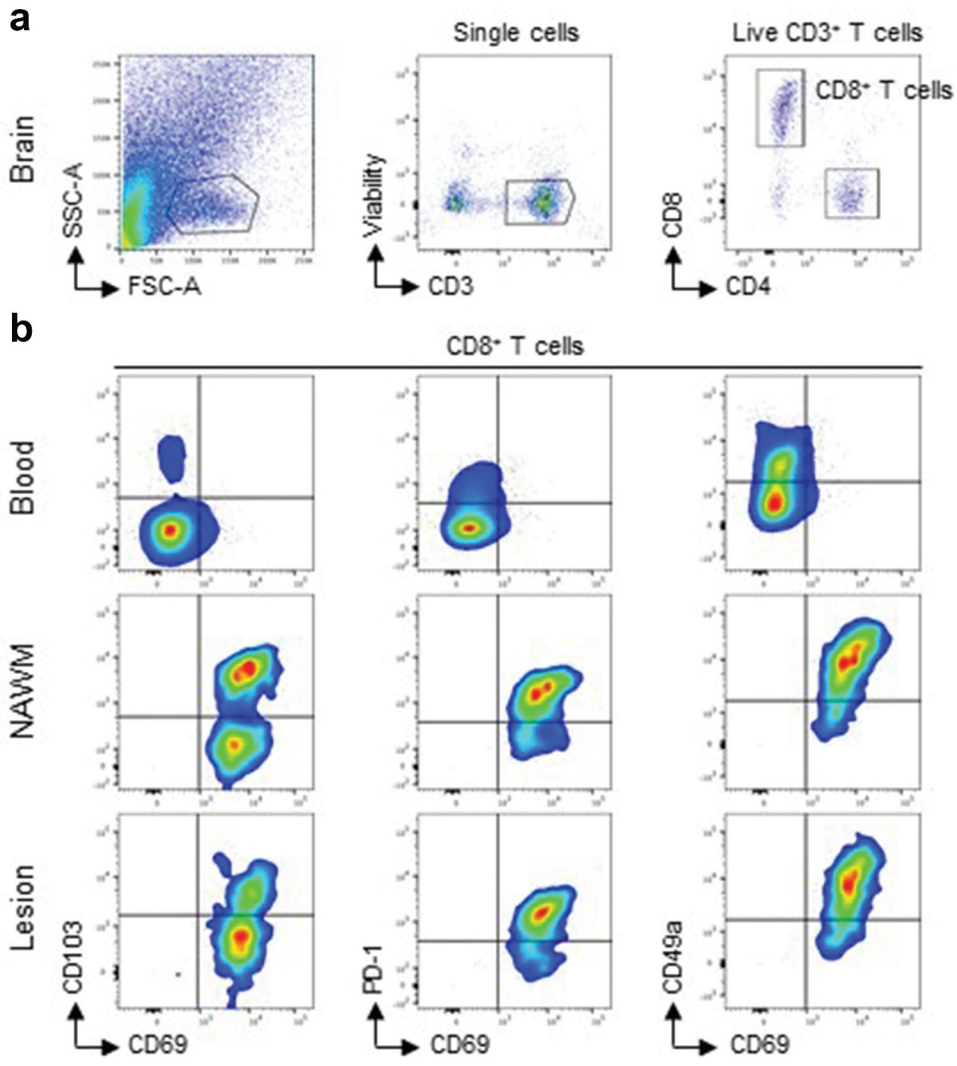

Figure 3. Flow-cytometric analysis of brain $CD8^+$ T_{RM} cells. (a) Gating procedure based on forward scatter (FSC), side scatter (SSC), viability, and expression of CD3, CD4, and CD8. (b) Expression of T_{RM} cell-associated surface markers on $CD8^+$ T cells isolated from blood, MS normal-appearing white matter (NAWM), and an active MS lesion [42,66].

5. The PVS as a physiological T_{RM}-cell niche in white matter

The PVS is the only compartment in the human body, which is bordered by two basal membranes, an endothelial and a parenchymal basement membrane (EBM and PBM), respectively[57]. These basement membranes are made of extracellular matrix molecules, including laminin, fibronectin, and collagen type IV[58]. On the luminal side, specialized endothelium with tight junction covers the EBM to form the BBB. On the parenchymal side, astrocyte end-feet form the glia limitans, covering the PBM. The glia limitans forms with astrocytic tight junctions a secluded barrier between the PVS and the parenchyma[59]. The PVS plays a crucial role in the drainage of the suggested CNS flow of interstitial fluid[60], which removes waste products from the parenchyma. Therefore, the PVS could be an excellent hub to screen for antigens. The PVS is populated by a variety of APCs, including specialized perivascular macrophages[61]. Although disputed, the presence of perivascular macrophages has been reported in the PVS of white matter venules[62]. The compartmentalization of T_{RM} cells in the PVS could be mediated by their signature surface markers. CD69 interferes with sphingosine 1-phospate receptor 1 (S1P1) to prevent tissue egress, CD49a is a receptor for collagen type IV, and the T_{RM}-associated molecule CD44 is a receptor for laminin[63]. Finally, a ligand for CD103 is E-cadherin, which has been described on activated $CD103^+$ lymphocytes, enabling cluster formation [57,63]. Interaction of these receptors with their ligands may mediate the homing and clustering of brain T_{RM} cells in the PVS. We assume them being under tight control by surrounding signals in the PVS, while awaiting potential reactivation. An interesting candidate for providing this local control of reactivation is the perivascular macrophage. The perivascular macrophage can present antigens yet can also express the inhibitory cytotoxic T lymphocyte-associated protein 4 (CTLA-4) ligand CD86, which may prevent activation of brain T_{RM} cells[64]. Moreover, activated astrocytes may present the

inhibitory programmed death-1 (PD-1) ligand PD-L1 to the T_{RM} cells via their end-feet at the glia limitans[42].

6. T cells in MS normal-appearing white matter and white matter lesions

In postmortem MS brain normal-appearing white matter, T cells are enriched[65]. On average 2–6 times as many $CD3^+$ T lymphocytes were encountered in MS normal-appearing white matter when compared to control white matter [33,36,66]. Like in control donors, these were more $CD8^+$ than $CD4^+$ T cells, and they were almost exclusively localized in the PVS (Figure 2). Perivascular cuffs of large clusters of lymphocytes, including T cells and B cells, are a known feature of neuroinflammatory disease, including MS[67]. Perivenular infiltrates, believed to contain infiltrating lymphocytes from the circulation, have been identified in white matter of both acute and chronic MS cases [68,69]. Despite advanced progressive MS not being associated with relapses or gadolinium-enhancing MRI lesions, perivascular cuffs were observed in some autopsy cohorts with advanced progressive MS [35,66]. Frischer *et al*. observed perivascular cuffs only in cases with active progressive disease[70]. In the NBB tissue collection, donors with perivascular cuffs in the brainstem had a higher brain stem lesion load and an overall higher proportion of mixed active/inactive lesions[66]. These observations suggest that presence of perivascular cuffing can be regarded as a detrimental phenomenon in advanced progressive MS, a clinical phenotypic entity not associated with attacks of infiltrating lymphocytes from the circulation.

The association of T cells with different white matter lesion types has been quantified both in the NBB and Vienna/Mayo postmortem MS-tissue collections, which show a comparable profile [66,70]. When compared to normal-appearing white matter, active MS lesions showed the most pronounced enrichment of T cells, followed by the mixed active/inactive lesion. Interestingly, there was no enrichment of T cells in inactive lesions. This enrichment comprised both $CD4^+$ and $CD8^+$ T cells, in which $CD8^+$ T cells were most prevalent [43,71–73]. Interestingly, the ratio of CD8/CD4 T cells was remarkably consistent within a donor between regions investigated[66]. Where brain T cells were located almost exclusively in the PVS in normal-appearing white matter, they infiltrated the parenchyma in both active and mixed active/inactive lesions (Figure 2) [43,66]. This was, however, not the case in inactive lesions. Altogether, these observations show that white matter lesion activity is associated with both T-cell number and distribution. Besides association with inflammatory lesions, a positive correlation between $CD8^+$ T cells and APP-positive neurons as marker of axonal damage has been reported [70,74,75]. This was not only the case in relapsing and secondary progressive cases but also in primary progressive cases[70].

7. MS white matter lesional T cells have a T_{RM}-cell profile

The phenotypic characteristics of T cells in MS white matter lesions in advanced MS have been analyzed by immunohistochemistry and by flow cytometry after rapid postmortem autopsies [41–43,76]. Several studies support a phenotypic profile consistent with T_{RM} cells, although there are several contrasting observations. Lesional T cells stained in autopsy cases lacked the T_{EM} cell-associated recirculation marker S1P1 [43,66]. Machado-Santos *et al*. reported absence of the lymph node homing receptor CCR7 on lesional T cells[43]. CD69 expression has been described by van Nierop *et al*. using immunostaining[76] and was confirmed by our group on all lesional T cells using flow cytometry[66], yet was not found with immunohistochemistry by Machado-Santos *et al* [43]. Immunostaining for CD103 was not observed on lesional T cells by van Nierop *et al*. but has been reported by Machado-Santos *et al*. and our group [43,66,76]. In our flow-cytometric studies, a sub-population of lesional T_{RM} cells expressed CD103[66]. Whether these inconsistencies between studies are contributable to technical issues or donor and tissue selection remains to be clarified. Expression of the T_{RM} cell-associated markers CD49a and PD-1 has been observed in lesional T cells with immunohistochemistry and flow cytometry [43,66]. Among the chemokine receptors expressed by these cells were CCR5, CXCR3, and CXCR6, which are all T_{RM}-cell phenotypic markers, possibly mediating homing into the parenchyma [43,66,77]. Previously, we showed the ligand for CXCR6, CCL16, to be upregulated by macrophages in the rim of mixed active/inactive lesions[78]. In the mouse experimental autoimmune encephalomyelitis model of neuroinflammation, the CCR5 and CXCR3 ligands CCL5 (RANTES), CXCL9, CXCL10, CXCL11, and CXCL12 were also expressed by resident macrophages[79]. Importantly, we were unable to identify clusters of cells lacking T_{RM}-cell characteristics among $CD8^+$ T-cell fractions isolated from MS white matter lesions[66]. When summarizing these characteristics, identification of white matter lesional T cells in autopsy tissue as T_{RM} cells appears valid. Recently, Bell *et al*. stained in n = 33 MS autopsy samples meningeal follicle-like structures for T cell-phenotypic markers[80]. Besides variable fractions of $CXCR5^+$ T-follicular helper cells and $CD27^+$ $CD8^-$ memory T cells, they observed meningeal follicle-like structures to be populated by $CD69^+$ $CD4^+$ T_{RM}-like cells. Further characterization of these T cells should reveal whether they also express other markers consistent with a T_{RM}-cell phenotype, and if and how they contribute to the cortical pathology of MS.

An important question is whether white matter T_{RM} cells are contributing to inflammation and demyelination in MS white matter lesions. In other tissues, re-encounter of T_{RM} cells with their antigen results in robust proliferation, cytokine release, and production of lytic enzymes[47]. With immunohistochemistry, Machado-Santos *et al*. observed low proportions of cells positive for the proliferation marker proliferating nuclear antigen (PCNA) in relapsing–remitting (median 1.45%) and progressive (median 0.5%) MS cases[43]. In our flow cytometry study, expression of proliferation marker Ki-67 was higher in $CD8^+$ T_{RM} cells isolated from MS lesions, compared to control white matter[66]. Immunohistochemical staining for Ki-67 revealed positive cells in the perivascular cuff in active lesions. These findings suggest antigen presentation and proliferation of T_{RM} cells in the context of mixed active/inactive lesion formation, but its extent is uncertain. An important site

of this reactivation could be perivascular cuffs, where CD103-positive T cells were observed in close association with HLA-DR positive cells[66]. These HLA-DR-positive cells double stained both with CD20 (B cells) and CD163 (perivascular macrophages). An increased number of $CD163^+$ $HLA\text{-}DR^+$ perivascular macrophages has been reported in active MS white matter lesions, in close association with perivascular T cells [64,81]. B cells have a well-characterized capacity of antigen uptake and MHC-dependent presentation[82], and could hereby serve an important role in the reactivation of brain T_{RM} cells.

The effector functions of white matter lesional T cells are uncertain. Although an increased rate of parenchymal infiltration suggests cellular cytotoxicity of small numbers of lesional $CD8^+$ T cells toward other parenchymal cells[66], diffusion of soluble molecules produced by the proportionally larger fraction of perivascular activated $CD8^+$ T cells has been proposed by Machado-Santos *et al*. as an effector mechanism[43]. Mixed results have been published on the role of granzyme B as lytic mediator in white matter lesions. Van Nierop *et al*. quantified immunohistochemical stainings for granzyme B in mixed active/inactive white matter lesions and reported perivascular and parenchymal T cells to express granzyme B[76]. The majority of cells displayed a punctate pattern of granzyme B immunostaining, with a fraction of cells showing evidence of granzyme B polarization. Machado-Santos *et al*. found with immunostainings a median average of 4.2% (range 0–30%) of $CD8^+$ T cells to express granzyme B, while this was in chronic MS cases only observed in 1.7% (range 0–27%)[43]. Salou *et al*., reported infiltration of granzyme B-positive $CD8^+$ T cells in white matter lesions, but showed no quantification[83]. Applying immunohistochemistry, we observed in active MS lesions a very low median number of 0.017 (IQR 0.012–0.026) granzyme B-positive cells/mm^2[66]. Additionally, flow-cytometric analysis of isolated $CD8^+$ T_{RM} cells showed no enrichment for granzyme B in white matter MS lesions, compared to normal-appearing white matter and control donors. These inconsistencies between studies warrant further investigation.

We showed lesional $CD8^+$ T_{RM} cells to upregulate the adhesion family G protein-coupled receptor GPR56[66], which on circulating lymphocytes indicates cytotoxic capacity[84]. It is uncertain whether non-circulating GPR56-positive $CD8^+$ T_{RM} cells bear cytolytic activity in the PVS and parenchyma. Human brain $CD8^+$ T cells expressed in our studies almost no perforin [41,42], although this lytic mediator is important in the control of neurotropic virus infections by brain T_{RM} cells in animal models [50,51]. Perforin and granzymes synergize to mediate apoptosis of target cells. Notably, Magliozzi *et al*. reported immunostaining of meningeal $CD8^+$ T cells for granzyme B, perforin, and the degranulation marker CD107 in association with Ig-positive cells in n = 5 MS cases[85]. Konjevic Sabolek *et al*. reported immunostaining for perforin in white matter lesional $CD8^+$ T cells of several cases with acute but also progressive MS[86]. Brain $CD4^+$ and $CD8^+$ T_{RM} cells did express granzyme K in our earlier studies [41,42]. Expression of granzyme K by lesional T cells remains to be shown, but a possible relevance of this lytic mediator is suggested by the expanded fraction of granzyme K-positive $CCR5^+$ $CD4^+$ T cells in the circulation of MS patients[87]. In sum, conflicting evidence exists regarding lytic molecule production by white matter $CD8^+$ T cells. Interestingly, Van Nierop *et al*. showed white matter lesion $CD8^+$ T cells to express high levels of Fas ligand (FasL, CD95 L), which may lead to Fas (CD95)-mediated target cell lysis[76]. Furthermore, production of cytokines is well possible, since human brain $CD4^+$ and $CD8^+$ T_{RM} cells rapidly make IFNγ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF upon stimulation *in vitro*[42]. Production of IFNγ by brain T_{RM} cells is a critical component in the control of neurotropic virus infections[51]. However, cytokine production by white matter lesional T cells *in situ* has not yet been investigated.

8. White matter T_{RM} cells as potential targets for MS therapies

The events leading to the establishment of T_{RM} cell-containing perivascular cuffs in MS are not known. These populations of T_{RM} cells most likely evolve from the circulating populations of T cells invading the perivascular space in early MS, and can possibly already be established at the earliest phases of MS[9]. Notably, the study by Machado-Santos *et al*. included also some donors with a fairly short disease duration[43], suggesting that population of the PVS by T_{RM} cells could be starting at an early stage of MS. We observed CD103-positive T cells in infiltrates of early MS biopsies, albeit less frequently when compared to autopsy material of advanced MS cases[66]. Since a high relapse rate and gadolinium-enhancing lesions are risk factors for developing progressive disease[5], a timely intervention on these endpoints could potentially reduce T_{RM}-cell formation in the course of MS and hereby their possible contribution to progressive disease. This is also suggested by the lower point estimate of secondary progressive MS in the DMT era[4], and the efficacy of early treatment with ocrelizumab in delaying disability progression in primary progressive MS[88]. Therefore, early treatment with DMTs could theoretically prevent the establishment or maintenance of perivascular T_{RM}-cell cuffs in the course of MS. It is unlikely that current DMTs affect the mobilization of perivascular T_{RM} cells from the PVS into the parenchyma in progressive MS. Regarding the limited penetrance of these compounds through the BBB, their effects on events in the PVS and parenchyma are presumably limited. Although fingolimod reaches the PVS[89], the absence of S1P1-receptor expression on T_{RM} cells [43,66] and the migration of parenchyma-invading lymphocytes away from the sphingosine phosphate gradient[90] makes a relevant functional interference of this drug with T_{RM} cell-migratory behavior unlikely. Metz *et al*. observed only very few $CD8^+$ T cells in the postmortem CNS of patients treated with autologous hematopoietic stem cell transplantation, suggesting at least some depletion by this treatment regimen[91]. Of note, besides a major role for local homeostatic proliferation, recruitment of memory T cells from the circulation to contribute to secondary T_{RM} cells has been described [47,92]. Via interference with this replenishment, DMTs could potentially

reduce the sustainability of the PVS T_{RM}-cell pool. Since reactivation, proliferation, and mobilization of brain T_{RM} cells can be a critical component in the maintenance of active and mixed active/inactive lesion in progressive MS, drugs interfering with these processes could be of benefit for patients with progressive disease. We just start to learn the exact phenotype of these cells and identify potential markers, which could be therapeutic targets.

In recent years, the therapeutic arsenal for T cells has been expanded by drugs that target molecules involved in activation, inhibition, and migration. In oncology, a major development has been the use of immune checkpoint inhibitors to enhance cytotoxicity. Several lines of evidence suggest that drugs that target the PD-1–PDL-1 and the CD28–CTLA-4 pathway also modulate the behavior of T cells in the CNS. The development of inflammatory CNS lesions as side effect is part of this evidence. Treatment with the CTLA-4 inhibitor ipilimumab has been associated with the occurrence of inflammatory demyelinated white matter lesions with T-cell infiltrates [93–95]. During treatment with the PD-1 inhibitor nivolumab, white matter T-cell infiltration with demyelination and macrophage activation has been described[96]. Also treatment with the PD-1 inhibitor pembrolizumab resulted in inflammatory demyelinating lesion of the CNS[97]. Potentially beneficial activation of CNS T cells has also been described. In a proportion of patients with a progressive multifocal leukoencephalopathy (PML) due to reactivation of the JC polyomavirus, treatment with pembrolizumab boosted JC-specific T-cell responses together with a down-regulation of PD-1 [98]. Additionally, despite not modulating total tumor-infiltrating cells quantitatively, treatment with neo-adjuvant pembrolizumab therapy resulted in potentially beneficial T-cell phenotypic changes in patients with recurrent glioblastoma[99]. Small molecules and viral vector-induced ligands boosting rather than inhibiting check points could reach and modulate T cells within the PVS in a beneficial way to suppress their reactivation in the CNS.

Several chemokine receptors, which are highly expressed by human brain T_{RM} cells and are part of their core phenotypic profile, have been targeted in the context of inflammatory diseases. $CD103^+$ T_{RM} cells highly express CCR5[42], which can also act as a receptor for infection of $CD4^+$ T cells by the R5-tropic human immunodeficiency virus (HIV). Maviroc is a drug, which blocks the CCR5 receptor and hereby prevents the virus from infecting T cells. In patients suffering from an immune reconstitution inflammatory syndrome (IRIS) after cessation of natalizumab due to PML, some case reports suggest an attenuation of the detrimental influx of inflammatory T cells in the CNS [100–102]. Small-molecule inhibitors for the CXCR6–CXCL16 pathway could potentially attenuate migration of reactivated T_{RM} cells into the parenchyma. Antibodies directed against CXCL6 are available but may not reach the PVS and lesions[103]. CXCR3 is a core phenotypic T_{RM}-cell marker, which is also expressed at high levels on brain T_{RM} cells [42,48]. In the murine skin, lack of CXCR3 expression in $CD8^+$ T cells was associated with a reduced T_{RM}-cell formation [104]. In an adoptive transfer but not an actively immunized experimental autoimmune encephalomyelitis model, treatment with anti-CXCR3 inhibited T-cell infiltration into the CNS and reduced disease severity[105]. CXCR3 has several ligands; CXCL4 is expressed by microglia[106], and CXCL9, CXCL10, and CXCL11 have been associated with the infiltration of the CNS by T cells in various inflammatory diseases including MS[107]. Targeting these chemokine receptors or their ligands with small molecules could hypothetically be of benefit for progressive MS.

9. Challenges for the upcoming years

Recent postmortem neuropathological studies made a case for mixed active/inactive lesions, fueled by reactivation of populations of T_{RM} cells in the PVS, as contributors to the disease process in advanced/progressive MS (Figure 4). The identification of T_{RM}-cell recruitment from the PVS offers possibilities to better understand the role of T cells in advanced MS, but also to develop new approaches to target the contribution of these cells disease process of progressive MS. There are however several questions that do still require clarification.

An urgent question is the identification of the antigen(s) against which T-cell responses in MS and specifically the T_{RM}-cell response is mounted. In other tissues, T_{RM} cells have been mostly studied in and associated with virus infections [45,46]. Therefore, a viral antigen appears tempting. A vast body of literature associates MS with Epstein-Barr virus (EBV) infection[108]. Accumulation of EBV-infected B cells and EBV-directed $CD8^+$ T cells has been described in MS CSF [109–111] and in MS lesions [76,85,112–114], although the reproducibility of these findings has also been debated [115–117]. Other viruses have also been associated with MS, including human herpesvirus (HHV)-6[118]. Moreover expression of endogenous retrovirus sequences has been described in MS lesions[119], which may also elicit a $CD8^+$ T-cell response[120]. Alternatively, a potential role of autoantigen-directed T_{RM} cells in autoimmune diseases has not been explored extensively yet.

Since brain $CD4^+$ and $CD8^+$ T_{RM} cells are physiological residents of the normal human PVS [41,42], therapeutic strategies interfering with their functional profile may disrupt physiological functions of these cells. In MS, the importance of physiological T-cell trafficking to the CNS became eminent with the development of PML in natalizumab-treated patients[121]. Therefore, a role of physiological T_{RM}-cell pools in immune surveillance of the CNS can be anticipated. The risks of interfering directly with this surveillance are not known. Although JC virus has been propagated to be retained in an inactive state in the kidneys[122], postmortem human studies also revealed JC virus genetic fragments in brains of 28–68% of asymptomatic cases [123–125]. The latter observations suggests JC virus to latently infect the human CNS, flaring up in the case of PML.

Lastly, there is a timeframe gap of knowledge in the immunopathology of MS. Thanks to the availability of biopsy material, the pathology of the earliest phases of MS has been extensively studied. Postmortem autopsy studies have provided much insight in the pathology of MS at its end-stage. Differences between these extreme groups can be identified, and the first group is likely to evolve into the

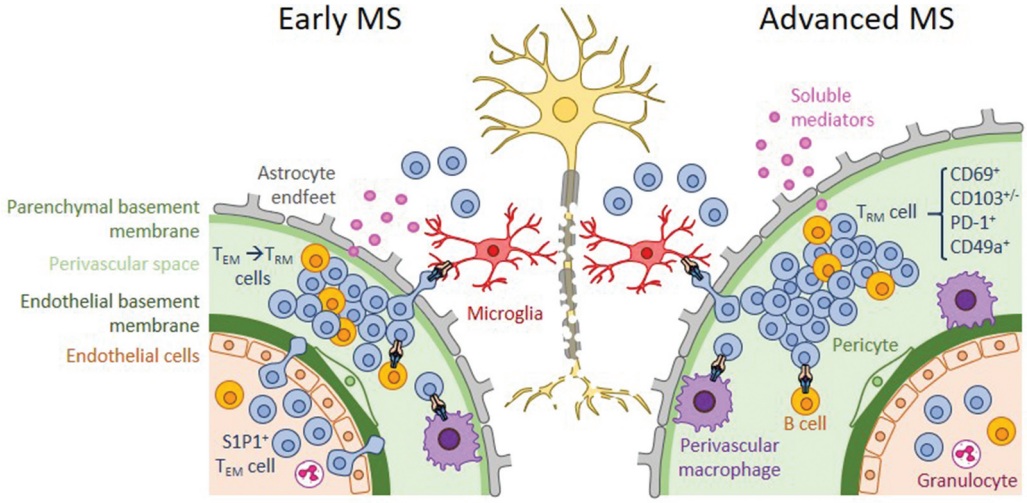

Figure 4. Concept of compartmentalized immune activation in advanced MS white matter lesions. In early MS, shown to the left, activated T_{EM} cells and effector T cells cross the endothelium of the blood brain barrier at the postcapillary venules and enter the perivascular space (PVS), forming perivascular infiltrates in acute lesions. These infiltrating T cells may give rise to a local T_{RM}-cell population. The extent to which T_{RM} cells contribute to acute infiltrates in early MS is incompletely understood. In chronic active lesions of advanced MS, shown to the right, T-cell trafficking is not evident, and perivascular cuffs are populated by T_{RM} cells. Perivascular T_{RM} cells are reactivated by APCs and contribute to inflammatory lesion formation, either locally in the PVS or upon entering the parenchyma, through producing soluble effector molecules and/or displaying cellular cytotoxicity.

latter. However, what happens in the intermediate years or even decades is uncertain and has been highlighted as 'black box' in MS-pathology research[126]. The dynamics of findings in human circulating lymphocytes must be interpreted in correlation with the natural history of MS and phenotypic characteristics of cells observed within the PVS. Circulating cell fractions associated with MS-disease activity must ultimately give rise to the T_{RM}-cell populations as they are encountered in MS. We have limited data on the presence of T_{RM} cells in white matter lesions at the early stages of MS, as well as their association with pathological patterns of early MS. Cells with T_{RM} cell-related characteristics have been observed in the blood and CSF of people with MS, as indicated by the enhanced presence of circulating $CD4^+$ T cells expressing high levels of CCR5 and granzyme K[87]. Additionally, clonally expanded $CD8^+$ T cells with T_{RM}-cell characteristics could be isolated from the CSF of twins with prodromal MS[127].

10. Conclusions

Our understanding of the pathology of MS has enormously benefited in recent years from studies of large tissue collections. These initiatives allowed to capture common elements as well as heterogeneous components of the pathology of MS. They also warrant a reflection of gratitude to all MS patients who donated CNS tissue for research to better understand the disease they suffer(ed) from. Pathological data on demyelination and myeloid cell activation point toward the mixed active/inactive lesion as a detrimental phenomenon in advanced progressive MS. Recent immunohistochemical and flow-cytometric studies revealed brain T_{RM} cells not only to be physiological residents in the human brain PVS but also to be numerically and spatially associated with mixed active/inactive MS lesions. Phenotypic changes of T_{RM} cells in correlation with these lesions suggests an active role of $CD8^+$ T_{RM} cells in lesion formation and/or maintenance. Further understanding of the functional dynamics of brain T_{RM} cells may offer intriguing new avenues to target mixed active/inactive lesion formation in advanced MS, for which current DMTs show in general a disappointing efficacy.

11. Expert opinion

The treatment of MS saw many important advances over the last decades, with an exponential growth of the number of DMTs registered. Except for interferon beta (IFNβ), glatiramer, and natalizumab, current DMTs have originally been developed within other fields in medicine. These drugs mostly target lymphocyte activation or migration. At present, T_{RM} cells are a subject of study in many organs. Although a role in the control of (viral) infections is best consolidated [45,46], a contribution of T_{RM} cells to local inflammatory reactions in autoimmune diseases has not been extensively explored. Certainly, T_{RM} cells will receive attention in inflammatory diseases in other organs with the aim to affect their behavior. These approaches likely will elude new classes of treatments, targeting specifically local inflammatory cells and mechanisms. An important CNS-specific bottle-neck will be the development of drugs that cross the BBB and reach the PVS. Not only the activation, mobilization, and inflammatory potential of T_{RM} cells themselves may be a target of therapy but also the crosstalk with other inflammatory players in the PVS and brain parenchyma. In the PVS, perivascular macrophages and B cells can present antigens, provide co-stimulatory/inhibitory signals, and/or make cytokines controlling the activation and recruitment of T_{RM} cells. Likely, myelin-laden microglia/macrophages in mixed active/inactive lesions are particularly important. They not only may provide signals critical for T_{RM}-cell activation, but also may receive signals from T cells amplifying their phagocytic potential. The dynamics of microglia

morphology and phenotype in relation to demyelinating lesion formation is only poorly understood [11,128].

A challenge for therapies directly targeting brain T_{RM} cells will be to preserve their physiological roles. Attenuating their inflammatory potential without compromising too much normal immune surveillance may suppress mixed active/inactive lesion formation without reactivation of latent neurotropic viruses. Therefore, it is important to comprehensively unravel the phenotype and functional programs of T_{RM} cells associated with MS lesions. In the end, modulating T_{RM}-cell activation and migration into the CNS parenchyma may suppress a component of disease activity but likely will not cure MS. However, disclosure of critical antigens and the cells presenting them may bring the field closer to the cause of MS. As discussed above, neurotropic viruses as well as the lymphotropic virus EBV are likely candidates.

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