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Contribution of CD8 T lymphocytes to the immuno-pathogenesis of multiple sclerosis and its animal models

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Abstract

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by multi-focal demyelination, axonal loss, and immune cell infiltration. Numerous immune mediators are detected within MS lesions, including $CD4^+$ and $CD8^+$ T lymphocytes suggesting that they participate in the related pathogenesis. Although $CD4^+$ T lymphocytes are traditionally considered the main actors in MS immunopathology, multiple lines of evidence suggest that $CD8^+$ T lymphocytes are also implicated in the pathogenesis. In this review, we outline the recent literature pertaining to the potential roles of $CD8^+$ T lymphocytes both in MS and its animal models. The $CD8^+$ T lymphocytes detected in MS lesions demonstrate characteristics of activated and clonally expanded cells supporting the notion that these cells actively contribute to the observed injury. Moreover, several experimental *in vivo* models mediated by $CD8^+$ T lymphocytes recapitulate important features of the human disease. Whether the $CD8^+$ T cells can induce or aggravate tissue destruction in the CNS needs to be fully explored. Strengthening our understanding of the pathogenic potential of $CD8^+$ T cells in MS should provide promising new avenues for the treatment of this disabling inflammatory disease.

Keywords

T lymphocyte; Cytotoxic T cell; Autoimmunity; Central nervous system; Demyelination; Suppressor cell

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) characterized by multi-focal demyelination, axonal loss, and activation of glial cells.

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Whereas components of the immune system are detected within MS lesions, the contribution of each of these immune mediators to injury remains to be defined [1]. A vast body of evidence gathered from the experimental autoimmune encephalomyelitis (EAE) mouse models points to the crucial role of $CD4^+$ T cells in the disease pathogenesis. This considerable literature has led the scientific community to transpose these observations to the human disease MS [2]. EAE is an autoimmune demyelinating disease induced by the active immunization of animals with myelin protein extracts or immunodominant myelin peptides emulsified in complete Freund's adjuvant (CFA) [3]. The identification of $CD4^+$ T cells as main culprits in EAE pathogenesis comes from the fact that immunization with major histocompatibility complex (MHC) class II restricted peptides induces EAE in genetically susceptible hosts [4]. Alternatively, the adoptive transfer of activated myelin-specific $CD4^+$ T cells is sufficient to induce the disease in autologous hosts. Moreover, the implication of $CD4^+$ T cells in the pathogenesis of MS is supported by the strongest genetic risk factor so far described being conferred by specific MHC class II alleles [1]. However, MS patients treated with an anti-CD4 depleting antibody did not gain any clinical benefits although the depletion was efficient [5–8]. Thus, at least in the human disease, the picture is far more complex and $CD4^+$ T lymphocytes are not the only perpetrators involved in the pathogenesis. A growing body of evidence suggests that $CD8^+$ T lymphocytes partake in MS related CNS damage raising interest in the scientific community [9,10]. This review provides an overview of the recent literature documenting the potential roles of $CD8^+$ T lymphocytes both in MS and its animal models.

1. $CD8^+$ T lymphocytes as effector cells

1.1. $CD8^+$ T lymphocytes: crucial immune cells

$CD8^+$ T lymphocytes recognize, via their T cell receptor (TCR), antigens (peptides of 8–11 amino acids) that are presented by MHC class I molecules [11,12]. The activation of a naïve $CD8^+$ T lymphocyte requires at least two signals provided by a professional antigen presenting cell (APC): the first one being the TCR engagement by a recognized peptide–MHC class I complex and the second one provided by the interaction between co-stimulatory molecules and co-activating receptors. Such efficient stimulation triggers a complex cascade of intracellular signaling leading to the maturation (change in surface molecules), proliferation, and production of mediators by cytotoxic T lymphocytes (CTL). Once adequately activated, $CD8^+$ T cells survey the body and kill encountered target cells expressing the appropriate peptide–MHC class I complex. Since most nucleated cells express or can express MHC class I molecules, they represent potential targets recognized by $CD8^+$ T lymphocytes. Several mechanisms are deployed by CD8 T cells including the release of lytic enzymes such as perforin and granzymes and the Fas–FasL interaction [13,14]. Also, activated $CD8^+$ T cells secrete several pro-inflammatory cytokines including interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-2 (IL-2). Through these combined effector functions $CD8^+$ T cells play a crucial role in the control of intracellular pathogens and neoplasic cells [15,16]. A fraction of the activated $CD8^+$ T lymphocytes persists as memory cells, which provide protection with an enhanced response upon secondary challenge. Memory $CD8^+$ T cells are also subdivided into central and effector

memory subsets, each group bearing specific markers [17]. Finally, subsets of $CD8^+$ T cells carrying suppressor or regulatory properties have been described.

Numerous publications have documented that activated $CD8^+$ T cells can utilize distinct mechanisms to recognize and attack CNS cells [18,19]. Murine neurons are susceptible to $CD8^+$ T cell Fas/FasL mediated cytotoxicity but are protected from a perforin-induced cell death by the same effector cells [20] unless FasL is not expressed by these neurons [21]. In contrast, murine astrocytes quickly respond to degranulation (release of lytic enzymes) by cytotoxic $CD8^+$ T cells [21]. Cell death of human neurons has been observed upon the addition of granzyme B [22], another lytic enzyme than perforin. Moreover, cytotoxic $CD8^+$ T cells can selectively attack neuronal neurites supporting the notion that axonal damage observed in MS lesions could be mediated by these cells [23]. We have recently shown that primary cultures of human adult oligodendrocytes express ligands for NKG2D while neurons, microglia and adult astrocytes do not. By disrupting the NKG2D–NKG2D ligand interaction, we could specifically impede the CD8 T cell-mediated killing of oligodendrocytes, but not that of other CNS cells not expressing these ligands [24]. These observations illustrate the specific exchanges occurring between cytotoxic CD8 T cells and distinct CNS cells and the subsequent diversified cell death pathways induced.

1.2. Characteristics of peripheral effector CD8 T lymphocytes in MS

Although it is possible that in the highly inflamed CNS, APC on site could activate/reactivate T cells, the first aggressive T cells infiltrating this organ most likely would have been efficiently activated in the periphery. Therefore, assessment of immune responses in MS patients in the peripheral compartment is highly relevant and could identify specific immune properties attributed to this autoimmune disease. Moreover, the feasibility of obtaining repetitive peripheral blood samples from patients makes this approach an attractive tool to assess and monitor disease development. Several groups reported that the peripheral T cell repertoire in MS patients varies according to disease activity [25–27] supporting the notion that the brain inflammation is associated with changes in the peripheral lymphocytes. Very early during MS development, skewing of the TCR repertoire, more importantly in the blood derived $CD8^+$ T cell compartment is observed in these patients compared to controls. These observations suggest that aberrant responses in the $CD8^+$ T cell subset already exist at the beginning of the disease [28]. Moreover, in the peripheral blood of MS patients increased levels of $CD8^+CCR7^+CD45RA^-$ (central memory T cells) are detected compared to healthy controls [29], advocating that these patients carry an enhanced proportion of previously activated $CD8^+$ T cells compared to controls.

Killestein and colleagues examined the cytokine profile of $CD4^+$ and $CD8^+$ T cells upon a short *in vitro* stimulation in relationship to magnetic resonance imaging (MRI) features of tissue destruction and disability score in MS patients [30]. The cytokine profile of $CD8^+$ T cells rather than of their $CD4^+$ counterparts was more predictive of future lesion development in MS patients. Moreover, the expression of specific chemokine receptors: CCR5 and CXCR3 on $CD8^+$ T lymphocytes was enhanced in MS patients compared to controls [31] and this increase correlated with new lesion development as assessed by MRI. In order to invade the CNS from the periphery, T cells need to adhere to the CNS

endothelium and then cross the blood brain barrier. Interestingly, $CD8^+$ T cells but not $CD4^+$ T cells from relapsing-remitting MS patients exhibited an augmented capacity to roll and arrest on inflamed brain venules via a P-selectin glycoprotein ligand-1 dependent mechanism [32]. These observations are compelling to support the notion that $CD8^+$ T cells from MS patients have enhanced capacity to cross from the periphery to the CNS compare to their $CD4^+$ counterparts. Taken together, there is considerable evidence implicating distinct characteristics in peripheral $CD8^+$ T cells from MS patients that could predict the development of CNS lesions.

1.3. MHC class I association with MS

As reviewed above, $CD8^+$ T cells recognize specific antigens that are presented by autologous MHC class I molecules (HLA-A, B, and C in humans or H-2D, H-2K, and H-2L in mice). The MHC alleles carried by the host dictate which peptides (self or foreign) and the affinity with which they are presented to autologous $CD8^+$ T cells. Therefore, specific alleles could play a key role in the modulation of auto-aggressive immune responses. An increased prevalence of specific HLA-A alleles within the MS population compared to controls has been reported more than 30 years ago [33]. In the last decade, using modern genetic tools different groups confirmed the association of specific alleles with this disease. HLA-A*0301 has been shown to increase the risk of developing MS in addition to HLA-DR15 [34] or independently of the HLA-DRB1*15, DQB1*06 [35,36]. In contrast, the HLA-A*0201 allele decreases the risk of developing MS [37] and reduces the risk for DRB1*15, DQB1*06 carrier [35]. Overall, these observations suggest that specific MHC class I alleles could impact on the presentation of self-antigens to auto-aggressive $CD8^+$ T cells involved in MS.

1.4. Reactivity of $CD8^+$ T cells to CNS antigens

Most peptides loaded on MHC class I molecules originate from intracellularly transcribed proteins; however, some phagocytosed proteins can also gain access to the MHC class I loading machinery and be presented by cross-presentation, especially by professional APC [38]. These mechanisms allow for CNS specific self-antigens to be efficiently presented to CTL by professional APC, especially when the self-antigens are derived from cells under attack in MS lesions such as oligodendrocytes and neurons. Proteins from the myelin sheath, which is produced by oligodendrocytes in the CNS, have been studied as potential targeted antigens in the context of MS and include: myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and myelin associated glycoprotein (MAG). Identifying the myelin-derived target epitopes for a given HLA class I allele permits the identification and enumeration of antigen-specific $CD8^+$ T cells in a given patient. Early studies focused on HLA-A2, because it is the most frequent HLA class I allele among Caucasians. Using prediction-algorithms [39,40], epitopes derived from candidate target-antigens for MS were identified and, after synthesis, their HLA-A2 binding properties were established. HLA-A2-restricted $CD8^+$ T cell lines or clones could thus be generated against $MAG_{287–295}$, $MAG_{509–517}$, $MAG_{556–564}$, $MBP_{87–95}$ $MBP_{110–118}$, $PLP_{80–88}$ [41–43], and $Transaldolase_{168–176}$, (an enzyme of the pentose phosphate pathway expressed at high levels by oligodendrocytes) [44]. Moreover, the frequency of $CD8^+$ T cells specific for HLA-A*0201: $MBP_{110–118}$ or $Transaldolase_{168–176}$, as well as other myelin and neural-antigens,

appears more elevated in the peripheral blood of MS patients relative to healthy controls [41,43,45,46]. Importantly, human myelin-specific $CD8^+$ T cells recognizing the HLA-A*0201:$MBP_{110–118}$ complex could induce lysis of HLA-matched oligodendrocytes *in vitro*, in the absence of exogenous antigen [47], indicating that this epitope is naturally processed by this glial cell subset. Furthermore, myelin-specific $CD8^+$ T cells carried effector functions including production of IFN-γ and TNF, proliferation and cytotoxicity when exposed to various epitopes of MAG, MBP and PLP (among others) [41,43,45]. Moreover, myelin-specific $CD8^+$ T cells bore a naïve phenotype in control subjects whereas in MS patients, they were activated and had an effector or memory phenotype [43,45,48], suggesting that in patients these cells have already been actively exposed to their cognate antigen. However, a recent analysis of myelin-specific $CD8^+$ T cell responses reported that MS patients and healthy controls had the same frequency of IFN-γ secreting $CD8^+$ T cells using peripheral blood in response to a large array of myelin peptides derived from MOG, MBP and PLP and presented by several HLA class I alleles (A3, A2, B7, B27 and B44) [49]. Other studies failed to detect differences between MS and controls regarding the $CD8^+$ T cells responses to neuronal or oligodendroglial antigens [50]. Thus, an increased peripheral frequency of autoreactive $CD8^+$ T cells targeting myelin or neuronal epitopes has been occasionally observed in MS patients compared to controls [41,43,45,46]. Additional studies will be required to determine whether autoreactive $CD8^+$ T cells from MS patients exhibit distinct properties, not only in frequency but also regarding their effector properties, compared to controls.

1.5. Antigen presentation to $CD8^+$ T cells

Similarly to $CD4^+$ T cells, activation of naïve $CD8^+$ T cells requires an efficient interaction with a professional APC [14,51]. APC located in different areas in and around the CNS may present CNS-derived antigens to $CD8^+$ T cells during MS or its animal models. Despite the immune privileged status of the CNS, part of the cerebrospinal fluid (CSF) drains to the cervical lymph nodes located in the neck. At this location, antigens gathered from the CNS can be presented to the peripheral immune cells. Interestingly, cervical lymph nodes obtained from MS patients and animals (marmosets and mice) affected with EAE contain myelin-laden APC. These APC have engulfed myelin proteins (PLP, MOG, MBP, among others) and expressed molecules such as IL-12, CD40, and MHC class I and class II molecules suggesting that they are competent APC [52,53]. Indeed, a remarkable study indicated that after gathering antigen from a tissue, APC migrate to the draining lymph node and cross-present antigens to $CD8^+$ T cells [54]. Upon peptide presentation to antigen-specific $CD8^+$ T cells, these APC imprint a particular integrin profile that directs these $CD8^+$ T cells to the tissue from which the antigen was initially gathered. Accordingly, APC that collected antigen from the CNS and presented them in the cervical lymph nodes directed antigen-specific $CD8^+$ T cells back to the CNS [54]. This implies that competent myelin-laden APC located in the cervical lymph nodes of MS patients may efficiently present myelin epitopes and activate myelin-reactive T cells (including $CD8^+$ T cells) and guide them to the CNS. Once in the CNS, these $CD8^+$ T cells can be re-activated locally by the numerous myelin-laden APC (macrophages and microglia) that are around lesions [55]. Moreover, APC in the CNS have been shown to be able to phagocyte and then cross-present antigens gathered locally, activate peptide-specific $CD8^+$ T cells and trigger a cytotoxic

response [56]. Finally, MHC class I is normally low but constitutively expressed by microglia and endothelial cells in human CNS although absent on other cell types. However, astrocytes, oligodendrocytes, and neurons in acute MS lesions have been shown to express MHC class I [57] supporting the notion that virtually all CNS cell types can be targeted and potentially killed by $CD8^+$ T cells.

1.6. Antigen-driven retention of CD8 T lymphocytes in the CNS

$CD8^+$ T cells that have encountered their antigen in the presence of appropriate co-stimulation and cytokines undergo a specific differentiation program whereby they clonally expand and express various effector molecules [13]. Three reports indicate that the response of $CD8^+$ T cells in the CNS of MS patients bear key features of an antigen-specific response. Using $CD8^+$ T cells from the blood and CSF as well as by micromanipulation of $CD8^+$ T cells in CNS tissue sections, these groups applied complementarity-determining region (CDR) 3 spectratyping. This enabled them to determine the sequence of the β chain of the TCR, which confers the specificity of the TCR by the select combination of V, D and J gene segments. Babbe and colleagues [58] studied two MS cases in detail and reported that the majority of $CD8^+$ T cells recovered from MS lesions belonged to a few clones; one clone accounting for up to 35% of the $CD8^+$ T cells in the lesion. Examining a larger study group consisting of 36 MS patients, Jacobsen et al. [59] indicated that $CD8^+$ T cells isolated from the CSF of MS patients contained also mainly clonally expanded cells. These $CD8^+$ T cells had a memory phenotype and persisted over several months in a few patients studied longitudinally. Furthermore, a specific β chain was enriched in CD8 T cells in the CSF compared to the blood compartment (7.7% in the blood and 16.4% in the CSF) whereas the frequency of a specific β chain was similar in both blood and CSF compartments for the $CD4^+$ T cells. Skulina et al. [60] confirmed the occurrence and enrichment of clonal populations of $CD8^+$ T cells in the CNS of MS patients and the absence of such phenomenon for $CD4^+$ T cells. CSF samples taken 5 years apart from the same individual indicated the persistence of the same $CD8^+$ T cell clone during that period. In contrast, these three studies observed a heterogeneous repertoire in the $CD4^+$ Tcell subset. Interestingly, a similar clonal expansion of $CD8^+$ T cells but not of $CD4^+$ T cells has been detected in the CNS and blood of Rasmussen encephalitis patients [61]. Taken together, there is considerable evidence implicating antigen-driven $CD8^+$ T cell expansion in MS and potentially other neurological diseases. These studies strongly support the notion that $CD8^+$ T cells in the CNS of MS patients are not bystander cells, but rather have locally carried out active immune responses. Despite the importance of these findings, they do not point toward the antigen specificity of these cells. Strong evidence confirming the oligodendroglial or neuronal specificity of clonally expanded CD8 T cells in the CSF and CNS tissue of MS patients has yet to be provided. This is particularly challenging as very few cells can be isolated from the CSF and used in functional experiments. Also, to determine the antigen specificity of $CD8^+$ T clones detected in post-mortem tissues, their specific rearranged TCR sequence should be cloned back into cells [62] to functionally assess their antigen specificity. This has yet to be accomplished.

1.7. CNS associated CD8+ T lymphocytes bear effector functions

T cells are detected in MS lesions and their number is more important in post-mortem material from acute or relapsing-remitting MS patients compared to more chronic cases or lesions [63]. Moreover, reports from several laboratories confirmed that specifically $CD8^+$ T lymphocytes are present in MS lesions and that their number reaches or surpasses that of $CD4^+$ T lymphocytes [58,63–68]. There is a vast body of evidence supporting the notion that effector $CD8^+$ T cells are present on the 'crime scene' of MS lesions. $CD8^+$ T lymphocytes are particularly detected within the parenchyma and in close proximity to oligodendrocytes and demyelinated axons with polarization of their cytolytic granules in MS lesions [65,66,69]. Dendritic cells that have engulfed myelin (positive either for oil-red O or myelin basic protein) are detected in MS lesions [70]. Moreover, these myelin-laden professional APC have interactions with T cells, some of which are proliferating and these are mainly $CD8^+$ T cells, at the margin of the chronic and active lesions [70]. Recently, over 70% of T cells in acute and chronic MS lesions have been shown to express IL-17, the prototypic cytokine of the new pathogenic T cell subset Th/Tc17. Equal proportion of $CD4^+$ and $CD8^+$ T cells was detected producing this cytokine in situ [71], demonstrating that both T cell subsets infiltrating the target organ possess pro-inflammatory properties. Moreover, $CD8^+$ T cells bearing an activated effector memory phenotype were enriched in both the CSF [72] and CNS tissue of MS patients [48,69,72]. This memory subset usually resides in tissues and is able to mediate effector function upon antigen encounter [17]. In addition, $CD8^+$ T cells in MS lesions do not express CCR7 [48,73], the chemokine receptor that enables lymphocytes to migrate back to lymph nodes (accordingly with the effector memory profile) [74,75], indicating their possible retention in the CNS. Moreover, the CSF of early diagnosed MS patients has been shown to be enriched for $CD8^+$ T cells bearing a highly differentiated effector memory phenotype: CCR7− CD45RA+/−, and this enrichment was more important than in the $CD4^+$ compartment [72], suggesting their role early on during the development to relapsing-remitting MS. Finally, a significant correlation has been observed between the number of $CD8^+$ T cells and the extent of axon damage in MS lesions as measured by the accumulation of amyloid precursor protein [76,77], advocating that $CD8^+$ T cells actively contribute to the neuronal damage.

2. Effector CD8+ T cells in animal models

2.1. HLA — humanized mouse models

Various mouse lines have been generated in which murine MHC class I expression has been invalidated to impose the restriction of the $CD8^+$ T cell repertoire to a transgenic human HLA class I allele. These humanized mice have been used to study the pathogenic impact of myelin-specific $CD8^+$ T cells with direct relevance to the human pathology. To assess whether $CD8^+$ T cell responses targeting HLA-A*0201 binding myelin epitopes could aggravate autoimmune demyelination, we identified MOG epitopes that are conserved between mouse and man. Using humanized HLA-A*0201 transgenic mice, we could reveal *in vivo* that $CD8^+$ T cells targeting the immunodominant, naturally processed $MOG_{181–189}$ peptide can potentiate the autoreactive $CD4^+$ T cell response by accelerating the encephalitogenic process and worsening the disease evolution [78].

Other investigations have generated $CD8^+$ T cell lines against $PLP_{45–53}$ in association with the HLA-A3 molecule [35,79,80]. To study the functional contribution of HLA-A3 to MS pathogenesis, humanized $CD8^+$ TCR transgenic mice were created on a C57Bl/6 background [81]. These humanized mice express HLA-A*0301 together with an HLA-A*0301:$PLP_{45–53}$ specific TCR (2D1) isolated from an MS patient. A small fraction of these mice (4%) developed spontaneous EAE mediated by $CD8^+$ T cells and characterized by demyelination and axonal damage. Immunization with $PLP_{45–53}$ peptide emulsified in CFA led to a disease with a 25% incidence and a distinct biphasic evolution. The first bout of disease was mediated by $PLP_{45–53}$ specific $CD8^+$ T cells. The second phase, however, was mediated by an encephalitogenic $CD4^+$ T cell response targeting the $MOG_{35–55}$ peptide presented by mouse I-A^b. As such, these data prove that CD8-mediated autoimmune demyelination can drive epitope spreading [82], not only from one myelin-antigen to another, but also between T cell compartments. In addition to proving the pathogenicity of myelin-specific $CD8^+$ T cells in a humanized context, this study provides an interesting hypothesis concerning the protective impact of the HLA-A*0201 allele on MS. Indeed, to test the epistatic interactions between the disease conferring HLA-A*0301 allele and the protective HLA-A*0201 allele, humanized mice were created expressing both HLA transgenes together with the 2D1 TCR. These triple transgenic mice were fully protected from both spontaneous and induced disease. This was the result of a strong negative selection of 2D1 expressing $CD8^+$ T cells in the thymus induced by HLA-A*0201. This suggests that HLA-A*0201 might protect from MS by purging $PLP_{45–53}$ reactive $CD8^+$ T cells from the repertoire by presenting an, as yet unidentified, self-antigen. Lastly, this and previous studies [81,83], demonstrate the potential of introducing human disease associated genes into rodents to reveal their functional roles in the pathogenesis [84].

2.2. Pathogenicity of myelin and oligodendrocyte-specific $CD8^+$ T cells

To reassess the pathogenic potential of myelin-specific $CD8^+$ T cells, several groups established experimental protocols to isolate and expand myelin-specific $CD8^+$ T cells permitting their transfer into syngeneic recipient mice. This approach revealed that $CD8^+$ T cells targeting myelin antigens can be highly pathogenic. Joan Goverman's laboratory generated $CD8^+$ T cell responses against $MBP_{79–87}$ in C3H mice using a vaccination strategy, which is more prone to induce $CD8^+$ T cell responses than protein/peptide immunization in CFA [85]. The adoptive transfer of these H-$2K^k$: $MBP_{79–87}$-specific $CD8^+$ T cells induced a severe autoimmune disease that reproduced certain features of MS, not classically seen in $CD4^+$-mediated EAE. Clinically these mice exhibited upper motor neuron impairment, ataxia, spasticity and only occasionally hind limb paralysis. The disease evolution was rapid and severe, proving fatal to all mice by day 14. The inflammatory lesions were located exclusively in the brain, with focal involvement of grey and white matter that was most pronounced in the white matter of the cerebellum. $CD8^+$ T cell-mediated lesions associated vascular damage and perivascular tissue insult reminiscent of ischemic injury. Few inflammatory cells were found outside the perivascular cuffs. Demyelination was severe in the adjacent nervous tissue. Tissue destruction could be alleviated by injecting neutralizing anti-IFN-γ mAbs, while tumor necrosis factor receptor (TNFR)-Fc fusion proteins provided no clinical improvement. This is of interest as IFN-γ

plays a similar detrimental role in MS [86], by contrast, IFN-γ has a beneficial role in the conventional model of EAE as it reduces the severity of disease [87].

Other studies revealed that the transfer of $MOG_{35–55}$-specific $CD8^+$ T cells, into syngeneic recipients consistently induced chronic paralysis in C57Bl/6 mice [88,89]. The disease was independent of a secondary $CD4^+$ T cell response as both $RAG^{-/-}$ and SCID recipients developed disease. Inflammatory lesions were observed in both the brain and spinal cord. The inflammatory infiltrate was dominated by MOG-specific $CD8^+$ T cells [88] and rich in neutrophils [89]. Tissue damage was marked by pronounced destruction of nerve fibers, rather than selective demyelination. The pathogenic $CD8^+$ T cells recognize the minimal epitope $MOG_{37–46}$ in the context of $H\text{-}2D^b$ [88]. This indicates that $MOG_{35–55}$ contains at least 2 nested peptides that are encephalitogenic in C57Bl/6 mice. $MOG_{40–48}$ drives the encephalitogenic $CD4^+$ T cell response when presented in the context of $I\text{-}A^b$ [90], while $MOG_{37–46}$ activates $H\text{-}2D^b$ restricted $CD8^+$ T cells.

Whether myelin-reactive $CD8^+$ T cells cause disease by killing oligodendrocytes, or via bystander-mechanism driven by cross-presentation of myelin antigens by CNS resident APC has not been addressed in the models mentioned above. This issue was recently studied in two distinct transgenic mouse models in which a ‘neo-self-antigen’ was selectively expressed in oligodendrocytes [91,92]. In mice expressing ovalbumin (OVA) under the proximal MBP promoter (ODC-OVA mice), OVA protein was exclusively detected in the CNS and localized to the cytosol of oligodendrocytes [93]. Crossing the ODC-OVA mice with OT-I mice expressing a transgenic TCR recognizing $OVA_{257–264}$ in the context of $H\text{-}2K^b$ caused a lethal demyelinating disease. The disease was mediated by $CD8^+$ T cells as both $RAG^{-/-}$/OT-I/ODC-OVA triple transgenic mice and the adoptive transfer of naïve OT-I $CD8^+$ T cells into $RAG^{-/-}$ ODC-OVA mice caused disease in an IFN-γ dependent manner. The ‘neo-self’ specific $CD8^+$ T cells got activated during the first 10 days after birth, so early in life the blood–brain barrier is not completely developed therefore spontaneous release of CNS specific antigen is most likely happening. During this period OVA was accessible to $CD8^+$ T cells in the cervical and mesenteric lymph nodes, permitting their priming and differentiation. *In vitro* studies indicated that oligodendrocytes from ODC-OVA mice were direct targets for the antigen-specific $CD8^+$ T cells. In addition to oligodendrocyte loss, the oligodendrocyte-specific CD8 T cells were shown to cause axonal damage via a bystander-mechanism in IFN-γ treated organotypic cerebellar brain slices of ODC-OVA mice [94]. Interestingly, the spontaneous demyelinating disease observed in the OT-I/ODC-OVA double-transgenic mice can be blocked by the administration of a mAb specific for the $H\text{-}2K^b$: $OVA_{257–264}$ complex, indicating the efficacy of blocking antigen-presentation in preventing autoimmune CD8 T cell responses targeting oligodendrocytes, which express low levels of MHC class I [91].

We expressed an alternative neo-self-antigen, *Influenza* virus Hemagglutinin (HA), selectively in oligodendrocytes [92]. This was achieved using a double knock-in approach combining MOG-iCre mice, expressing the Cre recombinase selectively in oligodendrocytes and knock-in mice allowing the conditional (Cre-dependent) expression of HA. In the double knock-in ‘MOG-HA’ mice, Cre-mediated DNA recombination and HA transcription were observed selectively in the CNS, but HA protein expression was below detection levels

of immunohistochemistry. Crossing MOG-HA mice with Cl4 mice, in which most $CD8^+$ T cells express a transgenic TCR specific for $HA_{512–520}$ in the context of H-$2K^d$, resulted in a situation of immune ignorance characterized by the indifference of antigen-specific $CD8^+$ T cells to their cognate self-antigen in oligodendrocytes. This is in contrast to the spontaneous EAE observed in the ODC-OVA transgenic mice. The low level of HA expression, as well as the different genetic background might have contributed to these differences. In addition, the membrane embedded $HA_{512–520}$ peptide is less soluble than cytosolic OVA and might, therefore, be less efficiently drained to secondary lymphoid organs [95]. Strikingly, severe demyelinating lesions could be induced in the MOG-HA mice upon adoptive transfer of *in vitro* differentiated cytotoxic $CD8^+$ T cells specific for HA. Non-irradiated MOG-HA recipients developed weight loss, and in more severe cases tremors, reduced mobility, but no overt paralysis. The inflammatory lesions were dominant in the optic nerve and spinal cord, but also affected the brain. T cell infiltration initiated in the optic nerve and caused microglia activation, oligodendrocyte apoptosis and subsequent demyelination. Transducing the pathogenic $CD8^+$ T cells with GFP prior to transfer permitted to trace these cells in the demyelinating lesions. GFP^+ $CD8^+$ T cells were frequently found in close apposition with oligodendrocytes and some had their Granzyme B containing granules polarized towards the juxtaposed oligodendrocyte, suggesting directed degranulation. Oligodendrocytes displayed nuclear condensation indicative of subsequent apoptosis. This loss of oligodendrocytes preceded severe demyelination leading to severe axonal damage and eventually their destruction. These studies demonstrate the deleterious capacity of oligodendrocyte-specific $CD8^+$ T cells in an inflammatory demyelinating pathology resembling active MS lesions. Direct antigen-driven cytotoxicity of oligodendrocytes is implicated, although additional mechanisms may be at play. A similar $CD8^+$ T cell-mediated autoimmune response targeting HA-expressing astrocytes, caused their selective deletion without providing any evidence of secondary tissue damage [96]. This study indicates that $CD8^+$ T cells are less likely to induce bystander damage than $CD4^+$ T cells.

2.3. Spontaneous demyelination driven by $CD8^+$ T cells

Modifications to the homeostasis of the CNS can breach immune tolerance and cause secondary immune insult. Two transgenic mouse models have recently illustrated initiation of secondary $CD8^+$ T cell responses in the CNS. Proteolipid protein (PLP) transgenic mice express a construct encoding for multiple copies of the wild-type *Plp* gene. Homozygotes develop tremors and seizures due to dysmyelination causing rapid death. [97]. Hemizygous mice only moderately augment PLP expression and are clinically normal for long periods of time [98]. After 12–18 months, these mice develop progressive neurological signs including ataxia, tremor and seizures. Affected areas in the optic nerve and cerebral white matter exhibited prominent demyelination, while the spinal cord revealed intense axonal degeneration. Oligodendrocytes frequently persisted in degenerative regions. Interestingly, activated $CD8^+$ T cells and macrophages accumulated in the CNS of these mice while only limited B and $CD4^+$ T cells were present in the infiltrates [99]. These infiltrating $CD8^+$ T cells were shown to be pathogenic as $RAG^{-/-}$ PLP transgenic mice reconstituted with bone marrow from wild-type but not CD8-deficient mice developed disease [99]. The target antigen recognized by the pathogenic $CD8^+$ T cells has so far not been identified. PLP and other myelin antigens might not be the strongest candidates as no cytotoxicity was observed

towards oligodendrocytes that persist with the degenerating lesions. Taken together these observations indicate that primary myelin damage might predispose to secondary CD8-mediated tissue destruction. A similar set of observations were made in mice with peroxisome-deficiency [100].

The second model was serendipitously generated when transgenic mice expressing the co-stimulatory molecule CD86 under the H-2K^{b} promoter were created [101]. All lines were characterized by strong constitutive CD86 expression on T cells, while B cells of different sublines expressed either low, intermediate, or high levels of CD86 [101]. One subline, was found to express CD86 constitutively in the CNS on CD45^{lo}CD11b^{hi} microglia [102]. These mice developed spontaneous neurological deficits, including ataxia and a difficulty to right when overturned, ultimately leading to death. Inflammatory lesions were observed in both grey and white matter of the spinal cord and caused axonal damage and demyelination. The lesions comprised CD4^{+} and CD8^{+} T cells, MHC-II expressing cells, but no B cells. This spontaneous pathology is T cell dependent as CD86 transgenic *Tcrβ*$^{-/-}$ mice, which are deficient in αβ T cells, were fully protected from disease [102]. Importantly, the T cell-deficient CD86 transgenic mice retained the elevated CD86 expression on microglia indicating that it is transgene driven and not inflammation induced. Reconstituting these mice with TCR$^{+/+}$ bone marrow or the transfer of purified T cells restored the T cell compartment, however, only bone marrow or T cells from CD86 transgenic mice, and not wild-type mice, caused CNS inflammatory demyelination, indicating that the transgenic expression of CD86 on T cells also contributes to disease pathogenesis. The CD8^{+} T cell subset likely drives this immune pathology as reconstituting CD86 transgenic *Tcrβ*$^{-/-}$ mice with bone marrow from CD4 T cell-deficient (*I-Ab*$^{-/-}$ or *Cd4*$^{-/-}$CD86 transgenic mice) accelerated and aggravated the disease course [103]. In this model, activated oligoclonal CD8^{+} T cells infiltrated the CNS prior to disease initiation. The detrimental property of CD8^{+} T cells requires IFN-γ as invalidation of the IFN-γ receptor (*Ifn-r*$^{-/-}$) in the recipient CD86 transgenic mice prevented disease development. Defining the target antigen(s) and the relative contribution of the CD86 expression on T cells and microglia will provide further insight in CD8-mediated demyelination and the tolerogenic mechanisms protecting the CNS.

3. CD8^{+} T cell immunity to CNS viral infections

3.1. Virus-induced demyelinating models

Viral infections of the CNS readily generate effector CD8^{+} T cell responses that infiltrate the CNS parenchyma aiming to control viral spread via both lytic and non-cytolytic mechanisms [104]. Delayed or suboptimal anti-viral responses can exacerbate CNS infection causing severe tissue damage and potentiating the risk of secondary autoimmunity via determinant spreading or bystander immune activation. In this context the model of Theiler's murine encephalomyelitis virus (TMEV) is noteworthy [105]. TMEV is a picornavirus that naturally infects mice. In experimental conditions, following intracerebral inoculation of the DA strain of TMEV, the virus initially replicates in neurons and all mice develop an acute grey matter encephalomyelitis. In resistant mouse strains such as C57Bl/6, the infection is cleared within 3 weeks by anti-viral CD8^{+} T cells recognizing the immunodominant VP2$_{122–130}$ epitope presented by H-2D^{b}. After viral clearing this intense cytotoxic CD8^{+} T cell response

targeting infected neurons contracts and mice fully recover. In contrast, in susceptible strains such as SJL, the inflammatory response fails to clear the virus that persists in the spinal cord white matter where it resides in microglia and oligodendrocytes. There, the virus elicits chronic inflammatory and demyelinating lesions, which can lead to flaccid paralysis. At this chronic phase the lesions are very similar to those observed in MS. Both $CD8^+$ and $CD4^+$ T cells are thought to contribute to demyelination: $CD8^+$ T cells by killing infected oligodendrocytes and $CD4^+$ T cells via bystander mechanisms in response to either viral or myelin antigens [105–107]. However, $CD8^+$ T cells might play a unique role in causing the neurological manifestations associated with TMEV infection. When comparing TMEV infection in MHC-I-deficient ($\beta 2\text{-}microglobulin^{-/-}$), MHC-II-deficient ($I\text{-}A^{b-/-}$) and wild-type SJL mice the intensity and localization of demyelinating lesions were identical in the 3 groups, yet only SJL and MHC-II-deficient mice developed severe neurological deficits [108]. In contrast, MHC-I-deficient mice exhibited preserved hind limb motor-evoked conduction velocities, suggesting that in the absence of $CD8^+$ T cells demyelinated axons retain functionality. Histologically, demyelinated axons were indeed shown to resist in MHC-I-deficient mice. Moreover, these axons exhibited increased sodium channel densities augmenting the efficacy of impulse conduction. It was, therefore, proposed that reactivation of $CD8^+$ T cells in the vicinity of demyelinated axons may contribute to functional neurological impairment [108]. $Cd8^{-/-}$ mice similarly revealed extensive demyelination without developing neurological impairment, further supporting a role for $CD8^+$ T cells in neuronal dysfunction after TMEV infection [109]. These data substantiate the previously mentioned correlation between the number of $CD8^+$ T cells and the extent of axon damage in MS lesions [76,77].

$CD8^+$ T cells can cause demyelination by targeting oligodendrocytes. Using a model system in which lymphocytic choriomeningitis virus (LCMV) proteins were selectively expressed in oligodendrocytes by transgenesis, it was possible to test whether LCMV Armstrong infection, which does not infect the CNS parenchyma, could initiate CNS autoimmune demyelination due to the imposed mimicry. Indeed, $CD8^+$ T cell-mediated demyelination was clearly illustrated in this viral model but the effector mechanisms involved have remained unexplored [110].

$CD8^+$ T cells can cause demyelination via bystander mechanisms. This was shown using murine hepatitis virus (MHV), which infects oligodendrocytes, astrocytes and neurons in the CNS [111]. MHV causes immune-mediated demyelination driven by T and B cells, as indicated by the fact that infected $RAG^{-/-}$ mice fail to develop demyelination despite harboring a large viral load in the CNS [112,113]. Adoptive transfer of either $CD4^+$ or $CD8^+$ T cells restored the inflammatory demyelinating phenotype in these mice [114]. Interestingly, even activated $CD8^+$ T cells of irrelevant specificity can mediate bystander demyelination [115] involving IFN-γ [116]. Moreover, CNS viral infections have revealed the versatility of effector $CD8^+$ T cells in exerting their immune functions. Intracerebral LCMV infection leads to blood–brain barrier breakdown and convulsive seizures caused by virus-specific $CD8^+$ T cells [117]. Interestingly, when the virus-specific $CD8^+$ T cells target the infected stromal cells in the subarachnoid space the interaction was transient and insufficient to cause apoptosis of the infected target cells. Rather, the activated $CD8^+$ T cells released chemokines that stimulated the permeability of the blood–brain barrier and

triggered the influx of pathogenic monocytes and neutrophils that contributed directly to the lethal seizures [117]. A recent study has illustrated that viral infection could break tolerance of myelin-specific CD8 T cells not only via a molecular mimicry mechanism but also by activating CD8 T cells bearing dual TCRs that are able to simultaneously recognize both viral and myelin antigens [118].

3.2. Epstein–Barr virus-specific CD8$^+$ T cells in the context of MS

Thirty years ago, Prineas reported the detection of organized lymphoid tissue in the perivascular spaces of MS plaques, especially in ‘old’ plaques as stated by the author [119]. More recently, Aloisi and colleagues reported the detection of lymphoid follicle like structures containing B cells, T cells and plasma cells [120] and showed that these lymph node like structures contained B cells infected with Epstein–Barr virus (EBV) and that some of the CD8$^+$ T cells in these ectopic structures had their granules polarized toward these infected B cells. Whether CD8$^+$ T cells in the CNS of MS patients while targeting infected EBV-B cells actively contribute to the pathogenesis of MS or represent a bystander population of infiltrating cells attracted by the prolonged inflammatory state of the organ has not been elucidated. In contrast, others detected CNS EBV infection in only rare cases of MS [121,122] suggesting that the presence of this virus in the target organ of this autoimmune disease is not a common feature. Moreover, whether EBV-specific CD8$^+$ T cell responses are distinct in MS patients is still controversial. Increased or decreased frequency of EBV-specific CD8$^+$ T cell responses was detected in MS patients compared to controls [123,124]. When autologous EBV-infected lymphoblastoid cells were used as APC, the frequency of EBV-specific CD8$^+$ T cells was lower in MS patients than in controls [124]. However, using ELISPOT with peptides of 8–15 mers to stimulate CD8$^+$ T cell responses, greater frequency of EBV-specific IFN-γ producing CD8$^+$ T cells was found in clinically isolated syndrome than in any other form of MS (RR-MS, SP-MS and PP-MS), other neurological diseases or healthy controls [125]. CD8$^+$ T cell responses (studied in PBMC) to latent EBV proteins were higher in MS patients than in controls as measured by intracellular cytokine staining (IFN-γ) in response to EBV-infected and immortalized autologous B cells, but no difference was observed between MS and controls for the frequency of EBV-specific CD4$^+$ T cells [126]. Overall, the potential contribution of EBV-specific CD8$^+$ T cell responses both in the periphery and locally in the CNS in the context of MS remains to be elucidated.

4. Regulatory CD8$^+$ T cells in MS and EAE

The cellular immunity provided by both CD4$^+$ and CD8$^+$ T cells is essential to fight infections and eliminate potentially neoplasic cells. However, T cell responses have the potential to instigate immune-mediated pathologies, such as autoimmune diseases, including MS. Thus, a fine balance between protective and deleterious effects needs to be sustained. Both CD4$^+$ and CD8$^+$ T cells bearing suppressor or regulatory properties have been described. CD4$^+$ regulatory T cells have been extensively studied; however, knowledge about their CD8 T cell counterparts is not as extensive. Subsets of CD8$^+$ T cells in both humans and rodents have been shown to suppress immune responses [127,128]. Whether

suppressor $CD8^+$ T cells play a role in MS or are deficient in MS patients remains a topic of considerable debate.

More than twenty years ago, MS patients were shown to have defective $CD8^+$ T cell suppressor functions compared to healthy controls [129,130] using a proliferation assay in which anti-CD3 stimulated and enriched $CD8^+$ T cell cultures used as suppressor cells were added to fresh autologous peripheral lymphocytes in the presence of concavalin A. Later on, a single clone of $CD8^+$ T cells expanded from one healthy donor was reported to regulate autologous MBP-specific $CD4^+$ T cells [131]. More recently, treatment of MS patients with glatiramer acetate, a synthetic copolymer of four amino acids, increased the capacity of $CD8^+$ T cells to kill in a MHC class I and HLA-E dependent manner $CD4^+$ T cells of any specificity as long as these target cells were loaded with glatiramer acetate-derived peptide in their MHC groove [132]. The same group (Karandikar and colleagues) showed that upon glatiramer acetate therapy, the TCR repertoire of $CD8^+$ T cells responsive to this peptide mixture was oligoclonal over time and more limited than that in the CD4 compartment [133]. Furthermore, Correale and Villa advocated that $CD8^+$ T cells can kill myelin-specific $CD4^+$ T cells in a HLA-E restricted fashion [134]. *In vitro* expanded myelin-specific $CD4^+$ T cell clones from MS patients were used as antigen for the expansion of autologous $CD8^+$ T cells. The amplified $CD8^+$ T cell clones killed autologous myelin-specific $CD4^+$ T cell clones only when these target cells were activated. It is not possible to rule out that the long-term *in vitro* culture did trigger T cell functions that are not representative of their *in vivo* properties. Although the authors implicated HLA-E recognition in the cytotoxicity, they did not show whether their $CD8^+$ T cell clones express either HLA-E receptor (NKG2A or NKG2C), nor whether the target cells ($CD4^+$ T cell clones) expressed higher levels of HLA-E upon activation since these cells at a resting state could not trigger the $CD8^+$ T cell responses [134].

It has been suggested that within the $CD8^+$ T cell compartment, $CD28^+$ expressing cells are cytotoxic whereas $CD28^-$ are suppressor [135]. Interestingly, $CD8^+CD28^-$ T cells were detected in lower levels in the peripheral blood of MS patients compared to controls [136]. Moreover, a recent study underlined the possibility that activation of $CD8^+$ T cells with a single antigen, an immunodominant cytomegalovirus HLA-A2-restricted antigen, can lead to either cytotoxic $CD8^+$ T cells (that were $CD28^+$) or suppressor $CD8^+$ T cells (that were $CD28^-$) depending on the APC and environmental milieu [137]. However, the sole absence of CD28 is not a reliable marker of suppressor capacity since $CD8^+CD28^-$ T cells isolated from myeloma have been shown to release inflammatory cytokines and to possess cytotoxic capacity [138]. Unfortunately, a specific marker distinguishing suppressor or regulatory cells from other conventional $CD8^+$ T cells has yet to be identified, although expression of Foxp3, the transcription factor associated with CD4 regulatory T cells, may identify a suppressive CD8 T cell subset [139]. Thus, only suppressor capacity confirmed by functional assays could substantiate that any $CD8^+$ T cells possess regulator properties and whether these cells are deficient in MS patients remains to be fully investigated.

4.1. CD8+ T cell responses in active EAE

EAE is predominantly driven by distinct autoreactive $CD4^+$ T cell subsets, with a varying importance of the humoral response [140]. The magnitude of CNS tissue damage correlates most strongly with the frequency of activated macrophages/microglia [140]. The active immunization protocol with myelin proteins or peptides favors $CD4^+$ T cell responses resulting in only low frequency $CD8^+$ T cell infiltration within demyelinating lesions [141]. During active EAE $CD8^+$ T cells are thought to play a dual role. Indeed, CD8-deficient mice display reduced initial mortality but increased disease relapses, suggesting that $CD8^+$ T cells can sustain disease remission [142,143]. A similar aggravation of EAE was observed in $MOG_{35–55}$ or MBP immunized $\beta 2m^{-/-}$ mice, in which the deficiency in β2-microglobulin prevents the expression of both classical and non-classical MHC-I molecules [144]. Further studies revealed that $CD8^+$ T cells isolated from mice that had recovered from EAE could eliminate MBP-specific $CD4^+$ T cells *in vitro* and *in vivo* [145]. This regulatory $CD8^+$ T cell response is restricted by the non-classical MHC-Ib molecule Qa-1, the mouse equivalent to human HLA-E. These $CD8^+$ T cells are thought to target a peptide from the Vβ8.2 chain of the autoreactive TCR presented in the context of Qa-1 on the surface of pathogenic $CD4^+$ T cells causing their apoptosis [146–149]. The generation of $Qa\text{-}1^{-/-}$ mice, lacking these regulatory $CD8^+$ T cells, has since permitted to assess their precise impact on EAE. These mice developed a more severe disease due to the resistance of Qa-1-deficient $CD4^+$ T cells to $CD8^+$ T cell-mediated regulation [150]. Moreover, polyclonal activation (e.g. concanavalin A) of splenic $CD4^+$ T cells induces them to express Qa-1 such that they become susceptible to NKG2A-expressing suppressor $CD8^+$ T cells that can then block their capacity to induce EAE [151].

Other reports of regulatory $CD8^+$ T cells in the context of EAE have been published such as the identification of suppressor cells in the polyclonal $CD8^+CD28^-$ T cell subset that are thought to convey protection against EAE [152]. Also, $CD8^+$ T cells expressing CD122 (IL-2/IL-15 receptor β) have been shown to spontaneously arise during EAE. While depletion of these cells exacerbated EAE symptoms, the adoptive transfer of these cells alleviated the disease from naïve recipients [153]. Overall, regulatory $CD8^+$ T cells have been reported but their frequency and the mechanisms by which they suppress other immune cells remain unexplored.

5. Concluding remarks

Major advances have recently been made in the understanding of the pathophysiology of MS. Unraveling the complexity of the inflammatory response has identified various immune mechanisms involved in the disease pathogenesis. Notably, the humoral response has been implicated in direct antibody-mediated demyelination while new effector $CD4^+$ T cell subsets that contribute to CNS autoimmunity have been identified. This review considers the detrimental traits of the $CD8^+$ T cell lineage during inflammatory demyelinating responses in the CNS. Experimental models strongly support the idea that $CD8^+$ T cells can induce or aggravate tissue destruction in the CNS. However, the interactions of $CD8^+$ T cells with the other immune components implicated in the pathophysiology of MS, and how these interactions can account for the heterogeneity in lesion formation and clinical evolution

remains to be fully explored. Experimental models will therefore have to be employed to understand the migration, cytokine secretion profile, and relative pathogenic impact of each of the immune cell subsets individually and in synergy. Early indications suggest that $CD4^+$ T subsets exhibit a distinct migratory behavior and might differentially influence lesion localization in the CNS [154]. Such differential behavior might be extended to $CD8^+$ T cells, which benefit from a unique antigen-driven mechanism to infiltrate the CNS suggesting that the requirements for CNS entry might be distinct between the $CD4^+$ and $CD8^+$ T cell subsets [155]. Consequently, the dominance of $CD8^+$ T cells might vary between CNS regions and over time. Documenting the dynamics of the $CD8^+$ T cell response in MS might point to distinct disease stages or alterations in disease severity that correlate with $CD8^+$ T cell dominance.

A more detailed dissection of the functional variety of $CD8^+$ T cells in MS and its animal models seems warranted. Functional subsets of $CD8^+$ T cells characterized by distinct cytotoxic properties and cytokine profiles have been observed in different immune settings [156–160] and might express their pathogenic potential during inflammatory demyelination in the CNS [71]. Moreover, existing observations regarding CD8-mediated tissue damage during CNS viral infection might provide important leads to the potential mechanisms employed by $CD8^+$ T cells during CNS inflammation. For instance, the non-cytolytic function of $CD8^+$ T cells to recruit immune components by releasing chemokines and promote blood–brain barrier permeability might be indicative of a broader role for $CD8^+$ T cells in the development of inflammatory lesions. Similarly to the $CD4^+$ T cell compartment, $CD8^+$ T cell subsets portray regulatory properties. Whether these $CD8^+$ T lymphocytes play a positive or negative role in the inflamed CNS is still a matter of debate and controversy [9,128]. Assessment of antigen specificity but also of specific functions will be essential to unravel the contribution of $CD8^+$ T cells to the pathogenesis of MS.

Lastly, current and future therapies for MS must be assessed for their impact on $CD8^+$ T cells [161]. As reviewed in detail [162], therapies aimed at selectively targeting the $CD4^+$ T cell response have produced little efficacy in MS. In contrast, strategies impacting on both $CD4^+$ and $CD8^+$ T cells by anti-CD52 mAb-mediated depletion (Alemtuzumab) [163], anti-α_4-integrin mAb-mediated inhibition of CNS infiltration (Natalizumab) [164], and the immunomodulator FTY720 mediated inhibition of lymphocyte egress from lymphoid organs reduce relapses and limit the formation of new lesions. Strengthening our understanding of the pathogenic potential of $CD8^+$ T cells in MS should provide promising new avenues for the treatment of this disabling inflammatory disease.

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