Review



# The Contribution of Tumor Necrosis Factor to Multiple Sclerosis Progression Independent of Relapses

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Keywords: TNF biology; MS pathology; PIRA; MS lesions; chronic compartmentalized inflammation; neurodegeneration; biomarkers; disease progression. 1. Introduction

*Review*

# The Contribution of Tumor Necrosis Factor to Multiple Sclerosis Progression Independent of Relapses

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**Simple Summary:** Multiple sclerosis (MS), the leading cause of non-traumatic neurological disability in young adults, is a chronic immune-mediated disease of the central nervous system, characterized by multifocal acute and chronic inflammatory demyelination in white and gray matter, and neuroaxonal loss. Disability resulting from progression independent of relapse activity (PIRA) is a recently proposed concept in the MS field to overcome the dualistic view of MS as either a relapsing-inflammatory or a progressive-neurodegenerative disease. This concept is supported by several studies demonstrating that both processes are present from the early phase of the disease. One major cytokine that has been shown to play an important role in MS pathogenesis and disease progression is tumor necrosis factor (TNF), a pleiotropic cytokine regulating many immunological mechanisms. This review summarizes several studies on the contribution of TNF and its receptors to MS progression and investigates their role as promising therapeutic targets for PIRA.

**Abstract:** Tumor necrosis factor (TNF) is a pleiotropic cytokine regulating many physiological and pathological immune-mediated processes. Although initially described as a tumor-cytotoxic agent, over time, it has also been recognized as an essential pro-inflammatory cytokine implicated in the pathogenesis of different autoimmune diseases, such as multiple sclerosis (MS). Several studies have shown an increase in TNF expression both in acute and chronic active MS brain lesions, as well as its involvement in maintaining a chronic inflammatory intrathecal process known as "compartmentalized inflammation", which contributes to disease progression and disability accumulation in MS, even more than acute inflammatory activity. Indeed, high TNF levels were observed in the serum and cerebrospinal fluid of subjects with MS and correlated to disease severity, promoting axonal damage and, consequently, neurodegeneration. In this review we discuss the current knowledge of TNF and its receptors involvement in MS progression, focusing on disability progression independent of relapse activity (PIRA), meant as the disability accumulation evident in relapsing MS patients not related to acute inflammatory events.

**Keywords:** TNF biology; MS pathology; PIRA; MS lesions; chronic compartmentalized inflammation; neurodegeneration; biomarkers; disease progression

## 1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated and neurodegenerative disease of the central nervous system (CNS) affecting millions of people worldwide [1] and representing the most common cause of non-traumatic neurological disability in young adults [2].

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MS is a complex multifactorial disease caused by complex gene–environment interactions and characterized by multiple pathological hallmarks, ranging from immune dysregulation and neuroinflammation to neurodegenerative mechanisms [3].

Several molecular changes, including the increase of cytokines, chemokines, nitric oxide, reactive oxygen species, glutamate, and free radicals, affect the pathogenesis and the disease course of MS [4].

MS clinical course is highly variable, heterogeneous and unpredictable at an individual level. Generally, it is characterized by transient and recurrent episodes of focal acute CNS inflammation early on, with complete or partial resolution (relapsing-remitting MS - RRMS) and, over time, by a prominent process of neurodegeneration, resulting in a late slow steady progressive accumulation of physical disability and cognitive impairment in absence of relapses (secondary progressive MS - SPMS) [5]. On the other hand, few cases reported a slow continuous neurological deterioration without relapse from the earliest stages of the disease (primary progressive MS - PPMS) [5,6].

Beyond this traditional phenotypic categorization, it is now clear that MS progresses along a continuum from RRMS to progressive MS (PrMS), with differing levels of neurologic reserve explaining phenotypic differences [7].

This emerging view of MS disease as a single-stage disorder, where all patients exhibit a progressive course since the disease onset, which can be overlapped by relapses [7], is supported by the new concept of progression independent of relapse activity (PIRA) [8]. The term PIRA, proposed by Kappos et al., refers to the progressive clinical deterioration occurring in many RRMS patients without signs of inflammatory activity [8]. This notion aligns with several previous observational studies that show disability accumulation is largely independent of superimposed focal inflammation and undetectable by conventional clinical-radiological parameters [9,10].

Although the frequency of PIRA has been reported within the first 5 years following the first MS-related clinical attack, its identification in clinical practice remains unclear due to the lack of standardized definitions (such as a time window after the last relapse) and/or measures to detect it (such as based on EDSS score or increase in composite measure) [11].

The mechanisms driving PIRA are not fully elucidated but are undoubtedly associated with smoldering inflammatory and neurodegenerative processes. In a recent, prospective, large sample size study, Cagol et al. showed that RRMS patients with PIRA (defined as a 6-month confirmed disability progression with no relapse during the 90-days before and the 180-days after the initial EDSS increase) exhibit more pronounced diffuse cerebral cortical volume loss [12]. This finding aligns with several studies demonstrating that grey matter (GM) atrophy is predictive of long-term accumulation of physical and cognitive disability [13] and conversion to PrMS [14].

Cerebral GM damage, which manifests as both focal cortical lesion(s) and diffuse cortical and deep GM atrophy, provides one of the best clinical correlations with irreversible disability accumulation [13,15] and topographically associated with aberrant tertiary B-cell-enriched lymphoid structures affecting the cerebral meninges [16]. The extent of meningeal immune infiltration correlates with the degree of subpial GM demyelination, microglial activation, and axonal loss [16–19].

MS patients with a progressive and severe course of the disease also display chronic active lesions (CALs), a subset of white matter (WM) lesions characterized by an inactive core surrounded by a “rim” of activated microglia [20–22]. CALs are associated with nearby persistent demyelination and axonal loss, even in the absence of blood-brain barrier (BBB) damage [20–22].

Molecular-neuropathological studies on progressive MS cases supported the hypothesis that soluble factors (chemokines and cytokines) produced by meningeal tertiary lymphoid structures and/or circulating immune cells, may diffuse throughout the cerebrospinal fluid (CSF) into the cortex, inducing brain damage either directly or indirectly through microglia activation [23]. In this regard, Kosa and colleagues recently found that CSF biomarkers associated with immune-related pathways correlate with clinical and imaging MS severity outcomes and predict future rates of disability accumulation [24].

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All these findings suggest that chronic inflammation in the CNS continuously disturbs neuroaxonal homeostasis, leading to prominent neurodegeneration, even outside of MS relapses and especially at the progressive stage [25]. This confirmed that compartmentalized inflammation (involving CSF, meninges and parenchyma) is a major mechanism driving progressive multiple sclerosis.

Among the different cytokines found to increase in the CSF of MS patients [23], tumor necrosis factor (TNF) represents the major pro-inflammatory cytokine correlated with the degree of disability in patients with progressive MS [26].

Selmaj et al. have also provided significant evidence according to which an increase of TNF uniquely occurred locally within the CNS of MS cases and not in other neurodegenerative brain diseases, such as Alzheimer's or Parkinson's disease [27], further suggesting that the combination of inflammation, demyelination and neurodegeneration is an MS quite-specific process, as supported then also by a study by Fischer et al. [28]

TNF exerts its potent pro-inflammatory activity by the activation of TNF receptors type-1 (TNFR1) and type-2 (TNFR2) signalling [29,30]. Besides this inflammatory action, TNF has excitotoxic [31] and necro-apoptotic activities on oligodendrocytes [32,33] and neurons mainly through TNFR1 activation [34].

A post-mortem study has additionally revealed that an imbalance of TNF receptors type-1 (TNFR1) and type-2 (TNFR2) signalling plays a role in determining the severity of MS [35], demonstrating a strong correlation between compartmentalized inflammation and the high expression of genes involved in TNFR1 signal cascade [35].

This review summarizes the contribution of TNF and its receptors in MS progression and investigates their involvement in neurodegenerative mechanisms occurring during chronic inflammatory events.

## 2. TNF biology, cellular production and signalling pathways

The master pro-inflammatory cytokine, TNF, has been shown to have a broad spectrum of cellular effects, including inflammatory response, cellular activation, and programmed cell death [36]. TNF belongs to the TNF superfamily, which includes 19 ligands produced primarily by monocytes/macrophages but also by T and B lymphocytes, smooth muscle cells, adipocytes, osteoclasts and fibroblasts, although in smaller quantities [36,37].

TNF is expressed initially as a transmembrane protein (mTNF, 26 KDa 233-amino-acid), which requires proteolytic cleavage by the TNF converting enzyme (TACE) to release soluble TNF (sTNF, 17 kDa 157-amino-acid). mTNF and sTNF are produced by a wide range of peripheral and central immune cells, such as activated macrophages, effector CD4 and CD8 T cells and B lymphocytes and microglia as well as neurons, oligodendrocytes and astrocytes [38].

Both mTNF and sTNF are biologically active and exert their effects by modulating a complex signalling pathway with wide-ranging downstream responses through two distinct surface receptors belonging to the TNF receptor (TNFR) superfamilies (comprise 29 receptors): the TNF Receptor Superfamily Member 1A (TNFRSF1A-TNFR1; p55/60; CD120a) and TNF Receptor Superfamily Member 1B (TNFRSF1B-TNFR2; p75/80; CD120b) [36–38].

The two receptors differ significantly in structure, binding affinity, localization, function and signalling pathways activated [39,40].

TNFR1, expressed on the membrane of all cell types except erythrocytes, shows a high affinity towards sTNF, promoting both necrosis and apoptotic pathways as well as pro-inflammatory signalling [41], through its death domain (DD), which when activated by TNF binding, recruits the TNFR1-associated death domain (TRADD). TRADD can in turn recruit Fas-associated death domain (FADD) and receptor-interacting serine/threonine-protein kinase 1 (RIPK1), which can either lead to necroptosis trough RIPK3 and mixed lineage kinase domain-like pseudokinase (MLKL) activation, or apoptosis through caspase 8 and caspase 3 recruitment [42,43]. On the contrary, pro-inflammatory signalling is mediated by TNFR-associated factor 2 (TRAF2) activation of, mitogen-activated protein

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kinases (MAPKs), such as c-Jun-N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), and transcription factor nuclear factor-κB (NFκB) [42].

TNFR2, expressed only in fewer cell types (neurons, oligodendrocytes, microglia and T lymphocytes), mediates local homeostatic effects, such as cell survival, tissue regeneration and inflammation through preferentially binding mTNF [44,45]. Unlike TNFR1, TNFR2 does not have a death domain. Nevertheless, a recent study has shown that under some circumstances TNFR2 signalling also has pro-apoptotic effects, by amplifying TNFR1-mediated stimulation of apoptosis or cooperating in the binding of TNF to TNFR1 [38,46]. However, the mechanism of TNFR2-mediated cell death is still unclear and homeostasis and cell survival remain the primary functions exerted by TNFR2-mediated signalling through TRAF (1/2) activation of MAPKs (JNK and ERK), protein kinase B (Akt), and NFκB [42].

Therefore, albeit in different ways, both TNFR1 and TNFR2signalling, may lead to NF-κB and MAPK activation, increasing the expression of inflammatory genes encoding chemokines and cytokines (including TNF itself) [39,40] as well as inducing anti-apoptotic transcriptional programs promoting cell survival, cell proliferation and cell differentiation [47,48].

This duality of TNFR signalling, which can induce both survival and cell death, probably depends on the cellular environment, the relative surface levels of TNFR1 and TNFR2, and their cellular activation status (Figure 1). However, the effects of altering the TNFR1/TNFR2 balance in normal and altered physiology remain to be understood [49].

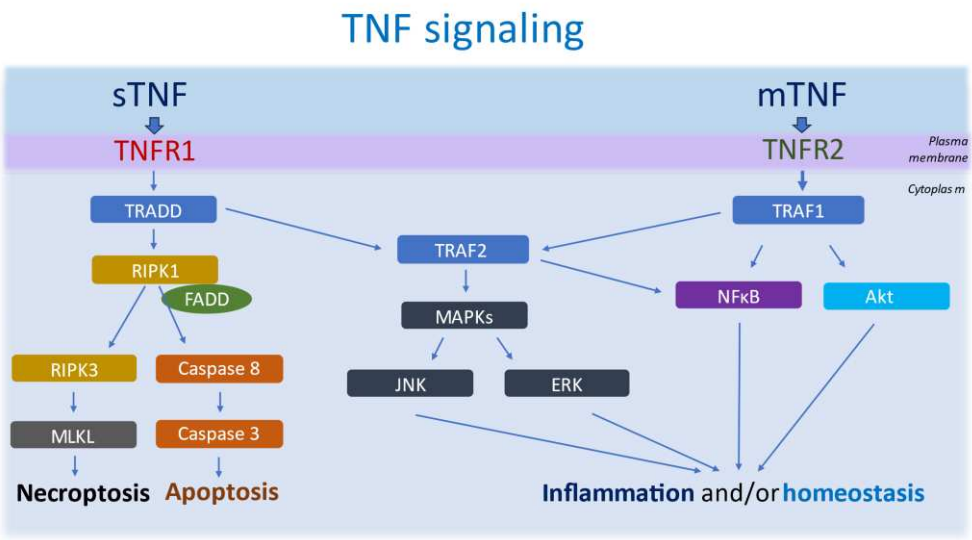


**Figure 1. TNF signalling.** TNF signalling is mediated by two isoforms (mTNF and sTNF) exerting their effects by modulating a complex signalling pathway through two distinct surface TNF receptors: TNFR1 and TNFR2. TNFR1 shows a high affinity towards sTNF, which once bound recruits TRADD. TRADD binds FADD and RIPK1, leading to necroptosis through RIPK3 and MLKL activation, or apoptosis through caspase8 and caspase 3 activation. On the other hand, mTNF interacts with TNFR2, inducing inflammation and homeostasis through TRAF1/2 activation of JNK, ERK MAPKs, Akt and/or NFκB.

## 3. Potential pathological implications of TNF/TNFRs impairment in MS and EAE

### *3.1. Neuroinflammation*

TNF exerts pleiotropic functions playing a crucial role in several immune-mediated conditions, including rheumatoid arthritis [50], systemic lupus erythematosus [51] and Crohn's disease [52]. As

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a potent mediator of inflammation, principally via TNFR1 signalling, TNF is considered the major cytokine involved in the pathogenesis of MS [29,30].

A relevant action of TNF is to activate T lymphocytes, enhancing their proliferation and recruitment and increasing pro-inflammatory cytokine production in the CNS by inducing the activation of NF-κB signaling pathways [53]. TNF-dependent T cell activation contributes to blood-brain barrier (BBB) damage via meningeal mast cells secondary activation and, therefore, promotes further inflammatory cell influx with consequent myelin and neuronal damage [54,55].

Not surprisingly, elevated production of TNF is found in MS patients [27,56] and in experimental autoimmune encephalomyelitis (EAE), the most used murine model of MS [56].

High TNF levels were found in active demyelinating lesions [26] as well as in the serum and CSF of MS patients [57–59], correlating with disease severity [59–62]. In EAE mice, TNF mRNA expression is upregulated in the CNS in parallel with disease progression, its external administration increases EAE severity [56,63] since it appears to be involved in immune cell (macrophages and T cells) activation and infiltration into the CNS [62].

The use of EAE transgenic mice for TNF and TNFRs has significantly contributed to understanding TNF's pathological role in MS [45].

TNF-gene knock-out (KO) EAE mice showed a milder disease course due to reduced leukocyte intrathecal trafficking and BBB permeability compared to wild-type (WT) EAE mice [64]. This evidence suggests that alterations in the TNF signaling are involved in the (early) pathological MS mechanisms typical of MS that occur in the CNS [64].

Beyond the cytokine, several studies have also investigated the role of the TNFRs in MS pathology. Specifically, TNFR1 KO EAE mice showed a reduction of immunopathological signs and symptoms of the disease compared to WT mice, whereas TNFR2 KO EAE mice showed more severe disease symptoms, an enhanced T cell infiltration in the CNS and diffuse demyelination [65].

Intriguingly, a crucial role of TNFR2 has recently been demonstrated in regulatory T cell ($T_{reg}$) biology, promoting their proliferation and expansion [66]. $T_{regs}$ are essential in maintaining immune homeostasis and preventing autoimmunity [63–69]. Not surprisingly, an impaired functional suppression of $T_{regs}$ in response to autoreactive T cells is typically reported in MS [70]. In line with this, a recent study on $T_{reg}$-restricted TNFR2 deficiency mice with induced EAE developed an aggressive disease, indicating the critical protective role of TNFR2 signalling [71]. However, the significance of intrinsic TNFR2 signalling in $T_{reg}$ cells in vivo remains incompletely defined [71].

These findings support the critical role of TNFR1 signalling in the induction of a pro-inflammatory environment in the CNS [62]. On the contrary, TNFR2 appears to be involved in neuroprotection and repair processes [62].

### *3.2. Neurodegeneration, Demyelination and Remyelination*

In addition to immune cell activation and infiltration, TNF signalling is involved in neurodegenerative processes. In particular, TNF promotes neuronal excitotoxicity and oligodendrocyte death, acting directly on neurons and glia, through TNFRs, with a further TNF release [42,72].

An elegant study by Centonze et al. has shown that increased concentration of TNF released by activated microglia induces changes in the expression and physiological properties of glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPRs) and NMDA (*N*-methyl-*D*-aspartate) receptors (NMDARs) in EAE mice [73]. Specifically, TNF acting on neuronal TNFR1 receptors causes excitotoxicity by increasing the surface expression of AMPARs and the activation of NMDARs, prolonging the duration of glutamate post-synaptic response [73].

In addition to excitotoxicity, TNF/TNFR1 signalling is involved in triggering oligodendrocyte apoptosis [32]. Consistent with this, TNF overexpressing transgenic mice developed spontaneous demyelinating lesions similar to those seen in MS [32,74,75]. On the contrary, TNF through TNFR2 facilitates remyelination by promoting oligodendrocyte differentiation in EAE [76]. Furthermore, TNFR2 KO mice develop more severe EAE motor disease than WT mice [76–78]. In particular, TNFR2

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conditional KO EAE mice, a novel transgenic mouse with selective TNFR2 ablation in oligodendrocytes, have shown that altered TNFR2 signalling results in impaired remyelination [78].

These findings suggest that TNF could have a bimodal action depending on the receptor subunit involved in its signalling cascade. TNFR1 signalling plays a harmful role in MS, while when TNF acts via TNFR2 it exerts a protective action resulting in an attenuation of aggressive course of the disease in EAE. In MS, TNF (through enhanced TNFR1- and weak TNFR2-signalling) contributes to disease pathogenesis and progression, leading to inflammatory demyelination, remyelination failure, and neuronal functional damage via synaptic damage.

**4. TNF and meningeal inflammation.**

Increased levels of pro-inflammatory cytokines and cytotoxic mediators are found in the CSF of MS patients [23]; specifically, CSF levels of TNF were correlated with the degree of disability in patients with PrMS [23,26,79,80] but not detected in patients with other neurodegenerative diseases [27]. This increase is determined by immune cell infiltration into the CNS; in particular, lymphocytes and macrophages enter the brain through the perivascular space and meninges, where they release cytokines and chemokines that trigger glial cells and neurons to release additional inflammatory mediators, such as IL-1β, TNF, and IFN-ɣ [81]. The resulting mounting intrathecal neuroinflammation induces a local and chronic immune response that alters synaptic transmission and neuroaxonal homeostasis [17], leading to an increasingly inflammatory environment in the CSF, which bathes the cortical layers [23,79]. In this regard, a strong correlation was found between CSF/meningeal inflammation and the degree of cortical damage, microglial activation, and axonal loss [17,23,79]. Chronic inflammation causes GM damage in MS from the earliest stages of the disease. It leads to disability accumulation independently from acute inflammation due to the decreased capacity of the compensatory mechanisms [17,25,82,83]. Early cortical GM damage is indeed related to a more severe and rapid disease course in terms of disability progression and cognitive impairment [25].

In this regard, Kosa and colleagues have recently demonstrated that neuroinflammation increases with MS progression, identifying specific inflammatory pathways correlated with MS progression, which include both innate and adaptive immunity of Th17 (IL17, GM-CSF and IL6), Th1 (IFNγ and TNF) and Th2 (IL13 and IL4) phenotype [24]. Moreover, Magliozzi et al. showed that meningeal inflammation specifically alters the balance between TNFR1 pro-cell death and TNFR1/TNFR2 pro-cell survival signalling, causing a more severe disease manifestation from the early stages [35]. In addition, this study not only confirmed the elevated TNF levels in the CSF of MS patients at the time of diagnosis but also showed a greater overexpression of the TNFR1 gene in MS patients, especially in cortical GM tissues of progressive subjects [35]. These results are in line with a recent study by Picon and colleagues that provides substantial evidence for TNF-mediated activation of necroptotic signaling via TNFR1 in cortical neurons of progressive MS cases [34]. In fact, the study demonstrates an increased expression of multiple steps in the TNF/TNFR1 signaling pathway leading to necroptosis, including the key proteins TNFR1, FADD, RIPK1, RIPK3 and MLKL [33].

All these results support the hypothesis that neurodegeneration in MS is mainly driven by chronic inflammation in the CNS, with a preponderant involvement of activated TNF/TNFR1 signalling.

**5. TNF and MS lesions**

Degenerative processes include demyelination, axonal injury and neuron loss, resulting in multifocal WM lesions and diffuse GM damage in subpial and subventricular regions close to CSF and meninges [84]. Significant upregulation of TNF and TNFR1 was found in white matter (WM) and subpial GM lesions [85].

WM lesions can be classified as active, chronic active (CALs; smouldering, slowly expanding, mixed active/inactive), remyelinating, and chronic inactive lesions [86]. Active lesions develop from normal appearing white matter (NAWM) and are characterized by areas of demyelination and activated macrophages and microglia. These lesions can remyelinate in the presence of activated

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microglia or evolve into CALs or inactive lesions [87]. CALs are characterized by a demyelinated hypocellular nucleus and rims of iron-laden activated microglia [88,89], while inactive lesions are well-defined areas of demyelination and axonal degeneration in the absence of inflammation [20,75,87].

Chronic compartmentalized inflammation leads to the formation of CALs, which increase in number as the disease progresses [28,90,91]. In fact, they represent more than half of all focal WM lesions especially in progressive MS patients [92], depicting a relevant pathological finding associated with a severe disease course mediated by neuroaxonal damage, in the absence of superimposed acute inflammatory activity [20,88,91].

TNF was identified in CALs but absent in inactive lesions with a consistent immunoreactivity reported principally in activated microglia and T cells at the lesion's edge [27,89]. In addition, a seminal study by Jackle et al. explored the immunological-molecular profile of CALs; through microarray analysis, they found an upregulation of different genes associated with immune functions including those for TNF and its receptors indicating its central role in the formation of CALs [93]. Specifically, the TNFR1 gene showed an almost 5-fold increased transcript expression in these lesion types [93].

TNF and TNFR1 also increased in cortical lesions [35]. GM damage, including cortical lesions and atrophy, is already present in the early disease phase [25,75,94,95], becoming more prominent during its progression [96]. Early cortical involvement is related to a more severe and rapid disease course in terms of disability progression and cognitive impairment. The transcriptional profile of chronic subpial GM lesions isolated from MS brain samples with prominent meningeal inflammation is consistent with skewing toward a detrimental, pro-inflammatory environment and microglia phenotype [85]. Such evidence is also supported by the above-mentioned study by Magliozzi et al., which demonstrates in subpial GM lesions of progressive MS cases higher levels of TNFR1 exclusively, but not TNFR2 [35]. Overall, these studies highlight TNF/TNFR1 signalling as potential future therapeutic targets for mitigating the impact of both CALs and GM lesions in MS.

## 6. TNF and PIRA

According to the newly proposed categorization, the different clinical MS phenotypes (RRMS, SPMS, PPMS, and PRMS) identified in 1996 [97] are summarized in relapsing–remitting disease versus progressive disease [98]. Both clinical forms of MS appear to reflect the same underlying disease process characterized by neuroinflammation and subsequent neurodegeneration [99,100] present in all MS lesions across the entire disease course [99–104]. In this context, compartmentalized neuroinflammation appears crucial in the onset and progression of neurodegenerative mechanisms that result in axonal loss and brain atrophy [105], strongly correlated with long-term functional and cognitive disability [106].

Several studies have also proven the association between focal inflammatory activity and diffuse and regional atrophic changes [12,107,109]. Specifically, MS lesions appear to cause brain volume loss through direct inflammatory damages leading to myelin and axonal loss and, indirectly, tissue loss following Wallerian degeneration [12].

In addition, evidence from neuropathological, imaging, and biomarker studies suggests a more continuous axonal loss across all clinically defined stages of MS, both in early and relapsing MS rather than in more advanced and progressive stages [8].

The classic RRMS/PrMS subdivision has been overcome since the emergence of a new concept of MS, the evidence of progression independent of relapse activity (PIRA) [12].

PIRA represents the first and main event responsible for irreversible disability accumulation in adult patients with RRMS, which occurs from 80% to 90% [8]. PIRA is already present in the early phases of disease and may even occur during disease-modifying treatments (DMTs) [12,106,109,110]. Two similar important studies investigating PIRA in early MS showed that about one-fourth of patients with RRMS may develop PIRA during the first ten years of the disease [111,112]. Patients who developed their first PIRA event very early in the disease course showed an unfavorable prognosis [112].

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PIRA occurs in roughly 5% of all patients with RRMS per annum, causing at least 50% of all disability accrual events in typical RRMS [113]. In this regard, a recent study confirms that up to 50% of the disability accumulation in adult patients with RRMS is not associated with evident relapses [114]. Relapses may mask disease progression and the loss of function over time may be as gradual in some patients that the patient or physician does not notice them; this would explain why PIRA is underestimated in RRMS [114].

Furthermore, patients with PIRA show significantly increased GM atrophy and CALs number, providing additional important evidence of the association between PIRA and diffuse neurodegeneration [12].

Currently, there are no specific biomarkers that can identify PIRA conditions. Overall, the only biomarker of ongoing neuronal damage taken into consideration, is serum neurofilament light chain (sNfLs). However, its association with long-term clinical outcomes or its ability to reflect slow and diffuse neurodegenerative damage in MS is not completely clear [115]. This lack of clarity is probably due to unstable measurements subject to physiological changes such as age or body mass index fluctuations [115,116].

In addition, although early treatment with DMTs is considered effective in preventing the transition to the progressive phase [115], several recent observational studies failed to confirm a beneficial association of DMT with PIRA [113,118,119].

Hence, the need to find a biological target that specifically reflects current and future prognostic disability and irreversible CNS tissue damage due to PIRA represents an urgent need.

## 7. Anti-TNF therapy and their potential use for PIRA

Based on TNF's strong pro-inflammatory activity, several anti-inflammatory drugs targeting TNF signalling have been developed and approved for treating inflammatory diseases, such as Crohn's disease, ankylosing spondylitis, and rheumatoid arthritis. Specifically, five TNF blockers are available for clinical use: infliximab, adalimumab, golimumab, certolizumab and etanercept. There is anti-TNF serum based on either anti-TNF antibodies (infliximab, adalimumab, golimumab and certolizumab) or TNFR fusion proteins (etanercept) that act as antagonists by blocking TNF (both mTNF and sTNF) interactions with TNFRs [120]. Despite being considered relatively safe and effective for the above-mentioned diseases, serious effects associated with immune suppression have been reported in MS [121,122]. In particular, a clinical trial of infliximab showed unfavorable results, whit increased disease activity and MRI lesion load, proving the association of TNF inhibitors with CNS demyelination [121,123]. Although the relationship between TNF blockers and demyelination remains uncertain, it is easy to speculate that it is probably since these blockers are not selective, i.e., they block the interaction TNFR1, which has a primarily pro-inflammatory action, and TNFR2, which has a primarily protective effect. This confirms the crucial role of TNF in the CNS, which exerts both potent pro-inflammatory effects (via TNFR1) and essential protective functions (via TNFR2) under pathological conditions [29,62]. Specifically, TNF through TNFR2 signalling modulates the reactivity of self-reactive T cells to self-antigens, promoting the expansion of Tregs cells and, subsequently, the preservation of myelin-oligodendrocytes [84]. Selective inhibition of TNFR1 and selective activation of TNFR2 through the use and even discovery of new antagonist and agonist antibodies could represent a new molecular target for developing therapeutic agents in MS [45]. In this regard, a recent preclinical study showed that treating atrosab, a human monovalent antibody against TNFR1 developed for treating inflammatory diseases, reduces disease severity. This preclinical evidence seems promising for finding novel effective drugs for MS and perhaps PIRA in the future [29].

## 8. Discussion and conclusions

TNF is considered the major cytokine involved in the pathogenesis of MS [29,30]. TNF exerts its pleiotropic effects by interacting with its receptors (TNFRs), TNFR1 and TNFR2, inducing respectively survival or cell death signals. TNFR1 signalling appears to be involved in the induction of neuroinflammatory processes, while TNFR2 is involved in neuroprotection and sustains homeostasis processes. The relative levels of TNFR1 and TNFR2 on the cellular surface and their

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activation status determine the complexity of the TNF/TNFRs signal. In MS, the alterations in TNFR1/TNFR2 balance have been confirmed [49] and associated with a more severe MS course, since the earliest stages of the disease [35]. In this regard, the use of transgenic mouse models has significantly contributed to understanding the pathological role of TNF in MS [45]. TNF/TNFR1 KO mice showed a milder disease course [64,65], whereas TNFR2 KO mice showed more severe EAE symptoms and diffuse demyelination [65]. Moreover, the selective ablation of TNFR2 in oligodendrocytes resulted in impaired remyelination [78]. Several studies confirmed that TNF/TNFR1 signalling mediates necroptosis and apoptosis in oligodendrocytes [32,33] and causes neuronal excitotoxicity [73] and necroptosis in cortical neurons [76], leading to inflammatory demyelinating processes and neurodegeneration. Demyelination and neurodegeneration are associated with the formation and expansion of typical pathological lesions, contributing to MS pathogenesis and progression. It is, therefore, not surprising that TNF and TNFR1 have been found overexpressed at the level of CALs and GM lesions in MS patients [27,35,89,93], especially in those with progressive MS [35]. In addition, increased levels of TNF were detected in the CSF of MS patients [23] to correlate with the degree of disability in patients with progressive disease [23,26,79,80]. TNF increase in the CSF is indeed associated with a chronic compartmentalized inflammation, which causes GM damage from the early stages, leading to more severe and rapid disease in terms of disability accumulation, disability progression and cognitive impairment [17,25]. All these results (summarized in Table 1) suggest a possible role of TNF/TNFR1 signal activation in disease progression independent of acute inflammation and in a decrease of compensatory mechanisms following a neuronal insult [17,25,82,83].

Progression independent of relapse activity, PIRA, is the main contributor to irreversible disability accumulation since the early phases of the disease and along the entire disease course [8,12,111–113]. However, disease progression can be extremely gradual and slow compared to relapses, making PIRA underestimated, not easily recognized [114], and, consequently, untreatable, although no recent observational studies confirm a beneficial association of DMT with PIRA [113,118,119].

Likewise, the TNF/ TNFRs blockers, currently available for the treatment of several inflammatory diseases, are ineffective but also harmful for MS patients [121,122]. TNF inhibitors' failure in MS therapy could be due to their unselective action, which, in attempting to inhibit TNFR1 signalling, fails to preserve the neuroprotective and cell survival processes associated with TNFR2 signalling.

For this reason, the use of selective modulators of TNFRs through TNFR2 activation and/or TNFR1 silencing (i.e., atrosab) represents a valid potential therapeutic option. Alterations in TNF/TNFRs signalling seem to be implicated not only in inflammatory but also in neurodegenerative processes, suggesting TNF and its receptors could be possible prognostic and therapeutic targets of PIRA.

**Table 1.** Studies on the role of TNF in MS and EAE.

| | | Type Cells Involved | Effects | Effects | Relevance | Relevance in MS | References |
|---|---|---|---|---|---|---|---|
| **MS patients** | TNF in the MS CSF | Macrophages, T cells | Infiltration of activated macrophages/T cells in the brain parenchyma | MS lesions formation | Inflammation | Disability accumulation | [53–55] |
| | | Neurons, Glial cells | Activation of neurons and glial cells | MS lesions formation | Neuroinflammation | | [23,26,79,80] |
| | TNF in MS Lesions | Neurons | Cortical lesions and Atrophy | GM damage | Neurodegeneration | Disability progression | [35] |

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|---|---|---|---|---|---|---|---|
| | | Microglia, T cells | Chronic active lesions | WM lesions | Demyelination | | [93] |
| | EAE mice | Macrophages, T cells | Infiltration of activated macrophages/T cells in the brain parenchyma | WM lesions | Neuroinflammation | Diffuse Demyelination | [56] |
| | | Neurons | AMPAR/NMDAR Overexpression | Neuronal Excitotoxicity | Neurodegeneration | Severe Disease Symptoms | [73] |
| | EAE TNF KO mice | Macrophages, T cells, Neurons, Glial cells | Infiltration reduction by macrophages and T cells; No AMPAR/NMDAR Overexpression; Enhanced Tregs cells | Neuronal Homeostasis | Neuroprotection | Severe Disease Symptoms | [64] |
| **EAE model** | EAE TNFR 1 KO mice | Macrophages, T cells, Neurons, Glial cells | No AMPAR/NMDAR Overexpression Enhanced Tregs cells | Remyelination Increase and Neurodegeneration Reduction | Neuroprotection | Reduction of disease signs and clinical symptoms | [65] |
| | EAE TNFR 2 KO mice | T regs | Suppression of $T_{regs}$ in response to autoreactive T cells | Impaired Remyelination | Demyelination | Aggressive Disease | [70,71] |
| | | Oligodendrocytes | Oligodendrocytes Death | Impaired Remyelination | Demyelination | Severe Disease Symptoms; Enhanced T cells infiltration in the CNS; Diffuse Demyelination | [65,78] |

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