The Dual Role of TNFRs in Multiple Sclerosis Treatment

Neuroprotection, Therapeutic Strategies, and Future Perspectives

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ABSTRACT

Multiple sclerosis (MS) is a neurological disease most prevalent in young adults. MS involves the immune system attacking the central nervous system (CNS), leading to demyelination, axonal damage, and neurodegeneration. Despite extensive research into the disease, the cause of MS remains unknown, however, it is believed that genetic and environmental factors are involved. Current treatment focuses mainly on reducing inflammation, slowing progression, and symptom relief, but none have successfully halted or reversed CNS damage. Studies have shown the effect of tumour necrosis factor-alpha (TNF-*a)* on MS pathology. TNF-*a* is a master cytokine that plays a role in inflammation and tissue regeneration. It works through two different receptors, TNFR1 and TNFR2, where TNFR1 stimulates inflammation and TNFR2 promotes neuroprotection and cell survival. TNF-a-based clinical trials have shown disappointing results on MS symptoms as a result of TNFR2 blocking. Recent research has demonstrated the potential of TNFR2-specific agonists to promote remyelination and neuroprotection while simultaneously inhibiting TNFR1-mediated damage to the CNS. The role of TNFR2 in regulating Treg cells and oligodendrocyte regeneration further shows its potential in therapeutics and promoting neuroprotection. An understanding of the pleiotropic effects of TNFR1 and TNFR2 could help in the development of new treatments that promote neuroprotection and tissue repair in MS and other inflammatory diseases. This review focuses on the dual role of TNFR1 and TNFR2 in MS therapy, with a focus on TNFR2's potential in neuroprotection.

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INTRODUCTION

Multiple sclerosis (MS) is a frequently occurring neurological disease most commonly found in young adults (Doshi & Chataway, 2016). Sub-Saharan Africa has the lowest prevalence of the disease, while North America and Europe have the highest prevalence (Doshi & Chataway, 2016). Patients with MS experience their immune system attacking the central nervous system (CNS), which leads to demyelination, axonal damage, and neurodegeneration (Kuhlmann et al., 2023). MS shows several neurological hallmarks, among which are inflammatory demyelinating plaques, which can be found in the CNS. These are marked by T-cell infiltration, activation of microglia and astroglia, and loss of synapses due to neurodegeneration (Fresenga et al., 2020). MS presents itself in various forms: relapsing-remitting (RRMS), primary progressive (PPMS), and secondary progressive (SPMS). In RRMS, patients experience acute attacks of symptoms followed by periods of recovery. PPMS is characterised by a gradual decline in motor functions after the onset of the disease. SPMS initially follows a relapsing-remitting pattern, but later in the disease, it turns into a slow decline (Kuhlmann et al., 2023).

Despite extensive research, the cause of MS remains unidentified. However, it is widely believed to be a multifactorial disease influenced by both genetic factors and environmental influences (Fresengna et al., 2020). Current therapies primarily focus on reducing inflammation, slowing disease progression, and managing relapses (Madsen et al., 2016). These treatments for MS focus on three main areas: managing acute relapses, disease-modifying treatments, and addressing symptom relief (Doshi & Chataway, 2016). Acute relapse management typically involves using *methylprednisolone* to treat infections that might trigger relapses, whereas disease-modifying treatments aim to halt new MS relapses or disease progression (Doshi & Chataway, 2016). Lastly, symptomatic treatments are used to treat symptoms occurring with MS, such as fatigue, spasticity, bladder or sexual dysfunction, or pain (Doshi & Chataway, 2016). These current treatments are mainly focused on silencing symptoms and disease progression. Current-day medicine does not yet allow the halting or reversing of myelin or axonal damage, which underlies the permanent disability of MS (Madsen et al., 2016). The goal of MS research is to find a therapeutic that will reduce inflammation and simultaneously promote remyelination and regeneration (Pegoretti et al., 2023).

An interesting topic of current-day research is the role of tumour necrosis factor-alpha (TNF) in the pathology and treatment of MS. TNFs play an important role in various signalling pathways, including cell survival, inflammation, and tissue regeneration (Pegoretti et al., 2023). This master cytokine plays an important role in the immune system and is often associated with MS. Research has shown a correlation between the TNF levels in the bloodstream and cerebrospinal fluid (CSF) and the disease severity in patients with MS (Vladić et al., 2002). Post-mortem studies show elevated TNF levels in the brains of individuals with MS, mostly at the site of active lesions, where its concentration correlates with the severity of the lesion (Fischer et al., 2019). TNF works through two receptors: TNFR1 and TNFR2. Each receptor plays a different role in MS pathology. TNFR1 is linked to signalling pathways that promote

inflammation and apoptosis. TNFR2 is associated with protective mechanisms and anti-inflammatory responses (Zahid et al., 2021). TNFR1 has been intensively researched in the past and has demonstrated its role in inflammatory processes. This research has highlighted the potential role of TNFR1 in tissue damage in MS. In contrast, research into TNFR2 has been relatively limited. Its role in protective mechanisms and anti-inflammatory signalling has been discovered in recent years. This discrepancy highlights the need for further research into TNFR2's role in MS.

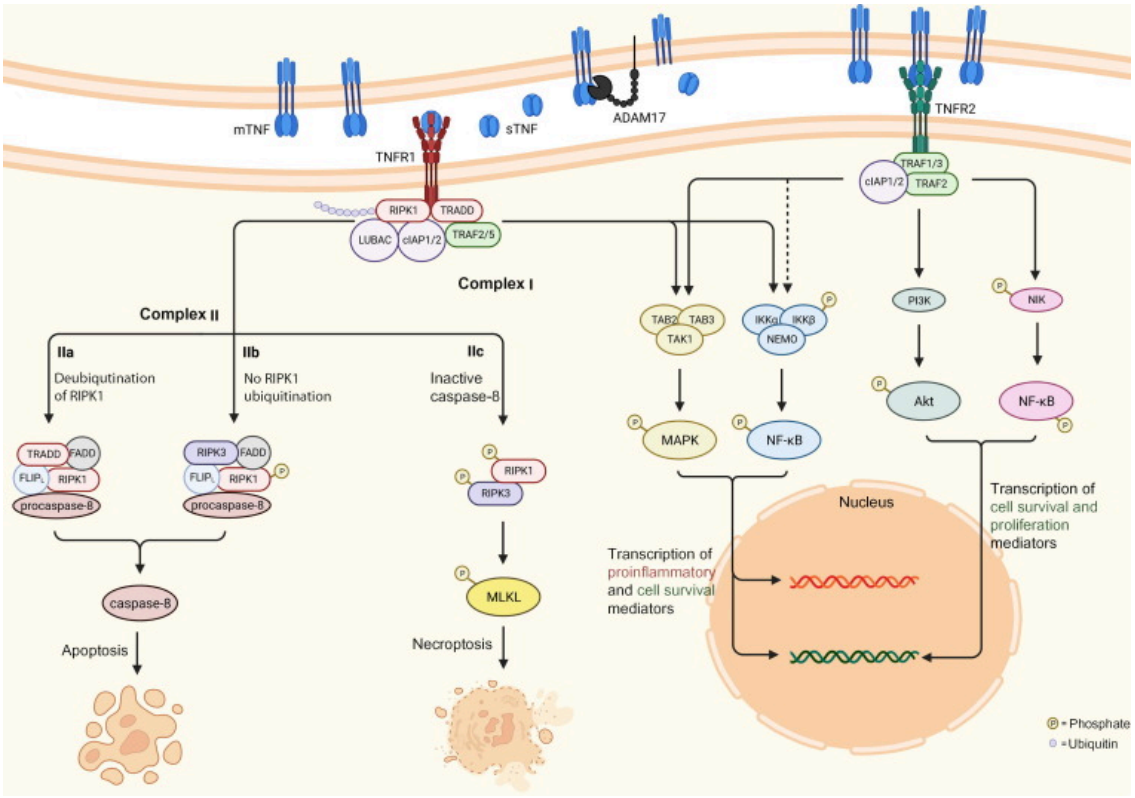
Various studies have investigated the potential therapeutic benefits of enhancing TNFR2 signalling. The effect of TNFR2 signalling has been researched using disease models and various TNFR2 agonists (Fiedler et al., 2023). An example of this is the TNFR2-specific agonist TNC-scTNF80. This agonist has shown positive effects in autoimmune disease models. These results show the possible use of TNFR2 signalling in MS treatments (Lamontain et al., 2018). TNFR1 and TNFR2 activate different signalling pathways. TNFR1 mainly acts on the NF-*k*B and MAPK pathways. When these pathways are triggered, they lead to inflammation and cell death. In contrast, TNFR2 activates the PI3K/Akt pathway. This pathway leads to increased cell survival and cell regeneration (Probert, 2015). Clinical trials targeting TNF have shown mixed results. Anti-TNF therapies, which primarily antagonise TNFR1, have failed in MS clinical trials and, in some cases, have worsened the disease. Research done on TNF-*a* antagonists (*Lenercept, Infliximab)* in two separate pieces of research showed negative effects during treatment (Wiendl et al., 2000). This failure is linked to the dual role of TNF, where blocking TNFR1 may reduce inflammation but simultaneously inhibits the protective effect mediated by TNFR2.

Current research on MS therapeutics is mainly focused on selectively targeting TNFR1 and TNFR2 signalling. Promising results have shown that the use of TNFR2 agonists may help in promoting neuroprotection and remyelination. Simultaneously inhibiting the negative effects of TNFR1 on MS pathology might result in a decrease in disease progression (Fresegna et al., 2020). Current studies are focused on understanding the complex interplay between these two receptors and their pleiotropic role in MS. Understanding the pleiotropic effect of TNFR1 and TNFR2 is important for the development of new MS treatments. Current treatments mainly focus on inflammation and symptom relief, whereas future medications should focus on encouraging tissue repair and regeneration. This review focuses on the interplay between TNFR1 and TNFR2 concerning multiple sclerosis treatment, with a focus on the neuroprotection effect of TNFR2.

I. THE ROLE OF TNFRs IN MS PATHOLOGY

Tumour necrosis factor-alpha (TNF-*a*) is a cytokine with pleiotropic effects in regulating the inflammatory response of the body (Jang et al., 2021). Research has demonstrated the role of TNF-*a* in the development of autoimmune and inflammatory diseases, including multiple sclerosis (MS), inflammatory bowel disease (IBD), and rheumatoid arthritis (RA) (Fiedler et al., 2023). This master regulator is involved in many physiological functions, including apoptosis, inflammation, autoimmunity, and demyelination (Pegoretti et al., 2018). TNF-*a* consists of 175 amino acids and is produced during acute inflammation by macrophages, T-lymphocytes, and natural killer cells (Idriss & Naismith, 2000). TNF-*a* contains two variants: soluble and transmembrane. The transmembrane form of TNF-*a* (tmTNF-*a*) is synthesised initially as a precursor form and acts through cell-to-cell contact (Jang et al., 2021). tmTNF-*a* is cleaved by TNF-*a* converting enzyme (TACE) to produce the soluble form of TNF (sTNF-*a*) (Fiedler et al., 2023). TNF signals through two surface receptors, TNF receptor-1 (TNFR1, p55/p60) and TNF receptor-2 (TNFR2, p75/p80) (Idriss & Naismith, 2000). These different receptors activate different signalling pathways downstream. TNFR1 is widely expressed in most tissues, whereas TNFR2 expression is more regulated and primarily limited to immune cells, endothelial cells, and glial cells (Fresegna et al., 2020). tmTNF signals mainly through TNFR1 and TNFR2, but mostly mediates its biological functions through TNFR2 (Horiuchi et al., 2010). On the contrary, sTNF signals mainly through TNFR1 due to a higher affinity for the receptor (Fischer et al., 2020). Both receptors initiate different biological processes, TNFR1 predominantly promotes inflammation and apoptosis, whereas TNFR2 mediates cell survival and tissue regeneration (Fresegna et al., 2020). The activity of these receptors is dependent on their intracellular structure, which differentiates them from each other. The pleiotropic effect of TNFa mediates the function of this cytokine through the ratio of expression of the different receptors. This ratio determines whether expression will shift to cell survival or apoptosis (Pegoretti et al., 2018). TNFR1 is part of the death domain (DD)-containing receptors, whereas TNFR2 is a receptor without DD (Wajant & Scheurich, 2011).

TNFR1 stimulates apoptosis through effector caspase and prevents apoptosis induced by TNF by activating the NF-*k*B pathway (Pegoretti et al., 2018). Signal transduction by the activation of TNFR1 is done by two intracellular complexes (complex I and complex II). Complex I activates the NF-*k*B and MAPK pathways, whereas complex II regulates programmed cell death (Chédotal et al., 2023). Upon TNF binding to TNFR1, a signalling complex is formed involving proteins like TRADD, RIP1, TRAF2, and cIAPs (Wajant & Scheurich, 2011). This complex I modifies intracellular proteins, leading to the activation of kinases like IKK and TAK1. Consequently, these kinases phosphorylate other proteins, eventually leading to the activation of transcription factors like NF-*k*B. This ultimately leads to the regulation of gene expression in inflammation and cell survival (Wajant & Scheurich, 2011). TNFR1 complex I can also bind MAP kinase kinases (MKK) and activate the MAP kinase pathway. Leading to the formation of a secondary signalling complex (complex II). This complex activates caspase 8,



(Figure 1). Visualisation of signalling pathways of TNFR1 and TNFR2. *Tumor necrosis factor receptor (TNFR) activation and signaling.* (2023, June). https://www.sciencedirect.com/science/article/pii/S1359644623000910

initiating the caspase cascade and ultimately inducing apoptosis (Maguire et al., 2021). However, TNFR1 signalling can prevent TNF-induced cell death by activating the classical NF-*k*B pathway (Pegoretti et al., 2018). The intracellular DD recruits RIP1 and TRADD after TNFR1 stimulation. TRADD forms complex I through its interaction with TRAF2 and cIAPs (Pegoretti et al., 2018). The activation of the NF-*k*B pathway is promoted by the IKK complex, which is activated by the ubiquitination of RIP1 and complex I (Pegoretti et al., 2018). If this signalling pathway fails, caspase-8-mediated apoptosis is activated by complex II.

TNFR2 signalling involves the formation of a complex with TRAF2, cIAP1/cIAP2, and HOIP. This complex activates NF-*k*B pathways, including both the canonical and non-canonical pathways. TNFR2 signalling also activates the JNK and p38 MAPK pathways, promoting cell survival and proliferation through the PI3K/Akt pathway (Maguire et al., 2021). Both TNFR1 and TNFR2 trigger the transcription factor NF-*k*B, despite their distinct roles. NF-*k*B is a regulatory transcription factor essential for the activation of genes related to inflammation. NF-*k*B initiates the transcription of proinflammatory cytokines, including TNF. This process results in a positive feedback loop that enhances and maintains inflammation as TNF activates and is activated by NF-*k*B (Marchetti et al., 2004).

Studies have highlighted that TNF levels can vary depending on the disease stage and correlate with MS progression (Fiedler et al., 2023). TNF-*a* is found at elevated levels in the cerebrospinal fluid, especially sTNFR (Madsen et al., 2016). Lesions of MS patients showed an increased amount of TNF expression, suggesting its involvement in the disease pathology (Madsen et al., 2016). Experimental models of MS have shown the effect of TNF overexpression on myelination. Increased TNF caused demyelination in MS patients, whereas blocking TNF signalling showed disease improvement (Madsen et al., 2016). Additional studies support the involvement of sTNFR in MS pathogenesis. Results from this study suggest that there is a positive correlation between plasma sTNFR1 levels and disease progression (Fiedler et al., 2023). On the contrary, a negative association was observed between sTNFR2 levels and the development of MS (Fiedler et al., 2023). TNF-*a* has been previously linked to inflammation and demyelination in the acute phases of MS, with TNFR1 playing a significant role in these processes. TNF-*a* has also been shown to have immunosuppressive properties in later stages of MS, possibly mediated by TNFR2 (Gregory et al., 2012).

TNF-*a* contributes to the breakdown of the blood-brain barrier (BBB), facilitating the infiltration of immune cells into the CNS and promoting inflammatory responses that lead to myelin and axonal damage (Fischer et al., 2020). An animal model of MS, experimental autoimmune encephalomyelitis (EAE), has been useful in providing insight into the role of TNF-*a* in MS. Results have shown the effect of blocking TNF-*a* and its receptors in reducing the severity of demyelination in EAE models (Raphael et al., 2019). This highlights the possible potential of TNF inhibition as a therapeutic target. However, clinical trials using TNF-*a* inhibitors, like Infliximab and Lenercept, have shown negative results in reducing MS pathology. (The Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group., 1999) These studies not only failed to demonstrate the benefits of TNF inhibition but also showed worsened disease symptoms in MS patients.

A promising therapeutic approach involving the inhibition of TNFR1 signalling and the agonism of TNFR2 for MS has been proposed (Fiedler et al., 2023). This may promote cell survival, proliferation, neuroprotection, and remyelination, offering new therapeutic perspectives for MS (Fiedler et al., 2023).

II. TNFR2: UNVEILING ITS ROLE IN MS

The role of TNFR1 has been largely known. Therapeutics targeting TNFR1 have been developed and have been successful in reducing inflammation in several studies. Anti-TNFR1 antibody antagonism has been demonstrated to minimise EAE in MS animal models (Gregory et al., 2012). An even more effective way of tackling the effects of TNF in MS is to block TNFR1 and promote TNFR2 signalling simultaneously (Fiedler et al., 2023). However, the workings of TNFR2 are still not completely understood.

In vivo, TNF signalling was thought to be predominantly mediated by TNFR1, as shown in TNFR1-deficient mice displaying the same defects as TNF-deficient mice (Fischer et al., 2015). However, knockout studies in mice have suggested that TNFR2 and its preferred ligand, tmTNF, may play a role in EAE concerning protection (Fischer et al., 2011). TNFR2 is found to be involved in various protective and regenerative pathways, mainly in the CNS (Dong et al., 2016). The effects of TNFR2 signalling have been investigated using in vivo models of neurotoxic damage, showing increased neuronal survival and oligodendrocyte regeneration (Dong et al., 2016). Additionally, research investigating the loss of function in mice showed the involvement of TNFR2 in EAE. The membrane-bound form of TNF showed a protective role in this mouse model for MS (Raphael et al., 2019). The experimental models of MS using EAE have provided insights into the functioning of TNFR2. Studies using TNFR2 knockout mice have demonstrated increased disease severity, indicating a protective role for TNFR2 in MS (Pegoretti et al., 2023). The demyelination processes in MS might be linked to the overexpression of TNF. Research involving the exclusive expression of tmTNF demonstrated that tmTNF inhibits the progression of EAE onset when sTNF is not present. (McCoy & Tansey, 2008). These findings are important for the therapeutic strategy for MS patients. It shows that while limiting TNF functions, tmTNF is still able to maintain self-tolerance and infection resistance (McCoy & Tansey, 2008).

TNFR2 is mostly expressed by immunosuppressive cells, like Tregs. Treg controls the development of autoimmune diseases by suppressing immune activity (Fischer et al., 2019). This effect is especially seen on CD4+FoxP3+Treg cells, which have been shown to be an essential part of immune homeostasis and autoimmunity suppression. (Fresegna et al., 2020). Multiple studies have shown the role of TNFR2 in Treg regulation, suggesting the potential for the development of therapeutics that target TNFR2 to enhance or suppress Treg's role in autoimmune diseases (Fresegna et al., 2020). TNFR2 is upregulated in Treg and can independently promote proliferation (Fresegna et al., 2020). Increased TNFR2 expression was seen in MS Tregs, this expression is assumed to play a role in MS patients' anti-inflammatory and regenerative responses (Chen et al., 2023). Treg cells in TNFR2 knockout mice failed to expand when stimulated in inflammatory conditions, even though the number and function were comparable with wild-type (Fresegna et al., 2020). A different study using double TNF/TNFR2 KO mice revealed significant impairment in Treg suppressive functions *in vitro* (Fresegna et al., 2020). The role of Treg-TNFR2 in CNS autoimmunity was shown using EAE mice. Deletion of Treg-TNFR2 showed worsened motor symptoms in this model, indicating that Treg-TNFR2

plays a role in the suppression of CNS autoimmunity (Fischer et al., 2019).
Treg cells regulate FoxP3 expression, and in this process, TNFR2 plays an essential role in maintaining it (Fischer et al., 2015). TNFR2 shows a preference for binding to tmTNF in comparison to TNFR1, which binds to both sTNF and tmTNF. TNFR2 signalling promotes inflammatory and pro-survival signalling pathways. TNF binding to TNFR2 starts a signalling pathway that uses TRAF1, which activates in response to NF-*k*B. This cascade results in neuronal growth and cell survival. TNFR2 activation does not lead directly to caspase activations as it does not contain a DD (McCoy & Tansey, 2008).
Various mouse models have demonstrated the positive effects of TNF receptor 2. Various researchers have investigated the role of TNFR2 using agonists to show its beneficial impacts. TNFR2-selective agonists only bind to TNFR2 but show no affinity to TNFR1 (Chen et al., 2023). Fischer et al., 2011 showed the workings of a human TNFR2-selective agonist in differentiated neurons. Results show that cell survival after exposure to H2O2 improved after being activated by TNFR2 agonists. This data showed that selective targeting of TNFR2 signalling might be a novel approach to treating neurodegenerative diseases. The effect of TNFR2 regulation has been investigated in EAE. EHD2-sc-mTNFR2 is an agonist for TNFR2 and has been proven to successfully improve EAE symptoms (Fiedler et al., 2023). This TNFR2-agonist showed beneficial effects in promoting the expansion of peripheral Treg cells and suppressing autoimmunity (Fiedler et al., 2023). Reserved effects were observed by the deletion of TNFR on Treg cells, which led to an increase in EAE severity. Concluding the role of TNR2 in suppressing autoimmunity (Fiedler et al., 2023).

III. NEUROPROTECTION MECHANISMS MEDIATED BY TNFR2

The role of TNFR2 has been investigated in the past. TNFR2 works through tmTNFR in tissue regeneration and neuroprotection. In vivo models have demonstrated that TNFR2 signalling promotes both neuronal survival and oligodendrocyte regeneration (Fontaine et al., 2002). TNFR2 activation promotes myelination and oligodendrocyte differentiation, as well as the protection of neurons and oligodendrocytes from oxidative stress (Dong et al., 2016). The phosphatidylinositol 3-kinase (PI3K) pathway plays an important role in neuroprotection against excitotoxicity (Long et al., 2021). The effect on the PI3K pathway in multiple sclerosis specifically has not been fully investigated. However, the effects of different neurodegenerative diseases have been identified. For example, stroke treatment has been studied using the PI3K pathway due to its involvement in ischemic injury (Khan et al., 2021). Activation of the PI3K pathway is linked to promoting neuronal survival and protecting against cell death in various neurodegenerative disorders. It acts as a regulator that initiates a cascade of events leading to the activation of Akt and NF-*k*B. These components are essential for the neuroprotective signalling pathway. One of the most significant effector kinases that occurs downstream of PI3K is Akt, which forms the core of the PKB pathway (Long et al., 2021). Activation of Akt can phosphorylate and regulate various downstream targets involved in cell survival and anti-apoptotic pathways. The expression of proteins involved in both apoptosis and cell survival is significantly regulated by the Akt pathway (Khan et al., 2021). Similarly, NF-*k*B activation influences the expression of genes that enhance cell survival and prevent cell death (Micheau & Tschopp, 2003). It has been discovered that sustained PI3K/PKB/Akt-mediated NF-kB activation results in neuroprotection. This pathway is enhanced by N-methyl-d-aspartate (NMDA) receptor co-stimulation (Pegoretti et al., 2018). A study using a human dopaminergic neuronal cell line (LUHMES) showed the effect of TNFR2 stimulation on the PI3K/PKB pathway. TNFR2 improved the state of the neurons that had stress-induced oxidative cell death (Pegoretti et al., 2018). TNFR2 neuroprotective properties are associated with PI3K-PKB/Akt activation (Fontaine et al., 2002). The same was seen in cortical neurons, where TNFR2 regulates protection against glutamate-induced cell death in a PI3k/PKB/Akt-dependent way (Marchetti et al., 2004). In primary cortical neurons exposed to glutamate-induced excitotoxicity, the neuroprotective signalling pathways of TNFR2 involve the activation of PKB/Akt and NF-*k*B (Marchetti et al., 2004). Specifically, TNFR2 facilitates long-term PI3K-dependent NF-*k*B activation, which is shown to be essential for neuronal survival. Susceptibility to excitotoxic stress is measured by the duration of NF-kB activation, which is dependent on how differently TNFR1 and TNFR2 use their respective upstream signal pathways (Marchetti et al., 2004).

In mouse models of neurodegenerative diseases, such as Alzheimer's disease, TNFR2 activation has been demonstrated to block neuroinflammation and promote neuronal survival, highlighting neuroprotective effects (Dong et al., 2016) Studies with *lovastatin* showed TNFR2 involvement in neuroprotection again. Lovastatin is a drug often used in Alzheimer's studies, however, its workings in neuroprotection might be useful in MS research. Lovastatin-mediated

neuroprotection resulted in increased PI3K-dependent PKB/Akt phosphorylation. In contrast, the blocking of PKB/Akt activation reduced lovastatin-induced neuroprotection. This result aligns with previous research showing the effect of TNFR2-mediated neuroprotection.

Furthermore, in the response of hippocampal neurons to TNF, TNFR1 and TNFR2 play different roles: TNFR1 stimulates NF-*k*B activation and cell death, whereas TNFR2 activates p38 mitogen-activated protein kinase (MAPK). It is evident that TNFR2 may be crucial for neuroprotection, even though the exact functional significance of TNFR2-mediated p38 activation and workings of apoptosis in the presence of strong NF-*k*B activation are still unclear (Marchetti et al., 2004).

A key feature of MS is demyelination. Autoreactive T-helper cells recognise myelin protein and myelin oligodendrocyte glycoproteins in MS patients as well as healthy individuals (Pegoretti et al., 2018). A shift is happening in the aetiology of MS; the disease is being investigated from a different angle. MS appears to be primarily an autoimmune disease, however, it is questioned whether or not MS might be a degenerative disorder (Stys et al., 2012). This proposed theory states that the degeneration of oligodendrocytes and myelin triggers pathology by releasing autoantigens. These antigens are responsible for the inflammatory response in the individual (Pegoretti et al., 2018).

In vitro studies reveal the effect of TNFR2 activation on protecting oligodendrocyte progenitor cells (OPCs) when induced by oxidative stress (Pegoretti et al., 2018). Previous studies have indicated the importance of OPCs in the remyelination process (Pegoretti et al., 2018). TNFR2 stimulation on astrocytes showed the differentiation effect of OPCs on mature oligodendrocytes (Fischer et al., 2013). An *in vivo* study in mice with either one of the TNF-*a* receptor knockout models was conducted to investigate the effect of TNFRs on demyelination (Arnett et al., 2001). Results showed that the remyelination process is delayed due to the lack of TNF-*a*, which causes a reduction in OPCs and mature oligodendrocytes. Similar results were obtained in TNFR2-knockout mice. But not in TNFR1, suggesting a function for TNFR2 in oligodendrocyte proliferation and myelin regeneration (Arnett et al., 2001). More evidence for this statement comes from an EAE study using a selective soluble TNF blocker (*XPro1595*) (Brambilla et al., 2011). Mice treated with this inhibitor showed increased expression of myelin-associated genes and an elevated number of oligodendrocyte precursors, which led to remyelination (Brambilla et al., 2011). This data demonstrates that inhibiting sTNF improved the recovery following EAE. The effect of this sTNF inhibitor was demonstrated again a few years later in oligodendrocyte-specific TNFR2 conditional KO mice (CNP-cre; TNFR2fl/fl mice) (Madsen et al., 2016). In these mice, TNFR2 expression in OPCs was significantly reduced compared to the control. When treated with the sTNF inhibitor, remyelination did not improve. These findings imply that TNFR2 in oligodendrocytes plays a positive role in remyelination and that the advantages of tmTNF-mediated signalling through TNFR2 outweigh those of sTNF blocking with Xpro1595.

IIII. THERAPEUTIC IMPLICATION AND FUTURE DIRECTIONS

The role of TNFs in MS has been extensively researched in the last few years. The pleiotropic cytokine is found to play an important role in the immune system and the central nervous system. TNF works through two receptors, TNF receptor type-1 (TNFR1) and type-2 (TNFR2), which complicates the understanding of the TNF signalling pathway. Several human and experimental studies show evidence for the involvement of TNF in MS pathology. TNF contributes to immune dysregulation by promoting autoreactive T cells and induces demyelination by causing oligodendrocyte apoptosis (Brambilla et al., 2011). Additionally, TNF plays an important role in neuroinflammation by provoking tissue damage in the CNS (Brambilla et al., 2011). These findings on TNF underscore the potential of targeting TNF in MS. The use of tumour necrosis factor *a*-inhibitor therapy is an essential therapeutic option in various inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases (Kaltsonoudis et al., 2014). These biologics have shown positive effects in the treatment of chronic inflammatory conditions. The inhibition of TNF-*a* prevents its binding to TNF receptors 1 and 2, therefore reducing inflammation and tissue damage (Kaltsonoudis et al., 2014). However, TNF-*a* antagonists may not have the same effect in the CNS due to the blood-brain barrier preventing TNF-*a* from being drawn out of the CNS. This way, TNF-*a* inhibition will not have the same effect in MS (Kaltsonoudis et al., 2014). Despite promising results in studies using anti-TNF therapies for different autoimmune diseases, the same effects were not established in MS. One of the first clinical trials for MS using a non-selective TNF inhibitor was Lenercept. Lenercept was shown to reduce clinical signs in patients with rheumatoid arthritis (Toussirot & Wendling, 2004). The recombinant TNF receptor was administered to patients with stable RR- or SPMS. These patients showed the development of new symptoms and increased neurological deficits after the Lenercept administrations, showing the deleterious effects of inhibiting TNF in MS. These findings in pre-clinical models suggest that the inhibition of TNFR2 signalling by Lenercept was the cause of the symptom increase (McCoy & Tansey, 2008). This increased severity of MS symptoms led to the rejection of this TNF inhibitor as a therapy for this immune disease (The Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group., 1999). The anti-TNF antibody (cA2), infliximab (McCoy & Tansey, 2008b), has also shown promising effects in studies of rheumatoid arthritis. However, when administered to MS patients, increased lesions and no improvement in disease severity were observed. This clinical trial ended as it did not show promising results. These failed results were unexpected, a plausible explanation for them might be linked to the pleiotropic effects of TNF. The cytokine has pro- and anti-inflammatory properties, which makes understanding its workings difficult. The interplay between nonselective TNF inhibitors and MS has been shown further in clinical cases with TNF inhibitors in other diseases. Patients with rheumatoid arthritis showed MS-like symptoms like demyelinating lesions in the CNS when treated with TNF inhibitors

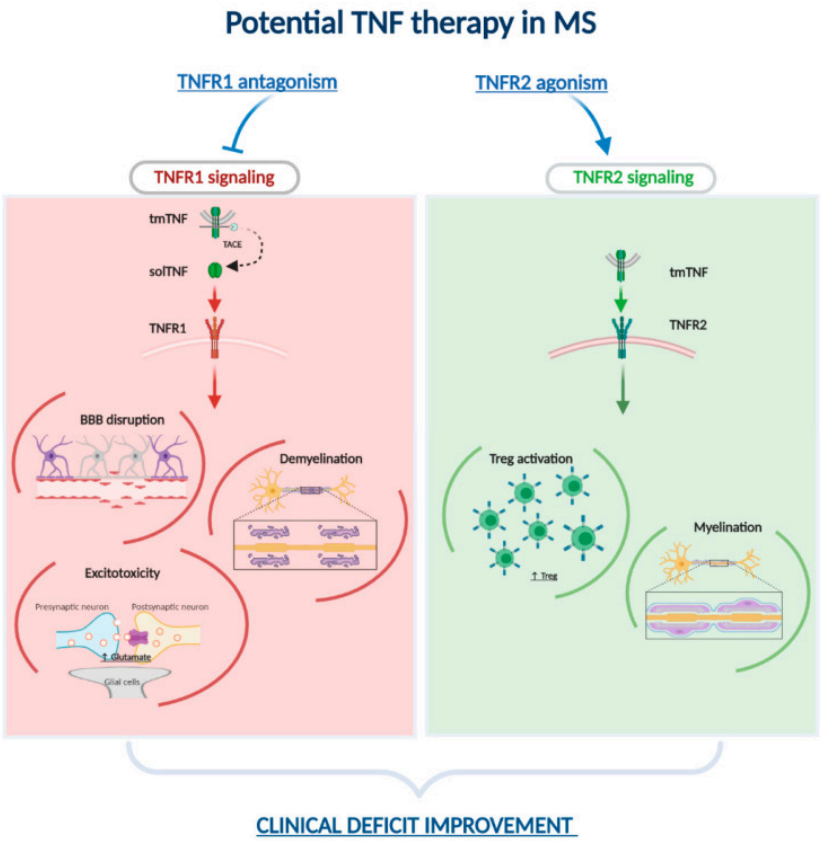


Figure 2. Potential TNF therapies in MS. TNFR2 agonists increase TNFR2 signalling to improve Treg activation and remyelination. TNFR1 antagonists block TNFR1 signalling to inhibit processes like demyelination. *Potential TNF therapy in MS. (2020, October 14). https://www.mdpi.com/2073-4409/9/10/2290*

etanercept or infliximab (Mohan et al., 2001). These results show the role of TNF in MS pathology but also demonstrate the harmful effects of non-selective TNF inhibition in MS and other inflammatory diseases (Fresegna et al., 2020). It has been established that non-selective TNF inhibition is not an effective way to treat MS, but TNF has been found to play an important role in MS pathology. Several theories have been conducted about the ineffectiveness of TNF inhibition in MS. The first theory, known as the "lack of entry" hypothesis, suggests the inability of TNFa-blockers to enter through the blood-brain barrier (BBB). This barrier is a selective membrane that protects the CNS from potentially harmful cytokines in the blood. According to this theory, the inability of TNF-a blockers to penetrate the BBB might explain the failure to reduce TNF-a-mediated demyelination in MS (Robinson et al., 2001). A second theory, the "sponge effect" hypothesis, explains the effect of TNFa blocker's ability to deactivate TNF-*a* throughout the body but fails to do so within the CNS due to the BBB (Robinson et al., 2001). The selective permeability of the BBB causes TNF-*a* to be neutralised in the peripheral bloodstream, but it remains active and accumulates in high concentration within the CNS. Consequently, the elevated TNF-*a* within the CNS continues to promote inflammation and demyelination, leading to the progression of MS (Robinson et al., 2001). Another theory explains that TNF-*a* blockers might increase demyelination within the CNS by inhibiting TNFR2

(Kemanetzoglou & Andreadou, 2017). TNFR2 plays an important role in the regeneration of oligodendrocytes and myelin repair processes. Upon administration, TNF-*a* blockers do not distinguish between TNFR1 and TNFR2, leading to the inhibition of both. This theory highlights the importance of developing a targeted therapy that selectively inhibits TNFR1 signalling and enhances TNFR2. This co-modulation of receptors has been investigated in an animal model of MS recently (Fiedler et al., 2023). This study showed the effect of human TNFR1-selective antagonists and mouse TNFR-specific agonism in humanised mice in an MS animal model (Fiedler et al., 2023). Key findings suggest that the conjoining approach of inhibiting TNFR1 and stimulating TNFR2 signalling leads to improvements in disease symptoms, neuroprotection, and oligodendrocyte survival. This dual targeting strategy might provide a more comprehensive and effective treatment option for MS patients by simultaneously addressing the inflammatory and neurodegenerative components of this disease.

DISCUSSION

Multiple sclerosis is a neuroinflammation disease that is most common among young adults. The disease pathology includes mostly inflammation and demyelination. Current therapeutics are mainly focused on halting disease progression and symptom relief, however, none of the current treatments have succeeded in positively improving the disease course. TNF-*a* is an interesting topic of research at the moment due to its well-researched role in other immune diseases like rheumatoid arthritis and inflammatory bowel disease. Clinical trials in these diseases using anti-TNF-*a* therapies have shown positive effects. However, when implementing these findings into clinical trials in MS, the same positive results are not perceived. The investigation into the role of TNF receptors, TNFR1 and TNFR2, in the pathology and potential treatment of multiple sclerosis (MS) reveals complex interplay that has significant implications for the development of therapeutic strategies. TNF-*a* is a key cytokine that is known for its pleiotropic effects in promoting both inflammation and tissue regeneration. Multiple studies have shown the involvement of TNF-*a* in MS pathology. Understanding the functions of TNFR1 and TNFR2 is important for the development of targeted therapies that can effectively treat MS.

Therapeutic targeting of TNF-*a* in MS has shown mixed results, mainly due to the pleiotropic effects of TNFR1 and TNFR2. Therapies using TNFR1 antagonists have failed to show improvement in MS symptoms and, in some cases, even exacerbated the disease. For example, Lenercept was previously shown to improve disease pathology in patients with rheumatoid arthritis, but when administered to MS patients, opposite results were perceived. This TNF-*a* inhibitor caused the inhibition of TNFR2, resulting in increased neurological deficits. Many theories have been hypothesised about the ineffectiveness of TNF inhibition in MS. The main problem is thought to be the inability of TNF-*a*-inhibitors to enter through the blood-brain barrier. This might explain the failure of reducing TNF-*a*-mediated demyelination in MS. Anti-TNF-*a* drugs are often not distributed in the CNS, resulting in an ineffective way of battling neurodegenerative diseases (Chédotal et al., 2023). Further research should focus on finding ways to infiltrate the BBB to selectively inhibit TNF-*a* in the CNS. Genome-wide association studies (GWAS) have shown a genetic variant in the TNFRSF1A gene as a potential causal factor in MS (Atretkhany et al., 2018). This gene encodes for TNF-receptor 1, which has previously been proven to be involved in the development of MS. A single nucleotide polymorphism (SNP) in this TNFRSF1A gene might interfere with the response to anti-TNF therapy. The variant of TNFR1 might play a role in stimulating the effects of TNF-blocking drugs. Further research has demonstrated that this SNP leads to the expression of a different form of sTNFR1 that might antagonise TNF. These findings align with the effects found in patients on anti-TNF therapies (Fiedler et al., 2023).

One of the main models of MS is the EAE mouse model. Most of the research in this review uses the MS mouse model to create MS-like symptoms in mice. Studies showed that EAE was a good model to show the same clinical, neuropathological, and immunological symptoms as MS. This led to the belief that this model was a good way to test therapeutics for MS.

However, this view led to disappointing results and questioned whether or not EAE is a suitable model to study MS pathology (Gold, 2006). Given the complex nature of this disease, it might be that this model does not fully suffice, as there is variation among patients. Therefore, the use of EAE models in research should be critically looked at, and a suitable model should be used. Another major limitation in research into drug targeting was the limited availability of suitable preclinical animal models (Fischer et al., 2015). Many of the TNFs derived from humans did not engage with murine TNFs and TNFR2, leading to insufficient results (Fischer et al., 2015). However, recent research has shown the usage of humanised TNFR knock-in mice, where exogenous human TNFs were able to bind and trigger the endogenous signalling pathways in mice (Pegoretti et al., 2023). This advancement in animal models will open up new pathways for therapeutic options in MS and other immune disorders.

The use of anti-TNF drugs is found to be a promising therapeutic target in other diseases. primarily due to the pleoitropic effect of TNF-*a* in regulating different processes and signalling pathways. Not just in inflammatory conditions like psoriasis, inflammatory bowel disease, and rheumatoid arthritis, but also in neurodegenerative conditions like Alzheimer's. Research demonstrates that patients with Alzheimer's disease have elevated TNF-a levels (Dhapola et al., 2021), which is also observed in MS patients. Because the properties of TNF-a are similar, therapeutic advances made in MS pathology may have application in Alzheimer's disease. The COVID-19 pandemic recently highlighted how critical it is to comprehend TNF's function in various illnesses. Research has found evidence for the role of TNF-*a* in the onset of COVID-19 disease. Although TNF-a inhibitor therapy has demonstrated encouraging results in patients with severe symptoms, it is not yet clinically approved (Leone et al., 2023).

The use of anti-biologics in other inflammatory diseases has shown positive effects in reducing disease pathology. One of the main inflammatory diseases that anti-TNF is used for is rheumatoid arthritis. However, in several cases, these biologics have caused severe side effects after administration. Due to the nonspecific immune inhibition by these anti-TNF therapeutics, there is an increased risk of side effects such as heightened susceptibility to infections or the development of autoimmune or demyelinating diseases, such as MS (Fischer et al., 2019). Therefore, patients who use anti-TNF drugs have been tested for a history of MS or other demyelinating diseases to prevent severe side effects from occurring. To prevent the onset of MS in these patients with inflammatory diseases, regulatory MRI scans should be mandatory to monitor the clinical status of these patients (Fresegna et al., 2020). Currently, anti-TNF biologics are the only drugs targeting TNF signalling. However, these drugs have intricate manufacturing processes and need to be temperature-controlled, making them not the ideal therapeutics to use due to expensive production costs (Chédotal et al., 2023).

A promising study using TNFR1 antagonists and TNFR2 agonists showed insight into a new possible avenue for MS treatment. The improved effects seen in disease symptoms, neuroprotection, and oligodendrocyte survival are promising for the future of anti-TNF therapeutics in MS. Further research is still very necessary for the development of treatments for MS and other inflammatory diseases. However, the fundamentals of TNFRs and their role in MS

pathology have been established through research, providing a solid basis for future studies to build on. This knowledge will ensure research to design more targeted and efficient therapeutics, which could potentially lead to a breakthrough in MS treatment. Continued investigation into the clinical applications of TNF-targeted therapies is essential to improving the lives of individuals affected by inflammatory diseases.

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