Diverging Effects of the Pro-Inflammatory Cytokine Tumor Necrosis Factor-alpha in the Neurodegenerative Disease Multiple Sclerosis

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Abstract

In the autoimmune and neurodegenerative disease Multiple Sclerosis (MS), the targeting of myelin sheaths by the immune system together with chronic inflammation affects the brain parenchyma eventually resulting in the degeneration of nerve cells. The pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) has been linked to MS, where single nucleotide polymorphisms (SNPs) in the TNFR1-encoding gene *TNFRSF1A* and increased TNF-α content of both serum and cerebrospinal fluid (CSF) of MS patients are associated with a higher risk and disease severity. In this article, the established contributions of TNF-α to blood-brain barrier (BBB) dysfunction, leukocyte transmigration into the brain parenchyma and the differentiation process of oligodendrocyte-precursor cells (OPCs) will be highlighted as they comprise crucial aspects that are affected in MS. In addition, based on the differences between TNF-α receptor signalling (TNFR1 vs TNFR2) during these processes, current clinical therapies and trials will be discussed and compared in an attempt to establish the most suitable TNF-α associated mechanism that may be beneficial to this neuropathology.

Abbreviations

AJ	*Adherens Junction*
ATROSAB	*Antagonistic TNF receptor one-specific antibody*
BBB	*Blood Brain Barrier*
BBB-EC	*Blood Brain Barrier Endothelial Cell*
Ciap-1/2	*Cellular Inhibitor of Apoptosis protein 1/2*
CNS	*Central Nervous System*
CSF	*Cerebrospinal fluid*
CYLD	*Cylindromatosis*
DAMP	*Damage associated molecular pattern*
EAE	*Experimental Autoimmune Encephalomyelitis*
Etv5	*ETS translocation variant 5*
FADD	*Fas-associated death domain*
FGF-2	*Fibroblast growth factor 2*
$FLIP_L$	*FLICE (FADD-like IL-1β converting enzyme) inhibitor protein*
Hes5	*Hes family bHLH transcription factor 5*
HSP27	*Heat shock protein 27*
ICAM-1	*Intercellular adhesion molecule-1*
IFNγ	*Interferon γ*
IGF-1	*Insulin-like growth factor*
IkBα	*Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha*
IKKα/β	*Inhibitor of kB kinase α/β*
IL-1β	*Interleukin 1β*
JAM	*Junction Adhesion Molecule*
JNK	*c-Jun N-terminal kinase*
LINGO-1	*Leucine rich repeat and Immunoglobin-like domain-containing protein 1*
LUBAC	*Linear ubiquitin chain assembly complex*
MAPK	*Mitogen-activated protein kinase*
MLC	*Myosin light chain*
MLCK	*Myosin light chain kinase*
MLKL	*Mixed lineage kinase domain like pseudokinase*
MMP-9	*Matrix metalloproteinase 9*
MS	*Multiple Sclerosis*
NEMO	*NF-kappaB essential modulator*
NeuroD4	*Neurogenic differentiation factor 4*
NF-kB	*Nuclear factor kappa-light-chain enhancer of activated B cells*
NOX2	*NADPH oxidase 2*
NPC	*Neural progenitor cell*
NVU	*Neurovascular Unit*
OPC	*Oligodendrocyte Progenitor Cell*
PAMP	*Pathogen associated molecular pattern*
Pax6	*Paired box protein 6*
PDGFRα	*Platelet-derived growth factor receptor A*

Abbreviations

Rac1	*Ras-related C3 botulinum toxin substrate 1*
RIPK-1/3	*Receptor Interacting Protein Kinase 1/3*
ROS	*Reactive oxygen species*
SNP	*Single Nucleotide Polymorphism*
SODD	*Silencer of Death Domains*
Sox6	*SRY-box 6*
sTNF	Soluble TNF
TAB 2/3	*TGFβ-activated kinase 2/3*
TAK-1	*Mitogen-activated protein kinase kinase kinase 7*
TGFβ-1	*Transforming growth factor β 1*
TJ	*Tight Junction*
tmTNF	*Transmembrane Tumor Necrosis Factor α*
TNF-α	*Tumor Necrosis Factor α*
TNFR 1/2	*Tumor Necrosis Factor Receptor 1/2*
TRADD	*TNF-receptor associated death domain*
TRAF 1/2/5	*TNF-receptor associated factor 1/2/5*
VCAM-1	*Vascular cell adhesion molecule 1*
VLA-4	*Very Late Antigen-4*
ZO 1/3	*Zona Occludens 1/3*

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Introduction

The Pathological Hallmarks of Multiple Sclerosis

A rapid transmission of electrical signals throughout the entire body is established via axons and the myelin sheaths that enclose them[1] allowing for an efficient communication between the central and peripheral nervous system. Myelin sheaths not only encapsulate axons and protect them from environmental stresses, but also behave as an insulating layer that forms the foundation for the saltatory conduction of signals from one node of Ranvier to the next.

In the autoimmune and neuro-degenerative disease Multiple Sclerosis (MS), these insulating myelin sheaths are targeted by the immune system resulting in a reduction of signal transmission and eventually the degeneration of nerve cells[2]. Dysfunction of the blood-brain-barrier (BBB)[3-5], chronic inflammation of the central nervous system (CNS)[2] and the inability of oligodendrocyte precursor cells (OPCs) to differentiate into mature oligodendrocytes (myelin-forming cells)[6] all contribute to the complexity of this neuropathology , making MS one of the most studied neurodegenerative diseases while still lacking an effective treatment. It therefore is essential to understand the multiple cellular processes that may be affected in this pathology and their contribution to disease progression.

Here, the primary focus will lie on the pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) and its association with the disease-related cellular malfunctions (e.g. BBB-disruption, chronic inflammation, arrest of OPC differentiation) that can be noticed in Multiple Sclerosis. Moreover, as a single nucleotide polymorphism (SNP) in the TNFR-1 translating gene *TNFRSF1A* was discovered to be associated with MS[7], an increasing amount of evidence highlights the fact that TNF-α signalling may be disrupted in MS resulting in its neurodegenerative pathology. However, as TNF-α signalling is associated with both neuroprotective and neurodegenerative effects, depending on both the TNF-receptor type and the target cell-type, the question remains to what extend TNF-α contributes to disease progression. In an attempt to provide a sufficient answer to this question, an overview is given comprising the established role of TNF-α and its receptors in the dysfunction of the BBB, initiation of chronic inflammation and the inhibition of OPC differentiation. Based on these assumptions, a 'potential' approach regarding TNF-α signalling in MS is discussed thereby attempting to solve at least some of the complexity of this neurodegenerative disease.

Tumor Necrosis Factor-α: the Basics

TNF-α is a multifunctional cytokine whose actions are regulated by a delicate web of controlling mechanisms that mediate the most adequate response to pathogens[8]. Secreted by various cell-types (e.g. activated macrophages, monocytes, microglia and astrocytes) it can orchestrate an inflammatory response in both the periphery and the CNS that is critical for an effective innate immune response. Nevertheless, anti-inflammatory functions have also been linked to TNF-α activity. An explanation for these contradictory effects can be found in the existence of two bio-active forms of TNF-α; transmembrane TNF (tmTNF) and soluble TNF (sTNF) and their associated receptors (TNFR1 and TNFR2)[9]. Here, tmTNF is synthesized as a 26 kDa transmembrane protein and sTNF is formed upon proteolytic cleavage of tmTNF by the TNF-converting enzyme TACE/ADAM17. Distinct signalling pathways can be initiated by these isoforms where TNFR1 can be activated by both sTNF and tmTNF (although with a higher affinity for sTNF) and is primarily associated with pro-inflammatory responses. TNFR2, on the other hand, can be activated mainly by tmTNF and mediates both pro-apoptotic and pro-survival signals, thus balancing the immune response of the CNS[7]

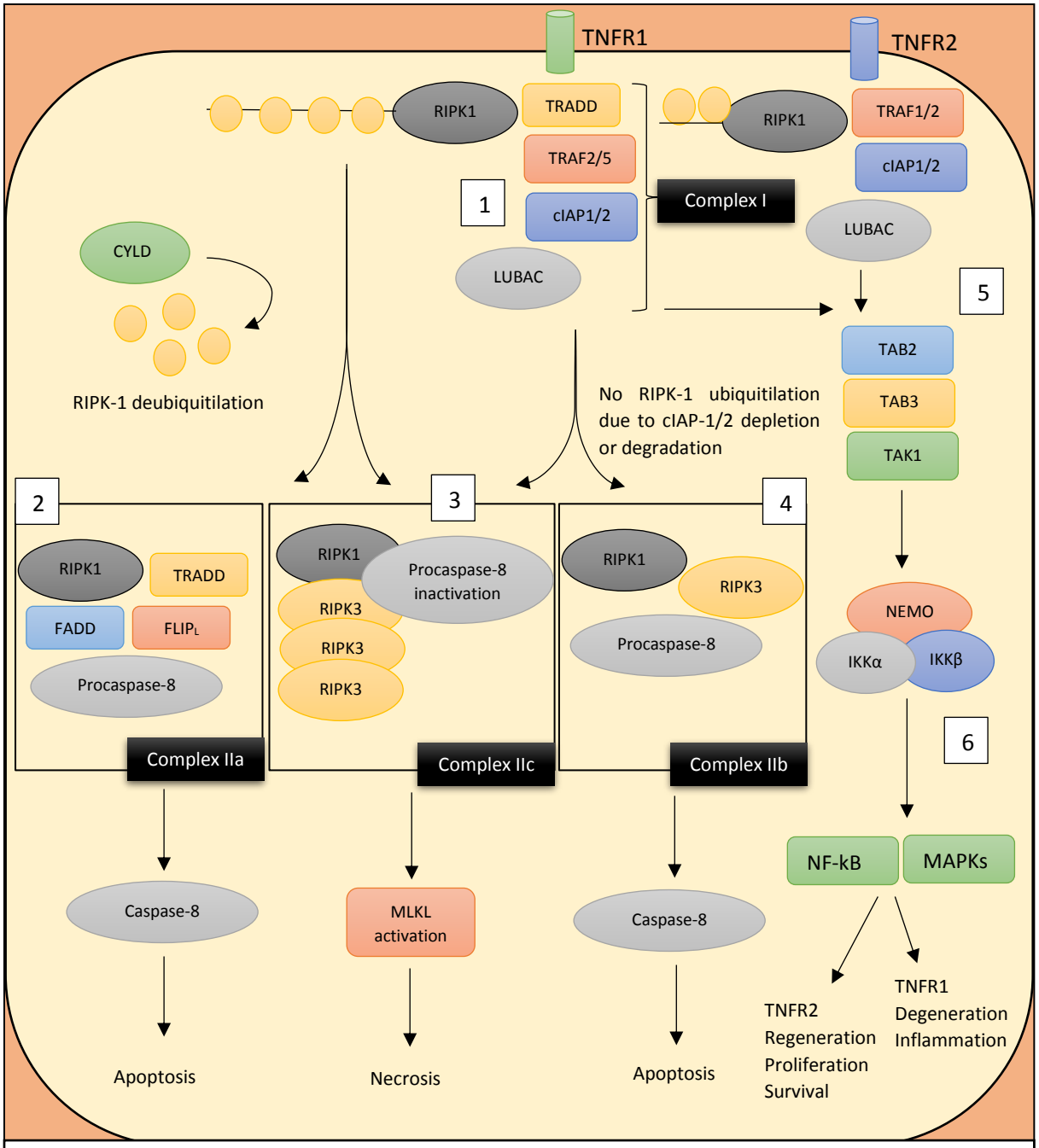

Figure 1. Schematic overview of the molecular pathways following TNFR1 and TNFR2 activation.

1) Upon TNFR1 activation, TRADD and RIPK-1 are recruited to the cytoplasmic death domain due to conformational changes. In turn, TRAF2/5, cIAP-1/2 and LUBAC can bind to and ubiquitinate RIPK-1, thereby forming Complex 1[10-17].

2) When the ubiquitin chains from RIPK-1 are removed by the tumour suppressor protein CYLD, RIPK-1 will dissociate from Complex I, after which cytosolic TRADD, FADD, $FLIP_L$ and procaspase 8 will bind to it, generating Complex IIa. Consequently, procaspase-8 will become active and initiates the caspase cascade leading to apoptosis[26-30].

3) When RIPK-1 and RIPK-3 are not cleaved from procaspase-8, it will remain inactive resulting in the aggregation of RIPK-1/3-procaspase-8 complexes, referred to as Complex IIc. Formation of this complex will activate MLKL which, in turn, will initiate necrosis, hence causing collateral damage to surrounding tissues[31].

4) Loss of RIPK-1 associated ubiquitin chains can also be caused by cIAP-1/2 depletion or degradation resulting in the formation of Complex IIb. This complex consists of RIPK-1, RIPK-3 and procaspase-8 and can initiate apoptosis, similar to Complex IIa[,18,26-30].

5) Both TNFR-1 and TNFR-2 activation will result in the formation of Complex I, even though TNFR-2 associated Complex I lacks TRADD. Ubiquitination of RIPK-1 by LUBAC and TRAF2/5 + cIAP-1/2 enables the recruitment of the TAK-1 and IKK complexes[21-25].

6) The IKK complex will enable NF-kB activation through ubiquitinating and subsequent degradation of IkBα, the natural inhibitor of NF-kB subunits. Interestingly, distinct effects upon NF-kB activation can be noticed between TNFR-1 and TNFR-2 signalling, where TNFR-1 is associated with inflammation and TNFR-2 with cell-survival[24,25].

TNFR1 signalling (Figure 1) is initiated upon binding of its ligand sTNF, which will release the attachment of the inhibitory protein silencer of death domains (SODD) to the intracellular domain of TNFR1. Consequently, the adaptor protein TNF receptor-associated death domain (TRADD)[10] and receptor-interacting serine/threonine-protein kinase 1 (RIPK-1)[11,12] can bind to this intracellular domain and, in turn, will initiate the formation of distinct protein complexes, respectively complex I, IIa, IIb and IIc, depending on the ubiquitination status of RIPK-1[13,14].

Here, Complex I is formed by the recruitment of TRAF-2/5, cIAP-1/2 and the linear ubiquitin chain assembly complex (LUBAC) to TNFR1, TRADD and RIPK-1[10,11,13,15-17]. TRAF-2/5 and cIAP-1/2 will enable the K63-linked ubiquitination and LUBAC the M1-linked polyubiquitination of RIPK-1, thereby stabilizing the complex which is essential for further signalling[18-20]. Consequently, the RIPK-1 associated ubiquitin chains promote the recruitment and activation of two signalling complexes, the transforming growth factor (TGF)-activated kinase (TAK) 1 complex and the inhibitor of kB (IKK) complex[16,21]. The first complex, consisting of TAK1, TAB-2 and TAB-3[22,23], can phosphorylate mitogen-activated kinase (MAPK) resulting in the activation of the c-JUN N-terminal kinase (JNK) and p38 MAPK pathways which are both associated with apoptosis and cell survival. The latter complex, the IKK complex, consist of nuclear factor kB essential modulator (NEMO), IKK-subunit α (IKK-α) and IKK-β and is crucial for activating NF-kB signalling[24,25]. In more detail, the IKK complex mediates the release of NF-kB subunits by ubiquitinating IkBα, the kinase that binds to these subunits in the absence of a stimulus, followed by it degradation. This, in turn, allows the NF-kB subunits to relocate towards the nucleus where they will activate NF-kB and regulate the transcription of their downstream target genes, associated with cell survival and proliferation

. Formation of the complexes IIa and IIb depends on the presence of non-ubiquitylated RIPK-1, which is formed either through the removal of the ubiquitin chains by cylindromatosis (CYLD)[26-30] or the depletion/degradation of cIAP-1/2[18]. Removal of the ubiquitin chains by CYLD will result in the dissociation of RIPK-1 from complex-I followed by the recruitment of cytosolic TRADD, FAS-associated death domain protein (FADD), pro-caspase-8 and FLICE-like inhibitory protein ($FLIP_L$) forming complex IIa. In the case of cIAP-1/2 depletion or degradation, deubiquitinated RIPK-1 will bind to RIPK-3, pro-caspase-8 and $FLIP_L$, thereby forming complex IIb. As both complex IIa and IIb can promote the conversion of pro-caspase 8 into active caspase 8, their generation is often associated with apoptosis or programmed cell death, which enables the restriction of cellular damage to a single cell. However, in order to induce apoptosis, RIPK-1 and RIPK-3 have to be cleaved by the pro-caspase-8- $FLIP_L$ heterodimer or the active caspase-8[31]. If RIPK-1 or RIPK-3 cannot be cleaved, complex IIc is formed, consisting of complex IIa/IIb aggregates and inactivated caspases. Complex IIc will activate mixed lineage kinase domain-like protein (MLKL) resulting in the more harmful initiation of necroptosis, which leads to an uncontrolled release of cellular products that may cause injury to neighbouring cells.

Similar effects regarding NF-kB and MAPK activation have been associated with TNFR2 stimulation even though TNFR2 lacks TRADD[34]. Upon TNFR2 activation, TRAF1/2 bind to TNFR2 and recruit cIAP-1/2, thus forming a complex similar to the TNFR1-associated complex I. The formation of this complex will also result in the activation of NF-kB and MAPK, yet large differences can be seen in the NF-kB associated effects of TNFR1 and TNFR2, where TNFR2 signalling is primarily associated with cell proliferation/survival and TNFR1 signalling with inflammation. Nonetheless, cytotoxic activity can be witnessed upon TNFR2 activation, which is believed to occur through

two distinct mechanisms. The first mechanism implies the activation of NF-kB, which will result in an increased production of TNF-α followed by a rise in both TNFR1-activity and inflammation. The second mechanism includes the degradation/depletion of the TRAF-2-cIAP-1/2 complexes. This may result in the decline of the TNFR1-associated complex 1 formation thus promoting the generation of the complexes IIa and IIb which are linked to apoptosis[33].

Due to the complexity of these signalling pathways, the effects and contributions of TNF-α in Multiple Sclerosis are still not completely understood. Nevertheless, multiple studies emphasize an effect for TNF-α in this neuropathology as elevated TNF-α content in both serum and cerebrospinal fluid (CSF) of MS patients and mutations in the TNFR1 gene *TNFRSF1A* seem to correlate with the severity of symptoms[7,34-36]. Non-selective blocking of TNF-α in a therapeutic trial consisting of relapse-remitting MS patients, however, resulted in the worsening of pathological symptoms compared to patients receiving placebo[37], thus adding a certain complexity to TNF-α signalling under these conditions. Selective-blocking of TNFR1, on the contrary, was shown to be beneficial in mice with experimental autoimmune encephalomyelitis (EAE), a mouse model for MS. Here, it was hypothesized that TNFR2 may be essential in established MS pathology where it limits the severity of autoimmune pathology, while TNFR1 may promote inflammation at disease onset[38-40]. In addition, TNFR1 may promote the infiltration of autoimmune cells into the CNS through a VCAM-1-associated mechanism thus linking its activity to the dysfunction of the Blood-Brain-Barrier[41,42].

The Blood-Brain Barrier and TNF-α

The Blood-Brain Barrier forms a physical barrier between the CNS and the systemic circulation, although still allowing the exchange of metabolites, such as nutrients, oxygen, ions and vitamins, between these two systems[43].

The BBB functions due to the presence of the Neurovascular Unit (NVU), which is a multi-cellular structure consisting of BBB-endothelial cells (BBB-ECs), perivascular astrocytes and pericytes. The NVU is crucial for a healthy CNS as it regulates and maintains a specific micro-environment, both biochemically and immunologically (Figure 2)[44]. In addition, the existence of multiprotein complexes called tight junctions (TJs)[45] and adherens junctions (AJ's)[46],that reside between the BBB-EC's, allow for an optimal regulation of BBB permeability by interacting with the actin cytoskeleton through the zona occludens-1 (ZO-1) scaffolding proteins (Figure 2). The Tight Junction protein complexes contain the three transmembrane proteins (I) Occludin, (II) Claudin and (III) Junctional adhesion molecules (JAMs) which form a paracellular and impermeable fence through interaction of their extracellular loops. This connection enables the BBB to withhold certain substances from entering the brain[47]. The Adherens Junctions consist primarily of transmembrane protein cadherins and cytoplasmic protein catenins. Here, the calcium-dependent cadherins can form homophilic interactions between their extracellular domains, while the catenins form connections with the actin cytoskeleton. The cadherins therefore contribute to the 'impermeable fence', while the catenins are able to control BBB-permeability through delicate changes in the morphology of the BBB-ECs[47]. A tight coordination of these transmembrane proteins is therefore important to maintain a proper functioning BBB.

In MS, BBB dysfunction and leakage have been observed and are linked to the rise of immune cells that is infiltrating the CNS where they contribute to the development of MS lesions (or plaques)[48]. Evidence shows that elevated levels of TNF-α and other cytokines are responsible for this effect by deregulation of the TJ's and AJ's in the BBB-EC's of both EAE-treated mice and MS patients[49,]. It is suggested that both TNFR1 and TNFR2 activation in BBB-

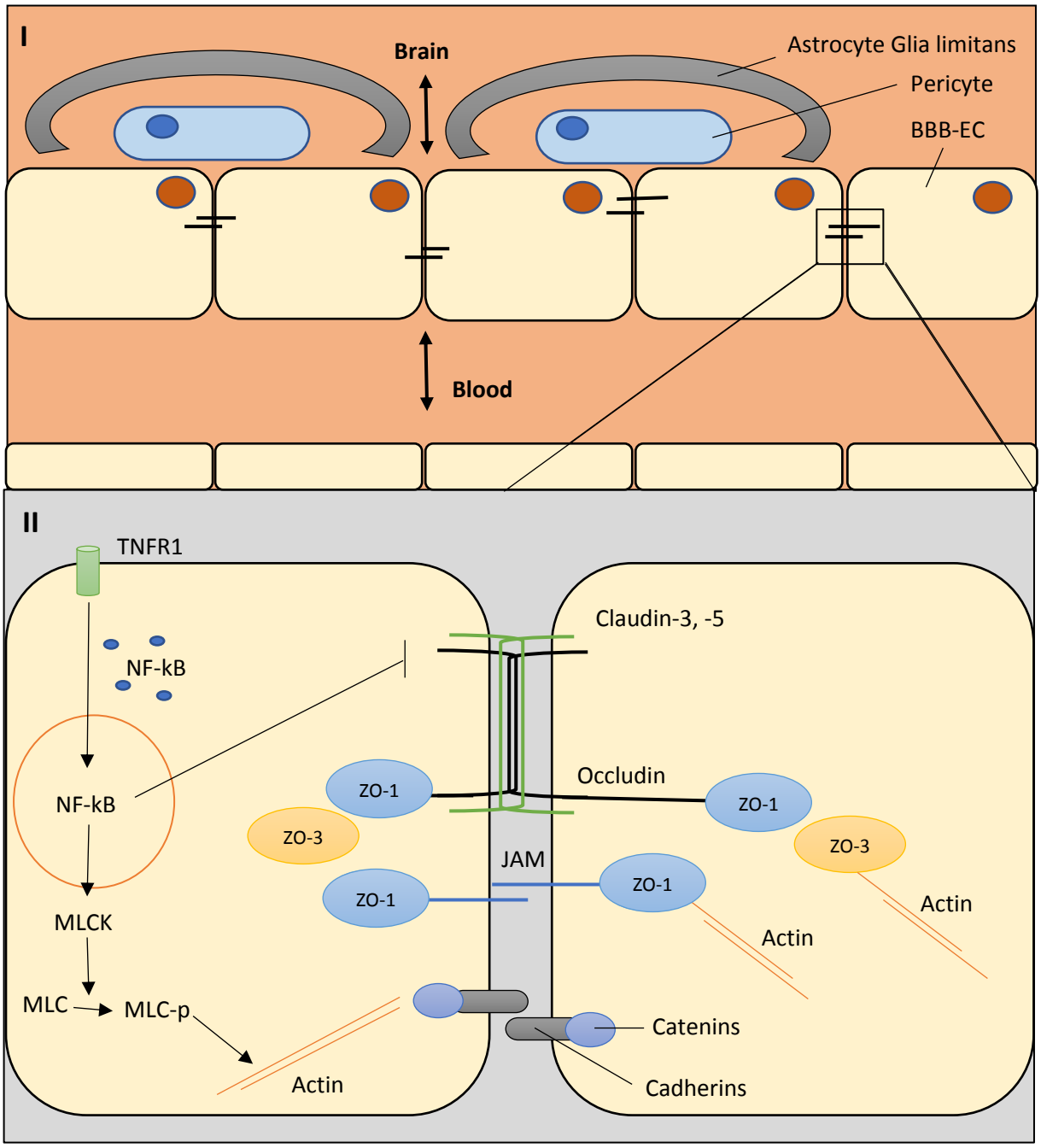

Figure 2. Schematic overview of TNF-α signalling in BBB-ECs associated with BBB permeability and transmigration of leukocytes.

I) Schematic view of the Neurovascular Unit (NVU) comprising Blood-Brain-Barrier endothelial cells (BBB-ECs), pericytes and the glia limitans of astrocytes. Arrows indicate the bidirectional transport of oxygen, hormones, nutrients across the BBB[44].

II) Detailed scheme of the Tight Junctions (TJ's) and Adherens Junctions (AJ's) that reside between the BBB-EC's. The TJ's comprise of Claudin 3/5, Occludin and Junctional Adhesion Molecules (JAMs) that are connected to the actin cytoskeleton through the Zona Occludens (ZO) 1/3. The Adherens Junctions (AJ's) consist of cadherins and cytoplasmic catenins, the latter of which are also connected to the actin cytoskeleton. BBB hyperpermeability is initiated upon TNFR-1 and TNFR-2 activation through a NF-kB-dependent signalling pathway. Here, NF-kB activation directly targets Occludin and VE-cadherin and can indirectly affect actin remodelling through the Myosin Light Chain Kinase (MLCK). MLCK phosphorylates its downstream target MLC, thus forming MLC-p which can form a mechanochemical interaction with actin that will cause a rise in cytoskeletal tension, changing BBB-EC morphology and resulting in hyperpermeability of the BBB[47-54].

ECs is responsible for this effect through an NF-kB-dependent mechanism that is associated with I) the downregulation of occludin[50] and VE-cadherin[51] protein expression, II) the increase in the internalization of TJ-proteins[52] and III) cytoskeletal retraction. Consequently, these effects may all contribute to the widening of the TJ's and AJ's and the subsequent increase in BBB permeability that can be observed in MS. Here, occludin and VE-cadherin protein expression is directly targeted by NF-kB, whereas cytoskeletal retraction is associated with the activity of the myosin light chain kinase (MLCK), a downstream target of NF-Kb[53]. NF-kB-dependent stimulation of MLCK leads to the phosphorylation of myosin light chain (MLC) thus forming MLC-p. In turn, MLC-p allows an ATP-dependent mechanochemical interaction between myosin and actin which may lead to a rise in cytoskeletal tension and subsequent hyperpermeability of the BBB[54].

Interestingly, it has been illustrated that this increase in BBB permeability may occur when TNF-α mediates beneficial effects during inflammatory conditions, but in situ production of TNF-α in the CNS is not extensive enough[55]. As a result, peripheral TNF-α is desired and needs to be transported across the BBB. TNF-α may mediate this process by increasing BBB permeability, and both receptors (e.g. TNFR1/2) are equally important in this as inhibition of either one results in a significant reduction in transported TNF-α[55].

However, despite the beneficial effects that this increase in cerebral TNF-α may have, a hyperpermeable BBB may also have deleterious effects as peripheral leukocytes can transmigrate towards the CNS more easily[56]. Here, peripheral leukocytes can have favourable effects as they can promote the clearance of waste products such as myelin debris from demyelinated areas hence promoting the remyelination process. However, it is also believed that the increased influx of leukocytes contributes to plaque formation and thus disease progression. Limiting leukocyte influx could therefore be considered as a strategy to control disease progression and one cell-type in particular is suggested to be fundamental for achieving this, the pericyte[57-59].

Effects of TNF-α signalling on leukocyte infiltration

Being an important component of the NVU and in close contact with BBB-EC's, pericytes have gained an increasing amount of interest regarding their role in Multiple Sclerosis. Under physiological conditions, pericytes are involved in the formation of the BBB and the regulation of the blood flow towards the CNS by stimulating the contraction or dilation of cerebral micro-vessels[57,60]. They also possess PDGF-Rβ, a receptor that allows pericytes to sense PDGF-B secreted by immature BBB-EC's. When sensing PDGF-B, pericytes are attracted to the brain endothelium, where they will promote the maturation of the BBB.

Pericytes have also been found to promote the transmigration of leukocytes during inflammation, where they support their rapid navigation to the sites of injury (Figure 3). They do so by increasing the presence of the adhesion molecules VCAM-1 and ICAM-1 on their membranes following inflammatory stimuli (e.g. IL-1β, TNF-α, PAMPs, DAMPs)[66,67]. In turn, leukocytes can bind to these adhesion molecules using integrin receptors situated on their outer membranes[64,65] and this binding will result in the activation of the G-protein Rac1 together with a Ca^{2+} influx[68]. Subsequently, the membrane complex NADPH oxidase (NOX2), known for the production of reactive oxygen species (ROS)[69], is formed and will start producing low levels of superoxide that rapidly converges into hydrogen peroxide (H_2O_2, 1μM). It is believed that the specific concentration of this H_2O_2 forms the foundation for distinct cellular signalling pathways that promote the leukocyte transmigration process. The low levels of H_2O_2 are, for instance, associated with a rise in the activation and secretion of matrix metalloproteinase-9 (MMP-9)[70], a pericyte-

derived product that is upregulated in MS patients[71] and linked to BBB hyperpermeability[72]. MMP-9 expression may facilitate the transmigration of leukocytes due to its abilities to degrade the extracellular matrix surrounding the BBB-ECs, affecting VE-cadherin functioning and rearranging both zona occludens-1 and occludin[73,74]. In addition, the low levels of H_2O_2 may also cause junctional disruptions by affecting actin structures. Here, H_2O_2 can promote p38 MAPK, which, in turn, can regulate actin polymerization through its downstream target HSP27[75].

In MS, a decrease in pericyte coverage and their detachment from BBB-EC's has been observed and is associated with the dysfunction of the BBB[76]. It is believed that TNF-α and other cytokines are involved in this process where they alter the phenotype of pericytes. Reduced secretion of BBB formation promoting factors (e.g. angiopoietin-1, TGF-β1) and a rise in Interleukin (IL)-6 and macrophagic inflammatory protein-1a secretion can be seen upon TNF-α activation, indicating the switch towards a more inflammatory-promoting phenotype that enables microglial activation[77].

This, however, has caused a lot of discussion since microglial activation can have both beneficial and deleterious effects in MS. For example, upon their activation, microglia will secrete high amounts of ROS that may cause oxidative damage to oligodendrocytes and this has been associated with active demyelination and axonal injury[78]. However, paradoxically, microglia and macrophages have also been found to promote remyelination as they 'clean-up' myelin debris in demyelinated areas and promote OPC proliferation through the secretion of TNF-α, IGF-1 and FGF-2[79,80]. In addition, microglia depletion was observed to decrease OPC differentiation[81], thus emphasize their importance during the process of remyelination.

Due to these contradictory effects, it remains uncertain whether microglial activation should be prevented in order to decrease plaque formation or should be

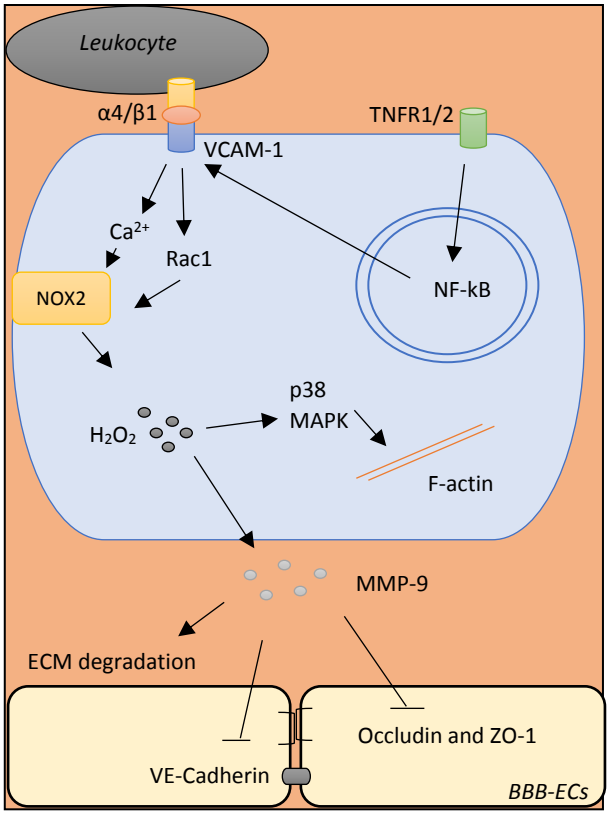

Figure 3. TNF-α-associated signalling in pericytes.

Upon TNF-α signalling, the adhesion molecules VCAM-1 and ICAM-1 are upregulated in a NF-kB-dependent manner. Binding of the integrin receptor (e.g. α4/β1) to either VCAM-1 or ICAM-1 will activate the G-protein Rac1 and promote the influx of Ca^{2+}. Both will initiate the formation of NOX2 which, then, will generate low levels of ROS. This, in turn, will result in actin remodelling and upregulated matrix metalloproteinase-9 (MMP-9) secretion, both of which result in the hyperpermeability of the BBB hence facilitating the transmigration of leukocytes into the CNS[64-75].

stimulated as it promotes both OPC proliferation/differentiation and the clearance of the myelin debris. Nevertheless, what is certain is that TNF-α plays a tremendous role during the remyelination process, where it can promote proliferation and differentiation of OPCs into myelin-forming oligodendrocytes.

TNF-α signalling in OPCs: contributions to apoptosis and remyelination

Oligodendrocytes remain the only cell-type in the entire brain parenchyma with the ability of forming myelin sheaths. By extending their plasma membrane and subsequently wrapping

it around the axons of nerve cells, they can insulate them, enabling the rapid transmission of electrical signals through saltatory conduction. In addition, by forming myelin sheaths, oligodendrocytes can support and protect nerve cells from environmental stresses and are thus essential for a proper functioning CNS[82].

In MS, oligodendrocytes experience multiple environmental stresses ranging from chronically active immune cells to cytotoxicity and these effects may contribute to the necroptotic sightings that can be observed in oligodendrocytes populations in MS patients[83]. These effects could possibly be ascribed to disturbances in TNF-α signalling as multiple studies show the activation of RIPK1, RIPK3 and MLKL together with reduced caspase-8 levels in cortical lesions of both EAE and cuprizone models for MS[84]. It therefore would seem as if TNF-α is unable to promote apoptosis in these cells thus giving rise to the activation of the necroptotic signalling pathway. It is believed that this may be due to defective activation of caspase-8, resulting in the clustering of RIPK-1 and RIPK-3 leading to the activation of MLKL and the initiation of necrosis (Figure 1). Since TNFR1 is primarily associated with this signalling pathway, its inhibition is believed to offer a therapeutic strategy to prevent this necrosis-associated pathway hence rescuing oligodendrocyte populations[84].

Interestingly, as was previously mentioned, TNF-α can promote remyelination through TNFR2-signalling, where it is believed to stimulate the proliferation and/or differentiation of oligodendrocyte precursor cells (OPCs). It was discovered that TNFR2 activation may promote OPC differentiation by regulating certain microRNAs (e.g. miR-7a, miR-219, miR-138, miR-338)[85], which are assumed to be crucial during oligodendroglial lineage specification. MicroRNAs are small non-coding RNAs that can bind to the 3' UTR of their target mRNAs thereby repressing their stability and translation. An increasing amount of evidence supports the idea that specific microRNAs become active during distinct phases of OPC differentiation and strict regulations of these microRNA's is essential in order to give rise to a mature, myelin-forming oligodendrocyte.

TNFR2 has been identified as a regulator of this process, where it can regulate the expression of certain microRNA's. TNFR2 activity, for example, was observed to reduce expression of miR-7a, which is associated with the arrest of oligodendrocyte differentiation. miR-7a is believed to be of great importance during the guidance of NPCs (Neural Progenitor Cells) towards the oligodendroglial lineage were it can inhibit the expression of the proneural differentiation factors Pax6 and NeuroD4[86]. However, its activity has also been linked to the inhibition of OPC maturation and should therefore be suppressed at a later stage of the oligodendroglial lineage in order to initiate OPC differentiation. In addition, TNFR2 may also elevate the expression of miR-219, miR-138 and miR-338, which are all required for OPC differentiation[85-89]. It is believed that these microRNAs should be active during a later stage of OPC differentiation as they can directly suppress the negative regulators of OPC differentiation Lingo1, Etv5, Sox6, Hes5 and PDFRα.

Nonetheless, it should be emphasized that OPC proliferation/differentiation is a highly complex process that can be affected by a large variation of stimuli and factors and therefore may not only rely on TNFR-2 signalling. TNFR-2 signalling, however, may still be of importance as it could be considered as one of the many potential therapeutic strategies to promote the remyelination process, as will be discussed further.

Discussion

In the complex neuropathology of MS, BBB dysfunction, chronically active leukocytes and incompetence of OPCs to differentiate are all examples of cellular processes that malfunction. While a solid explanation for these impairments is this lacking, an increasing amount of risk factors is being recognized that may initiate or contribute to the nerve degeneration that eventually can be seen. As tumor necrosis factor-α (TNF-α) is an important component of the auto-immune response and a SNP in the *TNFRSF1A* gene has been discovered to improve MS susceptibility, evidence supports the idea that TNF-α fulfils a fundamental role in this neuropathology by signalling through its two receptors (e.g. TNFR-1/2). However, as the receptor-associated effects are complicated and cell-type dependent, it has been a challenge to determine whether TNF-α contributes to neuroprotective or neurodegenerative output.

Here, the currently established views of TNF-α signalling in BBB disruption, leukocyte transmigration and OPC apoptosis/-differentiation are summarized in an attempt to uncover its implication in MS. It was seen that both TNFR1 and TNFR2 activation can contribute to BBB disruption and leukocyte transmigration by initiating NF-kB. This, however, is believed to be beneficial as peripheral TNF-α, together with microglia and macrophages can enter the CNS, where they contribute to myelin debris clearance. On the contrary, chronically active immune cells are also considered to be inducing oxidative damage to oligodendrocytes by secreting high levels of ROS hence contributing to myelin loss. In addition, TNFR-1 signalling has also been associated with oligodendrocytes death where it may initiate apoptosis/necrosis.

Due to these paradoxical effects, an effective treatment is still absent although potential clinical approaches have been rapidly emerging. In 1999, for example, an experiment using non-selective TNF-α blockers was performed on relapse-remitting MS patients. However, as disease progression was significantly worsened compared to patients receiving placebo, this experiment was stopped prematurely. Consequently, multiple theories were proposed that could explain these observations, where it was believed that these TNF-α blockers I) could not penetrate the BBB and therefore could not suppress demyelination but instead enhanced demyelination by enabling CNS access to autoreactive T-cells[90], II) aggravated demyelination by decreasing TNFR2 signalling and thus blocking OPC differentiation[91], or III) could decrease TNF-α concentrations in the periphery, but enhanced TNF-α concentrations in the CNS due to BBB impermeability[90].

Consequently, a change was seen within the field, where it was believed that selective blocking of TNFR-1, while promoting TNFR-2, could be beneficial in MS where it decreases demyelination. And indeed, selectively blocking TNFR-1 using ATROSAB was sufficient to ameliorate disease severity, where a decrease in immune cell infiltration into the CNS was observed[92]. In addition, it was observed that a reduced expression of the adhesion molecules ICAM-1 and VCAM-1 was associated with this decline in infiltration. However, as TNFR-2 activation in aortic EC's was discovered to increase the expression of VCAM-1 and ICAM-1 via a NF-kB-dependent pathway[93], it remains uncertain whether their reduced expression following ATROSAB treatment was crucial for the reduction of leukocyte transmigration.

Another strategy may imply the inhibition of TNFR-1 downstream targets associated with apoptosis/necrosis. RIPK1 and c-FLIP, for instance, have been linked to the defective inactivation of caspase-8 in EAE-associated oligodendrocytes where they are believed to contribute to the inflammatory environment through the initiation of necroptosis[84]. Selectively targeting non-ubiquitinated RIPK-1 and c-FLIP will therefore decrease necroptosis and thus prevent the

spreading of cellular damage throughout the brain parenchyma that follows upon the initiation of necroptosis.

Additionally, selective TNFR-2 activation, using the EHD2-scTNF$_{R2}$ agonist, has also been proposed to have beneficial effects in MS. Here, activation of TNFR2 in mouse microglia promoted the anti-inflammatory and neuroprotective expression of G-CSF, ADM, IL-10 and IFN-y. In line with this observation are the remyelination promoting and neuroprotective effects of astrocyte- and neuronal TNFR-2 [95-97]. However, in contrast, ablation of monocyte/macrophagic TNFR-2 resulted in EAE suppression[94]. This emphasizes the complexity of TNFR-2 signalling depending on its location, as it can have both beneficial and deleterious effects.

To conclude, TNF-α signalling in MS is complex and paradoxical, where it can be associated with both neurodegenerative and neuroprotective effects, depending on the receptor type and its localization. However, as our knowledge regarding these signalling pathways continuous to grow, more therapeutic and more precise strategies will be developed that will bring us closer to a suitable treatment for this highly complicated neuropathology.

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