

# TNF- $\alpha$ : A Paradigm of Paradox and Complexity in Multiple Sclerosis and its Animal Models

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**Abstract:** TNF- $\alpha$  (tumour necrosis factor- $\alpha$ ) is a pleiotropic cytokine with wide-ranging actions on the immune system and is an important mediator in immune-mediated inflammatory disease states, including multiple sclerosis. TNF- $\alpha$  and its receptors are part of a large and complex superfamily of homologous ligands and receptors, whose many biological functions overlap. Investigations have demonstrated the effects of TNF- $\alpha$  at various stages of pathology in multiple sclerosis (MS), including oligodendrocyte death, demyelination, immune cell trafficking, cellular proliferation and major histocompatibility (MHC) antigen expression. Targeting the TNF- $\alpha$  immunobiological pathway successfully ameliorates disease severity in a number of autoimmune inflammatory conditions except for multiple sclerosis. Anti-TNF- $\alpha$  therapy in experimental autoimmune encephalomyelitis (EAE) showed mixed results, whereas in MS trials it was deleterious. It is clear that TNF- $\alpha$  also has a beneficial role, especially in neuroprotection and regeneration. A clearer understanding of the protective role of TNF- $\alpha$  may be extrapolated from studies in other inflammatory conditions such as stroke and traumatic brain injury.

**Keywords:** Autoimmunity, central nervous system, demyelination, experimental autoimmune encephalomyelitis, lymphotoxin, multiple sclerosis, tumour necrosis factor.

## INTRODUCTION

The discovery of macrophage and lymphocyte-derived substances that could induce haemorrhagic necrosis of transplanted tumours in mice led to the description and isolation of the cytokines tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), along with lymphotoxin- $\alpha$  (LT- $\alpha$ , also known as TNF- $\beta$ ), more than three decades ago [1]. TNF- $\alpha$ , then termed cachectin, was soon implicated in other biological processes including endotoxic shock [2, 3], tissue injury [2, 4] and cachexia [5].

We now know that TNF- $\alpha$  and LT- $\alpha$  are in fact members of an extensive and growing superfamily of membrane-bound and soluble protein ligands which bind to one or more corresponding receptor(s) from the TNF receptor superfamily. Members of TNF superfamily ligands include, but are not limited to, TNF- $\alpha$ , LT- $\alpha$ , Fas ligand (FasL), CD40 ligand (CD40L), TNF-related apoptosis inducing ligand (TRAIL) and OX40 ligand (OX40L). Many more TNF ligands and receptors have been identified, and the list is growing [6].

The exact physiological roles of the TNF superfamily of ligands and receptors are still far from being fully elucidated, although it has become clear that they are the principal mediators in immune defense, inflammation and the development and maintenance of the immune system [7-10]. In particular, TNF- $\alpha$  has been shown to promote inflammation, mediate cell growth and differentiation and induce apoptosis in a variety of cell types including tumour cells, T-cells, viral host cells, oligodendrocytes, endothelial and epithelial cells. Crucially, its dysregulation is implicated in autoimmune

inflammatory diseases, most notably in autoimmune arthritis and inflammatory bowel disease, whereby antagonism of TNF- $\alpha$  has major therapeutic benefit. TNF- $\alpha$  levels have also noted to be elevated in a variety of other central nervous system (CNS) disorders such as multiple sclerosis (MS), cerebral ischaemia, traumatic brain injury, Parkinson's disease and Alzheimer's disease. In contrast however, TNF- $\alpha$  antagonism in MS has deleterious effects.

## 1. TNF- $\alpha$ LIGAND/RECEPTOR AND SIGNALING

TNF- $\alpha$  is produced predominantly by macrophages and monocytes, and additionally by B and T lymphocytes, natural killer (NK) cells, astrocytes, microglia, fibroblasts, adipocytes, and many other cells from immune and non-immune lineages. Its counterpart, LT- $\alpha$  on the other hand, is mainly produced by lymphocytes, although their biological actions may overlap [7]. In the normal physiological state, expression of TNF- $\alpha$  is low, but is significantly upregulated following exposure to a variety of stimuli including infective agents, tumour cells, complement and cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) [7, 11]. It is biologically active as a trimeric 26-kDa membrane-bound pro-protein, and in soluble 17-kDa form (sTNFR) following proteolytic cleavage by matrix metalloproteinases, primarily the TNF- $\alpha$  converting enzyme (TACE). TNF- $\alpha$  shares characteristic structural similarities with rest of the TNF superfamily of ligands, specifically in possessing the TNF homology domain (THD), which binds to cystein-rich domains (CRD) of TNF receptors [12]. TNF- $\alpha$  is encoded within the major histocompatibility complex (MHC) in the chromosomal segment 6p21[7].

Likewise, the TNF superfamily of receptors are mainly type I transmembrane proteins which may also be proteolysed into a biologically active soluble form. Although homologous in their extracellular structures, variations in the

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number of CRDs and their constituent primary amino acids allow for ligand-binding specificity [13]. TNF- $\alpha$  binds with varying affinity to two receptors, i.e. TNF-RI (p55TNFR) and TNF-RII (p75TNFR), which are also shared with lymphotoxin. Transmembrane TNF- $\alpha$  has been shown to be the primary binding ligand of TNF-RII, whereas soluble TNF- $\alpha$  mainly binds TNF-RI [14]. TNF-RI and TNF-RII are expressed on most nucleated cells, accounting for the wide-ranging effects of TNF- $\alpha$ . TNF-RI is thought to be the main receptor through which most of the known inflammatory effects of TNF- $\alpha$  is exerted.

TNF- $\alpha$  binding to its receptors induces several different signalling pathways. TNF-RI contains a death domain (DD) binding structure, which recruits intracellular adaptor molecules, in particular TNFR-associated death domain (TRADD) adaptor protein, involved in pro-apoptosis signalling. TNF-RI is also able to activate anti-apoptotic mechanisms *via* the transcription of nuclear factor  $\kappa$ -B (NF- $\kappa$ B), and is further regulated by other factors such as the silencer of death domain protein (SODD), which inhibits apoptosis [13, 15].

TNF-RII recruits TNF receptor-associated adaptor factors (TRAF), a family of adaptor proteins which in turn, regulate different cellular processes. The exact role of TNF-RII is less well known, although this may include enhancement of TNF-RI effects *via* distinct mechanisms [16, 17], proliferation of CD4 and CD8 T-cell subsets, including CD4+CD25+ regulatory T-cells in the periphery [18, 19] and other pro-inflammatory effects [20].

TNF- $\alpha$  expression and its signalling are regulated at the transcriptional and post-transcriptional level. TNF- $\alpha$  gene transcription is induced by various extracellular stimuli such as lipopolysaccharide, viruses and other antigens in a tightly-controlled, cell type- and stimulus-dependent manner [21-23]. Numerous transcription factors and complexes that facilitate and control this process have been identified, including NF- $\kappa$ B (nuclear factor kappa-B), activating protein 1, cyclic AMP (adenosine monophosphate) response element, NFAT (nuclear factor of activated T-cells), Ets, C/EBP $\beta$  (CCAAT-enhancer binding proteins beta) and LI-TAF (lipopolysaccharide-induced TNF- $\alpha$  factor) [24-29]. In the post-transcriptional stage, regulation is achieved by the presence of AU sequences in the 3' region of TNF- $\alpha$  messenger RNA (mRNA) and by the downstream induction of corticosteroids, prostaglandins and IL-10 [7]. The circulating extracellular domains of membrane-bound TNF receptors, which are shed following proteolytic cleavage, may bind to TNF- $\alpha$ , thereby acting as a natural antagonist [30].

## 2. TNF- $\alpha$ IN THE IMMUNOPATHOLOGY OF MS

Multiple sclerosis (MS) is a primary demyelinating disease of the central nervous system. The characteristic acute demyelinating plaques are the result of a T-cell mediated immune attack against myelin constituents, involving activated macrophages and microglia which damage the myelin sheath through secretion of toxic compounds, phagocytosis and the loss of myelin-producing oligodendrocytes [31-34]. Further damage is propagated *via* the secretion of pro-inflammatory cytokines, including TNF- $\alpha$  from autoreactive T cells, microglia and astrocytes, and by myelin/oligodendrocyte-specific antibodies *via* antibody-dependent cell cyto-

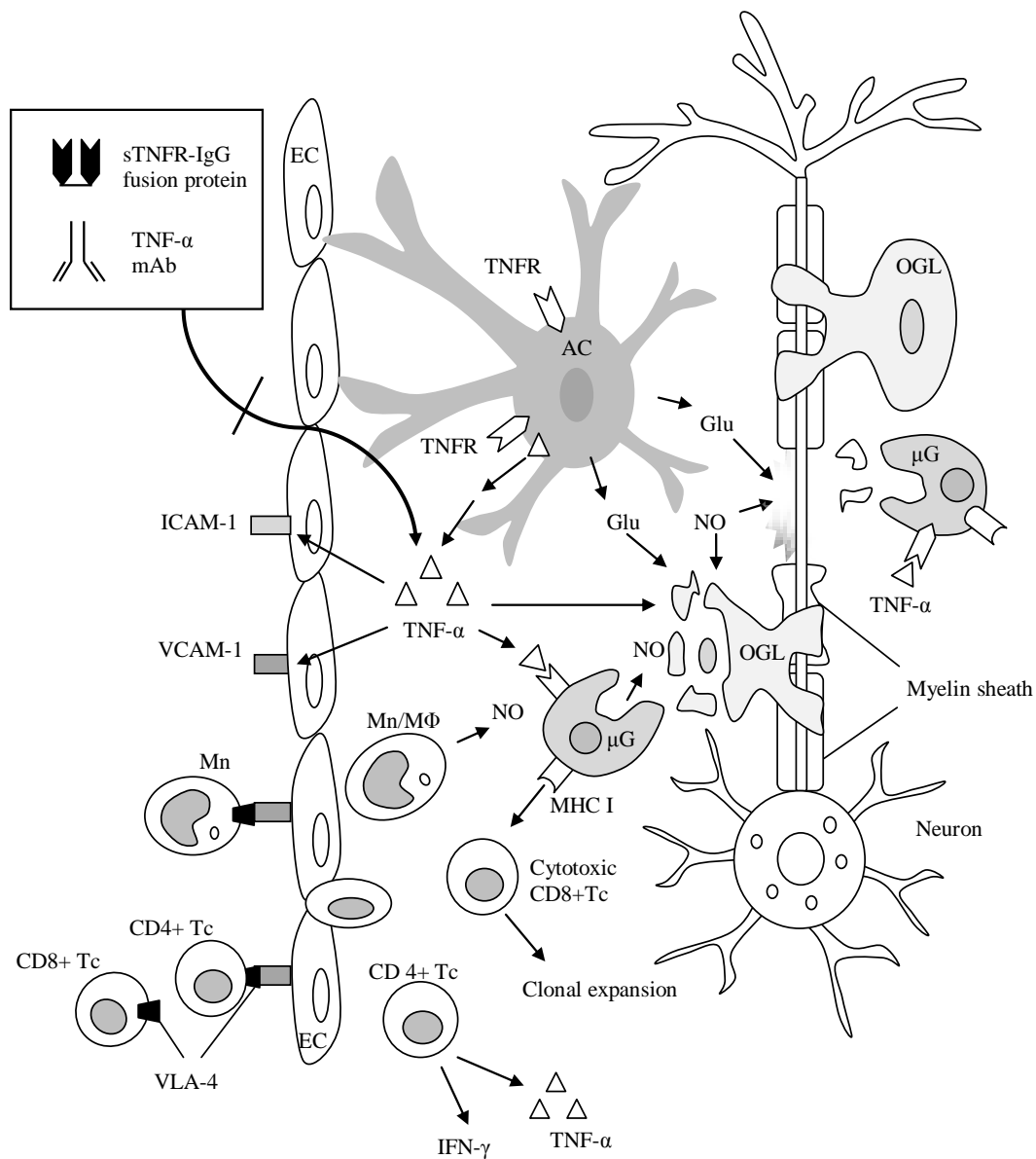
toxicity (ADCC) effector mechanisms [31, 33, 35]. The putative inflammatory actions of TNF- $\alpha$  in the CNS are illustrated in Fig. (1).

TNF- $\alpha$  and LT- $\alpha$  were detected in MS lesions using immunocytochemistry techniques on brain tissue samples of MS patients [36, 37]. Also, TNF- $\alpha$  mRNA has been detected in active MS lesions, but not in inactive or remyelinating lesions, using samples from diagnostic brain biopsies of MS patients [38]. Elevated levels of TNF- $\alpha$  have been detected in the cerebrospinal fluid (CSF) of MS patients and those with acute disseminated encephalomyelitis (ADEM) [39-41]. Sharief *et al.* (1991) observed a higher level of TNF- $\alpha$  in the CSF of patients with chronic progressive MS, compared to those with stable MS. Additionally, the levels of TNF- $\alpha$  in the CSF correlated with the degree of disability and rate of deterioration in progressive MS patients [40]. TNF- $\alpha$  production has also been shown to correlate with MS disease activity [42, 43]. In a longitudinal study of 20 MS patients [42], increased production of TNF- $\alpha$  and IFN- $\gamma$  as measured from whole-blood assays was noted to precede the onset of relapses, and their persistence correlated to clinical sequelae following the relapses. This was supported by the finding of an increase in TNF- $\alpha$  messenger RNA (mRNA) expression in peripheral blood mononuclear cells prior to relapses in a separate study [43].

### 2.1. TNF- $\alpha$ Causes Damage to Oligodendrocytes, Myelin and Axons

This may be achieved through cytotoxic mechanisms and/or apoptosis [44-48]. In an early study, the damaging effects of TNF on oligodendrocytes and myelin were shown in organotypic cultures of mouse spinal cord and recombinant human TNF (rhTNF) [46]. Exposure to rhTNF induced oligodendrocyte necrosis and subsequent dilatation of the myelin sheath by water influx into the periaxonal space, leading to demyelination. The team later observed that both TNF- $\alpha$  and LT- $\alpha$  caused time and dose-dependent injury to oligodendrocytes cultured from mature bovine brain. This occurred *via* an apoptotic process as evidenced by the finding of nuclear disintegration, although LT- $\alpha$  had more potent cytotoxic actions than TNF- $\alpha$  [47]. In another study, transgenic mice which expressed anti-apoptosis protein specifically in oligodendrocytes, and those deficient in caspase-11 (an essential effector molecule in apoptosis), were resistant to experimental autoimmune encephalomyelitis (EAE), the animal model of MS [49].

Although apoptotic mechanisms are seen to play a damaging role in pathological states linked to dysregulated TNF- $\alpha$ , apoptosis is essential to physiological processes and the resolution of inflammation. Binding of TNF- $\alpha$  to its receptors induces the caspase cascade which promotes cell apoptosis, and also the transcription of NK- $\kappa$ B and activation of Jun N-terminal kinase (JNK) pathway which conversely, inhibits apoptosis [15]. Intracellular and extracellular regulatory mechanisms exist to control the relative dominance of the apoptotic and non-apoptotic pathways, which determines cell survival or death [50-52]. Several alternative mechanisms of TNF-induced apoptosis have been also described [53]. Other death domain-containing TNF receptors such as TNF-RI, Fas and TRAIL receptors are capable of inducing apoptosis and have been shown to be preferentially expressed in MS lesion [54-56].



**Fig. (1).** Simplified diagram showing the putative actions of TNF- $\alpha$  in EAE and MS, including CNS demyelination, apoptosis and leukocyte transmigration as described in the text. AC = astrocyte, EC = endothelial cell, ICAM-1 = Intercellular adhesion molecule 1, Glu = glutamate, IFN- $\gamma$  = Interferon- $\gamma$ , mAb = monoclonal antibody,  $\mu$ G = microglia, MHC = major histocompatibility complex, Mn = monocyte, M $\Phi$  = macrophage, NO = nitric oxide, OGL = oligodendrocyte, Tc = T-cells, TNF- $\alpha$  = Tumour necrosis factor- $\alpha$ , TNFR = Tumour necrosis factor- $\alpha$  receptor, VCAM-1 = vascular cell adhesion molecule-1, VLA-4 = very late antigen-4.

Apart from direct oligodendrocyte damage, TNF- $\alpha$  has been shown to mediate indirect excitotoxic damage to oligodendrocytes and neurons by modulating the accumulation and release of glutamate from astrocytes [48]. Glutamate excitotoxicity is recognized as a mechanism for oligodendrocyte and axonal damage in MS models. Antagonism of glutamate receptors (e.g. NMDA, AMPA and kainate types) may protect neuronal structures from excitotoxic damage, potentially reducing disease progression and reversing axonal damage in MS [57-59].

## 2.2. TNF- $\alpha$ Mediates Leukocyte Trafficking into the CNS by Upregulating Cell Adhesion Molecules

Cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are cell surface glycoproteins that regulate immune-cell contact, antigen presentation and extravasation of immune cells to sites of inflammation. In particular, VCAM-1 facilitates lymphocytic migration and crossing of the blood-brain barrier by interacting with the VLA-4 integrin ( $\alpha$ 4 subunit) on blood mononuclear cells [60], a mechanism

targeted by Natalizumab, a monoclonal antibody against  $\alpha 4$  integrin used in the treatment of MS [61].

Upregulation of adhesion molecules is observed in inflammation and immune-mediated inflammatory diseases [62]. Elevated levels of VCAM-1 have been detected in MS lesions but is almost absent in normal brain tissue [63]. High levels of VCAM-1 have been measured in the serum of patients with active MS which correlate to magnetic resonance imaging (MRI) evidence of blood-brain barrier breakdown [64]. Serum and CSF ICAM-1 levels are also raised in MS patients with active disease [65-67]. Both are found to be upregulated in the EAE [68-70]. *In vitro* studies have demonstrated the ability of TNF- $\alpha$  to stimulate the expression of these cellular adhesion molecules on vascular endothelial cells [60] and astrocytes [71-73]. Barten *et al.* (1994) further demonstrated that anti-TNF- $\alpha$  therapy downregulated the expression of vascular endothelial VCAM-1 in passively transferred EAE, which was accompanied by disease inhibition [74]. More recently, it has been demonstrated that TNF- $\alpha$  signalling through TNF-RI is necessary for VCAM-1 expression on astrocytes, which in turn was essential for the transmigration of autoreactive T-cells into the CNS parenchyma in the passively-transferred EAE model [75]. These studies further support the role of TNF- $\alpha$  in the various stages of the inflammatory process in MS.

### 2.3. TNF- $\alpha$ Causes Astrocytic Activation and Proliferation

Astrocytic activation in the CNS results in a reactive gliosis, a pathological hallmark of MS lesions. The implications of gliosis in MS are wide-ranging and are reviewed in detail elsewhere [76]. In a study using *in vitro* cultures of mature bovine brain, TNF- $\alpha$ , and to a lesser extent interleukin-6 and lymphotoxin, induced proliferation of astrocytes, suggesting a key role of TNF- $\alpha$  in the development of CNS gliosis [47]. In a similar experiment, TNF- $\alpha$  stimulated the proliferation of adult human astrocytes derived in a dose-dependent manner [77].

### 2.4. TNF- $\alpha$ Induces MHC I and II Expression on Neurons and Glial Cells

The expression of MHC class I and II molecules, which are crucial for antigen presentation and subsequent T-cell responses, is normally tightly regulated in the CNS but can be induced by the activation or production of transcription factors that regulate MHC gene expression. The mechanisms by which this is triggered have not been fully elucidated. Immunohistochemical analysis of post-mortem brain tissue in MS patients has shown an increased expression of MHC antigens and its transcription factors in white matter lesions and in pre-lesional microglial clusters within the normal-appearing white matter [78]. MHC Class I antigens in particular, are highly expressed on neurons and glial cells in MS, which are targets for Class I MHC restricted cytotoxic T cells [79]. In various *in vitro* studies, TNF- $\alpha$  has been shown to induce or promote the expression of MHC class I and/or class II antigens on neurons and glial cells [80-83]. In contrast, TNF- $\alpha$  has been noted to inhibit the expression of MHC class II antigens on microvascular endothelial cells in the murine CNS [84]. This provides one example of a paradoxical role of TNF- $\alpha$  in the regulation of autoimmunity and inflammation in the CNS.

### 2.4.1. TNF- $\alpha$ in EAE

Our present understanding of the role of TNF- $\alpha$  in MS has been helped significantly by studying its effects in EAE using transgenic and gene knock-out mice. The histology, immunology and clinical symptomatology of various EAE models closely resemble that of MS. EAE can usually be induced in various species of animals either *via* immunization with exogenous myelin components, including myelin basic protein (MBP) and myelin oligodendrocyte protein (MOG), or by adoptive transfer of T-cells sensitized to myelin proteins [85]. TNF- $\alpha$  in EAE is mainly expressed by local microglia and infiltrating macrophages [85].

Studies in EAE have shown that encephalitogenic T-cells express higher levels of TNF- $\alpha$  [86, 87]. TNF- $\alpha$  is produced and upregulated at various stages of EAE evolution [88-91] and in parallel with disease progression [92]. Systemic administration of TNF- $\alpha$  increases the severity of EAE, prolongs its duration and induces relapses [93-95]. Furthermore, direct intravitreal injection of TNF- $\alpha$  has been shown to cause demyelination of the optic nerve in mice [96, 97].

### 2.5. EAE Development in TNF- $\alpha$ Overexpression

Transgenic mice have been developed to overexpress TNF- $\alpha$  or its receptors in specific cell lines in the CNS, whereas knock-out mice lacking the ability to express TNF- $\alpha$ , LT- $\alpha$  or both, or one or both of its receptors. Probert *et al.* (1995) utilised a mouse model containing a murine TNF- $\alpha$ -globin hybrid transgene expressed in CNS neurons. The mice spontaneously developed a chronic demyelinating inflammatory CNS disease with 100% phenotypic penetrance consisting of ataxia, seizures, paralysis and early mortality. Histological examination of CNS tissues showed lymphocytic infiltration, astrocytosis, microgliosis and focal demyelination [98]. In a transgenic mouse model with high expression of TNF- $\alpha$  in oligodendrocytes, immunization with MBP induced a severe form of EAE compared to non-transgenic controls [99].

Another team used transgenic mice which expressed either soluble human or transmembrane TNF- $\alpha$  in neurons or astrocytes [100]. The soluble TNF- $\alpha$  expressing- mice and those that expressed transmembrane TNF- $\alpha$  in astrocytes spontaneously developed CNS inflammation with a classic phenotype and histological appearance. However, neuronal overexpression of transmembrane TNF- $\alpha$  did not cause EAE. This could indicate that in order to trigger CNS inflammation, transmembrane TNF- $\alpha$  signalling requires its host cell to directly interact with other cells. Unlike neurons, astrocytes are able to participate in direct intercellular contact, e.g. with endothelial cells at the blood-brain barrier, and may induce critical changes in blood-brain barrier function and integrity, a crucial step in the inflammatory cascade [100]. EAE caused by overexpression of TNF- $\alpha$  was further shown to be independent of the adaptive immune response, as shown to occur in mice lacking CD4,  $\beta 2$  microglobulin, immunoglobulin  $\mu$  and RAG-1 [101].

### 2.6. EAE in the Absence of TNF- $\alpha$

It should be noted that EAE can still occur in the absence of TNF- $\alpha$  expression, although the disease severity may vary quite considerably between experiments. Single TNF- $\alpha$  knockout mice showed a milder form of EAE with shorter

duration and delayed onset, possibly related to reduced leukocyte trafficking into the CNS in the early stages of disease development [102, 103]. TNF- $\alpha$  and LT- $\alpha$  double knockout mice demonstrated delayed disease onset of MOG-induced EAE and less demyelination, albeit with increased inflammation [104].

Although some of these studies suggest a pathologic effect of TNF- $\alpha$ , there is also evidence of a protective effect, such as that seen in TNF- $\alpha$  knockout mice of the 129 or C57BL/6 strain, whereby immunization with MOG led to extensive inflammation, demyelination and high mortality [105]. Other experiments show a varied response to EAE induction in the absence of TNF- $\alpha$ , depending on the strain of mice and antigen used. In the 129xC57BL/6 mouse strain, where both TNF- $\alpha$  and LT- $\alpha$  were knocked out, a mild form of EAE occurred when immunised with mouse spinal cord homogenate (MSCH) [106]. However in the same study, SJL/J mice which lacked TNF- $\alpha$  and LT- $\alpha$  expression had a more severe and lethal form of EAE when immunised with MSCH. In addition, these mice also suffered from pronounced cachexia and demonstrated the pathological hallmarks of EAE despite the absence of TNF- $\alpha$  and LT- $\alpha$ . In the same mouse strain, when immunised with proteolipid protein, a milder form of EAE was induced compared with the non-knockout SJL/J mice.

### 2.7. EAE in the Absence of TNFR

Mice expressing TNF- $\alpha$  in CNS glial cells developed primary demyelination and an oligodendropathy in the presence of intact TNF-RI receptor and either intact or knocked-out TNF-RII receptor [107]. However, mice lacking the TNF-RI receptor either did not develop EAE or had a milder disease course, suggesting that TNF-RI is the primary receptor for the induction of EAE. TNF-RII on the other hand, may have a protective role in the clinical course of EAE. In separate experiments, TNF-RII knockout mice developed more severe demyelination and disease phenotype in MOG-induced EAE when compared to TNF-RI single and TNF-RI/II double knock-outs [104, 108].

The role of TNF- $\alpha$ /TNF-RI signalling alongside Fas ligand/Fas in the induction of EAE was further examined in mice which were unable to express oligodendrocyte-specific TNF-RI (through lack of TNF-RI gene) or Fas (through homologous recombination and LoxP-Cre methods), or both [109]. When immunised with MOG, inactivation of TNF-RI or Fas alone partially protected mice from EAE, and inactivation of both conferred complete resistance to EAE, indicating that both receptors are key signallers in the induction of apoptosis in EAE.

### 3. ANTI- TNF $\alpha$ THERAPY IN EAE AND MS

TNF- $\alpha$  inhibition by the administration of a monoclonal antibody or soluble TNF- $\alpha$  receptor-Ig G fusion protein has proved a successful strategy in the treatment of rheumatoid arthritis, psoriatic arthritis, inflammatory bowel disease, ankylosing spondylitis, juvenile rheumatoid arthritis and a variety of other immune-mediated inflammatory conditions [110]. Two anti-TNF- $\alpha$  monoclonal antibodies (Infliximab and Adalimumab) and a soluble TNF- $\alpha$  receptor-IgG fusion protein (Etanercept) are currently in commercial use, with newer anti-TNF antibodies (Certolizumab and Gollimumab) emerging.

Studies in EAE hint at the potential of TNF inhibition in reducing disease severity and progression in MS. Neutralisation of TNF- $\alpha$  by administration of an anti TNF- $\alpha$  antibody [111-113], soluble TNF-RI receptor [114] or TNF receptor-IgG fusion protein [111, 115-117] prevented the onset of EAE. It is worth noting however, that most of the studies of anti TNF- $\alpha$  antibodies in EAE used the adoptive transfer model. In a study using SJL/J mice immunised with MBP to induce EAE, the administration of an anti-TNF- $\alpha$  antibody at induction had no effect on the development of the disease, in contrast to the adoptive transfer mice which became resistant to EAE [118]. This suggests a differential effect between the two models of EAE to TNF- $\alpha$  neutralisation with antibodies.

Pharmacological agents such as phosphodiesterase inhibitors (e.g. rolipram, mesopram, pentoxifylline), which are known to reduce the expression of TNF- $\alpha$  and LT- $\alpha$ , can suppress EAE [119-122]. Blocking the conversion of TNF- $\alpha$  from its transmembrane form to its soluble form (which mainly binds the primary TNF- $\alpha$  receptor, TNF-RI) by inhibiting the actions of matrix metalloproteinases can also prevent EAE [123-125]. Interferon beta and glatiramer acetate, two immunomodulatory agents currently licensed for the treatment of relapsing-remitting MS, have wide-ranging effects on the immune system, part of which include the reduction of TNF- $\alpha$  production [126, 127] and bioavailability [128]. Both agents have been shown to reduce clinical relapses by about 30% in relapsing-remitting MS. In addition, corticosteroids, which are used in the treatment of MS relapses, also reduce transcription of TNF- $\alpha$  [129].

The effects of direct TNF- $\alpha$  inhibition in MS were first evaluated in an open-label study of two rapidly progressive MS patients who were treated with a chimeric anti-TNF- $\alpha$  monoclonal antibody (cA2). This was administered as two 10 mg/kg intravenous doses 2 weeks apart [130]. Both patients had an established diagnosis of MS with active relapsing disease. Unexpectedly following each infusion, the patients developed an increase in the number of gadolinium-enhancing lesions on brain magnetic resonance imaging (MRI) and elevation of CSF IgG index and lymphocyte count. These findings suggest that TNF- $\alpha$  inhibition increased CNS immune activation and disease activity in MS patients.

A later study investigated the safety and therapeutic efficacy of a soluble recombinant TNF-RI fusion protein (sTNFR-IgG p55; Lenercept) in MS [131]. Lenercept was previously shown to be effective in blocking the onset of clinical symptoms in EAE [116] and in treating rheumatoid arthritis [132]. The trial was conducted as a double-blind, placebo controlled phase II study involving 168 patients, the majority of which had relapsing-remitting disease. Patients were randomized to 4-weekly 10, 50 or 100mg of intravenous Lenercept or a placebo for 24 weeks, followed by an additional 24 week follow-up period. Outcome measures consisted of MRI and clinical markers of disease activity. The results did not show a significant difference in MRI outcomes between the study groups, however the relapse rate and was significantly higher and time to first relapse significantly earlier in the Lenercept group compared to the placebo. Exacerbations in the Lenercept group also tended to be more severe and of a longer duration. The conclusions of this study were that anti TNF- $\alpha$  therapy with Lenercept failed to

improve disease activity and in fact, worsened MS severity in a dose-dependent manner.

Subsequently, cases of CNS demyelination and new-onset MS following treatment with anti TNF- $\alpha$  agents have been reported in the post-marketing period [133-137]. All three commercial anti-TNF $\alpha$  agents have been implicated. Mohan *et al.* (2001) reviewed the cases of 20 patients with inflammatory arthritis who developed neurological events which were reported to the US Food and Drug Administration (FDA) between 1998 and 2000 [133]. The cases were temporally related to anti-TNF $\alpha$  therapy. Duration of treatment with anti-TNF- $\alpha$  varied between 2 and 18 months, with symptoms occurring between 1 week and 15 months from initiation of therapy. Clinical features included sensory disturbance, paresis, optic neuritis and cognitive dysfunction. MRI changes consistent with white matter demyelination were noted in most cases. On discontinuation of therapy, most patients showed partial or complete resolution of symptoms. However, despite their obvious implications, these cases do not definitively prove a causal relationship between anti TNF- $\alpha$  therapy and new-onset MS or CNS demyelination. Patients suffering from diseases within the spectrum of systemic immune-inflammatory disorders such as systemic lupus erythematosus and Behcet's Disease, may have CNS demyelination which can be difficult to differentiate from MS [138]. Yet, it remains possible that anti-TNF $\alpha$  therapy could cause CNS demyelination and may unmask MS in some cases.

#### 4. TNF- $\alpha$ : A DOUBLE-EDGED SWORD IN MS

The reasons for the disparity in response to TNF- $\alpha$  inhibition between MS, EAE and other immune-mediated inflammatory conditions are yet uncertain. The key is likely to lie in the pleiotropic nature of TNF- $\alpha$  and possibly also the lack of specificity in some of the TNF- $\alpha$  inhibitory treatments used in human trials thus far. Variability between the animal models used in experiments and the interpretation of experimental results may also play a part in the different responses seen [85].

Because of its actions, TNF- $\alpha$  has often been likened to a double-edged sword. TNF- $\alpha$  is highly pleiotropic and its actions in EAE and MS are not restricted to promoting inflammation. In fact, the downstream induction of anti-inflammatory prostaglandins, glucocorticoids and IL-10 by TNF- $\alpha$  serves to counter its own pro-inflammatory effects [7].

TNF- $\alpha$ /TNF-RI-mediated apoptosis is important for the deletion of autoreactive T-lymphocytes in the periphery, which subsequently downregulates the inflammatory response and promotes immune tolerance [139]. Genetic deletion of TNF-RI in mice resulted in significantly reduced *in vivo* apoptosis of activated cytotoxic T-cells and prolonged their persistence in the periphery [140]. In a later experiment supporting this finding, mice lacking TNF- $\alpha$  exhibited abnormally prolonged myelin-specific T-cell reactivity resulting in the exacerbation of EAE [141]. In addition, the immunosuppressive properties of TNF- $\alpha$  were found to be independent of TNF-RI. These findings suggest that TNF- $\alpha$  plays a dual role in EAE, i.e. in the initiation of a myelin-directed immune response and later, in the depletion of autoreactive lymphocytes and suppression of inflammation, possibly in-

dependent of TNF-RI activation. It was recently shown that mice lacking the ability to cleave transmembrane TNF- $\alpha$  into its soluble form exhibited resistance to EAE while retaining autoimmune suppressive properties and resisting intracellular bacterial infections [142]. It may therefore be possible to achieve anti-inflammatory effects without inhibiting the immune-regulatory capabilities of TNF- $\alpha$  by specifically targeting soluble TNF- $\alpha$ , which is the main binding ligand for TNF-RI.

In a separate experiment, mice lacking TNF- $\alpha$  or TNF-RII showed impaired remyelination in a cuprizone-induced demyelination/remyelination model. On histological examination, reduction in oligodendrocyte precursors and mature oligodendrocytes were noted [143]. The findings suggest that TNF- $\alpha$  mediating *via* TNF-RII may have a reparative role in oligodendrocyte regeneration and remyelination.

TNF- $\alpha$  is involved in the development and normal functioning of the nervous system. High levels of TNF- $\alpha$  are expressed in embryonic brain cells [144, 145], although TNF- $\alpha$  knockout mice seem to be able to develop normally [146]. Neuroprotective functions of TNF- $\alpha$  have been observed in response to a variety of cerebral insults such as ischaemia and trauma. Activation of the NK- $\kappa$ B pathway and the induction of antiapoptotic proteins are recognized neuroprotective effects of TNF- $\alpha$  following brain injury [52, 147-149]. In another neuroprotective role, TNF- $\alpha$  helps maintain intracellular calcium haemostasis, *via* the upregulation of calbindin, and subsequently reduce glutamate excitotoxicity following ischaemic and traumatic brain insult [150, 151].

TNF- $\alpha$  mediated activation of the NK- $\kappa$ B pathway can exert further beneficial effects by stimulating neurotrophic factor production essential for the survival, growth and function of neurons [152-154]. Importantly, TNF- $\alpha$  has been shown to induce the proliferation of neuronal progenitors in the CNS subventricular zone of adult rodents [155], whose equivalent in the human brain is a source of neural stem cells and is altered in neurodegenerative conditions [156]. TNF- $\alpha$  has also been noted to play a role in neuronal plasticity by improving synaptic strength *via* the upregulation of synaptic AMPA-type glutamate receptor (AMPA) expression, shown in *in vitro* hippocampal neuronal cultures [157]. On the other hand, an excess of AMPAR may make neurons more susceptible to glutamate-induced excitotoxicity [158].

TNF- $\alpha$  may enhance other neuroprotective pathways. For example, we have recently shown the up-regulation of cannabinoid receptors by TNF- $\alpha$  in an NF $\kappa$ B-dependent fashion (Jean-Gilles *et al.*, manuscript submitted). As shown by animal experiments, endocannabinoids promote neural stem cell proliferation [159, 160]. Another study eloquently demonstrated the co-dependent interactions between endocannabinoid and TNF- $\alpha$  signalling pathways, crucial to neural stem cell proliferation [161]. These protective, regenerative and plasticity mechanisms are particularly necessary for recovery in the post-injury period, whereby a lack of TNF- $\alpha$  has been shown to be markedly detrimental in the later stages of brain trauma [162].

The dual role of TNF- $\alpha$  is further reflected in the pathophysiology of stroke. TNF- $\alpha$  promotes the formation of atherosclerotic plaques [163]. Experiments in animal models showed that TNF- $\alpha$  expression is quickly upregulated fol-

lowing acute ischaemia [164] and the direct administration of TNF- $\alpha$  following acute stroke worsened focal ischaemic injury [165]. Furthermore, the biological effects of TNF- $\alpha$  on vascular endothelium can adversely affect microvascular perfusion following ischaemia [166]. TNF- $\alpha$  inhibition in various preclinical animal models of ischaemia conferred protective effects [167]. In contrast, TNF-RI/II knockout mice developed more severe focal ischaemia and increased oxidative stress following middle cerebral artery occlusion [168]. Another team showed that TNF-RI knockout mice suffered more damage than wild-type controls following an occlusive hypoxic stimulus, whereas the presence of TNF-RI directly protected neurons from apoptosis, an effect which was further enhanced by exogenous TNF- $\alpha$ . This was achieved through the activation of the anti-apoptotic NK- $\kappa$ B pathway and upregulation of the anti-apoptotic protein, FLIP<sub>L</sub> [169]. Furthermore, the inhibition of TACE, which proteolyzes TNF- $\alpha$ , reduced the proliferation of subventricular zone neural progenitor cells following cerebral ischaemia in mice [170]. Other experiments have shown the protective role of TNF- $\alpha$  in the induction and maintenance of ischaemic tolerance, as reviewed in more detail by Hellenbeck *et al.* (2005) [167].

In their recent review, Taoufik and Probert *et al.* (2007) speculate on other protective mechanisms of TNF- $\alpha$  in immune-mediated CNS diseases, including the effects of TNF- $\alpha$  on the proliferation of microglia, which have highly pluripotent functions themselves, and the potential effects of TNF- $\alpha$  on regulatory T-cells in the suppression of inflammation, which warrant further study [171].

## CONCLUSION

We now know that TNF- $\alpha$  not only exerts pro-inflammatory and cytotoxic effects but is also essential for the subsequent suppression of inflammation, repair and regeneration in the CNS. However, extending the findings from anti TNF- $\alpha$  treatments in EAE into MS has so far proven difficult. With regards to treating MS, the mechanism of TNF- $\alpha$  inhibition may be of particular importance, especially in view of the need to simultaneously preserve its many helpful functions. Even if this can be addressed, the overlapping biological functions of TNF- $\alpha$ /TNFR with that of other ligand/receptor superfamily members and the presence of genetic polymorphisms in individuals make this a very challenging prospect.

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