TNF in neuroinflammation; what can be learned from TNF research in non-neuronal autoimmune diseases

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Abstract

Tumor necrosis factor (TNF) plays an important role in inflammation. It is thought to be a key mediator in neurodegenerative disorders such as Alzheimer's disease (AD), Multiple sclerosis (MS), and ischemic stroke. TNF's role as immune modulator makes it a potential target for therapy in neurodegenerative disorders. Despite that there have been many studies regarding the effect of TNF and its signaling pathways via its two receptors, namely TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2), there is still much unknown. TNF research is not only limited to neuroscience on the contrary, TNF is a potent modulator in most inflammatory responses including autoimmune diseases. There have been loads of studies and trials regarding the role of TNF in autoimmune diseases such as: granulomatosis with polyangiitis (GPA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), diabetes, and Sjögren's syndrome (SS). Anti-TNF therapy is widely used as treatment of RA and this gives insights on the effect of treatment on inflammation. The following essay focuses on the role of TNF in neurodegenerative diseases and how TNF and anti-TNF treatment in non-neurodegenerative autoimmune disorders may contribute to a better understanding of its involvement in neuroinflammation.

Introduction

Tumor necrosis factor (TNF) is one of the most prominent mediators of inflammation and plays a key role in instigating the immune response. Although researchers have been trying to elucidate TNF signaling and the role it plays in inflammation for over 40 years, there is still much unknown about TNF biology [1]. Because of TNF's essential role as immune deregulator, either primary or secondary following medication, may greatly affect the pathology of inflammatory diseases.

There are two distinct TNF receptors; TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). It is generally thought that the two transmembrane receptors have opposing effects. The antagonistic effect allocates a pleiotropic effect to TNF which is yet to be fully understood[2]. The immense biological role that TNF plays in the physiology of inflammation implicates that a better understanding of its pathways could prove to be beneficial of treatment in inflammation related diseases.

In recent years there has been a growing interest in TNF and its role in neurodegenerative diseases. TNF is thought to be involved in diseases such as Alzheimer's disease (AD), Multiple sclerosis (MS),

and other neurodegenerative disorders [3]. A lot of progress has been made in understanding the role of TNF and its function in neurodegenerative disorders. However, the divergent role of TNF makes for a complex system which is still not fully unraveled.

While TNF intervention is not yet implementable for neurological conditions it has been intensively studied in non-neurodegenerative autoimmune diseases such as; Granulomatosis with polyangiitis (GPA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), diabetes, and Sjögren's syndrome (SS) [4–8]. Anti-TNF therapy is used as therapy for patients with RA and there have been several trials focusing on other autoimmune disorders [5]. Because of the fundamental role of TNF in inflammation these results might give insights into the role of TNF in various diseases including neuroinflammation.

This essay focuses on research of neurodegenerative disorders and non-neurodegenerative autoimmune disorders in order to shed light on how the advances in immune therapy contribute to the understanding of TNF in neurodegenerative disorders.

TNF signaling

TNFR1 and TNFR2 pathways

There are two different TNF receptors; TNFR1 and TNFR2, both with distinctive signaling pathways (Figure 1) [9]. TNFR1 activates a more apoptotic pathway and is activated by both membrane bound TNF (mTNF) as soluble TNF (sTNF) [10]. After activation TNFR1 recruits the TNFR-associated death domain (TRADD). Subsequently TRADD recruits various proteins including receptor-interacting protein 1 (RIP1), inhibitor of apoptosis protein 1 (cIAP1), and inhibitor of apoptosis protein 2 (cIAP2) to form the membrane bound signaling complex I [11]. Signaling complex I can in term activate catalytic IκB kinase (IKK) that's able to phosphorylate and degrade the Kappa-B inhibitor (IκB). With the loss of inhibition of IκB, nuclear factor- κB (NF-κB) gets released and translocated to the nucleus where it initiates transcription of anti-apoptotic genes including cellular FLICE-like inhibitory protein (cFLIP)[12]. If NK-κB is insufficiently activated a second complex, signaling complex II can induce apoptosis. Signaling complex II is essentially a dislocated and slightly modified complex I. The cytoplasmic signaling complex II has instead of a cIAP1 protein a Fas-associated death domain (FADD). FADD activates pre-caspase 8 and subsequently initiates apoptosis. This is mainly regulated by cFLIP levels within the cell [13]. Recent studies show that TNF is also able to induce necrosis via TNFR1 where the inhibition of caspases leads to an alternate signaling complex (Complex IIb). This complex is able to induce programmed necrosis via the mixed lineage kinase-domain like protein (MLKL) [14].

The signaling pathway activated by TNFR2 is less well studied than that of TNFR1 but is generally considered to promote cell survival [15]. TNFR2 is mainly activated by mTNF and recruits TNFR-associated factor 2 (TRAF2) upon activation [10]. TRAF2 recruits TNFR-associated factor 1 (TRAF1) and subsequently cIAP1 and 2 which are able to activate NF-κB via IKK [16]. A second way for the TRAF2 complex to promote cell survival is to activate phosphatidylinositol 3-kinase (PI3K) which in turn phosphorylates protein kinase B (PKB) and subsequently NF-κB, promoting cell survival [17]. Thirdly, TNFR2 is able to activate mitogen-activated protein kinase kinase kinase (MEKK1 or MAP3K) via TRAF2, which then activate c-Jun N-terminal kinase (JNK) by triggering JNK-activating kinase (JNKK1) [18]. Although acute activation of JNK leads to cell survival it is thought that long term activation can in fact promote apoptosis[19].

The basic signaling pathways of both receptors are thought to be understood, in principle there seems to be an opposing role between the two. TNFR1 is able to instigate apoptosis and even programmed necrosis while TNFR2 promotes cell survival [20]. And to make matters more complex, TNFR1 and TNFR2 are both able to instigate cell survival as well as apoptosis [15]. To make matters more complex TNFR1 and TNFR2 have been found to influence each other as well [21].

TNF and immune activation

In addition to the apoptosis and cell survival regulation, TNF is also an immune-stimulatory cytokine. TNF stimulates inflammatory cytokine and cell adhesion molecules essential for the recruitment of inflammatory cells [22]. Also, TNFR1 signaling is required for T cell modulation and subsequently granuloma forming [23]. TNF does not only act locally but it also exerts systemic functions, and can induce fever [24]. These and other functions of TNF, vital for instigating the innate immune response via cytokine production and induction of cell adhesion molecules, explain the precarious role of TNF regulation [25]. In the brain, TNFR1 promotes pro-apoptotic m1 microglia and at the same time prevents the switch to the pro-repair m2 microglia, leading to a more apoptotic driven cellular response [26,27]. TNF1 also promotes glutamate release in microglia. This leads to excitoneurotoxicity and may cause neuronal damage in neurodegenerative diseases [28].

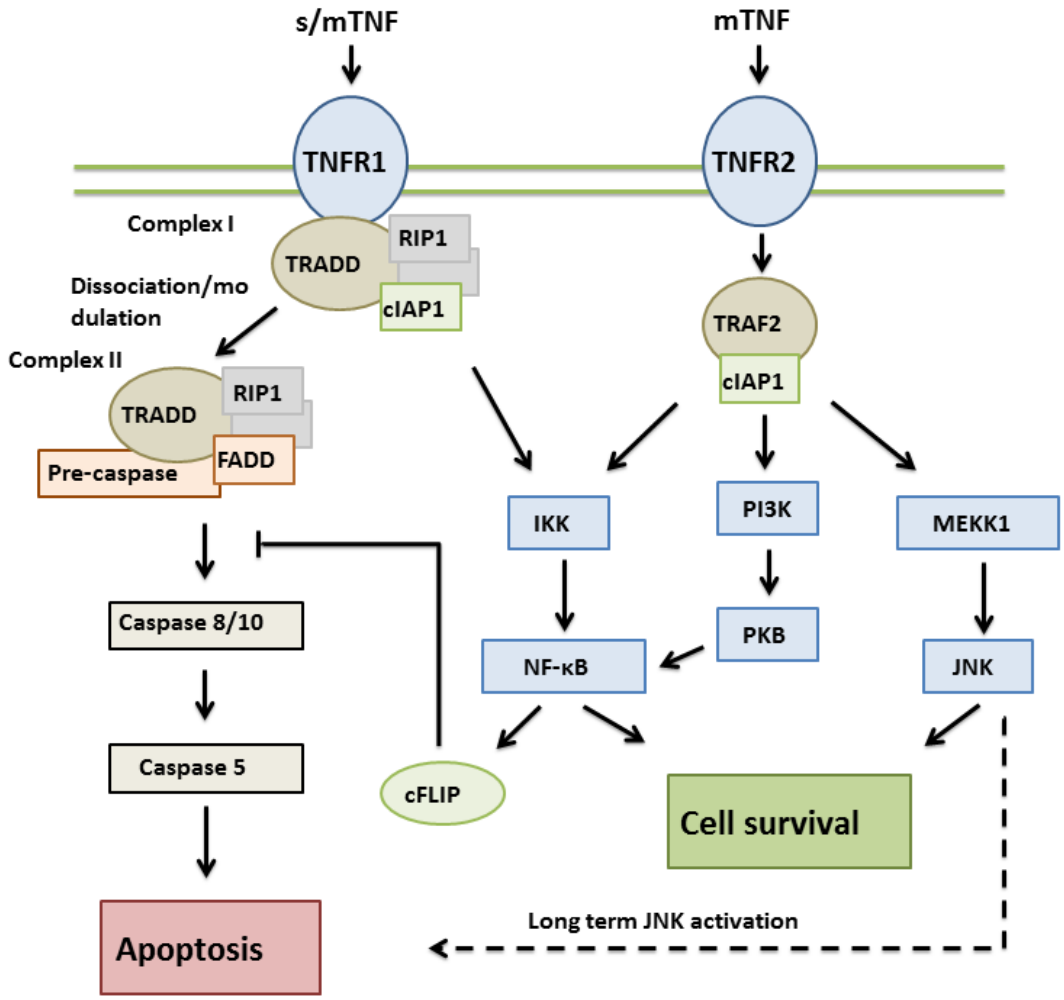

Figure 1. TNF signaling via TNFR1 and TNFR2. After activation of TNFR1 by either soluble TNF (sTNF) or membrane bound TNF (mTNF), TNFR associated death domain (TRADD) gets recruited. Subsequently receptor-interacting protein 1 (RIP1) and inhibitor of apoptosis protein 1 (cIAP1) are bound to form signaling complex I. Complex I is able to activate nuclear factor κB (NF-κB) via IκB kinase (IKK), NF-κB promotes cell survival by activating protein transcription, including FLICE-like inhibitory protein (cFLIP) which is able to inhibit apoptosis via complex II. When complex I gets dissociated from TNFR1 it forms complex II, loses cIAP1 and gains Fas-associated death domain (FADD). FADD recruits pre-caspase followed by a caspase cascade involving caspace 8/10 and caspace 3, resulting in apoptosis.TNFR2 activated by mTNF recruits TNFR-associated factor 2 (TRAF2) and activates NF-κB via cIAP1 and IKK. A secondary pathway activates phosphatidylinositol 3-kinase (PI3K) which activates protein kinase B (PKB) and subsequently NF- κB. Thirdly, TNFR2 activates mitogen-activated protein kinase kinase kinase (MEKK1) which activates c-Jun N-terminal kinase (JNK). Acute JNK activation promotes cell survival while prolonged activation may lead to apoptosis [3,18,19].

TNF in neurodegenerative diseases

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid beta (Aβ) plaques and hyperphosphorylated tau protein aggregates in the brain [29]. Besides the pathological hallmarks of AD, evidence raises that neuroinflammation and subsequently TNF plays a key role in AD and that these pathways are activated in the early stages of the disease [30,31]. Elevated TNF levels were found at the Aβ plaques and hyperphosphorylated tau protein sites of AD brain tissue [32]. The importance of TNF regulation in inflammation and the immune response is also supported by several genetic association studies done in neurodegenerative diseases. AD is associated with a polymorphism in the TNF promoter sequence that may cause higher levels of TNF [33,34]. Also, a polymorphism in the TNFR2 gene is associated with late-onset AD (Perry 2001).Interestingly there seems to be a shift towards the TNFR1 pathway in AD, with TNF showing increased binding affinity towards the TNFR1 receptor [35].

Multiple studies in AD mouse models showed that a knockout or silencing of TNFR2, even in combination with a TNFR1 knockout, exacerbated AD pathology [36,37]. In addition, overexpression of TNFR2 or a TNFR1 knockout were shown to have beneficial effects [38,39]. There have been limited reports of anti-TNF therapy trials in AD patients. One trial where Etanercept was subcutaneous administered to patients did not show to have any significant effect on disease pathology [40]. This was most likely due to the systemic administration and that Etanercept is not able to pass the blood-brain barrier (BBB) [41]. A case report showed that perispinal administration of Etanercept caused cognitive improvement in an AD diseased patient [42]. Although this was only reported once, combined with the theoretical knowledge and experimental data of animal studies, it does implicate anti-TNF might be a possible therapy for AD patients.

Multiple sclerosis

Multiple sclerosis (MS) is a demyelinating neurodegenerative disease. Demyelination of axons results in various neurological symptoms. Although it was first thought to be primarily caused by auto-antibodies, recent studies show that these antibodies might come secondary to the destruction of oligodendrocytes [43]. This implies that TNF might be a key mediating factor. Genetic association studies in MS patients showed various polymorphisms related to the immune system including in the TNF region [44]. Although not one polymorphism is directly linked to the disease, it seems that mutations present in the TNF gene may lead to a predisposition for MS. Also, polymorphisms in the TNF-β gene were found to be predictive of disease progression [45].Studies using experimental autoimmune encephalomyelitis (EAE) mice treated with the sTNF-α inhibitor Xpro-1595 showed less

clinical symptoms and pathology [46]. These promising results led to a phase II trial where MS patients were treated with the TNF-α antagonist lenercept showing that total TNF blockage in patients caused an increase of symptoms and adverse events [47]. This effect may be attributed the ability of lenercept to block both sTNF and mTNF, blocking not only TNFR1 but also the protective properties of TNFR2. This is supported by experiments in TNFR2 knockout EAE mice who developed more severe symptoms than normal EAE mice [48]. In addition in another mouse model, using cuprizone to induce demyelination, TNFR2 was found to be accountable for remyelination of oligodendrocyte precursor cells [49].

Other neurodegenerative disorders

TNF is not only a likely mediator in AD and MS but may also contribute to the pathology of Parkinson's disease (PD), ischemic stroke, and several other diseases involving neurodegeneration [50,51]. For example, TNF levels in the cerebrospinal fluid (CSF) are elevated in PD patients, implying the involvement TNF [52]. Animal studies using different models showed contradictory results. One study showed that induced PD by overexpression of a-synuclein increased TNF secretion. In addition, an *in vitro* study using cultured neurons showed TNFR2 activation led to the protection of neurons [53]. Another study showed that mice lacking both TNFR1 and TNFR2 were protected against neurotoxicity while a knockout of TNFR1 or TNFR2 did not protect against neurotoxicity [54]. TNF involvement in ischemic stroke is relatively well studied in various mouse models [51]. Interestingly, TNF was shown to be both beneficial and detrimental in a timing dependent manner [55]. Inhibition of TNF after induced ischemic stroke, using Etanercept, was shown to reduce neuroinflammation [56]. At the same time pretreatment with TNF had a neuroprotective effects and reduced infarct size [57]. Moreover TNFR1 knockout mice showed larger infarct sizes compared with TNFR2 and wild-type mice, which suggests that TNFR1 is needed for initial protection of neurons [58]. Next to these diseases TNF has been thought to play a role in other neurodegenerative diseases such as traumatic brain injury and epilepsy [59,60].

TNF in autoimmunity

Granulomatosis with polyangiitis

TNF is thought to be an important mediator in granulomatosis with polyangiitis (GPA), GPA is an autoimmune disease characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) and is part of a larger group of ANCA-associated vasculitis (AAV). GPA pathology includes inflammation of the small blood vessels, which often leads to kidney and respiratory inflammation [4]. Because of the efficacy of anti-TNF treatment in RA, Etanercept was thought to be a treatment option for GPA as well. In contrary, a trial involving 180 patients treated with Etanercept showed that it was not effective as treatment in GPA. Furthermore, there were multiple malignancies found in the Etanercept treated group [61]. And although the malignancies were most likely caused by a combination of disease and co-medication of cyclophosphamide, which is known to increase the risk of cancer; the combination with anti-TNF treatment is probably why more malignancies were found in the Etanercept trial [62]. Although the malignancies could not solely be ascribed to Etanercept, it does illustrate the complexity of TNF signaling In conclusion, anti-TNF treatment in GPA patients did not improve symptoms but could cause complications, thus it is not suited as therapy for GPA.

Rheumatoid arthritis

RA is a chronic inflammatory autoimmune disorder which affects approximately 0.5%-1% of the world population [63]. RA starts with synovial inflammation leading to cartilage and bone destruction, subsequent inflammation can cause systemic inflammatory disorders. The origin of RA is still unknown, although several genetic and, environmental factors have been associated with the disease. Most polymorphisms associated with RA involve T cell regulation, but mutations in the NF-κB pathway have also been described [64]. TNF-α blockage is widely used as treatment in RA, and is found to relieve patients of symptoms and showed considerable improvements compared to standard treatment [5]. The two main anti-TNF agents used as treatment of RA are Infliximab, a monoclonal anti-TNF antibody, and Etanercept, a dimeric TNR receptor linked by the human Fc part [65,66].

Adverse effects are not only seen in GPA but also in RA patients treated with anti-TNF-α therapy. Despite the fact that anti-TNF therapy is commonly used, and is considered safe as treatment for RA, there have been reports of SLE like symptoms and some cases of neurological damage and demyelination [67]. It is not inconceivable that demyelination is caused by anti-TNF therapy considering the Lenercept trial in MS patients previously described [47]. For patients who already have a predisposition for neurological or demyelinating diseases anti-TNF treatment may be enough to instigate the disease [68]. Despite these reports anti-TNF is widely used as a treatment for RA and only rare cases of neurological side effects are known [69].

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of antinuclear antibodies (ANA) and anti-dsDNA antibodies [70]. The main pathology of SLE involves inflammation, vasculitis, and immune complex aggregates [71]. SLE is a complex disease associated with a multitude of genetic mutations and factors such as sex and hormone balance [6,72]. SLE can also be induced by environmental factors including UV light, radiation chemicals or therapy. There have been noted over 80 drugs with drug-induced SLE [73].

The earlier mentioned successes achieved with anti-TNF treatment in RA patients led to believe anti-TNF therapy could very well be able to treat SLE. There have been several clinical trials conducted using TNF-blocking agents in patients with SLE that resulted in both positive and negative results. One trial showed higher ANA levels but clinical improvement in SLE patients treated with infliximab [74], while another trial with Infliximab showed that several patients developed antibodies directed at chromatin components. One patient developed anti-dsDNA antibodies with reversible anti-TNF induced lupus (ATIL) [75]. ATIL in RA patients treated with Etanercept after discontinuation of therapy SLE-related symptoms ameliorated [76]. Another trial showed that infliximab caused higher ANA levels but did not affect the disease score [77]. These findings suggest a precarious balance between auto-antibodies and TNF blockage in SLE patients treated with anti-TNF drugs [78]. This is also illustrated findings in RA patients with pre-existing anti-dsDNA antibodies, where patients developed ATIL after switching from infliximab to another TNF-inhibiting drug called adalimumab [79]. These trials, and findings of SLE like symptoms in RA patients treated with Infliximab, led to a contraindication of anti-TNF treatment in SLE patients [80].

Diabetes

There are two main types of diabetes; type 1 diabetes (T1D), and type 2 diabetes (T2D). T1D is characterized as an autoimmune disease which leads to the destruction of insulin-producing β cells, often antibodies directed at the islet cells are present in sera of patients [81]. T2D is not an autoimmune disease but patients develop an insulin resistance which is mainly caused by obesity and lack of exercise. Adipose tissue is responsible for various inflammatory markers which might be the cause of insulin resistance [82].

Non-obese diabetes (NOD) mice are used as a model to study diabetes. These mice mimic pathogenesis of type 1 diabetes (T1D). The pleiotropic nature of TNF is clearly illustrated by studies involving NOD mice and the effect of TNF in T1D development. One study showed that local expression of TNF in the islets prevented autoimmunity and diabetes [7]. A second study showed that local TNF in neonatal NOD mice was able to enhance antigen presentation and subsequently

promoting diabetes [83]. Another study showed that TNF administered to NOD mice from birth to days 21-24 caused earlier onset of diabetes whereas anti-TNF administered in the same period reduced the incidence of diabetes. When TNF was given after week 4 for a period of 3 weeks, the incidence of diabetes decreased while at the same time anti-TNF treatment led to earlier onset of diabetes [84]. NOD mice seem to be an applicable model for T1D, sharing the same pathological hallmarks. But there are limitations to this model, as it is relatively easy to prevent diabetes in NOD mice and hundreds of studies were successful in preventing diabetes development [85].

There have been studies that showed TNF is able to induce insulin resistance in mouse models [86]. Another study showed that T2D patients treated with an anti-TNF antibody did not affect insulin sensitivity [87]. In addition, a more recent study showed that there are no significantly elevated levels of TNF in healthy subjects compared to both obese persons without T2D and obese T2D patients. The same study showed that several inflammation markers such as interleukin-6 and C - reactive protein were elevated, suggesting a subordinate role for TNF in T2D [88].

Sjögren's syndrome

Sjögren's syndrome (SS) is a chronic autoimmune disease that involves abnormalities in T and B lymphocytes, and inflammation of the exocrine glandular cells. SS can be primary (pSS) or secondary (sSS) when associated with other autoimmune diseases [89]. Patients show decreased tear and saliva secretion causing dry eyes and mouth which results in poor quality of life. In later stages SS can cause inflammation in other organs including skin, lungs, gastrointestinal tract, blood vessels, bladder, kidneys, and vagina. 4-10% of SS patients develop B cell lymphomas as result of disturbances in B cell homeostasis [90]. Characteristic of SS is infiltrates of T and B cells, overexpression of TNF caused by CD4+ T lymphocytes, epithelial cells, and mononuclear cells have been found within these infiltrates [8].There is evidence that intraglandular TNF synthesis increases type 2 and 9 matrix metalloproteases (MMP-2/9) and expression of Fas on the cell surface. MMP-2, MMP-9 and Fas have been shown to induce destruction of salivary cell lines and acinar structure of SS salivary glands [91]. For this reason anti-TNF therapy was considered a possible therapeutic target in SS.

An early pilot study with a one year follow-up, using Infliximab in pSS presented promising results with patients showing clinical improvement [92,93]. But surprisingly, a second larger randomized, controlled trial using Infliximab showed no differences between the placebo and Infliximab treated group [94]. Finally, an Etanercept trial on 15 pSS patients showed no reduction of clinical symptoms [95]. In the year 2013 a notice of retraction appeared in Arthritis & Rheumatism reporting of methodological errors in the first pilot study, and that treated patients did in fact not differ from non-

treated patients [96]. This showed that, although SS has all the hallmarks of a TNF mediated disease, anti-TNF therapy could not be proven beneficial for SS patients.

Discussion

TNF has been a prime suspect in inflammation and autoimmunity for some time now, and although successful in treating RA patients, it still seems that the complexity and a specificity of TNF itself make it a difficult target in neuroinflammation. The involvement of TNF in autoimmune diseases differs greatly, whereas therapy was found to be beneficial in RA, it did not reduce symptoms GPA and SS patients. Implying that even in diseases relatively similar, underlying causes greatly affect TNF involvement.

It seems that TNF is generally pro-inflammatory but not necessarily pro-apoptotic and thus a relatively non-specific cytokine. Blocking TNF is shown to improve RA symptoms but TNF treatment in the brain might need more specific targeting of TNFR1. Considering that apoptosis may even be induced via TNFR2, TNF interference might need to look beyond TNFR1 and TNFR2 and target other modulating factors that favor a cell survival directed pathway [97]. It might be interesting what factors, besides cross talk between TNFR1 and TNFR2, are responsible for TNF receptor expression.

Some studies have been carried out on TNFR1 and TNFR2 knockout mice. It should be noted that early studies showed that proper TNF signaling is a requirement for germinal center forming and B cell follicle development, and thus the humoral immune response [98]. And although T cells appear almost unaffected in their development without TNF, some infectious agents were shown to trigger a disorganized infection subsequently leading to death in TNF-deficient mice, while causing only a mild response in normal mice [99]. This makes it hard to estimate the clinical relevance of these studies. Also, mice models are not perfect representations of disease development; in most cases they merely imitate disease pathology. This causes differences in outcome when animal studies are translated to human trials, as has been the case in MS treatment with TNF blockage. EAE mice treated with the sTNF-α inhibitor Xpro-1595 showed less clinical symptoms and pathology [46]. While in contrary a phase II trial where MS patients were treated with the TNF-α antagonist Lenercept showed that total TNF blockage in patients led to an increase of symptoms [47].

The case report where Etanercept was administered via a perispinal injection in a patient with AD is promising for ischemic stroke and traumatic brain injury patient [42]. Although this report only invlolves one treated patient, it does suggest an important role for anti-TNF treatment. Further research will be necessary to investigate if Anti-TNF treatment might help to prevent additional neuronal damage

when administered directly after an ischemic stroke or traumatic brain injury. There is one other report of perispinal Etanercept treatment, administered to three patients with chronic stroke deficits months after the initial stroke. All three patients are said to have improved in motor skills, special perception, speech and cognition[100]. However, the patients did not improve in such matter that the placebo effect of the treatment can be ruled out. This effect cannot be ruled out since it has been shown that in diseases such as PD, MS, and RA there is a significant effect, some not unlike the improvements seen in the three cases [101–103].

Maybe the most promising mechanism which seems to be involved in both autoimmune, and neurodegenerative disorders is the involvement of MMP-9. MMP-9, as discussed earlier, might be responsible for TNFR1 induced destruction of salivary glands in SS [91]. But MMP-9 is not only of interest in SS but has also been described to promote pathological processes in the brain. MMP-9 was shown to be able to disrupt the blood-brain barrier causing deterioration of the extracellular matrix [104]. TNF treatment however did not show to be instrumental in treating SS which implies that there are more factors regulating MMP-9 in inflammation, at least in SS but possibly also in neurodegenerative diseases [95]. One possible factor is neutrophil gelatinase-associated lipocalin (NGAL), a downstream regulator activated by TNFR1 [3]. NGAL silences the TNFR2-PI3K pathway and is known to form complexes with MMP-9 increasing its stability [105]. In SLE anti-NGAL IgG serum levels are associated with general inflammatory markers and the disease itself [106]. Suggesting NGAL may play a role in disease pathology. Surprisingly, a study using lipocalin 2 (the murine orthologue of human NGAL) deficient mice showed that the deficient mice had significantly higher levels of IgG compared to normal mice [107]. This suggests that a basal level of NGAL may be a requirement for the inhibition of antibody levels and in this way inhibiting the immune response. This in term might be an (partial) explanation for the fact that MS patients showed exacerbation of disease symptoms when treated with anti-TNF agents. This means that, although NGAL targeting in neurodegenerative diseases might be promising, the effects of NGAL on the adaptive immune response must be taken into consideration.

In conclusion, several studies have shown that anti-TNF therapy can be beneficial in inflammatory diseases but the differences in underlying pathology attributes greatly in the efficacy of TNF interference. Specific TNFR1 targeting, or targeting of its downstream protein NGAL, might hold the key for more specific treatment with possibly less complications. Although autoimmune diseases have many similarities among them the effect of anti-TNF treatment illustrates that there are key differences, the same might hold true for neurodegenerative disorders.

Literature

1. Wajant, H., Pfizenmaier, K. & Scheurich, P. Tumor necrosis factor signaling. *Cell Death Differ.* **10,** 45–65 (2003).

2. Kollias, G., Douni, E., Kassiotis, G. & Kontoyiannis, D. On the role of tumor necrosis factor and receptors in models of multiorgan failure, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. *Immunol. Rev.* **169,** 175–194 (1999).

3. Yun Dong, Doortje Dekens, Peter Paul De Deyn, Petrus J. W. Naudé, U. L. M. E. targeting of tumor necrosis factor alpha receptors as a therapeutic strategy for neurodegenerative disorders. *Antibodies* **4,** 369–408 (2015).

4. Land, J., Rutgers, A. & Kallenberg, C. G. M. Anti-neutrophil cytoplasmic autoantibody pathogenicity revisited: pathogenic versus non-pathogenic anti-neutrophil cytoplasmic autoantibody. *Nephrol. Dial. Transplant* **29,** 739–745 (2014).

5. Druce, K. L., Jones, G. T., Macfarlane, G. J. & Basu, N. Patients receiving anti-TNF therapies experience clinically important improvements in RA-related fatigue: results from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis. *Rheumatology (Oxford).* **54,** 964–971 (2015).

6. Somers, E. C. *et al.* Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. *Arthritis Rheumatol. (Hoboken, N.J.)* **66,** 369–378 (2014).

7. Grewal, I. S. *et al.* Local expression of transgene encoded TNF alpha in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J. Exp. Med.* **184,** 1963–1974 (1996).

8. Meijer, J. M., Pijpe, J., Bootsma, H., Vissink, A. & Kallenberg, C. G. M. The Future of Biologic Agents in the Treatment of Sjögren's Syndrome. *Clinical Reviews in Allergy & Immunology* **32,** 292–297 (2007).

9. Locksley, R. M., Killeen, N. & Lenardo, M. J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104,** 487–501 (2001).

10. Grell, M. *et al.* The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* **83,** 793–802 (1995).

11. Chen, G. & Goeddel, D. V. TNF-R1 signaling: a beautiful pathway. *Science* **296,** 1634–1635 (2002).

12. Micheau, O., Lens, S., Gaide, O., Alevizopoulos, K. & Tschopp, J. NF-kappaB signals induce the expression of c-FLIP. *Mol. Cell. Biol.* **21,** 5299–5305 (2001).

13. Micheau, O. & Tschopp, J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114,** 181–190 (2003).

14. Ofengeim, D. *et al.* Activation of necroptosis in multiple sclerosis. *Cell Rep.* **10,** 1836–1849 (2015).

15. Okamoto, H., Kimura, M., Watanabe, N. & Ogihara, M. Tumor necrosis factor (TNF) receptor-2-mediated DNA synthesis and proliferation in primary cultures of adult rat hepatocytes: The involvement of endogenous transforming growth factor-alpha. *Eur. J. Pharmacol.* **604,** 12–19 (2009).

16. Sun, S.-C. & Ley, S. C. New insights into NF-kappaB regulation and function. *Trends Immunol.* **29,** 469–478 (2008).

17. Marchetti, L., Klein, M., Schlett, K., Pfizenmaier, K. & Eisel, U. L. M. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *J. Biol. Chem.* **279,** 32869–32881 (2004).

18. Faustman, D. & Davis, M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat. Rev. Drug Discov.* **9,** 482–493 (2010).

19. Dhanasekaran, D. N. & Reddy, E. P. JNK signaling in apoptosis. *Oncogene* **27,** 6245–6251 (2008).

20. Van Herreweghe, F., Festjens, N., Declercq, W. & Vandenabeele, P. Tumor necrosis factor-mediated cell death: to break or to burst, that's the question. *Cell. Mol. Life Sci.* **67,** 1567–1579 (2010).

21. Naude, P. J. W., den Boer, J. A., Luiten, P. G. M. & Eisel, U. L. M. Tumor necrosis factor receptor cross-talk. *FEBS J.* **278,** 888–898 (2011).

22. Roach, D. R. *et al.* TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. *J. Immunol.* **168,** 4620–4627 (2002).

23. Ehlers, S., Kutsch, S., Ehlers, E. M., Benini, J. & Pfeffer, K. Lethal granuloma disintegration in mycobacteria-infected TNFRp55-/- mice is dependent on T cells and IL-12. *J. Immunol.* **165,**

483–492 (2000).

24. Clauss, M. *et al.* A permissive role for tumor necrosis factor in vascular endothelial growth factor-induced vascular permeability. *Blood* **97,** 1321–1329 (2001).

25. Hehlgans, T. & Pfeffer, K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology* **115,** 1–20 (2005).

26. Ghosh, M., Xu, Y. & Pearse, D. D. Cyclic AMP is a key regulator of M1 to M2a phenotypic conversion of microglia in the presence of Th2 cytokines. *J. Neuroinflammation* **13,** 9 (2016).

27. Kroner, A. *et al.* TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron* **83,** 1098–1116 (2014).

28. Takeuchi, H. *et al.* Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J. Biol. Chem.* **281,** 21362–21368 (2006).

29. Qiu, C., Kivipelto, M. & von Strauss, E. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues Clin. Neurosci.* **11,** 111–128 (2009).

30. McAlpine, F. E. & Tansey, M. G. Neuroinflammation and tumor necrosis factor signaling in the pathophysiology of Alzheimer's disease. *J. Inflamm. Res.* **1,** 29–39 (2008).

31. Zhao, M. *et al.* The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain. *Neurochem. Res.* **28,** 307–318 (2003).

32. Benzing, W. C. *et al.* Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. *Neurobiol. Aging* **20,** 581–589 (1999).

33. Di Bona, D. *et al.* Systematic review by meta-analyses on the possible role of TNF-alpha polymorphisms in association with Alzheimer's disease. *Brain Res. Rev.* **61,** 60–68 (2009).

34. Lee, Y. H., Choi, S. J., Ji, J. D. & Song, G. G. Association between TNF-alpha promoter -308 A/G polymorphism and Alzheimer's disease: a meta-analysis. *Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol.* **36,** 825–832 (2015).

35. Cheng, X., Yang, L., He, P., Li, R. & Shen, Y. Differential activation of tumor necrosis factor receptors distinguishes between brains from Alzheimer's disease and non-demented patients. *J. Alzheimers. Dis.* **19,** 621–630 (2010).

36. Montgomery, S. L. *et al.* Ablation of TNF-RI/RII expression in Alzheimer's disease mice leads to an unexpected enhancement of pathology: implications for chronic pan-TNF-alpha suppressive therapeutic strategies in the brain. *Am. J. Pathol.* **179,** 2053–2070 (2011).

37. Montgomery, S. L. *et al.* Chronic neuron- and age-selective down-regulation of TNF receptor expression in triple-transgenic Alzheimer disease mice leads to significant modulation of amyloid- and Tau-related pathologies. *Am. J. Pathol.* **182,** 2285–2297 (2013).

38. Jiang, H. *et al.* Genetic deletion of TNFRII gene enhances the Alzheimer-like pathology in an APP transgenic mouse model via reduction of phosphorylated IkappaBalpha. *Hum. Mol. Genet.* **23,** 4906–4918 (2014).

39. He, P. *et al.* Deletion of tumor necrosis factor death receptor inhibits amyloid beta generation and prevents learning and memory deficits in Alzheimer's mice. *J. Cell Biol.* **178,** 829–841 (2007).

40. Butchart, J. *et al.* Etanercept in Alzheimer disease: A randomized, placebo-controlled, double-blind, phase 2 trial. *Neurology* **84,** 2161–2168 (2015).

41. Tobinick, E. Perispinal etanercept: a new therapeutic paradigm in neurology. *Expert Rev. Neurother.* **10,** 985–1002 (2010).

42. Tobinick, E. L. & Gross, H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. *Journal of Neuroinflammation* **5,** 2 (2008).

43. Cudrici, C., Niculescu, T., Niculescu, F., Shin, M. L. & Rus, H. Oligodendrocyte cell death in pathogenesis of multiple sclerosis: Protection of oligodendrocytes from apoptosis by complement. *J. Rehabil. Res. Dev.* **43,** 123–132 (2006).

44. Kallaur, A. P. *et al.* Genetic polymorphisms associated with the development and clinical course of multiple sclerosis (review). *Int. J. Mol. Med.* **28,** 467–479 (2011).

45. Kallaur, A. P. *et al.* Genetic, Immune-Inflammatory, and Oxidative Stress Biomarkers as Predictors for Disability and Disease Progression in Multiple Sclerosis. *Mol. Neurobiol.* (2016). doi:10.1007/s12035-015-9648-6

46. Brambilla, R. *et al.* Inhibition of soluble tumour necrosis factor is therapeutic in experimental autoimmune encephalomyelitis and promotes axon preservation and remyelination. *Brain* **134,** 2736–2754 (2011).

47. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI

Analysis Group. *Neurology* **53,** 457–465 (1999).

48. Kassiotis, G. & Kollias, G. Uncoupling the Proinflammatory from the Immunosuppressive Properties of Tumor Necrosis Factor (Tnf) at the P55 TNF Receptor Level: Implications for Pathogenesis and Therapy of Autoimmune Demyelination. *The Journal of Experimental Medicine* **193,** 427–434 (2001).

49. Arnett, H. A. *et al.* TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat. Neurosci.* **4,** 1116–1122 (2001).

50. Mogi, M. *et al.* Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci. Lett.* **165,** 208–210 (1994).

51. Dziewulska, D. & Mossakowski, M. J. Cellular expression of tumor necrosis factor a and its receptors in human ischemic stroke. *Clin. Neuropathol.* **22,** 35–40 (2003).

52. Mogi, M. *et al.* Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *J. Neural Transm.* **107,** 335–341 (2000).

53. Fischer, R. *et al.* A TNF receptor 2 selective agonist rescues human neurons from oxidative stress-induced cell death. *PLoS One* **6,** e27621 (2011).

54. Sriram, K. *et al.* Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **16,** 1474–1476 (2002).

55. Pan, W. & Kastin, A. J. Tumor necrosis factor and stroke: role of the blood-brain barrier. *Prog. Neurobiol.* **83,** 363–374 (2007).

56. Wu, M.-H. *et al.* Inhibition of Peripheral TNF-alpha and Downregulation of Microglial Activation by Alpha-Lipoic Acid and Etanercept Protect Rat Brain Against Ischemic Stroke. *Mol. Neurobiol.* (2015). doi:10.1007/s12035-015-9418-5

57. Nawashiro, H., Tasaki, K., Ruetzler, C. A. & Hallenbeck, J. M. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. *J. Cereb. Blood Flow Metab.* **17,** 483–490 (1997).

58. Lambertsen, K. L. *et al.* Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. *J. Neurosci.* **29,** 1319–1330 (2009).

59. Woodcock, T. & Morganti-Kossmann, M. C. The role of markers of inflammation in traumatic

brain injury. *Front. Neurol.* **4,** 18 (2013).

60. Li, G. *et al.* Cytokines and epilepsy. *Seizure* **20,** 249–256 (2011).

61. Silva, F., Cisternas, M. & Specks, U. TNF-alpha blocker therapy and solid malignancy risk in ANCA-associated vasculitis. *Curr. Rheumatol. Rep.* **14,** 501–508 (2012).

62. Etanercept plus standard therapy for Wegener's granulomatosis. *N. Engl. J. Med.* **352,** 351–361 (2005).

63. Gibofsky, A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *Am. J. Manag. Care* **18,** S295–302 (2012).

64. McInnes, I. B. & Schett, G. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* **365,** 2205–2219 (2011).

65. Markham, A. & Lamb, H. M. Infliximab: a review of its use in the management of rheumatoid arthritis. *Drugs* **59,** 1341–1359 (2000).

66. Haraoui, B. & Bykerk, V. Etanercept in the treatment of rheumatoid arthritis. *Therapeutics and Clinical Risk Management* **3,** 99–105 (2007).

67. Fleischmann, R., Iqbal, I., Nandeshwar, P. & Quiceno, A. Safety and efficacy of disease-modifying anti-rheumatic agents: focus on the benefits and risks of etanercept. *Drug Saf.* **25,** 173–197 (2002).

68. Theibich, A., Dreyer, L., Magyari, M. & Locht, H. Demyelinizing neurological disease after treatment with tumor necrosis factor alpha-inhibiting agents in a rheumatological outpatient clinic: description of six cases. *Clin. Rheumatol.* **33,** 719–723 (2014).

69. Day, R. Adverse reactions to TNF-alpha inhibitors in rheumatoid arthritis. *Lancet (London, England)* **359,** 540–541 (2002).

70. Manson, J. J. & Isenberg, D. A. The pathogenesis of systemic lupus erythematosus. *Neth. J. Med.* **61,** 343–346 (2003).

71. Mok, C. C. & Lau, C. S. Pathogenesis of systemic lupus erythematosus. *Journal of Clinical Pathology* **56,** 481–490 (2003).

72. Lahita, R. G., Bradlow, H. L., Kunkel, H. G. & Fishman, J. Alterations of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum.* **22,** 1195–1198 (1979).

73. Vasoo, S. Drug-induced lupus: an update. *Lupus* **15,** 757–761 (2006).

74. Aringer, M. & Smolen, J. S. Tumour necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. *Lupus* **13,** 344–347 (2004).

75. Charles, P. J., Smeenk, R. J., De Jong, J., Feldmann, M. & Maini, R. N. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum.* **43,** 2383–2390 (2000).

76. Shakoor, N., Michalska, M., Harris, C. A. & Block, J. A. Drug-induced systemic lupus erythematosus associated with etanercept therapy. *Lancet (London, England)* **359,** 579–580 (2002).

77. Aringer, M. & Smolen, J. S. Efficacy and safety of TNF-blocker therapy in systemic lupus erythematosus. *Expert Opin. Drug Saf.* **7,** 411–419 (2008).

78. Almoallim, H., Al-Ghamdi, Y., Almaghrabi, H. & Alyasi, O. Anti-Tumor Necrosis Factor-alpha Induced Systemic Lupus Erythematosus(). *Open Rheumatol. J.* **6,** 315–319 (2012).

79. van Rijthoven, A. W. A. M., Bijlsma, J. W. J., Canninga-van Dijk, M., Derksen, R. H. W. M. & van Roon, J. A. G. Onset of systemic lupus erythematosus after conversion of infliximab to adalimumab treatment in rheumatoid arthritis with a pre-existing anti-dsDNA antibody level. *Rheumatology (Oxford, England)* **45,** 1317–1319 (2006).

80. Zhu, L.-J., Yang, X. & Yu, X.-Q. Anti-TNF-α Therapies in Systemic Lupus Erythematosus. *Journal of Biomedicine and Biotechnology* **2010,** (2010).

81. Gillespie, K. M. Type 1 diabetes: pathogenesis and prevention. *CMAJ : Canadian Medical Association Journal* **175,** 165–170 (2006).

82. Moller, D. E. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol. Metab.* **11,** 212–217 (2000).

83. Green, E. A., Eynon, E. E. & Flavell, R. A. Local expression of TNFalpha in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens. *Immunity* **9,** 733–743 (1998).

84. Yang, X. D. *et al.* Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J. Exp. Med.* **180,** 995–1004 (1994).

85. Kachapati, K., Adams, D., Bednar, K. & Ridgway, W. M. The non-obese diabetic (NOD)

mouse as a model of human type 1 diabetes. *Methods Mol. Biol.* **933,** 3–16 (2012).

86. Hotamisligil, G. S. Mechanisms of TNF-alpha-induced insulin resistance. *Exp. Clin. Endocrinol. Diabetes* **107,** 119–125 (1999).

87. Ofei, F., Hurel, S., Newkirk, J., Sopwith, M. & Taylor, R. Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* **45,** 881–885 (1996).

88. Hansen, D. *et al.* Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. *European Journal of Applied Physiology* **109,** 397–404 (2010).

89. Hansen, A., Lipsky, P. E. & Dorner, T. Immunopathogenesis of primary Sjogren's syndrome: implications for disease management and therapy. *Curr. Opin. Rheumatol.* **17,** 558–565 (2005).

90. Nguyen, C. Q. & Peck, A. B. Unraveling the Pathophysiology of Sjogren Syndrome-Associated Dry Eye Disease. *The ocular surface* **7,** 11–27 (2009).

91. Azuma, M. *et al.* Suppression of tumor necrosis factor alpha-induced matrix metalloproteinase 9 production by the introduction of a super-repressor form of inhibitor of nuclear factor kappaBalpha complementary DNA into immortalized human salivary gland acinar cells. Preven. *Arthritis Rheum.* **43,** 1756–1767 (2000).

92. Steinfeld, S. D., Demols, P., Salmon, I., Kiss, R. & Appelboom, T. Infliximab in patients with primary Sjogren's syndrome: a pilot study. *Arthritis Rheum.* **44,** 2371–2375 (2001).

93. Steinfeld, S. D., Demols, P. & Appelboom, T. Infliximab in primary Sjogren's syndrome: one-year followup. *Arthritis Rheum.* **46,** 3301–3303 (2002).

94. Mariette, X. Emerging biological therapies in rheumatoid arthritis. *Joint. Bone. Spine* **71,** 470–474 (2004).

95. Zandbelt, M. M. *et al.* Etanercept in the treatment of patients with primary Sjogren's syndrome: a pilot study. *J. Rheumatol.* **31,** 96–101 (2004).

96. Steinfeld, S. D., Demols, P., Salmon, I., Kiss, R. & Appelboom, T. Notice of retraction of two articles ('Infliximab in patients with primary Sjogren's syndrome: a pilot study' and 'Infliximab in patients with primary Sjogren's syndrome: one-year followup'). *Arthritis and rheumatism* **65,** 814 (2013).

97. Feldmann, M. & Maini, R. N. Lasker Clinical Medical Research Award. TNF defined as a

therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat. Med.* **9,** 1245–1250 (2003).

98. Pasparakis, M., Alexopoulou, L., Episkopou, V. & Kollias, G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J. Exp. Med.* **184,** 1397–1411 (1996).

99. Marino, M. W. *et al.* Characterization of tumor necrosis factor-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* **94,** 8093–8098 (1997).

100. Tobinick, E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. *CNS Drugs* **25,** 145–155 (2011).

101. Goetz, C. G. *et al.* Placebo response in Parkinson's disease: comparisons among 11 trials covering medical and surgical interventions. *Mov. Disord.* **23,** 690–699 (2008).

102. La Mantia, L., Eoli, M., Salmaggi, A. & Milanese, C. Does a placebo-effect exist in clinical trials on multiple sclerosis? Review of the literature. *Ital. J. Neurol. Sci.* **17,** 135–139 (1996).

103. Pollo, A. & Benedetti, F. Placebo response: relevance to the rheumatic diseases. *Rheum. Dis. Clin. North Am.* **34,** 331–349 (2008).

104. Lakhan, S. E., Kirchgessner, A., Tepper, D. & Leonard, A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front. Neurol.* **4,** 32 (2013).

105. Candido, S. *et al.* Roles of NGAL and MMP-9 in the tumor microenvironment and sensitivity to targeted therapy. *Biochim. Biophys. Acta* (2015). doi:10.1016/j.bbamcr.2015.08.010

106. Li, Y. N. *et al.* Serum anti-lipocalin 2 IgG is a novel biomarker in the diagnosis of systemic lupus erythematosus. *Lupus* **23,** 868–875 (2014).

107. Pawar, R. D. Serum Autoantibodies in Pristane Induced Lupus are Regulated by Neutrophil Gelatinase Associated Lipocalin. **154,** 49–65 (2014).