university of groningen / faculty of science and engineering

How to mediate neuroinflammation in traumatic brain injury for a better patient outcome? The role of microglia and TNF-alpha.

Emma Leinenga
15 July 2024
Bachelor's Thesis Life Sciences
Department: Groningen Institute for Evolutionary Life Sciences
Supervisor: Prof. Ulrich L.M. Eisel
Second examiner: Dr. Niki Gervais

Summary

This thesis aims to create an overview of the role of microglia and TNF-alpha in neuroinflammation, a common secondary injury mechanism in traumatic brain injury (TBI). Neuroinflammation is often overlooked as TBI patients receive short-term care and may suffer consequences in terms of chronic inflammation in the brain, leading to neurodegeneration and cognitive impairment. Experimental models are assessed, and spinal cord injury (SCI) models can be taken into consideration when researching neuroinflammation after TBI. It can be concluded that over-the-counter anti-inflammatory medications do not improve the outcome of secondary TBI injury, which paves the way for TNF-alpha as a focus of anti-inflammatory research. TNF-mediating compounds that mediate neuroinflammation and show a neuroprotective effect are TNFR2 agonists, direct TNF inhibitors and TNFR1 antagonists. Microglia depletion therapy has yielded mixed results, but activated microglia can be manipulated from an M1-like, inflammatory phenotype to an M2-like, inflammation-mediating phenotype using pharmacological treatment or genetic engineering. While these therapies are still in their experimental phase, more research is encouraged as they are not only promising as treatment of the much-overlooked neuroinflammation in TBI, but also other neurodegenerative and inflammatory conditions.

Abbreviations

Alzheimer's disease	AD
Antagonistic TNF Receptor One-Specific Antibody	ATROSAB
Glasgow Coma Scale	GCS
TNF receptor	TNFR
activator protein-1	AP-1
blood-brain barrier	BBB
central nervous system	CNS
cerebrospinal fluid	CSF
controlled cortical impact	CCI
cyclooxygenase	COX
damage-associated molecular patterns	DAMPs
diffuse axonal injury	DAI
dominant-negative TNF	DN-TNF
experimental autoimmune encephalomyelitis	EAE

glucocorticoid receptors	GRs
heavy chain domain 2 of immunoglobulin E	EHD2
immunoglobulin G	IgG
interferon regulatory factor	IRF
interferon gamma	IFNγ
interleukin	IL
lateral fluid percussion	LFP
lipopolysaccharides	LPS
long-term potentiation	LTP
mild TBI	mTBI
multiple sclerosis	MS
neural progenitor cells	NPCs
nitric oxide	NO
nitric oxide synthase	iNOS
nonsteroidal anti-inflammatory drugs	NSAIDs
nuclear factor kappa-light-chain-enhancer of activated B-cells	NF-kB
over-the-counter	OTC
pattern recognition receptors	PRRs
peroxisome proliferator-activated receptor α	PPARα
reactive oxygen species	ROS
regulatory T cells	Tregs
rheumatoid arthritis	RA
selective mouse TNF-based agonist of TNF receptor 2	STAR2
soluble TNF	solTNF
spinal cord injury	SCI
traumatic brain injury	TBI
tumour necrosis factor	TNF

Table of contents

Introduction

Traumatic brain injury (TBI) is an acute injury to the brain that anyone can sustain. Any type of accident or other situation, such as sports, causing trauma to the head can result in a TBI, be it mild or severe. In fact, around half of all people will suffer at least one TBI during their life (Maas et al., 2017). Broadly speaking, there are two types of TBI: penetrating or open TBI, and non-penetrating or closed TBI. Examples of common injuries are diffuse axonal injuries, concussions and contusions (NINDS, 2023). Globally, approximately 64-74 million people suffer from TBI every year (Dewan et al., 2019). This number only accounts for the initial injury; what is often forgotten are the long-term effects a TBI can have on a person's health. This is due to what is called the secondary brain injury, which is sustained as a reaction to the primary (initial) injury. Examples of secondary injuries include haemorrhages, increased intracranial pressure and infections in the brain (NINDS, 2023). These injuries can lead to chronic inflammation and other long-term health problems.

Neuroinflammation is essential for the healing process after TBI. Microglia are the resident innate immune cells in the brain, which activate upon injury in order to repair the local tissue. Microglia account for approximately 5-12% of brain cells in mouse models (Lawrence et al., 1990), which is seemingly similar to human brains, although highly dependent on the area of the brain (Mittelbronn et al., 2001). In their non-activated phenotype, microglia scavenge for debris in the brain. Activation through proinflammatory stimuli such as lipopolysaccharides (LPS), interferon gamma (IFNγ) or damage-associated molecular patterns (DAMPS) from damaged cells leads to an M1-like activation, causing the microglia to release proinflammatory stimuli such as tumour necrosis factor alpha (TNF-alpha), interleukin 12 (IL-12), chemokines and reactive oxygen species (ROS). In this way, peripheral immune cells are recruited: upon arrival at the target tissue, proinflammatory cytokines are released, attracting leukocytes. When microglia are activated by Th2 cytokines such as IL-4 or IL-13, their phenotype becomes M2-like instead, promoting tissue regeneration and inhibiting nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-kB) (Gordon, 2003, Sica and Mantovani, 2012).

While the function of the immune response is to repair damages, excess or chronic inflammation can have the opposite effect. The brain is generally an immune privileged organ, however, when the blood-brain barrier (BBB) is ruptured due to trauma, the influx of peripheral immune cells into the brain is increased further and contributes to the excess inflammation (Simon et al., 2017, Yang and Zhou, 2019). Thus, it is important that the microglia, as residents of the brain, mediate the immune response in such a way that it does not become excessive. Release of TNF-alpha and IL-6 in brain tissue had been proven in contusion models as early as 1994 by Shihami et al. Now it is clear that TNF-alpha plays an important role in neuroinflammation in TBI, and researchers are looking to sway the balance of activated microglia from neurodestructive to neuroprotective by reducing TNF-alpha levels, for example by reducing M1-like activation and increasing M2-like activation (Loane et al., 2016). Other methods target microglia directly. The aim of this thesis is to use current literature to research how neuroinflammation can be mediated after TBI, focusing on the role of microglia and TNF-alpha.

Main chapters

Traumatic Brain Injury (TBI) and neuroinflammation

It is commonly accepted that around 90% of TBI cases are mild TBI (mTBI), identified as a score of 13-15 on the Glasgow Coma Scale (GCS) presented in **Table 1** (Teasdale and Jennett, 1974). However, between the scores of mTBI there is a lot of room for variety, as some patients may not suffer any actual brain trauma and some can lose consciousness for up to 30 minutes with up to 24 hours post-traumatic amnesia. It has been suggested that the term “complicated mild TBI” ought to be used for cases where a focal lesion is visible after imaging, or that “moderate TBI” (which is usually a score from 12-9) should be used because the prognosis of those with a visible lesion is more similar to those with moderate TBI than those with mild TBI (Lefevre-Dognin et al., 2021). This shows that, while a TBI may be classified as mild, it may still have long-term effects on the patients depending on the severity of their injury. However, there is not much of an overview in terms of long-term care for these patients. A much-cited study showed that of 131 patients, 14.5% showed symptoms of mTBI at least 12 months after the incident (Rutherford et al., 1979). It is however clear that long-term support is often not provided while the patient does deem it necessary; in one study, this referenced about half of the patient population (Lefkovits et al., 2021).

Common symptoms of mTBI are headaches, dizziness, fatigue, sleep disturbance, concentration and memory problems, irritability, anxiety, depression and dissociation (Van Gils et al., 2020). Injuries in more severe TBI such as diffuse axonal injury (DAI) and hematomas can cause neuronal degradation, resulting in long-term effects such as cognitive impairment. In response to the initial injury, secondary injuries such as brain swelling and inflammation occur, along with a breakdown in the BBB that can exacerbate inflammation and has shown to be chronic (Van Vliet et al., 2020, NINDS, 2023). Neuroinflammation causes a range of degenerative changes in the brain, including damage to the synapses, loss of synaptic plasticity and loss of long-term potentiation (LTP) in the dentate gyrus of the hippocampus (Cumisky et al., 2007, Jamjoom et al., 2021). LTP is a strengthening of synapses allowed by synaptic plasticity, the inhibition of which leads to an impairment of memory formation and learning. Additionally, axonal damage such as DAI can persist months to years after the injury; axons can disconnect from each other and degenerate, leading to chronic cognitive deficiencies, and progressive axonal degeneration is linked to neurodegenerative diseases at a later stage in life (Johnson et al., 2013). The severity of axonal damage can also be related to synaptic damage (Canty et al., 2013). Another symptom of neurodegeneration that comes into play as a consequence of neuroinflammation is the inhibition of neurogenesis. Early inhibition of neurogenesis is linked to neurodegenerative disorders such as Alzheimer’s disease (AD) (Lui and Ozguner, 2005). It is clear that chronic neuroinflammation causes serious pathology in terms of neurodegenerative processes, and that there should be more attention to these secondary effects of TBI, as opposed to the current short-term treatments of especially mTBI. The mechanisms by which neuroinflammation induces damage will be discussed below, as well as possible treatments, focusing on modulation of TNF-alpha or the microglia directly.

Table 1: Glasgow Coma Scale (GCS), adapted from Teasdale and Jennett, 1974. The GCS includes scoring of eye, motor and verbal response behaviours after brain injury. Mild TBI is classified as a score of 13-15, moderate TBI being scored 12-9, and severe TBI being 8-3.

Score	Behaviour
Eye response	
4	Eyes open spontaneously
3	Eyes open to a verbal command
2	Eyes open to a pain stimulus
1	No eye opening
Motor response	
6	Obeys a movement command
5	Moves to localised pain
4	Reflexively withdraws from pain
3	Spastic flexion to pain
2	Rigid response to pain
1	No motor response
Verbal response	
5	Well-orientated
4	Confused but with appropriate responses
3	Inappropriate responses
2	Incomprehensible sounds
1	No verbal response

Mechanisms of neuroinflammation: microglia and TNF-alpha

Microglia are the primary defence mechanism in TBI, initiating the inflammatory response. After TBI, the first to release are DAMPs, which are inflammatory signals picked up by pattern recognition receptors (PRRs). PRRs are abundant in the cells of the central nervous system (CNS), such as in microglia, astrocytes, neurons and oligodendrocytes (Reyes and Shinohara, 2022). Via the PRRs, downstream signalling pathways lead to upregulation of pro-inflammatory

transcription factors such as NF-kB and interferon regulatory factor 3 and 7 (IRF3/7) which increase the release of pro-inflammatory cytokines and type 1 interferons (IFN-alpha, IFN-beta) (Takeuchi and Akira, 2010). Two pro-inflammatory cytokines, TNF-alpha and IL-18 have shown to be upregulated in the dentate gyrus after induction of neuroinflammation, thereby - as mentioned earlier - affecting synapse mechanisms such as LTP (Butler et al., 2004, Cumisky et al., 2007). TNF-alpha, IL-18 and IL-6 have also shown to inhibit cell differentiation and cause neuronal death of neural progenitor cells (NPCs) (Lui and Ozguner, 2005). Elevated levels of TNF-alpha, as seen in chronic neuroinflammation, have the ability to directly impair cognitive function (Belarbi et al., 2012), underlining the potential of TNF-alpha to contribute to cognitive impairment in chronic neuroinflammation.

It is clear that TNF-alpha has many direct effects on the state of the CNS during inflammation. The release of TNF-alpha is mediated by cells of the immune system. While peripheral influx of immune cells is common after TBI, microglia play an important role in the regulation of TNF-alpha. Upon M1-like activation of microglia, they will release an exaggerated amount of TNF-alpha, IL-6 and IL-1beta, but also inducible nitric oxide synthase (iNOS), which leads to an increase in the ROS nitric oxide (NO). In turn, the produced ROS and cytokines stimulate the pro-inflammatory environment and induce more activation of microglia to the M1-like phenotype. M1-like microglia, activated by pro-inflammatory factors, are induced through “classical activation”, while the activation of M2-like microglia is called “alternative activation” (Colton et al., 2010). Alternative activation happens via anti-inflammatory cytokines IL-4 and IL-13, and M2-like microglia assist in tissue repair and the stimulation of neurotrophic factors (**Figure 1**). However, in chronic neuroinflammation, the M1-like phenotype far outweighs the M2-like phenotype, perpetuating the problem of consequential neurodegeneration. In chronic inflammation, microglia can be primed, meaning the microglia become overly sensitive and produce an exaggerated inflammatory response when triggered. Microglial priming was first discovered in prion disease and found relevant in neurodegenerative disorders such as AD, but has been shown to appear in chronic neuroinflammation where it thus contributes to neurodegeneration (Perry and Holmes, 2014). Therefore, researchers are looking to balance the M2-like phenotype of microglia against the M1-like phenotype as a treatment for chronic neuroinflammation.

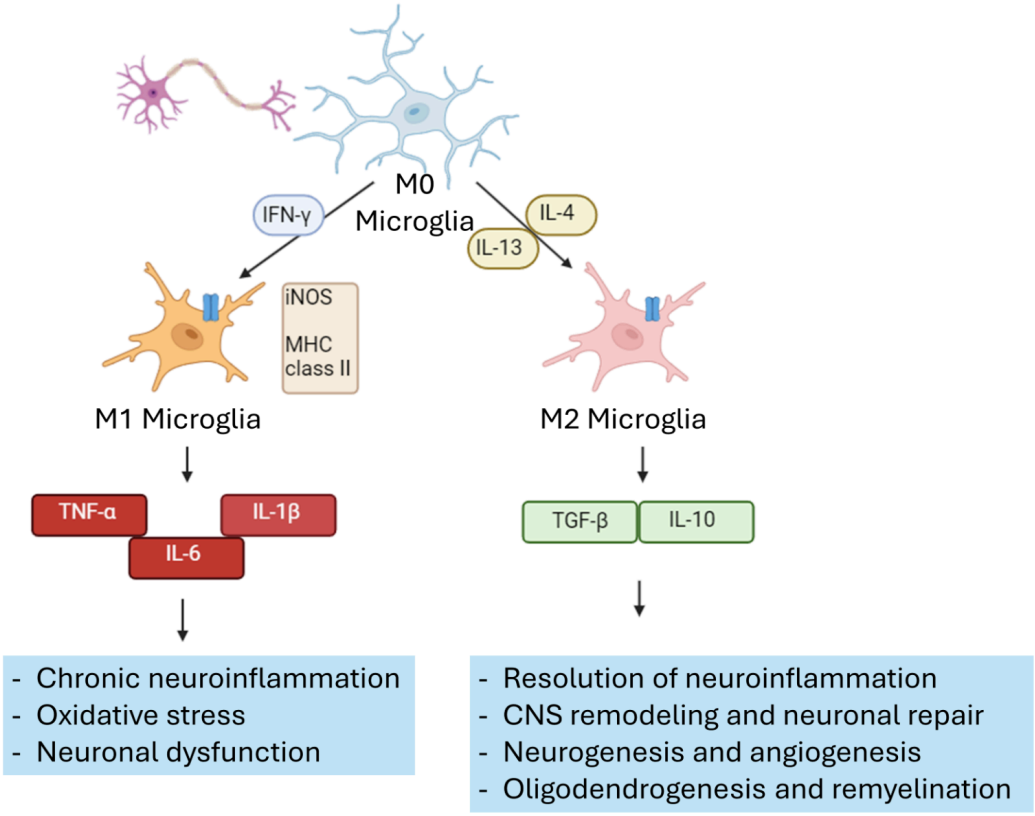

Figure 1. Differentiation of microglia into M1- and M2-like microglia. M1-like microglia are activated via IFN-gamma and start expressing MHC class II as well as iNOS. Together with secreted factors TNF-alpha, IL-6 and IL-1beta, M1 microglia contribute to chronic neuroinflammation, oxidative stress and neuronal dysfunction. On the other hand, M2-like microglia are activated through IL-4 and IL-13, interleukins that activate T-helper cells. M2 microglia secrete anti-inflammatory factors TGF-beta and IL-10, leading to resolution of neuroinflammation, CNS remodelling and neuronal repair, neurogenesis and angiogenesis, oligodendrogenesis and remyelination. Adapted from a review by Shao et al., 2022. *Image created using BioRender.com.*

Experimental models of TBI

Due to the complexity of the primary and secondary injuries in TBI, it is challenging to design animal models for the condition. Depending on the methods used, different severities and locations of the TBI (e.g. focal lesions vs. diffuse injury) can be modelled, leading to a large variety of models (O'Connor et al., 2011, Fesharaki-Zadeh and Datta, 2024). Therefore, research on the mechanisms of neuroinflammation using spinal cord injury (SCI) models is also important to consider. SCI models are much more precise in their location compared to TBI models due to the localisation of the damage in one part of the spinal cord compared to a head injury, where one or several parts of the brain can be damaged in different ways and severity. The differences and similarities between SCI and TBI and what this means for the outcomes of neuroinflammatory research, will be discussed.

A well-known model of TBI in mice is lateral fluid percussion (LFP), in which a craniectomy is followed by a single pulse with an LFP device. LFP is the most commonly used technique to

simulate TBI, and it mostly causes contusions and axonal injuries. The advantage of this model is that the grade of injury is highly adjustable (Dixon et al., 1987, Alder et al., 2011, O'Connor et al., 2011). However, this model usually causes focal injuries, along with the controlled cortical impact model (CCI) (Dixon et al., 1991), in which a pneumatic impactor is used to cause damage to a target area of the brain. Other models such as the weight drop model aim to cause diffuse brain injury, another outcome of TBI (Marmarou et al., 1994, Kalish and Whalen, 2016). Weight drop models are also used to simulate SCI. However, in the spine it is common to make lesions in different, more efficient ways that produce higher accuracy results as reviewed by Nakae et al. (2011). All these models were developed in either mice or rats, and have been optimised for other animal species as well. While human models are being developed by focusing on the inflammatory environment in stem cells (Thelin et al., 2018) or digital brain finite element models (Atsumi et al., 2018), animal models are capable of showing behaviour after TBI and are the most efficient models to date.

TBI and SCI are similar in that they are both injuries to the CNS, which is relatively immune privileged tissue. Upon injury, the immune system will be triggered and have both neuroprotective and neurodegenerative effects on the nerves. Due to the normal immune privileged status of the tissue, neuroinflammation can quickly become excessive and cause secondary injury to the neurons, leading to axonal damage and tissue destruction (Schwab et al., 2014). While their origins may be different, TBI and SCI thus have a similar outcome in terms of neuroinflammation, which is why SCI models can also be taken into account when focusing on neuroinflammation in TBI.

Attempts to ameliorate neuroinflammation

1. Over-the-counter inflammatory mediators

Anti-inflammatory medications such as glucocorticoids (steroids) and nonsteroidal anti-inflammatory drugs (NSAIDs) are much used in daily life. Glucocorticoids are a class of corticosteroids, steroid hormones produced in the adrenal cortex. They are anti-inflammatory and immunosuppressive by nature, by binding to glucocorticoid receptors (GRs) that are abundant in most cell types, including cells of the immune system such as microglia. This way, pro-inflammatory transcription factors such as NF-kB and activator protein-1 (AP-1) are inhibited by glucocorticoids. Glucocorticoids are therefore often used in animal models to examine their neuroprotective effect (Coutinho and Chapman, 2011, Sunishtha et al., 2022). However, it has been shown that glucocorticoids as treatment for TBI increase apoptosis of neurons in the hippocampus, which can cause cognitive impairment or memory loss (Chen et al., 2009, Zhu et al., 2013, Komoltsev and Gulyaeva, 2022). Therefore, while glucocorticoids are popular in animal experiments, treatment of neuroinflammation with glucocorticoids in TBI patients is currently not recommended.

NSAIDs are antipyretic and analgesic on top of their anti-inflammatory function, and work via the inhibition of the cyclooxygenase (COX) enzyme. The COX enzyme consists of isoenzymes COX-1 and COX-2 which synthesise prostaglandins, which are pro-inflammatory compounds, as well as stimulating levels of IL-10 and IL-1beta. One study by Harrison et al. (2014) found that, similar to taking an NSAID at home after mTBI, administration of an over-the-counter

(OTC) dose of an NSAID after LFP brain injury in mice did not mediate symptoms of concussion, concluding that NSAIDs do not have an immediate alleviating effect after mTBI. Other studies have found specific but mixed results such as non-selective COX inhibition alleviating neurological symptoms within 24 hours while COX-2 specific inhibition does not (Girgis et al., 2013) and IL-1β decrease through both COX pathways (Keshavarzi et al., 2012). It is clear that glucocorticoids and NSAIDs, while being anti-inflammatory, should not be recommended to treat neuroinflammation in TBI. Therefore, more specific methods should be targeted, such as mediating the pro-inflammatory cytokine TNF-alpha. Over the years, many therapeutic compounds targeting TNF-alpha and microglia have been developed, which are discussed below.

2. TNF receptor 2 agonists

TNF receptor 2 (TNFR2) is expressed on cells of the immune system, microglia, endothelial cells and cardiomyocytes (Medler and Wajant, 2019). Even though TNFR2 activates the NF-kB pathway, it has shown neuroprotective effects, for example through stimulation of regulatory T cells (Tregs) (Chen et al., 2007), which is also reviewed thoroughly by Medler and Wajant (2019). It can also support neurogenesis (Chen and Palmer, 2013), as opposed to TNFR1, which is often shown to have the exact opposite effect. A study by Gao et al. (2017) also showed that when TNFR2 is ablated in microglia, it worsens the outcome of neuroinflammation in mice. There seems to be clear evidence that microglial TNFR2 mediates neuroinflammation. Therefore, TNFR2 agonists have been researched to counter neuroinflammation in diseases such as TBI and multiple sclerosis (MS).

One such TNFR2 agonist is selective mouse TNF-based agonist of TNF receptor 2 (STAR2), developed by Chopra et al. (2016). Although it was tested in graft-versus-host disease (GvHD) instead of a neuroinflammatory disease, it was highly successful in activating Treg cells and it drastically improved the outcome of the disease. NewSTAR2 was developed after (Vargas et al., 2022), and was shown to have superior serum retention over STAR2 with improved pharmacokinetic properties. STAR2, also called TNC-sc(mu)TNF80, was upgraded into the fusion protein irrIgG1(N297A)-HC:sc(mu)TNF80 (NewSTAR2), meaning that essentially the single-chain TNF80 domain was fused to the C-terminal heavy chain domain of an immunoglobulin G (IgG) molecule (**Figure 2A**). A single injection of NewSTAR2 reached a 3-fold stronger bioluminescence signal and upregulated suppression-related markers compared to two weeks of STAR2 injections (Vargas et al., 2022). Since then, research has shown that NewSTAR2 mediates inflammation and improves outcomes in acute GvHD (Vargas et al., 2022b), stroke (Thougaard et al., 2023), and AD (Ortí-Casañ et al., 2022, Ortí-Casañ et al., 2023). Therefore, NewSTAR2 proves to be a valid candidate for treatment of neuroinflammation in TBI and other disorders.

Another TNFR2 agonist that has gained attention is the protein EHD2-sc-mTNFR2. A TNFR2-selective single chain TNF protein is used as three linked monomers and by linking it to the heavy chain domain 2 of immunoglobulin E (EHD2), a hexamer is formed (**Figure 2B**) (Fischer et al., 2018). Since it is primarily membrane-bound TNF that binds to TNFR2 (Sedger and McDermott, 2014), Like NewSTAR2, it causes selective activation of TNFR2 which leads to an increase in Treg cells, in this case alleviating experimental arthritis in vitro (Fischer et al., 2018). EDH2-sc-mTNFR2 is therefore another candidate against neuroinflammation, however, it

seems less attractive than NewSTAR2 when comparing the amount of publications on the two compounds. There are however publications demonstrating the effectiveness of EDH2-sc-mTNFR2 in mediating neuropathic pain (Fischer et al., 2019) and SCI (Gerald et al., 2019), suggesting that the neuroprotective effect of EDH2-sc-mTNFR2 may mitigate secondary injury in TBI as well.

3. TNF inhibitors

Many monoclonal antibodies have been developed against TNF, such as infliximab and adalimumab. They bind to TNF to inhibit it from binding to the TNFRs, mediating inflammation (Smolen and Emery, 2011, Miyasaka, 2009). Although both of these antibodies have a long history, etanercept was one of the first fusion protein TNF inhibitors to be brought onto the market (Weinblatt et al., 1999, Goldenberg, 1999, Moreland, 1999), and the first to be approved for rheumatoid arthritis (RA) while the others followed soon after (Zhao et al., 2018). Unlike the monoclonal antibodies, etanercept consists of two Fc portions of human IgG1 connected to two portions of extracellular TNFR2 (**Figure 2C**). It binds to TNF with greater affinity than the monoclonal antibodies and endogenous TNFRs, making it an effective drug. Membrane TNF binds strongly to TNFR1 and TNFR2 while soluble TNF binds to both but preferentially activates TNFR1 (Medler and Wajant, 2019). Etanercept preferentially binds to membrane TNF, leaving TNFR1 more vulnerable to TNF binding than TNFR2. This could lead to maintenance of inflammatory symptoms, which can be hypothesised for example in a study by Van den Brande et al. (2013) where etanercept showed less efficacy in mediating inflammation in Crohn's disease compared to infliximab. Etanercept is still much-prescribed for RA and proven to be a safe and effective drug with renewed popularity (Zhao et al., 2018). While it is not officially indicated for any neurological diseases, research has been done to assess the outcome of TBI with etanercept. An LFP model of TBI in mice by Chio et al. (2010) found that microglial activation and activated TNF-alpha levels along with motor and cognitive deficits were all decreased by administration of etanercept into the cerebrospinal fluid (CSF). Similarly, the same results were found with etanercept injections in an LFP model of TBI in rats (Cheong et al., 2013). An observational study in humans treated with perispinal injections of etanercept after chronic neurological deficits following stroke or TBI showed significant improvement in their condition compared to treatment with a placebo (Tobinick et al., 2012). All in all, etanercept has proven itself to be an effective TNF inhibitor that could be considered for official use in patients with TBI, both directly after TBI and when struggling with long-term neurological problems.

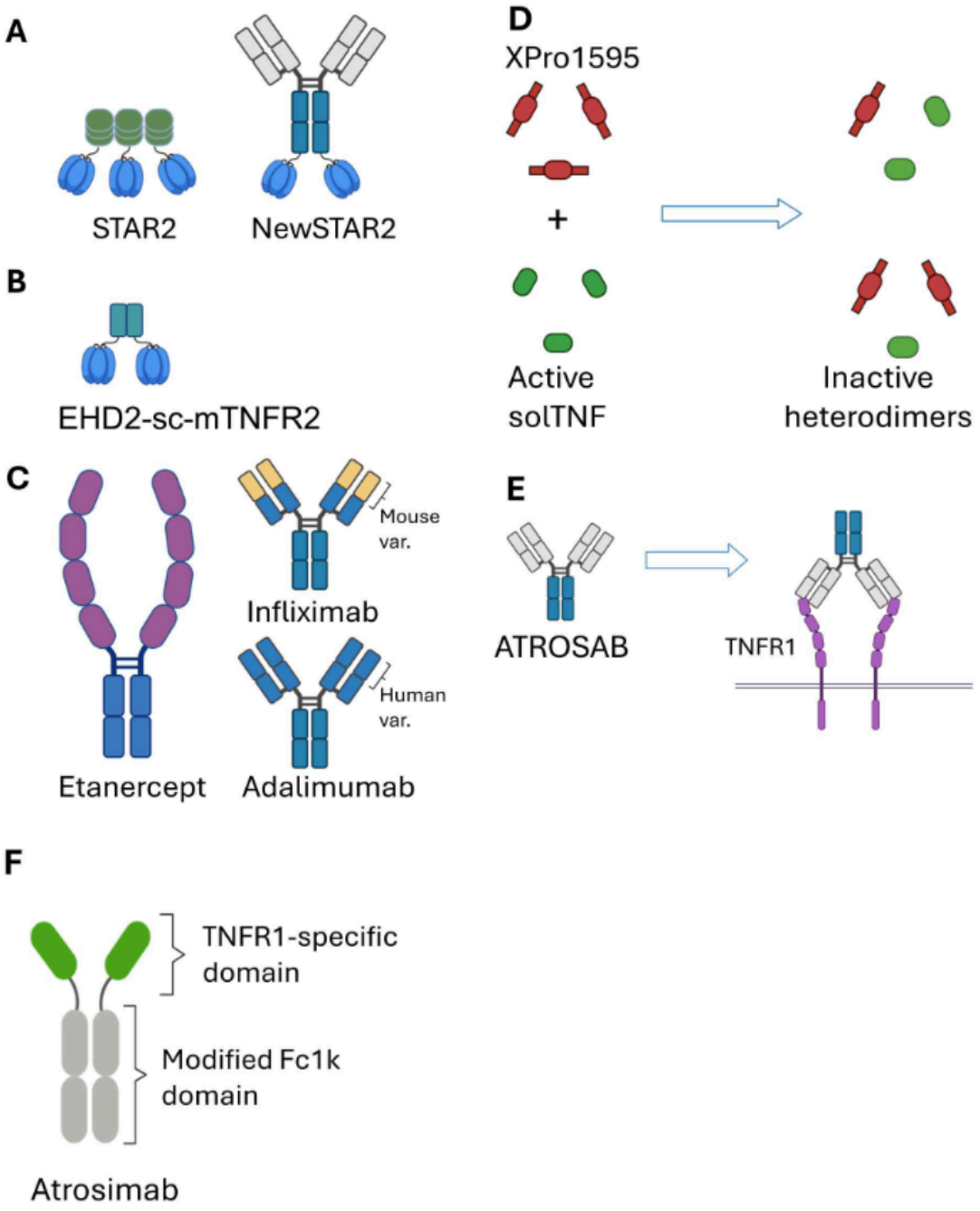

Figure 2. Schematic representations of TNFR2 agonists, TNF inhibitors and TNFR1 antagonists. **a)** selective mouse TNF-based agonist of TNF receptor 2 (STAR2) and NewSTAR2, both TNFR2 agonists. In NewSTAR2, the single-chain TNF80 domain is fused to the C-terminal heavy chain domain of an IgG molecule. Figures adapted from Vargas et al., 2022. **b)** EDH2-sc-mTNFR2, TNFR2 agonist. A TNFR2-selective single chain TNF protein is used as three linked monomers and by linking it to the heavy chain domain 2 of immunoglobulin E (EHD2), a hexamer is formed. Figure adapted from Fischer et al., 2018. **c)** The fusion protein TNF inhibitor etanercept next to TNF antibodies infliximab and adalimumab. Etanercept consists of two Fc portions of human IgG1 connected to two portions of extracellular TNFR2. Figure adapted from Zhao et al., 2018. **d)** solTNF-selective TNF inhibitor XPro1595, a dominant-negative variant of TNF. Binding to solTNF forms an inactive heterodimer which

cannot bind to TNFRs. Figure adapted from INmune Bio Inc. **e)** TNFR1 antagonist ATROSAB, consisting of a humanised anti-human TNFR1 antibody converted into IgG1. TNFR1 blockage is demonstrated. Figure adapted from Richter et al., 2013. **f)** TNFR1 antagonist Atrosimab, upgraded from ATROSAB. ATROSAB is converted to a new anti-TNFR1 antibody fragment named Fab 13.7, then the variable chains are fused to heterodimer Fc chains, a technique called Fc1k ("Fc-one/kappa"). This creates a protein with a TNFR1-specific domain and a modified Fc1k domain. Figure adapted from Richter et al., 2013. *Image created using BioRender.com.*

Another TNF inhibitor is the compound XPro1595, developed as a dominant-negative variant of TNF (DN-TNF). By binding to native soluble TNF (solTNF), an inactive heterodimer is formed, which cannot bind to TNFRs (Steed et al., 2003) (**Figure 2D**). XPro1595 is also known as DN-TNF, XENP345, XENP1595 and INB03 among others, and has steadily been worked on since 2003, making great progress as a TNF inhibitor and proving itself to be an effective anti-inflammatory. As early as 2006, its protective effect on dopaminergic neurons has been demonstrated (McCoy et al., 2006). Since XPro1595 selectively binds to solTNF, its anti-inflammatory effects are enhanced due to the preferential binding of solTNF to TNFR1 (Medler and Wajant, 2019). This gives XPro1595 an advantage over etanercept, since etanercept does not selectively target solTNF and therefore also blocks the anti-inflammatory effects of TNFR2. XPro1595 has shown to be neuroprotective in rodent studies of Parkinson's (Barnum et al., 2014), AD (MacPherson et al., 2017), SCI (Novrup et al., 2014, Lund et al., 2023) and even TBI (Larson et al., 2023). In this last study, a CCI mice model with XPro1595 administration 60 minutes post-injury, where it prevented reactivity of microglia, reduced synaptic plasticity and neuronal dendritic degeneration. Recently, the role of TNF-alpha in acute subdural hematoma in TBI was highlighted, with intracranial venous return disorder as an important part of the prognosis in TBI patients (Wang et al., 2023). Here, the possible therapeutic role of XPro1595 was also highlighted, showing its popularity and potential in mediating TBI from different perspectives.

4. TNF receptor 1 antagonists

Next to TNF inhibitors, which work optimally when solTNF is targeted due to their preference for TNFR1, there are also TNFR1 antagonists. Antagonistic TNF Receptor One-Specific Antibody (ATROSAB) is one such TNFR1 antagonist dating from 2010 (Zettlitz et al., 2010). It consists of a humanised anti-human TNFR1 antibody, converted into IgG1 (**Figure 2E**). Compared to other TNFR1 antagonists, ATROSAB has shown greater values of binding and inhibition, suggesting it to be the better compound in countering inflammation (Richter et al., 2013), along with better efficacy in mediating inflammation in monkey and mice models of RA and MS (Guenzi et al., 2013). ATROSAB has also improved the outcome of neuroinflammatory models in combination with EHD2-sc-mTNFR2; TNFR2 was stimulated first using the TNFR2 agonist to increase the anti-inflammatory response, after which ATROSAB was used to inhibit the effects of TNFR1 (Dong et al., 2016, Pegoretti et al., 2023).

ATROSAB was later converted to a new anti-TNFR1 antibody fragment named Fab 13.7, then fused to heterodimer Fc chains. The product is called atrosimab, which has shown an increased binding affinity to TNFR1 and reduced cross-linking interactions, making it a potentially improved anti-inflammatory drug over ATROSAB (Richter et al., 2019) (**Figure 2F**). Since then, Atrosimab has indeed shown therapeutic effects in mouse models of experimental arthritis,

experimental autoimmune encephalomyelitis (EAE) and non-alcoholic steatohepatitis, suggesting its potential in treating chronic inflammation (Richter et al., 2021). Neuroprotective effects of Atrosimab have also been demonstrated through the reduction of neuronal cell death, neurodegeneration and cognitive deficits in a neurodegenerative mouse model (Ortí-Casañ et al., 2023). As Atrosimab is a current focus as an anti-inflammatory therapeutic, more implications for its use in neuroinflammatory disease will probably be published, including TBI.

5. Microglia-targeted therapies

It is clear that mediation of the TNF receptors on microglia has great therapeutic potential for neuroinflammatory disease. However, microglia themselves are also targeted in research. The microglia depletion model is a well-used model that has served as an observatory for scientists to find out the importance of microglia in inflammatory settings. Microglia can be pharmacologically and genetically depleted. Pharmacological treatment focuses on the inhibition of the CSF-1 receptor, since CSF-1 is critical and selective for the survival of microglia (Elmore et al., 2014). In genetic depletion, immunotoxins or “suicide genes” are expressed upon activation by a prodrug, which has supposedly higher efficiency than the pharmacological approach (Han et al., 2017). Since microglia are protective under normal circumstances, there is a lot of debate and variety in outcomes of microglia depletion research. For example, pharmacological microglial depletion has shown a neuroprotective effect in intracerebral haemorrhage (Li et al., 2017), while depletion with the same compound shows exacerbation of neuronal damage in brain ischaemia (Szalay et al., 2018, Han et al., 2017). Many such results have led to critical review articles debating the value of microglia in neuroinflammation. Another concern is the ability of microglia to repopulate after artificial inhibition. It currently cannot be ascertained if repopulated microglia can take over the full functions of depleted microglia in the CNS. Nevertheless, research on microglial depletion seems to be popular and there are abundant studies showing overactivation of microglia in neurodegenerative and neuroinflammatory diseases, as illustrated by reviews (Parajuli and Koizumi, 2023, Zhang et al., 2023).

Regarding TBI, the microglia depletion model has shown general improvement of symptoms. In a study by Henry et al. (2020), a mice CCI model was treated with pharmacological depletion of microglia in the chronic phase, where depletion and repopulation showed similar morphology to uninjured mice. Inhibition of microglia decreased chronic neuroinflammation and neurodegeneration while improving cognitive function in the long term, mitigating the secondary effects of TBI. However, a study by Hanlon et al. (2019) found that immediate depletion of microglia after TBI led to an increase in neuroinflammation in the early post-TBI period, perhaps due to decreased clearance of dead and dying cells in the brain. All in all, review articles seem to agree that the timing of microglia depletion is an important factor in its effectiveness against neuroinflammation and this should be researched more thoroughly in different models.

Another way to encourage neuroprotective mechanisms using microglia is manipulation of the balance of primed microglia from M1-like to M2-like, to reduce pro-inflammatory and increase anti-inflammatory mechanisms. One drug that is used to achieve this, is fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist. This drug is used on the current market to treat high blood cholesterol, however, it could also have a future as neuroprotective medicine after TBI. PPARs regulate the activation of M2-like macrophages and microglia

(Chawla, 2010), and LFP-injured mice treated with fenofibrate showed a decrease in neurological deficits both in the early and late phase of recovery, indicating the neuroprotective effect of fenofibrate (Besson et al., 2005). Another model of TBI showed anti-inflammatory and antioxidant effects, decreasing oxidative stress (Chen et al., 2007). Since then, several studies have been conducted on the effects of fenofibrate in brain haemorrhage and ischemia models, showing a neuroprotective effect, reduced brain swelling and edema, and amelioration of learning and memory deficits (Wang et al., 2017, Vahidi et al., 2021, Xuan et al., 2015). Based on current experimental evidence, fenofibrate could possibly be used in treatment of TBI in the future.

Discussion

This thesis has focused on the role of microglia and TNF-alpha in mediating neuroinflammation following TBI. It is clear that the microglia play a big role in TBI and subsequent inflammation is caused by M1-like activation of microglia and consequentially, release of pro-inflammatory cytokines such as TNF-alpha (Loane et al., 2016, Shao et al., 2022). An overview of M1-like and M2-like microglia and the role of microglia and TNF-alpha were presented, and experimental models of TBI were discussed. It can be concluded that while there are different kinds of models for TBI, models for SCI can also be used to study neuroinflammation in the same way. In this thesis, several therapies against neuroinflammation have been discussed. It can be concluded that OTC anti-inflammatory medications are not effective in treating the secondary inflammatory injury after TBI (Chen et al., 2009, Zhu et al., 2013, Komoltsev and Gulyaeva, 2022, Harrison et al., 2014). While experiments with animals show an effect of glucocorticoids, patient studies have not had the same success. NSAID studies have not yielded beneficial results either. This indicates that another type of therapy should be developed against neuroinflammation, and TNF-alpha is a prime target. TNF-alpha can inhibit cell differentiation and cause neuronal death in neuroinflammation as well as impairing cognitive function (Lui and Ozguner, 2005, Belarbi et al., 2012). Therefore, three types of TNF-alpha-mediating compounds have been discussed: TNFR2 agonists, TNF inhibitors and TNFR1 antagonists. TNFR2 has demonstrated to be anti-inflammatory when activated, which is why TNFR2 agonists have been developed (Chen et al., 2007, Chen and Palmer, 2013, Gao et al., 2017). NewSTAR2, successor of STAR2, is a TNFR2 antagonist which is currently popular in research. It has shown to be effective in GvHD (Vargas et al., 2022b), stroke (Thougaard et al., 2023), and AD (Ortí-Casañ et al., 2022, Ortí-Casañ et al., 2023), making it a promising candidate for TBI as well. EDH2-sc-mTNFR2 is another TNFR2 inhibitor and has shown effect in neuropathic pain (Fischer et al., 2019) and SCI (Gerald et al., 2019). TNF inhibitors such as monoclonal antibodies infliximab and adalimumab have been discussed, but fusion protein etanercept has shown even more promise, as an anti-inflammatory medicine currently on the market for RA but with great experimental success in TBI (Chio et al., 2010, Cheong et al., 2013, Tobinick et al., 2012). Etanercept is yet outdone by XPro1595, a TNF inhibitor specific for solTNF, essentially blocking TNFR1 activation while not affecting TNFR2 (Medler and Wajant, 2019). XPro1595 has shown effectiveness in many neurodegenerative conditions and recently in TBI, making it a promising compound for the future (Barnum et al., 2014, Lund et al., 2023, Novrup et al., 2014, Larson et al., 2023). Lastly,

TNFR1 antagonists were discussed, with the popular ATROSAB being replaced by atrosimab. It has not yet been tested in TBI models, but its neuroprotective effects are clear, and additional research is needed (Richter et al., 2019, Richter et al., 2021, Ortí-Casañ et al., 2023). Microglia-targeted therapies were discussed as final remedy for neuroinflammation, first explaining the microglia depletion model followed by a potential shift from M1-like microglial activation to M2-like microglia. In the case of the microglia depletion model, while research uncovers valuable information about the role of microglia in neuroinflammation, results are still conflicting (Li et al., 2017, Szalay et al., 2018, Han et al., 2017, Parajuli and Koizumi, 2023, Zhang et al., 2023). However, a medication that is used for high blood cholesterol, fenofibrate, has shown effective in shifting the balance of primed microglia from M1-like to M2-like (Besson et al., 2005, Chen et al., 2007, Wang et al., 2017, Vahidi et al., 2021, Xuan et al., 2015). All in all, there are many possible ways to treat neuroinflammation just based on TNF-alpha and active microglia, and all of these suggestions have yet to be clinically assessed.

As summarised, not all discussed therapies show the expected outcome. OTC medications such as glucocorticoids and NSAIDs are anti-inflammatory in nature, but yield negative results in animal experiments on secondary injury in TBI (Chen et al., 2009, Zhu et al., 2013, Komoltsev and Gulyaeva, 2022, Harrison et al., 2014). This shows that TBI is a complex condition and neuroinflammation should be countered at its root instead of via generic inflammatory mechanisms. The microglia depletion model is another therapy that yields mixed results. The main takeaway from these results is that microglia have complex functions that lead to both inflammation-mediating and pro-inflammatory outcomes, depending on the timing of microglial depletion. It is suggested that primed, “overactive” microglia be depleted in a manner that allows new microglia to take over and put the brain in a state of normality to avoid overactivity of the immune system that would otherwise lead to exacerbated neuroinflammation. However, there are many theoretical and practical obstacles in the microglia depletion model and it will probably not be suggested for TBI therapy any time soon (Hanlon et al., 2019). Manipulation of microglia in other ways, such as balancing M1-like and M2-like activation, and TNF-focused therapies seem to be preferred in the current landscape of experimental TBI therapies.

With TBI being a global health problem and one of the leading acute health problems in many developed countries (Dewan et al., 2019), it is no wonder that researchers are focused on mediating secondary injury and neuroinflammation. However, experimental evidence does not automatically lead to treatment, as clinical trials have to be done on compounds to assess their safety and efficacy in human subjects. Many of the therapies mentioned in this thesis are not yet at such a stage, and thus will not be used in ameliorating TBI for many years to come. Currently, two XPro1595 clinical trials are ongoing for AD (ClinicalTrials.gov ID: NCT05522387, NCT05318976), and there are many etanercept trials being conducted for RA. In this regard, TNF inhibitors are closest to approved human treatment compared to the other discussed therapies. According to ClinicalTrials.gov, monoclonal antibodies infliximab and adalimumab are being tested for many inflammatory conditions including psoriasis and Crohn’s disease, however, no TBI trials were found. Similarly, fenofibrate is mainly being tested in type 1 diabetes. It can be concluded that while TNF-mediating and microglial therapies show great experimental promise for neuroinflammation and treatment of TBI, treatment with these compounds is far from reality due to lack of clinical trials. Many of the therapies are relatively new ideas and repeat experiments need to be done on their effectiveness, as well as additional

experiments on safety and comparison to other types of medications. Translational research is much needed in this area as safe dosing, compound uptake and metabolism and entry through the BBB pose significant obstacles in potential human administration.

As demonstrated by overwhelming experimental evidence, the therapies discussed in this thesis are not specific to TBI. Experiments cover several inflammatory and neurodegenerative diseases, mainly AD. Indeed, TNF-mediating therapies should be considered for AD and other neurodegenerative conditions such as Parkinson's disease. Other inflammatory diseases such as RA and encephalomyelitis should also be considered. Next to that, the closest mentioned pathology to TBI is SCI. While experimental models of neurodegenerative and inflammatory diseases such as AD and RA have inflammation as a common factor, TBI and SCI both shed light on neuroinflammation in the CNS, an immune-privileged tissue. It has been discussed and concluded that similar animal models are used for TBI and SCI. While the two are not interchangeable, SCI models can certainly be included when gathering information on TBI research, and perhaps the other way around. A model that is used in both TBI and SCI is the weight drop model, causing diffuse brain injury as a TBI model, less often used as SCI model due to its relative inaccuracy to other available methods (Nakae et al., 2011). However, it needs to be emphasised that the available experimental models for TBI, whether they cause focal lesions or diffuse injury, may be significant but not completely accurate to human TBI. TBI is a complicated condition which, while it can be categorised into several kinds, is eventually unique for everyone, as it most often occurs due to accidents. While animal models are necessary for behavioural tests and optimal for studies of the brain, future research should encourage the use of digital 3D models and simulations of TBI to shift away from the use of animals towards a model that can be both accurate and complicated.

To conclude, mediation of microglia or TNF-alpha seems to be an excellent opportunity to mediate secondary injury and neuroinflammation in TBI. It is clear that the role of microglia cannot be ignored, but manipulation of microglia should be approached with caution due to the complexity of the microglia's functions. TNF-alpha is a popular target for anti-inflammatory compounds, with several strategies being tested in experimental research such as TNFR2 agonism, direct TNF inhibition and TNFR1 antagonism. While many compounds show a lot of promise in experimental settings, more research needs to be conducted before human use and clinical trials can be considered. While many neuroinflammatory models can be used when researching secondary injury in TBI, it needs to be considered that animal TBI models may not capture the entire complexity of the condition, and that other secondary injury mechanisms such as brain swelling, haemorrhage and edema can indirectly influence real-life results. Hopefully, computer models of TBI can decrease these limitations in the future. The benefits of TNF-mediating therapies over therapies such as OTC medications should not be overlooked and future research should focus on TNF-mediating compounds in increasingly accurate TBI models to eventually mitigate neuroinflammation after TBI.

References

Alder, J., Fujioka, W., Lifshitz, J., Crockett, D. P., & Thakker-Varia, S. (2011). Lateral fluid percussion: model of traumatic brain injury in mice. *Journal of Visualized Experiments : JoVE*, *54*. https://doi.org/10.3791/3063

Atsumi, N., Nakahira, Y., Tanaka, E., & Iwamoto, M. (2018). Human Brain Modeling with Its Anatomical Structure and Realistic Material Properties for Brain Injury Prediction. *Annals of Biomedical Engineering*, *46*(5), 736–748. https://doi.org/10.1007/s10439-018-1988-8

Barnum, C. J., Chen, X., Chung, J., Chang, J., Williams, M., Grigoryan, N., Tesi, R. J., & Tansey, M. G. (2014). Peripheral Administration of the Selective Inhibitor of Soluble Tumor Necrosis Factor (TNF) XPro®1595 Attenuates Nigral Cell Loss and Glial Activation in 6-OHDA Hemiparkinsonian Rats. *Journal of Parkinson's Disease*, *4*(3), 349–360. https://doi.org/10.3233/JPD-140410

Belarbi, K., Jopson, T., Tweedie, D., Arellano, C., Luo, W., Greig, N. H., & Rosi, S. (2012). TNF-α protein synthesis inhibitor restores neuronal function and reverses cognitive deficits induced by chronic neuroinflammation. *Journal of Neuroinflammation*, *9*(1), 23. https://doi.org/10.1186/1742-2094-9-23

Besson, V. C., Chen, X. R., Plotkine, M., & Marchand-Verrecchia, C. (2005). Fenofibrate, a peroxisome proliferator-activated receptor α agonist, exerts neuroprotective effects in traumatic brain injury. *Neuroscience Letters*, *388*(1), 7–12. https://doi.org/10.1016/j.neulet.2005.06.019

Butler, M. P., O'Connor, J. J., & Moynagh, P. N. (2004). Dissection of tumor-necrosis factor-α inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early—but not late—phase LTP. *Neuroscience*, *124*(2), 319–326. https://doi.org/10.1016/j.neuroscience.2003.11.040

Canty, A. J., Teles-Grilo Ruivo, L. M., Nesarajah, C., Song, S., Jackson, J. S., Little, G. E., & de Paola, V. (2013). Synaptic Elimination and Protection after Minimal Injury Depend on Cell Type and Their Prelesion Structural Dynamics in the Adult Cerebral Cortex. *Journal of Neuroscience*, *33*(25), 10374–10383. https://doi.org/10.1523/JNEUROSCI.0254-13.2013

Chawla, A. (2010). Control of Macrophage Activation and Function by PPARs. *Circulation Research*, *106*(10), 1559–1569. https://doi.org/10.1161/CIRCRESAHA.110.216523

Chen, X., Bäumel, M., Männel, D. N., Howard, O. M. Z., & Oppenheim, J. J. (2007). Interaction of TNF with TNF Receptor Type 2 Promotes Expansion and Function of Mouse CD4+CD25+ T Regulatory Cells. *The Journal of Immunology*, *179*(1), 154–161. https://doi.org/10.4049/jimmunol.179.1.154

Chen, X. R., Besson, V. C., Palmier, B., Garcia, Y., Plotkine, M., & Marchand-Leroux, C. (2007). Neurological Recovery-Promoting, Anti-Inflammatory, and Anti-Oxidative Effects Afforded by Fenofibrate, a PPAR Alpha Agonist, in Traumatic Brain Injury. *Journal of Neurotrauma*, *24*(7), 1119–1131. https://doi.org/10.1089/neu.2006.0216

Chen, X., Zhang, K.-L., Yang, S.-Y., Dong, J.-F., & Zhang, J.-N. (2009). Glucocorticoids Aggravate Retrograde Memory Deficiency Associated with Traumatic Brain Injury in Rats. *Journal of Neurotrauma*, *26*(2), 253–260. https://doi.org/10.1089/neu.2007.0504

Chen, Z., & Palmer, T. D. (2013). Differential roles of TNFR1 and TNFR2 signaling in adult hippocampal neurogenesis. *Brain, Behavior, and Immunity*, *30*, 45–53. https://doi.org/10.1016/j.bbi.2013.01.083

Cheong, C.-U., Chang, C.-P., Chao, C.-M., Cheng, B.-C., Yang, C.-Z., & Chio, C.-C. (2013). Etanercept Attenuates Traumatic Brain Injury in Rats by Reducing Brain TNF- *α* Contents and by Stimulating Newly Formed Neurogenesis. *Mediators of Inflammation*, *2013*, 1–9. https://doi.org/10.1155/2013/620837

Chio, C., Lin, J., Chang, M., Wang, C., Kuo, J., Yang, C., & Chang, C. (2010). Therapeutic evaluation of etanercept in a model of traumatic brain injury. *Journal of Neurochemistry*, *115*(4), 921–929. https://doi.org/10.1111/j.1471-4159.2010.06969.x

Chopra, M., Biehl, M., Steinfatt, T., Brandl, A., Kums, J., Amich, J., Vaeth, M., Kuen, J., Holtappels, R., Podlech, J., Mottok, A., Kraus, S., Jordán-Garrote, A.-L., Bäuerlein, C. A., Brede, C., Ribechini, E., Fick, A., Seher, A., Polz, J., … Beilhack, A. (2016). Exogenous TNFR2 activation protects from acute GvHD via host T reg cell expansion. *Journal of Experimental Medicine*, *213*(9), 1881–1900. https://doi.org/10.1084/jem.20151563

Colton, C. A., & Wilcock, D. M. (2010). Assessing Activation States in Microglia. *CNS & Neurological Disorders - Drug Targets*, *9*(2), 174–191. https://doi.org/10.2174/187152710791012053

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, *335*(1), 2–13. https://doi.org/10.1016/j.mce.2010.04.005

Cumiskey, D., Curran, B. P., Herron, C. E., & O'Connor, J. J. (2007). A role for inflammatory mediators in the IL-18 mediated attenuation of LTP in the rat dentate gyrus. *Neuropharmacology*, *52*(8), 1616–1623. https://doi.org/10.1016/j.neuropharm.2007.03.006

Dewan, M. C., Rattani, A., Gupta, S., Baticulon, R. E., Hung, Y.-C., Punchak, M., Agrawal, A., Adeleye, A. O., Shrime, M. G., Rubiano, A. M., Rosenfeld, J. v., & Park, K. B. (2019). Estimating the global incidence of traumatic brain injury. *Journal of Neurosurgery*, *130*(4), 1080–1097. https://doi.org/10.3171/2017.10.JNS17352

Dixon, C. E., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A., & Hayes, R. L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods*, *39*(3), 253–262. https://doi.org/10.1016/0165-0270(91)90104-8

Dixon, C. E., Lyeth, B. G., Povlishock, J. T., Findling, R. L., Hamm, R. J., Marmarou, A., Young, H. F., & Hayes, R. L. (1987). A fluid percussion model of experimental brain injury in the rat. *Journal of Neurosurgery*, *67*(1), 110–119. https://doi.org/10.3171/jns.1987.67.1.0110

Dong, Y., Fischer, R., Naudé, P. J. W., Maier, O., Nyakas, C., Duffey, M., van der Zee, E. A., Dekens, D., Douwenga, W., Herrmann, A., Guenzi, E., Kontermann, R. E., Pfizenmaier, K., & Eisel, U. L. M. (2016). Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proceedings of the National Academy of Sciences*, *113*(43), 12304–12309. https://doi.org/10.1073/pnas.1605195113

Elmore, M. R. P., Najafi, A. R., Koike, M. A., Dagher, N. N., Spangenberg, E. E., Rice, R. A., Kitazawa, M., Matusow, B., Nguyen, H., West, B. L., & Green, K. N. (2014). Colony-Stimulating Factor 1 Receptor Signaling Is Necessary for Microglia Viability, Unmasking a Microglia Progenitor Cell in the Adult Brain. *Neuron*, *82*(2), 380–397. https://doi.org/10.1016/j.neuron.2014.02.040

Fesharaki-Zadeh, A., & Datta, D. (2024). An overview of preclinical models of traumatic brain injury (TBI): relevance to pathophysiological mechanisms. *Frontiers in Cellular Neuroscience*, *18*. https://doi.org/10.3389/fncel.2024.1371213

Fischer, R., Proske, M., Duffey, M., Stangl, H., Martinez, G. F., Peters, N., Kraske, A., Straub, R. H., Bethea, J. R., Kontermann, R. E., & Pfizenmaier, K. (2018). Selective Activation of Tumor Necrosis Factor Receptor <scp>II</scp> Induces Antiinflammatory Responses and Alleviates Experimental Arthritis. *Arthritis & Rheumatology*, *70*(5), 722–735. https://doi.org/10.1002/art.40413

Fischer, R., Sendetski, M., del Rivero, T., Martinez, G. F., Bracchi-Ricard, V., Swanson, K. A., Pruzinsky, E. K., Delguercio, N., Rosalino, M. J., Padutsch, T., Kontermann, R. E., Pfizenmaier, K., & Bethea, J. R.

(2019). TNFR2 promotes Treg-mediated recovery from neuropathic pain across sexes. *Proceedings of the National Academy of Sciences*, *116*(34), 17045–17050. https://doi.org/10.1073/pnas.1902091116

Gao, H., Danzi, M. C., Choi, C. S., Taherian, M., Dalby-Hansen, C., Ellman, D. G., Madsen, P. M., Bixby, J. L., Lemmon, V. P., Lambertsen, K. L., & Brambilla, R. (2017). Opposing Functions of Microglial and Macrophagic TNFR2 in the Pathogenesis of Experimental Autoimmune Encephalomyelitis. *Cell Reports*, *18*(1), 198–212. https://doi.org/10.1016/j.celrep.2016.11.083

Gerald, M. J., Bracchi-Ricard, V., Ricard, J., Fischer, R., Nandakumar, B., Blumenthal, G. H., Williams, R., Kontermann, R. E., Pfizenmaier, K., Moxon, K. A., & Bethea, J. R. (2019). Continuous infusion of an agonist of the tumor necrosis factor receptor 2 in the spinal cord improves recovery after traumatic contusive injury. *CNS Neuroscience & Therapeutics*, *25*(8), 884–893. https://doi.org/10.1111/cns.13125

Girgis, H., Palmier, B., Croci, N., Soustrat, M., Plotkine, M., & Marchand-Leroux, C. (2013). Effects of selective and non-selective cyclooxygenase inhibition against neurological deficit and brain oedema following closed head injury in mice. *Brain Research*, *1491*, 78–87. https://doi.org/10.1016/j.brainres.2012.10.049

Goldenberg, M. M. (1999). Etanercept, a novel drug for the treatment of patients with severe, active rheumatoid arthritis. *Clinical Therapeutics*, *21*(1), 75–87. https://doi.org/10.1016/S0149-2918(00)88269-7

Gordon, S. (2003). Alternative activation of macrophages. *Nature Reviews Immunology*, *3*(1), 23–35. https://doi.org/10.1038/nri978

Guenzi, E., Stroissnig, H., Vierboom, M., & Herrmann, A. (2013). FRI0231 Atrosab, a humanized antibody directed against tnf-receptor 1, hold great promises for the treatment of rheumatoid arthritis. *Annals of the Rheumatic Diseases*, *72*(Suppl 3), A451.2-A451. https://doi.org/10.1136/annrheumdis-2013-eular.1358

Han, J., Harris, R. A., & Zhang, X.-M. (2017). An updated assessment of microglia depletion: current concepts and future directions. *Molecular Brain*, *10*(1), 25. https://doi.org/10.1186/s13041-017-0307-x

Hanlon, L. A., Raghupathi, R., & Huh, J. W. (2019). Depletion of microglia immediately following traumatic brain injury in the pediatric rat: Implications for cellular and behavioral pathology. *Experimental Neurology*, *316*, 39–51. https://doi.org/10.1016/j.expneurol.2019.04.004

Harrison, J. L., Rowe, R. K., O'Hara, B. F., Adelson, P. D., & Lifshitz, J. (2014). Acute over-the-counter pharmacological intervention does not adversely affect behavioral outcome following diffuse traumatic brain injury in the mouse. *Experimental Brain Research*, *232*(9), 2709–2719. https://doi.org/10.1007/s00221-014-3948-3

Henry, R. J., Ritzel, R. M., Barrett, J. P., Doran, S. J., Jiao, Y., Leach, J. B., Szeto, G. L., Wu, J., Stoica, B. A., Faden, A. I., & Loane, D. J. (2020). Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. *The Journal of Neuroscience*, *40*(14), 2960–2974. https://doi.org/10.1523/JNEUROSCI.2402-19.2020

Jamjoom, A. A. B., Rhodes, J., Andrews, P. J. D., & Grant, S. G. N. (2021). The synapse in traumatic brain injury. *Brain*, *144*(1), 18–31. https://doi.org/10.1093/brain/awaa321

Johnson, V. E., Stewart, W., & Smith, D. H. (2013). Axonal pathology in traumatic brain injury. *Experimental Neurology*, *246*, 35–43. https://doi.org/10.1016/j.expneurol.2012.01.013
Kalish, B. T., & Whalen, M. J. (2016). *Weight Drop Models in Traumatic Brain Injury* (pp. 193–209). https://doi.org/10.1007/978-1-4939-3816-2_12

Kalra, S., Malik, R., Singh, G., Bhatia, S., Al-Harrasi, A., Mohan, S., Albratty, M., Albarrati, A., & Tambuwala, M. M. (2022). Pathogenesis and management of traumatic brain injury (TBI): role of

neuroinflammation and anti-inflammatory drugs. *Inflammopharmacology*, *30*(4), 1153–1166. https://doi.org/10.1007/s10787-022-01017-8

Keshavarzi, Z., Khaksari, M., Razmi, Z., Soltani Hekmat, A., Naderi, V., & Rostami, S. (2012). The effects of cyclooxygenase inhibitors on the brain inflammatory response following traumatic brain injury in rats. *Iranian Journal of Basic Medical Sciences*, *15*(5), 1102–1105.

Komoltsev, I. G., & Gulyaeva, N. v. (2022). Brain Trauma, Glucocorticoids and Neuroinflammation: Dangerous Liaisons for the Hippocampus. *Biomedicines*, *10*(5), 1139. https://doi.org/10.3390/biomedicines10051139

Larson, K., Damon, M., Randhi, R., Nixon-Lee, N., & Dixon, K. J. (2023). Selective Inhibition of Soluble TNF using XPro1595 Improves Hippocampal Pathology to Promote Improved Neurological Recovery Following Traumatic Brain Injury in Mice. *CNS & Neurological Disorders - Drug Targets*, *22*(9), 1378–1390. https://doi.org/10.2174/1871527321666220610104908

Lawson, L. J., Perry, V. H., Dri, P., & Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience*, *39*(1), 151–170. https://doi.org/10.1016/0306-4522(90)90229-W

Lefevre-Dognin, C., Cogné, M., Perdrieau, V., Granger, A., Heslot, C., & Azouvi, P. (2021). Definition and epidemiology of mild traumatic brain injury. *Neurochirurgie*, *67*(3), 218–221. https://doi.org/10.1016/j.neuchi.2020.02.002

Lefkovits, A. M., Hicks, A. J., Downing, M., & Ponsford, J. (2021). Surviving the “silent epidemic”: A qualitative exploration of the long-term journey after traumatic brain injury. *Neuropsychological Rehabilitation*, *31*(10), 1582–1606. https://doi.org/10.1080/09602011.2020.1787849

Li, M., Li, Z., Ren, H., Jin, W.-N., Wood, K., Liu, Q., Sheth, K. N., & Shi, F.-D. (2017). Colony stimulating factor 1 receptor inhibition eliminates microglia and attenuates brain injury after intracerebral hemorrhage. *Journal of Cerebral Blood Flow & Metabolism*, *37*(7), 2383–2395. https://doi.org/10.1177/0271678X16666551

Liu, Y., & Ozguner, U. (2005). Quantitative evaluation of inter-vehicle communication effect in highway transportation from micro and macro views. *International Journal of Vehicle Information and Communication Systems*, *1*(1/2), 152. https://doi.org/10.1504/IJVICS.2005.007592

Loane, D. J., & Kumar, A. (2016). Microglia in the TBI brain: The good, the bad, and the dysregulated. *Experimental Neurology*, *275*, 316–327. https://doi.org/10.1016/j.expneurol.2015.08.018

Lund, M. C., Ellman, D. G., Nielsen, P. V., Raffaele, S., Fumagalli, M., Guzman, R., Degn, M., Brambilla, R., Meyer, M., Clausen, B. H., & Lambertsen, K. L. (2023). Selective Inhibition of Soluble Tumor Necrosis Factor Alters the Neuroinflammatory Response following Moderate Spinal Cord Injury in Mice. *Biology*, *12*(6), 845. https://doi.org/10.3390/biology12060845

Maas, A. I. R., Menon, D. K., Adelson, P. D., Andelic, N., Bell, M. J., Belli, A., Bragge, P., Brazinova, A., Büki, A., Chesnut, R. M., Citerio, G., Coburn, M., Cooper, D. J., Crowder, A. T., Czeiter, E., Czosnyka, M., Diaz-Arrastia, R., Dreier, J. P., Duhaime, A.-C., … Zumbo, F. (2017). Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *The Lancet Neurology*, *16*(12), 987–1048. https://doi.org/10.1016/S1474-4422(17)30371-X

MacPherson, K. P., Sompol, P., Kannarkat, G. T., Chang, J., Sniffen, L., Wildner, M. E., Norris, C. M., & Tansey, M. G. (2017). Peripheral administration of the soluble TNF inhibitor XPro1595 modifies brain immune cell profiles, decreases beta-amyloid plaque load, and rescues impaired long-term potentiation in 5xFAD mice. *Neurobiology of Disease*, *102*, 81–95. https://doi.org/10.1016/j.nbd.2017.02.010

Marmarou, A., Foda, M. A. A.-E., Brink, W. van den, Campbell, J., Kita, H., & Demetriadou, K. (1994). A new model of diffuse brain injury in rats. *Journal of Neurosurgery*, *80*(2), 291–300. https://doi.org/10.3171/jns.1994.80.2.0291

McCoy, M. K., Martinez, T. N., Ruhn, K. A., Szymkowski, D. E., Smith, C. G., Botterman, B. R., Tansey, K. E., & Tansey, M. G. (2006). Blocking Soluble Tumor Necrosis Factor Signaling with Dominant-Negative Tumor Necrosis Factor Inhibitor Attenuates Loss of Dopaminergic Neurons in Models of Parkinson's Disease. *The Journal of Neuroscience*, *26*(37), 9365–9375. https://doi.org/10.1523/JNEUROSCI.1504-06.2006

Medler, J., & Wajant, H. (2019). Tumor necrosis factor receptor-2 (TNFR2): an overview of an emerging drug target. *Expert Opinion on Therapeutic Targets*, *23*(4), 295–307. https://doi.org/10.1080/14728222.2019.1586886

Mittelbronn, M., Dietz, K., Schluesener, H. J., & Meyermann, R. (2001). Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathologica*, *101*(3), 249–255. https://doi.org/10.1007/s004010000284

Miyasaka, N. (2009). Adalimumab for the treatment of rheumatoid arthritis. *Expert Review of Clinical Immunology*, *5*(1), 19–26. https://doi.org/10.1586/1744666X.5.1.19

Moreland, L. W. (1999). Etanercept Therapy in Rheumatoid Arthritis. *Annals of Internal Medicine*, *130*(6), 478. https://doi.org/10.7326/0003-4819-130-6-199903160-00004

Nakae, A., Nakai, K., Yano, K., Hosokawa, K., Shibata, M., & Mashimo, T. (2011). The Animal Model of Spinal Cord Injury as an Experimental Pain Model. *Journal of Biomedicine and Biotechnology*, *2011*, 1–11. https://doi.org/10.1155/2011/939023

Novrup, H. G., Bracchi-Ricard, V., Ellman, D. G., Ricard, J., Jain, A., Runko, E., Lyck, L., Yli-Karjanmaa, M., Szymkowski, D. E., Pearse, D. D., Lambertsen, K. L., & Bethea, J. R. (2014). Central but not systemic administration of XPro1595 is therapeutic following moderate spinal cord injury in mice. *Journal of Neuroinflammation*, *11*(1), 159. https://doi.org/10.1186/s12974-014-0159-6

O'Connor, W. T., Smyth, A., & Gilchrist, M. D. (2011). Animal models of traumatic brain injury: A critical evaluation. *Pharmacology & Therapeutics*, *130*(2), 106–113. https://doi.org/10.1016/j.pharmthera.2011.01.001

Ortí-Casañ, N., Boerema, A. S., Köpke, K., Ebskamp, A., Keijser, J., Zhang, Y., Chen, T., Dolga, A. M., Broersen, K., Fischer, R., Pfizenmaier, K., Kontermann, R. E., & Eisel, U. L. M. (2023). The TNFR1 antagonist Atrosimab reduces neuronal loss, glial activation and memory deficits in an acute mouse model of neurodegeneration. *Scientific Reports*, *13*(1), 10622. https://doi.org/10.1038/s41598-023-36846-2

Ortí-Casañ, N., Wajant, H., Kuiperij, H. B., Hooijsma, A., Tromp, L., Poortman, I. L., Tadema, N., de Lange, J. H. E., Verbeek, M. M., de Deyn, P. P., Naudé, P. J. W., & Eisel, U. L. M. (2023). Activation of TNF Receptor 2 Improves Synaptic Plasticity and Enhances Amyloid-β Clearance in an Alzheimer's Disease Mouse Model with Humanized TNF Receptor 2. *Journal of Alzheimer's Disease*, *94*(3), 977–991. https://doi.org/10.3233/JAD-221230

Ortí-Casañ, N., Zuhorn, I. S., Naudé, P. J. W., de Deyn, P. P., van Schaik, P. E. M., Wajant, H., & Eisel, U. L. M. (2022). A TNF receptor 2 agonist ameliorates neuropathology and improves cognition in an Alzheimer's disease mouse model. *Proceedings of the National Academy of Sciences*, *119*(37). https://doi.org/10.1073/pnas.2201137119

Parajuli, B., & Koizumi, S. (2023). Strategies for Manipulating Microglia to Determine Their Role in the Healthy and Diseased Brain. *Neurochemical Research*, *48*(4), 1066–1076. https://doi.org/10.1007/s11064-022-03742-6

Pegoretti, V., Bauer, J., Fischer, R., Paro, I., Douwenga, W., Kontermann, R. E., Pfizenmaier, K., Houben, E., Broux, B., Hellings, N., Baron, W., Laman, J. D., & Eisel, U. L. M. (2023). Sequential treatment with a TNFR2 agonist and a TNFR1 antagonist improves outcomes in a humanized mouse model for MS. *Journal of Neuroinflammation*, *20*(1), 106. https://doi.org/10.1186/s12974-023-02785-y

Perry, V. H., & Holmes, C. (2014). Microglial priming in neurodegenerative disease. *Nature Reviews Neurology*, *10*(4), 217–224. https://doi.org/10.1038/nrneurol.2014.38
Reyes, E. Y., & Shinohara, M. L. (2022). Host immune responses in the central nervous system during fungal infections. *Immunological Reviews*, *311*(1), 50–74. https://doi.org/10.1111/imr.13101

Richter, F., Liebig, T., Guenzi, E., Herrmann, A., Scheurich, P., Pfizenmaier, K., & Kontermann, R. E. (2013). Antagonistic TNF Receptor One-Specific Antibody (ATROSAB): Receptor Binding and In Vitro Bioactivity. *PLoS ONE*, *8*(8), e72156. https://doi.org/10.1371/journal.pone.0072156

Richter, F., Seifert, O., Herrmann, A., Pfizenmaier, K., & Kontermann, R. E. (2019). Improved monovalent TNF receptor 1-selective inhibitor with novel heterodimerizing Fc. *MAbs*, *11*(4), 653–665. https://doi.org/10.1080/19420862.2019.1596512

Richter, F., Williams, S. K., John, K., Huber, C., Vaslin, C., Zanker, H., Fairless, R., Pichi, K., Marhenke, S., Vogel, A., Dhaen, M.-A., Herrmann, S., Herrmann, A., Pfizenmaier, K., Bantel, H., Diem, R., Kontermann, R. E., & Fischer, R. (2021). The TNFR1 Antagonist Atrosimab Is Therapeutic in Mouse Models of Acute and Chronic Inflammation. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.705485

Rutherford, W. H., Merrett, J. D., & McDonald, J. R. (1979). Symptoms at one year following concussion from minor head injuries. *Injury*, *10*(3), 225–230. https://doi.org/10.1016/0020-1383(79)90015-9

Schwab, J. M., Zhang, Y., Kopp, M. A., Brommer, B., & Popovich, P. G. (2014). The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury. *Experimental Neurology*, *258*, 121–129. https://doi.org/10.1016/j.expneurol.2014.04.023

Sedger, L. M., & McDermott, M. F. (2014). TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants – past, present and future. *Cytokine & Growth Factor Reviews*, *25*(4), 453–472. https://doi.org/10.1016/j.cytogfr.2014.07.016

Shohami, E., Novikov, M., Bass, R., Yamin, A., & Gallily, R. (1994). Closed Head Injury Triggers Early Production of TNFα and IL-6 by Brain Tissue. *Journal of Cerebral Blood Flow & Metabolism*, *14*(4), 615–619. https://doi.org/10.1038/jcbfm.1994.76

Sica, A., & Mantovani, A. (2012). Macrophage plasticity and polarization: in vivo veritas. *Journal of Clinical Investigation*, *122*(3), 787–795. https://doi.org/10.1172/JCI59643

Simon, D. W., McGeachy, M. J., Bayır, H., Clark, R. S. B., Loane, D. J., & Kochanek, P. M. (2017). The far-reaching scope of neuroinflammation after traumatic brain injury. *Nature Reviews Neurology*, *13*(3), 171–191. https://doi.org/10.1038/nrneurol.2017.13

Smolen, J. S., & Emery, P. (2011). Infliximab: 12 years of experience. *Arthritis Research & Therapy*, *13*(S1), S2. https://doi.org/10.1186/1478-6354-13-S1-S2

Steed, P. M., Tansey, M. G., Zalevsky, J., Zhukovsky, E. A., Desjarlais, J. R., Szymkowski, D. E., Abbott, C., Carmichael, D., Chan, C., Cherry, L., Cheung, P., Chirino, A. J., Chung, H. H., Doberstein, S. K., Eivazi, A., Filikov, A. v., Gao, S. X., Hubert, R. S., Hwang, M., … Dahiyat, B. I. (2003). Inactivation of TNF

Signaling by Rationally Designed Dominant-Negative TNF Variants. *Science*, *301*(5641), 1895–1898. https://doi.org/10.1126/science.1081297

Szalay, G., Martinecz, B., Lénárt, N., Környei, Z., Orsolits, B., Judák, L., Császár, E., Fekete, R., West, B. L., Katona, G., Rózsa, B., & Dénes, Á. (2016). Microglia protect against brain injury and their selective elimination dysregulates neuronal network activity after stroke. *Nature Communications*, *7*(1), 11499. https://doi.org/10.1038/ncomms11499

Takeuchi, O., & Akira, S. (2010). Pattern Recognition Receptors and Inflammation. *Cell*, *140*(6), 805–820. https://doi.org/10.1016/j.cell.2010.01.022

Teasdale, G., & Jennett, B. (1974). ASSESSMENT OF COMA AND IMPAIRED CONSCIOUSNESS. *The Lancet*, *304*(7872), 81–84. https://doi.org/10.1016/S0140-6736(74)91639-0

Thelin, E. P., Hall, C. E., Gupta, K., Carpenter, K. L. H., Chandran, S., Hutchinson, P. J., Patani, R., & Helmy, A. (2018). Elucidating Pro-Inflammatory Cytokine Responses after Traumatic Brain Injury in a Human Stem Cell Model. *Journal of Neurotrauma*, *35*(2), 341–352. https://doi.org/10.1089/neu.2017.5155

Thougaard, E., Nielsen, P. V., Forsberg, A., Phuong, V., Velasco, A. M., Wlodarczyk, A., Wajant, H., Lang, I., Mikkelsen, J. D., Clausen, B. H., Brambilla, R., & Lambertsen, K. L. (2023). Systemic treatment with a selective TNFR2 agonist alters the central and peripheral immune responses and transiently improves functional outcome after experimental ischemic stroke. *Journal of Neuroimmunology*, *385*, 578246. https://doi.org/10.1016/j.jneuroim.2023.578246

Tobinick, E., Kim, N. M., Reyzin, G., Rodriguez-Romanacce, H., & DePuy, V. (2012). Selective TNF Inhibition for Chronic Stroke and Traumatic Brain Injury. *CNS Drugs*, *26*(12), 1051–1070. https://doi.org/10.1007/s40263-012-0013-2

Vahidi, H., Shahyad, S., Noroozzadeh, A., & Mohammadi, M. T. (2021). Effect of fenofibrate on brain infarction, brain swelling and edema in focal-transient cerebral ischemia in rat. *Scientific Journal of Kurdistan University of Medical Sciences*, *26*(4), 1–16. https://doi.org/10.52547/sjku.26.4.1

van den Brande, J. M. H., Braat, H., van den Brink, G. R., Versteeg, H. H., Bauer, C. A., Hoedemaeker, I., van Montfrans, C., Hommes, D. W., Peppelenbosch, M. P., & van Deventer, S. J. H. (2003). Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology*, *124*(7), 1774–1785. https://doi.org/10.1016/S0016-5085(03)00382-2

van Gils, A., Stone, J., Welch, K., Davidson, L. R., Kerslake, D., Caesar, D., McWhirter, L., & Carson, A. (2020). Management of mild traumatic brain injury. *Practical Neurology*, *20*(3), 213–221. https://doi.org/10.1136/practneurol-2018-002087

van Vliet, E. A., Ndode-Ekane, X. E., Lehto, L. J., Gorter, J. A., Andrade, P., Aronica, E., Gröhn, O., & Pitkänen, A. (2020). Long-lasting blood-brain barrier dysfunction and neuroinflammation after traumatic brain injury. *Neurobiology of Disease*, *145*, 105080. https://doi.org/10.1016/j.nbd.2020.105080

Vargas, J. G., Kanzler, C., Scheller, L., Kreckel, J., Shaikh, H., Lang, I., Medler, J., Anany, M., Steinfatt, T., Hartweg, J., Haack, S., Holler, E., Büttner-Herold, M., Tabares Gaviria, P., Wajant, H., Beilhack, A., & Einsele, H. (2022). Harnessing the Expression of TNFR2 on Tregs to Prevent Agvhd. *Blood*, *140*(Supplement 1), 4465–4466. https://doi.org/10.1182/blood-2022-168592

Vargas, J. G., Wagner, J., Shaikh, H., Lang, I., Medler, J., Anany, M., Steinfatt, T., Mosca, J. P., Haack, S., Dahlhoff, J., Büttner-Herold, M., Graf, C., Viera, E. A., Einsele, H., Wajant, H., & Beilhack, A. (2022). A TNFR2-Specific TNF Fusion Protein With Improved In Vivo Activity. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.888274

Wang, C., Xian, L., Zheng, S., Li, J., Chen, X., & Wang, S. (2023). Cranial venous-outflow obstruction promotes neuroinflammation via ADAM17/solTNF-α/NF-κB pathway following experimental TBI. *Brain Research Bulletin*, *204*, 110804. https://doi.org/10.1016/j.brainresbull.2023.110804

Wang, Y., Yu, M., Ma, Y., Wang, R., Liu, W., Xia, W., Guan, A., Xing, C., Lu, F., & Ji, X. (2017). Fenofibrate Increases Heme Oxygenase 1 Expression and Astrocyte Proliferation While Limits Neuronal Injury During Intracerebral Hemorrhage. *Current Neurovascular Research*, *14*(1), 11–18. https://doi.org/10.2174/1567202613666161014161943

Weinblatt, M. E., Kremer, J. M., Bankhurst, A. D., Bulpitt, K. J., Fleischmann, R. M., Fox, R. I., Jackson, C. G., Lange, M., & Burge, D. J. (1999). A Trial of Etanercept, a Recombinant Tumor Necrosis Factor Receptor:Fc Fusion Protein, in Patients with Rheumatoid Arthritis Receiving Methotrexate. *New England Journal of Medicine*, *340*(4), 253–259. https://doi.org/10.1056/NEJM199901283400401

Xuan, A.-G., Chen, Y., Long, D.-H., Zhang, M., Ji, W.-D., Zhang, W.-J., Liu, J.-H., Hong, L.-P., He, X.-S., & Chen, W.-L. (2015). PPARα Agonist Fenofibrate Ameliorates Learning and Memory Deficits in Rats Following Global Cerebral Ischemia. *Molecular Neurobiology*, *52*(1), 601–609. https://doi.org/10.1007/s12035-014-8882-7

Yang, Q., & Zhou, J. (2019). Neuroinflammation in the central nervous system: Symphony of glial cells. *Glia*, *67*(6), 1017–1035. https://doi.org/10.1002/glia.23571

Zettlitz, K. A., Lorenz, V., Landauer, K., Münkel, S., Herrmann, A., Scheurich, P., Pfizenmaier, K., & Kontermann, R. (2010). ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody. *MAbs*, *2*(6), 639–647. https://doi.org/10.4161/mabs.2.6.13583

Zhang, L., Wang, Y., Liu, T., Mao, Y., & Peng, B. (2023). Novel Microglia-based Therapeutic Approaches to Neurodegenerative Disorders. *Neuroscience Bulletin*, *39*(3), 491–502. https://doi.org/10.1007/s12264-022-01013-6

Zhao, S., Mysler, E., & Moots, R. J. (2018). Etanercept for the Treatment of Rheumatoid Arthritis. *Immunotherapy*, *10*(6), 433–445. https://doi.org/10.2217/imt-2017-0155

Zhu, H., Zhao, Z., Zhou, Y., Chen, X., Li, Y., Liu, X., Lu, H., Zhang, Y., & Zhang, J. (2013). High-dose glucocorticoid aggravates TBI-associated corticosteroid insufficiency by inducing hypothalamic neuronal apoptosis. *Brain Research*, *1541*, 69–80. https://doi.org/10.1016/j.brainres.2013.10.002

Afterword

I have always been especially interested in neuroscience and immunology, which is why I chose the topic of neuroinflammation for my thesis. Traumatic brain injury is an interesting phenomenon to me, one which is closely relatable to some people in my personal life. One would expect an injury and then a healing process, as opposed to much-researched neurodegenerative diseases; however, it is crucial that people understand that the impact of TBI on people's lives can be chronic as well. Therefore, I wanted to contribute to this awareness with my thesis, to show that neuroinflammatory reactions can influence the lives of patients for decades and highlight the current state of certain experimental treatments. I am thankful for the opportunity to end my degree with this thesis, and so I'd like to thank my supervisor prof. Ulrich L.M. Eisel for guiding me.

I'd like to thank my friends at university with whom I have spent the past three years, Umar, Leon, Sydney, Gerben, Can, Youwei, and my special person Hidde. They helped in making this degree very welcoming and fun, and I must say I have no regrets studying Biology in Groningen. I am sure I have a bright future ahead of me thanks to the personal development and educational experiences I have encountered here!