

An insight into lymphotoxin and TNF- α role in neuroinflammatory neurodegenerative diseases



Essay

Raquel Flores Hernández

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Supervised by prof. dr. Ulrich L. M. Eisel and dr. Robbert
Havekes

Department of Molecular Neurobiology

Groningen Institute of Evolutionary Life Sciences



Abstract

Cytokines are signalling molecules that regulate inflammation and modulate cellular activities including differentiation, growth, and survival. They coordinate systemic immune response, being also actively involved in the regulation of the interaction between the central nervous system and the immune system. Both pro-inflammatory and anti-inflammatory cytokines play a role in neuroinflammation processes that can be caused by different pathological states. The recognized pro-inflammatory cytokine TNF- α has been identified in patients suffering from Alzheimer's Disease (AD) and Multiple Sclerosis (MS), two neurodegenerative diseases causing neuroinflammation. The aim of this essay is to study the role of TNF- β (or lymphotoxin), a TNF- α homologue, in these neuroinflammatory neurodegenerative diseases. Both TNF- α and TNF- β can bind and activate TNFR1 and TNFR2. Signalling pathways triggered by TNFR1 activation mainly cause neurotoxicity, while TNFR2 activation is neuroprotective in AD and MS models. This suggests that the studied cytokines play a redundant role. Additionally, TNF- β can activate LT β R, whose signalling pathway causes demyelination during MS, suggesting a complementary function of TNF- β by means of its specific receptor. Even though confirmation studies are needed in order to completely understand the signalling pathways and the functions that TNF- α and TNF- β play in neuroinflammatory neurodegenerative diseases, the research on drugs that selectively inhibit or activate the involved receptors is promising. Furthermore, the presence of the cytokines during these processes could serve as an early diagnosis tool.

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1. Introduction

1.1. The role of cytokines in neuroinflammation

Cytokines are proteins that function as signalling molecules at nanomolar or picomolar concentrations to regulate inflammation and modulate cellular activities including differentiation, growth, and survival (Ramesh et al., 2013). They are mainly released from immune cells such as macrophages, monocytes, and lymphocytes, in addition to microglia and astrocytes. Cytokines are activated during situations in which infection, inflammation, and/or immunological alterations occur, in which they mediate signals between immune cells (Kim et al., 2016). Cytokines are important for the coordination of immune responses throughout the body, and they are actively involved in the regulation of central nervous system (CNS)-immune system interactions. Under physiological conditions, these molecules are usually maintained at low levels, but when the environment in the CNS is altered by injuries, cytokines are activated and their levels increase 100-fold over physiological conditions (Lee et al., 2002).

Mid-20th century Peter Medawar's studies positioned the brain as an immune-privileged organ. This immune privilege is partially dependent on the blood-brain barrier (BBB), which acts as a physical obstacle for the entry of solutes and ions into the CNS (Amor et al., 2014). In addition, several physiological characteristics of the CNS (such as the absence of professional antigen presenting cells in the brain parenchyma or apparent absence of classical lymphatic drainage from the CNS) play a role in the limited capacity of triggering an immune response to CNS-derived antigens (Louveau et al., 2015). However, cytokines can cross the BBB through a BBB transporter and also by means of the leakage occurring in the circumventricular organs (Kim et al., 2016). Once in the brain, cytokines can facilitate or inhibit inflammatory responses, being respectively denominated as pro-inflammatory or anti-inflammatory cytokines and generally viewed as neurotoxic or neuroprotective (Allan and Rothwell, 2003).

1.2. Neuroinflammation and neurodegeneration

Despite the immune-privileged environment, both innate and adaptive inflammatory responses occur in the CNS. Microglia play a critical role as resident immunocompetent cells in the CNS (Lee et al., 2002), being activated in pathological states including ischemic stroke, infection and injury (Ramesh et al., 2013). This activation usually causes the release of pro-inflammatory cytokines including IL-1 β , IL-6 and tumor necrosis factor-alpha (TNF- α). The main aim of the pro-inflammatory mediators is to defend and restore neural integrity in the CNS, as microglia do when clearing debris after myelin damage or removing necrotic cells following ischaemia (Amor et al., 2014; Kim et al., 2016). Thus, neuro-inflammatory response is beneficial to the CNS, minimizing the injury by activating the innate immune system. By contrast, chronic inflammation generates the long-standing activation of microglia that continuously release inflammatory mediators causing a magnification of the neuronal injury (Allan and Rothwell, 2003; Chen et al., 2016; Kim et al., 2016).

Neurodegeneration is a major contributor of injury in the CNS, since by definition it "disturbs the properties of the CNS and therefore affects neuronal function, as well as the structure or survival of neurons" (Ransohoff, 2016). In this way, neuroinflammation and

neurodegeneration can play a dual role. On the one hand, as mentioned above, injury can trigger neuroinflammation in the CNS. One of the most characteristic events occurring in Alzheimer's disease (AD) patients' CNS is the presence of extracellular plaques formed by aggregates of amyloid beta protein (A β). In culture, microglia have been shown to migrate to aggregated A β spots and to internalize portions of the aggregated structure, thus initiating the process of neuroinflammation (Wyss-Coray and Rogers, 2012). The pro-inflammatory cytokine TNF- α has been reported to contribute to both processes of neuroprotection and neurodegeneration in AD pathology (Montgomery et al., 2013). On the other hand, neuroinflammation can trigger neurodegeneration. This is the case of Multiple Sclerosis (MS), in which reactivity against autoantigens is thought to be the driven force of the disease. Pathological changes are dominated by widespread microglial activation, leading to damage of myelin and axons (Compston and Coles, 2008). The presence of pro-inflammatory cytokine TNF- α and TNF- β has been identified in MS lesions (Selmaj et al., 1991a).

The pathology of AD and MS is different. However, the symptoms of both neurodegenerative diseases are accompanied by certain cognitive impairment. Patients suffering from AD experience language difficulties, memory loss and executive dysfunction (Burns and Iliffe, 2009). The cognitive signs present on MS patients are deficits in reasoning, attention and executive function (Compston and Coles, 2008; Rahn et al, 2012). Cognitive impairment has negative impact on patient's daily life, often making them dependent on relatives and caregivers. If possible, this circumstance increases the importance of the research into the neuroinflammatory neurodegenerative diseases, whose comprehension could improve the quality of life of patients, relatives and caregivers.

1.3. Research question

A better understanding of the mechanisms underlying neuroinflammation in neurodegenerative diseases could lead to the finding of an effective therapeutic approach aiming to ameliorate the symptoms and the final outcome of these disorders.

Since its discovery, TNF- α cytokine has been popularly studied, but little research has focused on its homologous TNF- β . With this essay, I aim to provide insight into the possible relation between both tumor necrosis factors. Based on the known role of TNF- α in AD, could also TNF- β play a dual part in neurodegeneration and neuroprotection? Regarding MS, do they have a redundant, a complementary, or an agonistic function? The comprehension of the functions of these cytokines could broaden the scope of the therapies addressing these diseases.

2. TNF family

The TNF superfamily is formed by a complex network of receptors (TNFRs) and ligands (TNFLs). To date, 29 receptors and 18 ligands have been identified (Albarbar et al., 2015). However, the immediate TNF family includes the four receptors TNFR1, TNFR2, LT β R and HVEM (herpesvirus entry mediator) and the five ligands TNF- α , lymphotxin-alpha (LT- α), LT α 1 β 2, LT α 2 β 1 and LIGHT (LT-related inducible ligand that competes with glycoprotein D for binding to HVEM on T cells; Figure 1; Schneider et al., 2004). LIGHT can bind to both LT β R and HVEM receptors, but its functions and signalling pathways are out of the scope of this essay, which is focused on LT- α and its relation to TNF- α .

2.1. TNF- α

One century ago, P. Brunes observed that some cancer patients experienced tumour regression after suffering an acute bacterial infection (Tseng et al., 2016). This finding led William B. Coley to start his experiments injecting the so called Coley's toxins to induce infections in patients with far advanced cancer (Old, 1985; Tseng et al., 2016). TNF name was used several decades later, when Carswell and colleagues confirmed *in vivo* the ability of the previously named tumor necrosis serum (TNS) to induce necrosis to malignant cells (Albarbar et al., 2015). Nowadays, TNF is referred to as TNF- α .

TNF- α can be maintained as a membrane-bound ligand or it can be secreted by several immune and non-immune cell types, including T cells, mast cells, granulocytes, neutrophil, monocytes, macrophages, natural killer cells, as well as keratinocytes, fibroblasts, smooth muscle cells, endothelial cells and neurons (Albarbar et al., 2015). It can activate different signal transduction pathways, but TNF- α is the main ligand for the TNFR1 and TNFR2 receptors, through which it can regulate the balance between cell survival and apoptosis (Figure 1). In addition, TNF- α plays a role in differentiation, growth regulation, inflammation, tumorigenesis, viral replication, autoimmune diseases and the response to bacterial, fungal, viral, and parasitic infections (Aggarwal, 2000).

2.1.1. TNF- α signalling pathways: TNFR1 and TNFR2

TNFRs are transmembrane proteins characterized by the presence of a four cysteine-rich domain in their extracellular region, which is responsible for the affinity and specificity for their ligands (Albarbar et al., 2015). Trimeric TNFLs are needed for the activation of TNFRs, whose trimerisation is induced in order to trigger the signal transduction via recruitment of adaptor proteins (Cabal-Hierro and Lazo, 2012; Sedger and McDermott, 2014; Albarbar et al., 2015). TNFRs can be divided into two main groups based on the specific structural features present on their intracellular region. The first one, in which TNFR1 is included, is characterised by the presence of a death domain (DD) involved in the induction of cell death. TNFR2 belongs to the second group, characterised by the lack of a DD. Instead, these receptors present the TIM domain (TRAF-interacting motif), involved in the recruitment of TNFR-associated factors (TRAF) participating in each corresponding signalling pathway (Cabal-Hierro and Lazo, 2012; Sedger and McDermott, 2014; Albarbar et al., 2015).

TNF- α can bind its ligands either on the membrane-bound form or released as a soluble form after the cleavage produced by the metalloprotease TACE (TNF-alpha-converting enzyme; Sedger and McDermott, 2014; Albarbar et al., 2015). While membrane TNF can bind and activate both TNFR1 and TNFR2, soluble TNF, which convers TNF with its endocrine function, can only efficiently activate TNFR1 (Cabal-Hierro and Lazo, 2012; Puimège et al., 2014; Tseng et al., 2016).

Activation of TNFR1 (expressed ubiquitously on almost all cell types) induces distinct signalling pathways, which can lead to the induction of apoptosis, necroptosis or proliferation processes depending on the microenvironment conditions and the cellular context (Cabal-Hierro and Lazo, 2012). These activities depend on the formation of two different TNF receptor-signalling complexes separated temporally and spatially (Figure 1). TNF- α binding induces the activation of TNFR1 and the recruitment of the TNFR-associated DD (TRADD) adaptor protein through the DD. TRADD acts as a platform adapter, since it allows the binding of cellular inhibitor of apoptosis 1 and 2 (cIAP-1,-2), receptor interacting protein 1 (RIP1) and TNFR associated factor 2 or 5 (TRAF-2, -5), forming the so called membrane-bound complex I. Now, cIAP is able to ubiquinate RIP1 and TRAF-2, -5, making possible the activation of the inhibitor of κ B ($I-\kappa$ B) kinase complex (IKK). IKK ensures the ubiquitination and degradation by the proteasome of $I-\kappa$ B, thus activating the canonical NF- κ B transcription factor pathway. The phosphorylation cascade mediated by the mitogen activated protein (MAP) kinase is also activated, allowing the activation of c-Jun N-terminal kinsases (JNK) and the transcription factor AP-1. In this way, complex I promotes different pathways, which lead to the activation of NF- κ B and AP-1 and induction of anti-apoptotic and pro-inflammatory genes. Upon endocytosis of complex I, TNFR1 dissociates from TRADD, which associates with Fas-associated protein with death domain (FADD) in order to form the intracellular complex II. Now, deubiquitination of RIP1 causes the recruitment and autocatalytic cleavage of pro-caspase 8, being initiated the process of apoptosis (Naudé et al., 2011; Cabal-Hierro and Lazo, 2012; Puimège et al., 2014; Sedger and McDermott, 2014; Albarbar et al., 2015; Tseng et al., 2016).

TNFR2 expression is restricted to particular cell types including specific neuronal subtypes, microglia, oligodendrocytes and astrocytes in the brain and endothelial cells, cardiac myocytes, human mesenchymal stem cells, thymocytes and certain T-cell subpopulations, including lymphocytes (Naudé et al., 2011). Taking into account that TNFR2 lacks the DD, the binding of TRADD does not take place, but instead, upon TNF- α binding to TNFR2, TRAF2 acts as a key mediator, activating TNFR2 and leading to the transcriptional activation of genes related to cell survival and proliferation (Figure 1). TNFR2 can activate the canonical NF- κ B pathway, but it mainly stimulates the noncanonical NF- κ B one. Once TRAF2 is associated to TNFR2, the recruitment of cIAP-1, -2, TRAF1 and TRAF3 takes place. This complex allows the accumulation of NF- κ B-inducing kinase (NIK) on the cytosol, which is able to stimulate IKK α . This results in the proteolytic processing of p100, which causes the translocation of the transcription factor to the nucleus, thus activating the noncanonical NF- κ B pathway. MAPK signalling cascade is also initiated, leading to the activation of JNK (Naudé et al., 2011; Cabal-Hierro and Lazo, 2012; Sedger and McDermott, 2014; Tseng et al., 2016).

2.2. TNF- β or LT- α

Lymphotoxin (LT) was the name given to the factor discovered by Granger et al. secreted by lymphocytes after mitogenic or antigenic stimulation (Calmon-Hamaty et al., 2011; Ruddle, 2014). With the passage of time, LT name was changed to TNF- β because of its structural and functional similarity to TNF- α , but the realization that its biologic activity is different resulted in the rename of TNF- β back to LT (Ruddle, 2014; Albarbar et al., 2015). In fact, LT- α is the closest homologue to TNF- α , since they are 30% homologous in their primary amino acid sequence (Calmon-Hamaty et al., 2011). Nowadays, LT- α is the term used to describe the soluble homotrimeric LT α 3 molecule, whereas LT- α can bind to non-cleavable membrane LT- β ligand and form the two transmembrane heterotrimeric complexes LT α 1 β 2 and LT α 2 β 1 (Ware, 2005; Ruddle, 2014; Albarbar et al., 2015).

LT- α is secreted by macrophages, natural killer cells, resting B cells, activated lymphocytes, non-hematopoietic and myeloid lineage cells (Calmon-Hamaty et al., 2011; Albarbar et al., 2015). It can activate different signal transduction pathways, having specific roles in the development and function of the immune system, organization and maintenance of lymphoid microenvironments, inflammation, host defence, and regulation of intestinal microbiota (Calmon-Hamaty et al., 2011; Upadhyay and Fu, 2013). LT- α is crucial for secondary lymphoid organ development, since LT- α knockout mice lack Peyer's patches (PP), lymph nodes (LNs) and present defective nasal associated lymphoid tissue and highly disorganized spleens (Ying et al., 2005; Mounzer et al., 2010; Ruddle, 2014). LT- α also induces the formation of tertiary lymphoid organs during chronic immune stimulation in atherosclerosis, microbial infection, autoimmunity and graft rejection, being an important effector molecule present in graft-versus-host disease (Markey et al., 2010). Regarding inflammation, LT- α seems to play a disease-promoting role in rheumatoid arthritis (Calmon-Hamaty et al., 2011) and insulin-dependent diabetes mellitus (Schneider et al., 2004).

2.2.1. LT- α signalling pathways: TNFR1, TNFR2 and LT β R

LT- α binds both TNFR1 and TNFR2, activating the signalling pathways described above (Figure 1; Schneider et al., 2004; Ruddle 2014; Albarbar et al., 2015). No significant differences in signalling through TNFR1 between TNF- α and LT- α (Etemadi et al., 2013) or in their affinity for the two TNFRs (Schuchmann et al., 1995; Medvedev et al., 1996) have been reported, which explains the similar activities that both of the cytokines present regarding the induction of cell death or activation of cellular proliferation. However, they differ in their potency exerting biological effects in many cell types. For example, TNF- α is more potent than LT- α in inducing cytotoxicity toward MCF7 carcinoma cells; both TNF- α and LT- α are equally cytotoxic toward L929 fibroblastic cell line; LT- α is more potent than TNF- α in inducing cytotoxicity and apoptosis in oligodendrocyte cultures (Medvedev et al., 1996). Additionally, LT- α binds to the herpes virus entry mediator (HVEM) receptor (Ruddle, 2014; Albarbar et al., 2015), whose study is out of the scope of this essay.

LT α 1 β 2 and LT α 2 β 1 heterotrimers cannot activate TNFR1 and TNFR2, but they act through LT β R stimulation (Figure 1). LT β R belongs to the group of TNFRs lacking a DD, thus utilizing TRAFs such as TRAF -2, -3, and -5 for the transduction of the signal (Ware, 2005). While the ligation of TRAF3 to the cytoplasmic tail of the receptor is a negative regulator for

NF-κB activation, being associated with induction of cell death (VanArsdale et al., 1997), TRAF2 and TRAF5 ligation leads to NF-κB activation (Albarbar et al., 2015). LT β R can activate both the canonical and noncanonical NF-κB pathways. Regarding the noncanonical stimulation, LT β R signalling can activate the NF-κB2/p100 pathway. This activation causes the translocation of the transcription factor to the nucleus, leading to gene transcription involved in the maintenance of architecture in secondary lymphoid organs and the development of lymphoid organs (Schneider et al., 2004; Ware, 2005). In contrast, LT β R activation can also cause a mixture of cell death features depending on the cell type due to the induction of caspase-dependent and caspase-independent mediated death. For example, WEHI164 suffer apoptosis while HT29 cells suffer both apoptosis and necrosis (Albarbar et al., 2015).

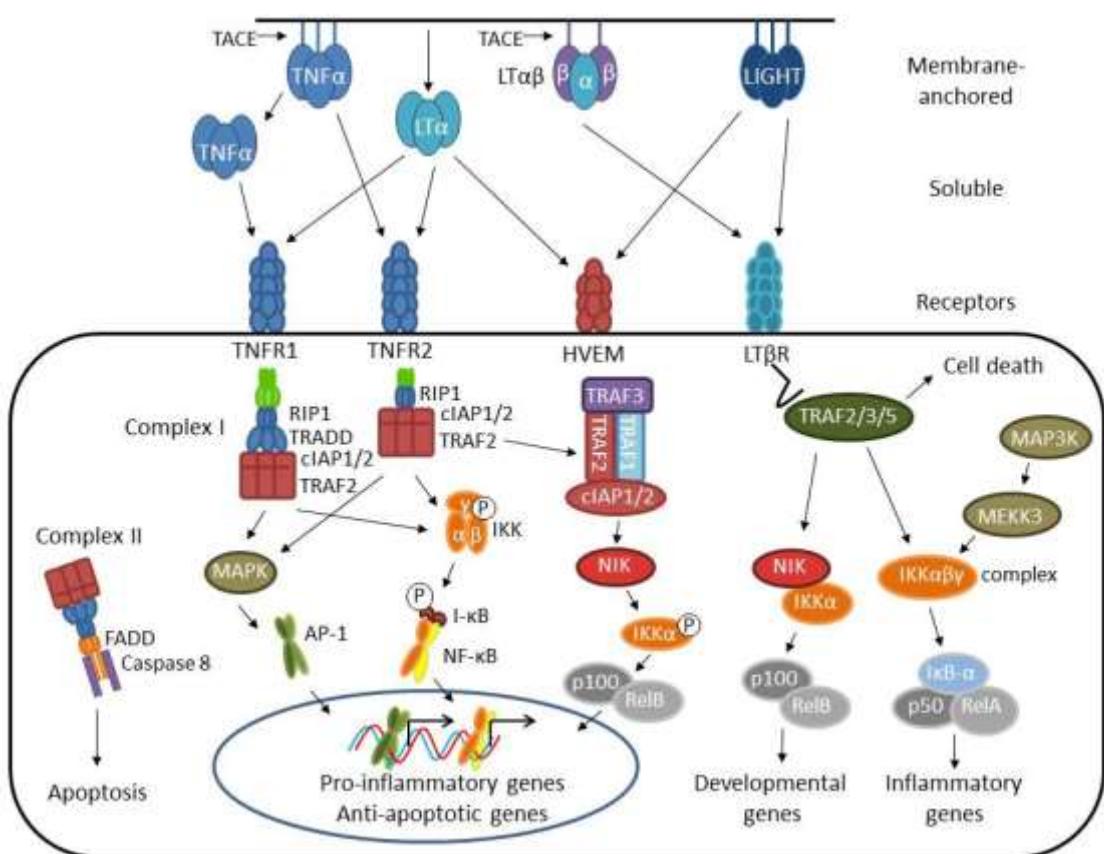


Figure 1. Schematic depiction of the members of the ligands and receptors of the immediate TNF family. Detailed TNFR1, TNFR2 and LT β R signalling pathways as described on the text. Adapted from Ware, 2005, Naudé et al., 2011, Puimège et al., 2014, and Ruddle, 2014.

3. The role of TNF- α and LT in neurodegeneration

3.1. Alzheimer's Disease

In AD patient's brains, microglia co-localize with A β plaques (Wyss-Coray and Rogers, 2012), initiating the process of neuroinflammation with the release of proinflammatory mediators, including cytokines. Elevated levels of TNF- α in AD patient's serum in comparison to controls have been reported, being the excess of production of TNF- α simultaneous to the increase of A β plaque deposition (Fillit et al., 1991; Paganelli et al., 2002). TNF- α has been reported to increase the production of A β by enhancing β -secretase (BACE-1) expression via NF- κ B dependent pathways (He et al., 2007). Additionally, in monocyte-derived macrophages it has been shown that TNF- α could directly decrease fibrillary amyloid A β degradation (Yamamoto et al., 2008). Furthermore, three TNF- α polymorphisms have been related to late-onset AD, being two of them linked to elevated TNF- α production (Montgomery et al., 2013). In this way, this cytokine has been connected to the intensified chronic inflammatory status in AD patients. TNF- α inhibition and modulation therapy has become a strategy for AD treatment, showing improved pathologic and functional outcomes in the short-term (Tobinick et al., 2006; Shi et al., 2011; Chang et al., 2017). However, the long-term consequences of global TNF- α inhibition have not been deeply examined, and they should be taken into account since neuroprotective effects of TNF- α have also been reported. Both TNF- α and LT- α have proved protective function in cultured neurons against glucose deprivation-induced damage (Cheng et al., 1994) and attenuation of A β -induced neuronal degeneration (Barger et al., 1995). In fact, long-term TNF- α inhibition has shown to accelerate AD progression due to the divergent roles that TNFR1 and TNFR2 play upon TNF- α activation (Montgomery et al., 2013). The same way, global inhibition of TNFR signalling reported an exacerbated pathogenesis in mice (Montgomery et al., 2011). First studies in retinal ischemia mouse model showed that TNFR1 signalling increased neuronal death while TNFR2 signalling promoted neuroprotection (Fontaine et al., 2002). This finding was supported by hippocampal neurons (Yang et al., 2002) and primary cortical neurons (Marchetti et al., 2004) studies, in which persistent NF- κ B activation by TNFR2 was reported to be essential for the promotion of neuronal survival. AD mouse studies have shown that TNFR1 activation promotes formation of AD hallmarks due to the increased apoptosis, whereas TNFR2 activation inhibits AD hallmarks exerting a neuroprotective effect (Li et al., 2004; He et al., 2007; Montgomery et al., 2013; Jiang et al., 2014). Together, these findings could explain the failure of long-term success of the TNF- α inhibition therapy. In view of these results, taking into consideration that soluble TNF- α signals mostly via TNFR1, an alternative therapeutic approach aiming to specifically inhibit soluble TNF- α has reported to prevent the effects of neuroinflammation on early pathology in AD mice model (McAlpine et al., 2009).

3.1.1. TNFR cross-talk

In addition to the already elaborated signalling pathways activated upon TNFR1 and TNFR2 stimulation, signal cross-talk between both of the receptors exists in terms of competition for

the common signal transducer TRAF2. Regarding TNFR1 signalling pathway, TRAF2 plays an important role in the formation of the complex I, which allows the induction of pro-inflammatory and anti-apoptotic genes. However, TNFR2 activation promotes TRAF2 degradation, thus preventing the activation of the anti-apoptotic and anti-inflammatory pathways by complex I and enhancing the cytotoxicity triggered by complex II (Fotin-Mleczek et al., 2002; Naudé et al., 2011; Cabal-Hierro and Lazo, 2012). The balance between apoptosis and cell survival is determined by the different activation of the signalling pathways, dependent on the cellular context and the microenvironment conditions (Cabal-Hierro and Lazo, 2012).

In conclusion, in view of the different cellular responses triggered by TNFR1 and TNFR2 activation, and taking into account their cross-talk, the study of TNFR-selective therapies is arising. The use of a TNFR2 agonist and a TNFR1 antagonist have shown to promote neuronal survival and block neuroinflammation in a mouse model of neurodegeneration related to AD (Dong et al., 2016). This finding opens opportunities to new treatment possibilities for neuroinflammatory neurodegenerative diseases, avoiding the use of anti-TNF therapies with undesirable long-term effects.

3.2. Multiple Sclerosis

Pathological changes produced during MS disease in the CNS are focal. They involve the attachment of microglia on myelin and axons, which results in their damage and the formation of the so called MS plaques (Compston and Coles, 2008; Rome Christensen et al., 2012). The presence of elevated levels of TNF- α and LT- α protein (Selmaj et al., 1991a) and mRNA (Woodroffe and Cuzner, 1993; Matusevicius et al., 1996; Navikas et al., 1996) in MS plaques, cerebrospinal fluid, and mononuclear cells of MS patients has been reported, suggesting their involvement in lesion formation. Additionally, *in vitro* studies have confirmed a cytotoxic role of TNF- α and LT- α on oligodendrocytes, being LT- α more effective than TNF- α in triggering apoptosis of this cell type (Selmaj and Raine, 1988; Selmaj et al., 1991b; Medvedev et al., 1996). TNF- α and LT- α have also shown detrimental effects on studies employing the experimental autoimmune encephalitis (EAE) model of demyelination in mice (Ruddle et al., 1990; Klinkert et al., 1997). Additionally, mice overexpressing TNF- α in CNS have reported to spontaneously develop a chronic inflammatory demyelinating disease (Probert et al., 1995).

These reasons, together with the known involvement of TNF- α in other inflammatory processes, led to the development of different clinical trials aiming to inhibit TNF- α signalling in MS patients. However, some of the patients showed exacerbated disease symptoms (van Oosten et al., 1996; Lenercept, 1999). Later findings demonstrated the implication of the TNFR1 signalling pathway activation on the induction of oligodendrocyte apoptosis and the resulting development of demyelination (Akassoglou et al., 1998; Probert et al., 2000; Jurewicz et al., 2005). In further EAE studies in which TNFR1 signalling was selectively inhibited, amelioration of the symptoms in mice was reported (Nomura et al., 2011; Williams et al., 2014). In this context, research in a cuprizone model for demyelination verified the exacerbation of demyelination caused by TNF- α , at the same time that this cytokine was reported to play a role in oligodendrocyte proliferation by means of TNFR2 signalling pathway activation (Arnett et al., 2001). Additionally, TNFR2 knockout mice reported an extensive

demyelination and aggravated EAE disease symptoms (Eugster et al., 1999; Suvannavejh et al., 2000). Taken together, these findings, which are in line with similar results reported from studies performed focused on AD pathology, may explain the worsening of symptoms observed during the clinical trials. They could also explain the contradictory findings described using EAE model in which complete knockout of TNF- α increased pathology of the disease, suggesting an anti-inflammatory role of TNF- α in MS (Liu et al., 1998; Kassiotis and Kollias, 2001). In view of these results, new therapies aiming to selectively inhibit soluble TNF- α , which mainly signals through TNFR1, were developed. Different studies have been performed using XPro1595, a selective soluble TNF- α inhibitor. Treatment with XPro1595 improved axon remyelination and preservation in both EAE (Brambilla et al., 2011; Taoufik et al., 2011; Evangelidou et al., 2014) and cuprizone mouse models (Karamita et al., 2017).

3.2.1. Susceptibility to MS and TNF genes

The major histocompatibility complex (MHC) or human leukocyte antigen (HLA) gene cluster has been identified as the strongest susceptibility locus for MS genome wide (McElroy and Oksenberg, 2008; Oksenberg et al., 2008). TNF- α and LT- α genes are localized in tandem within the MHC III region in the human chromosomal segment 6p21, suggesting that polymorphisms in these genes may be associated with MS susceptibility or progression. *Ncol* polymorphism consists on a single nucleotide polymorphism (SNP) in the first intron of the LT- α gene. The study of this polymorphism has drawn attention because variations in the region responsible for transcriptional regulation may be connected with variability on synthesis and expression of TNF- α and LT- α (Messer et al., 1991; Kallaur et al., 2014b). *Ncol* polymorphism has been evaluated in several studies involving different populations with conflicting results. However, most recent investigations have associated this polymorphism with MS in Caucasian patients from Southern Brazil, considered the most heterogeneous population in the world (Kallaur et al., 2014a) and with metabolic and inflammatory markers in MS patients (Kallaur et al., 2014b). Confirmation studies are needed in order to understand the implication of this polymorphism, which could be used as a genetic marker for MS patients.

3.2.2. LT β R role in MS

The location of the *Ncol* polymorphism in LT- α gene and the increased ability of this cytokine to induce apoptosis in oligodendrocytes in comparison with TNF- α , motivated a more detailed LT study concerning MS. First research was performed in LT- α and LT- β knockout mice which were immunized with rat myelin oligodendrocyte glycoprotein (MOG) generating the so called MOG induced EAE model. While *LT α* ^{-/-} mice showed reduced demyelination and minimal CNS inflammation, *LT β* ^{-/-} mice developed MOG induced EAE with reduced extent compared to wild-type animals (Suen et al., 1997). These results suggest that LT- α may play a pivotal role in the development of EAE but not the heterotrimers LT α 1 β 2 and LT α 2 β 1. Additionally, LT- α has been reported to be detrimental to the inflammatory demyelinating process in mouse cuprizone-induced EAE model, but unlike TNF- α , not necessary for reparative remyelination and oligodendrocyte progenitor proliferation (Plant et al., 2005). For these reasons, inhibition of LT- α signalling seems to present a promising strategy for MS treatment. Nevertheless, the interpretation of these experiments is challenging due to the phenotype of *LT α* ^{-/-} and *LT β* ^{-/-} mice. LT- α -deficient mice have profound defects in lymphoid organ development, since they

lack all PP, LNs, germinal centres and show splenic disorganization. LT- β -deficient mice also lack PP and germinal centres, and exhibit splenic disorganization. Although $LT\beta^{-/-}$ lacks most peripheral LNs, the mesenteric and cervical ones are present (De Togni et al., 1994; Matsumoto et al., 1996; Alimzhanov et al., 1997; Koni et al., 1997; Kuprash et al., 1997). Moreover, $TNFR1^{-/-}$ studies have reported loss of germinal centres and PP in mice (Matsumoto et al., 1996; Neumann et al., 1996; Kuprash et al., 1997), suggesting the involvement of TNFR1 signalling pathway in development of the immune system. Nonetheless, more extensive lymphoid organ development failure is exhibited in $LT\alpha^{-/-}$ mice despite normal TNF- α expression, suggesting that the function of LT- α in development cannot be compensated by TNF- α . This conclusion stresses the need of the formation of functional LT α 1 β 2 and LT α 2 β 1 heterotrimers, and thus the importance of LT β R signalling pathway, for lymphoid development.

The controversial interpretation of the results from knockout research regarding MS arises from the use of control animals with normal immune systems. Hence, the differences observed in the experimental outcomes from LT knockout mice in relation to wild-type cannot be attributed to the activities of the cytokine alone. In this regard, a study in which immunodeficiency was corrected by reconstitution with bone marrow cells in $TNF-\alpha^{-/-}$ and $LT-\alpha^{-/-}$ mice was carried out. Results showed that EAE developed normally in $LT\alpha^{-/-}$ but not in $TNF-\alpha^{-/-}$ mice, suggesting that TNF- α plays a pivotal role and not LT- α . Thus, the abundant expression of LT- α in MS patients could just reflect the cellular processes of chronic inflammatory plaques (Sean Riminton et al., 1998). An alternative strategy for studying the involvement of LT signalling pathways in MS while avoiding the complicating developmental defects observed in the knockouts is the use of a specific LT β R fusion protein (LT β R-Ig). LT β R-Ig blocks LT β R, interfering with LT α/β and LIGHT binding. First investigations revealed that specific LT α/β heterotrimer activation of LT β R was sufficient to induce disease in both rat and mice EAE models in the absence of LIGHT binding (Gommerman et al., 2003; Columba-Cabezas et al., 2006). Detrimental effects of $LT\beta R^{-/-}$ in cuprizone-induced demyelination were subsequently reported. $LT\beta R^{-/-}$ mice showed delayed demyelination due to a postponed loss of oligodendrocytes similar to the delay seen in $LT\alpha^{-/-}$ mice (Plant et al., 2005; Plant et al., 2007). Additional results proved that the use of LT β R-Ig in wild-type mice reduced demyelination at the same time that enhanced remyelination (Plant et al., 2007), suggesting that LT α/β may play a pivotal role in the pathological processes of MS. In this way, LT β R-Ig seems to be a new promising strategy to treat not just MS but also other demyelinating disorders and autoimmune diseases such as rheumatoid arthritis or inflammatory bowel disease (Browning, 2008).

In summary, both TNF- α and LT seem to contribute to the pathogenesis of MS. Their redundant role on the activation of TNFRs suggests that therapies aiming to inhibit TNFR1 or activate TNFR2 could help to treat neuroinflammation on MS patients. Additionally, inhibitors of soluble TNF- α and LT β R seem to ameliorate the symptoms of the studied disease models, demonstrating a complementary function of both cytokines. In this way, it is likely that the interference of different TNF superfamily signalling pathways can help to treat MS patients.

4. Conclusion

The involvement of cytokines in the regulation of the immune response within the CNS is irrefutable. These molecules have been extensively reported to have pro-inflammatory and anti-inflammatory CNS effects, playing a dual role in neurodegeneration and neuroprotection. For this reason, it is important to deeply study cytokines' signalling pathways and to completely understand their functions in different neuroinflammatory neurodegenerative diseases before an effective and side-effect-free treatment can be developed.

TNF- α has been widely studied because of its firstly discovered systemic pro-inflammatory effects, while its elevated presence in neuroinflammatory processes was later reported. Fewer studies have aimed to particularly understand the role of LT- α , in spite of the fact that it shares homology and some functions with TNF- α . Both cytokines have shown to bind to TNFR1 and TNFR2, having redundant roles regarding the induction of cell death and activation of cellular proliferation. Moreover, LT- α and TNF- α cooperate during the development of lymphoid tissue. Studies in which different members of TNF superfamily have been knocked out have shown the great involvement of LT β R signalling pathway in lymphoid organ development, and organization and maintenance of lymphoid microenvironments. Additionally, LT- α signalling through TNFR1 supplements this function, presenting cross-talk between TNFR1 and LT β R signalling triggered by LT during the development of the immune system. In this way, both cytokines seem to play a complementary role.

Concerning AD, no specific research has been performed for LT. Anti-TNF therapies have shown favourable short-term outcomes but undesirable long-term effects. This is due to the dual part that TNF- α plays in neurodegeneration and neuroprotection during AD pathogenesis by means of the activation of either TNFR1 or TNFR2 signal transduction pathway, respectively. Since LT- α and TNF- α share these signalling pathways, it is possible that both of the cytokines play the same dual role during the development of AD, having a redundant function. Taking into account that TNFR1 activation is mainly triggered by soluble TNF- α , its inactivation could also be considered as a therapeutic approach to treat AD. Similar principle could be applied to LT- α inhibition, but no significant differences in the affinity of LT- α for the two TNFRs has been reported so far. Thus, the best approach which can be used in order to find an appropriate therapy for neuroinflammation in AD patients is the selective activation of TNFR2 or blockage of TNFR1.

Regarding MS, LT and TNF- α seem to have both, a redundant and a complementary function. Similar results to the ones found in AD research have been reported when TNFR1 and TNFR2 are selectively activated or inhibited in EAE models, suggesting that LT- α and TNF- α may be redundant in their neurotoxic activation of TNFR1 and neuroprotective activation of TNFR2. In this way, similar therapies aiming to selectively inhibit soluble TNF- α , TNFR1 or activate TNFR2 could help to treat neuroinflammation in MS patients. In addition, LT appears to play a complementary function to TNF- α inducing MS pathogenesis by means of the activation of LT β R signalling pathway. Thus, therapies aiming to selectively inhibit LT β R could be used together with the previously mentioned ones, providing a double approach to treat neuroinflammation in MS patients. However, the lack of a single experimental model that

reproduces all aspects of MS is the main limitation of the study of this disease. The failure in human clinical trials of treatments previously tested positively in experimental models is often reported, as it has been addressed during this essay for anti-TNF- α drugs. Still, such studies in disease models are indispensable to recognize the potential harmful side-effects of new drugs, which emphasises the need of comprehension of the underlying mechanisms and signalling pathways targeted with the therapies.

In spite of the fact that the complete understanding of the different functions that TNF- α and LT play in neuroinflammatory neurodegenerative diseases has not yet been achieved, their involvement in the pathogenesis of AD and MS has been made clear. Thus, in addition to the promising selectively activation or inhibition of their signal transduction pathways, the presence of polymorphisms in the genes encoding for these cytokines could serve as a genetic marker that may be used for the early diagnosis of the diseases.

To the best of my knowledge, this is the first essay aimed to investigate the role of lymphotoxin and TNF- α in different neuroinflammatory neurodegenerative diseases. These cytokines have shown to play redundant and complementary functions during AD and MS pathogenesis, providing parallel strategies for the treatment and diagnosis of these disorders. Furthermore, the experience achieved by studying these cytokines could help to increase the knowledge about the underlying mechanisms of different neuroinflammatory, autoimmune and neurodegenerative diseases and not just AD and MS.

5. References

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