university of groningen

TREM-2 expressing microglia in neurodegeneration

Master Essay

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2013-2014

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Abstract

At present, it is still unclear how the innate immune response relates to several neuroinflammatory and neurodegenerative diseases such as Multiple sclerosis (MS) and Alzheimer's diseases (AD). Genome-wide association studies have revealed several genes and/or mutations which correlate with the risk for neurodegeneration. One receptor found to be involved, is the in 2000 discovered triggering receptor expressed on myeloid cells-2 (TREM-2). The receptor is expressed by microglia in the central nervous system (CNS) and TREM-2 expressing microglia function as phagocytic cells to clear neuronal debris, and can initiate an anti-inflammatory response. Furthermore, TREM-2 seems to be involved in neurodegeneration since loss-of function mutation TREM-2 found to causes the chronic neurodegenerative disease, Nasu-Hakola. Recent, researchers have found mutation in AD patients which was correlated with the risk to develop Alzheimer's disease. Both, in AD and in MS, high expression of TREM-2 was found on activated microglia. In this essay, the role of TREM-2 on microglia in Alzheimer's diseases and Multiple Sclerosis is discussed.

Abbreviations

AD	Alzheimer's disease
BM-MC	bone marrow-derived myeloid cells
CNS	central nervous system
CSF	cerebrospinal fluid
DCs	dendritic cells
EAE	Experimental autoimmune encephalomyelitis
iNOS	inducible nitric oxide
ITAM	immunoreceptor tyrosine based activation motif
LPS	lipopolysaccharide
MAF	minor allelic frequency
MBP	myelin basic protein
MS	Multiple Sclerosis
PLOSL	lipomembranous osteodysplasia with sclerosing leukoencephalopathy
SIRβ1	signal regulatory protein-β1
SNP's	Single Nucleotide Polymorphisms
TLR	toll-like receptor
TREM-2	myeloid cells-2

1. Introduction

The CNS is historically considered to be an immune privileged organ, shielded from circulatory system by the blood-brain barrier (BBB). The BBB regulates passage of molecules and cells into the CNS, therefore protecting entry of most plasma proteins and peripheral immune cells. Although the CNS is protected from its surroundings by the BBB, recent studies have clearly shown that CNS is a highly immunologically active organ. It shows complex immune responses, which are mostly based on the innate immune responses (Carson, Doose, Melchior, Schmid, & Ploix, 2006; Lampron, Elali, & Rivest, 2013). During traumas and viral and bacterial infection, the CNS recognizes specific proteins present on a large numbers of microorganisms. These pathogen-associated molecular patterns (PAMPs), for example the component lipopolysaccharide (LPS), from bacterial membranes, are recognized by the innate immunity (Kumar, Kawai, & Akira, 2011). Upon activation of the innate immune system, PAMPs recognition by mononuclear phagocytes will initiate a cascade of signalling event to produce and secrete cytokines (Anderson, 2000).

Microglia is an example of mononuclear phagocyte and plays a key role in the first line defence. Microglia are the resident macrophages of the CNS system, they controls early stage of infection and stimulates cells activation required for the adaptive immune system. In normal healthy brain, microglia are in their 'resting' ramified stage, with long branched processes to monitor their environment, but once environment triggers them, microglia becomes activated and change their morphology to amoeboid shape with thicker cellular body without long branched processes (Kennedy & Abkowitz, 1997). Microglia response to CNS infection by phagocytosis of the invading microorganism, this is associated with pro-inflammatory cytokines release. Conversely, microglia clearance of neuronal debris is associated with production and secretion of anti-inflammatory cytokines. Therefore, researchers hypothesized microglia to be neuroprotective during acute injury but during chronic activation may play a role in the mechanism of neurodegenerative disorders (Hanisch et al., 2001; Tim Magnus, 2001)

Indeed, patients with chronic neurodegenerative diseases, including Alzheimer's disease (AD), Multiple sclerosis (MS), Parkinson, Huntington's disease and amyotrophic lateral sclerosis, have large numbers of activated microglia (Takahashi, Prinz, Stagi, Chechneva, & Neumann, 2007). These activated microglia are thought to be the activated by toxic molecules secreted by degenerating neurons, which in turn promotes further neurodegeneration (Akiyama et al., 2000). However, the functional role of microglia in neurodegenerative diseases is not fully understood, since they can either be harmful or benign for the CNS.

Several genome-wide association studies have revealed genes which are correlated with the risk for neurodegenerative diseases like in AD and MS (Mowry et al., 2013). During the search for DAP12-associated receptors, in 2000, Bouchon et al. found the triggering receptor expressed on myeloid cells-2, which were expressed on macrophages and dendritic cells (DCs). Further research has shown that TREM-2 mutation leads to a chronic neurodegenerative diseases, therefore in this essay; the function of TREM-2 on microglia in neurodegeneration will be discussed, with respect to AD and MS.

2. TREM-2

2.1 molecular characterizations of TREMs

Mononuclear phagocytes, as mentioned above, are important cells for the innate immune system to survive infection by microbes. Pathogen recognition is performed through several pathogen recognition receptors like; Toll-like receptor (TLR) and C-type lectin family, to activate the innate immune system, which in turn will activate the adaptive immune system.
Example of another innate immune receptor is TREM, who since the discovery in 2000 is extensively investigated. The TREM gene, is located on human chromosome 6p21.1 and encodes for a structurally related protein family (Sharif & Knapp, 2008). This receptor family comprise of one inhibitory and two activating receptors of the immunoglobulin gene family. Human TREM1 and TREM2 are transmembrane glycoproteins with a single extracellular immunoglobulin-like domain and a cytoplasmatic tail, which couples with DAP12 for signalling and function (Bouchon, Dietrich, & Colonna, 2000; Bouchon, Hernandez-Munain, Cella, & Colonna, 2001). DAP12 contains an immunoreceptor tyrosine based activation motif (ITAM), which become phosphorylated after activation of DAP12-associated receptors. Phosphorylation leads to a cascade of tyrosine kinase and protein activation and triggering of several pathways will ultimately lead to cellular response like Ca^{2+} mobilizations, actin cytoskeleton rearrangement and regulation of cytokine production (Gingras, Lapillonne, & Margolin, 2002; McVicar, 1998; Takaki, Watson, & Lanier, 2006). TREM-1 has the ability to increase chemokines production causing lymphocytes to recruit to the site of inflammation. Therefore TREM-1 acts as a positive regulator on granulocyte and monocytes/macrophages, following microbe infection (Colonna, 2003). Conversely, TREM-2 mainly regulates the development and functions of other myeloid cells, like dendritic cells, osteoclasts and microglia (Bouchon et al., 2000). They are known to suppress inflammatory responses, by negative regulation of TLR-responses in dendritic cells as well as cytokine synthesis in macrophages and suppression of TNF and iNOS secretion by microglia (Bajramovic, 2011; Ito & Hamerman, 2012; Turnbull et al., 2006). Loss of function of TREM2 will lead to an autosomal recessive disorder called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) or Nasu-Hakola disease, and eventually to severe neurodegeneration. In addition, TREM-2 was found to regulate phagocytic pathways that are responsible for the removal of neuronal debris (Takahashi, Rochford, & Neumann, 2005). Therefore, in this essay we will focus on the TREM-2 variant of the TREM receptor family.

2.2 TREM-2 function

Originally, TREM-2 was identified as a key player in mononuclear phagocyte biology and the innate immune system, regulating inflammation in a negative way (Turnbull et al., 2006). But recent studies indicated it might have an important role in brain function and bone modelling. Loss of function of TREM-2 leads to PLOSL/Nasu-Hakola disease, which was originally described independently in Finland and Japan. Multiple bone cyst-like lesions and progressive

encephalopathy characterize the Nasu-Hakola disease. There is accumulation of lipid material, demyelination, axonal loss and massive gliosis present in the brain (Verloes et al., 1997). The original study in Finland reported the linkage of the disease to a region of chromosome 19q13, and analysis of potential candidate genes showed a homozygous deletion of exon 1-2 of the gene encoding DAP12 (Paloneva et al., 2000). Five out of six Japanese patients with Nasu-Hakola disease found to have loss-of-function DAP12 mutations, which confirmed the role of DAP12 (Kondo et al., 2002). Recent analysis of patients with Nasu-Hakola, found to have an intact gene encoding DAP12 and normal DAP12 gene expression, which indicated loss-of-function mutations in TREM-2 (Paloneva et al., 2002). TREM-2 and DAP12 seems to be important in pathogenesis of Nasu-Hakola diseases. Myeloid cells, including osteoclasts and microglia, express them; both cells are crucial for bone modelling and brain functionality.

2.3 TREM-2 expression on microglia

The microglia expression of TREM-2 was first described in a study of Schmid et al., they screened gene transcripts of molecules expressed by microglia and regulated by inflammatory signals. They found TREM-2 expression on inactivated microglia, which could be down-regulated by LPS-IFN-γ (Schmid et al., 2002). Several other studies now confirmed the expression of TREM-2 on microglia in mouse and human (Sessa et al., 2004; Takahashi et al., 2005). In addition, TREM-2 was the first receptor shown to be expressed on microglia and to associate with the ITAM-containing adaptor DAP12. Signalling of TREM-2 stimulation via cross-linking antibodies goes via DAP12 in microglial cells. Microglia are kept in their inactive silent state, by getting inhibitory signals via their receptors that contain an immunoreceptor tyrosine based inhibition motif (ITIM) (Hayakawa et al., 2005). Activation of microglia is regulated by activation signals that are transduced by the adapter molecule DAP12. Stimulation of TREM-2 leads to changes in actin polymerization, cytoskeleton organization and up-regulation of cell surface expression of chemokine receptor CCR7. Furthermore activation of TREM-2 by antibodies will stimulate microglial phagocytosis and the protein tyrosine kinase ERK via DAP12. Microglia with TREM-2 knockdown shown to have increased gene expression of pro-inflammatory cytokines, compared to microglia with intact TREM-2 receptors. This establishes the thought of TREM-2 receptor being involved in anti-inflammatory responses and TREM-2 provides a counterbalance to the pro-inflammatory activity of microglia (Takahashi et al., 2005).

TREM-2 also found to have a role in promoting removal of apoptotic cells, organic matrix components and macromolecules by microglia. Hsieh et al. reported the interaction of TREM-2 on microglia with endogenous ligands on neurons. This lead to formation of receptor-ligand pair and connecting microglia with apoptotic neurons, triggering removal of damaged cells (Hsieh et al., 2009). Furthermore, another study shows reduced phagocytosis of apoptotic cells when TREM-2 where knock-downed, while overexpression enhanced the phagocytosis (Bajramovic, 2011). The first evidence of TREM-2 role in Alzheimer's Disease (AD) was provided by the study of Melchior et al, where they found that increased TREM-2 expression promote amyloid phagocytosis in unstimulated microglia (Melchior et al., 2010).

In addition, they found that high TREM-2 expression was correlated with decreased motility of BV-2 microglial cells *in vitro*. They also demonstrated that increased levels of TREM-2 corresponded with higher antigen-presenting cell function in microglia, without triggering T-cell production of IFN-γ (Melchior et al., 2010).

This all indicate that defects in TREM2-DAP12 complex can lead to accumulation of toxic product that can cause brain damage. And increased expression have influence on the functionally of microglia. Hence we will discuss the role of TREM-2 on microglia activation in neurodegeneration diseases in the next chapter.

3. TREM-2 in neurodegeneration

3.1 Alzheimer's disease

3.1.1 Pathogenesis of Alzheimer's disease

Alzheimer's disease is the most common form of neurodegeneration characterized by progressive memory loss and cognitive function. The accumulation of extracellular amyloid-β (Aβ) peptides plaques within the brain parenchyma and intracellular neurofibrillary tangles seems to be key pathological hallmarks of AD (Hardy & Selkoe, 2002). Aβ plaques are generated by cleavage of amyloid precursor protein (APP), which results in formation of 20-42 amino acids peptides. These peptides can aggregate to form Aβ oligomers and fibrils that are deposited within the brain (Selkoe et al., 1988). Neurofibrillary tangles are composed of the tau protein, which is a component of microtubules in healthy neurons. In AD, tau becomes hyperphosporylated and will bind together to form neurofibrillary tangles (Braak, Braak, & Strothjohann, 1994). In AD, activated astrocytes and microglia are found near neurons and plaques, producing pro-inflammatory cytokines, growth factors, chemokines, complement molecules and cell adhesion molecules (Bales, Du, Holtzman, Cordell, & Paul, 2000; Mrak & Griffin, 2005; Tuppo & Arias, 2005). Activation is thought to results from chronic deposition of Aβ (Town, Nikolic, & Tan, 2005).

3.1.2 Microglia in Alzheimer's disease

In the AD brain, microglia was found to be activated and to interact with amyloid deposits by extending their processes into the plaques. Microglia can induce an immune response to Aβ plaques and migrate to the amyloid deposition areas. Despite their ability to interact with these deposits, microglia are not capable of clearing these plaques from the brain (Akiyama et al., 2000; Lee & Landreth, 2010). In addition, the amount and size of microglia seems to correlate to plaque dimension (Wegiel et al., 2003). Normally, the brain possesses an intrinsic mechanism to suppress interaction of microglia with neurons and other glial cells, controlling the microglia activation. However, in AD several ligands for these receptor seems to be lost due to loss of neurons and this mechanism fails to suppress microglia activation (Walker, Dalsing-Hernandez, Campbell, & Lue, 2009). In AD patients and APP transgenic animals, an increased level of inflammatory cytokines like IFN-γ, TNF-α, IL-6 and IL-1β is observed. IFN-γ and TNF-α, both found to reduce levels of insulin degrading enzyme, which is a protease to degrade Aβ-plaques. They also shown to increase production of Aβ from APP and affect the microglia ability to degrade (Rojo, Fernandez, Maccioni, Jimenez, & Maccioni, 2008; Wyss-Coray, 2006). These mechanisms can all contribute to increased deposition or decreased degradation of Aβ-plaques through inflammation.

3.1.3 TREM-2 loss of function in Alzheimer's disease

Recently, two groups of researchers have reported independently a mutation in TREM-2 gene, encoding the TREM-2 protein, which causes susceptibility to late onset AD. In 2012, Jonsson et al. carried out whole-genome sequencing on samples of 2261 Icelanders. They found a rare missense mutation on the T allele of rs75932628, which has an allelic frequency of 0.63% in Iceland. This region encodes a substitution of histidine for arginine at position 47 (R47H) in the gene encoding TREM-2 on chromosome 6p21.1 and could significant enhance the risk of Alzheimer's disease (odds ratio, 2.92; 95% confidence interval, 2.09 to 4.09; $P= 3.42\times10^{-10}$). They successfully replicated this association by genotyping rs75932628 in cohorts from the United States (Emory), Germany (Munich), the Netherlands (Rotterdam) and Norway. They found that rs75932628 conducted a risk of AD in all replication cohorts, and the combined odd ratio was 2.83 (95% CI, 1.45 to 5.40; P = 0.002). Furthermore, they found that each copy of rs75932628-T was associated with the age of onset from patients that was lowered with 3.18 years and 3.65 years in cohorts from Iceland and the Netherlands comparing to the controls without the variant (P=20 and P=0.13). In addition, they investigated how rs75932628 influence the cognitive function of elderly controls without AD. They found a worse cognitive function of rs75932628 carriers between 80 and 100 years compared to the non-carriers (P=0.003). In clinic, AD is partially based on the progressive loss of cognitive function, so the observation in rs75932628-T carriers can indicate early decline of cognitive function that ultimately result in AD (Jonsson et al., 2012).

Another study of Guerreiro et al. also reported a strong association of R47H variant in TREM-2 with Alzheimer's disease (P<0.001). In addition, they observed six other variant (H157Y, R98W, D87N, T66M, Y38C, and Q33X) that were present in cased and not in controls. Among these variant, D87N was significantly associated with the disease (P=0.02). The homozygous variant of T66M, Y38C, and Q33X were previously observed in patients with frontotemporal dementia-like syndrome and in addition homozygous Q33X is also identified in Nasu-Hakola patients (Guerreiro et al., 2012). These three variant were also found to be more common in persons with Alzheimer's disease compared to unaffected persons (P=0.01) (Guerreiro et al., 2012).

3.1.4 TREM-2 expression in Alzheimer's disease brain

Guerreiro and colleagues also demonstrated the expression of TREM-2 in human control brain was distributed widely, with high levels in the white matter, substantial abundance in the hippocampus and neocortex but low levels in cerebellum. The expression of TREM2 mRNA was increased in TgCRND8 mice, a transgenic mouse model of Alzheimer's disease. This enhancement was also observed in another transgenic mouse model of AD (Takahashi et al., 2007). Furthermore, they found increased expression of TREM-2 in activated microglia and mainly on microglia surrounding the outer border of amyloid plaques. This was also reported by Frank et al., showing 6.9 fold upregulation of TREM-2 mRNA in plaques areas when comparing to areas without plaques and 3.9 fold upregulation of DAP12 mRNA using qPCR analysis. In correlation with the mRNA expression they found increased expression of

TREM-2 protein in the cortex of aged APP23 transgenic mice, compared to controls (Frank et al., 2008).

Later, in 2010, Melchior et al. again used APP23 mice to examine both TREM-2 and Tmem176b for vaccine-based therapies for AD. They found both TREM-2 and DAP12 to express in the cortex and hippocampus of mice and this pattern was co-incident with deposition of amyloid plaques. They also tested whether other TREM molecules could be detected in APP23 mice, but did not observed amyloid-associated expression of TREM-1, TREM-3 and TREM-4.

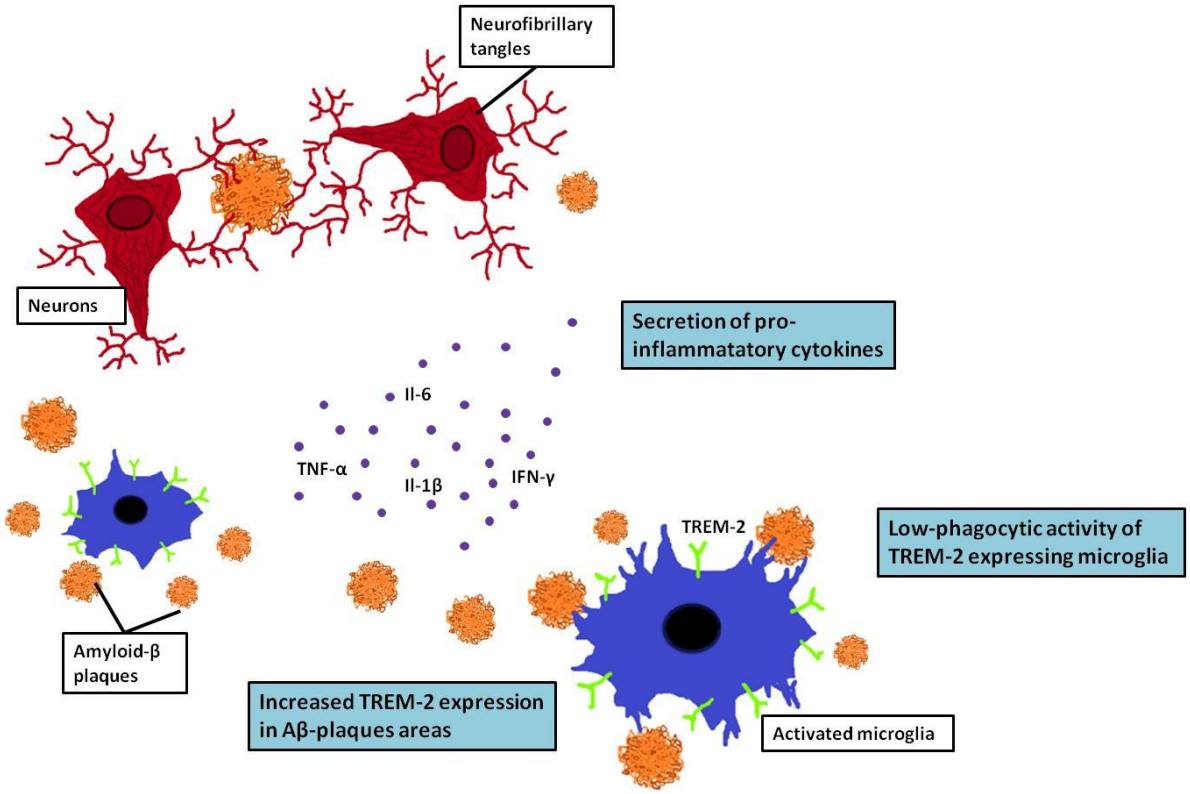

Figure 1) TREM-2 expression on activated microglia in Alzheimer's disease. High levels of mutated TREM-2 on activated microglia are found in AD models. These activated TREM-2 expressing microglia are mainly found around the amyloid-β plaques in the cortex and hippocampus of diseased brain. Although high expression of TREM-2 is found, microglia are not able to clear the plaques and other neural debris from the brain. Furthermore, TREM-2 is not able to suppress the pro-inflammatory response by microglia and high levels of pro-inflammatory cytokines are found which causes damages to neurons.

3.1.5 The functional role of TREM-2 on microglia in Alzheimer's disease

In the progression of AD, microglia found to adopt a classical activated M-1 like phenotype, with secretion of pro-inflammatory cytokines leading to damage of surrounding neurons. In contrast, if activated microglia adopt a M2-like phenotype, they will secrete anti-inflammatory cytokines, increase the Aβ-plaques phagocytosis and enhance tissue remodelling (Varnum & Ikezu, 2012). TREM-2 is known to have anti-inflammatory properties on the innate immune response. They suppress inflammatory responses by repression of cytokines secretion and production. Takahashi et al., found increased expression of pro-inflammatory cytokine TNF-α and NOS-2 when TREM-2 expression was knock-downed. Conversely, TREM-2 overexpression shown to decreased the microglial pro-inflammatory responses (Takahashi et al., 2005). Microglia in AD is known to be low in phagocytosis, and previous study has examined the consequences of increased TREM-2 expression on this property. Increased expression of TREM-2 was shown to correlate with increased phagocytosis of Aβ-plaques. Pre-treatment with LPS decreased the correlation between TREM-2 and phagocytosis drastically (Melchior et al., 2010). This is in line with previous finding of Takahashi et al., where knockdown of TREM-2 on microglia inhibited phagocytosis of apoptotic neurons, while overexpression of TREM-2 increased phagocytosis.

Recent studies have found the important role of microglia in inflammation during neurodegeneration, mainly in Alzheimer's disease. Based on their normal function, TREM-2 is believed to be able to play a protective role in AD, by suppressing pro-inflammatory response and favours phagocytosis of Aβ-plaques. TREM-2 mutation may be an important factor of activating microglia to favours pro-inflammatory response and no longer able to phagocytes neural debris (figure 1).

3.2 Multiple Sclerosis

3.2.1 Pathogenesis of Multiple Sclerosis

Multiple Sclerosis is the most common central nervous system disease that causes neurological impairment and disability in young adults. Chronic inflammation in the CNS leads to demyelination and axonal degeneration (Goldenberg, 2012). Patients can be grouped into categories according to their course of the disease and 80-90% of all cases start with relapsing-remitting course. In time, the numbers of relapsed decreased and patients will get in the secondary progressive phase, which means that the disease continues to worsen. In 10-20% of cases, MS begins in a primary progressive course, without relapses or remission. This form of MS is more resistance to treatment compared to other courses.

The main cause is unknown, but it is believed that combination of genetic susceptibility and non-genetic influences, like environmental factors, infectious agent and metabolism can lead to the autoimmune disorder (Ramagopalan & Sadovnick, 2011). The finding of genetic predisposition to MS, have lead to large number of studies to identify disease loci and alleles. Genome wide studies have resulted in the finding of over 100 genes which all seems to contribute in MS. The main pathological hallmark in the progression of MS is demyelination in areas of the brain and spinal cord. Studies have shown that areas with loss of myelin are possessed with infiltrating T-and B-cells and macrophages, as well as activated microglia. In addition, axonal injury and loss is observed in MS and it found to be correlated with the degree of inflammation (Kuhlmann, Lingfeld, Bitsch, Schuchardt, & Bruck, 2002).

3.2.2 Microglia in Multiple Sclerosis

Activated microglia in MS are found in abundance in white matter lesions, which is undergoing demyelination. The triggering of microglia activation is not known, although there are some possibilities proposed. Triggering of pro-inflammatory cytokines released from T-and B-cells can activate microglia or the phagocytosis of myelin and debris of demyelination can leads to activation. Also, activation of TLR pathway by pathogen is suggested to activate microglia, which is also in line other studies were they claim a role for infectious agents in MS (Sriram, 2011). Activation of microglia may play a harmful or protective role in MS. Several studies demonstrated some mechanism that supports microglia to be detrimental in the demyelinated lesion. Recruitment and reactivation of T-cells through the release of proteases, secretion and production of pro-inflammatory cytokines and reactive oxygen as well as toxicity to neurons and oligodendrocytes precursor cells may all contribute to worse progression of MS (Rawji & Yong, 2013). However, recent evidence indicates that microglia could also have beneficial role in MS. Mechanism underlying these beneficial function can be divided into three pathways. The first beneficial function is secretion of cytokines, chemokines, and growth factors that can triggers repair and promote remyelination. Second, microglia can phagocytes inhibitory debris and remove apoptotic cells and thirdly, stem cells populations can be recruited and induce neurogenesis (Napoli & Neumann, 2010).

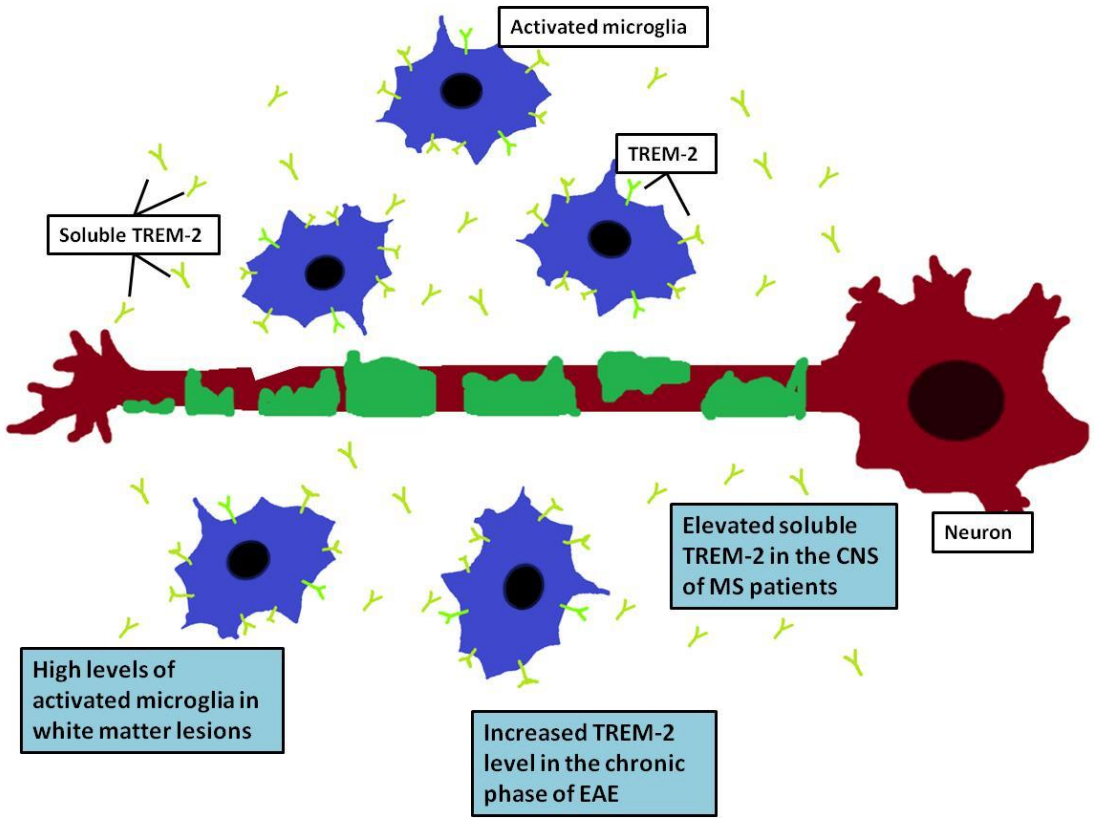

Figure 2) TREM-2 expression on activated microglia in Multiple Sclerosis. High levels of activated microglia are found in the white matter lesions, which are undergoing demyelination. TREM-2 expression is increased on activated microglia in the chronic phase of EAE while during early inflammation TREM-2 is hardly detectable. The soluble form of TREM-2 increased in the CSF of patients with MS and other inflammatory diseases. Furthermore, high expression of the TREM-2 receptor is found on macrophages in the demyelinated lesions of MS patients.

3.2.3 TREM-2 expression in Multiple Sclerosis brain and animal model

Animal models are used for examination of MS; one example is the experimental autoimmune encephalomyelitis (EAE) model. EAE is an inflammatory demyelination disease in the CNS, mediated by an autoimmune attack against myelin proteins. This model shares several clinical and pathological features with MS and therefore is broadly used (Rosenling et al., 2012).

In 2007, Piccio and colleagues has examined the TREM-2 protein expression on microglia in EAE mice, using their own produced monoclonal antibody against mouse TREM-2 receptor. During early inflammatory phases of EAE, TREM-2 expression was hardly detectable in the spinals cords, while in the chronic (later) phase TREM-2 was up-regulated and remained increased. This expression pattern was also seen in DAP12 transcript and from the analysis of expression in the brain. Quantification shown instant upregulation of TREM-2 expression, 18 days post-immunization, this is in accordance with the peak of inflammation in the disease. Using immunofluorescent, they investigated the TREM-2 protein expression, and found high and diffuse expression, 20 days post-immunization, on

microglia/macrophages and dendritic cells. Furthermore, they isolated mononuclear cells from the brain and spinal cord of EAE affected mice. Findings show TREM-2 expression primarily on resident microglia, identified as $CD45^{low/}CD11b^{+}$, and in a lower extend on infiltrating macrophages/activated microglia, $CD45^{high}/CD11b^{+}$ cells (Piccio et al., 2007) (figure 2).

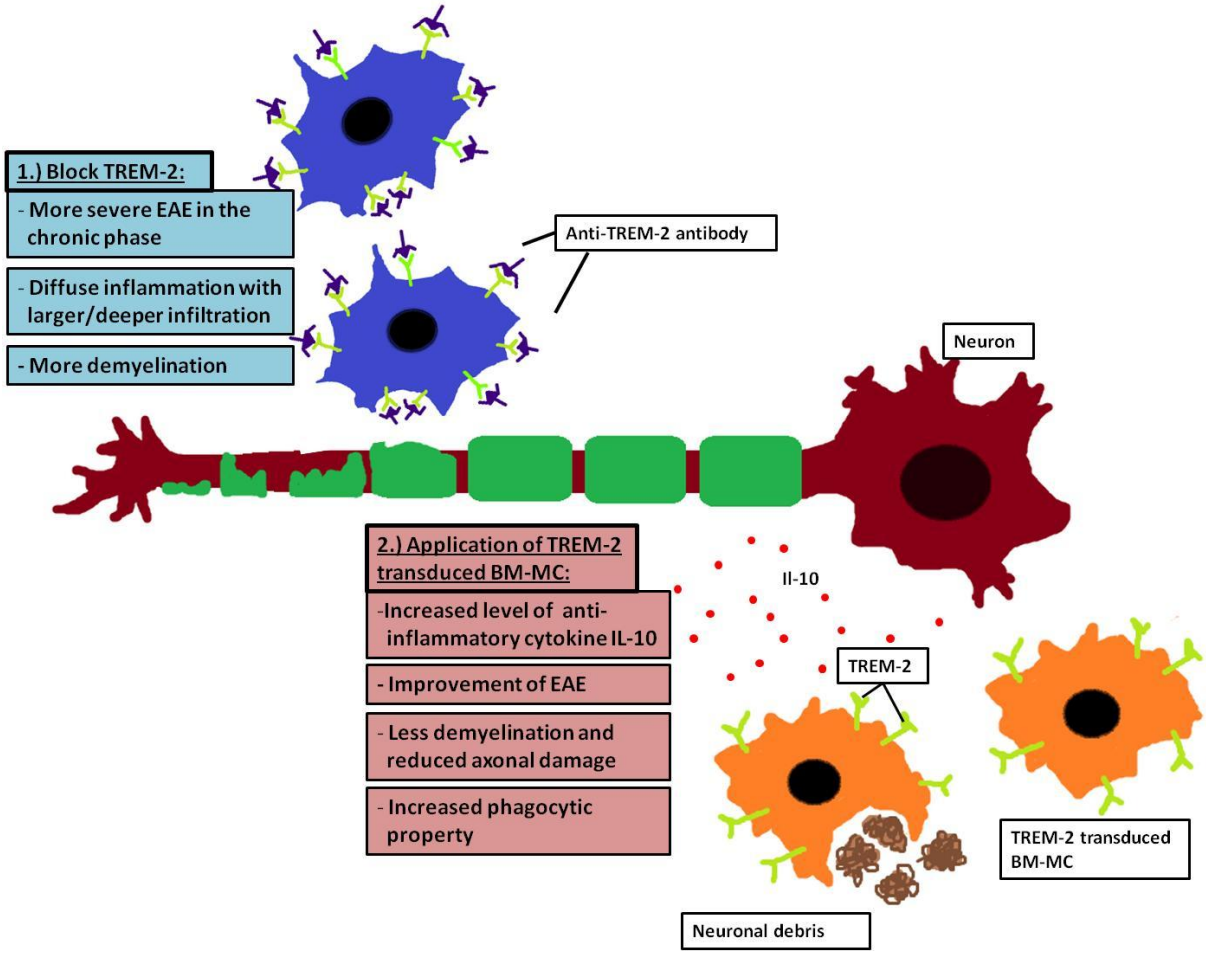

Figure 3) The role of TREM-2 in the progression of MS. 1) Blocking TREM-2 with anti-TREM-2 antibody clone 1 will lead to a more severe EAE during the chronic phase of the disease. In addition, a more diffuse inflammation is reported with larger and deeper cellular infiltration and more demyelination. **2.)** When TREM-2 transduced bone-marrow derived myeloid cells are applied in EAE models, an improvement of EAE progression is observed. Reduced level of pro-inflammatory cytokines, like TNF-α and Il-1β, and increased level of anti-inflammatory cytokine IL-10 is shown. Reduced degeneration of MBP is accompanied with less demyelination and axonal damage. Furthermore researchers found increased phagocytosis of apoptotic neurons of TREM-2 transduced BM-MC *in vitro* as well as *in vivo*.

One year later, the same research group reported for the first time soluble human TREM-2 protein expression in the cerebrospinal fluid (CSF) and serum from MS and other CNS inflammatory diseases patients. They found elevated soluble TREM-2 levels in the CSF of subject with relapsing-remitting MS (P=0.004), primary-progressive MS (P=0.0005) and other inflammatory disease subjects (P=0.0005) compared to controls non-inflammatory disease subjects (figure 2). However, they did not observed significant differences in soluble TREM-2 levels in serum among the four groups. They identified monocytes as $CD14^+$ from the CSF by flow cytometry and found the expression of TREM-2 on a subpopulation of CSF monocytes. TREM-2 was not detected on $CD14^-$ CSF cells and on circulating blood monocytes. Though, when they compared TREM-2 expression on CSF monocytes among the MS group, other inflammatory diseases group and control non-inflammatory diseases group, they found significant higher expression of TREM-2 in subject with non-inflammatory diseases. They also tested whether factors within the CSF could regulate TREM-2 on monocytes, but they concluded that factors from the CSF are unlikely modulate TREM-2 expression on monocytes. Furthermore they found that soluble TREM-2 levels do not correlate with the number of $CD14^+$monocytes or with the number of TREM-2^+monocytes, proposing that CSF monocytes are probably not the main source of soluble TREM-2. They also reported high expression of TREM-2 receptor on macrophages in actively demyelinated multiple sclerosis lesions from human CNS (Piccio et al., 2008).

3.2.4 TREM-2 in Multiple sclerosis progression

Two research groups have shown a protective role of TREM-2 in progression of EAE. The first group, Piccio et al., investigated the effect of blocking TREM-2 receptor in EAE, by using anti-TREM-2 antibody clone 1, to block the interaction of TREM-2 with its ligand, *in vivo*. Mice treated with antibody before clinical onset of EAE developed a significant more severe EAE. At the peak of the disease they had a higher clinical score and a more persistent symptomatic chronic phase. Moreover, diffuse inflammation was observed, compared to controls, showing larger and deeper cellular infiltration in the CNS parenchyma and more demyelination (Piccio et al., 2007) (figure 3).

The second research group, Takahashi and colleagues have shown the effect of intravenously application of TREM-2 transduced bone marrow-derived myeloid cells (BM-MC) in EAE mice. *In vitro*, they found increased phagocytosis properties of TREM-2 transduced BM-MC compared to control BM-MC and monocytes in culture. Phagocytosis of both microsphere beads and apoptotic neurons were increased, but could be neutralized by ERK inhibitor PD98059, indicating that ERK signalling was involved in the phagocytosis process. Simultaneous, they found suppression of pro-inflammatory gene transcript in TREM-2 transduced BM-MC and therefore examined their *in vivo* potentials. Intravenous injection of TREM-2 cells, four days after disease onset, showed higher level of lysosomal degradation that was associated with phagocytosis of tissue debris. Next, they investigated the degeneration of myelin basic protein (MBP), which is phagocytosed and degraded in lysosomes by myeloid cells in inflammatory lesions during MS. Immunostaining, showed decreased degeneration of MBP in the spinal cord after treatment with TREM-2 transduced

BM-MC compared to controls. Furthermore, q-PCR analysis of the spinal cord showed reduced gene transcription of pro-inflammatory cytokines TNF-α, IFN-γ and IL-1β, while there was increased IL-10 gene transcript. The most important finding was that TREM-2 transduced BM-MC showed an improvement of EAE progression. Injection of mice, four days after onset of clinical symptoms reduced the cumulative clinical score from 22.8 (SD 2.5) to 17.6 (SD6.0), while no differenced were observed in PBS or GFP vector control mice (figure 2). Treatment of TREM-2 found to be only effective in the recovery and repairs phase and not in preventing the initiation of EAE. Furthermore, reduced axonal damage and demyelination were observed by immunostaining of the spinal cord tissue (Takahashi et al., 2007).

4. Discussion

TREM is a family of receptors, discovered in 2000, which in human comprise of TREM-1 and TREM-2 variant (Bouchon et al., 2000). TREM-1 found to be upregulated during inflammation, modulating myeloid-cell differentiation and function (Bouchon et al., 2001). In contrast, TREM-2, which under normal homeostatic condition promotes dendritic cells, osteoclasts, microglia and oligodendrocytes differentiation, was found to be down-regulated in inflammatory conditions. The first evidence of TREM-2 playing a role beyond immune system was discovered by the finding of TREM-2 loss-of-function mutation causing chronic neurodegenerative disease (Paloneva et al., 2002). Nasu-Hakola disease/ PLOSL is characterized by TREM-2/DAP12 loss-of-function, which lead to accumulation of lipidic material, demyelination, axonal los and massive gliosis. Patients suffer from multifocal bone cysts and inflammatory neurodegeneration. These findings initiated discussion about TREM-2 functionality in relation to innate immunity during neurodegeneration.

A defect TREM-2 or incorrect formation of TREM-2/DAP12 complex can affect microglia functionality. The TREM-2/DAP12 receptor-signalling complex is necessary for microglial removal of apoptotic cells, organic matrix components and macromolecules. A defect complex leads to accumulation of toxic product in the brain, which might causes brain damage. Besides, production of pro-inflammatory cytokines is increased and chronic inflammation can induce neural cell death and increase apoptotic material. Further research has found other families, which possesses homozygous TREM-2 mutation, presenting early onset dementia or frontotemporal dementia without bone-cysts associated symptoms (Chouery et al., 2008; Guerreiro et al., 2012). This suggests that TREM-2 mutation play an important role in dementia even in the absence of bone cysts.

As TREM-2 deficiency has shown to provoke dementia, different research teams had raised the question of a possible role for TREM-2 in the molecular mechanism of other neurodegenerative diseases, like Alzheimer's disease and Multiple Sclerosis. During chronic inflammation, microglia remains longer activated compared to acute inflammation; therefore production of mediators is sustained. Increased secretion of mediators as IL-1β, TNF-α and IL-1α may cause neuronal degradation. Indeed, researchers found microglia to produce and secrete higher levels of pro-inflammatory cytokines, causing worse progression of both AD and MS (Rawji & Yong, 2013; Wyss-Coray, 2006). Knowing TREM-2/DAP12 complex to be anti-inflammatory, it is obvious to predict decreased expression of TREM-2 during disease progression. However, in mouse models of both diseases, researcher reported increased expression of TREM-2 and DAP12 mainly on activated microglia. The elevated TREM-2 expression during chronic inflammation in patients and mice models can be explained by a mechanism of the brain to compensate for the sustained pro-inflammatory response of microglia. Chronically activated microglia can contribute to progression of the disorder; therefore the brain will try to oppose this by expressing several molecules that can induce a contrary response. Increase of TREM-2 on microglia can induce anti-inflammatory response by suppressing TNF-α expression and inducible nitric oxide (iNOS) secretion by microglia. Knock-down of TREM-2 receptor shown to cause increased gene transcript on microglia of pro-inflammatory cytokines in present of apoptotic neural cells. However, when TREM-2

receptor is intact, it can promote anti-inflammatory response, counterbalancing the pro-inflammatory response of chronically activated microglia (Takahashi et al., 2005). TREM-2 expression and functionality on microglia in AD and MS will be further discussed separately in the next sections.

4.1 TREM-2 in Alzheimer's disease

The most common form of neurodegeneration is Alzheimer's disease, being the sixth leading cause of death in the United States (Alzheimer's, Thies, & Bleiler, 2011). In 2006, an Italian research team has screened three Single Nucleotide Polymorphisms (SNP's) in TREM of AD patients, but none of the SNPs were present, and they failed to detect the presence of novel polymorphism. Later, in 2012, two other research groups successfully demonstrated missense mutation in TREM-2 gene, associated with higher risk of AD development (Guerreiro et al., 2012; Jonsson et al., 2012). After this, several studies have successfully replicated this work on different ethnic cohorts. All the studies have reported increased risk for AD when patients carried the R47H variant. However, several findings have not been take into consideration to which extends TREM-2 can be helpful in treating AD at present.

In the latest study (June 2013), researchers have genotyped 2974 samples, being the largest designed study in finding R47H mutation variant in AD and like other studies, they concluded that R47H variant increases the risk of late-onset AD (Gonzalez Murcia et al., 2013). However, they reported a minor allelic frequency (MAF) of 0.0029 and a population-attributable fraction of 0.004. The minor allele frequency refers to the allele, which has the least frequency among all the alleles within a population. Genome Wide Association Studies will exclude variant with MAF<0.05 and for the HapMap project, researchers only included variant with minor allele frequency ≥0.05 (International HapMap, 2005). They compared the MAF of R47H with the most common risk factor for AD, the ApoE ε4 gene, and found a minor allele frequency of 0.20 for ApoE ε4 gene (M.-S. Tsai & Emre Kokmen, 1994). So although several studies have shown the risk of R47H variant in AD, the effect on the population of this new variant is much lower comparing to those of the ApoE ε4 gene. Furthermore, the minor allele frequency of R47H is as low that in genome wide studies researchers will exclude this variant.

As mentioned above, several studies have identified the R47H variant to be associated with higher risk for AD. Studies included different ethnic groups; Northern-Europe (Iceland, Norway and United Kingdom), Western-Europe (Germany, The Netherlands and France), Southern-Europe (Spain and Portugal), Canada, Colombia and the United States (Benitez et al., 2013; Giraldo et al., 2013; Gonzalez Murcia et al., 2013; Guerreiro et al., 2012; Jonsson et al., 2012; Pottier et al., 2013). Although they all found significant risk of R47H, comparable with that ApoE ε4 allele, results need further conformation in studies of other racial and ethnic groups, like Africans and Asians. A more comprehensive and in-depth genetic screening should be performed to uncover potential other variant of TREM-2 and a possibility to find variants with MAF higher than 5%.

Right now, the consequence of R47H variant is not clear; decreased affinity of TREM-2 for its ligand can potentially be the outcome. However several studies has reported potential

ligands, but they all differ dramatically in their structures and it is still unclear how many types of ligands are able to trigger TREM-2 activation (Turnbull et al., 2006). Therefore, some researchers have used a fusion protein to detect TREM-2 expression or binding activity. But since the endogenous ligand expressed in the CNS is unidentified, it is hard to conclude these findings to be similar in AD patients. Another study has shown TREM-2 to associate with decreased motility of microglia, TREM-2 expression correlates with increased antigen-presenting cell function and increased TREM-2 expression promotes phagocytosis. However, they performed these experiments on BV-2 microglial cell line, which are derived from primary mouse microglial cells (Melchior et al., 2010). Cell lines may behave different comparing to primary cells in response, therefore the findings needs to be taken in consideration. Furthermore, they used mouse cell lines, which may react differently compared to human microglia cells. All the above-mentioned studies, to find R47H variant, are performed on human genome, but expression examination is all performed on Alzheimer's disease mice model. Therefore researchers need to focus on experiment of human AD brain material, to see whether they will find the same expression profile and pattern as in AD mice, and functional activity assays have to determine the effect of R47H.

Clearance of damaged or apoptotic neurons may improve AD pathogenesis, by reducing pro-inflammatory response to damage the brain. More important in AD is the removal of amyloid plaques, being the most important pathogenic hallmark. Melchior et al., reported that increased TREM-2 expression was correlated with higher amyloid phagocytosis in unstimulated cells (Melchior et al., 2010). Though, carriers of homozygous TREM-2 mutation with Nasu-Hakola disease did not show increased amyloid plaques phagocytosis (Singaraja, 2013). Therefore, there is no evidence that TREM-2 enhance phagocytosis of apoptotic neurons in plaque lesion of the brain, suggesting TREM-2 not improving AD trough plaques clearance.

4.2 TREM-2 in Multiple sclerosis

Multiple sclerosis is a chronic inflammatory neurodegenerative disease, characterized by demyelination and axon degeneration. Activated microglia has been found during EAE, indicated by morphologic changes and expression of cell surface markers, like MHC-II and CD45 (Piccio et al., 2007). The main function of microglia in MS is not clear, some researcher propose a harmful role, while other indicates protective properties of microglia. Therefore activated microglia during EAE can either promote inflammation in the CNS by antigen presenting to T-cells and production of pro-inflammatory cytokines or they can protect the CNS from inflammation by secreting immunosuppressive cytokines and through this, controlling severity of inflammation and autoimmune response (Napoli & Neumann, 2010; Rawji & Yong, 2013). The second hypothesis is supported by the findings of microglia subset with expression of CD45, MHC-II and co-stimulatory molecules are able to induce tolerance and cytokine production is increased in the absence of TREM-2 in DAP12 signalling (Ponomarev, Shriver, Maresz, & Dittel, 2005; Turnbull et al., 2006).

Piccio and colleagues have reported increased expression of TREM-2 in MS mouse model EAE as well as the soluble TREM-2 in human CSF from MS patients. However, they

did not examine what triggered the up-regulation of TREM-2. During the acute phase of EAE, IL-4 level shown to be elevated, and in line with this, previous study have shown that alternative activation with IL-4 can induce TREM-2 expression on infiltrating macrophages and rapidly down-regulated by treatment with either LPS or IFN-γ (Okuda, Sakoda, Bernard, & Yanagihara, 1998; Turnbull et al., 2006). Therefore IL-4 can be a potential factor, which contributes to up-regulation of TREM2 on microglia and macrophages during EAE. Besides finding elevated expression of TREM-2 in EAE mouse model, researchers have found the soluble form of TREM-2 to be significantly upregulated in the CSF of MS subjects. The TREM-2 receptor was highly expressed on myelin-laden macrophages in the active demyelinated MS lesions. These 'foamy' macrophages originate from resident microglia and infiltrating monocytes (Li, Cuzner, & Newcombe, 1996). They found to have anti-inflammatory properties and function in clearing myelin and cell debris, resembling the alternative activated type II macrophages (Boven et al., 2006). Expression of TREM-2 on these 'foamy' macrophages support the idea of alternative activated macrophages induces TREM-2 expression

TREM-2 receptor is believed to rapidly translocate to the cell surface upon activation of microglia, suggesting an intracellular pool of TREM-2 (Sessa et al., 2004). But the soluble form of TREM-2 might originate from two other ways: first, TREM-2 can be shed by proteolytic cleavage of TREM-2 molecules anchored on the membrane; second, it can be produced by alternative splicing of TREM-2, leading to secretion of soluble TREM-2. However, the molecular mass of the soluble TREM-2 in this study was 20kDda, while previous studies have shown alternative spliced TREM-2 to have molecular weight of 27kDa (Begum et al., 2004; Schmid et al., 2002). Since TREM-1 has been demonstrated to result from cleavage and shedding, researchers suggest TREM-2 may exist from similar mechanism (Gomez-Pina et al., 2007). Increased expression of TREM-2 has found to stimulate anti-inflammatory response and enhance phagocytic activity of microglia. But how does the soluble form of TREM-2 play a role in this mechanism? Soluble TREM-2 may act as an attractant for the endogenous ligand, and therefore blocking the ligand for binding to the TREM-2 receptor. This mechanism may balance the inflammatory reaction of TREM-2 by secreting soluble form of TREM-2 to attenuate the TREM-2 response. Supporting this idea is the soluble TREM-1, who had been shown to weaken the inflammatory response and thereby improving survival of animal models of septic shock (Bouchon et al., 2001; Gibot et al., 2004). However, at present, the ligand(s) for TREM-2 is still unknown, so examination of the function by using the ligand is not possible.

Several studies have shown the important role of TREM-2 in the immune damage to CNS, by blocking or intravenous application TREM-2 in EAE (Piccio et al., 2007; Takahashi et al., 2007). Blocking of TREM-2 activation before the clinical onset of EAE, have shown to increase severity of disease by higher clinical score at the peak of inflammation. Conversely, intravenous application of TREM-2 transduced myeloid precursor cells reduced severity and induced recovery of EAE in mice. Deterioration as well as improvement of EAE *in vivo* may results from inflammatory response and phagocytic function of microglia. TREM-2 transduced myeloid cells migrated into the MS lesions and induced tissue debris clearance and created anti-inflammatory environment. Macrophages/ microglia are known to clear myelin debris during EAE, as is required since components of myelin can prevent axonal remodelling

and regeneration. Therefore, treatment with TREM-2 transduced BM-MC improved remyelination and axon remodelling most probably by phagocytosis of myelin debris. Furthermore, after TREM-2 application, TNF-α transcript was reduced while IL-10 was increased, indicating TREM-2 created an anti-inflammatory cytokine milieu in the spinal cord. Knowing IL-10 is increased during EAE recovering and remission is impaired in IL-10 deficient mice, it can be concluded that TREM-2 application can induce recovery by creating anti-inflammatory environment.

As TREM-2 have shown to be up-regulated in EAE model as well as in human, and TREM-2 functionality is examined *in vivo*, it can be concluded that TREM-2 demonstrated to be a very interesting novel approach for therapy of MS. TREM-2 expression not only found to be increased during EAE, in other neuroinflammatory diseases TREM-2 also found to be elevated. Therefore TREM-2 may not only be interesting for MS, but also for other neuroinflammatory diseases. Expression of TREM-2 was specific detected on human monocytes recruited to the CSF but not on blood monocytes and creation of anti-inflammatory environment was only detected in the spinal cord but not in spleen and lymph nodes. These findings indicate that TREM-2 expression is restricted to specific cell types and operation of TREM-2 functionality is in specific areas. Furthermore, TREM-2 found to only be effective when applied before clinical onset, and not in preventing the initiation of EAE.

4.3 TREM-2 in other neurodegeneration and possibility of other receptors

Since several groups have reported the R47H variant to increase the risk for AD, Rayaprolu and colleagues raised the question, whether TREM-2 may play a more generalized role in neurodegeneration. Therefore, they designed the study to examine genetic association of R47H variant in other neurodegenerative disorders. They observed a significant higher risk for frontotemporal dementia, which was also found in another study, and Parkinson diseases when patients carried the R47H variant (Guerreiro et al., 2012). But they did not observed significant risk for amyotrophic lateral sclerosis, progressive supranuclear palsy or ischemic stroke (Rayaprolu et al., 2013). In line, Sieber et al., shown attenuated inflammatory response in TREM-2 knock-out mice following stoke. Mice showed decreased expression of pro-inflammatory cytokines associated with reduced activation of microglia. They expected the pro-inflammatory response to increase in the absent of TREM-2, since this was shown in previous study. These data indicate that TREM-2 does not play an important role in all neurodegeneration.

TREM-2 is a signal regulatory receptor, function trough association with DAP12. There is evidence that other DAP12 associated receptors also play a role in neurodegeneration. The signal regulatory protein-β1 (SIRβ1) is a microglial receptor with phagocytic ITAM signalling capacity like TREM-2. SIRβ1 was found to be up-regulated in APP-transgenic mice and in AD patients. In addition, elevation caused increased phagocytosis of microsphere beads, neural debris and fibrillary amyloid-β (Gaikwad et al., 2009). Further investigation to SIRβ1 is needed, since the concrete *in vivo* relevance for direct pathogenesis of neurodegeneration is missing (Linnartz, Wang, & Neumann, 2010).

4.4 TREM-2 contribution to therapy development

In Alzheimer's disease, at present, R47H variant in TREM-2 may not be a very helpful target to develop novel treatment since the frequency of this mutation is very low and the precise consequence of this mutation is unknown. This variant may lead to loss-of-function of TREM-2, but this mutation can also be benign. Knock out of TREM-2 has shown to enhance pro-inflammation and increased expression of TREM-2 shows to decrease pro-inflammatory response of microglia. However, there is no evidence yet, to approve TREM-2 to enhance phagocytosis of death neurons in AD and whether TREM-2 can induce anti-inflammatory cytokine milieu in AD model needs to be investigated. More important is, decreased pro-inflammatory reactions due to high expression of TREM-2 has only been shown in mouse models, and yet have to be proven in human. Also, several functional activity assays are performed, but all *in vitro*, using microglial cell lines, which in most of time differ from primary cells and *in vivo* behaviour of cells. In contrast, studies to TREM-2 expression in MS are performed in EAE model as well as human CSF. Elevated expression of TREM-2 has shown *in vitro* and *in vivo* to enhance phagocytosis and creation of anti-inflammatory response. Moreover, blocking of TREM-2 has shown to enhance severity of EAE while intravenous application of TREM-2 transduced BM-MC cause recovery of EAE with remyelination and axonal remodelling. There was recovery of EAE only when TREM-2 was applied at the peak of inflammation, meaning that TREM-2 play a role in the severity of EAE. Thought, the ligand(s) for TREM-2 is still under investigation, therefore TREM-2 may show to be more important in MS, not knowing the ligand(s) causes it to premature for speculating development of novel therapy.

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