**Synaptic pruning in Alzheimer’s disease in a classic complement- and microglia dependent manner**

**Written by Ronja Wabeke, supervised by Ulrich Eisel**

**Abstract**

Synaptic pruning is one of the most important hallmarks of Alzheimer’s disease (AD). Synaptic pruning during development is initiated by the complement system and microglia. In genome wide association studies microglia and complement where identified in AD. To find out, among other things, if the synaptic pruning in AD works comparable to the synaptic pruning in development, this review will address the question: what is the relation between the classical complement system and microglia, in synaptic pruning in AD. It was found that the classical complement has an important role in the pruning of synapses in AD and that the underlying mechanisms are comparable to the synaptic pruning in development. The complement system tags the synapses, microglia recognize these tags and phagocytosis is followed. Inhibiting the complement system could prevent synaptic pruning in AD. C1q, a protein of the complement system, is suggested to be a possible target in AD therapy.

**Introduction**

Alzheimer’s disease (AD) is a neurodegenerative disorder with progressive memory loss and cognitive impairment (Hong, Dissing-Olesen and Stevens, 2016). Since AD was first described in 1906, a lot of researchers put their focus on this disease but still, after more than 110 years, plenty remains to be discovered (Alzheimer’s Association, 2017). It is projected that in 2050, 88 million people of age 65 and older will have Alzheimer, and this number is for Americans only, let alone how many people in the whole world will have AD. This makes finding the solution on how the disease can be prevented, slowed or stopped, crucial.
One of the characteristics of AD is the loss of synapses (Leshchyns’Ka *et al.*, 2015). This loss is stronger correlated with cognitive decline, rather than the well-known characteristics like Amyloid-Beta (Aβ) plaques, Tau tangles and even the loss of neurons (Hong *et al.*, 2016). The complement system is known to mediate synaptic pruning during development in the retinogeniculate system, where synapses are eliminated to create eye-specific regions (Michailidou *et al.*, 2015). Recent genome wide association studies (GWAS) implicated microglia and complement related mechanisms in AD (Hong, Dissing-Olesen and Stevens, 2016). This raises the question, if synaptic pruning in AD occurs by similar mechanisms as synaptic pruning in the developmental pathway in the retinogeniculate system. In addition, soluble oligomeric amyloid beta peptides (oAβ) are correlated with the loss of synapses (Price *et al.*, 2014). Could oAβ play a role in complement- and microglia mediated synapse loss? Could the complement be a new target in AD therapy? To address these questions, the main question of this review is: what is the relation between the classical complement system and microglia in synaptic pruning in AD?

**Loss of synapses in AD**

AD is a progressive neurodegenerative disorder and it is one of the most common forms of dementia (Fettelschoss, Zabel and Bachmann, 2014). The disease is characterized by the loss of memory, cognitive impairment and personality changes (Puzzo *et al.*, 2015). The well-known hallmarks of this illness are aggregates of Aβ peptides called Aβ plaques, neurofibrillary tangles containing hyperphosphorylated tau proteins, and neuronal loss.
Additionally, neuroinflammation is a hallmark for AD and one of the crucial players in neuroinflammation are microglia, the innate immune cells of the brain (Navarro *et al.*, 2018). However, the role of microglia in AD has controversial findings, just as there is no consensus whether neuroinflammation is beneficial or not, to AD patients: chronic microglial activation could help reduce the levels of Aβ, but the inflammatory response caused by cytokines, which are released by microglia, is associated with neurotoxicity.
Microglia, the phagocytes of the central nervous system (CNS), play a key role in the early neuronal development and circuit formation (Paolicelli *et al.*, 2017). During neural circuit maturation, microglia can eliminate profuse synapses and maintain brain homeostasis. Recent GWAS studies have revealed multiple immune-associated pathways to be a risk factor in late-onset AD (Hong, Dissing-Olesen and Stevens, 2016). It is suggested that abnormally phagocytose by microglia can eliminate synapses (Paolicelli *et al.*, 2017).
The severity of dementia in AD is more correlated to the loss of synapse function than it is to any of the hallmarks above (Pozueta, Lefort and Shelanski, 2013; Hong *et al.*, 2016). This loss of synaptic function in AD was first described in 1967 by Gonatas and colleagues (Gonatas, Anderson and Evangelista, 1967), and follow-up studies concluded that synaptic boutons, in particular in the hippocampus and the neocortex, are lost 45% more in AD patients compared to cognitively healthy control patients (Pozueta, Lefort and Shelanski, 2013). The importance of microglia in the elimination of synapses is shown in multiple studies (Hong *et al.*, 2016; Hong, Dissing-Olesen and Stevens, 2016; Thion and Garel, 2018).

One of the hallmarks of AD is the aggregation of Aβ proteins in the brain (Mohamed, Shakeri and Rao, 2016). These aggregations originate from the processing of amyloid precursor protein with beta and gamma secretase, resulting in Aβ proteins in the extracellular space. These proteins can stick together and form aggregations of Aβ. The assembling of Aβ in the brain is random and spontaneous, which causes different states of conformation, one of these is the oAβ (Price *et al.*, 2014). This oAβ is correlated with the loss of synapses and, when injected, with loss of memory and difficulty in learning. Furthermore, it is suggested that oAβ is associated with the impaired functioning of synapses in AD (Mroczko *et al.*, 2018).

Schafer and his colleagues provided a study demonstrating the underlying mechanisms of synaptic pruning by microglia in the early development of neural circuits in the retinogeniculate system (Schafer *et al.*, 2012). Proteins of the complement system were identified participating in the interactions between microglia and synapses, and synapse pruning by microglia. The study also demonstrated that in absence of one of the proteins, complement component 1q (C1q) or complement component 3 (C3), there is a disrupted microglial function, which results in errors in the synaptic remodelling. Additionally, GWAS insinuated microglia and complement-related pathways in AD (Hong *et al.*, 2016).

**The complement system**

The complement system is an essential part of the innate immune system, where it can eliminate invading pathogens, respond to injury and infection, limit the damage to host tissue by the immune system and help keeping the body in homeostasis (Barnum, 2017). The activation of the complement system is a cascade of cleavage of pro-enzymes, until two convertases are generated. Convertases are multi-molecular enzyme complexes. The complement system can be activated through the classical, lectin or alternative pathway. In this article the focus will be on the classical pathway, since it seems to be that proteins of the classical pathway, like C1q, C3 and C3b, are involved in synapse pruning (Stevens *et al.*, 2007; Harvey and Durant, 2014; Hong *et al.*, 2016; Hong, Dissing-Olesen and Stevens, 2016; Thion and Garel, 2018)
The activation components of the classical complement system are C1, C2, C3 and C4 (Barnum, 2017). The cascade of the classical complement starts by activating the C1 complex, which consists of C1q, C1r and C1s, and will become active after C1q binds to the surface of, for example, a pathogen (Presumey, Bialas and Carroll, 2017). After activation, the C1 complex cleaves C2 and C4 in respectively C2a and C2b, and C4a and C4b. A complex of C4b and C2a is formed and then cleaves C3 in C3a and C3b, which then becomes activated. C3b can bind to targets and tags them to initiate phagocytosis clearance or promote downstream effects that lead in lyses of the target cell. *Figure 1* gives a schematic representation of the cascade. In the rest of this review, often C3 is mentioned as the active protein but by that C3b is meant.

Figure 1: The classical complement cascade (Presumey, Bialas and Carroll, 2017)

The liver is the main producer of complement proteins, and for a long time it was thought that the presence of complement proteins in the CNS was because these proteins could by-pass the blood brain barrier (BBB), until multiple studies around 1990 confirmed that the proteins of the complex system are locally produced by astrocytes and microglia (Veerhuis, Nielsen and Tenner, 2011).

Figure 2:The elimination of synapses after being tagged by complement components (Presumey, Bialas and Carroll, 2017)

**Complement system in CNS**

The development of a mature neural circuit demands the elimination of profuse or inappropriate synapses (Stevens *et al.*, 2007). Multiple studies have proposed the complement system to be an underlying mechanism for the pruning of synapses (Stevens *et al.*, 2007; Hajishengallis *et al.*, 2017; Presumey, Bialas and Carroll, 2017; Thion and Garel, 2018). In the retinogeniculate system of adult mice, the neurons of the retina of each eye have non-overlapping regions in the dorsolateral geniculate nucleus of the thalamus, even though the neurons in new-born mice do have overlapping eye regions and the neurons of the retina of both eyes form synapses onto the same neurons in de dorsolateral geniculate nucleus (Presumey, Bialas and Carroll, 2017). To discard this overlap, synapses must be removed. This is where the complement cascade comes in. In a short period of time in post-natal mice, weaker synapses in the CNS are tagged by complement proteins and eliminated by macrophages (Stevens *et al.*, 2007). C1q and C3 are required for this refinement of the retinogeniculate network. It is proposed that synapses are eliminated comparable to how pathogens are eliminated by the immune system. C1q binds to the synapse and activates C1, by which the classical complement cascades follows and C3b deposits can activate C3 receptors (CR3) on the surface of microglia, initiating the removal by phagocytosis. *Figure 2* gives a schematic representation of the elimination of synapses via microglia. Activated C3 can also lead to the activation of the terminal complement cascade, which leads to a membrane attack complex that makes the cell, or possibly synapse, go in to lysis (Barnum, 2017).

The phagocytosis of synapses by microglia works via the recognition of C3b by the CR3. This receptor is a dimer of CD11b and CD18, also known as Mac-1 (Lukácsi *et al.*, 2017). Functions of this receptor are cell adhesion and the elimination of pathogens, apoptotic- and tumour cells via phagocytoses. The phagocytosis mediated by CR3 is a result from the ligation of the receptor to complement component-coated particles (Choucair *et al.*, 2006). This is a type II phagocytosis and leads to a so called ‘passive phagocytotic response’, whereby the target particles sink into the membrane of the microglia, after which they will be digested.

The importance of the complement system in the development is proven in different knock-out (KO) studies, where C1q-, C3-, and C4-deficient mice have defects in synaptic pruning (Stevens *et al.*, 2007; Sekar *et al.*, 2016). Beside deficits in the proteins of the complement system, are impaired microglia, induced by CR3 KO, correlated with defects in connections between synapses. These deficiencies were all in the retinogeniculate system.

Paolicelli and her colleagues proved the importance of microglia in the pruning of synapses in another region: the hippocampus (Paolicelli *et al.*, 2011). It was shown that the fractalkine receptor was involved in dendritic spine removal. Even though this isn’t evidence for a role of the complement system in synaptic pruning, it does show the importance of microglia in brain wiring in the healthy brain.

The role of C1q in synaptic pruning during development is shown in different studies: C1q is upregulated during the time window of the visual development, C1q is localized to synapses during this development and C1q is required for the development of the retinogeniculate system (Stevens *et al.*, 2007; Hong *et al.*, 2016). Yet, little is known about the precise mechanism underlying the attachment of C1q to the synapses. In the immune system, different C1q receptors can activate the complement system through C1q (Stephan, Barres and Stevens, 2012). Via its globular head domains, C1q can bind to cell surfaces or pathogens, where it binds directly to surface proteins, lipids or other molecules that coat the surface of the cell or pathogen. When C1q binds to one of these molecules, the classical complement cascade can be initiated, and phagocytose or lysis can be induced. Because C1q can interact with a diversity of molecules, it is thought that this mechanism works similar in the CNS. However specific C1q receptors on synapses have not yet been found, so the precise mechanism of C1q binding to synapses remains unknown.

**Complement system in AD**

As mentioned above, the loss of synapses is an important hallmark in AD, and since the complement system plays an important role in synapse pruning, it does not come as a surprise that more than 20 years ago the complement system was already suggested to play a role in AD (Fischer *et al.*, 1995). In this study of Fischer and his colleagues evidence of the upregulation of C1q in AD was found. Even three years before that, an increase in C3 and C4 in AD patients compared to healthy controls was shown (Walker and McGeer, 1992). Since then, interest in the role of the complement system in AD has increased and more studies have put their focus on this. It is suggested by multiple studies that the developmental pathway of the microglia- and complement dependent synaptic pruning is activated in AD, and can lead to the unnecessary loss of healthy synapse and cognitive impairment (Stevens *et al.*, 2007; Hong *et al.*, 2016; Hajishengallis *et al.*, 2017).

Hong and his colleagues provided evidence for the role of the complement system in synaptic pruning in AD (Hong *et al.*, 2016). In their study they found that soluble oAβ and Aβ monomers induce C1q upregulation. Besides, in C1q KO mice the oAβ alone do not cause synapse loss, implying C1q is necessary for synapse loss induced by oAβ, shown in *figure 3*. It is suggested that C1q and oAβ perform in a common mechanism to induce the complement system, and drive microglia via CR3 to eliminate synapses. If oAβ weakens the synapse and reveals a C1q receptor or that they work indirectly together via cytokines, is something that should be studied further. Additionally, the authors showed that the deletion of C3 improves the loss of synapses, and CR3 deletion protected the mice from synapse loss, as shown in *figure 4*. All these data suggested an activation of the normal developmental pruning pathway as main mechanism underlying oAβ dependent synapse loss, in pre-plaque AD brains in AD mice models.

Figure 3: Aβ oligomers mediate synapsin and PSD95 immunoreactive puncta loss in hippocampus (left) but failed to mediate this in C1qaKO mice (right), suggesting C1q is necessary for oAβ induced synapse loss (Hong et al., 2016).

Figure 4: Deletion of C3 prevents the loss of synapses (Hong et al., 2016)


In a study focused on the role of C3 in neurodegeneration, done by Shi and her colleagues, was found that C3 deficiency leads to an improvement of synapse loss in the hippocampal Cornu Ammonis region 3 (CA3) (Shi *et al.*, 2017). Furthermore, C3 deficiency protects against cognitive decline, tested with water T-maze test reversal learning as shown in *figure 5*. A surprizing founding in this study was that the deficiency of C3 leads to increased plaque load. It is suggested that this increased plaque load is due to a muted response of the glial cells, also caused by the C3 deficiency. Despite the increased plaque load, the deficiency of C3 does protect against synapse loss, related with age and AD, shown in *figure 6*. This indicates that plaque load is less dangerous to the AD patient than the reaction of glial cells to the plaques. Another possible theory is that the deficiency of C3 leads to increased oAβ forming into plaques, this reduces the amount of oAβ that can bind to synapses and by that, loss of synapses will be prevented. Nevertheless, this is still just a theory that must be further examined. Overall the results proposed C3 or C3 signalling to be a probable therapeutic target in AD treatment.

Figure 5: Percentage is mice that reached reversal criterion, more than 80% correct choices each day, suggests C3 KO in APP/PS1 protects the mice from cognitive impairment (Shi et al., 2017)

Figure 6: Colocalization of pre- and post-synaptic puncta suggests partial protection against synapse loss in C3 KO (Shi et al., 2017)

**Aβ and its relation with microglia and the complement system**

From the earlier mentioned researches the relation between Aβ, microglia and the complement system comes forward, where oAβ seems to work together with C1q in the synaptic pruning (Hong *et al.*, 2016) and where is speculated that the C3 deficiency leads to increased oAβ forming into plaque, decreasing free oAβ that can bind to synapses (Shi *et al.*, 2017).

The relation between Aβ, microglia and the complement system has been examined before, for example by Wyss-Coray and his colleagues, who showed that the complement system plays a role in the clearance of Aβ plaques, since increase of C3 production was correlated with the reduction of Aβ deposition (Wyss-Coray *et al.*, 2002). This is in agreement with the study of Shi and her colleagues, as stated above (Shi *et al.*, 2017). Wyss-Coray and his colleagues also suggested that the activation of complement components may protect against the toxicity of Aβ, but no behavioural study was done to prove the effect on cognitive decline.

Additionally, a later study of Fu and his colleagues showed that the phagocytosis and clearance of fibrillary Aβ is manifested by microglia, whereby the C3 and its receptor CR3 are involved (Fu *et al.*, 2012). They found that in C3 KO mice the phagocytosis of fibrillary Aβ was lessened. The role of microglia in the clearance of Aβ is variously studied and proven, reviewed by Zuroff and her colleagues (Zuroff *et al.*, 2017). However, the deficiency of C3 leading to decreased fibrillary Aβ is in contrast to the study of Shi and her colleagues, who stated that deficiency of C3 increases plaque load (Shi *et al.*, 2017). An explanation for this difference in results is that the study of Shi is done with insoluble Aβ in APP/PS1 mice, while the study of Fu is performed with C57BL/6 mice with injected Aβ fibrils (Fu *et al.*, 2012; Shi *et al.*, 2017).

A more recent study showed that the absence of CR3 leads to reduction of Aβ deposition and extracellular Aβ (Czirr *et al.*, 2017). Additionally, it was proposed that targeting CR3 with a small molecule could be a possible treatment in AD, since this decreases the Aβ levels. This is in contrast to the study of Shi (Shi *et al.*, 2017). An explanation for this could be, that the study of Czirr did not base these results on APP/PS1 mice but on WT mice with injected Aβ (Czirr *et al.*, 2017).

In a study of Tacnet-Delmore, Chelvallier and Arlaud, the focus was on the interaction between Aβ and complement proteins (Tacnet-Delorme, Chevallier and Arlaud, 2001). This study showed that Aβ fibrils can induce the activation of C1 and thereby activate the classical complement pathway. This activation is done by binding C1q, the classical complement activator, to Aβ fibril through interaction with the recognition site of Aβ in its peripheral globular region. Even though this does not concern synapse pruning, it does show the link between the complement system and the pathogenesis of AD.

**The complement system as a medical target in AD**

In absence of C1q or C3, the loss of synapses is lessened (Hong *et al.*, 2016; Shi *et al.*, 2017). This could mean that when C1q or C3 is blocked, the loss of synapses could be reduced. C1q or C3 as possible therapeutic targets in AD has been an idea proposed by multiple studies (Harvey and Durant, 2014; Hong *et al.*, 2016; Presumey, Bialas and Carroll, 2017; Shi *et al.*, 2017).

C1q and C3 have a lot of important roles regarding the immune system (Barnum, 2017) and are proven to be critical in the development of neuronal circuits (Stevens *et al.*, 2007). Therefore, deficiency of C1q and C3 is not an option as a therapy for AD. Nevertheless, studies with C1q and C3 deficiency can give insight in the possibilities of blocking C1q or C3.
Since C1q lies upstream of C3 in the classical complement system, a lot of the effects of deficient C1q are similar to deficient C3. A few of the effects on mice with deficient C1q and C3 regarding the CNS are that they have impaired eye-specific segregation leading to overlap between the eye-specific regions (Thion and Garel, 2018), they have defects in synaptic pruning (Stevens *et al.*, 2007), or from a different view, can protect synapses from age dependent loss (Schafer *et al.*, 2012; Shi *et al.*, 2017) and they have improved hippocampal function (Morgan, 2017).
In addition, C3 deficiency alone can alter the response of microglia to plaques and can lead to downregulation of TNF-a, IFN-g, and IL-12p70, which are normally elevated in AD brain (Shi *et al.*, 2017). C3 inhibition can also lead to a higher plaque load but meanwhile it protects against cognitive decline. Infections in C3 deficient species are mostly induced by encapsulated bacteria that cannot be tagged by C3 due to a low or absent concentration of C3.

The deficiency of C1q apart can lead to spontaneous seizures (Presumey, Bialas and Carroll, 2017). In C1q deficient mice, the defect in synapse pruning can lead to an overconnected brain and susceptibility to epileptic seizures (Morgan, 2017). In humans, deficiency of C1q at birth is a genetic disorder called Lupus, with symptoms that vary between asymptomatic to having autoimmune diseases and/or infections that are life threatening (Stegert, Bock and Trendelenburg, 2015). In C1q deficiency, approximately 40% suffers from bacterial infections.

These effects of C1q or C3 deficiency are just a few of all the known effects, and they are not all positive. Therefore, it is important to examine the possible side effects that C1q or C3 inhibition can have.

As mentioned earlier, C1q is the first molecule in the classical complement cascade. By inhibiting C1q, only the downstream activation of the classical complement system will be inhibited (McGonigal *et al.*, 2016). This means that the other complement pathways are still intact to tackle the infection of pathogens and prevent tissue damage. A possible antibody for C1q is M1, which binds to C1q and neutralises it, preventing the activation of the classical complement system. In the study of Hong and his colleagues, they already used a comparable antibody against C1q in vitro, called ANX-M1, and found that this binding prevented synapse loss induced by oAβ (Hong *et al.*, 2016).

The C3 receptor is the connection between complement and the microglia (Presumey, Bialas and Carroll, 2017). C3 on synapses is recognised by microglial C3R, after which the synapse is phagocytosed by the microglia. This means, when C3 is blocked, microglia will not recognize the tagged synapses and will not phagocytose them. A possible inhibitor of C3 is Compstatin, which inhibits the cleavage of C3 in C3a and C3b (Egge *et al.*, 2014). The problem with blocking C3 is that it is involved in not only the classical complement system but it is one of the two main proteins of the complement system (Barnum, 2017). Thereby, blocking C3 will cause problems in the alternative and lectin pathways as well, and the chances of getting an infection can rise tremendously.

During development, when C1q and C3 are inhibited, the synaptic deletion and separation of eye-specific regions is impaired, which lead to overlap between the projections of the neurons in the brain (Thion and Garel, 2018). Therefore, the timing of the therapy is crucial, and could only take place after complete development of the circuits of the retinogeniculate system. When exactly, is something that can be clarified by biomarker- and imaging studies (Morgan, 2017).

The biggest problem in finding a treatment for AD is the drug delivery (Morgan, 2017). Right now, the only two anti-complement agents that are currently approved for use, both functioning by disassociating the C1 complex, are no candidate in AD because both agents cannot cross the BBB. The previously mentioned ANX-M1, designed by Annexon biosciences, too cannot cross the BBB. Crossing the BBB is a crucial step in finding a cure for AD, which should be addressed to by researchers.

**Discussion**

In AD, the severity of dementia is mostly correlated to the loss of synapse function. Multiple studies showed a connection between the loss of synapses, microglia and the complement system. It is thought that synapse pruning in AD functions the same way as it does in the development of the retinogeniculate system, where the complement system tags weak synapses, by which the classical complement cascade is activated and leads to the assembling of C3. Whereas CR3 on microglia can bind C3, and phagocytosis of the synapse is followed. Two recent studies demonstrate this in different ways. Hong and his colleagues proved the involvement of oAβ in this synaptic pruning cascade, where is shown that C1q is necessary for the loss of synapses induced by oAβ, and the deletion of C3 or CR3 protects the mice from synapse loss in pre-plaque AD (Hong *et al.*, 2016).
Shi and her colleges focused more on the specific role of C3 in the loss of synapses, and they showed that C3 deficiency protects against cognitive decline and synapse loss, despite the increased plaque load (Shi *et al.*, 2017). They propose C3 or C3 signalling as a probable therapeutic target in AD treatment. Next to C3, C1q is also proposed as probable therapeutic target in AD.
A possible way to target these components of the classical complement pathway is by inhibiting them. C1q is the first protein in the classical complement cascade and inhibiting C1q does not affect the other complement pathways compared to C3 that does, when inhibited. Therefore, it is best to focus on the inhibition of C1q instead of the inhibition of C3. An inhibitor for C1q is ANX-M1, but unfortunately it cannot pass the BBB. The inability of crossing the BBB by medicine is one of the biggest problems in finding a cure for AD and should be focussed on in follow-up studies.

Some of the studies discussed in this review are not in consensus with each other. For instance, the in- or decrease of plaque burden in C3 deficient mice, where Shi and her colleagues found an increase in plaque load with C3 deficient mice, Fu and his colleagues found a decrease (Fu *et al.*, 2012; Shi *et al.*, 2017). An explanation for these findings can be that they both used different mouse models of AD. The same applies to the study of Czirr and his colleagues that showed too, that a deficiency of C3 leads to reduction of Aβ deposition (Czirr *et al.*, 2017). Each model can have a different outcome of a study, just like each species reacts differently to a treatment. When looking for answers about human AD, it is important to take the right model, the one that is the most similar to AD in humans.

AD is not the only disease where complement is involved in synapse pruning. Complement plays a role in synaptic pruning in other forms of dementia, schizophrenia, epilepsy, West Nile infection, Systemic Lupus Erythematosus, Multiple Sclerosis and not to forget disease concerning the visual system like Glaucoma and age-related macular degeneration (Michailidou *et al.*, 2015; Presumey, Bialas and Carroll, 2017; Koenig and Dulla, 2018). In some of the diseases, the mechanisms are thought to be comparable to those of AD, regarding the complement system, like in Multiple Sclerosis, where the C1q-C3 axis of the complement system is activated in the MS hippocampus and the synaptic alterations are thought to be involved with this axis (Michailidou *et al.*, 2015). Other diseases are thought to have a different mechanism, like Schizophrenia where it is not C3 but C4 that tags the synapses, after which the synapses are phagocytosed (Presumey, Bialas and Carroll, 2017). This suggests that the complement, when functioning abnormally, has a general role in neurodevelopmental and -degenerative diseases and it is therefore important that more research is done regarding the relation between the complement and neurodevelopmental and -degenerative diseases.

Unfortunately, a lot about the role of the complement- and microglia dependent synaptic pruning in AD remains unexplained. For instance, the binding of C1q to synapses is mentioned in multiple articles but a C1q receptor on synapses is not yet discovered. This is important in the understanding of how the classical complement system ‘tags’ synapses.
Furthermore, contradictory studies call the role of Aβ in the complement- and microglia dependent synaptic pruning into question (Wyss-Coray *et al.*, 2002; Fu *et al.*, 2012; Czirr *et al.*, 2017; Shi *et al.*, 2017). Does Aβ work together with the complement system in synaptic pruning or plays the complement system a role in clearance of Aβ.
This contradiction also brings into question how to use of the complement in therapy, should it be inhibited to reduce the loss of synapses or should it be activated to enhance Aβ clearance.
The biggest obstacle now in the search for a therapy for AD is finding medicine that can cross the BBB. Only then a drug as a therapy can be an option for AD.

From this review it can be concluded that the classical complement system, in specific C1q and C3, plays an important role in de elimination of synapses in AD. Whereby it is thought and partially proven that the mechanisms of synaptic pruning work similar in the development of the retinogeniculate system as they do in AD. It is suggested that the inhibition of C1q could be used as a potential therapy in AD.

**References**

Alzheimer’s Association (2017) ‘2017 Alzheimer’s Disease Facts and Figures’, *Alzheimers Dement*, 13, pp. 325–373. doi: 10.1016/j.jalz.2017.02.001.

Barnum, S. R. (2017) ‘Complement: A primer for the coming therapeutic revolution’, *Pharmacology and Therapeutics*. Elsevier Inc., 172, pp. 63–72. doi: 10.1016/j.pharmthera.2016.11.014.

Choucair, N. *et al.* (2006) ‘Phagocytic functions of microglial cells in the central nervous system and their importance in two neurodegenerative diseases: Multiple sclerosis and Alzheimer’s disease’, *Central European Journal of Biology*, 1(4), pp. 463–493. doi: 10.2478/s11535-006-0038-y.

Czirr, E. *et al.* (2017) ‘Microglial complement receptor 3 regulates brain Aβ levels through secreted proteolytic activity’, *The Journal of Experimental Medicine*, 214(4), pp. 1081–1092. doi: 10.1084/jem.20162011.

Egge, K. H. *et al.* (2014) ‘Post challenge inhibition of C3 and CD14 attenuates Escherichia coli-induced inflammation in human whole blood’, *Innate Immun*, 25(4), pp. 68–77. doi: 10.1016/j.cogdev.2010.08.003.Personal.

Fettelschoss, A., Zabel, F. and Bachmann, M. F. (2014) ‘Vaccination against Alzheimer disease: An update on future strategies.’, *Human vaccines & immunotherapeutics*, 10(4), pp. 1–5. doi: 10.4161/hv.28183.

Fischer, B. *et al.* (1995) ‘Complement C1q and C3 mRNA expression in the frontal cortex of Alzheimer’s patients’, *Journal of Molecular Medicine*, 73(9), pp. 465–471. doi: 10.1007/BF00202265.

Fu, H. *et al.* (2012) ‘Complement Component C3 and Complement Receptor Type 3 Contribute to the Phagocytosis and Clearance of Fibrillar Aβ by Microglia’, *Glia*, 60(6), pp. 993–1003. doi: 10.1111/j.1743-6109.2008.01122.x.Endothelial.

Gonatas, N. K., Anderson, W. and Evangelista, I. (1967) ‘The contribution of altered synapses in the senile plaque: An electron microscopic study in alzheimer’s dementia’, *Journal of Neuropathology and Experimental Neurology*, pp. 25–39. doi: 10.1097/00005072-196701000-00003.

Hajishengallis, G. *et al.* (2017) ‘Novel mechanisms and functions of complement’, *Nature Immunology*, 18(12), pp. 1288–1298. doi: 10.1038/ni.3858.

Harvey, H. and Durant, S. (2014) ‘The role of glial cells and the complement system in retinal diseases and Alzheimer’s disease: common neural degeneration mechanisms’, *Experimental Brain Research*, 232(11), pp. 3363–3377. doi: 10.1007/s00221-014-4078-7.

Hong, S. *et al.* (2016) ‘Complement and Microglia Mediate Early Synapse Loss in Alzheimer Mouse Models’, *Science*, 352(6286), pp. 712–716. doi: 10.1126/science.aad8373.Complement.

Hong, S., Dissing-Olesen, L. and Stevens, B. (2016) ‘New insights on the role of microglia in synaptic pruning in health and disease’, *Current Opinion in Neurobiology*. Elsevier Ltd, 36, pp. 128–134. doi: 10.1016/j.conb.2015.12.004.

Koenig, J. and Dulla, C. (2018) ‘Complements to the chef: Are microglia eating neurons in the epileptic brain?’, *Epilepsy Currents*, 18(2), pp. 128–130. doi: 10.5698/1535-7597.18.2.128.

Leshchyns’Ka, I. *et al.* (2015) ‘Aβ-dependent reduction of NCAM2-mediated synaptic adhesion contributes to synapse loss in Alzheimer’s disease’, *Nature Communications*, 6. doi: 10.1038/ncomms9836.

Lukácsi, S. *et al.* (2017) ‘The role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in complement-mediated phagocytosis and podosome formation by human phagocytes’, *Immunology Letters*. Elsevier, 189(May 2017), pp. 64–72. doi: 10.1016/j.imlet.2017.05.014.

McGonigal, R. *et al.* (2016) ‘C1q-targeted inhibition of the classical complement pathway prevents injury in a novel mouse model of acute motor axonal neuropathy’, *Acta neuropathologica communications*. Acta Neuropathologica Communications, 4, p. 23. doi: 10.1186/s40478-016-0291-x.

Michailidou, I. *et al.* (2015) ‘Complement C1q-C3-associated synaptic changes in multiple sclerosis hippocampus’, *Annals of Neurology*, 77(6), pp. 1007–1026. doi: 10.1002/ana.24398.

Mohamed, T., Shakeri, A. and Rao, P. P. N. (2016) ‘Amyloid cascade in Alzheimer’s disease: Recent advances in medicinal chemistry’, *European Journal of Medicinal Chemistry*. Elsevier Masson SAS, 113, pp. 258–272. doi: 10.1016/j.ejmech.2016.02.049.

Morgan, B. P. (2017) ‘Complement in the pathogenesis of Alzheimer’s disease’, *Seminars in Immunopathology*. Seminars in Immunopathology, pp. 113–124. doi: 10.1007/s00281-017-0662-9.

Mroczko, B. *et al.* (2018) ‘Amyloid β oligomers (AβOs) in Alzheimer’s disease’, *Journal of Neural Transmission*. Springer Vienna, 125(2), pp. 177–191. doi: 10.1007/s00702-017-1820-x.

Navarro, V. *et al.* (2018) ‘Microglia in Alzheimer’s disease: Activated, dysfunctional or degenerative’, *Frontiers in Aging Neuroscience*, 10(MAY), pp. 1–8. doi: 10.3389/fnagi.2018.00140.

Paolicelli, R. C. *et al.* (2011) ‘Synaptic Pruning by Microglia Is Necessary for Normal Brain Synaptic Pruning by Microglia Is Necessary for Normal Brain Development’, *Science*, 333(September), pp. 1456–1459. doi: 10.1126/science.1202529.

Paolicelli, R. C. *et al.* (2017) ‘TDP-43 Depletion in Microglia Promotes Amyloid Clearance but Also Induces Synapse Loss’, *Neuron*. Elsevier Inc., 95(2), p. 297–308.e6. doi: 10.1016/j.neuron.2017.05.037.

Pozueta, J., Lefort, R. and Shelanski, M. L. (2013) ‘Synaptic changes in Alzheimer’s disease and its models’, *Neuroscience*, 251(2013), pp. 51–65. doi: 10.1016/j.neuroscience.2012.05.050.

Presumey, J., Bialas, A. R. and Carroll, M. C. (2017) *Complement System in Neural Synapse Elimination in Development and Disease*. 1st edn, *Advances in Immunology*. 1st edn. Elsevier Inc. doi: 10.1016/bs.ai.2017.06.004.

Price, K. A. *et al.* (2014) ‘Altered synaptic structure in the hippocampus in a mouse model of Alzheimer’s disease with soluble amyloid-β oligomers and no plaque pathology’, *Molecular neurodegeneration*, 9, p. 41. doi: 10.1186/1750-1326-9-41.

Puzzo, D. *et al.* (2015) ‘Rodent models for Alzheimer’s disease drug discovery Daniela’, *Expert Opinion on Drug Discovery*, 10(7), pp. 703–711. doi: 10.1517/17460441.2015.1041913.Rodent.

Schafer, D. P. *et al.* (2012) ‘Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner’, *Neuron*. Elsevier Inc., 74(4), pp. 691–705. doi: 10.1016/j.neuron.2012.03.026.

Sekar, A. *et al.* (2016) ‘Schizophrenia risk from complex variation of complement component 4’, *Nature*. Nature Publishing Group, 530(7589), pp. 177–183. doi: 10.1038/nature16549.

Shi, Q. *et al.* (2017) ‘Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice’, *Science Translational Medicine*, 9(392). doi: 10.1126/scitranslmed.aaf6295.

Stegert, M., Bock, M. and Trendelenburg, M. (2015) ‘Clinical presentation of human C1q deficiency: How much of a lupus?’, *Molecular Immunology*. Elsevier Ltd, 67(1), pp. 3–11. doi: 10.1016/j.molimm.2015.03.007.

Stephan, A. H., Barres, B. A. and Stevens, B. (2012) ‘The Complement System: An Unexpected Role in Synaptic Pruning During Development and Disease’, *Annual Review of Neuroscience*, 35(1), pp. 369–389. doi: 10.1146/annurev-neuro-061010-113810.

Stevens, B. *et al.* (2007) ‘The Classical Complement Cascade Mediates CNS Synapse Elimination’, *Cell*, 131(6), pp. 1164–1178. doi: 10.1016/j.cell.2007.10.036.

Tacnet-Delorme, P., Chevallier, S. and Arlaud, G. J. (2001) ‘β-Amyloid Fibrils Activate the C1 Complex of Complement Under Physiological Conditions: Evidence for a Binding Site for A on the C1q Globular Regions’, *The Journal of Immunology*, 167(11), pp. 6374–6381. doi: 10.4049/jimmunol.167.11.6374.

Thion, M. S. and Garel, S. (2018) ‘Microglia Under the Spotlight: Activity and Complement-Dependent Engulfment of Synapses’, *Trends in Neurosciences*. Elsevier Ltd, 41(6), pp. 332–334. doi: 10.1016/j.tins.2018.03.017.

Veerhuis, R., Nielsen, H. M. and Tenner, A. J. (2011) ‘Complement in the brain’, *Molecular Immunology*, 48(14), pp. 1592–1603. doi: 10.1016/j.molimm.2011.04.003.

Walker, D. G. and McGeer, P. L. (1992) ‘Complement gene expression in human brain: comparison between normal and Alzheimer disease cases.’, *Brain research. Molecular brain research*, 14(1–2), pp. 109–16. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1323007.

Wyss-Coray, T. *et al.* (2002) ‘Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer’s mice’, *Proceedings of the National Academy of Sciences*, 99(16), pp. 10837–10842. doi: 10.1073/pnas.162350199.

Zuroff, L. *et al.* (2017) *Clearance of cerebral Aβ in Alzheimer’s disease: reassessing the role of microglia and monocytes*, *Cellular and Molecular Life Sciences*. Springer International Publishing. doi: 10.1007/s00018-017-2463-7.