Nervous immunity: A study on the role of complement system in neuronal degeneration and regeneration

Sta, M.

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Chapter 1:

Complement activation in neurodegenerative disorders

M. Sta and F. Baas
Introduction: chapter 1
Complement activation in neurodegenerative disorders

Structures of the nervous system

The nervous system is commonly divided into central and peripheral components. The central nervous system (CNS), located within the skull and spinal canal, consists of the brain, retinæ, optic nerves, optic tracts and the spinal cord. The peripheral nervous system (PNS) consists of cranial nerves, afferent dorsal roots and efferent ventral root contiguous with peripheral nerves that run back and forth between CNS and muscles, organs and skin.

It is not only the location and the connections with the target organs that make a difference between CNS and PNS. There are also differences in structure: the brain contains layers of neurons and supportive cells that form networks. In the spinal cord large bundles of neurons run from top to bottom and vice versa.

The PNS is different; it consists of nerves, or nerve bundles, which are made up by long axons that connect muscle, skin or organs to the central nervous system. Neuronal cell bodies are located in two distinct locations, the sensory neuron cell bodies lie close to spinal cord in so called dorsal root ganglions (DRGs); the cell bodies of motor neurons are located in the ventral part of the spinal cord. Their axons extend from this location all the way to their target. Axons can be over a meter long. Peripheral nerves consist of axon bundles, myelinated and unmyelinated, surrounded by the perineurium. Multiple bundles together form the nerve, which is enclosed by another layer of connective tissue, called the epineurium (for a schematic view of a peripheral nerve see Fig. 1).

Both the CNS and PNS are able to regenerate their axons after damage. However, their capacities to do so are different. The nerves in the PNS readily regenerate after injury or damage, but the axons in the CNS are often incapable of regeneration. A part of this difference is attributed to the differences in the interneural connections between the CNS and the PNS. As stated above the CNS is build up as a complex network of neuronal interactions and supportive cells, whereas the PNS contains long “single” axons connecting in a single lined fashion.

There are other factors that influence the capacity of nerves to regenerate. The myelin associated glycoprotein (MAG), present in CNS myelin, inhibits neurite outgrowth (Tang et al., 1997). MAG is not present in the myelin of the PNS. Furthermore, the space inside the tissues of the CNS is limited, therefore there could be more hinder of edema, within the damage area. In the PNS growth factors and inhibitors direct the axons towards their target (Cai et al., 1999; Xu et al., 1997). If the epineurium is not broken, the neurites will reside inside the endoneurial tube and grow in the right direction, where they eventually reconnect with a target. As with all new axons, only the ones that have reconnected will eventually survive (de Medinaceli L. and Rawlings, 1987).

When the peripheral nerve is damaged, it will degenerate distally from the point of injury. During this process myelin is degraded and axons are lost. Schwann cells dedifferentiate into a more progenitor-like phenotype. This process of degeneration was first described by Augustus Waller in 1850 and therefore called Wallerian Degeneration (WD) (Waller, 1850). It is recognizable under the microscope, because the myelin forms ovoids during degeneration; these ovoids are taken up by phagocytes, such as macrophages or in the absence of phagocytes by Schwann cells themselves. After the axons and myelin sheaths have broken down, the regeneration process starts. During this process the Schwann cells reprogram to a more progenitor-like state. They secrete extracellular matrix proteins such as laminin and they will align inside the endoneurial tube (Chen et al., 2005; Chen and Strickland, 2003).
 Axons sprout from the damaged tip and grow towards the target (Koltzenburg and Bendzus, 2004; Lichtman and Fraser, 2001; Verma et al., 2005). Guidance proteins, a combination of growth factors and repellant proteins, are expressed along the endoneurial tract to force the growth into one direction (Pan et al., 2003). Axons of the peripheral nerves can grow with a maximum speed of 1-3 mm a day (Wood et al., 2011; Waller, 1850). During this axonal regeneration phase the Schwann cells engage in a one to one relationship with an axon and start the remyelination process (Chen et al., 2005). At the end, ideally, each neuron will have established contact with its target.

**Neuro-inflammation**

It has long been thought that the nervous system, especially the central nervous system was immune-privileged, meaning shielded from blood-derived inflammatory cells and factors by the blood-brain-barrier. However, it becomes more and more clear that this is not the case. In the CNS the immune system is activated during disease and neurodegeneration, this will be discussed in further detail below. Additionally, it is shown that during WD in peripheral nerves the innate system is activated. Many studies show that endoneurial immune cells become activated whilst inflammatory factors and cells from the blood stream are found in the diseased nervous system (Ginsberg et al., 2004; Karanth et al., 2006; McGeer and McGeer, 2001; Wang et al., 2006; Zilka et al., 2006; Zipp and Aktas, 2006). In nervous tissue many inflammatory proteins are found during disease and degeneration. These proteins can either be produced by endoneurial or blood-derived cells, lymphoid tissue or liver; some of the inflammatory proteins are produced by nervous tissue itself. One of the important factors in neural inflammation is the complement system.

**Complement system**

The complement system, a highly conserved part of the innate immune system, consists of a cascade of self-cleaving proteases that act as a first line of defense against pathogens (Sjoberg et al., 2009; Ricklin et al., 2010; Morgan, 2000). The complement system, a key component of innate immunity, defends against infections and disposes of dead or dying cells. Because complement can harm self-tissue, activation is tightly controlled by regulators to eliminate pathogens or damaged cells without injuring the host. In addition, complement activation may link innate to adaptive immune responses.
Complement activation in neurodegenerative disorders

The complement system can be activated via three pathways, the classical, lectin and alternative pathway (Fig. 2). The classical and lectin pathways are initiated by pattern recognition proteins (PRPs). PRPs recognize repeating patterns on membranes, so called pathogen-associated membrane patterns (PAMP). In case of the classical activation pathway this is done by C1q which recognizes antigen/antibody complexes. Upon recognition the proteins become active and associate with proteolytic components, C1r and C1s. The lectin pathway is activated by the binding of mannose binding lectin, which recognizes mannose (sugar) patterns. Upon binding the membrane associated serine protease 2 (MASP2), a proteolytic component, becomes active. The proteases, C1q-r-s and MBL-MASP2 complexes, cleave C2 and C4 protein that can in turn associate and form the C3 convertase (C4b2C3b complex). C3 is the main effector molecule of the complement cascade. All pathways converge at the level of C3 cleavage. C3 convertase cleaves the C3 in C3a and C3b, C3b associates with the C4b2C3b complex forming C5 convertase (C4b2C3b2C3b), which cleaves C5 into C5a and C5b. C5b acts as an anchor molecule for the membrane attack complex, a pore forming structure consisting of C5b-C6-C7-C8 and C9 (MAC or C5b-9). Additionally, anaphylatoxins C3a and C5a are powerful inducers of proinflammatory cytokines and induce attraction of phagocytes. The activators of both the classical and the lectin pathway are membrane bound. In contrast, the alternative pathway is activated in fluid phase by spontaneous conversion of the C3 molecule into its active component. The C3b alone is highly unstable, but can associate with factor B, which is cleaved by factor D, to form the active C3bBb convertase. This C3bBb is a C3 convertase, which binds to membranes; this binding is stabilized by Properdin. On foreign cells the amount of C3bBb rapidly increases, but is strictly regulated on self-targets. A potent regulator of the alternative pathway is factor H, which induces a fast turn-over of the C3bBb complex, by breaking the link between C3b and the Bb proteins. Additionally, factor H also works on membrane-bound C3bBb by allowing binding of yet another regulator of the complement system factor I, which degrades C3b (for a detailed review on the complement system see Ricklin et al., 2010). When this balance between activation and inhibition is disrupted, complement activation causes injury and contributes to pathology in various diseases (Harris et al., 2012). One must keep in mind that activation of the alternative pathway accounts for approximately 80% of the complement activity, even if the initial activation occurred via either the classical or the lectin pathway (Brouwer et al., 2006).

Complement activation during neurodegenerative processes is mainly activated via the classical and the alternative pathways.

Complement and neuro-Inflammation

Activation of the complement system is often seen in the nervous system after damage or in disease. In the PNS activation of the complement system is found after damage by traumatic injury or in diseases, such as Charcot-Marie-Tooth (CMT) (Gabriel et al., 2002). After traumatic injury or in CMT, deposition of components of the complement system, such as C1q, C3 and MAC is observed on neuronal cells (Ramaglia et al., 2007; Ramaglia et al., 2008a). There are indications that initiation of the complement system and signaling via several receptors contribute to the damage. For example, complement activation can attract and activate phagocytes and induce a full-blown immune response. Complement activation can trigger attraction and diapedesis of adaptive immune cells and induce production of several pro- and anti-inflammatory cytokines, chemokines and interleukins (Ruohonen et al., 2005). The primary goal, to obtain clearance of dead cells and debris, might overshoot and, as a consequence, excess damage to the neuronal structure can occur.
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Figure 2: Schematic representation of the complement system: Direction of reactions is shown by arrows, inhibitors are shown in the gray squares, and cofactors are shown in the grey rounds. Both Classical pathway and Lectin pathway are mostly membrane bound. The Alternative pathway initiation takes place in the fluid phase.

**Complement activation modifies neurodegeneration**

Evidence that complement plays a dual role in neurodegeneration is confirmed by findings that report a beneficial effect of initial complement activation, but during later stages of disease, activation of the complement system results in more degeneration (Alexander et al., 2008).

In a rat sciatic nerve crush injury model, the complement system is responsible for rapid Wallerian degeneration (WD) (Ramaglia et al., 2007). These studies also showed that complement activation also influences the subsequent regeneration after injury of the peripheral nerves. Degeneration and regeneration were determined in a crush injury model of the sciatic nerve in rats that are C6 sufficient (WT) and rats which are C6 deficient (C6−/−). The C6−/− animals have a 31-bp deletion in the C6 gene resulting in production of a non-functional mRNA. These C6−/− animals are able to activate the upstream complement system including C3 and C5 splicing, but are unable to form MAC. This experiment demonstrated that active complement components, C1q and C3 appeared within one day after sciatic nerve crush in WT animals. MAC was found as early as 24h PI. The degeneration was quite profound at 72h PI and sensory recovery took almost 4 weeks. The C6−/− rats displayed a slower WD than the WT animals. C6−/− animals also showed a decreased amount of infiltrated and activated macrophages in affected
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nerve (Ramaglia et al., 2007). Surprisingly, regeneration was faster in C6\(^{-/-}\) animals compared to WTs; Time to full recovery in the C6\(^{-/-}\) animals was approximately 2-3 weeks in contrast to WTs in which the recovery time was approximately 3-4 weeks. Additionally, at the end of the experiment the regenerated nerves of the C6\(^{-/-}\) showed a better morphology; they exhibited more large myelinated fibers and smaller inter axonal spacing, resembling uninjured controls rather than WT animals. When the C6\(^{-/-}\) animals were reconstituted with functional C6, WD, regeneration and morphology returned to pattern found in WT animals (Ramaglia et al., 2009b). A delay in WD was also found when complement inhibition was obtained by pharmacological inhibition with a recombinant soluble complement receptor 1 (sCR1), which resulted in protection against early axon loss during nerve degeneration (Ramaglia et al., 2008b).

Another study demonstrated that mice lacking the complement regulator CD59a, which regulates MAC deposition, showed an exacerbated WD phenotype (Ramaglia et al., 2009a) after nerve crush. This was characterized by a more rapid degeneration and more influx and presence of activated macrophages in the damaged nerves.

The findings on the modifying role of the complement system in neurodegeneration are supported by various genetic studies. This demonstrates how regulation of complement activation and the activation state of the complement system affect degeneration and regeneration of the nerve. Variations in complement activation state might affect the susceptibility to and the outcome of peripheral nerve disease. Polymorphisms in complement inhibitors can affect their regulatory capacity (Ferreira et al., 2010). For example, age-related macula degeneration (ARMD) can be triggered by an inappropriate activation of the complement system (Anderson et al., 2010), linked to a single amino acid polymorphism in the CFH (Y402H), as shown by GWAS (Haines et al., 2005). Studies done on the Y402H polymorphism in a population of ARMD patients showed that it may account for a substantial portion of the ARMD in individuals (Despriet et al., 2006). Especially, when the Y402H mutation coincides with mutations in other genes of the complement system, for example C3. The C3 polymorphisms R102G and P314L are also associated with ARMD outcome (Despriet et al., 2009). In contrast, the presence of isoleucin on codon 62 (Ile62) in CCP1 domain of CFH is protective for the development of ARMD. In contrast the presence of a valine on codon 62 (Val62) is associated with a poor prognosis (Tortajada et al., 2009).

**Neuro-inflammation**

Not only complement is activated during the disease or damage processes of the nervous system. It is known is that the complement system can act as a switch between the innate and adaptive immune systems. Induction of the adaptive immune response results from signaling via complement factors and their receptors on specific cells, resulting in production and secretion of interleukins, chemokines and cytokines, secondary complement effects. One of the effects of complement activation is the attraction and activation. Other effects that complement activation can have is the induction of antigen presentation, the attraction of leukocytes and trigger activation of these cells as well as the production of various inflammatory factors such as cytokines, chemokines and interleukins, via direct and indirect signaling.

A genetic study in the rat nerve crush model has demonstrated production of various interleukins (Ruohonen et al., 2005), amongst which IL1, IL2, IL12, IL17 and IL18 (Menge et al., 2001) as well as an array of cytokines, for example Ccl2, also known as MCP1, a powerful macrophage attractant. All these factors contribute to or are an indication of the evoking of an adaptive immune response.
Benefits of complement inhibition?

We have previously described that inhibition of the terminal complement complex (MAC) in crush injured nerves of rats resulted in a less severe degeneration and improved regeneration. It is unclear why the inability to form MAC causes those effects, but there are several possible explanations. One is that long term (over)activity of the complement system, possibly induces collateral damage secondary to the initial physical damage. Another explanation is that reduction in influx of hematogenous macrophages results in a more subtle clearance of myelin by Schwann cells, which contributes to minimize secondary damage. Along the same line, we can argue that the lack of macrophage activation in the degenerating C6^{-/-} nerve is also another reason for the faster recovery. Maybe the macrophages destroy too much of the structure of the peripheral nerve that would give guidance to the regenerating axons.

In this thesis we investigate the mechanism via which complement deficiency results in improved regeneration of peripheral nerves after injury.

In chapter 2 of this thesis we describe the role of macrophages in degeneration of the peripheral nerve after crush injury. We wanted to determine whether macrophages are responsible for the effect that MAC has on degeneration and regeneration of the peripheral nerve after crush injury or if MAC itself is responsible for this effect? To determine the attribution of macrophages to WD we used a sciatic nerve crush injury model in WT rats. In these rats we depleted the hematogenous macrophages by administration of clodronate-containing liposomes. This study showed that following sciatic nerve crush injury myelin clearance was delayed in nerves of macrophage-depleted animals. Macrophage depletion had no effect on the axonal degeneration after injury. We found that at 72h after the crush injury all nerves showed complement activation, with or without the presence of macrophages. This indicates that after crush injury the complement system is activated regardless of macrophage presence. However, the amount of C9 deposition was lower in macrophage-depleted animals than PBS treated animals. The levels of C9 deposition positively correlated with the amount of macrophages in the nerve at 5 mm distal of the crush. This suggests that macrophage activation contributes to MAC deposition in neurodegeneration.

In chapter 3 we analyzed expression profiles of uninjured and crushed sciatic nerves and their DRGs from WT and C6^{-/-} rats. We isolated mRNA from the uninjured and injured nerves and DRGs at 72 h PI. A microarray analysis was used to determine the changes in gene expression after crush injury. We used mRNA from DRGs to analyze the changes in sensory neuron gene expression and isolated mRNA of the sciatic nerve that represents the changes in gene expression at the crush site. Many biological pathways are regulated after crush. We focused our analysis to the pathways and genes associated with the immune system and inflammatory responses. Additionally we looked at changes in genes associated with neurite outgrowth and extracellular remodeling. This genetic analysis revealed a difference between WT and C6^{-/-} animals in their response to damage. The data showed that WT animals display an M1 macrophage phenotype and presence of Th1 cells. Both associated with a pro-inflammatory response. C6^{-/-} animals showed an M2 macrophage response to nerve crush. Presence of IL10 suggests a role for Th2 cells. Additionally, markers for pro-inflammatory Th17 cells suggest the C6^{-/-} animals use T cell driven clearance of damaged structures rather than macrophage-driven clearance of damaged structures. Presence of CD25 in C6^{-/-} animals indicates that regulatory T cells are also present in the damaged nerves, probably to inhibit a pro-inflammatory T cell response.
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Next to the analysis of neurodegeneration and regeneration by morphological and molecular techniques, we developed a model for ex vivo analysis of nerve function. This new method to study functional changes during the degeneration and regeneration process is described in chapter 4. We compared the new method of ex vivo electrophysiology with traditional methods to study peripheral nerve degeneration and regeneration after injury. We delivered a crush with a standardized intensity and measured the ability of the nerve to generate a compound action potential along its entire length at several time points PI. The findings were compared with morphological data obtained by immunohistochemistry and with functional recovery data obtained using an in vivo test for sensory recovery. We concluded that our method corresponds with findings obtained with traditional methods. Our method, however, is a good addition to the current tools to study the degeneration and subsequent regeneration of peripheral nerves after injury, because it provides more detailed information on early functional recovery and allows researchers to monitor the functional recovery along the entire length of the nerve starting as early as 48 h PI (Sta et al., 2014).

This method is used in chapter 5 to determine whether the observed effect of faster sensory recovery in C6\(^{-}\) rats after crush injury is caused by the inability to form MAC or if it is attributable to an intrinsic difference in regrowth properties between the two rat strains. We used a sciatic nerve crush injury model in which the activation of the complement system is minor, while still sufficient damage is delivered to induce WD. In this way, the crush delivered in this experiment could be used to determine the contribution of C6 status and genetic background on axonal regrowth. We found no difference in regrowth properties, onset and propagation between WT and C6\(^{-}\) animals. WT and C6\(^{-}\) animal showed similar morphology throughout the degeneration and regeneration process. The functional characteristics of the nerves and sensory recovery were similar throughout the experiment as well. The data presented in this chapter shows that complement C6 deficiency on its own does not alter axonal regrowth after sciatic nerve injury. This proves that the faster recovery after nerve crush in the C6\(^{-}\) animals previously observed is due to the inability to form MAC and not due to an underlying trait that causes C6\(^{-}\) peripheral nerves to regenerate faster after injury.

In chapter 6 we determined the effect of C6 deficiency on the degeneration and regeneration of peripheral nerves after a transection-resuture injury. A transection-resuture injury differs from a crush injury, because transection disrupts the epineurium and the blood-nerve barrier. The area of initial damage to the axons and myelin is much smaller in the transection-resuture (0.1 mm wide), than in the crush injury (2.0 mm wide).

In this model WD progressed slowly from the site of injury distally. At 72 h PI, degradation of myelin and axons was observed at and right adjacent to the transection site, but had not spread further distally. The influx of blood-derived cells was also limited to the transection site, but the numbers of cells were very high. The complement system was less activated in transected-resutured nerves than in crush-injured nerves. Functionally and morphologically WT and C6\(^{-}\) crushed nerves recovered equally well.

The complicated role of complement in the central nervous system

In the CNS complement activation is also observed. In the CNS complement activation has a dual role. In the healthy central nervous system, complement activation is necessary for synaptic remodeling, especially during the embryonic development (Stevens et al 2007a). Complement activation is also observed during various neurodegenerative diseases of the CNS, such as Alzheimer’s disease (AD) (Eikelenboom et al., 1988; Farkas et al., 2003; Kolev et al.,...
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2009; Nerl et al., 1982; Veerhuis et al., 1998; Webster et al., 2000; Zhou et al., 2008), Parkinson’s disease (PD) (Goldknope et al., 2006), Huntington disease (HD), amyotrophic lateral sclerosis (ALS) (Nerl et al., 1984; Goldknope et al., 2006; Morgan et al., 1984; Sta et al., 2011; Woodruff et al., 2008), multiple sclerosis (MS) (Morgan et al., 1984), traumatic brain injury (TBI) and age related macular degeneration (ARMD) (Camara-Lemarroy et al., 2010; Zipp and Aktas, 2006). The activation of complement during neurodegeneration in the CNS is either neuroprotective or destructive (Joebberg et al., 2009). This has been described in various animal models mimicking neurodegenerative diseases, in which complement activation was found at different stages of disease progression (Alexander et al., 2008; Martini and Toyka, 2004; Morgan and Gasque, 1996; Ramaglia and Baas, 2009). A short overview of the animal models and the findings on complement timing are shown in table 1.

The protective and destructive nature of complement activation in neurodegenerative disorders is illustrated by a mouse model of AD (Alexander et al., 2008). During the early stages of the disease, complement activation is beneficial. In the mouse model for AD C1q and C3 target amyloid beta plaques. Maier et al. (2008) found that deficiency for C3 accelerates amyloid plaque deposition and modifies microglia and macrophage phenotypes.

In later stages of AD, chronic complement activation is associated with more neurodegeneration (Alexander et al., 2008). In addition, mice that received C5aR antagonists showed better memory scores and less plaque deposition (Fonseca et al., 2004). Similar findings were obtained in the SOD1 mutant rat, a model of ALS where C5aR antagonist treatment increased neuronal survival (Woodruff et al., 2008). In contrast, deficiency for C1q did not alter neuronal survival in a SOD1 G37R mutant mouse (Lobsiger et al., 2013).

Complement activation in the nervous system is not always destructive, it helps to maintain homeostasis in the brain (Nayak et al., 2010). Additionally, it has been described that C1q plays an important role in the developing brain, where it is involved in synapse remodeling. This process is necessary to decrease the amount of initial synapses formed during development (Yuzaki, 2010; Fourgeaud and Boulanger, 2007). For example, mice with a deletion in the C1q gene (C1q−), showed abnormal projection of retinal ganglion cells (RGCs) on the neurons of the lateral geniculate nucleus (LGN). Normally, the LGN neurons are innervated by only 1-2 RGCs, whereas in the C1q− mouse the embryonic situation is maintained, LGN neurons that are innervated by multiple (ten or more) RGCs (Chu et al., 2010; Stevens et al., 2007; Fourgeaud and Boulanger, 2007). Similar results were obtained from C3 deficient animals. The findings in the animal models are also true for the human diseases. Studies showed that brain and spinal cord tissue of ALS patients, both sporadic (sALS) and familial (fALS) showed positivity for complement components C1q, C3 and MAC. We also found that C1q and C4 were produced by neurons and microglia inside the spinal cord of these patients (Sta et al. 2011 (chapter 7). Additionally, it is shown that remaining muscle endplates of deceased ALS patients showed positivity for MAC (unpublished data: N. Bahia El idrissi et al. 2014). In AD patients depositions of C1q and C3 were found on plaques in brain, Complement activation is also observed spinal cord of MS patients and in brain from epileptic patients.

The complement system is able to trigger the adaptive immune response in the CNS as well; various studies report evidence of an adaptive immune response during neurodegenerative disease. For example: in MS evidence of a massive macrophage/phagocyte influx is observed in the lesions of patients’ spinal cord tissue (Vogel et al., 2014).
Table 1: Examples of Complement system activation in animal models of neurodegenerative diseases.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Disease mimic</th>
<th>Complement</th>
<th>References</th>
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<tbody>
<tr>
<td>Mouse (SOD1 G93A)</td>
<td>Amyotrophic lateral sclerosis (ALS)</td>
<td>- C1q, C3 found in spinal cord during asymptomatic and symptomatic stages of the disease. - Unable to detect MAC during any stage of the disease in the spinal cord. - MAC positivity observed on neuromuscular junctions in the SOD1 mouse model.</td>
<td>(Heurich et al., 2011), unpublished results M. Sta El Baha al Idrissi et al., 2014 (unpublished)</td>
</tr>
<tr>
<td>Mouse (SOD1 G37A)</td>
<td>ALS</td>
<td>No improvement of neuronal survival in absence of C1q, during disease progression.</td>
<td>(Lobsiger et al., 2013)</td>
</tr>
<tr>
<td>Rat (human SOD1 (G93A))</td>
<td>ALS</td>
<td>- Inhibition of complement activity by treatment with C5aR antagonist increases neuronal survival.</td>
<td>(Woodruff et al., 2008)</td>
</tr>
<tr>
<td>Rat (PVG), mouse (BalbC)</td>
<td>Traumatic brain injury (TBI)</td>
<td>- Brains show positivity for C1q, C3 and MAC as early as 24h PI. - Inhibition of complement improves neuronal survival after TBI.</td>
<td>(Fluiter et al., 2014), (Leinhase et al., 2006)</td>
</tr>
<tr>
<td>Mouse (C57 Bl6) with multicopy PMP22</td>
<td>Chronic demyelinating poly neuropathy</td>
<td>- Nerves are positive for C1q, C3 and MAC.</td>
<td>(de Jonge et al., 2004)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Alzheimer’s disease</td>
<td>- C1q is found on plaques in preclinical AD - Absence of C1q is neuroprotective in AD - Early and late phase of the disease C3 deposition on amyloid beta plaques, - C3 deposition protective during early phases; absence of C3 exacerbated disease progression. During late stages of disease presence of complement is associated with rapid progression. - Treatment of mice with anti-C5aR antagonists is protective.</td>
<td>(Alexander et al., 2008; Fonseca et al., 2004a; Fonseca et al., 2004b)</td>
</tr>
<tr>
<td>Mouse - Experimental autoimmune encephalitis (EAE)</td>
<td>Multiple sclerosis (MS)</td>
<td>- Complement positivity was observed in the affected areas. - MBL mediated complement activation - A key factor in the neurodegenerative processes ongoing in disease and trauma. - Genetic deletion of inhibitors accelerates disease progression.</td>
<td>(Ingram et al., 2009), (Kwok et al., 2011), (Hundgeburth et al., 2013; Rus et al., 2005), (Ramaglia et al., 2012)</td>
</tr>
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</table>

In the cerebrospinal fluid of relapsing remitting (RR) MS patients, regulation of IL1beta, IL6, IL12, IL4, IL10, IL17, TNF-alpha and IFN-gamma is reported. These cytokines are associated with the disease state in RR MS patients. IL6, IL12, IL4 and IFN-gamma are upregulated in RR MS
patients; IL10 and TNF alpha are associated with the inactive phase of the disease (Kallaur et al., 2013).

**Uncertainties and our research:**

In this thesis project, we studied complement activation in a neurodegenerative disease of the CNS. We studied whether complement activation was associated with the progression rate, motor neuron loss, in sporadic amyotrophic lateral sclerosis (sALS).

ALS is a motor neuron disease, hallmarked by the progressive loss of motor neurons in the spinal cord and motor cortex. Until now the mechanisms of the disease are largely unknown. Some genetic mutations are associated with the development of ALS, such as mutations in superoxide dismutase 1 (SOD1), FUS, DCTN1, VAPB, VCP, ANG, FIG4, C9orf72, OPTN, TARDBP, SQSTM1, UBQLN2, PFN1, DCTN1, ERBB4, UNC13A, Alsin, SETX, SPG11, SigMAR1, CHMP2B, DAO (for a full list of known genes, see review Finsterer et al 2014). Mutations found in familial ALS (fALS) are associated with increased tendency to form misfolded or aggregated proteins (Trippier et al., 2012) as well as genes that are associated with RNA-processing proteins (Verma and Tandan, 2013; Verma, 2013). Other factors also play a role in disease development, like glutamate excitotoxicity (Milanese et al., 2013). Findings in SOD1 mutant rats showed that activation of the complement system occurs and that inhibiting signaling of the complement 5 protein and its receptor improved motor neuron survival (Woodruff et al., 2008). In contrast, studies in a SOD1 G37R mutant mouse showed that there was no effect of the depletion of C1q, and the inhibition of the classical complement activation pathway, on the motor neuron degeneration and synapse loss. Additionally, this study showed that depletion of C3 did not affect the progression of motor neuron degeneration either (Lobsiger et al., 2013). So there is no simple explanation for the role of complement in ALS in animal models.

In chapters 7 and 8 we discuss the activation of the innate and adaptive immune system in sALS patients, as well as findings on the expression and presence of toll-like receptors (TLRs) in ALS patients. TLRs, first described in fruit flies, are part of the innate immune system and trigger adaptive immune responses specific for the type of infection. TLRs are so-called pattern recognition receptors (PRRs). The different TLRs all recognize their own specific target for example some TLRs recognize single stranded RNA, whereas others recognize bacterial factors such as Lipo-poly-saccharide (LPS) (Medzhitov and Janeway, Jr., 2000; Medzhitov, 2013; Iwasaki and Medzhitov, 2004; Barton and Medzhitov, 2002; Barton and Medzhitov, 2003). Because all TLRs have their specific target, determining which TLRs are activated might be a good tool to predict what process or infection with what pathogenic agent triggers ALS. Our findings in the studies of the innate and adaptive immunity in ALS showed that there was evidence of complement activation in the spinal cord and motor cortex of all sALS patients. Presence of C1q, C3c and C5b-9 was observed on motor neurons in spinal cord and motor cortex. We found that C1q and C4 are produced by neurons and microglia in the ALS patients. Additionally, we found evidence of a cellular adaptive immune response in the sALS patients. Immature and mature dendritic cells, CD3+, CD8+ positive T lymphocytes, HLA-DR and CD68+ cells were found in spinal cord and motor cortex (Sta et al., 2011).

We found higher levels of complement activation in fast progressing ALS compared to cases with a slow progression. In both forms, complement activation is a general indicator of neurodegeneration. We also found that the presence of adapted immune cells were associated with disease progression. Dendritic cells inside the spinal cord were associated with a rapid
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progression of the disease, whereas the presence of CD8\(^+\) T cells was higher in slowly progressing ALS patients.

In the last chapter, chapter 9, of this thesis we discuss our findings against the background of what we know about complement in neuronal degeneration and regeneration. How our data contribute to the knowledge of the function of complement activation during neurodegeneration and how these processes can be influenced by complement deficiency or inhibition. There are more indications that the amount of initial complement activation is important for the outcome of disease progression (Lobsiger et al., 2013). There are indications that in a crush injury model, the amount of initial complement activation is, at least partially, dependent on the amount of myelin and axonal damage in the nerve. We should consider the factors amount and timing of complement activation before using complement inhibition to promote peripheral nerve regeneration, since it is only applicable in a case of substantial and/or chronic activation of the complement system. In summary: we conclude that controlling complement activation might provide a powerful tool to delay neurodegenerative diseases, by allowing early activation and inhibition at later stages. We speculate that variation in the activity of complement inhibitors will affect disease outcome.

Amount and timing

The data presented in this thesis combined with the existing knowledge on complement in the nervous system suggest that the amount and timing of complement activation affects the outcome of neuronal degeneration and regeneration. From our work and that of others, we propose the following model: Early complement activation in neurodegenerative diseases slows down the disease progression because the amount of complement activation is low. Long-term activation of the complement system, or complement activation at a later stage in the disease is almost always associated with poorer outcome. Thus the timing of complement activation is important, because during later stages of disease, more target areas are available which might result in a massive over-activation of the complement system. Additionally, chronic activation of the complement system will trigger unwanted responses of the adaptive immune system or other cellular responses that lead to collateral damage (Zipfel, 2009; Morgan, 2000).

Our hypothesis predicts that it is important to prevent long term activation of the complement system. This is supported by the findings that defects in regulatory molecules of the complement system, which result in excess activation, are associated with susceptibility for neurodegenerative disease (Alexander et al., 2008; Morgan and Gasque, 1996; Morgan et al., 1997). The findings on ARMD show that in those cases, where there is a deficiency for one of the inhibitors of the membrane attack complex, like factor H, disease progression is more severe (Despriet et al., 2006; Ferreira et al., 2010).

The main message of this hypothesis is: The timing, duration and amount of complement activation should be considered if complement inhibition is used in therapy for neurodegenerative disorders.

Implications for future treatments

Therapeutic intervention in the complement cascade is possible. There are many separate targets and inhibition can be done at several stages in the cascade. In view of the important role of the complement system in the defense against infections, complete inhibition is not preferable. In fact, individuals deficient for components of the classical, lectin and
alternative pathway, C3 or C5 have a compromised immune system. However, individuals with a deficiency in components of the terminal pathway (C6, C7, C8 and C9) have no complaints, except a slightly increased susceptibility for meningitis.

Thus for therapeutic intervention, one could target all enzymatic cleaving steps to inhibit complement activation. A prime target is at the terminal part of the complement cascade, the step of MAC formation. This will only inhibit MAC formation while retaining all the upstream properties of complement activation. Complement activation as described above has several functions, like attracting and activating macrophages and bridging between the innate and adaptive immune systems. Inhibiting just the terminal part of the complement cascade is therefore less intrusive for patients. An additional advantage is that all three the complement activation pathways are inhibited, because the final common pathway of complement activation is MAC formation.

Future studies should focus on the genetic variation in complement regulators and their effect on the disease progression. This will not only provide more insight on the contribution of complement regulators on neurodegeneration, but also allow a personalized approach to complement targeted therapies to improve patients’ conditions. A condition where complement inhibition might be directly applied to is traumatic brain and nerve injury. In these cases complement inhibition might prove especially powerful to improve the outcome of neuronal regeneration, since substantial initial damage may occur when an overshoot of complement activation is present.

Reference List


Complement activation in neurodegenerative disorders


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PART I

Peripheral nerves
Chapter 2

Macrophage depletion delays myelin clearance after peripheral nerve injury

M. Sta, V. Ramaglia, K. Fluiter, J.M.B.V. de Jong and F. Baas
Abstract

Background: Deficiency for complement component C6 results in slower Wallerian degeneration (WD) and rapid nerve regeneration after crush injury in rats. C6 is essential for the formation of the membrane attack complex. C6 deficient rats are unable to form this complex, but retain upstream complement effector functions. Though capable to attract macrophages to the damage site, C6 deficient animals show significantly lower amounts of activated macrophages in the damaged nerve compared to WT animals.

Hypothesis: The lowered amount of activated macrophages in injured peripheral nerves is responsible for the delayed WD in C6−/− rats.

Method: WT animals were depleted of circulating macrophages by treatment with clodronate-containing liposomes before and after the sciatic nerve crush injury. PBS-containing liposomes and PBS were used as controls.

Results: Macrophage-depleted animals showed delayed myelin clearance, but the extent of axonal damage was unchanged compared to animals treated with PBS or liposomes only. In addition, depletion of macrophages reduced the amount of complement deposition in the injured nerves.

Conclusion: Activated macrophages in the nerve are responsible for rapid myelin clearance during WD and increase complement activation in the injured nerve. However, macrophage depletion did not delay axonal degeneration during WD.
Macrophage depletion delays myelin clearance after peripheral nerve injury

Introduction

After injury, peripheral nerves undergo Wallerian degeneration (WD), a process in which the nerve degenerates completely from the first node of Ranvier proximal of the damage site, all the way to the distal end (Waller, 1850). During WD the nerve undergoes profound changes: axons degenerate, myelin is removed and Schwann cells dedifferentiate. Furthermore, macrophages are attracted to the nerve and activated to remove debris (Hirata and Kawabuchi, 2002). The dedifferentiated Schwann cells will align inside the endoneurial tube, where they provide a framework along which new axon sprouts can grow (Chen et al., 2005). Eventually, Schwann cells proliferate, engage in a one to one relationship with an axon and start the remyelination process.

The complement system, part of innate immunity, is activated during WD (Alexander et al., 2008; Bonifati and Kishore, 2007; de Jonge et al., 2004; Morgan et al., 1997; Ramaglia and Baas, 2009). The complement system is a cascade of self-cleaving proteases. The cleaved complement proteins have several effector functions (for a full description see (Ricklin et al., 2010)); they opsonize damaged structures (e.g. C3b), attract phagocytes (e.g. C3a and C5a), and form the membrane attack complex (MAC), a pore-forming structure, also known as the terminal complement complex or C5b-9. It is thought that activation of the complement system facilitates efficient clearance of the debris in the degenerating nerve.

We previously showed that in a rat sciatic nerve crush injury model WD was delayed when complement activation or MAC formation was prevented (Ramaglia et al., 2007; Ramaglia et al., 2008). Deletion or inhibition of complement protected the injured nerve from loss of myelin and axonal damage. In addition, the number of macrophages in the damaged nerves was reduced and the macrophages were not activated as measured by the absence of the cd11b/cd18 surface marker. Despite the delayed clearance of myelin debris, the complement inhibited or C6 deficient animals showed fast regeneration (Ramaglia et al., 2009); a surprising finding, in view of the notorious inhibitory properties of myelin debris on axonal outgrowth (Shen et al., 1998).

Furthermore, the morphology of the regenerated nerves in the complement inhibited or deficient animals was improved, showing more large caliber fibers and better myelination of the nerve fibres (Ramaglia et al., 2009).

The mechanism that is responsible for the improved regeneration of injured nerves in these complement inhibited or deficient animals is not well understood. Possible explanations for the observed improved regeneration are: 1) complement-mediated damage to the nerves is reduced or 2) macrophages-mediated damage to the nerves is reduced in the complement deficient animals.

In this study we addressed the role of infiltrating macrophages during post-traumatic WD. Therefore, we depleted complement sufficient rats of hematogenous macrophages 24 hours prior to and 18 hours after crush injury of the sciatic nerve by intravenous administration of clodronate containing liposomes. Nerve degeneration was studied by quantifying the amount of myelin and axonal damage, complement activation and macrophages. To exclude effects from liposomes, we included control groups, which received either PBS-containing liposomes or PBS alone. Furthermore, the spleen and lymph nodes were analyzed for the presence of macrophages in order to determine the effect of clodronate on peripheral macrophages.
Part I: chapter 2

These experiments allowed us to determine the role of macrophages in post-traumatic sciatic nerve degeneration. Our findings suggest that macrophages are responsible for rapid myelin clearance, but macrophage depletion does not alter axonal damage and clearance.

Materials and methods

In vivo depletion of macrophages:

The experiment was approved by the Animal Ethical Committee of the AMC (DNL1000 – 100729). 24 Wild type PVG rats (HSD, Germany) were injected intravenously with 2ml of the treatment solution. For depletion of macrophages a solution of liposomes containing dichloromethylenediphophonate (also known as clodronate) (Clodlip b.v., clodronateliposomes.com, the Netherlands) was used (9 rats), as a control for the liposomes a PBS liposome solution (Clodlip b.v.) was used (9 rats). Six rats, treated with PBS only were included as placebo group. Intravenous injections were given twice; 24 hours (hrs) before and 18 hrs. after crush injury. Crush injury: the sciatic nerve of the right hind leg was crushed under isoflurane anesthesia and tempesic analgesia, by crushing for 3 times 10 seconds (Ramaglia et al., 2007). Animals were housed in filter top Perspex cages with food and water ad libitum. To determine the extent of nerve degeneration, 3 animals per group were killed at 3 days (d) after crush; left and right sciatic nerves, spleen and lymph nodes from the groin and thorax were processed for paraffin embedding. The effectiveness of the treatment was assessed by immunohistochemical analysis for CD68^+ cells which confirmed reduction of macrophages in spleen and lymph nodes. Immunohistochemical analysis was done to verify whether the depletion of macrophages with the clodronate liposomes was successful.

Immunohistochemistry

Paraffin embedded tissue was processed. Sections of 6µm for nerve, 5µm for lymph node and 3µm for spleen were mounted on Superfrost slides and kept to dry overnight at 37°C.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Type</th>
<th>Dilution</th>
<th>Art. #/ company</th>
</tr>
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<tr>
<td>Rat-C68 (ED1)</td>
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<tr>
<td>C9</td>
<td>Rabbit polyclonal</td>
<td>1:300</td>
<td>B.P. Morgan (Cardiff, UK)</td>
</tr>
</tbody>
</table>

Table 1: Primary antibodies used for immunohistochemistry.

Sections were deparaffinized and endogenous peroxidase activity was blocked using methanol/0.3% H2O2. Slides were washed and antigens were retrieved by heat in Tris/EDTA buffer [100 mM Tris, 10mM EDTA pH 6.5] for 13 minutes (‘) in microwave. Antigen was targeted with primary antibodies (see table 1) for 90’ at room temperature (RT). The primary antibody was detected by incubation with a secondary antibody, either biotinylated (DAKO, Glostrup, Denmark) or directly fluorescent labeled (Invitrogen, Carlsbad, USA), for 30’ at RT. In case of a biotinylated secondary antibody, slides were washed and incubated with streptavidin-HRP (DAKO) for 20’ at RT. Detection was done by incubating the slides with 0.05% 3-amino-9-ethylcarbazole in acetate buffer (pH 5) for 5 minutes, followed by a counterstain with hematoxylin for 30 seconds. Slides were mounted in Kaiser’s glycerin gelatin (Merck, Darmstadt, Germany). In case of a directly fluorescent labeled secondary antibody, slides were
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counterstained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich, St Louis, USA) and enclosed in Vectashield Hard Set mounting medium (Vector Laboratories Inc., Burlingame, USA).

Quantification of sections
To determine the amount of CD68 positive cells per area, we stained and analyzed 5 non-consecutive sections, with a minimum interspace of 30µm to avoid double counting. CD68+ cells that contained a nucleus in the same plane were counted manually per nerve section. The area analyzed was measured in square microns using ImagePro Plus 7.0 “measurement” tool. Per section, the total amount of CD68+ cells per mm² was calculated [total count of CD68+ cells per section/ area of the section]. Per nerve, all CD68/mm² were averaged and used for statistical analysis.

Quantification of C9 positivity: Low magnification pictures of sections stained with the C9 antibody were processed in ImagePro, using the measurement setting “percentage positive per area”. The nerve area was encircled and the color, which identified C9 positivity, was assigned. The percentage of positive area was calculated and exported to Excel for statistical analysis.

Statistics
For statistical analysis on macrophage count we performed a one way ANOVA with Bonferonni correction for multiple testing, assuming normal distribution. Changes were considered statistically significant if P < 0.05. To calculate the correlation between the amount of C9 deposition and the amount of CD68+ macrophages we used the Pearson’s Rho correlation coefficient.

Results
Clodronate liposome treatment depletes macrophages in peripheral lymphoid organs
Animals received intravenous injections of clodronate-containing liposomes, PBS-containing liposomes or PBS alone 1 day prior to the crush and 18 hours after the crush. Nerves were harvested at 3 days post-injury (d PI). The efficiency of the macrophage depletion was assessed by the reduction of macrophages in spleen and lymph nodes. The immunohistochemical assessment of spleen (Fig. 1) and lymph nodes (not shown) showed macrophage depletion in clodronate treated animals was successful. The amount of observed CD68+ cells is much lower in clodronate treated animals (1C) compared to PBS (1A) and PBS liposome (1B) treated animals. A similar decrease of CD68+ cells was observed in the lymph nodes (not shown).

Clodronate liposome treatment decreases influx of blood-derived macrophages in the injured nerve
To determine the amount of infiltrated and phagocytic (activated) macrophages inside the injured peripheral nerve at 5 mm distal of the crush site, we stained for macrophages and myelin (Fig. 2). PBS only treated animals showed enlarged and foamy macrophages with inclusions of myelin positive debris, as shown by the colocalization of the CD68 and the MBP signals, indicating phagocytosis of myelin by active macrophages (arrows, 2B). Similar amounts of CD68+ cells and signs of phagocytosis (arrows, 2C) were observed in animals treated with PBS.
In contrast to the two control groups, animals treated with clodronate liposomes showed reduced amount of CD68\(^+\) cells with a small and quiescent morphology, lacking signs of phagocytosis (arrow: Fig. 2D).

**Figure 2:** Cross sections of sciatic nerves stained for myelin (MBP; Green) and macrophages (CD68; Red) 5mm distal of crush injury site. A) Uninjured nerve, does not show any CD68 staining and shows normal myelin; B) Injured nerve of PBS treated animals, many CD68 positive (\(^+\)) cells are found, containing myelin (arrows); C) Injured nerve of PBS liposome treated animal, shows CD68\(^+\) cells containing myelin (arrows). The amount of damaged myelin is consistent with the quantification shown in figure 2 (B > C > D); D) Injured nerve of clodronate liposome treated animal, some CD68\(^+\) cells are found, but much smaller in size than those found in B and C. Additionally, the CD68\(^+\) cells in the clodronate liposome treated animals did not contain myelin (arrow).

Quantification of the amount of macrophages at 3 d PI, showed a robust and significant (77%, P < 0.01) reduction in the amount of CD68\(^+\) cells in the nerves of clodronate liposome...
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Treated animals (88 ± 39 CD68+ cells/mm²) compared to PBS (387 ± 201 CD68+ cells/mm²) and PBS liposome (290 ± 82 CD68+ cells/mm²) treated controls (fig. 3). PBS loaded liposomes had no significant effect on the amount of activated macrophages in the nerve.

Figure 3: Quantification of the amount of CD68+ cells in 5 non-consecutive sections of sciatic nerves 5mm distal of the crush site. Bars represent absolute count of positive cells divided by the scored surface area of the nerve per treatment condition. One-way ANOVA showed clodronate liposome treated animals had significantly less CD68+ cells per mm² compared to controls (* = p < 0.01). Residual intraneural CD68+ cell count in clodronate liposome treated animals indicates that clodronate liposomes likely did not affect the resident endoneurial macrophage population but depleted blood and lymphoid organs from hematogenous macrophages without crossing the blood-nerve barrier.

Figure 4: Cross sections of sciatic nerves stained for myelin (MBP; Green) and neurofilament (SMI31, Red), 5mm distal of crush injury site at 3 d PI. A: uninjured nerve, same level as injured nerve sections. Normal dense, dotted neurofilament, ensheathed with ring-shaped myelin is visible within the nerve area. B) Injured nerve of PBS treated rat; C) Injured nerve of PBS containing liposome treated rat; D) Injured nerve of clodronate containing liposome treated rat. In all injured nerves similar axonal damage is found, swollen and damaged neurofilament (red arrows). Damaged myelin is observed in all injured nerves (B, C, D: white arrows), but the amount of myelin loss is different; PBS and PBS liposome treated animals show reduced myelin staining compared to clodronate treated animals, indicating that myelin clearance is reduced in clodronate treated animals.
Part I: chapter 2

Macrophages are responsible for rapid myelin clearance

To determine the contribution of macrophages to axonal damage and myelin clearance, crushed nerves were analyzed for the presence and morphology of neurofilament, as a marker of axons, and MBP as a marker of myelin, (Fig. 4) in nerve sections at 5 mm distal of the crush at 3 d PI. In PBS (4B) and PBS liposome treated (4C) animals we found a decrease in the amount of myelin staining compared to controls (4A). Myelin that is still found in these nerves is partly collapsed (white arrows Fig. 4). We also observed damaged axons as indicated by the annulated neurofilament staining, consistent with collapsed axonal content (red arrows Fig. 4).

Similar axonal damage was observed in the macrophage depleted animals (4D). However, in macrophage depleted animals less myelin degradation was found in the injured nerves compared to animals treated with PBS or PBS liposomes.

Complement activation depends partly on presence of macrophages

To determine the amount of complement activation in the injured nerves we stained for C9 a marker for the membrane attack complex (MAC) in tissue (Fluit et al., 2014) and we quantified the amount of C9 immunoreactivity per area. All injured nerves showed C9 deposition. The amount of C9 deposition differed between PBS treated compared to macrophage-depleted animals at 3 d PI. Quantification of the C9 staining showed that the amount of C9 deposition was slightly reduced in macrophage depleted animals injured nerves compared to both control groups (Fig. 5). In addition, a trend was observed found between the C9 positivity (Fig. 5) and the amount of activated macrophages in the nerves at 3 d PI (Fig. 3), in which it seems positively correlated, calculation of R^2 showed a coefficient of 0.96.

Discussion

Our findings show that macrophages are responsible for rapid myelin clearance in the injured nerves, but do not affect the amount of axonal damage during WD.

Initial activation of the complement system was similar in all animals. All injured nerves showed C9 deposition at 3 d PI. However, the amount of C9 deposition remained low in macrophage depleted animals. More C9 deposition was found in animals treated with PBS. It is
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Known that macrophages themselves are attracted by complement activation and become activated via complement and downstream effects of complement. Previous studies showed that in the absence of C9 (MAC) there was reduced influx and activation of macrophages. We found that in WT animals the amount of C9 deposition positively correlates with the amount of activated macrophages present in the injured nerve at 3 d PI (Fig. 3 and 5). We propose that in addition to being attracted and activated by complement, once activated, macrophages induce more complement activation in the injured nerves. The complement activation can be induced, either directly or indirectly, by exposing more complement activating epitopes.

We found no difference in axonal damage after sciatic nerve injury between all treatment groups. This indicates that axonal damage after peripheral nerve injury is not directly mediated by macrophages.

Previous studies comparing WD in WT and C6−/− rats showed that the C6−/− rats had a delay in both axonal and myelin degeneration during WD, as well as a lack of activated macrophages in the nerve 5 mm distal of the crush area at 3 d PI. Comparing the findings of the previous study to the current findings in the macrophage depleted animals we can conclude: the delay in WD observed in the C6−/− animals is only in part due to the lack of activated macrophages, which are responsible for rapid myelin clearance. The observed delay in axonal degradation in the C6−/− animals is not caused by the lack of activated macrophages but might be due to the inability to form MAC.

**Figure 6:** Schematic representation of the events after peripheral nerve damage. Damage induces slow axonal degeneration and triggers Schwann cells to dedifferentiate and proliferate; damage induces complement activation as well. Upstream components of activated complement system attract macrophages and induce influx. Downstream components of activated complement, MAC, induce macrophage activation and rapid axonal degeneration. Activated macrophages are responsible for rapid myelin degradation, myelin clearance and induce more MAC deposition. Dedifferentiated, proliferated Schwann cells can display phagocytic properties, they can degrade and clear myelin too, but this process is slow.

A schematic representation of the events, which occur after damage of the peripheral nerves by the key players, macrophages, complement and Schwann cells, is shown in figure 6. The complement system, specifically MAC, directly attacks the axons, resulting in axonal damage. This is underscored by the differences found in WD between wild type and complement deficient animals (Ramaglia et al., 2007). The difference in myelin clearance between WT and C6−/− animals previously found is partly dependent on activation of the complement system and especially MAC formation. Activation of the complement system is most likely responsible for the attraction (via the upstream components C3a and C5a) and activation of macrophages (via the MAC). Rapid clearance of myelin is done by macrophages. Additionally, the presence of
activated macrophages results in more complement activation and more axonal damage as consequence of complement activation. The delayed WD observed in C6−/− rat reported in the study by Ramaglia et al. (2007) might be the result of the absence of activated macrophages, normally responsible for rapid myelin clearance. In the C6−/− animals other cell types like Schwann cells, must be responsible for myelin clearance (Fernandez-Valle et al., 1995; Gray et al., 2007; Hirata and Kawabuchi, 2002; Kobsar et al., 2006). Contributing to the reported difference in degeneration between WT and C6−/− animals is that activated macrophages themselves appear to induce more complement activation.

Propagation of damage and degradation in injured sciatic nerves is initiated by complement activation; rapid degradation of axons is mediated by MAC. Inhibition of the complement system at the level of MAC formation, decreasing macrophage influx and inhibition of rapid axonal degradation might therefore be the most powerful tool to inhibit post-traumatic peripheral nerve degeneration.

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Reference List


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