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Reducing pneumococcal meningitis related brain damage by inhibition of the host innate immune response.

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Abstract

Pneumococcal meningitis is an infectious disease of the central nervous system (CNS) caused by the Gram positive bacterium *Streptococcus pneumoniae*. The immune response in the CNS against *S. pneumoniae* is thought to be a cause of the pneumococcal meningitis related brain damage that results in long-term sequelae including deafness and cognitive impairment. In normal situation there are, due to the blood-brain barrier (BBB), no immune modulators present in the CNS, with the exception of microglia, the macrophages of the CNS. Pattern-recognition receptors (PRRs) on and in microglia, including Toll-like receptors (TLR; mainly 2, 4 and 9), Nod-like receptors (NLR) and SIGN-R1 (a C-type lectin receptor) are thought to be responsible for the activation of microglia. These microglia are then able to secrete immune modulatory factors including cytokines, chemokines, matrix metalloproteinases (MMPs) and complement factors. Various and some contradictory effects of the secreted cytokines have been seen in pneumococcal meningitis. While chemokines appear to be primarily responsible for recruitment of leukocytes, MMPs (especially MMP-9) are mainly responsible for the breakdown of the BBB what makes it possible for leukocytes to enter the brain. Complement factors seem to have various functions in the immune response during pneumococcal meningitis, but one important function is to activate leukocytes. Microglia and some leukocytes are able to phagocytize pathogens and secrete reactive nitrogen (RNS) and oxygen (ROS) species, with the intention to eliminate *S. pneumoniae* out of the CNS. In summary, the host innate immune response during pneumococcal meningitis is very complex and is formed by many immune modulators which can both induce and prevent brain injury. In the future long-term sequelae as a result of pneumococcal meningitis could be reduced by inhibition of pro-inflammatory immune modulators or by stimulation of anti-inflammatory immune modulators.

Key words: Streptococcus pneumoniae, pneumococcal meningitis, innate immune response

Introduction

The Blood-Brain-Barrier (BBB) is a physical barrier that has several roles in favor of the brain. By supplying essential nutrients and protection against changes in osmolarity and potential threats coming from the periphery, the BBB ensures that neurons work optimal (1). The BBB consists mainly of endothelial cells which are closely linked with each other via tight junctions (TJ) (1). It is seen that besides endothelial cells, also astrocytes play an important role in maintaining the BBB. One important function of astrocytes is to tighten the TJ between endothelial cells (1, 2). Another outcome of the presence of the BBB is the difference between the immune system of the central nervous system (CNS) and the periphery. In a normal and healthy situation the BBB ensures that (almost) no immune system related molecules, or immune modulators like complement factors and lymphocytes, are present in the brain (3). Basically, the presence of these immune modulators would be unnecessary, because the BBB also prevents the entry of pathogens and other potential threats into the brain. To make sure the CNS is still able to respond appropriately to potential threats, which are able to cross the BBB, the CNS contains special macrophages: microglia. In their active state microglia have the pivotal role in the defense against infections in the CNS by initiating the immune response (4).

The Gram positive bacterium *Streptococcus pneumoniae* is one of those potential threats which are able to cross the BBB. So besides causing many types of pneumococcal infections in the periphery, this bacterium is also able to infect the brain. This infection of the CNS by *S. pneumoniae* is called pneumococcal meningitis. As a consequence of this disease not only a high mortality rate (10%), but also long term morbidity (in 30% of the surviving patients) is seen (5). Especially long term sequelae, including deafness and cognitive impairment are common consequences of pneumococcal meningitis(6-8). Last twenty years several studies have been carried out to investigate the detailed cause of these CNS complications. Often these studies make use of several *in vitro* and *in vivo* models. In most cases, those *in vivo* models of pneumococcal meningitis are obtained by infecting animals, most often rats and mice, directly into the cerebrospinal fluid (CSF). Depending on the approach of a study the effect of this infection of the CNS can be measured in the brain. For example, the amount of immune modulators in the CSF can provide information about the immune response against *S. pneumoniae*. Information about the course of the disease can be obtained by looking at the amount of brain injury (the amount of apoptotic and/or necrotic cells) and the amount of bacteria, the bacterial load, in the CNS.

It is thought that the pneumococcal meningitis related complications of the CNS do not only develop as a consequence of the presence of *S. pneumoniae*, but rather as a detrimental result of the immune response directed against this pathogen (9). Several immune modulators which become activated by microglia might have, besides fighting *S. pneumoniae,* negative effects on the CNS. In this review the host innate immune response after exposure to *S. pneumoniae* to the CNS will be discussed in detail. In this way the immune modulators of the innate immune response that are responsible for the brain damage as seen in pneumococcal meningitis can be identified.

PRRs are responsible for the recognition of *S. pneumoniae* and the initiation of an immune response

Microglia, the immune response initiating cells of the CNS, can be found in several forms including the ramified or resting state and the active state (4). During the ramified state microglia have a supportive role, which contributes to the well-being of neurons. In this state the microglia do express certain immune regulatory markers, such as major histocompatibility complex II (MHC II), but do not act as immune modulators. To become active, and therefore initiate an immune response, ramified microglia have to be exposed to antigens, derived from a potential threat, like *S. pneumoniae*. In addition, many, mostly *in vitro*, studies have been achieved to investigate the antigen presenting role of astrocytes, as reviewed by P. Shrikant and E.N. Benveniste (10). Some of those studies claim that astrocytes are important antigen-presenting cells (APC), because they express the T-cell activating MHCII receptor and are able to respond to stimuli like lipopolysaccharide (LPS). However other *in vitro* and *in vivo* studies, mentioned in that same review, question this observation, because they were not able to detect MHCII on astrocytes. Therefore the antigen-presenting role of astrocytes in the activation of microglia is doubtful. Because active microglia initiate the immune response against *S. pneumoniae*, these cells are for a large extent indirectly responsible for the pneumococcal meningitis related brain injury. Once microglia have become active they will up-regulate pattern-recognition receptors (PRR) (4). These receptors are able to recognize pathogen-associated molecular patterns (PAMPs) like unmethylated CpG DNA or LPS from bacteria (11). PRRs consist of several classes of receptors, including Nod-like receptors (NLR) and C-type lectins.

Another class of PRRs is the family of Toll-like receptors (TLR) (4). TLRs are an evolutionary conserved family of PRRs which are expressed on many cell types (11). Once these receptors recognized a PAMP they will activate transcription factors which are important for an innate immune response against the detected pathogen (11). It is known that Toll-like receptor 2 (TLR2) is involved in cell activation in presence of Gram-positive bacteria cell wall and cell membrane components (12). Therefore Echchannaoui et all. designed a study in which they investigated *in vivo* the severity of pneumococcal meningitis in TLR2 deficient mice ($TLR2^{-/-}$) compared to wild type mice (wt) (12). They determined the severity of the disease by looking at the bacterial load in the brains and the meningeal inflammation, which was measured by the amount of TNF (a cytokine with a central role during immune responses) and the infiltration of leukocytes into the brain. Echchannaoui et all. concluded that $TLR2^{-/-}$ mice have a higher bacterial load in the brains and a higher meningeal inflammation, what means that TLR2 is important in the clearing of *S. pneumoniae* in meningitis (12). A study of Koedel et all. confirms this, by showing that TLR2 deficiency led to increased bacterial titers in the cerebellum (9). In that same study Koedel et all. also tried to prove the effect of TLR2 deficiency on the amount of immune modulators. Those amounts did differ moderate, but unfortunately not significantly between $TLR2^{-/-}$ and the wt mice, so the role of TLR2 in the initiation of an immune response is not entirely clear yet.

As an explanation for the only moderate change in amount of immune modulators in $TLR2^{-/-}$ mice Koedel et all. put another, yet unidentified PRR forward. Because they saw that certain non-immune cells (HEK293) with a TLR4 over-expression were able to react properly to *S. pneumoniae;* this TLR4 was mentioned as a potential candidate (9). Unfortunately, in that particular study Koedel et all. were not able to deliver convincing evidence to confirm this (9). In contrast, Klein et all. did (13). In that study Klein et all. used mouse models which were deficient in several (combinations of) TLRs:

TLR4 single deficiency ($TLR4^{-/-}$), TLR2/TLR4 double deficiency ($TLR2/4^{-/-}$) and TLR2/TLR4/TLR9 triple deficiency ($TLR2/4/9^{-/-}$). After infection with *S. pneumoniae* into the cisterna magna (a part of the brain which is directly in contact with the CSF) it seemed that $TLR4^{-/-}$ mice were better able to fight *S. pneumoniae* than $TLR2/4^{-/-}$ mice. The evidence for this statement is that $TLR2/4^{-/-}$ mice showed a decreased amount of immune modulators (leukocytes and cytokines) and an increased amount of bacteria in the brain (13).

In that same study of Klein et all. the role of TLR9 in the recognition of pathogens and subsequently the activation of microglia and the initiation of an immune response was also studied (13). TLR9 is located on the endosomal membrane inside the cell and is able to recognize unmethylated CpG DNA of bacteria and viruses (11). In the case of pneumococcal meningitis this DNA rich in CG dinucleotide motifs is available for the TLR9 due to phagocytosis or ingestion of autolyzed bacteria. Autolysis is a common process for bacteria when they have reached a stationary phase: a balance between multiplication and autolysis (14). The *in vitro* experiments of Klein et all. showed that TLR9 was able to activate cells after recognition of heat-inactivated *S. pneumoniae* (13). On the other hand the *in vivo* study showed that $TLR2/4/9^{-/-}$ triple deficient mice did not suffer more from CNS complications, such as BBB destruction and development of brain edema than $TLR2/4^{-/-}$ double deficient mice. This implicates that TRL9 does not have a pivotal role in the recognition of *S. pneumoniae*. Still remains the fact that also other *in vitro* studies showed that TLR9 can activate cells after binding of unmethylated CG dinucleotide motife-rich DNA of bacteria (15, 16). However these studies are not really convincing about the role of TLR9 in development of pneumococcal meningitis related brain injury because these studies were not specific aimed at *S. pneumoniae* infections.

Besides TLRs, it is thought that there are more PRRs involved in recognition of *S. pneumoniae* and therefore microglia activation. An example are the Nod-like receptors (NLR). These receptors are cytoplasmatic molecules with certain conserved domains which are able to sense bacterial infections (11). One subset of this family is called nucleotide-binding oligomerization domains (NOD), which are able to recognize peptidoglycan (a component of bacterial cell walls) (11, 17). Especially NOD2 and in lower extent NOD1 seem to be important in the immune response to *S. pneumoniae*. First of all, this is shown as an increase in the amount of NOD1 and NOD2 mRNA due to *in vitro* and *in vivo* exposure to *S. pneumoniae* (17). But most importantly, the study of Liu et all. showed that NOD2 deficient mice suffered from a higher bacterial load and decreased amount of two immune modulators: CCL3 (a chemokine) and TNF-α (a cytokine) (18). This indicates that at least one of the NLRs, namely NOD2, is involved in the initiation of an immune response. This means that NOD2 might indirectly cause brain injury as a response to pneumococcal meningitis.

A third potential receptor related to *S. pneumoniae* recognition is the C-type lectin SIGN-R1. C-type lectins are PRRs which recognize carbohydrate structures found on cell walls of microorganisms (11). It is shown that SIGN-R1 is present on the membrane of several cells, including microglia (19). Kang et all. showed that macrophages in the marginal zone of the spleen were able to clear *S. pneumoniae* from the bloodstream (20). For this clearing the interaction of the SIGN-R1 receptor with certain polysaccharides from the capsule of the *S. pneumoniae* and the complement factor C1q is necessary. Because active microglia act just like macrophages this mechanism could also be important in pneumococcal meningitis. Park et all. investigated this hypothesis and they proved that SIGN-R1 (or its rat homologue CD209D) was necessary for the recognition of *S. pneumoniae* (19). In short, they visualized the uptake of the polysaccharide CPS14 derived from the capsule of *S. pneumoniae* by

using immunolabeled CD209D positive rat microglia. Based on their, but also other studies Park et all. strongly suggest that SIGN-R1 and CD209D mediate complement activation. Given the fact that one of the complement factors (C1q) is involved in the activation of these receptors, that hypothesis seems quite plausible. The role of the complement system in pneumococcal meningitis will be discussed later.

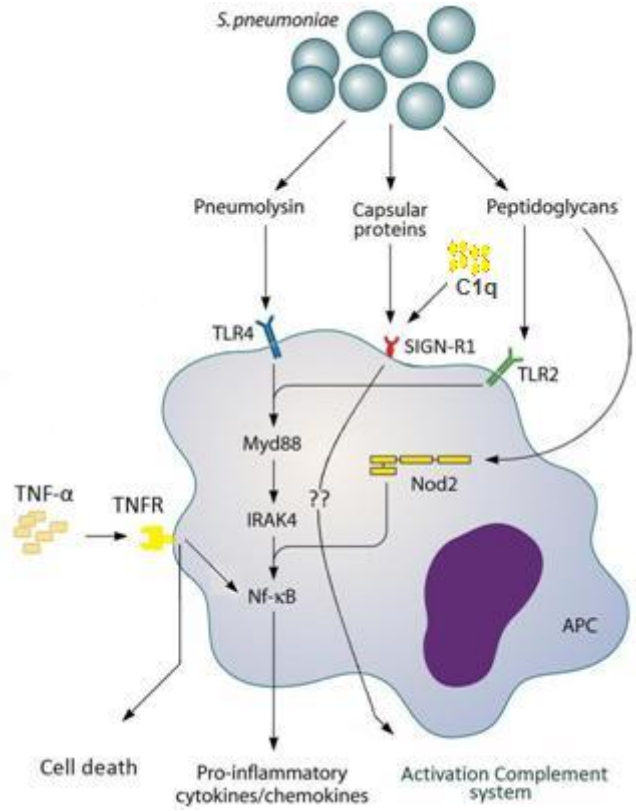

Figure 1. Activation pathways of microglia after recognition of *S. pneumoniae* via several receptors. TLR2 and the intracellular NOD2 receptor become activated by the pneumococcal cell wall protein peptidoglycan. TLR4 is activated by the pneumococcal toxin pneumolysin. These three receptors activate the transcription factor Nf-κβ, inducing transcription of several pro-inflammatory cytokines and chemokines. SIGN-R1 is activated by pneumococcal capsular proteins together with the complement factor C1q and activates thereby the complement system. During the immune response the binding of TNF-α to the TNFR initiates NF-κβ activation and cell death.
Adapted from Mook-Kanamori et all. (7).

So several receptors seem to be related with the recognition of *S. pneumoniae* and thereby the initiation of an immune response via microglia activation (Fig. 1). The intracellular activation route initiated by the TLR and the NOD receptors leads eventually to the activation of the transcription factor NF-κβ (7). It is known that this transcription factor is responsible for the induction of several pro-inflammatory cytokines. As mentioned earlier, the activation of microglia via the SIGN-R1 is related to the complement pathway and will therefore be discussed later on in this review (11). Beside these complement factors and pro-inflammatory cytokines, activated microglia secrete many other products as shown in table 1. The secretory products which are thought to play a role in the immune response during pneumococcal meningitis and which might therefore cause brain injury will be discussed in this review.

Table 1 Immune modulating secretory products of Microglia*
Cytokines (IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-16, IL-23, TNF-α, TGF-β)
Chemokines CxC: CXCL8 (IL-8), CXCL9(MIG), CXCL10(IP-10), CXCL12(SDF-1α) CC: CCL2(MCP-1), CCL3(MIP-1α), CCL4(MIP-1β), CCL5(RANTES) CX_3C: CX_3CL1(fractaline)
Matrix metalloproteinases (MMP-2, MMP-3, MMP-8, MMP-9, TIMP)
Free radicals: superoxide, nitric oxide
Eicosanoids: PGD_2, leukotriene C_4
Growth factors: nerve growth factor, fibroblast growth factor
Proteases: elastase, plasminogen
Cathepsins B and L
Quinolinic acid, glutamate
Amyloid precursor protein
Complement factors, C1, C3, C4, C5

*Secretory products reported in the literature, whose generation is influenced by the state of activation as well as by the anatomic location, age and animal species from which the microglia are derived. Adapted from Rock et all. (4).

The contribution of cytokines has both positive and negative effects

Activated microglia are able to secrete several pro-inflammatory cytokines, which all have their own function in modulating the immune response against *S. pneumoniae*. The cytokines tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) are called the major early-response cytokines, because these molecules seem to be up-regulated in the first hours after exposure to *S. pneumoniae* (21). Once released these cytokines trigger the release of many other immune modulators like other cytokines, chemokines, reactive nitrogen and oxygen intermediates and proteases (21). Besides, TNF-α seems to have an important role in the recruitment of cells from the adaptive immune response, like leukocytes. The detailed mechanism is not clear yet, but it is thought that TNF-α stimulates the expression of chemokines, adhesion molecules and MHC receptors, that eventually leads to the facilitation of (cytolytic) T cells (21).

That TNF-α plays an important role in the immune response against *S. pneumoniae* has been shown by several studies, which observed an up-regulation of this molecule during the first hours after *S. pneumoniae* infection directly into the CSF(6, 22-24). Gerber et all. showed that as a result of a higher amount of necrotic cells the mortality of TNF-α deficient mice was significantly higher compared to wild type mice after the induction of pneumococcal meningitis (23). Based on the fact that TNF-α has an essential role in the control of bacterial growth Gerber et all. concluded that TNF-α has a major protective role in pneumococcal infections. Though it seems that TNF-α is capable to do more than these early-response effects. A couple of studies showed that TNF-α is also present in a later phase of pneumococcal meningitis (21, 23). It is seen that compared to wild type mice, the rate of neurogenesis in TNF-α deficient mice is decreased (23). This can be the cause of the increased spatial memory deficits, which were observed in TNF-α deficient mice. This may indicate that TNF-α in the late phase is responsible for the recovery of the brain after pneumococcal meningitis, but more research in necessary to confirm this hypothesis.

As mentioned before IL-1β is one of the early-response cytokines. Several studies have shown that this cytokine is relevant in meningitis caused by *S. pneumoniae* (5, 21, 25, 26). Quagliarello et all. showed that IL1, both the α and β subunits, induces pleocytosis, an increased number of immune cells in the CSF (26). This is probably due to the fact that IL1 is related to the migration of neutrophils across the BBB. In addition, it is shown that the mortality of mice that lack the receptor for IL1 ($IL1R^{-/-}$) is significantly higher (5). This demonstrates that endogenous IL1 is required for the immune response against *S. pneumoniae* and might therefore contribute to the pneumococcal meningitis related brain injury. At last the third early-response cytokine: IL-6. This cytokine seems to have contradictory functions. First of all it is shown that IL-6 is able to increase the BBB permeability, what advances the entry of immune modulators into the CNS (27). On the opposite IL-6 seems to act anti-inflammatory by suppressing pleocytosis, possibly via the inhibition of TNF-α and IL-1 release (27). This anti-inflammatory function might be important in the prevention of brain damage, whereas the increase of immune modulators in the CNS might increase brain injury.

Besides these three early-response immune modulators, there is another cytokine produced by microglia which is active during the immune response against *S. pneumoniae*: IL-10. The role of this cytokine seems to be quiet clear. In 1995 Koedel et all. were already able to show that systemically administration of IL-10 leads to a down-regulation of pathophysiological changes, including CSF pleocytosis during the early-phase of the immune response against *S. pneumoniae* (28). The results of a more recent study of Zwijnenburg et all. confirmed this anti-inflammatory function of IL-10 (29). They showed that IL-10 regulates cytokine and chemokine production, but that it does not assist with the clearing of *S. pneumoniae* (29). In summary, there are at least four cytokines which are involved in the immune response against *S. pneumoniae*. IL-1, seems to contribute to the brain injury seen in pneumococcal meningitis, while IL-10 appears to prevent this. The other two cytokines, TNF-α and IL-6 seem to have multiple (contradictory) functions and need therefore more research before their exact role in the development of brain injury can be established.

Potential negative role of chemokines

Another group of secretory products released by active microglia is the chemokines. These molecules function is to attract immune cells via chemotaxis. Chemokines are divided into four subfamilies, based on their structure: the CXC, CC, CX_3C and C subfamilies (8). Several chemokines, including CXCL16, CXCL8 (or IL-8), CXCL2 (or MIP-2), CXCL1 (KC), CCL2 (or MCP-1), CCL3 (or MIP-1α) and CCL5 (or RANTES) seem to play a role in the immune response in pneumococcal meningitis, because their expression is increased (4, 24). However, the role of almost all of these chemokines is unknown. Ostergaard et all. discovered *in vivo* in a rabbit meningitis model that CXCL8 plays an important role in pleocytosis (30). This recruitment of extra immune modulators, in this case leukocytes might lead to increased brain injury. What has to be noted is that only treatment of anti-CXCL8 into the blood (intravenous), and not directly into the brain (intracisternal), led to an attenuated CXCL8 function (30). This indicates that CXCL8 probably performs its function on the bloodstream side of the BBB.

Secondly the role of the chemokine CXCL16 has been investigated slightly. Woehrl et all. showed that this chemokine is present in the brain and that neutrophils migrate towards CXCL16 in a dose-dependent manner (31). The study of Shimaoka et all. confirms this chemotactic function of CXCL16 towards immune cells (32). In addition, this last study also found that CXCL16 works as a scavenger

receptor: the facilitation of the uptake from several pathogens. However, this scavenger activities were questioned by Woehrl et all., because they did not see an effect on bacterial outgrowth in CXCL16 neutralized models (31). In short, a lot of research should be done before the role of this and other chemokines in the development of brain injury in pneumococcal meningitis will be clear.

Imbalance between MMP and TIMP leads to brain damage by proteolysis

A third group of secretions of active microglia is the matrix metalloproteinases (MMPs). This family of zinc-dependent enzymes is able to degrade proteins in the extracellular matrix (4, 33). The release of MMPs is seen to be induced by the earlier discussed cytokine TNF-α (33, 34). MMPs seem to play an important role in BBB breakdown (4, 33, 34). This increase in BBB permeability makes it possible for leukocytes to migrate into the CNS. Therefore these enzymes are likely candidates as effector molecules in the immune response in pneumococcal meningitis.

For several years many research has been done to figure out the effect of several MMPs in pneumococcal meningitis. Many of these studies show an up-regulation of various MMPs but in most cases the contribution of these MMPs is not unraveled yet. MMP-9 is an exception to this, because this member of the MMP family is shown to be up-regulated in most of the studies (6, 21, 33, 34). The mechanism in not clear yet, but it is seen that by inhibition of MMP-9, or MMPs in general, brain injury can be prevented (6, 34, 35).

However, the inhibition of MMPs is not an abnormal process in the brain. The tissue inhibitors of MMPs (TIMPs) have the function to bind MMPs and therefore prevent their proteolytic function. Studies aimed at the time-course of the concentration of MMP-9 and TIMP-1 showed that the peak of TIMP-1 is later than the peak of its substrate MMP-9 after the induction of pneumococcal meningitis (21, 35). This imbalance between MMP-9 and TIMP-1 leads to an excessive proteolytic activity in the brain (35). Thus, MMPs and TIMPs are related to the brain injury seen in pneumococcal meningitis, but more research has to be done to unravel their detailed contribution.

Leukocyte recruitment by complement factors

Besides cytokines, chemokines and MMPs the complement system might also have a role in the immune response against *S. pneumoniae* in the CNS. This complement system consists of several serum and cell surface proteins which all have their own function (11, 36). Together they are responsible for the opsonization of danger signals followed by the activation of cells to the uptake and destruction of the potential threats. The complement system can be activated via three different pathways: the alternative, the classical and the lectin pathway(11). Most studies concerning pneumococcal meningitis did only show the involvement of the classical pathway (Fig. 2). What has to be noted is that in healthy subjects almost no complement factors are detectable in the CSF. During *S. pneumoniae* infections the concentration of complement factors in the CSF increases. This implicates that the complement system plays a role in the immune response during pneumococcal meningitis and might therefore be responsible for the development of brain injury.

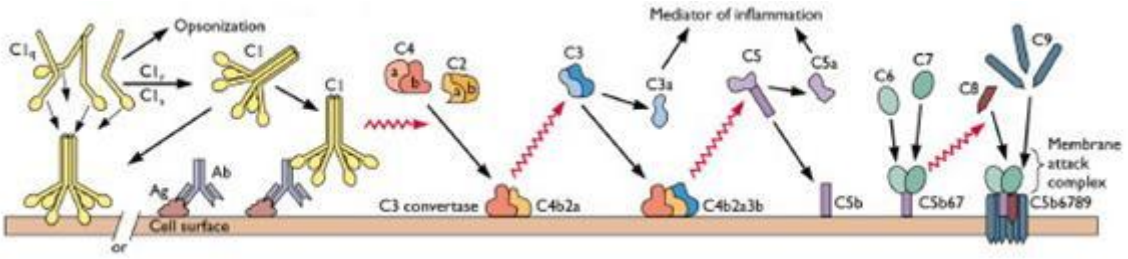

Figure 2. The activation of the classical complement pathway. The classical pathway is initiated by C1 binding to antigen-antibody (Ag-Ab) complexes. Via activation of several complement factors this cascade eventually mediates opsonization of pathogens, inflammation and the forming of the membrane attack complex (MAC). Adapted from (37).

For the activation of the complement system during pneumococcal meningitis the interaction of a SIGN-R1 receptor with *S.* pneumoniae and the complement factor C1q is required (19, 38). Normally C1q binds to antigen-antibody complexes, as shown in figure 2, but Kang et all. showed that the SIGN-R1 receptor even in absence of antibodies after binding with C1q is able to activate C3 (11, 39). It is seen that C1q deficient mice ($C1q^{-/-}$) have an attenuated immune response (for example seen as decreased amounts of leukocytes and IL-1β) and a higher bacterial load in the CNS compared to wild type mice (3, 40, 41). Besides, Lynch et all. showed that the concentration of C1q remained high after BBB recovery (41). Therefore this C1q is not only a complement factor which is important in the immune response in pneumococcal meningitis, but which also might play a role in the removal of cell debris.

C1q is not the only complement factor on which the immune response against *S. pneumoniae* depends. Early in the 1980s Ernst et all. showed that C5 also has an important role in fighting against *S. pneumoniae.* Their study indicated that C5 had a chemotactic function and thereby recruited the polymorphonuclear leukocytes (PMN) (42). The more recent study of Woehrl et all. confirmed this, by showing that in mice that lack the receptor for C5a ($C5aR^{-/-}$) the facilitation of white blood cells (WBC) decreased with circa 25% (43). Woehrl et all. were also able to deliver a reason for this lower WBC recruitment. They showed that the amount of two important chemokines, CXCL1 and CXCL2 was decreased in $C5aR^{-/-}$ mice (43). Apparently, C5 does not only act chemotactic on a direct manner, but also on an indirect manner, via the release of chemokines. Though, chemotaxis is not C5s only function. Ernst et all. were able to show that the presence of C5 led to an increase of the oxidative metabolism and the degranulation of PMNs (42). This last occurrence will be discussed later.

The last complement factor which has been studied in relation to pneumococcal meningitis is C3. Rupprecht et all. showed that in the absence of the factor C3 the bacterial load in the brains increases, the amount of leukocytes in the CSF decreases and that the expression of several cytokines also decreases (3). This indicates that also C3 is essential for a good immune response against *S. pneumoniae*. In summary, it is seen that the presence a couple of complement factors (C1q, C5 and C3) are important in the immune response during pneumococcal meningitis. The function which they have in common is the recruitment of leukocytes. That these cells of the adaptive immune response have an important role in the pneumococcal meningitis related brain injury will be discussed now.

Phagocytosis and intracellular killing by adaptive immune cells

Several immune modulators are responsible for the activation of the adaptive immune response by the recruitment of WBCs. These WBCs, or leukocytes, consist of a broad spectrum of cells which all originate from the hematopoietic stem cell. In a healthy situation these cells are not present in the CSF, due to the BBB. However, during pneumococcal meningitis leukocytes are able to enter the brain. This is mainly caused by the breakdown of the BBB and an increase of adhesion molecules caused by immune modulators from the innate response, like TNF-α. Once present in the CNS leukocytes generate a powerful immune response against *S. pneumoniae*.

However it seems that the function of leukocytes is not only to combat pathogens, they also seem to influence the function of innate immune cells, including microglia. One way leukocytes are able to do that is via interferon-γ (IFNγ). IFNγ is a protein produced by natural killer (NK) and T cells in response to antigens, for example *S. pneumoniae*, recognition (11). The main functions of this protein are the stimulation of phagocytosis and antigen presentation in macrophages and the initiation of differentiation from adaptive immune cells (11, 44). Diab et all. showed that the amount of IFNγ is increased in rats infected with *S. pneumoniae* compared to wild type rats (45). The exact role of IFNγ in pneumococcal meningitis is unknown, but research around the general role of IFNγ showed a possible relation. IFNγ appears to have an effect on the chemokine release of microglia and may therefore affect the immune cell infiltration into the CNS (44). However about the details of this effect of IFNγ on microglia is not known much yet, so more research is necessary.

Besides the production of IFNγ leukocytes have another very important function: phagocytosis of pathogens. Mainly neutrophils, but also microglia are able to mediate bacterial killing via phagocytosis. The ingestion of for example *S. pneumoniae* is facilitated by the opsonization of these organisms by complement factors and/or antibodies (7). Once *S. pneumoniae* is located in vesicles, called endosomes, inside the phagocyte these vesicles fuse with lysosomes. Lysosomes are vesicles which contain anti-bacterial mediators like nitric oxide (NO) and lysozymes, which kill the bacteria (7). Via this mechanism *S. pneumoniae* is cleared out of the CNS without too much harmful effects for the environment.

Reactive nitrogen and oxygen species as harmful defense mechanism

Unfortunately, in addition to intracellular killing microglia and some leukocytes, including neutrophils are also able to secrete reactive nitrogen (RNS) and oxygen (ROS) species to combat the free *S. pneumoniae* in the CSF (4, 7). Once released in the environment these RNS and ROS cause oxidative stress and are therefore at least for a part responsible for the neuronal damage seen in pneumococcal meningitis. Several studies aimed at the potential factors which induce the production of RNS and ROS have been achieved. One of those factors is the complement factor C5. As mentioned before the C5 is responsible for degranulation and an increase of the oxidative metabolism of PMNs (42). This last effect of C5 leads to a higher amount of ROS and RNS and therefore to more damage to the brain.

Another factor in relation to RNS and ROS production might be the stimulation of the receptor TLR9. As mentioned earlier this receptor is able to stimulate certain immune cells after binding unmethylated CG dinucleotide motife-rich DNA (15, 16). The stimulation of immune cells is seen to

result in the production of the frequently seen ROS nitric oxide (NO) (15). NO is a free radical gas and is not only important as a mediator of hemodynamic changes, but is also a potential cytotoxic agent. Especially when NO reacts with superoxide to form peroxynitrate, it is likely that this agent causes BBB damage (46, 47). According to Freyer et all. the release of NO is mediated via a TNF-α dependent autocrine pathway (47). This implies that TNF-α has indirect a role in the destruction of brain tissue.

For the production of NO, NO synthases (NOS) are necessary. Winkler et all. showed that in pneumococcal meningitis two important forms of these enzymes, iNOS and eNOS were up regulated (46). In addition, they showed that the BBB disruption was attenuated and that the amount of inflammatory mediators was decreased in iNOS deficient mice. Therefore RNS and ROS, but especially NO, seem to have an important role in causing brain damage in pneumococcal meningitis.

Conclusion

As reaction on an *S. pneumoniae* infection of the CNS a complex innate immune response is activated. This immune response consists of several factors, like microglia, cytokines and chemokines and so on. In the case of pneumococcal meningitis it is seen that these immune modulators do not only have advantageous functions. On one hand they are responsible for the clearing of *S. pneumoniae* out of the CNS, but on the other hand these factors cause brain damage. Therefore two groups of the mainly innate immune modulators can be distinguished. The first group of immune modulating factors has a pro-inflammatory function and causes thereby brain damage. Factors like ROS, RNS, MMPs and C5 are covered by this group. In addition, also factors with a chemotactic function, like IL-1β and CXCL8, which lead to the invasion of leukocytes and therefore to increased ROS and RNS amounts, belong to this second group. The second group consists of factors which act anti-inflammatory and therefore contribute to the prevention of brain damage. Two factors which definitely belong to this group are IL-10 and TIMP. By their respectively anti-inflammatory and MMP inhibiting function they prevent that certain immune modulators of the first group cause brain damage. What has to be taken into account is that not all pneumococcal related immune modulators can be divided into these two groups. For example TNF-α and IL-6, these two factors have various, but also contradictory functions and do therefore fit in both the groups. At last there are factors, like CXCL16, IFNγ and the receptor TLR9, from which the real function not has been unraveled yet and which require more research. In summary, the host innate immune response during pneumococcal meningitis is very complex and is formed by many immune modulators which can both induce and prevent brain injury. The detailed knowledge about the role of immune modulators might be useful for the treatment and/or prevention of brain damage caused by pneumococcal meningitis. The main idea is to invent treatments which either inhibit the first group or stimulate the second group of immune modulators. With the knowledge of nowadays, as described in this review, treatments which inhibit the complement system (in particular C5 either directly with antibodies or indirectly via inhibition of SIGN-R1) and the production of ROS and RNS and treatments which activate TIMP might be feasible and effectively. In this way the long term morbidity after pneumococcal meningitis, as a consequence of brain damage might be reduced in the future.

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