Can the peripheral innate immune system be involved in the onset of the development of Alzheimer’s disease?

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A.E.P. Kamphuis
Supervised by prof. dr. U. L. M. Eisel
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Abstract

Alzheimer's disease (AD) accounts for 60-80% of all dementia cases and is therefore the most common form. For a few decades now, the amyloid cascade hypothesis has been considered as the main concept in AD pathology research, stating that the accumulation as well as the deposition of Aβ peptides is the trigger of the onset of AD. However, recently, a new important pathological hallmark has emerged, the neuroinflammatory reaction mediated by innate immune cells. This review will answer if the peripheral innate immune system can be involved in the onset of the development of AD. The innate immune response in the central nervous system, the innate immune response in the periphery, the interaction between peripheral immune cells and microglia, and peripheral infections in AD will be discussed. The activation of peripheral innate immune cells most likely functions as an early biomarker of the onset of the development of AD. A large amount of data discussed is consistent with the involvement of the peripheral innate immune system, particularly in the early-stage of AD. Although, to further support the role of the peripheral innate immune system as a trigger, more thorough research is required. If the aforementioned hypothesis is correct, supporters of the amyloid cascade hypothesis will have to modify their theories about the AD pathology. Eventually, this may lead to the revelation of new therapeutic targets regarding the prevention and treatment of this still untreatable disease.
Introduction

At present, a new case of dementia develops every 70 seconds in the United States alone, which translates into 450,000 cases each year. Alzheimer's disease (AD) accounts for 60-80% of all those cases and is therefore the most common form [1]. The main pathological hallmarks of AD consist of amyloid plaques, existing of predominant Amyloid-β (Aβ) peptides derived from the β-amyloid precursor protein (APP), and neurofibrillary tangles (NFTs), which are formed by the hyperphosphorylated Tau protein. Other biological abnormalities that have been reported in AD are the loss of overall brain substance and damage to certain parts of the brain, such as the hippocampus, the amygdala and the association cortices of the frontal, temporal and parietal lobes. These parts are associated with learning and memory. Furthermore, Aβ accumulation in small blood vessels is observed, as well as impaired regulation of free radicals (oxidative stress), metabolic impairment, inflammation, loss of synapses through loss of synaptophysin immunoreactivity, and cell loss, especially of large neurons [2;3].

AD can be distinguished in two major categories: the sporadic late-onset (LOAD) and the early-onset familial (FAD). LOAD manifests itself at ages higher than 65 years, whereas FAD manifests itself earlier, which can be as early as twenty years old in some cases. FAD is expected to be less than 1% of all AD cases [1].

For a few decades now, the amyloid cascade hypothesis has been considered as the main pathogenic concept in AD research. This hypothesis states that the accumulation as well as the deposition of Aβ peptides is the trigger of the initiating events resulting into AD and the onset of synaptic and neuronal dysfunction and loss [4,5]. Several lines of evidence emerged supporting the amyloid cascade hypothesis [6]. It all began with the analysis of Down syndrome patients, who are carriers of the additional AβPP allele. This allele leads to the onset of FAD, probably due to a dose-dependent effect. In 2006, the duplication of the AβPP locus had been studied and was reported to result in FAD, linking the AD manifestation to APP processing [7]. Subsequently, APP mutations had been found in families with a history of early-onset AD, causing the impairment of the aggregation propensity of Aβ peptides [8].

Besides the accumulation and deposition of Aβ peptides and the formation of NFTs, a third component in AD has risen as an important pathological hallmark, the neuroinflammatory reaction mediated by innate immune cells [9]. Over the past few decades, several studies have investigated the role of anti-inflammatory drugs in the treatment of AD and whether this hallmark could be a cause or a consequence [10;11;12].

The innate immune system is responsible for non-specific inflammatory responses in order to terminate extra- and intracellular pathogens. It consists of neutrophils, monocytes/macrophages, dendritic cells and natural killer (NK) cells. For the brain, the innate immune system includes the microglia, which play a leading role in neuroinflammation [13]. AD is seen as a chronic inflammatory disease of the brain, where the driving factor is the neuroinflammation in the central nervous system (CNS). It was believed that AD was limited to the brain, although neuroinflammation was seen as an important contributor [14]. A study of Busse et al. showed that alterations of the blood brain barrier (BBB) in AD caused recirculation of inflammation mediators like cytokines, chemokines and complement activation products. There is a cross-talk between the periphery and the brain [15]. If we accept AD as a systemic disease, the role of the whole immune system, central and peripheral, in the development or progression of AD can be suggested [14].

New therapeutic targets are highly needed in the battle against AD, because till this day there has not been an effective therapy nor a preventative cure available [16]. To gain more insight in possible new therapeutic targets, we will have to clarify whether neuroinflammation by peripheral innate immune cells can be a cause or a consequence in AD. Accordingly, in this review, I will answer the following main question: can the peripheral innate immune system be involved in the onset of the development of Alzheimer’s disease?
The innate immune response in the central nervous system

Microglia and Astrocytes

From the $10^{12}$ cells which the human brain consists of, approximately nine times $10^{11}$ is occupied by glia cells [17]. Based on morphology, function and location, different classes can be distinguished between glia. Macroglia and microglia are the two most important glial subsets of the CNS. Macroglia can be further classified as astrocytes, among other things [18]. Microglia and astrocytes are the key factors in the innate immune responses of the CNS [19].

The most persistent cells of the brain are astrocytes. Astrocytes were believed to mainly be a supporting cell type. However, in the last decade this view shifted to astrocytes being multifunctional housekeeping cells, with specializing neurons for information processing as its main function [20]. Astrocytes play an important role in inflammatory processes and therefore most likely also in neurodegenerative disorders like AD [21]. Astrocytes can, in contrast to microglia, remove and degrade Aβ without the help of mediators or stimuli, such as cytokines [21]. When astrocytes are activated, they release cytokines and growth factors, which are similar to the ones produced by microglia [22]. They share some of the immune-related functional properties of microglia [23]. In this thesis, I will focus on the microglia as a part of the innate immune system of the CNS.

Microglia occupy about 5-15% of all brain cells [24]. They are thought to be the major component of the brain’s innate immune system and often considered as the macrophages of the CNS [20]. Microglial cells can be found throughout the brain with various densities between the different areas of the brain. Depending on which area, the phenotype of microglia is either compact or ramified [25]. Initially, microglia were seen as resting resident immune cells. However, nowadays there is evidence showing microglia are highly dynamic cells, which regulate tissue homeostasis under pathological as well as physiological conditions [26].

The blood brain barrier keeps the immune system of the CNS private, but the possibility of an influx of peripheral immune cells is present [27]. When the innate immune system is stimulated, the microglial cells respond to signals received from the periphery and produce pro-inflammatory cytokines to coordinate a complementary reaction as a result [28]. In the presence of infectious pathogens, but also during brain injury and chronic disease, the innate immune system in microglia activates [29]. In case of neurodegeneration, the loss, abnormality or functional disruption of microglia can occur, which may lead to the pathogenesis of AD. During the process of neurodegeneration an increase of microglia occurs, as well as a dysfunction of microglial cells [30].

Microglial activation in AD

High levels of pro-inflammatory mediators, such as chemokines and cytokines, have been found in the brain of AD patients, which implies that inflammatory processes are activated [31].

The surface of microglial cells provides many transporters, channels and receptors. A high variety of neurotransmitters, neurohormones, neuromodulators, cytokines and chemokines can bind to these receptors [31]. Similar to peripheral innate immune cells, these receptors include Pattern Recognition Receptors (PRRs). Regarding neurodegeneration, the Toll-like receptors (TLRs) and inflamasomes are the best-known PRRs [32].

Exposure to immune activators during neurodegeneration leads to the triggering of PRRs, which in turn results in the activation of microglia [31]. PRRs can detect Pathogen Associated Molecular Patterns (PAMPs), which are sequences or structures presented on the surfaces of possible pathogens, and therefore function as one of the first lines of defence against potential infections. Besides PAMPs, PRRs are also capable of recognizing self-derived DAMPs (Danger-Associated Molecular Patterns) [33]. These signals activate TLRs and their co-receptors and cause oxidative and neuroinflammatory circumstances in AD through overproduction and release of pro-inflammatory cytokines, reactive oxygen species (ROS) and nitric oxide (NO) [34]. Identification of the DAMPs involved in the activation of microglia in relation to neurodegeneration is difficult [32].

The function of TLRs in the inflammatory response in AD has only recently taken an interest from researchers [35]. TLRs are characterized by an extracellular leucine-rich repeat domain and an intracellular Toll/Interleukin-1 receptor (TIR) domain [36]. When ligands bind, a conformational change is initiated in TLRs which, due to the recruitment of the adaptor proteins MyD88 or TIR-domain
Inflammasomes are innate immune system proteins involved in the assembly of large caspase-1 activating multiprotein complexes. They can be distinguished in four NLR subsets, from which the NLRP3 inflammasome can be characterized in microglia. Through proteolytic maturation caspase-1 activates. This activation leads to the release of IL-1β and IL-18 cytokines, promoting inflammatory cell death [37]. For inflammasome activation, priming is of importance. Before a second stimulus can cause the inflammasome activation, both pro-IL-1β and NLRP3 expression needs to be induced [38]. Through the use of mice with either a NLRP3 deficiency or a caspase-1 deficiency, the role of the NLRP3 inflammasome and IL-1β in AD development was confirmed in a study of Heneka et al. [39]. Furthermore, studies have shown that Aβ can activate the NLPR3 inflammasome in microglia, which describes a new pathway possibly leading to AD progression [37]

Recently, there has been evidence indicating that the aged brain is in a chronic state of neuroinflammation. Microglia are chronically activated. According to one hypothesis, this hyperreactivity is the result of microglial priming, which has circumstances similar to peripheral macrophages [40]. With the knowledge that peripherally-derived monocytes and macrophages can perform microglial-related functions after moving into the brain [35], a contribution of the peripheral innate immune system cannot be excluded.

**Microglial dysfunction during neuroinflammation**

The inflammatory cytokines released by active microglia possibly cause a dysfunction of microglial cells, especially with a loss of phagocytic functionality [32]. Differences between healthy and dysfunctional microglia are shown below [table 1].

<table>
<thead>
<tr>
<th>Healthy microglial state</th>
<th>Dysfunctional microglial state</th>
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<tbody>
<tr>
<td>Rarely proliferation under normal conditions</td>
<td>Increased proliferative capacity</td>
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<tr>
<td>Maintaining tissue homeostasis</td>
<td>Increased number and density of microglia</td>
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<tr>
<td>Ramified microglia with many thin processes</td>
<td>A reactive morphology with short, thick, and</td>
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<tr>
<td>Characterized by initial retraction and slight</td>
<td>poorly ramified processes</td>
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<tr>
<td>hypertrophy under injury conditions</td>
<td></td>
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<tr>
<td>Highly dynamic with extremely motile processes</td>
<td>Reduced response regarding motility, cellular</td>
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<tr>
<td>Inflammatory mediators enable intercellular</td>
<td>migration, and process thickening</td>
</tr>
<tr>
<td>communication in order to destroy harmful material</td>
<td>More easily responsive to defend against potential</td>
</tr>
<tr>
<td>Capable of clearing small numbers of dead cells</td>
<td>insults to the brain: disruption of the balance</td>
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<td>Normal protein folding and stability, protein</td>
<td>causing excessive activity</td>
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<td>trafficking, protein degradation, and autophagy</td>
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<td>Impairments in protein folding and stability,</td>
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<td>protein trafficking, protein degradation, and</td>
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<td>autophagy</td>
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*Table 1.* A normal microglial state in comparison with a dysfunctional microglial state, divided in six categories: proliferation, morphological transformation, motility and migration, intracellular communication, phagocytosis and proteostasis [46]. Proteostasis is protein homeostasis, necessary for protein quality control and prevention of protein accumulation [46]. A dysfunctional state can be caused by age and local and systemic inflammation, among other factors [32].

The term ‘inflammingaging’ is used for the imbalanced shift towards a pro-inflammatory state of aged microglia, causing impaired brain health [41]. A study with murine AD models showed that these cytokines lead to the downregulation of microglial receptors involved in phagocytosis, which resulted in an impaired clearance of Aβ [42].
Among these receptors, the TREM2 (Triggering receptor expressed on myeloid cells 2) receptor has been studied the most. Microglia greatly express TREM2, initiating functions such as phagocytosis, survival, proliferation as well as the secretion of cytokines. They also play an immunosuppressive role in TLR-induced inflammation in macrophages [32]. The link between TREM2, microglial dysfunction and neurodegeneration remains not fully understood [43]. However, an in-vitro study indicated that microglia with an overexpression of TREM2 were more effective in the removal of apoptotic neurons through phagocytosis, in comparison to normal microglia [44].

Microglial priming, the process in which microglia react excessively to a second inflammatory stimulus, is proposed as an explanation for microglial dysfunction in the process of aging [45]. Second stimuli reacting to primed microglia results in them being over reactive, which eventually leads to neurodegeneration and sometimes the progression of AD [46]. Microglial priming can be compared to a new concept called ‘trained immunity’, in which peripheral innate immune cells go through epigenetic and metabolic changes [47]. When these changes can be systematically defined in microglia, it can be determined whether microglial priming indeed has taken place and whether it belongs to peripheral trained immunity [32].

Systemic inflammaging could be a cause of neurodegeneration. Since activation of microglia is triggered by systemic immune abnormalities, chronic neuroinflammation could cause a pro-inflammatory and dysfunctional microglial state [46].

The innate immune response in the periphery

The cross-link between the brain and the periphery

During insults, the brain is responsible for sensing, processing and coordinating peripheral signals for the sake of maintaining homeostasis [40]. The brain is a reflection of the peripheral immune status, with only little direct contact [48]. The role of the blood-brain barrier (BBB) needs to be determined in order to understand how peripheral immune signals can access the brain [40].

The BBB is a highly specialized endothelial cell membrane within CNS micro-vessels. Its main functions are the regulation of the entry of plasma components and to ensure the export of possible neurotoxic molecules from the brain to the blood [49].

For a long time, it was thought that the CNS was an immune-privileged organ, with the BBB shielding the peripheral circulation. Now, however, it is known that the BBB is able to communicate with the peripheral immune system by responding to soluble factors and plasma proteins. These interactions support the role of neuroinflammation in the pathogenesis of AD [50].

In AD patients, degenerated endothelium in the brain vasculature is a common feature, as well as greater impairment of BBB transport systems [49].

In recent times, a necessary role of GLUT1, which regulates glucose uptake, for the maintenance of decent brain capillary networks, blood flow and BBB integrity has been indicated [51]. GLUT1 deficiency in endothelial cells causes a breakdown of the BBB with consequences such as extravascular accumulation of fibrin and immunoglobulin G in brain parenchyma, suggesting GLUT1 deficiency plays a part in AD pathology. Furthermore, a reduction of glucose uptake across the BBB may precede degenerative processes characterizing the transition of Mild Cognitive Impairment (MCI), a pre-stage of AD, to AD [49].

Oxidative stress in endothelial cells can also lead to cerebrovascular dysfunction in AD, where ROS modulates gene expression, proliferation, migration, angiogenesis, apoptosis, and senescence. Other inflammatory mediators are possibly involved in BBB dysfunction during AD progression. The secretion of increased levels of NO, pro-inflammatory cytokines, chemokines and prostaglandins by micro-vessels point out pro-inflammatory changes in the brain vasculature [49].

As mentioned above, the BBB seals the CNS from the changeable milieu of blood. However, there is a second barrier to be taken into account, the blood-cerebrospinal fluid barrier (BCSFB). The BCSFB is established by the choroid plexus at the level of epithelial cells [52]. The choroid plexus mainly consists of tight junctions, which reduce the permeability of intercellular adhesion areas [53]. The traditional function of the choroid plexus is to provide protection to the brain and to facilitate removal of brain metabolites by bulk drainage of CSF [54].
According to recent studies, the choroid plexus-CSF system plays a much bigger role in AD pathogenesis than expected. The alteration of the choroid plexus in AD can be compared to the abnormalities developed during aging, however highly increased. These altered functions may not be enough to initiate Aβ deposits in most AD cases, the choroid plexus condition is seen as an important factor in the beginning as well as the development of AD. Furthermore, studies suggested that altered functions of the choroid plexus may precede various AD hallmarks, for example Aβ accumulation [54].

In the choroid plexus stroma, all types of immune cells can be found, including interferon gamma (IFN-γ). The production of IFN-γ, a signal produced by local or systemic immune cells originating from the CNS, ensures a constant low level of cell trafficking necessary for screening and response to dangerous pathogens. Any insult, such as an infection or a trauma, triggers an enhanced production of IFN-γ, which causes a controlled migration of immune cells. However, in case of AD, the IFN-γ signal cannot be fully expressed. When insults occur, it was indicated that neutrophils not only enter the brain to contribute to pathogen removal, but also to uncontrolled inflammation, an AD hallmark [14].

As monocytes being the most studied circulating immune system cells in AD [49] and NK cells being considered as a biomarker of an early stage of AD [14], I will mainly focus on these cells of the peripheral innate immune system, together with neutrophils. An overview is given of the peripheral and the central innate immune system involved in AD pathogenesis [Fig. 1].

![Fig. 1](image.png)

**Fig. 1.** The cross-talk between the peripheral innate immune system and the central nervous system in AD pathogenesis. Pathological insults activate microglia in the brain, causing the migration of inflammatory mediators to the periphery, where they activate the peripheral innate immune cells. These activated peripheral innate immune cells enter the brain through alterations in the BBB and the choroid plexus characterizing AD, and mostly contribute to the uncontrolled inflammation.

**Monocytes in AD**

Peripheral monocytes/macrophages are also recruited in the CNS during AD pathogenesis, most likely to stop the formation and/or extension of Aβ plaques. Monocytes migrate from the peripheral blood into the CSF, crossing the BBB following chemotactic gradients [55].

As previously mentioned, the most studied peripheral innate immune system cells in AD are monocytes. Because these cells can easily be targeted by antibodies among other things, they are favoured candidates for therapeutic research regarding neurodegenerative diseases [56].

During AD, monocytes migrate through the BBB into the brain, a process which is dependent on C-C chemokine receptor type 2, CCR-2. This G protein-coupled receptor is highly expressed in monocytes of the pro-inflammatory subset. However, CCR-2 is found in much lower or undetectable levels in monocytes of the anti-inflammatory subset, otherwise called the ‘patrolling’ subset [57]. The main ligand of CCR-2 is CCL-2, C-C chemokine ligand type 2, and is the most important contributor in the signal transduction pathway, which leads to monocyte migration through the BBB. Studies have indicated that CCL-2 is upregulated in micro-vessels in the post-mortem brain of AD patients [49].
Furthermore, an increased number of monocyte chemotactic protein-1 (MCP-1), a protein bound by CCR-2, is found in AD brains [55].

The expression of CCR-2 is also upregulated in AD. Recently, it was shown that CCR-2 expression is a prerequisite for the transplantation of bone marrow-derived monocytic cells into AD brains. An increased CCR-2 expression promotes monocyte arrival within the BBB and the reduction of the expansion of Aβ plaques, and thus could act as a protective factor in AD pathogenesis. It is suggested that peripheral monocytes and the CCR-2/MCP-1 pathway may ease the influx of pro-inflammatory cells into the brain during neurodegeneration [55].

Neutrophils in AD
Neutrophils are highly reactive cells, releasing ROS, NFTs, enzymes and cytokines, which can lead to long-term tissue damage throughout chronic neuro-inflammation [49]. Studies have indicated circulating neutrophils to be pathogenic in brains of AD patients [56].

Accumulation of neutrophils in the CNS has been shown during all stages of AD-like disease, implying that the trafficking of neutrophils may be involved in chronic BBB damage and inflammation [58].

Lymphocyte function-associated antigen 1 (LFA-1) integrin regulates neutrophil arrest on endothelium inflammation. Interaction between LFA-1 and endothelial intercellular adhesion molecule 1 (ICAM-1) leads to changes in the cytoskeleton of brain endothelium, which increases vascular permeability, already observed in AD brains [59]. LFA-1 not only regulates vascular permeability, but also intraparenchymal migration. Together with Aβ deposits, LFA-1 promotes neutrophil migration into brain, causing tissue damage [58].

High-affinity LFA-1 and low-affinity LFA-1 can be distinguished. The high-affinity subset may as well stop arrested neutrophils in the parenchyma, suggesting high-affinity neutrophils are necessary for neutrophil accumulation and tissue damage in the CNS caused by neutrophils during AD [58].

When LFA-1 integrin is blocked at the onset of AD-like diseases, a reduction of pathological hallmarks and memory deficits were shown, indicating a role for neutrophils in the induction of cognitive dysfunction. Furthermore, when neutrophils were temporarily inhibited during the early stages of disease, it resulted in long-term positive effects [58].

Finally, the study of Zenaro et al. suggests a cross-link between microglia activation and neutrophil inhibition, which has never been suggested before in AD research. The connection between these two may create feedback loops that augment and preserve their activation.

Neutrophil trafficking as a therapeutic target to prevent early AD pathogenesis or AD progression is proposed [58].

Natural Killer cells in AD
When it comes to virus-infected cells and cancer cells, NK cells form the first line of defence. Two subsets of NK cells can be distinguished, based on the expression of the CD56 surface marker. Mature NK cells have a low CD56 expression, whereas the immature ones have high CD56 expression and have a cytotoxic activity [14].

Multiple studies have indicated that circulating NK cells in AD caused no changes in comparison with healthy controls. However, during MCI, there is data suggesting the activation of various NK functions. The state of this activation could contribute to the neuroinflammation process, as NK cells provide pro-inflammatory mediators [60].

Findings suggest that NK cells must be considered as markers for the early AD stage [14]. For more knowledge about the contribution of NK cells to the onset of AD, more research about NK cells in MCI needs to be executed.

Interaction between peripheral immune cells and microglia
Cytokines and chemokines seem to be critical players in AD, as they establish the communication between the periphery and the brain microglia. Both control the movement of immune cells into the CNS and instruct the final effector mechanisms, such as macrophage activation, glial-glial interaction
and glial-neuronal interaction [61]. According to recent studies, systemic cytokines and chemokines are most likely involved in AD pathogenesis [61].

**The role of cytokines**
Cytokines are small and non-structural proteins, secreted by different kinds of immune and non-immune cells. When cytokines are being secreted, a variety of effects are brought about, including cell proliferation, cell stimulation or cell inhibition, apoptosis, antiviral activity, cell growth and differentiation, inflammatory responses, and upregulation of expression of surface membrane proteins. It was indicated that a lot of these cytokines are produced by glia or neurons. Furthermore, many studies have shown changes in the cytokines levels in the brain, blood and CF of AD patients [63].

During inflammation, the balance between pro-inflammatory cytokines and anti-inflammatory cytokines is thought to be the determining factor in disease outcome. In a disease like AD, the pro-inflammatory cytokines outweigh the anti-inflammatory cytokines, leading to the characterizing amplification of cellular activation and cytotoxicity [63]. The focus will lie on the three most important circulating inflammatory cytokines, IL-1, IL-6 and TNF-α.

IL-1 is a key player in the onset and development of a hormonal and cellular inflammatory cascade. A high level of IL-1β is found in CF and brain parenchyma within the early hours of brain injury. It has been documented that IL-1 plays a role in neuronal degeneration. In addition, IL-1 augments microglial and astrocyte activation [63].

On the other hand, IL-6 is a key player in host defence, mediating immune responses and inflammatory reactions which affect the CNS cell growth and differentiation. Furthermore, IL-6 promotes astrocyte production, microglia activation and stimulates the production of acute phase proteins [63; 64].

At last, TNF-α is a central key player in the initiation and regulation of cytokine cascade during inflammatory responses. Expression levels of TNF-α in the brain is low, which makes it difficult to determine the exact role of TNF-α under physiological conditions [63]. It has been shown that TNF-α levels are lower in serum from patients with dementia [65]. During inflammation, TNF-α among other pro-inflammatory cytokines is secreted by activated microglia, mostly reacting on Aβ peptides and oxidative stress [64].

Regarding peripheral cytokine dysregulation, data of circulating cytokines have been inconsistent in AD research. It is a difficult task to point out the specific actions of single cytokines, because they are considered pleiotropic. Therefore, any conclusions about disease-related effects cannot be drawn based on the measurement of one individual cytokine [64].

**The role of chemokines**
More and more evidence is emerging that there is an upregulation of chemokines and their associated receptors in resident CNS cells, which may play a major role in neuroinflammation and neuronal death in neurodegeneration, such as AD [66;64]. Various chemokines are found in altered AD brains or AD models, supporting the pathogenic role of chemokines in AD [67].

Chemokines are a major inflammatory molecules family [61]. These molecules regulate the recruitment of microglia and astrocytes to the area of neuroinflammation, or the area of Aβ deposits [63]. An increase of several chemokines, including inducible protein 10 (IP-10), previously mentioned CCL-2 and CXCL-8, otherwise known as IL-8, is reported in both brain tissue and CSF during MCI and AD. Remarkably, increased IP-10 levels are shown in MCI, but decreased IP-10 levels in further progression of AD, whereas CCL-2 and CXCL-8 are augmented in both MCI and AD [66].

**Peripheral infections in AD**
Bacteria or bacterial products may be involved in the elevation of brain cytokines. Lipopolysaccharide (LPS) is a component of the cell walls of gram negative bacteria, as well as a strong pathogen-associated molecular pattern for the innate immune response. LPS increases peripheral cytokines production and upregulates CD14 receptors in the brain [68]. The CD14 receptor functions as a pattern recognition molecule in the innate immune system. It responds to pathogenic micro-organisms and exogenous and endogenous stress factors [69]. It can be activated by Aβ proteins or by LPS. In a research of Sheng et
transgenic mice that overexpress APP with mutations linked to familial AD were treated with LPS. This treatment lead to an elevation of APP and Aβ in the treated mice when compared to the controls [70].

It is possible peripheral LPS may enlarge the BBB permeability, resulting in the migration of bacteria into the brain [71]. A study analysed 47 papers including 74 studies examining BBB permeability change after LPS challenge in vivo. 60% of these studies indicated a disruptive BBB as a consequence of LPS challenge [72].

The pathogen hypothesis suggests some pathogens function as triggers in AD pathology. This hypothesis can be linked with the risen component in AD pathology, the neuroinflammatory reaction mediated by innate immune cells. The pathogen hypothesis can be supported by various clinical studies. For instance, an impaired cognitive function was reported in AD patients who suffered and recovered from a systemic infection at least two months before the research. Furthermore, it was reported that peripheral infections increased the risk of delirium in AD patients. In addition, it was shown that a history of past severe peripheral infections leads to a faster development of AD [73].

*Chlamydia pneumoniae, Helicobacter pylori* and *spirochetes* are gram negative bacterial species involved in the pathogenesis of AD. The presence of *C. pneumoniae* was reported in 17 out of 19 samples from AD individuals, and a presence in 1 out of 18 samples of non-AD controls. With regard to *H. pylori*, a higher serum IgG antibody against this bacterium was found in AD patients when compared to controls. Spirochetes were reported present in the blood, CSF and brain samples from AD patients, and were absent in the controls [73].

**Periodontal disease and AD**

There are many studies reporting indirect links between periodontal disease (PD) and AD. All of them suggest a higher risk of AD due to PD [73;74;75;76]. The group of PDs are diseases affecting the supporting structures of the teeth. Infectious forms of PD are the consequence of bacteria located in the dental plaque [73].

As previously mentioned in this review, inflammation increases the inflammatory state of the brain which may contribute to AD progression. PD is a chronic infectious disease leading to bacterial and inflammatory exposure, on a local as well as a systemic level. The study of Kamer *et al.* proposes two mechanisms on how PD contributes to an enhanced brain inflammation and to AD progression.

First, inflammatory molecules as a consequence of PD increase brain inflammation. Production of pro-inflammatory molecules such as IL-1β, IL-6, IL-8 and TNF-α is the response to the interaction between periodontal bacteria and the host. In cases of severe PD, these molecules can lead to systemic inflammation and may migrate into the brain through systemic circulation [73].

Second, PD increases brain inflammation through bacteria or bacterial products. Bacteria such as *A. actinomycetemcomitans, P. gingivalis, T. denticola* and *F. nucleatum* can invade the brain, resulting in changes in cytokine production. Once these bacteria arrive in the brain, the bacteria rich of LPS are capable of stimulating cytokine production. Unfortunately, the mechanism how periodontal bacteria invade the brain remains unknown till this day [73].

Another possibility for bacteria to reach the brain is through peripheral nerves. A presence of spirochetes was detected in ganglia, implying that oral spirochetes can invade the CNS through peripheral nerves. However, the presence of periodontal bacteria does not suggest migration into the brain via systemic circulation or peripheral nerves [77].

There is no direct evidence supporting the link between PD and AD [73].
Discussion and Conclusion

A role of neuroinflammation in the pathogenesis of AD has well been proven. Regarding the brain, it concerns a chronic state of neuroinflammation \textit{(microglial activation in AD)}.

Now, it is also accepted that the BBB is able to communicate with the peripheral immune system by responding to soluble factors and plasma proteins, supporting the role of neuroinflammation \textit{(the cross-link between the brain and the periphery)}. If that is true, the periphery may most likely contribute to this chronic neuroinflammation.

Concerning the innate peripheral immune cells, monocytes may ease the pro-inflammatory cell influx into the brain \textit{(monocytes in AD)}, neutrophils may augment and preserve their activation by feedback loops in communication with active microglia \textit{(neutrophils in AD)}, and NK cells may activate during MCI \textit{(Natural Killer cells in AD)}, all leading to neuroinflammation.

It has been reported that peripherally-derived monocytes and macrophages can perform microglial-related functions in the brain after migration \textit{(microglial activation in AD)}, and that microglial priming can be compared with peripheral innate immune cells going through epigenetic and metabolic changes \textit{(microglial dysfunction during neuroinflammation)}, both forming great evidence for the presence of peripheral innate immune cells in the CNS.

The question remains whether neuroinflammation is the cause or the consequence in the pathology of AD. Studies have provided evidence that neuroinflammation may precede AD, even may precede MCI, the pre-stage of AD. First, GLUT1 has a necessary role in the preservation of BBB integrity. GLUT1 deficiency plays a part in AD, leading to glucose uptake reduction, which may precede the transition of MCI into AD \textit{(the cross-link between the brain and the periphery)}. Furthermore, an altered choroid plexus characterizes AD, which also may precede various AD hallmarks, including Aβ accumulation \textit{(the cross-link between the brain and the periphery)}). This is contradictory with the amyloid cascade hypothesis. In addition, NK cells as markers for the early AD stage have been suggested \textit{(Natural Killer cells in AD)}. In order to know for sure whether NK cells contribute to the onset of AD, a closer look into the various functions which are activated during MCI is necessary. If possible, these functions must be inhibited, and their consequences must be examined. Researchers already inhibited neutrophils temporarily during early stages of AD, resulting in long-term positive effects. Neutrophil trafficking is proposed as a therapeutic target to prevent early AD pathogenesis or AD progression \textit{(neutrophils in AD)}. It requires more research to know the precise role of neutrophil trafficking as therapeutic targets. More research on the cross-link between microglia activation and neutrophil inhibition needs to be performed as well, in order to gain more insight in possible new therapeutic targets.

The role of circulating cytokines and chemokines in AD is also of importance. Unfortunately, data about circulating cytokines in AD have been inconsistent. Any conclusions about disease-related effects of specific cytokines cannot be drawn, because the actions of individual cytokines are hard to determine because of their pleiotropy \textit{(the role of cytokines)}. A variety of studies showed the increased presence of various chemokines in AD brains and their pathogenic contribution to AD. Increased levels of IP-10 are found during MCI, but there is a decrease of IP-10 during AD \textit{(the role of chemokines)}. More research on this chemokine is needed to determine its exact role in the onset of AD development. There is a possibility for IP-10 to play a more major role than expected. 

There is evidence indicating the link between peripheral infections and a higher risk of AD, supporting the pathogen hypothesis, which states these infections could act as a trigger in the onset of AD. 60% of studies examining a changing BBB permeability due to LPS challenge indicated a disruptive BBB, allowing the movement of bacteria into the brain causing neuroinflammation \textit{(peripheral infections in AD)}. Although many studies already examined the association of LPS with BBB permeability, more research on this topic is necessary to confirm whether LPS leads to a disruptive BBB. When it is clear LPS causes a more permeable BBB, and when the link between PD and AD is further examined, it would be more likely to accept the pathogen hypothesis. Acceptance of the pathogen hypothesis demonstrates that peripheral infections may play an important role in AD pathology, implies that presence of peripheral infections is accompanied with the risk of acquiring AD, and suggests early treatment of these infections will limit development and/or progression of AD or even prevent the onset of AD.
On the other hand, there is research indicating that Aβ deposits can activate the NLPR3 inflammasome in microglia, and therefore initiating a pro-inflammatory state by active microglia (microglial activation in AD). This describes a possible new pathway leading to progression in AD, supporting the amyloid cascade hypothesis, where the accumulation as well as the deposition of Aβ peptides are considered the trigger of the onset of AD development.

In reference to the question raised in the introduction, if the peripheral innate immune system can be involved in the onset of the development of Alzheimer’s disease, and taking all above into consideration, the answer is that the activation of peripheral innate immune cells most likely functions as an early biomarker for the onset of AD development. A large amount of data discussed is consistent with the involvement of the peripheral innate immune system, particularly in the early-stage of AD. Although, to further support the role of the peripheral innate immune system as a trigger, more thorough research is required. If the aforementioned hypothesis is correct, supporters of the amyloid cascade hypothesis will have to modify their theories about the AD pathology. Eventually, this may lead to the revelation of new therapeutic targets regarding the prevention and treatment of this still untreatable disease.
References

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