

Alzheimer's Disease & Treatment

Chapter 6

The Pivotal Role of Neuroinflammation in the Genesis and Evolution of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease with progressive and irreversible clinical course. Without effective treatments, Alzheimer's disease could reach epidemic proportions. This means that a global approach to the disease is based on the study of the interaction between three fundamental processes involved in neurodegeneration: neuroinflammation, amyloidogenesis and synaptic dysfunction.

For two years, there are two classic hypotheses to explain Neurodegenerative diseases. The hypothesis of amyloid plaques: in patients, beta-amyloid protein accumulates between neurons and forms senile plaques that compress neurons, which would be the cause of their destruction. The second hypothesis of tau protein: in patients, this protein accumulates inside the neuronal cells and causes their suicide. However, the experiments wanted to limit the formation of these compounds did not give results. The researchers have now been able to be stored in the brain.

Brain damage is caused by β -amyloid deposits ($A\beta$) and neurofibrillary tangles are responsible for neuronal death, particularly in the cortex and

hippocampus. These lesions are the central inflammatory reactions that participate in the process of neurodegeneration.

It is interesting to study in depth the fundamental principles that link neuroinflammation, amyloidogenesis and synaptic dysfunction in order to adopt an effective therapeutic strategy.

Abbreviations: A β : β amyloid; AD: Alzheimer's disease; ApoE: Apolipoprotein E; APP: Amyloid precursor protein; CNS: Central nervous system; IL-1 β : Interleukin 1 beta; IL-6: Interleukin-6; IFN- γ : Interferon gamma; NFT: Neurofibrillary tangles; ROS: Reactive oxygen species; TNF- α : Tumor necrosis factor alpha. BBB: blood brain barrier; NO: Nitric oxide; LPS: lipopolysaccharide.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, which is clinically characterized by progressive cognitive decline finally leading to the full-blown picture of dementia [1]. AD represents 50 to 70% of all dementia cases. Yet, it has no cure. Synaptic loss and dendritic loss have been observed in the hippocampus and neocortex of AD patients [2]. It is characterized by 3 stages according to the evolution and the severity of the symptoms. This disease is associated with an immune disorder, which appears to a significant rise in the inflammatory cytokines and increased production of free radicals such as nitric oxide (NO) [3]. Similar to peripheral inflammation, the process in the central nervous system (CNS) has both cellular and humoral mediated mechanisms. The primary cell of interest is the microglial cell, derived from myeloid precursors in the bone marrow during embryogenesis [4]. Under normal physiological conditions, microglia are in a resting state, evenly distributed throughout the brain with a characteristic star-like morphology. They have varied age-dependent functions, including brain development, synaptic plasticity, immune surveillance, and repair. These cells respond to a wide variety of stressors, including ischemia, trauma, and pathogens, in part via specific signaling molecules, such as proinflammatory cytokines, reactive oxygen species (ROS) and nitrogen species, chemokines, complement, and heat shock proteins, by becoming activated [5]. When so activated, they move to affected areas (such as areas of cell injury or apoptosis), and undergo morphological changes to resemble macrophages. This change heralds phagocytosis by the activated microglia, and the production of cytokines, chemokines, growth factors, and ROS [6]. The origin, fate and repletion of microglia are incompletely understood, but it is thought that certain cells (e.g., monocytes) can move from the periphery into the brain, especially in situations that disrupt the blood brain barrier (BBB), to participate in these processes, and perhaps become microglia. The other major brain cell type that responds to the same stressors is the astrocyte. Reactive astrogliosis is a common finding in areas of the brain damaged by ischemia, infection or misfolded protein deposits, focal lesions or trauma [7].

Neuroinflammatory responses can be both detrimental and beneficial [8]. On the one hand, activated microglia clear apoptotic or injured cells, dysfunctional synapses, and amyloid- β deposits, and with astrocytes, promote repair via secretion of neurotrophic factors

and produce immunoregulatory cytokines, such as interleukin-10 (IL-10). The timing and regionality of the humoral response are important to its success at protection. On the other hand, microglial activation is accompanied by an immune response and the expression of proinflammatory proteins, such as interleukin-beta and interleukin-6 (IL-1 β , IL-6) and tumor necrosis factor alpha (TNF- α), whose exuberance can lead to the damage of normal neurons, and signaling processes through recruitment of other cells that generate an ROS response. The result is synaptic and neuronal dysfunction, manifest ultimately by cognitive dysfunction. Cognitive disturbance resulting from systemic inflammation alone has been well documented [9]. The balance between the beneficial and detrimental effects of neuroinflammation is crucial to the outcome, and thus factors capable of modulating aspects of the process are important to understand.

Neuroinflammatory response is primarily a protective mechanism in the brain. However, excessive and chronic inflammatory responses can lead to deleterious effects involving immune cells, brain cells and signaling molecules. Neuroinflammation induces and accelerates pathogenesis of Parkinson's disease (PD), Alzheimer's disease (AD) and Multiple sclerosis (MS). Neuroinflammatory pathways are indicated as novel therapeutic targets for these diseases [10]. Nitric oxide (NO) is a free radical messenger molecule produced by neuronal nitric oxide synthase (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS or NOS2) [11]. The inducible isoform of NOS (iNOS) generates large amounts of NO [12]. The iNOS is induced by lipopolysaccharide (LPS) and/or proinflammatory cytokines, like TNF α , IL-1 β and IFN- γ . The nitric oxide can damage tissues in part by oxidative stress, the cytopathologic consequence of an imbalance between antioxidant defenses and free radical production leading to cellular death [13]. The nitric oxide has been implicated in neurodegeneration and neuronal cell death through its neurotoxicity in AD and other neurodegenerative dementias [14]. In AD patients, A β stimulates microglial and astrocytic NO production [15].

2. Cell mediators of Neuroinflammation in Alzheimer's Disease

2.1. Roles of glia in neuroinflammation

2.1.1. Microglia

Microglia are considered the resident immune cell of the brain. Under resting conditions, they exist primarily in a state of surveillance in the CNS and their major role is the maintenance of homeostasis within the brain microenvironment [16]. Maintenance of microglia in a relatively quiescent state is attributed in part to astrocyte and neuronal activity; for example, neurons can facilitate microglial quiescence by secreting signal factors including CD200, CX3CL1 and neurotrophins [17]. Microglia share phenotypic characteristics with peripheral monocytes cells and, during injury to the CNS, are polarized towards a pro-inflammatory phenotype (M1 state), induced mainly by exposure to pro-inflammatory cytokines, such as IFN- γ ,

TNF- α and cellular or microbial debris. The M1 state is characterized by production of pro-inflammatory cytokines, including TNF- α , IL-1 β and IL-6 and increased expression of inducible nitric oxide synthase (iNOS), inducing elevated production of NO and morphological change of microglia to an amoeboid shape [18]. However, in an effort at neuroprotection and repair, microglia can assume an 'alternative' activation, featured by an anti-inflammatory phenotype (M2 state). The M2 activation can be driven by anti-inflammatory cytokines, such as IL-4, IL-13 and IL-10 and is characterized by increased production of anti-inflammatory cytokines, including IL-4, IL-10, IL-13, as well as upregulation of Arginase-1 (Arg1), Chitinase-3-like-3 (Ym1, in rodents) and Mannose receptor C (MRC-1) [19]. Microglia activated towards the M2 state can also trigger inflammation resolution through the release of other anti-inflammatory factors, such as neurotrophins and growth factors (IGF-1 and TGF- β) [20]. The involvement of microglial activation has been identified in the pathophysiology of several neurodegenerative diseases, such as AD and Parkinson's Disease (PD), mainly by increasing neurotoxicity and cellular damage, thereby contributing to the degenerative process [21]. While polarization toward an M1 or M2 state can be readily induced in vitro, the complex nature of the brain microenvironment and the multiple signals that glia are exposed to makes it likely that a spectrum of intermediate transitional activation states exists in vivo. Nevertheless, the manipulation of microglial polarization is being actively investigated as a potential therapeutic strategy in a number of neurodegenerative conditions [22].

As a response to receptor ligation, microglia start to engulf A β fibrils by phagocytosis. As a consequence, these fibrils enter the endosomal/lysosomal pathway. In contrast to fibrillar A β , which is largely resistant to enzymatic degradation, soluble A β can be degraded by a variety of extracellular proteases [23]. In the microglial context, two proteases, neprilysin and insulin degrading enzyme (IDE) are of major importance. In sporadic cases of AD, inefficient clearance of A β has been identified as a major pathogenic pathway [24]. It has been suggested, that increased cytokine levels are responsible for the insufficient microglial phagocytic capacity by downregulating A β phagocytosis receptors [25]. Soluble oligomeric amyloid β (oA β) increased the processing of pro-IL-1 β into mature IL-1 β in microglia via ROS-dependent activation of NLRP3 inflammation. The production of IL-1 β depends on the activation of MAP kinases and NF- κ B signaling pathways. Subsequently, that overexpression of IL-1 β exacerbates tau phosphorylation and tangle formation through aberrant activation of p38-MAPK and synthase kinase 3 (GSK3) the increased expression of IL-1 β , was found to impair microglial A β clearance functions and increase BBB permeability, which can promote the accumulation of A β in the brain and increased neurotoxic factors. Thus, IL-1 β may play a complex role in AD pathogenesis (**Figure 1**) [26].

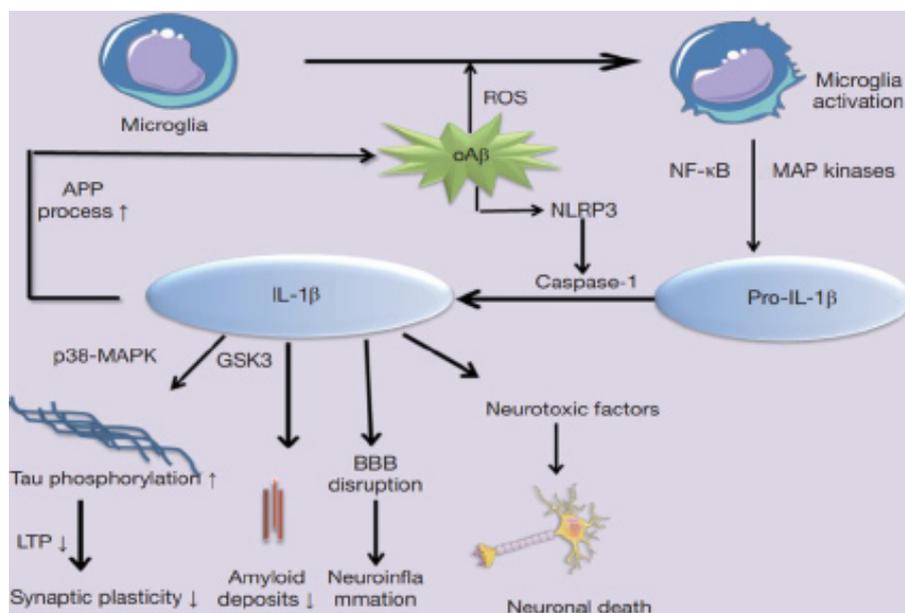


Figure 1: Hypothetical model linking the IL-1 β activation to AD pathogenesis [26].

AD, Alzheimer's disease; oA β , oligomeric amyloid β ; GSK3, glycogen synthase kinase 3; LTP, inhibiting long-term potentiation; BBB, blood-brain barrier; pro-IL-1 β , pro forms IL-1 β ; ROS, reactive oxygen species; $\uparrow\downarrow$, increase or decrease [26].

2.1.2. Astrocytes

Astrocytes are the most populous cells in the CNS, where they provide structural and functional support to neurons, form part of the blood brain barrier (BBB) and participate in synaptic formation [6]. While their main role is in neuronal support and brain homeostasis, it is accepted that they play an important role in neuroinflammation [27]. Similar to microglia, astrocytes can be activated from the resting state in response to insults and pathologies and this reactive astrogliosis is characterized by increased expression of GFAP [28]. Activated astrocytes have been shown to be a significant source of pro-inflammatory cytokines, including TNF- α , IL-1 β and IL-6 as well as other inflammatory mediators such as iNOS [29]. Most recently, a harmful/helpful A1/A2 classification, analogous to the microglial M1/M2 phenotypes, has been suggested though it is proposed that, again similar to microglia, a continuum of activation states is likely, especially in vivo [30]. Accordingly, astrocytes exposed to IL-4 and IL-10 show typical "alternative" activation (A2 phenotype), increasing expression of Arg-1, Mrc-1 and Ym1, while activated astrocytes can also help in tissue repair by releasing IL-10, which has been reported to suppress neuronal apoptosis through TLR/NF- κ B pathway activation [31]. In addition, astrocyte reactivity has been associated with several neurodegenerative diseases, including Huntingtons Disease, PD and AD and most recently, it was suggested that the normal process of ageing induces astrocytes to present A1-like astrocyte reactivity, with pro-inflammatory features [29]. The ability of reactive astrocytes and microglia to influence each other's morphology and function is now being painstakingly investigated, for example it has recently been shown that the A1-type astrocyte phenotype can be induced by neuroinflammatory microglia [32]. It is hoped that investigation of the cross-talk between microglia,

astrocytes and neurons will yield insights that may inform therapeutic interventions in diseases and disorders of the brain, including AD. A β aggregation and accumulation associated with AD pathogenesis trigger an inflammatory response in affected areas of the brain. Thus, active microglia and astrocytes are conspicuous around neuritic plaques [33]. Both microglia and astrocytes modify their morphology to adopt a reactive morphology and undergo functional changes [10]. Chronic inflammation states, characterized by sustained reactive gliosis, have been shown to worsen the AD pathology [34].

A β 1–42 and neurofibrillary tangles (NFTs) are the classic hallmarks of Alzheimer's disease can trigger neuroinflammatory changes, which induces the release of complement factors, cytokines and others inflammatory factors. PET uses biological surrogates for measuring neuroinflammation. Microglial activation is estimated by the expression of the 18-kDa translocator protein (TSPO), which is mainly found on the outer mitochondrial membrane of the microglial cells under inflammatory conditions. Monoamine oxidase-B (MAO-B), an enzyme usually located on the outer mitochondrial membrane of astrocytes, is proposed as an index of reactive astrocytosis. Radiolabeled arachidonic acid (AA), a phospholipid present in the cell membrane and cleaved by phospholipase A2 (PLA2), can estimate the AA metabolism. AA is the precursor of eicosanoids - prostaglandins and leukotrienes - which are potent mediators of the inflammatory response (**Figure 2**) [35].

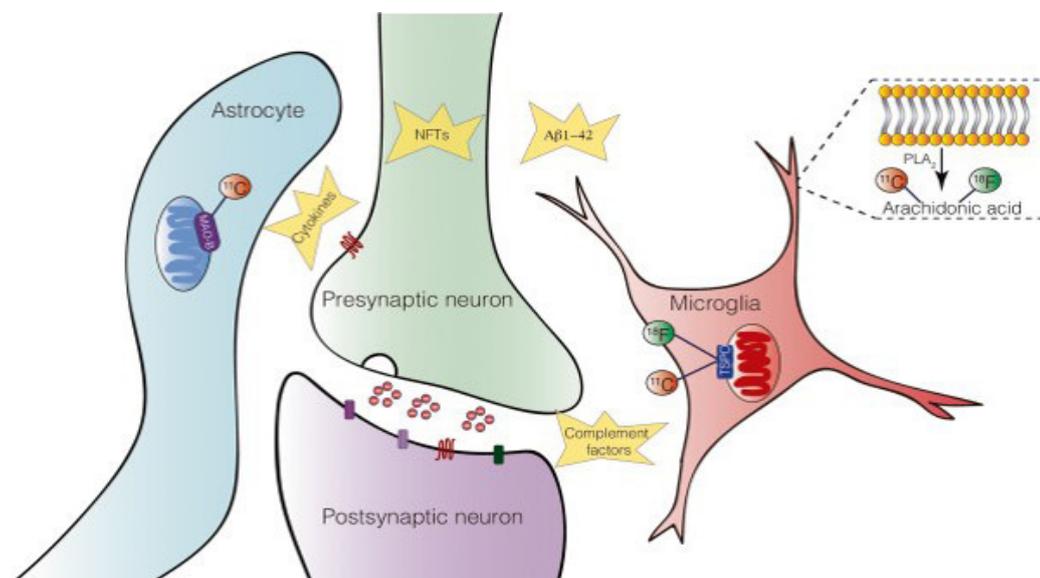


Figure 2: PET (positron emission tomography) biological targets for measuring neuroinflammation in AD [35].

Recently, a study demonstrated the involvement of microglia in synapse pathology at early stages of AD, preceding plaque formation, thus supporting the existence of a mechanism described during development and also modulating early pathological conditions during AD [36].

2.2. The Role of CD4+T Lymphocytes in Neuroinflammation

CD4+T cells are capable of activating and directing the functions of other cells. They participate in cellular mechanisms as antibody isotype switching and activation, and mobiliza-

tion of cytotoxic T lymphocytes. They also regulate phagocytic and lytic activity of mononuclear phagocytes (microglial and tissue macrophages) [37]. Activated CD4⁺T cells can easily cross the BBB [38]. Once they enter the damaged site, the cells exert several actions according to their phenotype [39]. Each cell subpopulation is specialized in coordinating immune responses against different types of threats and will produce particular effects. For example, in a medium where IL-12 is predominant, there will be a polarization toward the T helper 1 (Th1) phenotype, which has been associated to the elimination of intracellular microorganisms and causes neuroinflammation and neuronal damage in the CNS [40]. Th17 is another inflammatory phenotype whose differentiation is mediated by the presence of IL-23. These cells participate in intestinal immunity, autoimmune diseases and have been related to neuroinflammation and neurodegeneration mediated by the activation of apoptotic Fas/FasL pathway [41]. On the other hand, differentiation toward Th2 phenotype occurs in a microenvironment where IL-4 is predominant. This cell subpopulation directs immune response against helminths and in allergy, but it is also involved in the attenuation of neuroinflammatory processes. Tregs are a cell subpopulation that suppresses the effector function of Th cells [42]. These cells usually participate in the maintenance of peripheral tolerance to own molecules, limiting inflammatory responses against exogenous antigens. Within the CNS they attenuate neuroinflammation and, in consequence, neurodegeneration (**Figure 3**) [38].

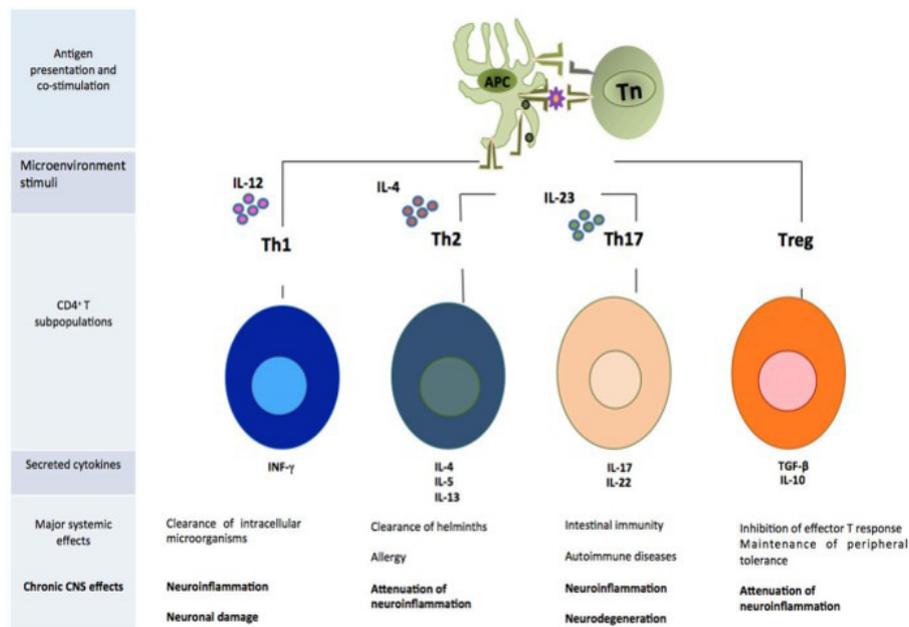


Figure 3: Subpopulations of CD4⁺T cells that play an important role in the development of neuroinflammation. APC, antigen presenting cell; Tn, T naive cell [9].

3. Inflammatory Mediators in Alzheimer's Disease

3.1. The complement system

The complement system is part of the innate immune system in multicellular organisms, and it is activated by three biochemical pathways. The classical complement pathway is activated when ligands bind to C1q triggering C1 complex activation. C3, a central protein

of the complement cascade, acts as downstream of C1q in the classical complement cascade and also activates the alternative pathway when ligands bind directly to it. Recent publications point towards a role played by microglia and astrocytes in early synapse pruning during development, presumably via the classical complement pathway [43]. They also showed that expression of C1q protein by retinal neurons modulated by astrocytes was a crucial event for synaptic pruning [44]. In AD, complement components have been associated with amyloid- β (A β) plaques [36]. It has also been reported that oligomeric/fibrillar A β and hyperphosphorylated tau (pTau) activate the complement pathway by binding to C1q [45]. C1q is upregulated and associated to synapses in the presence of oligomeric A β [36]. Under these circumstances, the classical complement pathway activates and results in synapse loss before A β deposition takes place [36]. C3 has been localized on reactive astrocytes in human AD cases [46] and they might contribute to synapse loss by releasing complement components themselves. In vitro and in vivo studies involving the use of mRNA expression and immunohistochemistry techniques have described the localization of C1q in neurons, both in synaptic puncta and axons during development. Also, astrocyte-secreted transforming growth factor- β (TGF- β) has been demonstrated to increase C1q expression in neurons [43]. Synapse loss is an early manifestation of pathology in Alzheimer's disease (AD) and is currently the best correlate to cognitive decline. Microglial cells are involved in synapse pruning during development via the complement pathway [47].

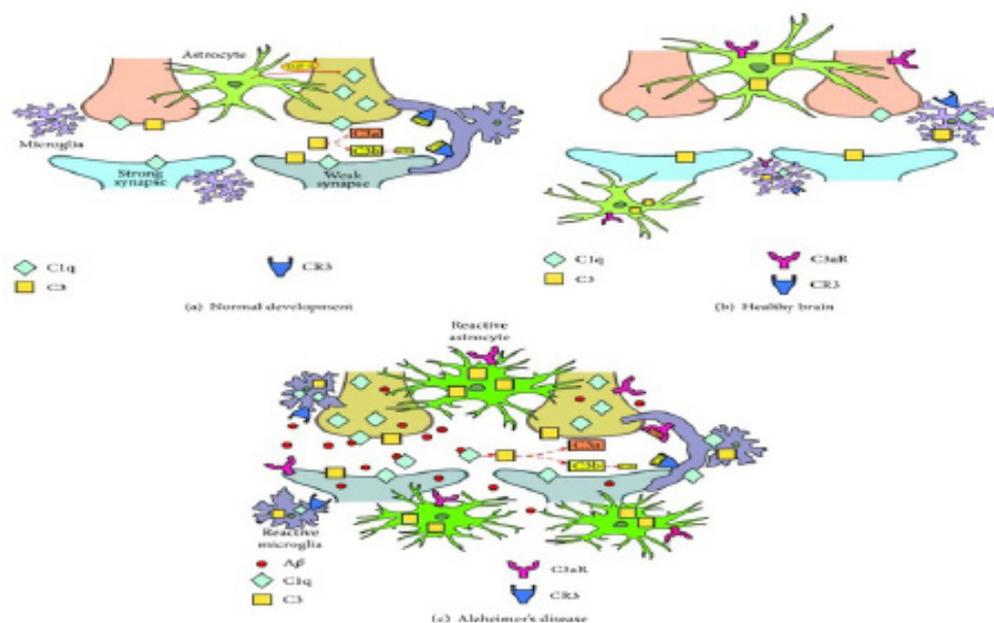


Figure 4: Model of complement-mediated synapse elimination during development, adulthood, and Alzheimer's disease [47].

(a) During early postnatal development, synaptic pruning takes place in order to eliminate excessive or weak synapses. Astrocytes induce the expression of C1q in neurons through TGF- β , and C1q colocalizes with synapses. The complement protein C3, which also colocalizes with synaptic puncta, is enzymatically cleaved to smaller fragments C3a and C3b. Finally, microglia engulf the synapse through the interaction of iC3b, the cleavage product of C3b, with its CR3 receptors [47].

(b) In the healthy brain, synaptic pruning decreases with age to basal levels and complement protein expression is reduced. Nonetheless, microglia and astrocytes continuously survey surrounding synapses [47].

(c) AD brain is characterized by progressive accumulation of extracellular and intracellular A β , gliosis, and neuroinflammation. Some studies have reported the role of microglia and complement pathway on synapse loss in AD models. Neuron-derived C1q and microglia-derived C1q are recruited to synapses and interact with A β . This triggers the activation of complement protein C3, expressed by both astrocytes and microglia. C3 is cleaved to smaller fragments such as C3b and iC3b that tag synapses and bind to CR3 on microglia. All these events lead to the removal of tagged synapses by the latter (**Figure 4**) [47].

3.2. Proinflammatory cytokines

Microglia and astrocytes are arguably the major source of cytokines in AD. Cytokines contribute to nearly every aspect of neuroinflammation, including pro- and anti-inflammatory processes, bystander neuronal injury, chemoattraction and response of microglia to A β deposits. Microglial activation is both characterized and modulated by cytokines. Increase in A β in aging TgAPPsw and PSAPP transgenic mice is associated with increased pro-inflammatory cytokines including TNF- α , IL-6, IL-1 α and granulocyte macrophage-colony stimulating factor (GM-CSF) [48]. This observation suggests that pathological accumulation of A β is a key factor that drives neuroinflammatory responses in AD. In addition, exposure of microglia to pre-aggregated A β 1-42 increases production of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α), macrophage inflammatory peptide (MIP-1 α) and macrophage colony-stimulating factor (M-CSF) [49]. Furthermore, MCSF levels in the plasma and CNS of AD patients are significantly elevated when compared to age-matched healthy controls or patients with mild cognitive impairment [50]. Caspase-1 activation, which is required to mature IL-1 β from its inactive pro-forms is similarly elevated in the brains of patients suffering from MCI and AD [51]. Consequently, high levels of the cardinal pro-inflammatory cytokine IL-1 β are detected in microglial cells surrounding A β plaques in AD patient brains and cerebrospinal fluid (CSF). In vitro, IL-1 β is released by activated microglia after stimulation with A β [52]. IL-1 β can, at least under certain circumstances, favor A β deposition by modulating APP expression and proteolysis. In addition to these cytokines, IL-12 and IL-23, well known from leukocytes, have been found to be produced by microglia in AD mouse models and the inhibition of IL 12/23 improves AD-like pathology [53], even so the regulation of IL-12 in human CSF is controversial [54]. There are multiple evidences suggesting that the pro-inflammatory environment present in the AD brain and in transgenic mouse models of cerebral amyloidosis assumes damaging proportions. For instance, risk for conversion from MCI to AD is higher in subjects with elevated CSF presence of the pro-inflammatory cytokine TNF- α and decreased anti-inflammatory TGF- β levels [55]. IL-1 β , TNF- α and other cytokines may impair neuronal func-

tion even before leading to structural changes [56]. Multiple interactions as well as elevated expression of additional cytokines/chemokines and innate immune receptors favor an M1- like activation state in AD. For example, in neuron-microglia co cultures, the synergistic action of A β with either interferon- γ (IFN- γ) or CD40 ligand triggers TNF- α secretion and production of neurotoxic reactive oxygen species [57]. In addition, the innate immune toll like receptor 4 is responsible for elevated levels of TNF- α and MIP-1 α in AD model mice.

Conversely, stimulation of some pro-inflammatory signaling pathways seems to be a beneficial approach in AD mouse models. Transgenic expression of IL-1 β in APP/PS1 led to robust neuroinflammation and a reduction of amyloid plaque pathology [58]. These findings implicate IL-1 β expression in activating a “good” form of neuroinflammation in APP/PS1 mice. In another study, (Adino Associated Virus, AAV) mediated expression of IFN- γ in the brains of the mouse model demonstrated the ability of this pro inflammatory cytokine to enhance clearance of amyloid plaques, alongside with a widespread increase astrogliosis and microgliosis [58]. In addition, these mice exhibited decreased levels of soluble A β and A β plaque burden, without altered APP processing. Similar results were obtained using AAV mediated expression of IL-6 and TNF- α [59]. Using the opposite approach, expression of the anti-inflammatory cytokine IL-4 resulted in the exacerbation of A β deposition [60]. These results suggest that certain “good” forms of pro-inflammatory microglial activation are potentially beneficial for reducing AD-like pathology in transgenic mouse models.

The source of the circulating cytokines in the plasma from patients is probably related to peripheral cells or endothelial cells and/or the brain [3]. Inflammatory mediators, including inflammatory cytokines, are highly expressed in the vicinity of A β deposits and neurofibrillary tangles. A wide range of inflammatory markers, typically absent in the normal elderly population, have been found in AD. Cytokines, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), when they are chronically produced, have been clearly implicated in the inflammatory process near the amyloid plaques inducing a cytotoxic effect. These cytokines could stimulate the production of A β peptides [61].

NO production is related to the increased levels of IFN- γ and TNF- α , in mild and severe stages of AD. Remarkably, significant IFN- γ level is only detected in mild stage of AD. NO production is IFN- γ dependent both in MCI and mild Alzheimer's patients. Further, high levels of NO are associated with an elevation of TNF- α levels in severe stage of AD. The proinflammatory cytokine production seems, in part, to be involved in neurological deleterious effects observed during the development of AD through NO pathway [3].

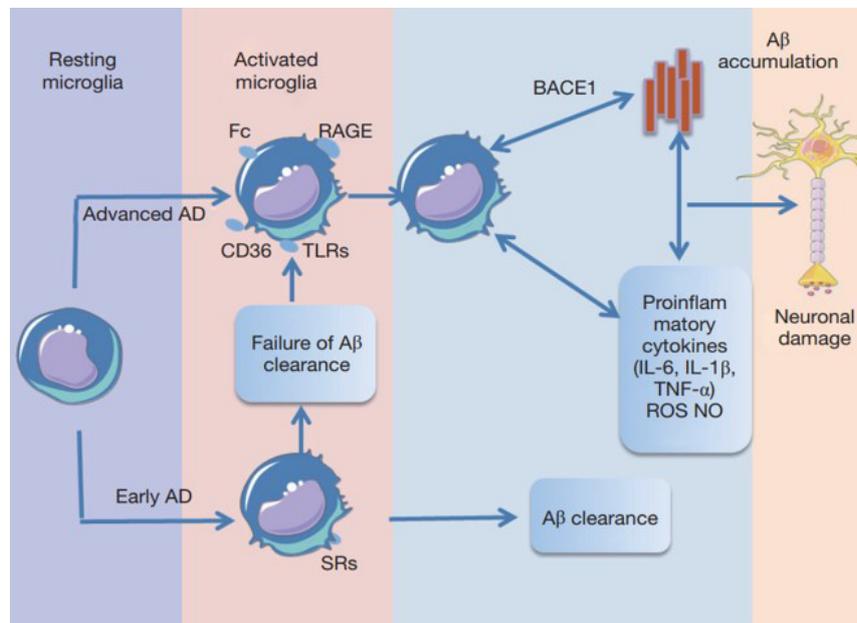


Figure 5: Possible mechanisms underlying microglial activation $A\beta$ deposition and subsequent pro-inflammatory cytokine release contribute to AD [26].

In the early stages of AD microglial activation can promote $A\beta$ clearance via microglia's SRs. The persistent microglial activation stimulated by $A\beta$ via the receptor for CD36, Fc receptors, TLRs and RAGE, creating a vicious circle between microglia activation, neuroinflammation, and $A\beta$ accumulation. A crucial role on pathogenesis of AD is an absolute culprit for both amyloid plaque and other pathologic change such as the neuronal damage. $A\beta$, amyloid- β ; AD, Alzheimer's disease; SRs, scavenger receptors; TLRs, toll-like receptors; RAGE, complement receptors advanced glycation end products; NO, nitric oxide; ROS, reactive oxygen species (**Figure 5**) [26].

2.3. Chemokines

Chemokines in AD have been suggested to regulate microglial migration to areas of neuroinflammation, thereby enhancing local inflammation [62]. In AD up-regulation of CCL2, CCR3 and CCR5 in reactive microglia has been reported [63], whereas CCL4 has been detected in reactive astrocytes near $A\beta$ plaques. In vitro, $A\beta$ causes the generation of CXCL8 (IL-8), CCL2, CCL3 and CCL4 in human macrophages and astrocytes [90], and microglia cultured from autopsies of AD revealed an increased expression of CXCL8, CCL2, and CCL3 after experimental exposure to $A\beta$ [64]. In AD mouse models a modulation of neuronal survival, plaque load, and cognition by the CX3CR1/CX3CL1 system has been shown. Further, the receptors CCR5 and CCR2 can modulate the course of the disease by influencing microglial positioning and function [65].

2.4. Nitric oxide and reactive oxygen species and oxidative stress

Next to their direct actions via surface receptors, cytokines stimulate inducible nitric oxide synthase (iNOS) in micro- and astroglia, producing high levels of NO that can be toxic to neurons. iNOS is upregulated in AD brains, and genetic knockout of iNOS is protective in

mouse models of AD [66]. Likewise, NADPH oxidase (PHOX) is highly expressed by microglia, upregulated in AD, and rapidly activated by inflammatory stimuli such as A β , resulting in hydrogen peroxide that further promotes microglial activation [67]. Superoxide from PHOX reacts with iNOS-derived NO forming peroxynitrite. Increased expression of iNOS in AD has also been shown to introduce NO-caused posttranslational modifications, which include nitration, S-nitrosylation and dityrosine formation, nitration of the A β peptide at tyrosine 10 has been recently shown to increase the propensity of A β to aggregate and has been identified in the core of the amyloid plaques [67]. More compelling, this modified peptide was able to initiate plaque formation in APP/PS1 mice, suggesting a central role during the early phase of AD. Nitrated A β suppressed hippocampal LTP more effectively when compared to non-nitrated A β , indicating that this posttranslational modification exerts functional as well as structural damage to the AD brain. There is evidence that oxidative stress supports the formation of this A β species [68]. Other NO-mediated modifications that may be relevant for AD have already been reported and it is to be expected that there are more to follow. Oxidative stress has been identified as a key feature in the pathogenesis of AD, and has been associated with the deposition of A β . The A β plaques have been related to cellular effects, such as the activation of p38 MAPK signaling pathway that leads to tau hyperphosphorylation, which, in turn, leads to intracellular NFT formation; mediation of apoptotic pathways by triggering the death promoter Bcl-2, which leads to the mitochondrial release of cytochrome C, and the infiltration of T cells into the brain parenchyma [69]. On the other hand, CNS or systemic inflammation positively feedback ROS over-accumulation.

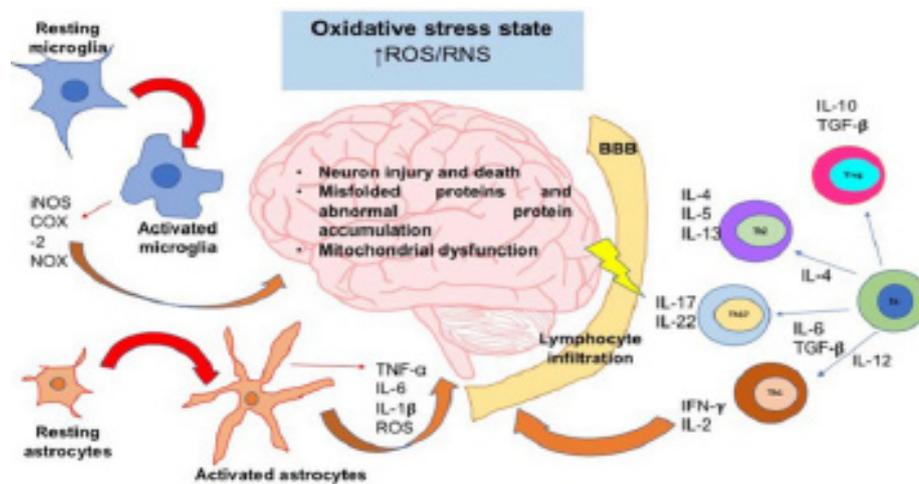


Figure 6: The oxidative stress state induces neuroinflammation and neurodegeneration [9].

In an oxidative stress state, ROS and RNS levels are augmented; these reactive species can activate signaling pathways that lead to the activation of the major glial inflammatory characters: microglia and astrocytes. These glial cells secrete proinflammatory factors which positively feedback the neuroinflammatory response. On the other hand, SNC-secreted factors and peripheral cytokines are able to disrupt the blood brain barrier (BBB) integrity; thereby, leukocytes such as T cells are able to infiltrate into SNC and take turn in the positive feedback of neuroinflammation. Inflammatory cells and secreted factors lead to neurodegeneration, in

which the most characteristic feature is the neuron injury and death. iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; NOX, NADPH oxidase; IL, interleukin; Th, T helper cell; Tn, T naive cell; Treg, T regulatory cell; ROS, reactive oxygen species; RNS, reactive nitrogen species; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor beta (Fig 06) [9].

2.5. Autoantibodies

The etiology of AD has not been fully defined and currently there is no cure for this devastating disease. Compelling evidence suggests that the immune system plays a critical role in the pathophysiology of AD. Autoantibodies against a variety of molecules have been associated with AD. The roles of these autoantibodies in AD, however, are not well understood [70].

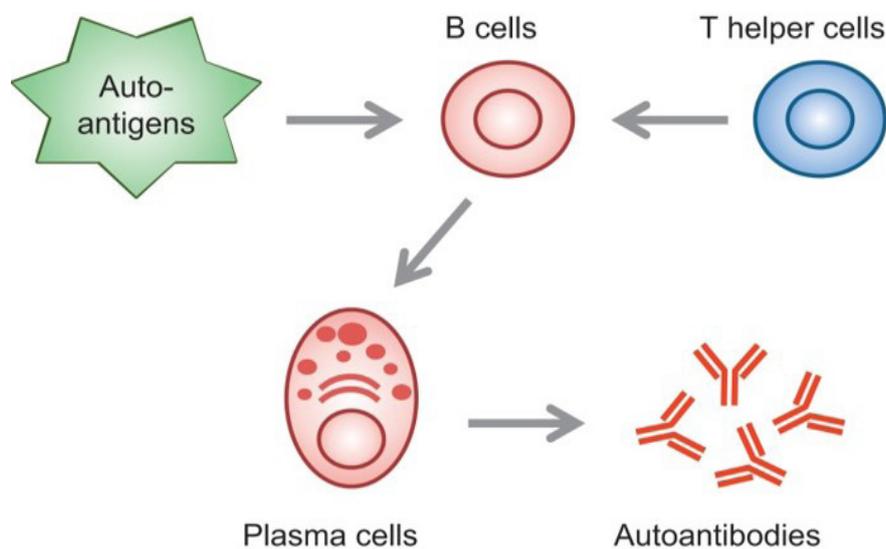


Figure 7: Schematic illustration of autoantibody production [70].

Under certain physiological and pathological conditions, B cells recognize endogenous constituents of the body as antigens (autoantigens). With the stimulation of the T helper cells, B cells differentiate into plasma cells that produce autoantibodies (**Figure 7**) [70].

Some of the autoantibodies described above are not merely markers but also contributors to the pathogenesis of AD. Early studies showed that AD brains had significantly more Ig-positive neurons, which showed neurodegenerative apoptotic features absent in Ig-negative neurons [71]. Immunoglobulin-positive neurons were frequently found in AD brains while they were rarely observed in the brains of healthy controls, suggesting a pathogenic role for autoantibodies in neuron death. Later studies reported that brain-reactive autoantibodies were nearly ubiquitous in human sera, which could contribute to neuropathology under the condition of BBB breakdown as in AD [48]. These studies confirmed the abundance of Ig-positive neurons in AD brains, and showed that treatment of cultured neurons with brain-reactive autoantibodies prevalent in human sera increased intraneuronal A β 42 accumulation, demonstrating a potential role of brain-reactive autoantibodies in the initiation and/or progression of AD.

Further studies suggested that protein citrullination, a post-translational protein modification that converts arginine to citrulline within proteins, may be involved in eliciting the production of brain-reactive autoantibodies [72]. The preclinical studies also support the pathogenic role of autoimmunity in AD. In a triple transgenic mouse model of AD, which develops both amyloid plaques and tau tangles, it was found that these mice exhibited manifestations of systemic autoimmune/inflammatory disease [73], including the elevation of autoantibodies. Further, the mice develop behavioral deficits in company with systemic autoimmune/inflammatory manifestations, prior to plaque and tangle pathology in the brain. These findings suggest a causal link between autoimmunity and abnormal behavioral function. The pathogenic role of some specific autoantibodies has also been investigated. For example, it has been shown that autoantibodies to ATP synthase are not only indicative of AD but also pathogenic in AD [74]. ATP synthase autoantibodies were capable of inducing the inhibition of ATP synthesis, alterations of mitochondrial homeostasis and cell death by apoptosis in SH-SY5Y neuroblastoma cell line. Further studies *in vivo* showed that intracerebroventricular administration of ATP synthase autoantibodies purified from AD patients caused poor cognitive performance and pronounced cell damage in the hippocampus in mice [75]. In addition, specific autoantibodies to ceramide were found to increase amyloid plaque burden in a transgenic mouse model of AD [35]. Natural autoantibodies against A β are generally considered protective in AD. Active and passive immunizations against A β have been explored as potential therapeutic approaches for AD. However, these immunotherapies have been associated with severe side effects related to A β anti-body-induced cerebral amyloid angiopathy (CAA) and perivascular inflammation [56]. Recent studies showed further evidence that A β autoantibodies causes CAA-related inflammation [73], similarly to what observed in A β -immunization trials. Thus, autoantibodies to A β could be pathogenic under certain conditions.

3. Neuroinflammation is the Result of the Interaction Between the Peripheral Immune System and the Central Nervous System

Further, inflammatory mediators from the brain can also enter into the peripheral system through defective BBB, recruit immune cells into the brain, and exacerbate neuroinflammation. We suggest that mast cell-associated inflammatory mediators from systemic inflammation and brain could augment neuroinflammation and neurodegeneration in the brain [10]. Systemic inflammation-derived proinflammatory cytokines/chemokines and other factors cause a breach in the blood brain-barrier (BBB) thereby allowing for the entry of immune/inflammatory cells including mast cell progenitors, mast cells and proinflammatory cytokines and chemokines into the brain. These peripheral-derived factors and intrinsically generated cytokines/chemokines, α -synuclein, corticotropin-releasing hormone (CRH), substance P (SP), beta amyloid 1–42 (A β 1–42) peptide and amyloid precursor proteins can activate glial cells, T-cells and mast cells in the brain can induce additional release of inflammatory and neurotoxic molecules

contributing to chronic neuroinflammation and neuronal death (**Figure 8**) [10].

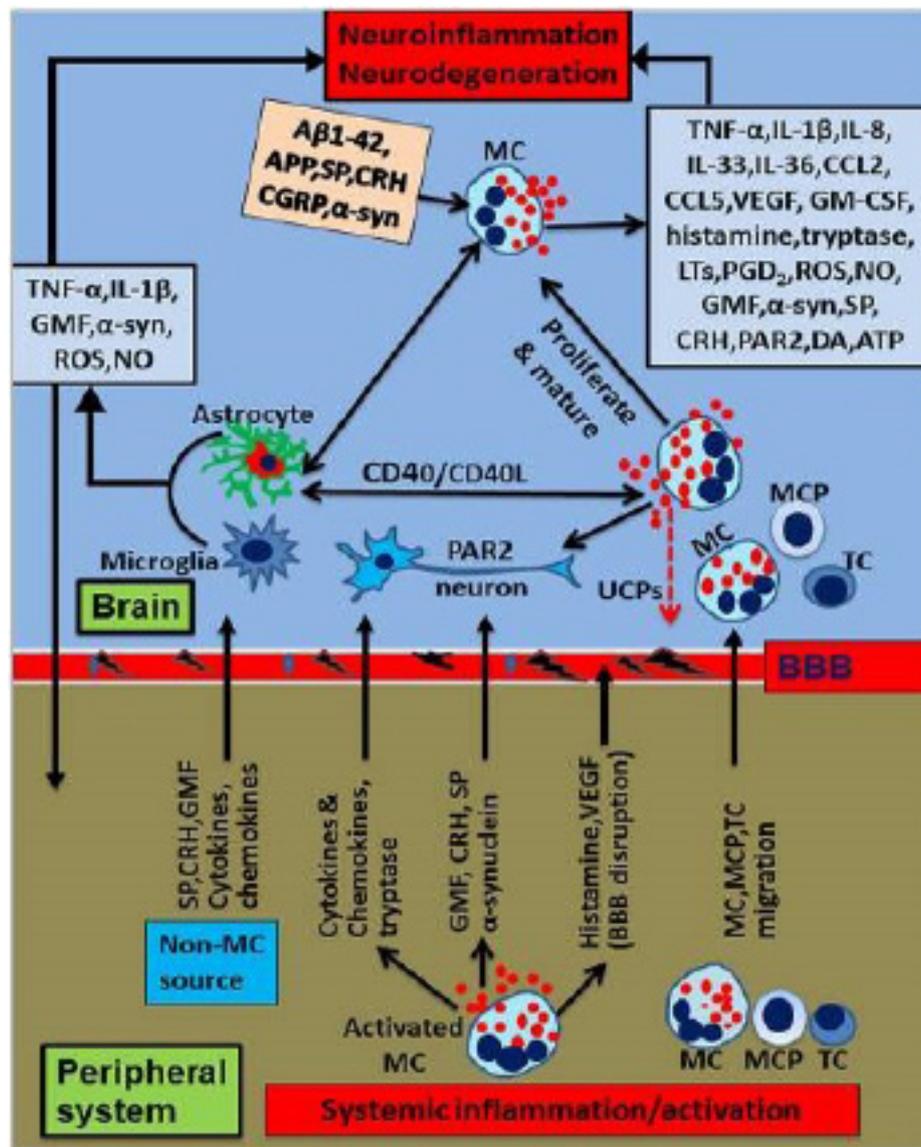


Figure 8: Schematic diagram showing peripheral inflammatory factors and cells on neuroinflammation and neurodegeneration [10]

Peripheral mast cell activation releases proinflammatory and neurotoxic mediators such as histamine, glia maturation factor (GMF), α -synuclein, corticotropin-releasing hormone (CRH), proteases, cytokines and chemokines. These mediators can induce neuroinflammation by inducing BBB breakdown, entering into the brain and activating glia and neurons to secrete various additional inflammatory mediators [10]. Peripheral mast cells and T-cells enter into the brain, proliferate and secrete proinflammatory mediators that activate glia and neurons to secrete more inflammatory mediators, reduce uncoupling protein (UCP) expression, and induce neurodegeneration. Further, glia and mast cells reactivate each other in the brain through co-stimulatory molecules CD40/CD154 or inflammatory mediators such as TNF- α , IL-1 β or IL-33 [10]. Mast cell tryptase acts on the neurons through PAR2. Mast cells can reactivate by their own mediators in an autocrine and paracrine manner to exacerbate inflammatory mechanisms [10]. The α -synuclein or MPP⁺ from glia/neuron or extracellular A β 1–42 can further activate mast cells to release neuroinflammatory mediators in Alzheimer's disease (AD) [10]. Additionally, several inflammatory mediators from the peripheral system can alter BBB, enter

the brain and activate the neuroinflammatory pathways. Inflammatory mediators released from activated microglia and astrocytes can enter into peripheral system through defective BBB; then, they can activate and recruit immune and inflammatory cells towards the inflammatory site in the brain [10]. MC, mast cell; MCP, mast cell progenitor; TC, T-cell; PAR2, protease activated receptor-2 [10].

MMP-9 is a secreted enzyme and member of the zinc metalloprotease (MMP) family. In general, MMPs are responsible for the degradation and maintenance of the extracellular matrix. MMP-9 has been shown to degrade compact plaques as well as soluble A β 42 and A β 40 [76]. In the CNS, MMP-9 is expressed by neurons, microglia, astrocytes, and infiltrating Iba+/CD45hi monocytes [77]. MMP-9 has also been shown to act as an α -secretase, favoring non-amyloidogenic processing of APP and the production of sAPP α [78]. In addition to its efficient degradation of A β , MMP-9 was shown to be involved in both TNF α -mediated pro-inflammatory and anti-inflammatory signaling in activated macrophages and microglia [79]. Elevated levels of MMP-9 have been correlated with BBB breakdown, demyelination, and cell death in other CNS disorders like multiple sclerosis and spinal cord injury [80]. These effects should be considered when modulating MMP-9 activity in vivo.

3.1. Mechanisms by which peripheral inflammatory factors and inflammatory cells augment neuroinflammation

The brain was originally considered as an immunologically privileged organ but now it is well known that the peripheral immune system and the brain communicate through several pathways [34]. We have previously shown that the presence of a tumor in the brain affects peripheral blood immune parameters [81]. Several peripheral inflammatory conditions could activate mast cells and release proinflammatory and neurotoxic mediators such as GMF. Systemic inflammation also increases BBB permeability in AD [82]. Normally BBB, formed by endothelial cells and astrocyte end-feet, restricts transfer of larger molecules and cells into the brain. Cytokines/chemokines and other proinflammatory molecules have been shown to cross BBB by an active transport mechanism [83] or through circumventricular organs that lack BBB [8]. The peripheral immune and inflammatory mediators can interact with brain BBB endothelial cells and induce the release of additional inflammatory molecules including PGD2 into the brain [84]. Systemic immune cells such as T cells can infiltrate into the brain through the BBB via choroid plexus or CSF that could induce neurodegeneration [85]. Peripheral inflammation is also translated to the brain through the vagus nerve by neural reflex [86]. As the BBB is disrupted in neurodegenerative diseases, the flow of immune cells and inflammatory molecules across the BBB is increased and thereby increases neuroinflammation [82]. Several previous reports indicate that mast cell activation [87], as well as peripheral inflammation influences BBB disruption to increase the permeability of inflammatory mediators and immune cell infiltration into the brain thereby spreading peripheral inflammation into the brain (Figure

09) [82].

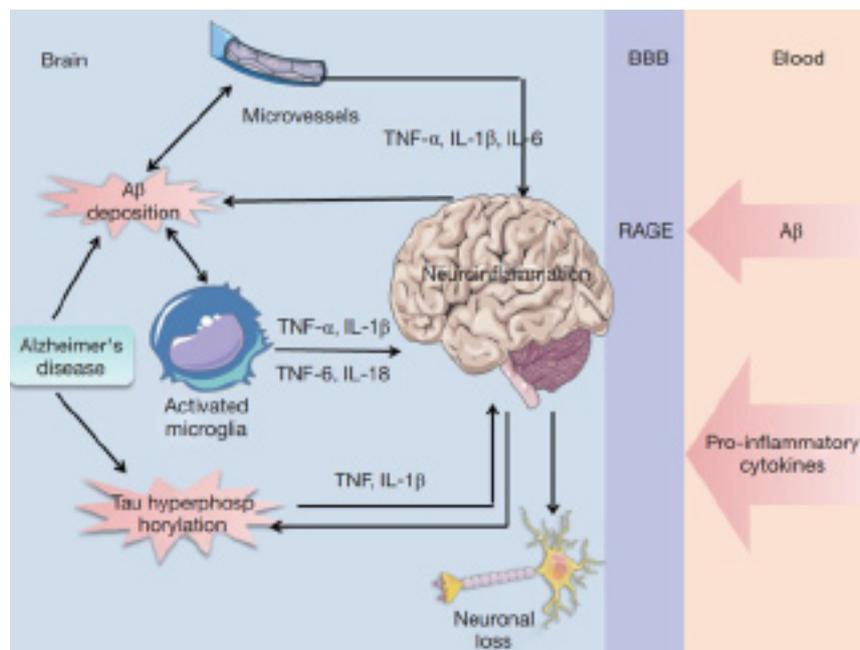


Figure 9: Speculative model of dysregulation of pro-inflammatory cytokines in the AD brain [82].

(I) A vicious circle between microglia activation, pro-inflammatory cytokines production, and A β , tau accumulation in AD brain; (II) AD cerebral microvessels participates in a destructive cycle of events where inflammation precedes A β deposition and A β in turn promotes release of proinflammatory cytokines; (III) pro-inflammatory cytokines and A β could cross the BBB from the periphery into brain, the latter is mediated by RAGE. AD, Alzheimer's disease; A β , amyloid- β ; RAGE, complement receptors advanced glycation end products; BBB, blood-brain barrier [26].

4. Conclusion

An early protective role of the immune system against Alzheimer's disease is identified in the early and even preclinical stages to the response of microglial immune cells. Increasing evidence has certified that the inflammation induced by A β plays a key role in AD pathogenesis. The inflammatory process itself is driven by microglial activation through the induction of pro-inflammatory molecules and related signaling pathways, thus leading to A β aggregation, tau formation, synaptic damage, neuronal loss, and the activation of other inflammatory participants. Thus, modulating neuroinflammation by targeting causing agents or/and trying to ameliorate their harmful effects could be of great importance to possibly, prevent AD pathology and contribute to stimulate endogenous repairing mechanisms as the formation of new neurons. A protective role of the inflammatory reaction during the disease, but only in the preclinical and asymptomatic stage, the progressive disease, inflammation that seems to have ignited. Finally, these findings underscore the importance of diagnosing the disease earlier and the new therapeutic perspective to slow down or even prevent its progression.

5. References

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