

Microglial Activation in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a devastating chronic neurodegenerative disease with currently no available disease modifying treatment. In recent years, the peptide amyloid- β has been proposed as the major pathogenic force in the development and progression of AD. Microglia, the resident immune and phagocytic cells of the brain, are known to constantly scan brain tissue and to respond to various pathological stimuli. Thus, newly formed plaque composed of A β seem to activate and recruit microglia in AD transgenic mice. However, the role of microglia is only poorly understood in AD. Microglia may act as a double-edged sword being either detrimental or protective depending on the context.

In this mini-review, we discuss the importance of microglia and its receptors in neuroinflammation and plaque clearance. A possible disease modifying role of blood-borne monocytes, which are close relatives of bone-marrow derived microglia, will also be addressed.

INTRODUCTION

Microglia are the phagocytic innate immune cells of the central nervous system (CNS). They survey their environment by extension and retraction of their processes [1, 2]. Upon detection of changes in the brain homeostasis, microglia undergo morphological, phenotypical and functional changes, a process described in the literature as "activation". Morphologically, microglia change from a small cell soma with long processes into a large cell body with almost no processes *in vivo* (Fig. 1). This is accompanied by upregulation of a plethora of surface receptors. Functionally, microglia react in diverse ways: they secrete inflammatory mediators, proteolytic enzymes or neurotrophic factors, and are able to take up soluble and insoluble molecules (Fig. 1). Because of their functional diversity, microglia can, depending on their environment, exhibit beneficial or detrimental effects on cells in their vicinity, thereby preventing or driving disease progression.

Microglia can become activated by amyloid- β (A β), a key peptide in the pathogenesis of Alzheimer's disease (AD) [3]. A β monomers of 40 to 42 amino acids are generated by neurons by cleavage of the membrane bound amyloid-precursor protein via β - and γ -secretases. While A β monomers do not seem to be toxic, production of A β oligomers containing especially A β ₁₋₄₂, has been considered to be synapto- and neurotoxic. A β dimers have been recently shown to impair synaptic plasticity by deficits in long term potentiation (LTP) and reduce dendritic spine density [4].

A β accumulation leads to the pathological hallmark of AD, the amyloid plaques. These can lead to neuritic abnormalities [5] and to pathological increases in intracellular Ca²⁺

in neurites [6]. During this process, microglia become activated and recruited to the plaque deposition sites [3]. After binding to A β , microglia respond with the secretion of inflammatory mediators and increased oxidative stress [7]. Microglia are thereby believed to be the main contributors to the observed neuroinflammation in the brains of AD patients and transgenic mouse models. Thus increased neuroinflammation can contribute to the observed changes in neuronal calcium homeostasis [8] and to the development of intracellular neurofibrillary tangles, composed of hyperphosphorylated tau protein in neurons [9]. These processes further accelerate neuritic and synaptic dysfunction and finally lead to the loss of neurons.

This review focuses on the role of microglia in AD, which has caused great dispute among scientists, since it was recognized that microglia are involved in the disease process. We will discuss the activation of microglia by A β and microglial's role in inflammation and A β clearance. We will also point towards beneficial effects of monocytes and other blood-marrow derived cells, which may preserve enhanced phagocytic activity. In the end, recent therapeutic developments that aim to use microglial features to prevent AD or delay disease progression will be highlighted.

MICROGLIAL ACTIVATION BY A β

Increased levels of A β can be found in the brain of AD patients and animal models. This is accompanied by the activation of microglia and an increase in the number of microglia in AD brains, referred to as microgliosis. Activated microglia can be found early in disease progression. In the APP/PS1 AD animal model, microglial activation can be observed as early as 2 months of age, which comes along with the deposition of amyloid plaques [10]. In the single transgenic APP mouse model, Heneka and colleagues were even able to detect microglial activation before the onset of amyloid deposition [11].

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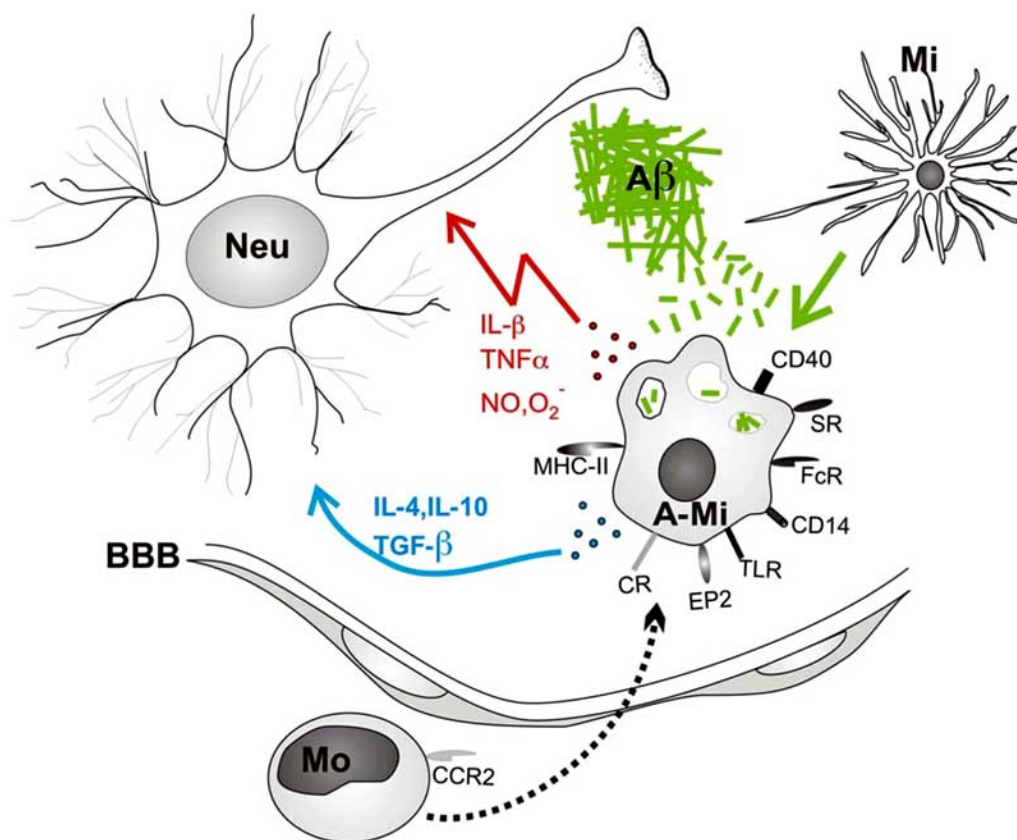


Fig. (1). Soluble A β (-), generated by neurons (Neu) is toxic to neurites and synapses. Soluble A β can accumulate into senile amyloid plaques (green), which alter calcium homeostasis in neurites and leads to neuritic dystrophy. Soluble A β also seems to be sequestered from amyloid plaques. “Resting microglia” (Mi) become recruited to the amyloid plaques. Two populations could account for the increase in microglia: First, endogenous brain microglia migrate to the lesion and proliferate. Second, peripheral blood cells, e.g. Ly-6ChiCCR2+ monocytes (Mo), are recruited from the periphery and cross the blood-brain-barrier. A β can bind to a plethora of microglial receptors. Microglia become activated (A-Mi) and secrete neurotoxic and/or anti-inflammatory molecules. In addition, some microglia may have the ability to take up soluble or insoluble forms of A β .

SR: Scavenger Receptor; TLR-Toll-like-receptor; CR-Complement receptor; MHC-II: major histocompatibility class II; BBB: blood brain barrier

Taken together, subtle changes in APP processing seem to be sufficient to initiate a pronounced and long lasting microglial response.

A β BINDING RECEPTORS IN MICROGLIA

Soluble and insoluble fibrillar A β can bind to a plethora of receptors expressed by microglia (Fig. 2). At least *in vitro*, microglia are able to take up A β after direct or indirect binding to its respective receptors [3, 12]. Traditionally, focus of intensive research was the inflammatory and oxidative response of microglia stimulated by fibrillar A β . Upon stimulation with fibrillar A β , microglia can respond by secretion of neurodetrital and neurobeneficial molecules (Fig. 2). Among the most prominent factors considered to be neurotoxic in AD are cytokines (e.g., TNF α , IL-1, IL-6), chemokines, complement factors, prostanoids, and reactive oxygen species like superoxide and nitric oxide [7]. But microglia can also produce “beneficial” cytokines such as IL-4, IL-10 and transforming growth factor - β (TGF β), which may reduce neuroinflammation and thereby modulate disease progression [13, 14].

A β binding to microglial receptors does not either result in secretion of inflammatory mediators or in phagocytosis, but results in both, linking inflammation with phagocytosis. Toll-like receptors, the prostanoid receptor EP2 and complement receptors are described here as examples that link inflammation to phagocytosis.

TLR, CD14

Toll-like receptors (TLRs) generally recognize and bind to various microbial products such as lipopolysaccharides (LPS) or hypomethylated DNA. TLRs on microglia and monocytes initiate an innate immune response and can bind to A β . Increased levels of certain TLRs can be found both in transgenic AD mouse models and human AD brains [15]. On one hand, activation of TLR2, TLR4, and TLR9 leads to increased production of nitric oxide (NO) and other inflammatory mediators [16]. A loss-of-function mutation in the TLR4 gene inhibits microglial and monocytic activation by aggregated amyloid peptide resulting in lower release of IL-6, TNF α and NO [17]. On the other hand, activation of cells of a microglial cell line with ligands for TLR2, TLR4, and TLR9 increases ingestion of A β *in vitro* [15]. In particular,

activation of TLR2 leads to an upregulation of p38 and ERK1/2 MAPKs and NF- κ B, signaling pathways which are involved in inflammation and phagocytosis [18]. TLR2 deficiency in APP/PS1 transgenic mice causes elevated levels of A β ₁₋₄₂ and TGF β in the brain accompanied by increased memory impairments. These deficits could be reverted by lentiviral delivery of TLR2 to bone marrow cells [19]. *In vivo*, transgenic AD mice with a TLR4 deficiency show an increase of insoluble A β in the cortex, which might be due to a decreased phagocytosis [15].

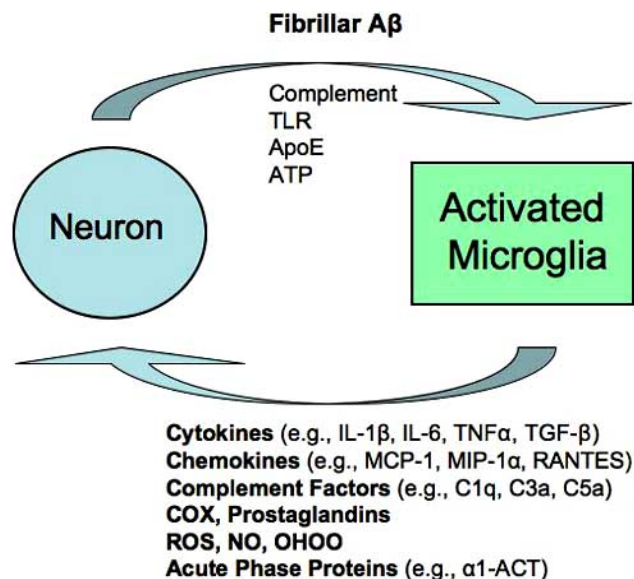


Fig. (2). A β is produced and secreted by neurons. Fibrils of A β can activate microglial cells. A β stimulated microglial cells become activated and respond with the secretion of a wide array of pro- and anti-inflammatory mediators. Increased secretion of proinflammatory mediators can further damage neurons. This increases in turn secretion of A β and other microglial activators and leads to the formation of a vicious cycle.

Related to TLRs is the lipopolysaccharide LPS receptor CD14, which is upregulated in AD brains. CD14 can bind to fibrillar A β *in vitro*. At higher concentrations of A β , microglial cells respond by increased secretion of IL-6 accompanied by an increased neurotoxicity. At lower concentration, A β is bound and internalized via CD14. Therefore the response of CD14 expressing microglia and macrophages depend on the concentration ranges of A β . While lower amount of may be internalized, higher concentrations elicit a neuroinflammatory response [20, 21].

EP

Mouse models support a role of cyclooxygenase (COX) enzymes and their products prostaglandins in AD. COX-2 overexpression in neurons induces neuronal apoptosis and cognitive deficits in transgenic mice as compared to control animals and cross-breeding with APP-PS1 leads to a doubling in A β plaques [22]. Increased prostaglandin E₂ (PGE₂) levels are found in the cerebrospinal fluid (CSF) of patients with probable AD. The prostanoid PGE₂ is released by over-activated microglia and PGE₂ can bind to the prostanoid receptor EP1 to EP4. EP receptors on microglial cells may be dynamically regulated as has been shown for the expression

of EP3 in microglial cells during neuronal cell death *in vivo* [23]. EP2 and EP4 stimulation by PGE₂ *in vitro* increases neuronal A β production [24]. Microglia from EP2 deficient mice showed increased phagocytosis of A β from human brain sections *in vitro* [25]. General basal uptake of A β in wildtype microglia seems to be EP2 independent [25]. Neurotoxicity was not observed in this model *in vitro* [25]. *In vivo*, deletion of EP2 reduces oxidative stress and β -secretase activity [26]. However, it is still unclear, if EP2 can modulate microglial A β phagocytosis *in vivo*.

Complement

Activation of the complement system is also observed in AD. The complement system is essential for immune-mediated defence against pathogens. Activation of the central complement C3, e.g., by fibrillar A β , may lead to the formation of the cytolytic membrane attack complex (MAC), which can cause lysis of pathogens but also healthy surrounding tissue. Alternatively, binding of C3 to complement activating structures like neuritic plaques can promote phagocytosis by microglia or monocytes expressing the corresponding complement receptors (CR) such as CR3 (CD11b/CD18). Inhibition of C3 activation by receptor-related protein γ (sCrry) [27] or cross-breeding of C3 knockout mice with APP mice increased soluble and insoluble A β levels [28]. The authors of the latter study pointed out that microglia in these mice showed a distinct phenotype with reduced levels of iNOS, TNF α but with higher expression CD45, and the so called anti-inflammatory cytokines IL-4 and IL-10 [28].

MICROGLIAL DEGRADATION OF A β

It is still unclear, if microglia have the capability of intracellular degradation of internalized fibrillar A β . In an early study, Frackowiak *et al.* did not observe any degradation of A β in microglia [29]. Halle *et al.* could recently show that internalized A β can activate the “inflammasome”, a cytosolic protein complex, which mediates the generation and release of IL-1 β by activation of caspase-1. This also leads to enhanced generation of other potentially proinflammatory and neurotoxic mediators [30]. In addition, the authors observed lysosomal damage, indicated by lysosomal swelling, loss of lysosomal integrity and the release of cathepsin B, a proteolytic enzyme, which can activate caspase-1 [30].

However, microglia seem to be capable of efficient degradation of soluble forms of A β . Recently, it was shown that apolipoprotein E (ApoE) can promote the proteolytic degradation of soluble A β *in vitro* [31]. ApoE is a lipid transporter and is mainly secreted by astrocytes but also microglia [32]. The ApoE4 allele has been implicated as a risk factor for the development for the disease [33], although the mechanisms whereby ApoE elevates the risk for AD has not been unraveled. Jiang *et al.* demonstrated *in vitro* that ApoE increases degradation of A β and that the rate of degradation depends on the lipidation status [31]. Enhanced expression of lipidated ApoE can be mediated by activation of liver x receptors (LXR). LXR are transcription factors, which are involved in lipid metabolism. Activation of LXR can repress microglial inflammatory response as evidenced by inhibition of inflammatory gene expression [34]. Treatment of Tg2576

AD mice with GW3965, a ligand for LXR, reduced brain A β load and reversed memory deficits [31].

EFFECT OF INFLAMMATION ON MICROGLIAL A β PHAGOCYTOSIS

Neuroinflammation seems to be a secondary response to the chronic progressive neurodegenerative process in AD. But does inflammation change the phagocytic activity of microglia?

The duration of inflammation seems to play a crucial role for the phagocytic activity of microglia. Transient overexpression of the cytokine Il-1 β in the hippocampus of APP-swe/PS1dE9 mice resulted in lower A β plaque deposition in the hippocampus accompanied by increased microglial activation in these animals compared to control APP-swe/PS1dE9 mice [35]. Similarly, acute application of LPS, a major component of the outer membrane of gram-negative bacteria and strong activator of microglial cells, ameliorates plaque load in transgenic AD models. In contrast to acute treatment, chronic intracranial delivery of LPS causes increased plaque deposition in transgenic AD models [36]. Moreover, microglia seem to depend on activation to be able to phagocytose A β . The physical attendance of microglia only does not seem to be sufficient, as shown in APP-MCP-1 (monocyte chemoattractant protein-1) double transgenic mice. MCP-1 is a chemoattractant for microglia. Although there was a strong increase in the number of "resting" microglia, as evidenced morphologically and by lack of expression of certain activation markers, an increase in diffuse A β deposition was observed [37]. These data hint at the notion that at least at some point in the disease process microglial activation accompanied by an inflammatory response may be beneficial, as evidenced in A β targeting therapy discussed below. The chronic inflammatory process seems to shift the function of microglial cells towards a neurodetritmental phenotype. At an early stage of the disease, microglia may be

beneficial, while later they may become dysfunctional (Fig. 3).

MICROGLIA AND AMYLOID PLAQUES

Activated microglia are found in the vicinity of compact plaques in the brains of AD patients and in transgenic AD mouse models [38]. The other plaque prototype, i.e. diffuse plaque composed either of none or of low-grade fibrillar A β , is not accompanied by neuritic or glial changes. In particular, diffuse plaques do not show microglial clustering. The role of diffuse deposits in AD is not known so far, however diffuse plaques are commonly found in elderly humans without dementia and are proposed to be an early stage of amyloid pathology.

By applying longitudinal multiphoton imaging technique, Meyer-Luehmann *et al.* recently showed in APP/PS1 transgenic AD mice, that amyloid plaque can form quickly over the course of a few days, accompanied with the appearance of activated microglia surrounding the plaques *in vivo* [39]. Amyloid plaques seem to attract and recruit microglia rather than microglia being responsible for the induction of new plaque development.

Still, only scarce information is available for the role of microglia before plaque deposition occurs. For example, do microglia play a role in removal of A β dimers and oligomers before they accumulate into plaques? This question is difficult to answer due to the limited technical possibilities to image non-fibrillated A β by multiphoton microscopy. A β dimers have been shown to be synaptotoxic [4] and a possible removal of these toxic molecules by microglia could be beneficial and thereby preventing neuro- and synaptotoxicity and delaying or even preventing amyloid deposition. In the course of the early stages of AD, microglia could either become dysfunctional or increased levels of A β surpass the capacity of microglia to remove and degrade toxic A β species, leading to the deposition of amyloid plaques. Once

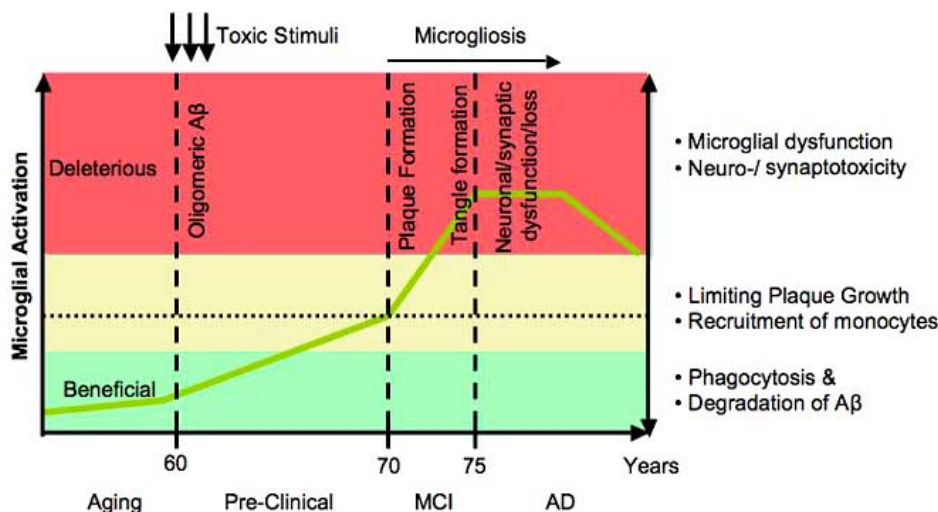


Fig. (3). Microglial activation involves beneficial and detrimental implications for brain homeostasis. In the healthy brain, microglia survey the brain environment and respond to new lesions by secretion of mediators, phagocytosis and proliferation. Years before the development of clinical symptoms for AD, microglia become activated by A β , leading to a chronic neuroinflammatory response in the brain. During this stage of the disease, microglia may actually prevent plaque formation or restrict plaque growth. As the disease progresses, microglia contribute to neuritic and synaptic dysfunction, generation of tangles, and the patient now develops "mild cognitive impairment". Over the following years, more plaques and tangles are generated, and the patient develops AD.

Table 1. Microglia-Target Therapy in AD

Therapeutic Agent	Effect on microglia	Clinical data
Immunization	- ↑ amyloid phagocytosis ([84] [83] [91] [82] [81] [92] [87]) - ↑ microglial activation ([90] [85])	Active Immunization: AN1792: ↑meningo-encephalitis ([93]) Clinical efficacy? ([94] [93]) Passive Immunization: clinical efficacy?
Non-steroidal anti-inflammatory drugs (NSAIDs)	- anti-inflammatory ([74] [95]) - PPAR γ activation ([96] [97]) ↔ phagocytic activity([98] [99])	- Ibuprofen protective against AD ([100]) - Indomethacin likely to slow progression of AD ([101]) - naproxen and celecoxib showed no effect on cognition ([76])
Minocycline	- anti-inflammatory ([77] [102] [103] [78]) - ↔ phagocytic activity ([80])	- clinical efficacy?
Statins	- anti-inflammatory ([104] [105] [106])	- Atorvastatin maybe beneficial in early AD ([107])
Cannabinoids	- anti-inflammatory ([108] [109] [110])	- clinical efficacy?
Botanical Polyphenols - Curcumin - Resveratrol	- anti-inflammatory, antioxidant ([111] [112]) - ↑ phagocytic activity? ([113]) - anti-inflammatory, antioxidant ([114] [115]) - ↔ phagocytic activity (own unpublished data)	- clinical efficacy? - clinical efficacy?

amyloid plaques have been formed, microglia could then try to restrict the growth of plaques and to remove A β dimers, which may be sequestered from amyloid plaques [4].

The interplay between amyloid plaques and microglia seems to be highly dynamic. Bolmont *et al.* showed in a transgenic AD mouse model, that the number of plaque associated microglia increased over time either via proliferation or infiltration [40]. Microglial cells can quickly migrate towards newly developed plaques and extend their processes towards the plaques. Microglia at the interface of the plaque were not static, but rather highly dynamic and showed membrane movement and process motility. Microglia around larger plaques were also enlarged in their size. The size of plaques corresponded over time to the volumes of the surrounding microglial cells. Interestingly, it seemed that there is a relationship between microglial cell volume and number: increase in the cell volume of microglia was accompanied with less cells in the vicinity of a plaque. The authors hypothesized, that this could be a compensatory mechanism. A limited number of microglia in the vicinity of a plaque could respond with larger cell bodies to cover the plaque. Moreover, the authors observed a change in plaque size over time, with small plaques increasing and large plaques getting smaller in size. The growing plaques had initially a smaller size with only little microglial coverage. The plaques that decreased in size over time had the highest number of microglial cells [40].

This could imply that a sufficient number of microglia can prevent plaque growth by preventing the transformation of local soluble A β to insoluble fibrillar A β and by an uptake of the sequestered soluble A β . Furthermore, it points towards

the potential of microglia to phagocytose and / or degrade A β extracellularly.

IS A SWITCH OF MICROGLIAL ACTIVATION ASSOCIATED WITH AD?

Takata *et al.* were able to demonstrate, that microglia transplanted into the lateral ventricle of rats are capable of taking up previously injected A β [41]. Also, microglia appear to phagocytose A β in the vicinity of an ischemic infarction [42]. Despite evidence of A β uptake *in vitro* evidence that microglia are able to phagocytose A β in AD patients under normal conditions is still lacking. However, it has been suggested that ageing and/or a chronic neuroinflammatory environment may alter the microglial state of activation [43, 44]. While the classical activation of microglia by LPS or A β leads to the production of a plethora of neurotoxic molecules, alternatively activated microglia may exert neuroprotective properties. IL-4- stimulated microglial cells respond with an increase in A β phagocytosis, reduction of A β toxicity and production of growth factors [45-47]. Colton *et al.* were able to detect gene expression profiles suggestive of alternatively activated microglia in human AD brain samples and in transgenic AD mouse models [48]. In support of these findings, Jimenez *et al.* found alternatively activated microglia in the hippocampus of young PS1xAPP transgenic mice, while at an older age both alternatively and classically activated microglia were detected immunohistochemically [49]. This may imply that microglia undergo a functional switch with an increased inflammatory burden during ageing. Ageing itself may effect microglial phagocytic activity. Floden and Combs isolated early postnatal and adult microglial cells in order to compare their phagocytic activity after stimula-

tion with A β [50]. Postnatal microglia appear more able than adult microglia to phagocytose fibrillar A β . In addition, adult microglial cells differ from the postnatal ones in expression and/or secretion of COX, IL-6 and IL-1 β after stimulation with A β [51] or LPS [52]. Indirect evidence for limited phagocytic ability comes from older transgenic AD mice, which exhibit a lower capability to clear plaques [53, 54].

Hickman *et al.* compared gene expression of various inflammatory/phagocytic key molecules in microglia of APP/PS1 double transgenic mouse with microglia from wild-type animals [55]. Microglia from older transgenic mice had a significant decrease in expression of the A β binding receptors SRA, CD36 and RAGE and a downregulation of A β degrading enzymes neprilysin and insulin-degrading enzyme, while these microglia showed an increase in the inflammatory cytokines IL-1 β and TNF α . In addition, incubation of N9 cells, a murine microglial cell line, with TNF α causes a decreased expression of A β binding receptors. Thus, an increase in inflammation in AD over time may therefore limit microglia's capacity to phagocytose and degrade A β due to a decrease in A β phagocytic receptors.

DO BONE-MARROW DERIVED CELLS PLAY A ROLE IN AD?

Resident parenchymal microglia originate from myeloid tissue and are recruited to the brain during embryogenesis and early postnatal days [56]. Microglia are similar in respect to the phenotypical and functional characteristics of their close cell line relatives in the periphery, namely monocytes and macrophages. However, it is unclear, whether bone-marrow derived cells like monocytes are able to migrate into the healthy brain and differentiate into microglia (Fig. 1). In AD, is the increase in the number of microglia due to increased proliferative activity of microglia or enhanced recruitment of peripheral hematopoietic cells? In regards to AD, it is of particular importance, whether these cells, in case they are able to migrate into the AD brain and differentiate into microglia-like cells, are beneficial in the disease process.

First, do bone-marrow derived blood cells contribute to the microglial population in the healthy adult brain? Indeed, blood cells of the myeloid lineage are able to acquire microglial characteristics [57, 58]. But are these cells able to infiltrate the healthy brain? To answer this question, Ajami *et al.* joined temporarily circulation systems of two animals together, the wild type mouse with a mouse expressing GFP-labeled peripheral blood cells [59]. By this approach, they found only a few GFP-positive microglia-like cells in the CNS of the other animal, suggesting that peripheral blood cells in the adult healthy brain only replenish microglial cells in a very limited way. Secondly, in damaged brain, e.g. after facial nerve axotomy or in an ALS transgenic animal model, microgliosis observed in these experimental approaches was not due to enhanced recruitment of bone-marrow derived cells [59]. These findings are corroborated by Mildner *et al.* [60] who described a certain population of monocytes in mice which is able to migrate into the lesioned CNS and adopt microglial characteristics *after* the BBB's integrity has been lost. Interestingly, monocytic cells expressing the chemokine receptor CCR2 and the cell surface glycoprotein

Ly6C, were found to be recruited to the brain and to differentiate into microglia [60]. It is unclear, however, if this microglia precursor population is able to invade the AD brain in large numbers. In the APP23 transgenic AD model, a weakening in the brain vessels has been described [61], thereby possibly enabling peripheral blood cells to infiltrate the brain parenchyma.

Second, can peripheral blood cells modify the course of the disease? To this aim, GFP labelled bone marrow cells were transplanted into mouse models of AD, who had undergone irradiation of their bone marrow cells prior to transplantation [62-64]. The number of engrafted cells in the brain were higher among younger transgenic animals, i.e. before plaque deposition has begun [62, 63]. This bone marrow chimeric AD transgenic mice, who had received intrahippocampal LPS injection, showed further recruitment of bone marrow cells and in addition a diminished plaque burden [62]. Simard *et al.* showed in irradiated mice that bone marrow derived microglia are recruited to the core of amyloid plaques *in vivo*, and that these cells in contrast to the resident microglia can take up A β [64]. Interestingly, not all plaques in these transgenic AD models were the target of the newly infiltrating cells [64].

If monocytes are able to invade the AD brain, how do they get attracted to the lesioned brain? Usually, peripheral monocytes and macrophages can get attracted by chemokines to the damaged site. One important chemokine is monocyte chemoattractant protein-1 (MCP-1, or CC-chemokine ligand 2, Ccl2), which is upregulated in plaques and activated microglia [65]. MCP-1 binds to the chemokine receptor CCR2, which is expressed by mononuclear cells. APP mice crossed with CCR2 knock-out mice resulted in a substantial increase in A β ₁₋₄₂ levels accompanied by a dramatic decrease in the number of microglia and monocytes in the brain [66]. This could mean that the reduced number of microglia or monocytes leads to an impaired removal of A β either by reduced phagocytosis or extracellular degradation of A β . So does an increase in the recruitment of monocytes/microglia to amyloid plaque deposition lead to an increase in amyloid removal? Overexpression of MCP-1 in AD transgenic mice indeed led to an increase in the number of monocytes/microglia. However, despite the increased number of potentially protective cells these animals showed an increase in A β accumulation with no signs for microglial activation [37]. It could be, that microglia and monocytes become desensitized by chronic overexpression of MCP-1, so that these cells are not responsive any longer to A β [67].

Recently, Town *et al.* showed that macrophages could be beneficial in removing amyloid plaques as opposed to microglia. They investigated the role of transforming growth factor β (TGF- β) in peripheral macrophages in different transgenic AD models [68]. TGF- β is a key innate immune cytokine, which is involved in immune cell activation, inflammation and repair after injury [69, 14]. Blocking of TGF- β and downstream Smad2/3 in innate immune cells led to increased infiltration of A β - containing macrophages around amyloid plaques and amyloid vessels. A β burden was reduced up to 90% in these animals [68]. Peripheral macrophages, not microglia, showed blockage of Smad 2/3 and

hyperactivation of Smad1/5/8 signaling and increased A β phagocytosis [68].

Similarly, macrophages seem to be distinguished from microglia by their ability to degrade A β intracellularly. Microglial lysosomes are found to be less acidic than macrophages lysosomes, thereby limiting the intracellular degradation of fibrillar A β . Treatment with Macrophage Colony Stimulating Factor (M-CSF) acidifies the lysosomes of microglia, facilitating A β degradation [70].

Besides monocytes and macrophages, dendritic cells may also be able to take up and utilize A β after treatment with glatiramer acetate [71, 72]. Administration of glatiramer shifts the population of T cells from pro-inflammatory Th1 cells to regulatory Th2 cells that suppress the inflammatory response. Abolishing the recruitment of dendritic cells by systemic administration of diphtheria toxin led to an increased number in amyloid plaques in the hippocampus of transgenic AD mice [73].

Taken together, the role of peripheral blood cells, especially monocytes, in AD pathogenesis is not clear. However, enhancing the recruitment of certain populations of peripheral myeloid cells may help to clear amyloid plaque deposition.

MICROGLIA AS TARGETS IN THERAPY OF AD

As previously mentioned, enhanced inflammatory response is considered to be detrimental in AD making a therapeutical approach aiming at dampening the inflammatory response of microglia tempting. Indeed, epidemiological studies revealed a reduction of risk to develop AD in people who frequently take NSAIDs. In fact, in transgenic animals ibuprofen and indomethacin treatment lead to diminished microglial activation and a reduction in A β plaque load [74, 75]. In contrast to these findings, recent data from a large randomized trial indicate that the selective COX-2 inhibition by celecoxib and naproxen did not improve cognitive function or delay the onset of AD [76]. These results corroborate the notion that the observed inflammatory response and activation of microglia are not necessarily detrimental at certain disease stages.

The ideal drug would be able prevent or attenuate microglial overactivation while promoting their phagocytic activity. In AD, minocycline, an antibiotic with high BBB penetration, may be beneficial through reducing the release of pro-inflammatory cytokines without affecting phagocytosis [77-80].

As suggested in immunization studies against A β , inflammation may serve to clear A β . Active and passive vaccination against A β ₁₋₄₂ leads to the clearance of A β deposits in the brains of transgenic mice models and human AD patients [81-84]. In human AD patients, Nicoll and colleagues identified CD68⁺ and HLA-DR upregulated amyloid containing cells in human brains immunized with amyloid-beta peptide [83, 84], suggesting that microglia and maybe microglia-like cells, f.e. monocytes, may be used actively in the therapy of AD.

The mechanisms of immunotherapy-mediated plaque clearance are however unknown. It is known, that immuniza-

tion leads to an increase in the number of activated microglia [85] and A β containing microglia have been observed both in animal models and immunized AD cases as confirmed by confocal microscopy [84]. Antibody-mediated A β clearance *in vitro* is partially mediated through Fc receptor (FcR) as proposed by Bard *et al.* [86]. FcR may bind to the Fc part of the antibody against A β ₁₋₄₂. However, antibody-induced A β clearance can occur in the absence of FcR, suggesting that other receptors and foremost other mechanisms may play a role in clearance of A β , e.g. disaggregation of A β deposits by antibodies and removal of A β passively into the CSF [87-89]. Indeed, Garcia-Alloza *et al.* were able to point out that microglia only account for some of the effect observed after anti-A β antibody treatment [85]. In addition, they showed that reduction in the activation of microglia leads to diminished A β removal, which is in concurrence with the results produced by Wilcock *et al.* They also observed limited plaque removal after immunization when microglial activation was diminished using dexamethasone [90].

CONCLUSIONS

The roles of peripheral blood cells and microglia in AD pathogenesis and possible disease therapy remain elusive. The development of specific and sensitive new biomarkers would be desirable to monitor microglial activity in AD patients. Related to AD pathology, a simplistic view of microglial as solely beneficial or detrimental cells does not reflect the complexity of microglial function. Some answers may lie in between. Most likely, a multimodal approach in the treatment of AD with amyloid and tau directed therapies accompanied by shifting microglia to phagocytic activity while limiting its neurotoxic characteristics may be a successful strategy in treating AD patients.

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