Acta Neuropathol (2014) 128:333–345
DOI 10.1007/s00401-014-1321-z

REVIEW

Microglia as a critical player in both developmental and late-life CNS pathologies

Noël C. Derecki · Natalie Katzmarski · Jonathan Kipnis · Melanie Meyer-Luehmann

Received: 28 February 2014 / Revised: 17 June 2014 / Accepted: 1 July 2014 / Published online: 24 July 2014

Abstract Microglia, the tissue-resident macrophages of the brain, are attracting increasing attention as key players in brain homeostasis from development through aging. Recent works have highlighted new and unexpected roles for these once-enigmatic cells in both healthy central nervous system function and in diverse pathologies long thought to be primarily the result of neuronal malfunction. In this review, we have chosen to focus on Rett syndrome, which features early neurodevelopmental pathology, and Alzheimer's disease, a disorder associated predominantly with aging. Interestingly, receptor-mediated microglial phagocytosis has emerged as a key function in both developmental and late-life brain pathologies. In a mouse model of Rett syndrome, bone marrow transplant and CNS engraftment of microglia-like cells were associated with surprising improvements in pathology—these benefits were abrogated by block of phagocytic function. In Alzheimer's disease, large-scale genome-wide association studies have been brought to bear as a method of identifying previously unknown susceptibility genes, which highlight microglial receptors as promising novel targets for therapeutic modulation. Multi-photon in vivo microscopy has provided a method of directly visualizing the effects of manipulation of these target genes. Here, we review the latest findings and concepts emerging from the rapidly growing body of literature exemplified for Rett syndrome and late-onset, sporadic Alzheimer's disease.

Keywords Alzheimer's disease · Rett syndrome · Mecp2 · Phagocytosis · Amyloid plaques · Microglia · In vivo two-photon microscopy · Genome-wide association studies (GWAS)

Introduction

For decades, microglia have been conspicuously absent from most neuroscientific discussions regarding the critical players in the workings of the brain. If microglia were mentioned at all, it was more often than not in terms of their pathological role in neuroinflammation. Only very recently have these cells come under more careful consideration as to their unique place in the network of the central nervous system (CNS). As such, microglia have recently been revealed to be surprisingly more nimble and nuanced than previously imagined in their ability to respond to their surrounding environment. In the review that follows, we will discuss key studies highlighting the extraordinary roles these cells have now been shown to play in both CNS development and decline. In the development, we focus on the role of microglia in Rett syndrome; in decline, we look at microglia as major players in Alzheimer's disease (AD) pathology.

N. C. Derecki and N. Katzmarski contributed equally.

N. C. Derecki (✉) · J. Kipnis
Department of Neuroscience and Center for Brain Immunology and Glia (BIG), School of Medicine, University of Virginia, Charlottesville, VA 22908, USA
e-mail: ncd3z@virginia.edu

N. Katzmarski · M. Meyer-Luehmann (✉)
Department of Neurology, Neurocenter, University of Freiburg, 79106 Freiburg, Germany
e-mail: melanie.meyer-luehmann@uniklinik-freiburg.de

N. Katzmarski
Faculty of Biology, Albert-Ludwigs-University of Freiburg, Freiburg, Germany

Microglia: the tissue-resident macrophages of the brain

Microglia are ubiquitous throughout the CNS, and are estimated to represent approximately 10 % of all cells found within the brain [70]. Microglia are unique within the brain in many ways, but perhaps primarily so in that they are true immune cells, originating during primitive hematopoiesis in the yolk sac as CX3CR1-expressing tissue-resident macrophage precursors [39]. Subsequently, precursors become migratory, move into the nascent brain, and proliferate in situ [1]. Recent lineage tracing experiments have suggested that expression of runt-related transcription factor (Runx)1, Pu.1 and Irf8 are critical, and that establishment of the brain vasculature is necessary for the infiltration of the CNS by these cells [39, 62, 63].

Cellular origin will frequently dictate function, and microglia share provenance with other tissue-resident macrophages [38, 45]. Thus, it can be expected that microglia should perform well as phagocytes, producers of cytokines, and of growth factors, as necessary [2]. Indeed, this has been shown to be the case. However, the nature of the tissue surrounding a cell will also impact upon its phenotype, and certainly microglia must ply their trade in immediate proximity to delicate neurons. Therefore, it is not surprising that molecules found in the CNS milieu act to modulate microglial function accordingly. TGF-β1, for instance, plays a major role in dampening microglial cytokine production, with loss having been shown to be associated with neuronal death and pathological microglial activation/microgliosis as measured at postnatal day 21 [15]. It is important to point out that the mouse model used succumbs to autoimmune-mediated lethality early in adulthood. In another recent work, TGF-β1$^{-/-}$ mice in which T cell production of TGF-β is preserved, thus preventing the abovementioned peripheral autoimmune wasting phenotype, were shown to suffer from severe microglia deficiency, indicating that CNS tissue-resident macrophages and their surrounding tissue may engage in dynamic and mutual support [18]. The fact remains, however, that certain aspects of these two works are in conflict, and clearly more research is needed to better define the precise role for TGF-β in microglial function and survival. Fractalkine is another such modulatory molecule produced by neural cells, suggested to act directly on the microglial CX3CR1 receptor to prevent neurotoxicity [19, 71]. Thus, microglia are bona fide immune cells, but like other tissue-resident macrophages, they are also uniquely positioned to provide benefit and homeostatic support to the tissue within which they reside, in this case, the CNS. Microglia must be dynamic, but also judicious and measured in their responses. Moreover, microglial phenotype has been suggested to be plastic [104]. Yet, as guardians of the brain, they need to also possess the ability to respond vigorously to pathological insults when appropriate. Along these lines, recently developed imaging techniques have revealed that microglia are surprisingly dynamic cells, rather than inactive unless provoked, as was long believed [89].

Microglia are active participants in developing brain

Microglia were long thought to be "quiescent" cells except during pathological insult; this notion, however, has lately been powerfully challenged by two-photon imaging studies indicating that microglia are in a state of perpetual surveillance even in healthy brain [89]. Resting microglia display highly ramified processes. Under physiological conditions, these processes are highly motile, constantly sampling their environment, while the soma remains stationary [28, 89]. In addition to performing active surveillance, microglia are also the professional phagocytes of the brain, and, as such, must be involved in unremitting cleanup of cellular corpses and debris. Phagocytosis of apoptotic cells is a necessary part of normal tissue development [101], and is thus integral to the role of microglia as tissue-resident macrophage. During the process of brain maturation, millions of neurons undergo apoptosis, yet leave barely a trace—thanks to vigilant corpse removal attributable largely to microglia [126]. Similarly, in experiments involving induced massive ablation of cells within the brain parenchyma, there is little—if any—detectable debris to be found even days after [95]. It is all the more striking that phagocytosis of apoptotic neurons is not merely a passive response. The active role of microglia in prompting apoptosis during development is well-illustrated by studies which demonstrate that deficiencies in microglial CD11b and DAP12, important in myeloid activation phenotype, were associated with impairment in hippocampal development [123].

Based on findings in these works and others, it is likely that impairment of normal microglial function—particularly during early life—could be expected to have serious implications regarding normal brain development. A dysregulated or deficient immune response could contribute significantly to pathologies seen in neurodevelopmental disorders involving abnormal dendritic arborization and dendritic spine maintenance. Along these lines, it was demonstrated in a series of studies that an interplay between several cell types in the CNS was ultimately responsible for the pruning of synapses by microglia in the visual system. Immune complement factors, namely C1q and C3, were shown to be expressed on neurons [113] as a result of TGF-β signaling by astrocytes [10] and recognized by cognate receptors expressed exclusively in brain parenchyma by microglia [103]. Pruning was suggested to be activity dependent [119] in works that correlated light deprivation and re-exposure with dynamic interactions between

microglia and synapses [103] and directly by showing increased microglial phagocytosis of synaptic inputs corresponding to tetrodotoxin-mediated silencing compared to those activated by forskolin [103]. While these studies were conducted largely with a focus on the developing visual system, these results imply that similar microglial dysfunction in other brain areas could impact powerfully on synaptic maintenance and neural network activity, as is suggested to be important in autistic spectrum pathologies. A recently published work indeed suggests that network function and social behavior are impaired as a result of microglial deficiency and compromised microglial–neuron signaling [130].

In vivo imaging of microglia in mouse models of AD

The same methodology that has revealed the surprisingly dynamic role of microglia—two-photon microscopy [29]—has enabled in vivo long-term imaging studies and allowed monitoring of morphological and functional changes in the living mouse brain under normal and pathological conditions [72, 111]. A recent in vivo imaging study revealed that microglia cells undergo age-related morphological changes such as increased soma volume and a shortening of the processes [46]. Interestingly, aged mice exhibited increased microglial soma movement in comparison to younger mice, but this effect was diminished in response to acute injury by laser lesion [46]. Under pathological conditions, such as AD and in mouse models of AD, microglial cells are tightly associated with and cluster around dense core amyloid plaques [54], which are the major neuropathological hallmark of AD and are thought to be toxic to the surrounding neural tissue [17, 67, 82, 83, 112]. In terms of morphology, microglia display a reactive phenotype in AD with typically short, thickened and less ramified processes [54, 81, 116]. In the aged human brain, 'dystrophic' de-ramified microglia have been described with fragmented processes and bulbous swellings [115]. However, a similar dystrophic phenotype resembling morphological signs of aging has not yet been seen in the rodent brain. Instead, microglia in the rodent brain appear to be less complex [26, 46, 120]. In a mouse model of AD with plaque pathology, microglia had shorter processes (Fig. 1b) and less process movement compared to younger pre-depositing transgenic mice indicating, that age may impact the aforementioned morphological changes [65]. Nonetheless, the functional consequences of these morphological changes remain poorly understood and need further study.

Acute two-photon imaging identified that microglia cells surrounding Aβ plaques were morphologically mainly hypertrophic and amoeboid, whereas in plaque-free areas of the brain, ramified microglia were predominantly evident [14]. Ramified microglia were shown to rapidly migrate

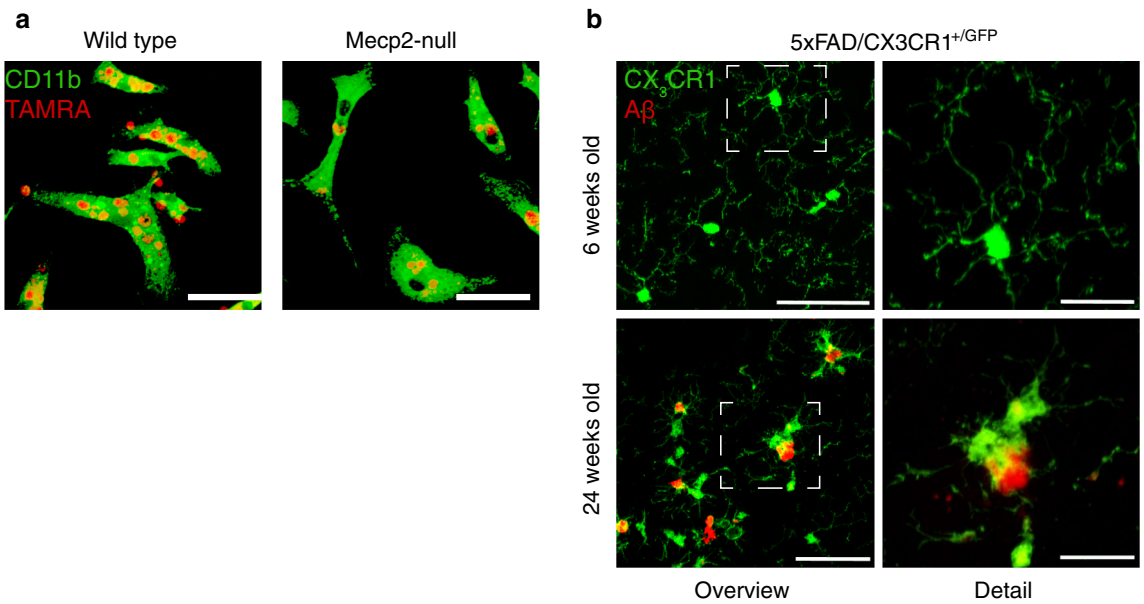

Fig. 1 Aberrant microglia in models of Rett syndrome and Alzheimer's disease. **a** Representative captures of phagocytosing microglia (labeled with anti-CD11b, green) incubated for 2 h with TAMRA-labeled UV-irradiated neural progenitor cells (*red*). **b** Representative confocal images of microglia in a pre-depositing 6-week-old 5xFAD transgenic mouse and activated microglia encompassing plaques in a 24-week-old 5xFAD transgenic mouse. Note the shortened and less ramified processes. *Green labeling* is GFP expressed under the control of the CX3CR1 promoter; red fluorescence depicts Aβ plaques labeled with an anti-Aβ antibody. *Scale bars*: **a** 25 µm and **b** left panel 50 µm; *right panel* 20 µm

towards newly formed plaques [83], suggesting that they might transform into a hypertrophic or even amoeboid cell type in response to Aβ plaque formation and could have a pathological role. Recently, several in vivo imaging studies have monitored the appearance and growth of Aβ plaques [16, 47, 80, 83], which is of importance for the pathological development of AD. Plaque growth might be restricted by microglia removing and phagocytosing Aβ fibrils [11, 110]. This idea is supported by two immunization studies that provided in vivo evidence that microglia are able to phagocytose Aβ [4, 65]. The precise role of microglia in the pathogenesis of AD remains, however, poorly understood and the microglia response to plaques remains controversial. Surprisingly, microglial depletion in a mouse model of AD changed neither the plaque count nor the size of plaques [40]. However, microglia were only depleted for 4 weeks, and in older mice, which might explain the lack of effect.

In addition to plaques, AD is also characterized by massive neurodegeneration and neuronal cell loss, and several studies have tried to unravel how microglial activation might influence this neuronal loss. A combination of two-photon microscopy and laser ablation demonstrated that microglia cells adjacent to the site of injury polarized their processes towards the lesion to contain the injury [28]. In the context of AD, the neuronal cell loss in another triple transgenic mouse model of AD [91] was rescued after cross-breeding with mice lacking the CX3CR1 receptor, indicating a crucial role of the microglial chemokine receptor in mediating neuronal apoptosis in AD [36]. Other studies using CX3CR1-deficient mice showed exacerbated levels of phospho-Tau and elevated Tau pathology in Tau transgenic mice [9, 22], while CX3CR1 deficiency reduced amyloid load in AD mouse models with numerous Aβ deposits [71, 74]. Interestingly, neuronal and behavioral deficits worsened in CX3CR1-deficient mice and were plaque independent [22]. Despite these compelling results, the precise role of microglia in the pathogenesis of AD remains enigmatic. We predict two-photon microscopy will help shed light on the contribution of microglia to this devastating disease.

In the following sections, we take a look at evidence from genetic analyses that suggest a basis for immune abnormalities in Rett syndrome, previously considered to be a disease solely of neurons, and also summarize genetic approaches that have led to the recent discovery of a number of new AD risk and susceptibility factors including inflammation.

Mecp2 as a regulator of immune-related genes

Rett syndrome is an X-linked CNS developmental disorder grouped with the autism spectrum disorders. Rett syndrome is linked in the majority of cases to mutations in the methyl-CpG-binding protein 2 (*MeCP2*) gene [3], which acts to modulate transcription at the level of chromatin remodeling by partnering with cofactors such as Sin3A in the formation of repressor complexes [21]. Interestingly, it was shown that Mecp2 functions as both activator and repressor of transcription, thereby complicating the predicted results of mutation of the gene. Regardless, mutation of *MeCP2* has been definitively linked to major disruptions of CNS and somatic function which together underly Rett syndrome pathology. Rett syndrome symptoms include psychomotor impairment, locomotor deficits, tremors, apneas, osteopenia, scoliosis, intestinal dysfunction and severe mental retardation [109]. *MeCP2* is an epigenetic regulator of thousands of downstream genes and is expressed in most cells, including at high levels in immune tissues [107]. While several investigations into genes dysregulated as a result of *Mecp2* deletion have been performed to date, the genes selected for further analysis have been those previously implicated in neuronal, rather than glial or immune function. However, microarray analyses of neural tissue from *Mecp2*-deficient mice have consistently included immune genes, e.g., Irak-1 [58], as among the most highly aberrant in transcription as a result of loss of *Mecp2*. Along these lines, *MeCP2* was recently named as a candidate susceptibility gene for systemic lupus erythematosus, an autoimmune disorder [124]. Nevertheless, research into immune system abnormalities in Rett syndrome has been surprisingly limited. In the few such studies conducted to date, however, it has become increasingly clear that mutations in *MeCP2* can greatly modify the expression of genes influencing the function of immune cells. Thus, it is not surprising that *Mecp2* has been directly connected to regulation of genes including *FoxP3*, a defining marker of T regulatory cells [68], and interferon (IFN)-γ, produced by several immune cell types including T lymphocytes and myeloid cells [118]. Notably, dysregulated IFN-γ production by T cells was recently suggested to be key to peripheral immune pathology common in *Mecp2* duplication syndrome, a disease similar in severity to Rett syndrome, but caused by overexpression of *Mecp2* rather than deficiency [129]. More recently, it was shown that *Mecp2* plays a role in the generation of T_H1 and T_H17 cells by the regulation of microRNA-124. This latest work is notable in that it elucidates a mode of gene regulation by *Mecp2* that is separate from its canonical function in chromatin modification [56].

Clinical data from Rett syndrome patients regarding immune function present a picture of overall dysregulation, but studies are few; thus, overarching patterns are still unclear. In general, the picture painted may be one of immune insufficiency rather than one implicating *Mecp2*-mutant immune cells as "bad actors". For example, CD25-positive T lymphocytes were undetected in blood analyzed

from Rett syndrome patients [98] indicating the possibility of activated effector T and T regulatory cell deficiency, both of which express CD25. Interestingly, an unrelated study showed increased serum levels of soluble CD25 [33] in serum from Rett syndrome patients. Increased soluble CD25 has been linked to both T regulatory and T effector cell repression. Additionally, a decrease in $CD8^+$ T lymphocytes and natural killer cells has also been shown.

In sum, while several groups have focused on elucidation of downstream genetic targets of *Mecp2* in neurons or whole brain tissue, these data, while intriguing, have not as yet revealed many potential critical regulatory molecules that might serve as targets for manipulation. However, one that has shown some promise, first in mouse models [121] and now in Stage two clinical trials, is insulin-like growth factor (IGF)-1 [61]. It is perhaps noteworthy that microglia are a major producer of IGF-1 in the brain, thus supporting the notion of a central role for these immune cells in Rett syndrome pathobiology.

GWAS studies for identifying risk genes in AD

Familial AD (FAD) is caused by specific, identifiable, autosomal dominant mutations in the Amyloid Precursor Protein (APP), Presenilin 1 and/or Presenilin 2 and is characterized by an early onset of the disease. However, the majority of AD patients suffer from sporadic AD, which has no definitive genetic cause. The etiology of sporadic AD is largely unknown, but environmental factors and genetic predisposition may be risk factors for the disease.

Recently, genome-wide association studies (GWAS) have emerged as an effective tool for identifying several new risk genes for Alzheimer's disease. The most common risk factor for sporadic AD is the ApoE4 allele, which increases the risk of developing the disease by three times for heterozygous carriers and by 15 times for homozygous carriers [64]. Other genes, such as CLU, Bin1 or PICALM have also been identified as risk genes for AD [44, 52, 69, 105]. Among the genes identified as risk factors for AD were genes expressed by microglia. For example CD33 was identified as a sporadic AD risk locus [8, 50, 87]. CD33 is a transmembrane protein and a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs). Its activity has been linked to triggering endocytosis and pathogen recognition, however, its function in the brain is still unknown [25]. Several new studies have recently examined the role of CD33 in AD. CD33 expression was found to be increased in circulating monocytes in carriers of the $rs3865444^C$ risk allele and was associated with diminished internalization of Aβ peptide [13]. CD33 expression is also increased in the brains of AD patients and protein levels are specifically increased in the frontal cortex by twofold. Furthermore, CD33 expression in microglia is correlated with AD pathology, whereas the deletion of CD33 in a mouse model of AD lowered cortical and hippocampal Aβ plaque burden. Finally, in vitro experiments in BV2 cells with increased levels of CD33 diminished microglial uptake of Aβ, while the degradation was unaffected [41]. The authors implicated CD33 as a regulator of microglial clearance of Aβ and proposed it as a target for the treatment and prevention of AD.

Two recent reports linked another innate immune receptor and known microglial Aβ clearing molecule, triggering receptor expressed on myeloid 2 cells (TREM2) to increased risk of sporadic AD [42, 57]. TREM2 acts through DAP12 and, loss-of-function mutations lead to presenile dementia with bone cysts, also known as Nasu–Hakola disease [20, 94]. The function of this receptor in general and specifically its role in the pathogenesis of AD is not well understood. In the human and mouse cerebral cortex, TREM2 is predominantly expressed in microglia and to a much lesser amount in neurons [106]. TREM2 seems to be crucial for brain homeostasis, since its activation stimulates the phagocytic activity in microglia without causing inflammation [117]. In APP transgenic mice, TREM2 is up-regulated in microglial cells in the vicinity of amyloid plaques [34]. However, Nasu–Hakola disease patients do not overly develop Aβ plaques and therefore the relevance of TREM2 in sporadic AD needs to be investigated further.

Interestingly, CD33 and TREM2 both were found to be linked to DAP12 [131]. As aforementioned, DAP12 is crucial for normal brain development and for the clearance of apoptotic neurons. The authors used an integrated systems approach and ranked network structures for their relevance in sporadic AD. DAP12 was identified as being in the center of this microglial network and unifying previous top GWAS risk loci including CD33.

In the final sections, we suggest immune approaches aimed at the possible amelioration of Rett syndrome and Alzheimer's disease, and highlight some critical functions of microglia, that, when impaired, may provide clues that link these seemingly disparate brain pathologies.

Immune-directed treatments in Rett syndrome

While MeCP2 is highly expressed in neurons, it is also expressed in many other cells and tissues, including microglia [78] (Kipnis Lab, personal communication). Rett pathology was originally believed to be solely due to alteration or loss of *Mecp2* expression in neurons; however, more recent data suggest that glia also play a major role in the disease [6, 78]. For example, expression of wild-type *Mecp2* in astrocytes of otherwise *Mecp2*-null mice was

shown to significantly block disease progression [73]. It was also proposed that *Mecp2*-null microglia might be directly damaging neuronal dendrites via supranormal production of glutamate [78]. In parallel, our analysis of primary microglia revealed a striking impairment of phagocytic capability by *Mecp2*-null microglia as compared to controls (Fig. 1a). Subsequent examination of brain tissue revealed increased levels of debris in *Mecp2*-null mice, suggesting that insufficient clearance of debris by *Mecp2*-null microglia could be a key factor contributing to Rett pathology [30].

If intrinsic impairment of microglia as a result of *Mecp2* deficiency were important to pathology, then supplementation of the CNS with wild-type microglia might be expected to yield benefit. A strategy of bone marrow transplant was previously shown by several groups to result in engraftment of microglia-like cells within brain parenchyma [84, 99] and such methods have been employed with some modest success in human disease in the treatment of globoid cell leukodystrophy (Krabbe disease) and related metabolic storage diseases associated with CNS pathology [51, 66]. Murine studies using the twitcher mouse model for Krabbe disease preceded clinical therapies, and showed CNS and peripheral engraftment followed by significant remyelination [51].

Indeed, *Mecp2*-null mice that received bone marrow transplant displayed remarkably blunted pathology. Although still different from wild-type mice, treated mice significantly outlived both *Mecp2*-null mice transplanted with *Mecp2*-null bone marrow and untreated controls. When brains from transplanted mice were examined, robust engraftment of microglia-like cells in brain parenchyma was observed, suggesting that arrest was associated with improved ability of wild-type microglia to support *Mecp2*-null neurons. Results from bone marrow transplant were supported by results from a complementary genetic/pharmacological approach using $Lysm^{cre}Mecp2^{lox\text{-}stop/y}$ mice, in which arrest was achieved wild-type *Mecp2* was driven in a significant proportion of myeloid cells, including microglia, on an otherwise *Mecp2*-null background. These mice were treated with chronic injections of annexin V, shown to be effective in binding to phosphatidylserine residues exposed on apoptotic cells and critical for corpse recognition and removal. As expected, blocking recognition and uptake of debris in these mice by annexin administration resulted in abolishment of disease arrest and significant increase in TUNEL-positive debris in brains. These data strongly suggested that arrest was being mediated in otherwise *Mecp2*-null mice by the ability of wild-type microglia to effectively clear debris in CNS.

A further possibility is a scenario in which microglia may also be deficient in production of growth factors needed to maintain neural cells. Along these lines, it was recently shown that acute ablation of microglia or ablation of BDNF production by microglia resulted in deficits in dendritic spine elimination and behavioral impairments in motor learning, fear conditioning, and novel object recognition [95]. Similarly, mice deficient in hematopoietic-specific BDNF production exhibited pathological levels of hyperphagia and insulin resistance. IBA1-positive cells in brain parenchyma of these animals were visualized in hypothalamus in contact with neurons that are shown to control feeding. Importantly, BDNF-competent cells engrafted into hypothalamus following intracerebroventricular injection were associated with reversal of pathological feeding phenotype [122].

These works and others suggest the strong possibility that pathologies conventionally considered to be the result of isolated neural dysfunction may in fact be influenced significantly—and even caused in some cases—by microglial dysfunction. Along these lines, recent studies now highlight a central role for microglia, and more specifically microglial receptors in Alzheimer's disease, a pathology classically associated with aging brain, but long assumed to be primarily of neuron-intrinsic etiology.

Immune-receptors on microglia as therapeutic target for the treatment of AD

Microglia are the major phagocytic cells in the brain which become activated upon contact with Aβ [81]. To date, compelling evidence is lacking as to whether microglia are truly able to phagocytose Aβ [100]. This occurrence would not only involve the engulfment of the protein, but also a degradation step (degradation of Aβ), which has yet to be proven. Without the capability to degrade Aβ, it seems unlikely that amyloid-containing microglia can clear additional Aβ but instead may even transform into toxic cells. Nevertheless, growing evidence suggests that microglia are able to prevent or decelerate AD by promoting the clearance of Aβ. Several microglial receptors seem to play a pivotal role in the clearance of Aβ (Fig. 2). One of these receptors involved in the clearance of Aβ is the scavenger receptor expressed on microglia Scara-1. Scara-1 promotes the binding and phagocytosis of Aβ in vitro [31], and in vivo in a mouse model of AD [35]. Isolated human microglia also bind Aβ via Scara-1 receptor [53]. Furthermore, microglia that decorate Aβ plaques in a mouse model of AD showed increased levels of Scara-1 [12], whereas microglia isolated from Scara-1 knockout mice had reduced Aβ clearance capacity compared to wild-type cells [23]. Scara-1 deficiency increased Aβ plaque pathology in APPPS1 transgenic mice and accelerated the disease whereas pharmacological up-regulation of Scara-1 on mononuclear phagocytes had the opposite effect and increased clearance of Aβ [35] emphasizing the relevance of Scara-1 in the pathogenesis of AD.

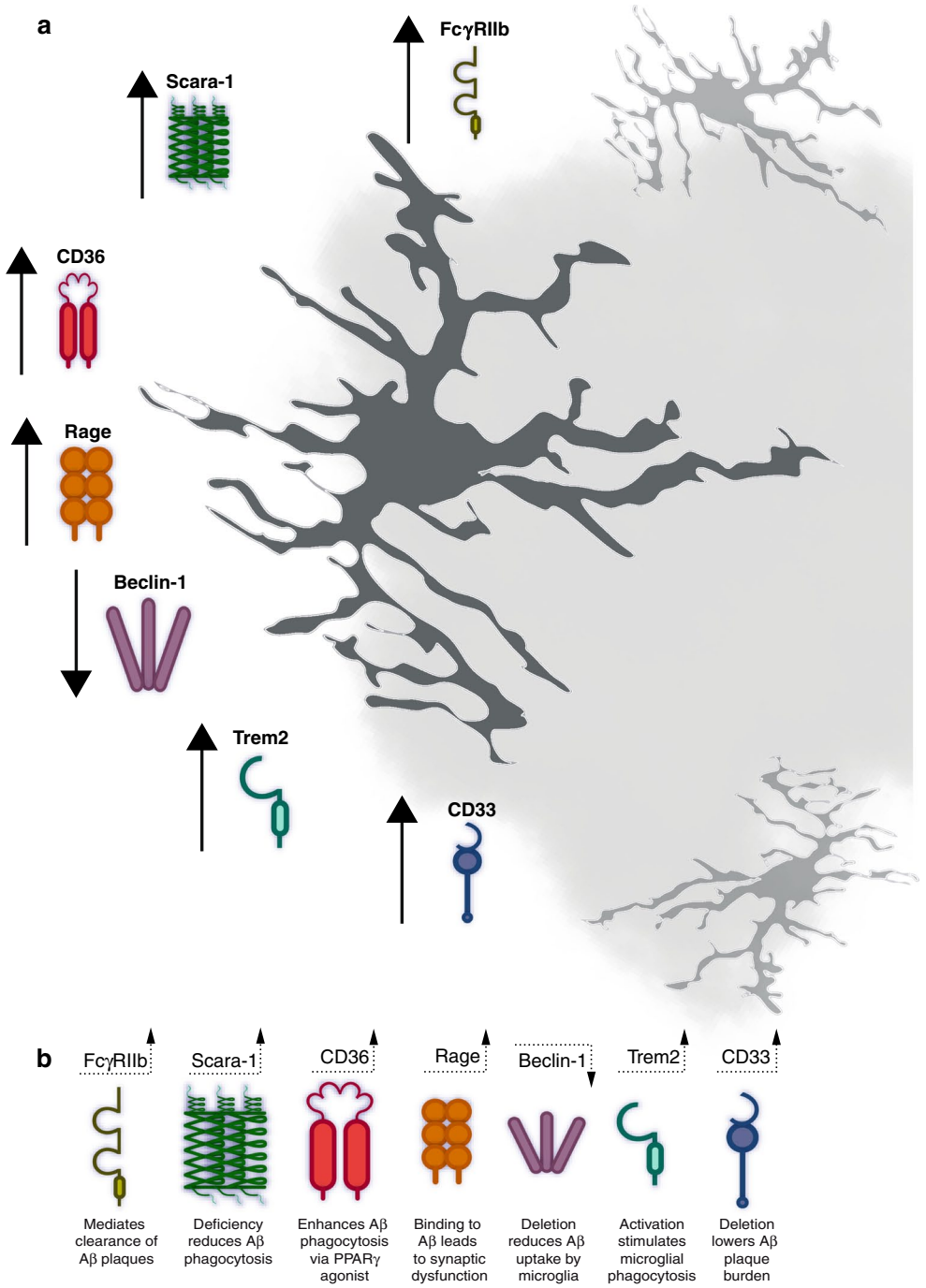

Fig. 2 Regulation of microglial phagocytosis via receptors. **a**, **b** Summary of known receptors expressed on microglia and an autophagic protein that are involved in the phagocytosis of Aβ and are described in the context of AD pathology. The *faint gray shadow* in the background represents a plaque surrounded by several microglia. *Arrows* indicate increased or decreased receptor expression in AD. *Receptor shapes* are not meant to represent actual receptor structures

Another microglial receptor for Aβ of the scavenger receptor family is CD36. CD36 binds Aβ fibrils and is expressed on microglia in the AD brain [24]. Additionally, CD36 mediates reactive oxygen species production in response to Aβ [24] and microglial recruitment to the site of aggregated fibrillar Aβ in vivo [32]. Investigation of the signaling molecules of microglial CD36 upon Aβ stimulation helped to identify Src family members such as Fyn and Lyn as well as MAPK [85]. There have been attempts to increase the phagocytic activity of microglia via peroxisome proliferator-activated receptor γ (PPARγ) agonists that reduced Aβ plaque burden in APP transgenic

mice. This enhancement of microglial Aβ phagocytosis was shown to be mediated by the CD36 receptor [127]. Interestingly, CD36 is a co-receptor as it can form a heterodimeric complex with TLR-4 and TLR-6. The interaction of Aβ with this receptor complex on microglia provokes an inflammatory response with IL-1β production indicative of inflammasome activation [114].

This is well in line with the finding that microglial Beclin-1 is also impaired in AD and regulates phagocytic receptor function. Beclin-1 is known for its role in the autophagosomal system, which is in turn implicated in AD. Early in AD progression, Beclin-1 expression and protein levels in the human enthorinal cortex decrease [97]. A heterozygous deletion of Beclin-1 in a mouse model for AD increased the accumulation of intraneuronal and extracellular Aβ, most likely due to the accumulation of APP and its metabolites [55, 97]. Beclin-1 is important for efficient phagocytosis by microglia in vitro and in vivo. Reduced Beclin-1 expression also caused microglial changes; microglia and microglia-like cell culture lines deficient for Beclin-1 were only able to clear a minor amount of Aβ aggregates [75]. The author further claims that together with its binding partner Vps34, Beclin-1 regulates a retromer complex which is among others involved in receptor recycling. Interestingly, reduced Beclin-1 levels also diminished phagocytic receptor recycling for TREM2 and CD36, which both are linked to microglial impairment in AD and are discussed as well in this review.

Another microglial receptor reported to bind to Aβ is FcγRIIb. FcγRIIb is expressed on most leucocytes excluding natural killer cells. It contains an ITIM in its cytoplasmic domain which recruits phosphatases in turn resulting in an inhibition of cell activation (for review see [92]). Like other FcRs, FcγRIIb is widely expressed in the central nervous system, and is important for cerebral function and development, especially for Purkinje cells [88]. However, its role in AD still remains elusive. The first hint came from a study by Peress et al. 1993, identifying FcγR immunoreactivity on microglia and in senile plaques distributed throughout the white matter and cortex in healthy and AD brains [96]. This was further extended and confirmed with the finding that FcγRIIb was specifically up-regulated in the hippocampus of AD brains [59]. From immunization studies, there is evidence for FcγRIIb-mediated phagocytosis by microglia that increases the clearance of Aβ plaques in vivo [5, 7]. However, another immunization study using FcγR knockout mice did not conclude that FcγRIIb-mediated phagocytosis was the crucial phagocytic mechanism [27]. A recent paper linked Aβ oligomers and FcγIIb receptor binding as a possible cause of neurotoxicity by activating caspase-12 and boosting endoplasmic reticulum stress markers [59]. Furthermore, oligomer-specific antibodies to Aβ lead to increased neurotoxicity of Aβ in a mixed culture of primary neuronal and glial cells. Both the depletion of the Fc region and the removal of microglia from the culture diminished this effect implicating microglia as a crucial mediator of neuronal death [86]. Inhibition of the Aβ–FcγR interaction could be a new therapeutic approach for AD and might bring a revival of immunization trials.

Yet another example of a receptor involved in the pathogenesis of AD is the receptor of advanced glycation end products (RAGE) that has also been shown to bind to Aβ [128]. RAGE is expressed in multiple cell types, including microglia and is up-regulated in hippocampal microglia of AD brains (see review by [77]). The interaction of RAGE with Aβ has diverse implications on inflammatory responses, neuronal function and the elevation of amyloidosis. Microglial activation elicited from the binding of Aβ to RAGE produced cytokines such as IL-1β and TNF-α, which in turn might lead to the clustering of microglia around Aβ plaques [76, 128]. Furthermore, microglial RAGE signaling through p38MAPK and JNK released IL-1β, leading to synaptic dysfunction [93]. In a mouse model for systemic amyloidosis, the use of an anti-RAGE antibody significantly reduced amyloid plaque formation. Although promising results have been achieved with the RAGE inhibitor PF-04494700 from Pfizer in preclinical studies in transgenic mice and in a 10-week Phase 2 trial [102], the follow-up Phase 2 trial over 18 months was halted because of serious side effects and worsened cognitive decline in the higher dose treatment group. In contrast, the lower dose group did not raise safety concerns and some analyses showed decreased decline on the Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog), but other clinical and biomarker measures failed to show significant differences between low-dose and placebo group (for information see http://clinicaltrials.gov) [37].

Developmental and age-related CNS pathologies: is microglia dysfunction a common element?

While Rett syndrome and Alzheimer's disease are clearly different in terms of time of age of onset and pathological sequelae, in terms of microglial dysfunction as a contributing factor, they may indeed have much in common. As was detailed previously, in a mouse model of Rett syndrome, data suggest that the ability of microglia to respond efficiently to a buildup of apoptotic corpses during development may be one of the many keys to the postnatal onset of pathology. Phagocytosis of apoptotic neurons has long been recognized as necessary to normal brain development, and more recently, it is suggested that microglia also play a critical role in the synaptic pruning process.

Similarly, as covered earlier in this review, GWAS studies and other genetic screening techniques have identified microglial receptors as strongly up- or down-regulated in the Alzheimer's disease brain. For instance, during the progression of AD, the expression of several Aβ binding receptors such as the Scara-1, CD36 and RAGE decreased by two- to fivefold in microglia, and thereby lost their ability to clear Aβ [49]. Taken together, these studies provide the basic rationale for the development of further therapeutic strategies targeting microglia, e.g., enhancing the ability of microglia to clear Aβ by an up-regulation of these microglial receptors (Fig. 2).

Dysregulated inflammatory response may also represent a common mechanism by which microglial impairment contributes to pathology in both diseases. *Mecp2* is a known regulator of NFκB though multiple pathways, including via the aforementioned Irak1, and also through direct regulation of the IκBα promoter [79]. Along these lines, it was recently shown that loss of *Mecp2* in immune cells, including neonatal mouse microglia and siRNA-treated human monocytes, led to supranormal production of glutaminase via an NFκB-dependent process [90]. Glutamate is well-recognized as a factor in neurotoxicity, and unrestrained production within the confines of the CNS by microglia would conceivably lead to adverse effects on neuronal function. The possibility of dendritic and synaptic damage mediated by microglia-derived glutamate was previously proposed as an underlying factor in Rett syndrome pathology [78].

In the context of AD, the NLRP3 inflammasome pathway in microglia has been elucidated over the last few years as possibly being of critical importance. Fibrillar Aβ was shown to activate the NLRP3 inflammasome with IL-1β secretion by microglia [43]. Interestingly, inhibition of phagocytosis reversed this effect and resulted in reduced NLRP3-mediated IL-1β release [43]. As mentioned above, CD36 is able to bind to Aβ [125], and its uptake was shown to promote NLRP3 activation and amyloid aggregation at least in cell culture experiments [108]. More in vivo evidence for the involvement of the NLRP3 inflammasome pathway in the pathogenesis of AD came recently from a study by Heneka et al. which clearly demonstrated an increase in caspase-1 activation in diseased human AD brains. Furthermore, NLRP3 deficiency in APP transgenic mice decreased Aβ plaque load, suggesting a prominent role of NLRP3 in the pathogenesis of AD and a potential target for therapeutic intervention [48].

Conclusion

The long-held view that microglia are at best inactive unless provoked by pathogens, and at worst "the enemy within" [60] has recently been challenged by several high-profile studies. Lineage tracing and genetic analysis have revealed that microglia share origins with other tissue-resident macrophages [38, 39], thus suggesting an important homeostatic role in tissue maintenance, a trademark function of resident macrophages. In support of this notion, in vivo imaging has revealed, that microglia are indeed dynamic surveyors of the brain's milieu, even during health [89]. During development, microglia are now shown to be necessary for pruning of supernumerary synapses and clearance of apoptotic neurons [103, 119]. Accordingly, deficiencies in phagocytic function may be linked to autistic spectrum disorders, which frequently present with dendritic pruning defects. In the aging brain, microglia have been similarly implicated as mediators of both healthy function and pathology. Aged microglia have been demonstrated to display an amoeboid morphology in particular when associated with Aβ plaques [14], a classical hallmark of AD pathology. Along these lines, it has become increasingly clear that expression of key receptors on microglia may be linked to their response to Aβ, shifting them to either ameliorative or aggressive phenotype.

Thus, the dogmatic view of microglial response solely as an indicator of pathology is becoming revised as it becomes clear that microglial inactivity is as dangerous as overreaction. We should seek to encourage a robust and well-tuned response from microglia, rather than a diminished response, as necessary for maintained CNS health.

Acknowledgments We would like to thank Joanna McCarter and Andrew Harborne for excellent comments on the manuscript. This work was supported by a grant from the Rett Syndrome Research Trust (to J. K.) and from the National Institutes of Neurological Disorders and Stroke NS081026 (to J. K), the Emmy Noether Program of the German Research Foundation (DFG) (to M.M.-L) and the Hans and Ilse Breuer Foundation (M.M.-L.).

References

1. Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117:145–152
2. Aloisi F, Ria F, Adorini L (2000) Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. Immunol Today 21:141–147
3. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188
4. Bacskai BJ, Kajdasz ST, Christie RH, Carter C, Games D et al (2001) Imaging of amyloid-beta deposits in brains of living

mice permits direct observation of clearance of plaques with immunotherapy. Nat Med 7:369–372
5. Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P et al (2002) Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. J Neurosci 22:7873–7878
6. Ballas N, Lioy DT, Grunseich C, Mandel G (2009) Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Nat Neurosci 12:311–317
7. Bard F, Cannon C, Barbour R, Burke RL, Games D et al (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 6:916–919
8. Bertram L, Lange C, Mullin K, Parkinson M, Hsiao M et al (2008) Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. Am J Hum Genet 83:623–632
9. Bhaskar K, Konerth M, Kokiko-Cochran ON, Cardona A, Ransohoff RM, Lamb BT (2010) Regulation of tau pathology by the microglial fractalkine receptor. Neuron 68:19–31
10. Bialas AR, Stevens B (2013) TGF-beta signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat Neurosci 16:1773–1782
11. Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA et al (2008) Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. J Neurosci 28:4283–4292
12. Bornemann KD, Wiederhold KH, Pauli C, Ermini F, Stalder M et al (2001) Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice. Am J Pathol 158:63–73
13. Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T et al (2013) CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. Nat Neurosci 16:848–850
14. Brawek B, Schwendele B, Riester K, Kohsaka S, Lerdkrai C et al (2014) Impairment of in vivo calcium signaling in amyloid plaque-associated microglia. Acta Neuropathol 127:495–505
15. Brionne TC, Tesseur I, Masliah E, Wyss-Coray T (2003) Loss of TGF-beta 1 leads to increased neuronal cell death and microgliosis in mouse brain. Neuron 40:1133–1145
16. Burgold S, Bittner T, Dorostkar MM, Kieser D, Fuhrmann M et al (2011) In vivo multiphoton imaging reveals gradual growth of newborn amyloid plaques over weeks. Acta Neuropathol 121:327–335
17. Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH et al (2008) Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. Science 321:1686–1689
18. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ et al (2014) Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 17:131–143
19. Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM et al (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917–924
20. Cella M, Buonsanti C, Strader C, Kondo T, Salmaggi A, Colonna M (2003) Impaired differentiation of osteoclasts in TREM-2-deficient individuals. J Exp Med 198:645–651
21. Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST et al (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320:1224–1229
22. Cho SH, Sun B, Zhou Y, Kauppinen TM, Halabisky B et al (2011) CX3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease. J Biol Chem 286:32713–32722
23. Chung H, Brazil MI, Irizarry MC, Hyman BT, Maxfield FR (2001) Uptake of fibrillar beta-amyloid by microglia isolated from MSR-A (type I and type II) knockout mice. Neuroreport 12:1151–1154
24. Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH et al (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. Am J Pathol 160:101–112
25. Crocker PR, Paulson JC, Varki A (2007) Siglecs and their roles in the immune system. Nat Rev Immunol 7:255–266
26. Damani MR, Zhao L, Fontainhas AM, Amaral J, Fariss RN, Wong WT (2011) Age-related alterations in the dynamic behavior of microglia. Aging Cell 10:263–276
27. Das P, Howard V, Loosbrock N, Dickson D, Murphy MP, Golde TE (2003) Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma-/- knock-out mice. J Neurosci 23:8532–8538
28. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y et al (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8:752–758
29. Denk W, Strickler JH, Webb WW (1990) Two-photon laser scanning fluorescence microscopy. Science 248:73–76
30. Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SB et al (2012) Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 484:105–109
31. El Khoury J, Hickman SE, Thomas CA, Cao L, Silverstein SC, Loike JD (1996) Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 382:716–719
32. El Khoury JB, Moore KJ, Means TK, Leung J, Terada K et al (2003) CD36 mediates the innate host response to beta-amyloid. J Exp Med 197:1657–1666
33. Fiumara A, Sciotto A, Barone R, D'Asero G, Munda S et al (1999) Peripheral lymphocyte subsets and other immune aspects in Rett syndrome. Pediatr Neurol 21:619–621
34. Frank S, Burbach GJ, Bonin M, Walter M, Streit W et al (2008) TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice. Glia 56:1438–1447
35. Frenkel D, Wilkinson K, Zhao L, Hickman SE, Means TK et al (2013) Scara1 deficiency impairs clearance of soluble amyloid-beta by mononuclear phagocytes and accelerates Alzheimer's-like disease progression. Nat Commun 4:2030
36. Fuhrmann M, Bittner T, Jung CK, Burgold S, Page RM et al (2010) Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. Nat Neurosci 13:411–413
37. Galasko D, Bell J, Mancuso JY, Kupiec JW, Sabbagh MN et al (2014) Clinical trial of an inhibitor of RAGE-Abeta interactions in Alzheimer disease. Neurology 82:1536–1542
38. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C et al (2012) Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 13:1118–1128
39. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P et al (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845
40. Grathwohl SA, Kalin RE, Bolmont T, Prokop S, Winkelmann G et al (2009) Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. Nat Neurosci 12:1361–1363
41. Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN et al (2013) Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78:631–643
42. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E et al (2013) TREM2 variants in Alzheimer's disease. N Engl J Med 368:117–127
43. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG et al (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 9:857–865

44. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41:1088–1093
45. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB et al (2013) Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38:792–804
46. Hefendehl JK, Neher JJ, Suhs RB, Kohsaka S, Skodras A, Jucker M (2014) Homeostatic and injury-induced microglia behavior in the aging brain. Aging Cell 13:60–69
47. Hefendehl JK, Wegenast-Braun BM, Liebig C, Eicke D, Milford D et al (2011) Long-term in vivo imaging of beta-amyloid plaque appearance and growth in a mouse model of cerebral beta-amyloidosis. J Neurosci 31:624–629
48. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S et al (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678
49. Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci 28:8354–8360
50. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435
51. Hoogerbrugge PM, Suzuki K, Poorthuis BJ, Kobayashi T, Wagemaker G, van Bekkum DW (1988) Donor-derived cells in the central nervous system of twitcher mice after bone marrow transplantation. Science 239:1035–1038
52. Hu X, Pickering E, Liu YC, Hall S, Fournier H et al (2011) Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease. PLoS One 6:e16616
53. Husemann J, Silverstein SC (2001) Expression of scavenger receptor class B, type I, by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and in Alzheimer's disease brain. Am J Pathol 158:825–832
54. Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 24:173–182
55. Jaeger PA, Pickford F, Sun CH, Lucin KM, Masliah E, Wyss-Coray T (2010) Regulation of amyloid precursor protein processing by the Beclin 1 complex. PLoS One 5:e11102
56. Jiang S, Li C, McRae G, Lykken E, Sevilla J et al (2014) MeCP2 reinforces STAT3 signaling and the generation of effector CD4 + T cells by promoting miR-124-mediated suppression of SOCS5. Sci Signal 7:ra25
57. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV et al (2013) Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 368:107–116
58. Jordan C, Li HH, Kwan HC, Francke U (2007) Cerebellar gene expression profiles of mouse models for Rett syndrome reveal novel MeCP2 targets. BMC Med Genet 8:36
59. Kam TI, Song S, Gwon Y, Park H, Yan JJ et al (2013) FcgammaRIIb mediates amyloid-beta neurotoxicity and memory impairment in Alzheimer's disease. J Clin Invest 123:2791–2802
60. Kempermann G, Neumann H (2003) Neuroscience. Microglia: the enemy within? Science 302:1689–1690
61. Khwaja OS, Ho E, Barnes KV, O'Leary HM, Pereira LM et al (2014) Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. PNAS 111:4596–4601
62. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C et al (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16:273–280
63. Kierdorf K, Prinz M (2013) Factors regulating microglia activation. Front Cell Neurosci 7:44
64. Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M et al (2009) Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular A beta clearance. Neuron 64:632–644
65. Koenigsknecht-Talboo J, Meyer-Luehmann M, Parsadanian M, Garcia-Alloza M, Finn MB et al (2008) Rapid microglial response around amyloid pathology after systemic anti-Abeta antibody administration in PDAPP mice. J Neurosci 28:14156–14164
66. Krivit W, Peters C, Shapiro EG (1999) Bone marrow transplantation as effective treatment of central nervous system disease in globoid cell leukodystrophy, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Maroteaux-Lamy, and Sly syndromes, and Gaucher disease type III. Curr Opin Neurol 12:167–176
67. Kuchibhotla KV, Goldman ST, Lattarulo CR, Wu HY, Hyman BT, Bacskai BJ (2008) Abeta plaques lead to aberrant regulation of calcium homeostasis in vivo resulting in structural and functional disruption of neuronal networks. Neuron 59:214–225
68. Lal G, Zhang N, van der Touw W, Ding Y, Ju W et al (2009) Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol 182:259–273
69. Lambert JC, Zelenika D, Hiltunen M, Chouraki V, Combarros O et al (2011) Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations. Neurobiol Aging 32(756):e11–e15
70. Lawson LJ, Perry VH, Gordon S (1992) Turnover of resident microglia in the normal adult mouse brain. Neuroscience 48:405–415
71. Lee S, Varvel NH, Konerth ME, Xu G, Cardona AE et al (2010) CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. Am J Pathol 177:2549–2562
72. Liebscher S, Meyer-Luehmann M (2012) A Peephole into the Brain: neuropathological features of Alzheimer's disease revealed by in vivo two-photon imaging. Front Psychiatry 3:26
73. Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD et al (2011) A role for glia in the progression of Rett's syndrome. Nature 475:497–500
74. Liu Z, Condello C, Schain A, Harb R, Grutzendler J (2010) CX3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-beta phagocytosis. J Neurosci 30:17091–17101
75. Lucin KM, O'Brien CE, Bieri G, Czirr E, Mosher KI et al (2013) Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease. Neuron 79:873–886
76. Lue LF, Walker DG, Brachova L, Beach TG, Rogers J et al (2001) Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer's disease: identification of a cellular activation mechanism. Exp Neurol 171:29–45
77. Lue LF, Yan SD, Stern DM, Walker DG (2005) Preventing activation of receptor for advanced glycation endproducts in Alzheimer's disease. Curr Drug Targets CNS Neurol Disord 4:249–266
78. Maezawa I, Jin LW (2010) Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. J Neurosci 30:5346–5356
79. Mann J, Oakley F, Akiboye F, Elsharkawy A, Thorne AW, Mann DA (2007) Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. Cell Death Differ 14:275–285
80. McCarter JF, Liebscher S, Bachhuber T, Abou-Ajram C, Hubener M et al (2013) Clustering of plaques contributes to

plaque growth in a mouse model of Alzheimer's disease. Acta Neuropathol 126:179–188
81. McGeer PL, Itagaki S, Tago H, McGeer EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neurosci Lett 79:195–200
82. Meyer-Luehmann M, Mielke M, Spires-Jones TL, Stoothoff W, Jones P et al (2009) A reporter of local dendritic translocation shows plaque- related loss of neural system function in APP-transgenic mice. J Neurosci 29:12636–12640
83. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A et al (2008) Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 451:720–724
84. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK et al (2007) Microglia in the adult brain arise from Ly-6ChiCCR2 + monocytes only under defined host conditions. Nat Neurosci 10:1544–1553
85. Moore KJ, El Khoury J, Medeiros LA, Terada K, Geula C et al (2002) A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. J Biol Chem 277:47373–47379
86. Morkuniene R, Zvirbliene A, Dalgediene I, Cizas P, Jankeviciute S et al (2013) Antibodies bound to Abeta oligomers potentiate the neurotoxicity of Abeta by activating microglia. J Neurochem 126:604–615
87. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN et al (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43:436–441
88. Nakamura K, Hirai H, Torashima T, Miyazaki T, Tsurui H et al (2007) CD3 and immunoglobulin G Fc receptor regulate cerebellar functions. Mol Cell Biol 27:5128–5134
89. Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318
90. O'Driscoll CM, Kaufmann WE, Bressler JP (2013) MeCP2 deficiency enhances glutamate release through NF-kappaB signaling in myeloid derived cells. J Neuroimmunol 265:61–67
91. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE et al (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409–421
92. Okun E, Mattson MP, Arumugam TV (2010) Involvement of Fc receptors in disorders of the central nervous system. Neuromolecular Med 12:164–178
93. Origlia N, Bonadonna C, Rosellini A, Leznik E, Arancio O et al (2010) Microglial receptor for advanced glycation end product-dependent signal pathway drives beta-amyloid-induced synaptic depression and long-term depression impairment in entorhinal cortex. J Neurosci 30:11414–11425
94. Paloneva J, Kestila M, Wu J, Salminen A, Bohling T et al (2000) Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet 25:357–361
95. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd et al (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155:1596–1609
96. Peress NS, Fleit HB, Perillo E, Kuljis R, Pezzullo C (1993) Identification of Fc gamma RI, II and III on normal human brain ramified microglia and on microglia in senile plaques in Alzheimer's disease. J Neuroimmunol 48:71–79
97. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R et al (2008) The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. J Clin Invest 118:2190–2199
98. Plioplys AV, Greaves A, Kazemi K, Silverman E (1994) Lymphocyte function in autism and Rett syndrome. Neuropsychobiology 29:12–16
99. Priller J, Flugel A, Wehner T, Boentert M, Haas CA et al (2001) Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat Med 7:1356–1361
100. Prokop S, Miller KR, Heppner FL (2013) Microglia actions in Alzheimer's disease. Acta Neuropathol 126:461–477
101. Ravichandran KS (2010) Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. J Exp Med 207:1807–1817
102. Sabbagh MN, Agro A, Bell J, Aisen PS, Schweizer E, Galasko D (2011) PF-04494700, an oral inhibitor of receptor for advanced glycation end products (RAGE), in Alzheimer disease. Alzheimer Dis Assoc Disord 25:206–212
103. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR et al (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705
104. Schwartz M, Butovsky O, Bruck W, Hanisch UK (2006) Microglial phenotype: is the commitment reversible? Trends Neurosci 29:68–74
105. Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V et al (2010) Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 303:1832–1840
106. Sessa G, Podini P, Mariani M, Meroni A, Spreafico R et al (2004) Distribution and signaling of TREM2/DAP12, the receptor system mutated in human polycystic lipomembraneous osteodysplasia with sclerosing leukoencephalopathy dementia. Eur J Neurosci 20:2617–2628
107. Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY (2002) Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. Hum Mol Genet 11:115–124
108. Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B et al (2013) CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nat Immunol 14:812–820
109. Shetty AK, Chatters R, Tilton AH, Lacassie Y (2000) Syndrome of microcephaly, mental retardation, and tracheoesophageal fistula associated with features of Rett syndrome. J Child Neurol 15:61–63
110. Simard AR, Soulet D, Gowing G, Julien JP, Rivest S (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 49:489–502
111. Spires-Jones TL, de Calignon A, Meyer-Luehmann M, Bacskai BJ, Hyman BT (2011) Monitoring protein aggregation and toxicity in Alzheimer's disease mouse models using in vivo imaging. Methods 53:201–207
112. Spires TL, Meyer-Luehmann M, Stern EA, McLean PJ, Skoch J et al (2005) Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. J Neurosci 25:7278–7287
113. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS et al (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164–1178
114. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J et al (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155–161
115. Streit WJ (2004) Microglia and Alzheimer's disease pathogenesis. J Neurosci Res 77:1–8
116. Styren SD, Civin WH, Rogers J (1990) Molecular, cellular, and pathologic characterization of HLA-DR immunoreactivity

in normal elderly and Alzheimer's disease brain. Exp Neurol 110:93–104
117. Takahashi K, Rochford CD, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201:647–657
118. Tong Y, Aune T, Boothby M (2005) T-bet antagonizes mSin3a recruitment and transactivates a fully methylated IFN-gamma promoter via a conserved T-box half-site. PNAS 102:2034–2039
119. Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527
120. Tremblay ME, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60:541–558
121. Tropea D, Giacometti E, Wilson NR, Beard C, McCurry C et al (2009) Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. PNAS 106:2029–2034
122. Urabe H, Kojima H, Chan L, Terashima T, Ogawa N et al (2013) Haematopoietic cells produce BDNF and regulate appetite upon migration to the hypothalamus. Nat Commun 4:1526
123. Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A (2008) Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J Neurosci 28:8138–8143
124. Webb R, Wren JD, Jeffries M, Kelly JA, Kaufman KM et al (2009) Variants within MECP2, a key transcription regulator, are associated with increased susceptibility to lupus and differential gene expression in patients with systemic lupus erythematosus. Arthritis Rheum 60:1076–1084
125. Wilkinson K, Boyd JD, Glicksman M, Moore KJ, El Khoury J (2011) A high content drug screen identifies ursolic acid as an inhibitor of amyloid beta protein interactions with its receptor CD36. J Biol Chem 286:34914–34922
126. Witting A, Muller P, Herrmann A, Kettenmann H, Nolte C (2000) Phagocytic clearance of apoptotic neurons by Microglia/Brain macrophages in vitro: involvement of lectin-, integrin-, and phosphatidylserine-mediated recognition. J Neurochem 75:1060–1070
127. Yamanaka M, Ishikawa T, Griep A, Axt D, Kummer MP, Heneka MT (2012) PPARgamma/RXRalpha-induced and CD36-mediated microglial amyloid-beta phagocytosis results in cognitive improvement in amyloid precursor protein/presenilin 1 mice. J Neurosci 32:17321–17331
128. Yan SD, Chen X, Fu J, Chen M, Zhu H et al (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 382:685–691
129. Yang T, Ramocki MB, Neul JL, Lu W, Roberts L et al (2012) Overexpression of methyl-CpG binding protein 2 impairs T(H)1 responses. Sci Transl Med 4:163ra58
130. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G et al (2014) Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17:400–406
131. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J et al (2013) Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell 153:707–720