INVITED REVIEW

Inflammation and Microglia Actions in Alzheimer's Disease

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Abstract A variety of studies have documented increased presence of reactive microglia in the brains of not only Alzheimer's disease (AD) patients but its transgenic mouse models. Since these cells are often characterized in association with fibrillar Aβ peptide-containing plaques, it has been assumed that plaque interaction provides one stimulus for the phenotype observed. The growing appreciation that microglia phenotype changes with age and that resident immune cells are comingled with blood-derived macrophage has complicated understanding of the behavior of these cells in AD. In addition, comparison of microglia within AD brains and the many rodent models suggests that there are population phenotype differences among these cells within any given brain during disease. Recent immunomodulatory strategies that have been employed, although effective at improving behavioral performance, decreasing Aβ plaque load, and altering immune molecule levels, have not yet resolved the details and dynamics of the microglial and macrophage responses. The heterogeneity of microglial presentation in AD brains and its transgenic mouse models and the outcomes of immunoregulatory efforts will be reviewed below along with the remaining question of how much understanding of microglial behavior is actually required in order to propose a microglia-related therapy for AD.

Keywords amyloid · inflammation · phagocytosis · macrophage · neurodegeneration

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Reactive microglia are present within the AD brain

Increased numbers of morphologically reactive microglia are a well-characterized histological observation from Alzheimer's disease (AD) brains compared to nondemented controls (Akiyama and McGeer 1990; Cras et al. 1990; McGeer et al. 1987; Styren et al. 1990). These have commonly been described in both white and gray matter with gray matter microglia often reported in association with compact Aβ peptide-containing plaques (Itagaki et al. 1989; Mackenzie et al. 1995; Mattiace et al. 1990; Sasaki et al. 1997). The majority of morphologically reactive microglia are within and around compact plaques but a small percentage of diffuse plaques also have associated, morphologically distinct microglia (Akiyama et al. 1999; Itagaki et al. 1989; Mackenzie et al. 1995; Mattiace et al. 1990; Sasaki et al. 1997). Ultrastructural analyses have demonstrated finger-like projections from individual microglia surrounding fibrillar Aβ within compact plaques, suggesting that a very specific microglia–fibril interaction occurs (Perlmutter et al. 1990; Wisniewski et al. 1992). These types of data have helped support the notion that microglia respond to fibrillar Aβ plaque deposition and the resultant changes in their phenotype are a component of disease progression.

However, several observations support the idea that microgliosis may actually be an early component of the disease process and not necessarily dependent upon Aβ plaque interaction as a stimulus. It has been reported that numbers of ferritin-immunoreactive microglia in the frontal cortex do not correlate with the age of onset or duration of disease allowing Hayes et al. (2002) to conclude that microgliosis is an early occurring event. A similar report of increased HLA-DR-immunoreactive microglia in probable AD cases versus control brains compared to no difference

for glial fibrillary acidic protein-immunoreactive astrocytes again suggests that microglial phenotype changes may begin early in disease progression (Vehmas et al. 2003). An earlier study following four AD patients from biopsy to autopsy over the course of nearly a decade demonstrated that, although plaque and tangle load increased in two of the four samples, increased microgliosis only occurred in one of them in spite of progressive cognitive decline for all (Di Patre et al. 1999). Also, increased microglial immuno-reactivity for HLA-DR is reportedly unique to mild to moderate AD brains compared to high plaque pathology nondemented controls and total reactive microglial load correlated inversely with performance on the mini mental status exam (Parachikova et al. 2007). These data demonstrate via immunodetection that elevated levels of microgliosis have been characterized in early or probable disease, end-stage disease, as well as, in a more limited fashion, progressive disease versus controls. On the other hand, microgliosis does not appear to be exquisitely dependent upon plaque deposition nor is it an absolute requirement for all cases of cognitive decline. When considering this ambiguity, it is important to consider that different brain region comparisons from study to study as well as different microglial and plaque detection strategies may well influence the conclusions drawn regarding microgliosis.

Recent imaging studies to visualize microglia have helped to clarify the relationship between microgliosis, disease progression, and plaque deposition that has remained unclear from the immunohistology. For example, positron emission tomography (PET) imaging to visualize reactive microglia via binding to ligands for the peripheral benzodiazepine binding receptor have demonstrated that reactive microglial load increases in AD versus control brains and does directly correlate with degree of cognitive deficit (Cagnin et al. 2001; Versijpt et al. 2003). This finding supports the limited immunohistological analysis described above (Di Patre et al. 1999) demonstrating that progressive microgliosis directly correlates with disease progression in some cases. Moreover, Edison et al. (2008), again using PET imaging, demonstrated that mini mental status exam scores correlated inversely with activated microglial density rather than plaque load in AD patients. Similar to conclusions drawn from immunodetection studies (Parachikova et al. 2007), this data suggests that microglial activation, at least as assessed by peripheral benzodiazepine binding receptor ligand interaction, can be independent of fibrillar Aβ interaction. Finally, a very recent additional PET study imaged reactive microglial load versus fibrillar amyloid burden in patients with mild cognitive impairment versus AD and control patients. The authors observed increased amyloid burden comparable to AD patients in seven out of 14 impaired individuals while only five out of 13 impaired individuals had increased reactive microglia load comparable to AD brains (Okello et al. 2009). Moreover, only three of these five had elevated amyloid burden comparable to those found in compared AD brains (Okello et al. 2009). Therefore, using the method of PET-based microglial detection, the data again supports the idea that microgliosis is increased in AD versus control brains but is not exclusively tied to plaque deposition and is not definitively required for all cases of AD-related behavioral decline. Although these recent imaging data are limited to assessing microgliosis via one method of detection, the studies support the idea that microglial phenotype changes are a component of the disease process and the source of their activation may be somewhat heterogeneous in nature.

This complexity of microglial response with regard to plaque load suggests that the stimuli for microglial phenotype change and microglial-mediated contribution to the disease process has yet to be fully determined. For example, it appears that there is nothing particularly unique about the Aβ fibril itself with regard to ability to stimulate microglia. Miyazono et al. (1991) have assessed another compact plaque pathology in kuru brains unrelated to Aβ fibrils and demonstrated abundant, associated reactive microglia. These results indicate that fibrillar extracellular aggregates, per se, rather than Aβ uniquely, are a potent source of microglial activation (Miyazono et al. 1991). On the other hand, Shepherd et al. (2000, 2005) compared early onset AD brains related to mutations in presenilin 1 (PS1) to sporadic AD brains to demonstrate no correlation between the unique, Aβ-negative "inflammatory" plaque pathology with abundant reactive microglia, in early onset disease and the degree of neuron loss, in spite of the significantly greater degree of neuron loss present in early onset versus sporadic disease brains. This suggests that microglia activation to fibrillar aggregates, per se, is also not necessarily contributing to neurodegeneration.

Independent of this possible ambiguity regarding the source of microglial stimulation in AD and the resultant effects on disease process, it has also become increasingly clearer that there is a normal age-associated dystrophy of subpopulations of HLA-DR-immunoreactive microglia in the brain that is independent of plaque association (Lopes et al. 2008; Streit et al. 2004). This suggests that Aβ-dependent or Aβ-independent microglial activation during disease is superimposed upon concomitant age-associated changes. Additional methods of detecting microglia have provided essentially the same conclusion that specific microglial phenotypes exist independent of fibrillar amyloid interaction. For instance, laminar distribution of IL-1α-immunoreactive microglia in the human brain parallels the eventual distribution of neuritic plaques in disease (Sheng et al. 1998). Similarly, Sheffield et al. (2000) demonstrated that microglial distribution visualized by *Ricinus communic*

agglutinin-1 labeling also predicts the eventual distribution not of plaques but of neurofibrillary tangle pathology. Collectively, there appears overwhelming evidence that microglial activation is a component of the AD process. The necessary details of determining variability in the nature of the response, the stimulus for the response, the ensuing consequences of the response, and the necessity of the response for neuron loss remain viable questions.

Reactive microglia are present in the brains of AD transgenic mouse models

Animal studies offer the potential, at least, to begin answering these temporal and mechanistic questions raised by the data collected from human studies. In particular, a reasonable approach has been to turn to the many available transgenic mouse models of AD to attempt to define the role of microglial activation in the disease process. Since these transgenic animal models are, by design, the result of exogenous expression of a mutant human protein(s) linked to early onset disease, they present the potential caveat of representing the rarer early onset form of disease rather than the majority sporadic form. Nevertheless, it has been noted that microglia phenotype, as assessed by varying immuno-detection choices, is quite diverse in response to mutant protein expression in the various transgenic models (Morgan et al. 2005). This is a conclusion not entirely different from those drawn above based upon data from human studies. Therefore, with regard to the complexity of microglial response, both plaque-related and nonplaque-related, the transgenic animals appear a reasonable model for study.

Without reiterating the summary of prior reviews documenting the microglia phenotype changes in the AD mouse models, a few comparisons will be made to the human disease with particular attention to the issue of microglia–plaque association. In general, observations regarding whether or not microglia associate with amyloid plaques are similar between human disease and the mouse models. For instance, using the Tg2576 transgenic line at 10–16 months of age expressing the human APP695 double Swedish mutation (K670N, M671L, APP770 numbering) under the control of the hamster prion promoter, Frautschy et al. (1998) described increased phosphotyrosine-immunoreactive microglial density and size that decreased with distance away from fibrillar plaques. Similar plaque-associated microgliosis has been reported from this same line using antibodies recognizing CD45 and CD11b (Benzing et al. 1999). These animals develop particularly large amyloid plaques that are associated with increasing numbers of microglia with size (Sasaki et al. 2002; Wegiel et al. 2001, 2003). Similar findings were reported by

Stalder et al. (1999) using the APP23 transgenic mouse that also expresses the human APP Swedish familial double mutation but under the control of the neuron specific Thy-1 promoter. Importantly, however, this study documented no association of reactive microglia with diffuse Aβ-immunoreactive plaques in the APP23 line (Stalder et al. 1999). This is in contrast to findings from human brain demonstrating that some microglia are associated with diffuse plaques (Akiyama and McGeer 1990; Itagaki et al. 1989; Mackenzie et al. 1995; Mattiace et al. 1990; Sasaki et al. 1997). A further separation from human disease was demonstrated by Schwab et al. (2004) using the APP23 line in which they demonstrated weakly CD11b-immunoreactive microglia surrounding the amyloid plaques in the mouse brain compared to strongly CD11b-immunoreactive microglia invested within plaques in human disease. Similarly, the plaques themselves in this mouse line differed dramatically with respect to several complement protein immunoreactivities when compared to human brains (Schwab et al. 2004). Nevertheless, ultrastructural analysis of plaque-associated microglia from the APP23 mice do demonstrate microglia with channel-like or finger-like extensions around Aβ fibrils similar to findings reported in the human brain (Stalder et al. 2001). This demonstrates that, although there may be some differences in specific immunoreactivity-based phenotypes, the microglia in the mouse models may be interacting with fibrils in a fashion similar to that occurring in human disease thus offering tentative validation of these models.

In support of continued use of the transgenic models to assess microgliosis, Matsuoka et al. (2001) demonstrated that CD11b-immunoreactive microglia associate with diffuse as well as compact plaques when the Tg2576 line was crossed with a mutant presenilin 1 (PS1$_{M146L}$) mouse. Two recent in vivo multiphoton imaging studies have offered rather definitive evidence of the ability of microglia to associate with fibrillar amyloid. Using PDAPP mice which express a human APP minigene encoding the APP$_{V717F}$ mutation under the control of the platelet-derived growth factor-β promoter (Games et al. 1995) crossed to a mouse with a targeted deletion of the CX$_3$CR1 fractalkine receptor replaced with green fluorescent protein (Jung et al. 2000), Meyer-Luehmann et al. (2008) demonstrated that plaques, both large and small diameter, form within a 24-h period followed by subsequent, rapid recruitment of microglia. However, using the same crossed line, Koenigsknecht-Talboo et al. (2008) demonstrated that microglia from 14- to 17-month-old mice compared to 3.5- to 6.5-month-old mice are less motile with fewer processes regardless of whether they are plaque-associated or not. Moreover, in this same study, the authors document that microglia that are directly plaque-associated are highly CD45-immunoreactive after passive immunization compared

to those further away (Koenigsknecht-Talboo et al. 2008). These data once again suggest that microglia are heterogeneous in nature both physiologically and during disease, not entirely different from the conclusions drawn from human data. More importantly, however, is the possibility that all transgenic mouse models may not be the same with regard to microglial activation state and response to fibrillar or diffuse plaque deposition. These possible differences based upon animal background or particular transgene expression strategy indicates that a specific temporal and spatial comparison with multiple assessments of microglial phenotype change across the various transgenic lines may ultimately be required to identify a model closest to human disease.

Modulating microglial phenotype to improve disease conditions in humans

In spite of the fact that the actual stimulating ligand(s) for microglial activation in the AD brain may be diverse and change with age and the possibility that transgenic mouse models may differ with respect to their own mechanisms and profile of microgliosis, it is generally accepted, based upon the preponderance of evidence, that microglia both in human brain and the mouse models exhibit some form of reactive phenotype when associated with the compact amyloid deposits. Therefore, the proximity of the cells to what appears to be undigested, extracellular Aβ aggregates has prompted the rationale to alter microglial phenotype in favor of increased phagocytic potential. Schenk et al. (1999) demonstrated the feasibility of using an Aβ immunization approach to attenuate both diffuse and compact Aβ-immunoreactive plaques in parallel with an increase in Aβ-immunoreactive, MHCII-positive microglia in the PDAPP mice suggesting stimulated microglial uptake. The logical extension of this approach was to attempt similar results by immunizing AD patients against Aβ peptide, termed AN-1792 (Schenk 2002). The double-blind phase II trial was terminated early due to what is believed to have been onset of aseptic meningoencephalitis in ultimately 18 of the treated patients (Orgogozo et al. 2003). However, the limited amount of histological analysis performed does indicate that immunization produced a decrease in parenchymal rather than vascular fibrillar plaque load in parallel with an increase in Aβ-immunoreactive microglia consistent with phagocytic uptake (Ferrer et al. 2004; Masliah et al. 2005; Nicoll et al. 2003). Although the mechanism remains unclear, it is intriguing to speculate that the beneficial effect may have been due, in part, to microglial-dependent phagocytosis of opsonized plaques simultaneously clearing fibrillar aggregates from the brain and altering the phenotype of the microglia involved. This tantalizing data supports the idea

that it may not be entirely necessary to understand the complexity of microglial behavior during disease in order to harness their responses to provide a measurable, therapeutic outcome such as plaque clearance.

Modulating microglial phenotype to improve disease conditions in AD transgenic mouse models

Based upon the presumed mechanisms of altering microglial behavior to promote plaque clearance after immunization, a plethora of microglial modulatory strategies have ensued in recent years with a common goal of decreasing fibrillar plaque load, minimizing markers of inflammatory change, and improving behavioral performance. Aβ vaccination, both active and passive, has been a logical and largely successful strategy to carry out exactly these goals. This has been the subject of many prior reviews and, therefore, will not be discussed at length (Brody and Holtzman 2008; Hawkes and McLaurin 2007; Maier et al. 2005; Morgan 2006; Okura and Matsumoto 2007; Schenk et al. 2005; Steinitz 2008). However, several recent rodent studies have either attempted to directly modulate microglial phenotype or have concluded that changes in microglial phenotype contribute to altered plaque load or inflammatory state in the brain independent of any active or passive vaccination strategy (Ding et al. 2008; El Khoury et al. 2007; Jiang et al. 2008; Jin et al. 2008; Li et al. 2008; Maier et al. 2008; Nichol et al. 2008; Richard et al. 2008; Scholtzova et al. 2009; Shaftel et al. 2007; Town et al. 2008). It is important to note that, these studies, in line with the immunization approaches, have directly or indirectly sought to alter microglial behavior rather than generally inhibit it. This is likely a reflection of the growing appreciation, as already mentioned, of the intrinsic heterogeneity of microglial response during disease.

For example, several studies have documented what appears to be a varied phenotype with age and disease in the transgenic mouse models. Using an APP/PS1 mouse line coexpressing a humanized Swedish amyloid precursor protein mutation (APP695S_{WE}) and an exon 9 deletion variant of human presenilin 1 (PSEN1/dE9) under the control of the mouse prion promoter, Hickman et al. (2008) demonstrated that, between 1.5 and 8 months of age, microglial mRNA for several putative Aβ fibril-interacting proteins decrease with age in both wild-type and mutant animals. However, mutant animals had a significantly decreased level compared to wild-type controls indicating, once again, that age-associated changes are superimposed upon events occurring during disease (Hickman et al. 2008). Microglial mRNA for Aβ-degrading enzymes also decreased significantly in mutant versus control animals over this time period (Hickman et al. 2008). Jimenez et al. (2008) used a different mouse line expressing human

mutant APP with the Swedish double and London (V642I) mutations under the control of the mouse Thy-1 promoter and human mutant presenilin 1 (PS1M146L) driven by the HMG-CoA reductase promoter to demonstrate a related observation of temporally changing immune state in the brain with age and disease. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis of whole-brain RNA demonstrated increased levels of chitinase 3-like 3 (YM-1) message, a putative alternative activation phenotype marker in peripheral macrophage, at 6 months of age maintained until 18 months of age (Jimenez et al. 2008). However, classic inflammatory markers, such as mRNA for TNFα, were only increased at 18 months of age (Jimenez et al. 2008). Immunolocalization at 18 months demonstrated that YM-1-positive cells were microglia exclusively surrounding and infiltrating the plaques while TNFα-positive cells were microglia that were not directly associated with plaques (Jimenez et al. 2008). This increase in TNFα-positive, YM-1-negative microglia with age corresponded to an increase in IL-4-immunoreactive, Th2-type CD3$^+$ T cells in the aged animals (Jimenez et al. 2008). Finally, Colton et al. (2006) also demonstrated by RT-PCR, this time from Tg2576 mice and AD brains, that mRNA for TNFα and alternative activation genes, arginase I, mannose receptor, and chitinase 3-like 3 (YM-1 in mice), and chitinase 3-like 1 and 2 (CHI3L1 and CHI3L2 in humans) were increased compared to wild-type mice and non-demented controls, respectively. Collectively, these data support the growing notion that microglial phenotype changes during disease and microglia are heterogeneously activated at any given point in the process.

However, as the vaccination strategies have proven, it is not necessary to entirely define microglial behavior to begin altering it to offer quantifiable, therapeutically attractive outcomes. For example, treatment of the prion promoter-driven APP/PS1 line with all-trans retinoic acid during 5–7 months of age resulted in a decrease in Iba-I-immunoreactive microglial volume and density in correlation with a decrease in Aβ plaque volume, neuronal marker protein loss, and spatial memory deterioration (Ding et al. 2008). One possible explanation for the therapeutic benefit was the suggested anti-inflammatory action of all-trans retinoic acid on microglia (Dheen et al. 2005). In another study, Maier et al. (2008) used mice deficient for complement protein 3 (C3$^{-/-}$) but expressing platelet-derived growth factor promoter-driven human APP with both the Swedish (K670N, M671L) and Indiana (V717F) mutations and compared them to animals that expressed endogenous C3 levels and the mutant APP. The authors demonstrated that microglia at 17 months but not 8 or 12 months of age had increased immunoreactivity for CD45 in the mutant APP;C3$^{-/-}$ animals compared to animals expressing mutant APP alone (Maier et al. 2008).

Interestingly, immunoreactivity for CD68 or Iba1, other common microglial markers, demonstrated no significant differences across the two lines (Maier et al. 2008). This change in microglial immunoreactivity correlated with increased IL-4 but decreased iNOS and TNFα levels in the APP;C3$^{-/-}$ animals compared to the mice expressing mutant APP alone (Maier et al. 2008). The microglial changes also correlated with increased Aβ plaque load in the 17-month-old APP;C3$^{-/-}$ animals compared to the mutant APP alone expressing mice (Maier et al. 2008). The authors suggested that one possibility for the changes in Aβ load might be due to decreased microglial uptake via decreased complement-mediated opsonization of plaques. Another study by Li et al. (2008) crossed a G protein-coupled receptor (GPCR) kinase 5 knockout (GRK5) mouse with the Tg2576 line to demonstrate that animals hemizygous for the knockout had increased amounts of CD45-immunoreactive microglia compared to the Tg2576 line alone at 18 months of age. A suggested mechanism for the increased microgliosis was that a loss of the dampening effect of the GRK5 on GPCR signaling had occurred in microglia. An additional microglial modulatory strategy was suggested by Jiang et al. (2008) in which the Tg2576 mouse line was treated with the liver X receptor (LXR) agonist, GW3965, from 12 to 16 months of age to decrease plaque load and improve contextual memory. These changes correlated with an increased ability of microglia to degrade Aβ both intracellularly and extracellularly upon stimulation of the LXR (Jiang et al. 2008).

Other microglial modulatory strategies and interpreted outcomes in the rodent models have been complicated by the possibility that the heterogeneity of microglial phenotype is a combination of resident microglial behavior and that of blood-derived macrophage migrating into the brain. For instance, Butovsky et al. (2007) demonstrated, using the prion promoter-driven APP/PS1 line crossed to animals with CDllc promoter-driven expression of human diphtheria toxin receptor–green fluorescent protein fusion protein, that T cell-based immunization with glatiramer acetate stimulated an increase in CDllc-positive blood-derived macrophage into the brain as an essential component of a subsequent decrease in plaque load. Another study using excisional activation of IL-1β in the APP/PS1 line demonstrated that localized expression of IL-1β in the hippocampus of these animals from 6 to 7 months of age significantly decreased Congo red-labeled plaque load and insoluble Aβ in conjunction with increased numbers of Iba1-immunoreactive and MCHII-immunoreactive microglia associated with plaques in the IL-1β-overexpressing hippocampus compared to the contralateral control (Shaftel et al. 2007). Interestingly, this increase in plaque-associated microglia was again heterogeneous with a population that was MHCII/Iba1 double labeled and a population that was Iba1-immunoreactive alone

(Shaftel et al. 2007). The authors suggested the possibility that the decrease in plaque load may have been due to increased phagocytosis by resident microglia or increased influx of blood-derived macrophage (Shaftel et al. 2007). An additional therapeutic strategy employed 3-week voluntary running wheel exercise for 17- to 19-month-old Tg2576 mice that apparently also altered microglial phenotype in conjunction with a decrease in soluble Aβ1–40 levels and increased cognitive performance (Nichol et al. 2008). Microglia from run versus sedentary Tg2576 animals displayed increased CD40, MHC, and CD11c immunoreactivity in addition to decreased levels of TNFα and IL-1β, supporting the idea that exercise promoted acquisition of an alternative reactive phenotype (Nichol et al. 2008). Much of this increased microglial marker immunoreactivity was vascular-associated as well as plaque-associated, supporting the conclusion that, not only was an altered microglia phenotype induced by exercise, but also increased infiltration of perivascular, blood-derived macrophage (Nichol et al. 2008).

Other studies have assessed the consequences of altering microglial phenotype to determine effects on Aβ deposition and disease pathology in the mouse models by directly altering microglial protein expression. For example, Town et al. (2008) crossed a CD11c promoter-driven dominant-negative TGF-β receptor-expressing mouse to the Tg2576 line to demonstrate that decreasing functional TGFβ signaling in peripheral macrophage resulted in a significant decrease in parenchymal and vascular Aβ deposition and increase in infiltrating peripheral macrophage in 17- to 18-month-old animals compared to the Tg2576 line alone. El Khoury et al. (2007) arrived at a similar conclusion regarding the importance of peripheral macrophage for plaque clearance. The authors found that Tg2576 mice crossed with animals deficient for CC-chemokine receptor 2 (Ccr2) resulted in earlier mortality, increased Aβ levels, and decreased microglial activation compared to the parent Tg2576 line. The authors also found a significant decrease in CD45-immunoreactive blood-derived macrophage in the Tg2576 Ccr2-deficient animals compared to the Tg2576 animals, suggesting that both resident microglia and peripheral macrophage require Ccr2 stimulation for chemotaxis into and within the brain to promote plaque clearance (El Khoury et al. 2007). Decreased or mutant expression of microglial/macrophage-related receptor, toll-like receptor 2 (TLR2) and mutant expression of toll-like receptor 4 (TLR4) in the prion promoter-driven APP/PS1 line resulted in increased Aβ accumulation compared to the APP/PS1 line alone (Jin et al. 2008; Richard et al. 2008). Importantly, expression of TLR2 via lentivirus in peripheral macrophage was sufficient to rescue the TLR2-deficient animals, again pointing towards a critical importance for blood-derived cells in mechanisms of plaque clearance (Jin

et al. 2008; Richard et al. 2008). Also of importance is the fact that increased reactive microglia in the mutant TLR4 APP/PS1 mice were highly CD11b-immunoreactive compared to the APP/PS1 animals while there was no difference in CD45 immunoreactivity between the lines (Jin et al. 2008). Targeting an additional microglia-related receptor in the brain, toll-like receptor 9 (TLR9) via administration of TLR9 agonists to the Tg2576 line from 6 weeks to 16 months of age improved working memory and decreased Aβ plaque load in both the parenchyma and vasculature in a study by Scholtzova et al. (2009). More importantly, these changes correlated with an overall decrease in both CD45-immunoreactive and CD11b-immunoreactive microglia but an increase in specifically plaque-associated CD45-immunoreactive cells (Scholtzova et al. 2009). Taken together, the growing consensus using strategies that indirectly or directly affect microglial phenotype is that the population of cells in the brain is a combination of both resident microglia and newly recruited blood-derived macrophage. Moreover, their responses during various plaque reduction strategies are varied and quite possibly distinct.

Immune changes are a component of AD

This recent demonstration of heterogeneity in the phenotype of microglia not only within the diseased brain but also across age in AD and its mouse models is not surprising, given the fact the peripheral immune cell traffic into the brain appears to be a potentially important part of both physiologic and pathologic processes. Moreover, it is now appreciated that microglia themselves have a heterogeneous morphology and phenotype within the brain in addition to their migratory and proliferative capacity. Therefore, a diverse, resident cell type to begin with coupled with a vasculature-derived peripheral influxing population of leukocytes lends full support to the idea that any type of phagocyte-mediated immune response in the brain has the potential to be quite heterogeneous. One simplified scenario that may be occurring, for example, is that microglial phenotype is altered early in disease via factors independent of Aβ such as vascular-derived or glial-derived cytokines or degenerating neuron-secreted factors. The resultant phenotype change, coupled with a change in age-dependent behavior, could then lead to an inability of the cells to clear Aβ thus promoting fibrillar accumulation and plaque deposition. The accumulated Aβ plaques could serve as an additional microglial stimulus to nearby or attracted cells again altering secretion and phenotype. This change could include increased chemotactic recruitment of blood-derived macrophage into the brain and plaques. The combined secretory presence of resident microglia-derived and blood-derived macrophage could now create yet another phenotype

alteration in both cell types now that is a combination of age-associated phenotype, brain versus peripheral leukocyte phenotype, and all the various disease process-stimulated phenotypes. Deciphering the temporal and specific details of such a process is daunting yet likely necessary to answer the question of how to increase phagocytic potential of microglia or macrophage while decreasing secretion of proinflammatory factors. Encouragingly, there appears to be significant supporting evidence that microglia/macrophage activation and the coincident changes in immune mediator status in the brain remains an integral characteristic of AD. For example, when brains of AD patients are compared to those of individuals with mixed dementia or vascular dementia alone, the proinflammatory environment of AD appears unique. Increased IL-1β levels are a characteristic of AD versus vascular dementia (Cacabelos et al. 1994). Indeed, in contrast to an overall impression of increased proinflammatory cytokines in brains of AD patients, gray and white matter quantitation from vascular and mixed dementia patients indicates decreased levels of MCP-1 and IL-6 compared to nondemented controls (Mulugeta et al. 2008). In fact, a population-based cohort study by in in t' Veld et al. (2001) demonstrated that long-term nonsteroidal anti-inflammatory drug (NSAID) use decreased the risk for developing AD but not vascular dementia again supporting the idea that immune changes, likely involving microglia and macrophage, are central to the AD process. More importantly, a more recent cohort study by Szekely et al. (2008) found that NSAID use reduced the risk of preferentially AD versus vascular dementia but only in those individuals with an APOE ε4 allele. This data indicates, as has been reiterated from the many microglial-related findings reviewed above, that the heterogeneity of the inflammatory response in AD and its mechanistic contribution to disease has yet to be fully resolved. As the human Aβ vaccination strategies have suggested, it may not ultimately be necessary to fully understand the subtleties of microglial behavior in AD for a beneficial therapeutic strategy to arise. However, should these overall immunomodulatory efforts fail to produce a beneficial outcome for disease therapy, a specific, targeted strategy for altering microglial behavior could be proposed when further biology of these cells is resolved during normal aging and disease.

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