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Experimental manipulations of microglia in mouse models of Alzheimer's pathology. Activation reduces amyloid but hastens tau pathology

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Abstract

The inflammation hypothesis of Alzheimer's pathogenesis has directed much scientific effort towards ameliorating this disease. The development of mouse models of amyloid deposition permitted direct tests of the proposal that amyloid-activated microglia could cause neurodegeneration in vivo. Many approaches to manipulating microglial activation have been applied to these mouse models, and are the subject of this review. In general, these results do not support a direct neuricidal action of microglia in mouse amyloid models under any activation state. Some of the manipulations cause both a reduction in pathology, and a reduction in microglial activation. However, at least for agents like ibuprofen, this outcome may result from a direct action on amyloid production, and a reduction in the microglial provoking amyloid deposits, rather than from reduced microglial activation leading to a decline in amyloid deposition. Instead, a surprising number of the experimental manipulations which increase microglial activation lead to enhanced clearance of the amyloid deposits. Both the literature and new data presented here suggest that either classical or alternative activation of microglia can lead to enhanced amyloid clearance. However, a limited number of studies comparing the same treatments in amyloid-depositing vs tau-depositing mice find the opposite effects. Treatments that benefit amyloid pathology accelerate tau pathology. This observation argues strongly that potential treatments be tested for impact on both amyloid and tau pathology before consideration of testing in humans.

Keywords

cytokines; chemokines; neuroimmunology; tauopathy; inflammation; toll-like receptors; complement; nonsteroidal anti-inflammatory drugs (NSAIDs)

Introduction

At the turn of the century, one hypothesis regarding Alzheimer's pathogenesis was that inflammation participated in a positive feedback loop which continued to increase amyloid deposition and ultimately resulted in neurodegeneration [1]. There were three primary observations supporting this contention. The first was the evidence in histopathology and neurochemistry from autopsied brains that Alzheimer's victims expressed all of the markers of associated with an innate immune system inflammatory reaction and that complement cascade proteins had been activated, including the cytotoxic membrane attack complex [2–4]. The second was the observation that individuals who had extended exposures to anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs,) had a reduced risk of Alzheimer's dementia [5, 6]. A third was the observation that in co-cultures, microglia activated by A β aggregates became toxic towards neurons [7]. After an initial period of challenges [8], valuable amyloid precursor protein (APP) overexpressing mouse models of amyloid deposition appeared in the mid 1990's [9–11]. This led to a variety of studies in transgenic mouse models to test the hypothesis that inflammation mediates some of the toxicity of pathologies found in Alzheimer's disease.

This review will describe a number of these studies, focusing on mouse models of select aspects of Alzheimer's pathology. The vast majority of these are amyloid-depositing models in mice transgenic for mutated forms of the human amyloid precursor protein (APP), sometimes in association with a presenilin-1 mutation (PS-1) to drive greater production of the more toxic A β 1–42 peptide. Critically, a few studies in tau-depositing mice are mentioned near the end. The review will focus primarily on studies specifically designed to modulate the microglial activation state in the mice, rather than to summarize other treatments that might indirectly impact this variable. The literature has been parceled into several categories, depending upon the method of modulating microglial activation.

Toll-Like Receptor (TLR) Activation

A vexing issue early on in the studies of mouse models of amyloid deposition was the general paucity of neuron loss observed in the transgenic models, in spite of considerable amyloid deposition [12–14]. One option, our group believed, was that the microglial activation in the mouse models was, for some reason, not as profound as that reported in Alzheimer's brains, and the degree of microglial activation required for neurocidal activity was not attained. Our group opted to test this by injecting directly into the CNS the prototypical proinflammatory agent lipopolysaccharide (LPS) [15]. We specifically chose the intracranial route as detection of systemic inflammation was not a common observation in Alzheimer's patients. Although we expected neuron degeneration under the combined assault of amyloid and LPS, we could find no evidence of neuron loss 3 or 7 days later. Instead we observed a considerable clearance of the diffuse A β deposits (detected by immunocytochemistry), but not compact deposits (labeled with Congo red or Thioflavin S). We have multiple replications of this observation using acute LPS injections which cause substantial increases in M1 cytokines (interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF]) [16]. We further observed suppression of the LPS-associated amyloid clearance and microglial activation by dexamethasone treatment, but not by NSAIDs or minocycline [17].

Quinn et al [18] also observed reduced amyloid loads 7 days after systemic LPS treatment. Malm et al [19] injected LPS intrahippocampally and observed reductions in diffuse, but not compacted amyloid deposits 7 days later. LPS transmits signals through the toll-like receptor 4 (TLR4). Consistent with the arguments that TLR4 signaling benefits A β pathology, Song et al [20] reported that APP mice bred onto a background lacking a functional TLR4 receptor had reduced levels of IL-1 and increased levels of A β . They also detected reduced levels of CCL3 and CCL4 in the mice with a mutated TLR4 gene.

However, there is equally convincing evidence to counter this notion that LPS can exacerbate amyloid pathology. Qiao [21] infused LPS intraventricularly for 2 weeks into young APP mice prior to deposit formation. They found appearance of Thioflavin S deposits in the LPS-treated mice, but not in those infused with vehicle. Sheng et al [22] used weekly injections of intracerebroventricular LPS in mice prior to formation of plaques and found increased A β and APP inside neurons, and increased β C-terminal fragments. Sly et al [23] found that a single systemic injection of LPS increased A β levels in brains of APP mice at 4 hours, but not at 18 hours after the injection (the longest time point measured). An unresolved question is whether these outcomes suggest different actions in mice that already have deposits compared to those that do not. It is also appropriate to consider that repeated administrations of LPS may induce tolerance, and the mechanisms mediating this tolerance may shift the impact of the LPS from one promoting clearance to one promoting deposition.

Another TLR-related manipulation regards deletion of CD14, a co-receptor for TLR2 and TLR4. These mice, like those with mutant TLR4, would be expected to lack signaling through TLR4. However, mice lacking CD14 exhibited a reduction in amyloid deposition when bred with an APP mouse [24]. This was associated with reduced expression of TNF and interferon-gamma, and increased expression of the M2 markers Fizz1 and YM1.

Another TLR examined in APP mice is TLR9 stimulated with CpG containing oligodeoxynucleotides. Sholtzova et al [25] demonstrated that monthly injections over 14 months dramatically reduced amyloid deposition and rescued memory impairments found in APP mice. Moreover, the few remaining plaques had increased CD45 immunostaining associated with them, although there was overall reduced microglial activation measured with CD11b and CD45 due to reduced plaque load. Consistent with this observation, Doi et al [26] found that a single intracerebroventricular injection of CpG oligodeoxynucleotides reversed fear conditioning deficits and reduced amyloid loads one week after injection of APP mice. Hence, it seems that the microglial activation associated with TLR9 has benefits in amyloid-depositing mice, similar to TLR4.

Myeloid Differentiation Factor 88 (MyD88) is a downstream adapter protein of most TLRs, leading to changes in transcription and generally promoting an inflammatory phenotype. Several groups have attempted genetic manipulation of this factor to understand the role of TLRs in amyloid processing. Hao et al [27] grafted bone marrow cells deficient and sufficient for MyD88 into irradiated amyloid-depositing mice. The MyD88 deficient bone marrow derived cells infiltrated into brain and led to reduction in the classical activation state (lower TNF and IL-1 levels), decreased A β deposition and improved memory (compared to MyD88 positive cells). A second approach used amyloid-depositing mice bred

onto a MyD88 homozygous null condition, which also reduced A β and reduced activation of microglia (CD45, CD11b) [28]. Both of these investigators concluded that MyD88 signaling normally exacerbates amyloid pathology. However, a third publication [29] bred amyloid-depositing mice onto a heterozygous null MyD88 background. They found reduced classical activation of microglia (IL-1) and reduced fibrillar A β deposits, but increased oligomeric A β and impaired memory compared to fully sufficient MyD88 mice. Because of the behavioral difference, these authors concluded that MyD88 normally protects from A β -induced memory impairments.

On the basis of the above studies, it is difficult to draw firm conclusions. Acute LPS, TLR4 knockout, and TLR9 activation support a role for microglial activation in clearing amyloid. However, the CD14 knockout and MyD88 knockout, plus chronic LPS data support the opposite conclusion, that reduced microglial activation is associated with less A β deposition (Table 1). A detailed characterization of the microglial phenotype has not been performed in the studies above, so it is possible that a more thorough analysis of microglial markers might suggest a specific function or activation state that is most beneficial for amyloid reduction.

Complement Activation

It has been known for some time that A β is capable of activating the complement cascade independent of antibody [30]. Several attempts have been made to intervene in the complement activation pathway in transgenic mice. Wyss-Coray, [31] inhibited complement activation by crossing APP mice onto those expressing the complement inhibitor sCrry. This led to a dramatic elevation of A β deposition, and the appearance of neuron loss. Intriguingly, in spite of an increased number of plaques, the microglial activation, assessed with F4/80, was significantly decreased. Another attempt to modulate complement activation is the deletion of the complement protein C3 [32]. When bred onto a C3 deficient background, APP mice had increased amyloid deposition at 17 mo of age (yet not earlier). Although CD45 microglia were increased, other indicators of M1 type activation (CD68, iNOS, TNF) were reduced, while markers of M2 like activation (IL-4 and IL-10) were elevated. These data would be consistent with an M1 activation state being associated with increased microglial clearance of A β . However, a third group manipulated the complement activation by using an inhibitor of the C5a receptor [33]. This caused a decrease in fibrillar A β , a reduction in microglial staining and a corresponding reduction CD45 staining of reactive cells. Furthermore, mice were rescued from a passive avoidance training deficit by the treatment. However, deletion of the C1q gene, the initiating factor in the classical complement pathway, in the Tg2576 mouse or APP/PS1 mouse had no impact on amyloid loads, although there was less microglial activation as the mice aged [34]. More recently, 3xTg mice (expressing both amyloid and tau pathology) with a deletion of the C1q gene had no alteration on the development of amyloid pathology or microglial activation (CD45, CD11b, Iba-1) [35].

In summary, four approaches to down-regulating complement generally succeeded in diminishing the microglial activation state (Table 2). However, the impact on amyloid deposition is mixed, with two approaches increasing deposition, one reducing and the other

without impact. This suggests the possibility that different components of the complement cascade may have opposing effects on amyloid deposition.

NSAID Drugs

NSAIDs are among the most commonly used small molecule drugs. Their long history of use and their overall efficacy in treating a range of maladies leads to frequent use by many, and occasional use by most of the population. Indeed, the apparent reduction in risk in those using these drugs from epidemiological studies is one of the three pillars supporting the inflammation hypothesis of Alzheimer's disease pathogenesis (see Introduction).

Based largely on this hypothesis, Greg Cole and Sally Frautschy tested the capacity of ibuprofen with a 6 mo treatment in Tg2576 APP mice, and found reduction in A β deposition and decreased phospho-tyrosine in activated microglia [36]. They further found reduced IL-1 β and GFAP (a marker for astrocyte activation). A subsequent paper reported that the ibuprofen treatment also reversed the excessive open field activity associated with the APP mouse phenotype, as well as reduced protein carbonyls [37]. Ibuprofen reduced A β deposition and production, and was associated with reduced microglial activation measured by CD45 or CD11b staining [38]. There was a further elevation of peroxisome-proliferation activating receptor gamma (PPAR-gamma), an anti-inflammatory transcription factor, although treatment with the PPAR-gamma agonist pioglitazone was without effect in this study. However, subsequent work with higher doses of these agents found significant amyloid lowering with pioglitazone and the related drug rosiglitazone, again, with reduced indications of microglial activation [39, 40].

A number of studies have largely confirmed these general findings of reduced A β , and reduced markers of glial activation and inflammation with this drug [41–43], and a similar outcome is observed with the COX-2 selective drug, celecoxib [44].

Our own work in this area examined a novel NSAID compound that was a combination of flurbiprofen, a nitric oxide generating group and a ferulic acid linker (NCX2216; [45]). The nitric acid group was included to reduce gastrointestinal tract toxicity and ferulic acid was included as an anti-oxidant. In head to head comparison, NCX-2216 was more efficacious than ibuprofen or celecoxib in lowering amyloid loads. Moreover, we found this agent had the unusual effect of increasing microglial activation near the few remaining plaques either measured by CD11b or MHC-2 immunostaining. A subsequent effort using a variant of NCX-2216 lacking ferulic acid, HCT-1026, also reported a reduced deposition of amyloid, but in this instance, the microglial activation was also reduced [46]. One possibility here is that the treatment reduced the amyloid deposits sufficiently that the stimulus for microglial activation (amyloid) was no longer present, hence reduced microglial activation (the treatment was from 8–14 mo). Using a measure that corrects for differences in plaque load may sometimes overcome this potential confound.

Curcumin, a major component of the curry spice tumeric, is diferulic acid. Treatment of rodents with this agent was reported to reduce amyloid loads and IL-1 in APP mice or rats infused with A β [47, 48]. Although this is associated with reduced IL-1, the microglia near the remaining deposits were activated to greater degree in the curcumin-treated mice. These

data suggest that ferulic acid can protect from amyloid toxicity [49] and can lead to activation of microglia in a form that may enhance the clearance of A β , without excess production of inflammatory cytokines. Unfortunately, complete characterization of the microglial phenotype produced by curcumin in APP mice has yet to be reported.

These NSAID data would superficially suggest that inflammation can promote amyloid deposition, and that NSAID drugs can reduce inflammation and thereby amyloid deposits. However, a key manuscript from Ed Koo and Todd Golde found that some of this action of NSAIDs, in particular ibuprofen, may be due to an action on gamma-secretase, rather than on cyclooxygenase (COX) activity [50]. They showed convincingly that high doses of some, but not all NSAIDs, could lead to a modulation of the gamma-secretase activity such that it biased APP C-terminal cleavage away from the A β 1–42 and towards the less amyloidogenic A β 1–38. The potency of NSAIDs in achieving this modulation of gamma-secretase did not equal their potency for COX inhibition. Moreover, the modulation of gamma-secretase was not stereoselective, and inactive enantiomers of chiral compounds which lacked COX inhibition were still effective gamma-secretase modulators. The most efficacious of these NSAIDs was flurbiprofen (as used in NCX-2216 above) [51]. Treatment of Tg2576 mice with the COX inactive form of this agent, R-flurbiprofen, resulted in reduction in A β deposits and rescue of the memory impairments [52]. This led to testing of R-flurbiprofen in human clinical trials (see below).

The identification that NSAID structures could modulate gamma-secretase cleavage sites led to development of a number of modifications of this scaffold to identify new drugs that share the modulation without possessing COX inhibition. Bruno Imbimbo has led one group working on these agents and has developed one compound, CHF5074, which, when administered to APP mice, leads to reduced A β deposition, reduced microglial activation and rescue of memory impairment [53]. Further work found that the agent could also restore neurogenesis, avoid reductions in synaptophysin staining and activate astrocytes (while still reducing microglial activation measured by CD11b) [54]. These effects are presumably due to actions at gamma-secretase, rather than on inflammation associated with microglial activation.

Thus, one would anticipate that NSAID drugs, given the epidemiology and the experimental data in mouse models, would be useful in the treatment of Alzheimer's disease. However, most trials have failed miserably. The first was that of Paul Aisen and Leon Thal [55]. This failure has been confirmed by Pasqualetti et al [56]. A similar failure was obtained using the more potent NSAID, indomethacin [57]. The R-flurbiprofen study similarly failed in phase 3, after some suggestive effects in phase 2, identified using stratification analysis [58]. Although the drug did enter the CNS, it is uncertain if it reached concentrations needed to modulate gamma secretase [59]. Even the ADAPT prevention trial, cut short after 2 years, suggested, if anything, an increased risk of memory decline in individuals randomly assigned to naproxen [60]. Consistent with this observation, a recent autopsy series study reports elevated amyloid plaque formation in participants that chronically used high dose NSAIDs [61]. A more recent analysis of these ADAPT trial subjects suggests the drugs may have some benefit once subjects stop taking them [61].

All of this leads to some confusion. The NSAID studies in mouse models support an amyloid lowering effect. Yet this may not be due to actions in reducing inflammation, but in modifying APP processing. An important caveat regarding glial activation in mouse models of amyloid deposition is that the increase in microglial activity found normally in APP mice is assumed to be related to the amount of A β present in one of several pools. This deposition is the presumed factor leading to activation. Thus, any treatment that reduces A β should be expected to result in less microglial activation, simply because the amount of activating stimulus is reduced. Thus, a direct action of NSAIDs on lowering A β production/deposition may lead to less microglial activity, not the other way around, as was the presumed rationale for the studies to begin with.

Summary. In general, studies with traditional NSAIDs are consistent with the prediction that reduced inflammation should reduce Alzheimer's disease (see table 3). However, the therapeutic clinical trials with NSAIDs failed...completely. Moreover, the prevention trial suggested the possibility of an increased risk. A recent pathology study reports elevated amyloid plaque formation in patients that used high dose NSAIDs [62]. However, the epidemiological data suggest those taking high dose NSAIDs for long time frames are protected, presumably because of lower inflammation both in the brain as well as the periphery. One alternative explanation is that individuals who use high dose NSAIDs for extended period have a physiological trait which leads to both increased use of NSAIDs and protects from development of Alzheimer's disease. In this sense the NSAID use is a surrogate marker for the trait. A tantalizing possibility in this regard is a recent paper from Park et al [63]. They found that inducing arthritis by collagen injections in an APP mouse model resulted in reduced amyloid deposition associated with increased activation of microglia measured by CD45 or Iba-1. They also found increased numbers of leptomeningeal and perivascular macrophages. This is consistent with the alternative hypothesis, that arthritis, because of a more active state of innate immunity, transfer some of this activation to the CNS where it assists in reducing the deposition of A β deposits. The use of NSAIDs in the human epidemiology studies merely identifies the cohort with this trait. Of course, all of the clinical data must be tempered by the possibility that once cognitive symptoms appear, the neuropathology of Alzheimer's disease has progressed to the point that it can no longer be managed by such agents.

Cytokines and Chemokines

Inflammation can be regulated by a series of secreted protein factors. Some attempt has been made to differentiate specific influences of these factors. With respect to activation of monocytic cells (such as microglia), one set of cytokines has been argued to induce a classical form of activation (M1, associated with bacterial infections; interferon [IFN]-gamma, TNF, IL-1, IL-6) while another set of cytokines has been argued to cause an alternative activation state (M2, often associated with parasitic infections; IL-4, IL-13) (reviewed in [64]). Recently a third state of 'deactivation' has been proposed, associated with TGF β and IL-10 [65]. Some have suggested in the periphery that classical activation may mediate the detrimental aspects of macrophage activation while the M2 condition might be considered a beneficial activation state. Such a conclusion has yet to be definitively demonstrated in the CNS.

IL-1 has been proposed to be a central mediator of the inflammation associated pathogenesis in Alzheimer's disease [66]. However, with respect to amyloid pathology in mouse models, this does not appear to be the case. Kerry O'Banion's group has shown that overexpression of IL-1 in the forebrain of APP mice leads to increases in astrocytic (GFAP) and microglial (Iba-1, MHC-2) activation, as well as increases in TNF and IL-6 expression, consistent with a classical M1 activation response [67]. These changes are associated with reductions in the A β loads in these mice by both histological and ELISA methods. Moreover, the few remaining plaques are more heavily invested with activated microglial than in mice lacking IL-1 overexpression. Importantly, this does not imply increased IL-1 signaling would help Alzheimer's patients, as such overexpression in nonAPP mice leads to spatial memory impairments [68, 69]. While this might suggest that inflammation promotes A β clearance through activation of phagocytosis, studies in vitro find that IL-1 can also shift the balance of APP processing away from β -secretase and towards α -secretase, thereby reducing A β production [70, 71].

Another M1 cytokine widely perceived as having detrimental influences on pathology is TNF. However, studies increasing TNF expression in APP mouse brain using viral transduction observe increased glial activation (Iba-1, MHC-2, GFAP) and reduced A β deposition [72]. Supporting this observation, breeding APP mice onto a background lacking TNF receptors results in reduced microglial activation, but increased A β pathology (as well as increased tau pathology in 3xTg mice)[73]. IFN-gamma overexpression mimics the effects of TNF overexpression in APP mice, with increased glial activation and reduced amyloid deposition [74]. This was further associated with increased infiltration of Ly6c positive cells and activation of the early components of the complement cascade. However, in the 3xTg mouse (with both A β and tau pathology) a slightly different outcome was observed, with increased IFN-gamma expression exacerbating A β , but benefiting tau pathology [75]. To confuse the picture a bit further, APP mice bred onto a background lacking the IFN-gamma receptor type 1 demonstrated reduced glial activation (Iba-1, GFAP) but also reduced amyloid deposition [76]. Finally, IL-6, another cytokine more associated with the M1 classical response, decreased amyloid deposition when overexpressed in an APP mouse model on a background of increased microglial activation [77], just like IL-1 and TNF.

Although less well examined, the M2 cytokines also appear to impact amyloid pathology. Viral transduction of IL-4 in APP mice leads to fewer astrocytes and microglia associated with amyloid plaques, but also fewer plaques and A β overall [78]. Moreover, this overexpression of IL-4 leads to rescue of neurogenesis in dentate gyrus and learning and memory performance in these mice. These overexpression studies have been replicated by direct injections of IL-4 with IL-13 into the brains of APP mice, which reduced intraneuronal A β [79]. This resulted in increased Iba-1, CD36 and CD68 staining of microglia, expression of YM1 and CD163, and increased expression of neprilysin.

One of the earliest indications of that microglial activation could reduce amyloid deposition was associated with transforming growth factor beta (TGF β) overexpression [80]. TGF β overexpression in the brains of APP mice led to reduced parenchymal amyloid deposits, and increased vascular deposits (with an overall net reduction in amyloid). This was associated

with increased staining for F4/80 in microglia, and increased expression of MIP-1 α , a microglial cytokine. More recently, IL-10 overexpression was associated with reduced microglial activation in APP mice, and rescued neurogenesis and learning and memory deficits in APP mice [81].

Peripheral macrophage activating factors have also shown some benefit in amyloid depositing mice. Injections of granulocyte colony stimulating factor (G-CSF) for 3 weeks reduced A β , improved memory, activated microglia, reduced inflammatory cytokines, and increased neurogenesis in an APP mouse model [82]. Similar results were obtained by another group that also included SDF1 α (a chemotactic recruitment agent [83]). A related protein, granulocyte-macrophage colony stimulating factor (GM-CSF), when administered centrally or peripherally, activated microglia and reduced amyloid deposition, while improving memory [84]. Conversely, another group found that centrally administering antibody to GM-CSF, inactivating the protein, also benefited APP mice, associated with increases in inflammatory cytokines and reduced intraneuronal A β [85].

Our studies injecting cytokine cocktails directly into the brain largely confirm the results found with overexpression using transgenesis or viral transduction. Figure 1 shows the effects of direct injection of a cytokine cocktail containing IL-1, TNF and IL-12 (CKT-1 to produce an M1 response), IL-4 plus IL-13 (CKT-2 to produce an M2 response) or vehicle into the frontal cortex of aged APP mice. Within 5 days, both cocktails caused a significant reduction in the diffuse amyloid deposits in the APP mice. This was evident with the immunohistochemical measurement of total A β or the A β ending at amino acid 42 (largely labeling diffuse and to a lesser degree compacted deposits; Figure 1B–G). However, immunostaining for A β ending at amino acid 40 or Congo red were largely unchanged (labeling compacted and vascular deposits; Table 5). This is qualitatively similar to the observations we made with LPS injections [15, 86], but unlike results with antibodies, where all forms of A β were rapidly cleared after central administration [87].

We also found activation of microglia with these treatments. We have identified a number of genes that are differentially activated by these cytokine cocktails using microarray expression analysis in nontransgenic mice. Surprisingly, YM1, used widely as a marker of the alternative activation phenotype, is significantly increased using both the M1 and the M2 cocktail. YM1 was also elevated by both cocktails in aged APP mice (Figure 2). Whether this reflects a lack of specificity of the cocktails, or the failure of these peripheral markers to accurately define a specific microglial phenotype in the CNS, is unclear. However, the results certainly confirm that exposure to either M1 cytokines or M2 cytokines can result in reductions in amyloid pathology in APP mice.

The effects found here both in terms of A β clearance and microglial activation were restricted to the region in the vicinity of the injection. There was not a brain-wide activation caused by these injections. We also used aged mice in these studies to mimic the circumstances found in Alzheimer's patients. Younger mice might be expected to reveal less activation, at least for the M1 cytokines. It is uncertain how long these effects might persist. However, in aged rats we found that glial activation in old animals persisted considerably longer than they did in young animals [88].

In conclusion, data from the many studies of the impact of cytokine overexpression or deletion of receptors are generally consistent with the suggestion that A β deposits can be reduced when microglia are activated (Table 5). The general perception that the phenotype of the activated cell is critical does not appear well supported, at least along the M1-M2 continuum. It appears that many forms of activation can enhance microglial responses sufficiently to engage in clearance of A β deposits.

Antibody Administration

Although not specifically intended to cause effects on microglia, administration of antibodies against the A β peptide do have profound impact on the microglial phenotypes found in APP mice [89]. There is considerable evidence that one mechanism by which some antibodies act to clear amyloid is to modify the microglial phenotype into one that is more adept at phagocytizing deposited A β [90]. This was first suggested by Schenk et al [91] in their description of the first active immunization study in mice. Subsequently, Bard et al [92] demonstrated that Alzheimer brain amyloid deposits could be phagocytized by microglia if they were first opsonized with antibody, but not in the absence of this opsonization. This same mechanism has been shown for another antibody in clinical testing, gantenerumab [93].

Our work supports the contention that some actions of antibodies require microglial engagement. We found that use of dexamethasone (but not NSAIDs or minocycline) to suppress microglia, partially blocked the ability of antibodies to clear amyloid, in particular the compacted forms of amyloid [94]. The same studies showed that deleting the Fc domain, negating the ability of antibodies to bind Fc receptors on microglia or activate complement, also partially blocked the ability of antibodies to clear brain amyloid. In other studies, we found that deglycosylating antibodies, which decreases their affinity for Fc receptors, also diminished, but did not eliminate, the ability of antibodies to clear amyloid deposits [95]. This deglycosylation also blunted the increase in vascular amyloid seen in aged APP mice treated with antibodies, suggesting that microglia were at least partially responsible for this result too (similar to the effects of TGF β treatment above).

The changes in microglial phenotype after antibody administration are complex, with some markers increasing in expression and others declining over time after the initiation of therapy [64]. Some of this probably results from the signaling caused by effector molecule activation, and other changes may result from declines in the provoking stimulus as A β is cleared. Recent work from Donna Wilcock and Carol Colton [96] has shown that prior to administration of antibody, the APP mouse brain has elevated expression of M2 markers (YM-1, arginase) and normal expression levels of M1 markers (TNF, IL-6) compared to age-matched nontransgenic mice. Antibody administration resulted in an elevation of the M1 markers TNF and IL-6, and a decline in the M2 markers YM-1 and arginase. It should also be noted these were older mice (treated from 19–22 mo during the study). There is discussion that the APP mice transition their microglial phenotype with age [97].

Related to this are studies showing that nonspecific activation of innate immunity can also reduce amyloid deposits. Administration of glatiramer acetate and a proteosome adjuvant

alone can decrease amyloid deposition on an APP mouse model [98]. The administration of the proteasome adjuvant alone was further shown to reduce amyloid deposits and improve behavioral performance in 24 mo old mice as well, demonstrating that this benefit is not lost in mice of advanced age [99].

Impact of microglial activation on tau pathology

Recently, several studies have examined tau-depositing mice using the same manipulations of microglia as used for amyloid-depositing mice. The neuronal protein fractalkine (CX3CL1) is released from neurons and suppresses microglial activation. Deletion of the receptor for fractalkine (CX3CR1) results in activation of microglia, because they are released from this tonic suppression. Two studies examined the effects of deleting the fractalkine receptor in APP mice and found reduction in amyloid in the mice lacking fractalkine signaling [100, 101]. Both manuscripts also indicate that the microglia lacking fractalkine receptors are more capable of phagocytizing various pools of A β . A surprising discrepancy in microglial activation is reported between these two studies. One manuscript observes an increased number of microglia per remaining plaque, while the other describes a decreased number. In both cases, the microglial marker was Iba-1.

Recently, the effects of deleting the fractalkine receptor were examined in a model of tau deposition. This resulted in the opposite effect; tau pathology was exacerbated by the deletion of the fractalkine receptor [102]. Our group has examined the effect of virally transducing neurons with fractalkine in a tau deposition model (Nash et al, 2012; in press). We find that increasing fractalkine receptor signaling reduces tau pathology in the Tg4510 model, consistent with the increased pathology found in the absence of fractalkine signaling. Interestingly, we do not find any change in amyloid pathology when transducing an APP mouse to overexpress fractalkine.

In the sections above, it was found that acute LPS administration and overexpression of IL-1 both benefited APP mice with reductions in amyloid deposits. However, as for fractalkine manipulations, these exact same manipulations in tau models have the opposite effect. LPS administration caused a dramatic increase in tau pathology in the Tg4510 mouse [103]. This exacerbation of tau pathology was also observed with systemic administration of LPS in the 3xTg mouse (with no effects on amyloid) [104]. Similarly, IL-1 overexpression in the forebrain of a 3xTg mouse model resulted in increased tau deposition [105]. This is consistent with earlier work in vitro showing that IL-1 promoted phosphorylation of tau [106, 107].

However, not all forms of microglial activation appear to accelerate tau deposition. Oddo et al [108] injected anti-A β antibodies into a triple transgenic mouse with both amyloid and tau pathology. They noted that the antibody led to microglial activation, reduction of amyloid pathology and diminution of early stage tau pathology (but not late stage). One option is that the activation phenotype induced by the antibody binding to antigen, does not exacerbate tau pathology. However, this would appear to be a classical activation phenotype [96], including IL-1 and TNF-alpha elevations. Additionally, it was recently demonstrated that formation of an immune complex in the CNS (against injected ovalbumin) causes microglial activation

and appears to transiently inhibit neuronal activity (assessed by rotational behavior) [109]. An alternative explanation of Oddo et al is that the reduced tau pathology resulted from diminished amyloid, and was independent of microglial activation caused by the antibody injection. Although the triple transgenic model has many advantages, the complex interplay between amyloid and tau pathology in this mouse makes identification of direct actions of agents upon tau difficult to interpret.

These results demonstrate the challenges of translating observations in mouse models to clinical testing. Available models are not true models of Alzheimer's disease, as widely and incorrectly implied in the scientific literature. Instead they model very select aspects of the pathology. The models can be useful and predictive regarding amyloid or tau deposition. However, none of the models reflects the full spectrum of changes in the Alzheimer brain. Moreover, most studies don't even study aged animals. It is clear that some effects found in aged mice and men are not present in younger mice [110]. Thus, until we recognize the limitations of any single model, and fully test potential therapeutic agents in the range of models available, including aged organisms, the predictability of outcomes in human trials will continue to suffer.

Conclusions

Although the data are not wholly consistent, in general, many routes of microglial activation appear capable of mobilizing microglia to assist in the process of clearing the A β peptide and reducing amyloid deposition. Importantly, many of the approaches that produce a pro-inflammatory state (M1 activation) have not examined behavioral outcomes. Because treatments like IL-1 overexpression and LPS administration are known to cause memory impairments in nontransgenic animals [69, 111], it seems unlikely that APP mice, much less Alzheimer's patients, will benefit from these treatments. This may not be an issue for the M2-activating treatments, as memory enhancements were obtained [78]. Thus, this review does not conclude that we should inject Alzheimer's patients with LPS or IL-1, even it were to benefit amyloid pathology.

Another limitation of the rodent literature on experimental manipulation of microglial is that investigators do not always include both chemical and histological measurement of the amyloid. As methods for measuring soluble variants of A β emerge, it will be important to note if the loss of histological amyloid might be associated with increased soluble forms of the peptide, that may cause harm, rather than relieving the brain of an irritant. In addition, more attention needs to be paid to the age of the animals being used. Chronological age is the greatest risk factor for Alzheimer's disease. Age also has dramatic impacts on both innate and adaptive immunity. Clearly studies attempting to model the role of innate immune activation in Alzheimer's pathogenesis need to consider the context and more work needs to be performed in aged animals.

One of the most important recent observations involves the opposite effects of these treatments on amyloid versus tau pathology. Without exception (albeit few manipulations have yet been attempted), the proinflammatory state has exacerbated tau pathology. Hence,

manipulations hoping to exploit the amyloid-clearing capability of activated microglia may be compromised by opposing actions on tau pathology.

Although premature based upon the existing data, it is tempting to speculate that one mechanism by which amyloid provokes tau pathology is through inducement of microglial activation. This could be a result of oligomeric A β aggregates or compacted amyloid deposits activating adjacent microglia or infiltrating macrophages. This may in turn lead to changes in tau localization or post-translational modification leading to aggregation and deposition. Understanding what factors are released by microglia that cause this exacerbation of tau pathology may suggest new drugs targeting the development of tau pathology, independent of amyloid pathology. It could well be that the pathogenic influence of inflammation in Alzheimer's is not on amyloid pathology, but instead on acceleration of tau pathology, leading to neurodegeneration and ultimately, dementia. In this context, the recent identification of self-propagating tau pathology in a prion like manner [112, 113] suggests that at a certain stage of disease development, alleviation of amyloid deposition and/or microglial activation may fail to slow the course of the disease. This highlights the need for early identification of those at risk, and aggressive treatment with agents that might break the cascade of events leading to neurodegeneration and brain atrophy in Alzheimer's dementia.

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Figure 1.

Effects of M1 or M2 cytokine cocktails on amyloid deposition in 22 mo old APP mice. Methods. APP Tg2576 mice on a mixed genetic background from our breeding colony [11] aged 22 mo were injected (unilaterally) with 2 μ l of either a) cytokine cocktail [CKT-1: M1 cocktail containing TNF- α (333ng), IL-12 (13ng) and IL-1 β (13ng) total mass, with b) CKT-2: M2 cytokine cocktail containing IL-4 (400ng) and IL-13 (120ng total mass), or with c) vehicle control (phosphate-buffered saline). Mice were injected into the right anterior cortex using convection enhanced delivery as described previously [114]. Five days post

cytokine injection, mice were intracardially perfused with 25 ml of 0.9% saline. The brain was removed, immersed in 4% paraformaldehyde in 100 mM PO₄ buffer (pH 7.4) for 24 hours. The tissue was cryoprotected in a series of 10%, 20% and 30% sucrose solutions. Horizontal sections were cut at 25 µm using a sliding microtome and processed for floating section immunocytochemistry as described previously [115]. Primary antibodies used were rat anti-mouse CD45, rat anti-CD68 (AbD Serotec, Raleigh, NC), rabbit anti-mouse chitinase 3-like-3 (YM1; StemCell Technologies, Vancouver, Canada), rabbit anti-Aβ (Total anti serum, gifted by Paul Gottschall), rabbit anti-Aβ 40, rabbit anti-Aβ 42 (Biosource/Invitrogen, Grand Island, NY). Immunoreaction product in the anterior cortex was quantified as described previously [115]. Sample size was 7–8 per group. Panel A indicates the region quantified by image analysis in the horizontal brain sections. Panels B, D and F show immunostaining using a polyclonal anti-Aβ antibody and panels C, E and G show immunostaining for an antibody specific for Aβ ending at position 42. Rows specify the treatment used. Panels H and I show the quantification of the Aβ staining for total and Aβ₄₂ respectively, measured as the ratio of the injected to the uninjected side. Data presented are mean ± sem. * indicate $P < 0.05$ compared to vehicle treated mice. ** indicates $P < 0.01$.

Figure 2.

Microglial activation after injection of M1 or M2 cocktails in 22mo old APP mice. Methods as in Fig 1. Panels A, C, and E are stained for CD-68. Panels B, D and F are stained for YM1. Rows specify the treatment conditions. Values are mean $\pm$ sem of the immunostained area for each marker in panels G and H.

Table 1

Manipulations affecting toll-like receptor signaling in amyloid deposition models

Experimental Manipulation	Plaque Microglial Response	Effect on Amyloid Load
Acute LPS	↑	↓
Chronic LPS	↑	↑
TLR4 null	↓	↑
CD14 null	↓	↓
CpG (TLR9 agonism)	↑	↓
MyD88 null	↓	↓

See text for references and detailed explanation.

Table 2

Manipulations affecting complement activation in amyloid deposition models

Experimental Manipulation	Plaque Microglial Response	Effect on Amyloid Load
sCrry (complement inhibitor)	↓	↑
Complement C3 null	↑↓	↑
C5a receptor inhibitor	↓	↓
Complement C1q null	↓	no change

Table 3

NSAID treatment in mouse models of amyloid deposition

Experimental Manipulation	Plaque Microglial Response	Effect on Amyloid Load
Ibuprofen	↓	↓
PPAR-gamma agonist	↓	↓
NCX-2216 (nitroNSAID)	↑	↓
curcumin	↑	↓
secretase modulator	↑	↓
induced arthritis	↑	↓

Table 4

Effects of injecting cytokine cocktails to produce M1 or M2 activation of microglia in APP mice

Injection	A β 40	Congophilic Deposits	CD45
	Ipsi/Contra Ratio	Ipsi/Contra Ratio	% Area
Vehicle	1.42 $\pm$ 0.17	1.21 $\pm$ 0.22	1.08 $\pm$ 0.42
CKT-1	1.11 $\pm$ 0.16	0.86 $\pm$ 0.07	1.85 $\pm$ 0.62
CKT-2	1.35 $\pm$ 0.15	0.95 $\pm$ 0.21	1.02 $\pm$ 0.23

No differences were significant statistically. See text and figure captions for description of study.

Values are mean $\pm$ sem.

Table 5

Manipulation of cytokine signaling in models of amyloid deposition

Experimental Manipulation	Plaque Microglial Response	Effect on Amyloid Load
IL-1 increase	↑	↓
TNF increase	↑	↓
TNF receptor null	↓	↑
IFN-gamma increase	↑	↓
IFN receptor null	↓	↓
IL-6 increase	↑	↓
IL-4 increase	↑	↓
TGF-beta increase	↑	↓
G-CSF increase	↑	↓
GM-CSF increase	↑	↓

Table 6

Manipulations of microglia differentially impacting amyloid versus tau deposits.

Manipulation	Microglial Activation	Amyloid Deposits	Tau Deposits
Fractalkine-R null	↑	↓	↑
Fractalkine increase	↓	no change	↓
LPS	↑	↓	↑
IL-1 increase	↑	↓	↑