Assessing the contribution of inflammation in models of Alzheimer’s disease

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Abstract
Inflammation has long been proposed as having a role in AD (Alzheimer’s disease), although it remains unclear whether inflammation represents a cause or consequence of AD. Evidence from the clinical setting in support of a role for inflammation in AD includes increased expression of inflammatory mediators and microglial activation in the post-mortem AD brain. Also, epidemiological studies on AD patients under long-term treatment with non-steroidal anti-inflammatory drugs suggest some benefits, although recent prospective trials showed no effect. Furthermore, in AD patients, infection and other systemic inflammatory events worsen symptoms. Finally, several inflammatory genes are associated with increased risk of AD. Therefore, to elucidate the underlying mechanisms of AD and the role of inflammation, researchers have turned to experimental models and here we present a short overview of some key findings from these studies. Activation of microglia is seen in various transgenic models of AD, with both a protective role and a detrimental role being ascribed to it. Early microglial activation is probably beneficial in AD, through phagocytosis of amyloid β-peptide. At later stages however, pro-inflammatory cytokine release from microglia could contribute to neuronal demise. A better understanding of microglial phenotype at the various stages of AD is therefore still required. Although most studies suggest a detrimental role for pro-inflammatory cytokines such as interleukin-1 and tumour necrosis factor in AD, contradictory findings do exist. Age-related and differential cellular expression of these inflammatory mediators is probably a key determinant of their exact contribution to AD. In conclusion, there is no doubt that inflammatory processes are part of the pathophysiology of AD, but a better understanding of the exact contribution at different stages of the disease process is still required before appropriate treatment strategies can be devised.

Introduction
AD (Alzheimer’s disease) is a common neurodegenerative disease, primarily affecting the elderly population. AD accounts for 50–70% of dementia patients and is characterized by impaired memory, visuo-spatial skills and complex cognition and changes in emotion and personality. Post-mortem analysis of AD patient brains reveals the now well accepted pathology of extracellular Aβ (amyloid β-peptide) plaques and intracellular hyper-phosphorylated tau protein NFTs (neurofibrillary tangles), as well as neuronal loss [1]. However, Aβ plaques and NFTs alone do not seem sufficient to explain all the features of AD, as the presence of abnormally high levels of Aβ plaques is also observed in control (i.e. non-demented) subjects [2]. Furthermore, recent clinical trials found no improvement in cognitive function in patients immunized against Aβ, despite evidence of plaque clearance on the brains post mortem [3]. Also, animal models of AD do not show significant neurodegenerative changes despite high levels of expression of Aβ and/or tau protein and behavioural deficits. These findings suggest that other factors besides Aβ plaques and NFTs are also required for AD to develop fully.

Inflammation has been highlighted as one possible key contributing factor to AD, and classic hallmarks of neuroinflammation, such as microglial activation, activation of the complement system and increased cytokine expression, are all observed in the tissue of AD patients [4]. In addition, retrospective studies of individuals under long-term treatment with NSAIDs (non-steroidal anti-inflammatory drugs) have demonstrated delayed onset and reduced severity of AD symptoms, although recent prospective trials have failed to confirm this [5]. Recent genome-wide association studies have also identified some inflammatory genes as having a role in AD [6].

However, despite the wide acceptance of the fact that inflammation contributes to AD, what remains ambiguous is how different inflammatory components interact and at what stages of AD, inflammation is beneficial or detrimental [7]. We therefore need a better understanding of inflammatory pathways and mechanisms in AD, which has led to the use of animal models of AD, particularly transgenic mice.

Animal models and AD
The most commonly used AD models are mice genetically modified to express genes associated with familial AD or
Inflammation and AD

Neuroinflammatory changes, such as microglial activation and cytokine expression, have been demonstrated in AD patients both post mortem [13] and in vivo [14]. However, the temporal profile and precise contribution of neuroinflammatory changes to AD remain to be determined, evidence being available for both beneficial and detrimental effects. As in AD patients, many mouse models of AD do show increased neuroinflammation, although typically this is at later ages and correlates with increased levels of Aβ deposition [15]. However, very few experimental studies have examined in detail the full temporal and spatial profile of neuroinflammation and Aβ pathology together in vivo [16].

Microglia: friend or foe?

Microglia are mononuclear phagocytes that patrol the brain for pathogens and show high levels of activation in the AD brain. Microglia can release a combination of both pro- and anti-inflammatory cytokines when activated, including IL-1 (interleukin-1), IFNγ (interferon γ), TNF (tumour necrosis factor) and IL-6 [17].

Although it is widely accepted that activated microglial cells surround Aβ plaques in both human AD and transgenic AD mouse brains, the exact contribution of microglia in the formation of plaque pathology is still unknown. Early studies mostly proposed a detrimental role for microglia in AD, but more recent work suggests it is more complex than this, with microglia being proposed as neuroprotective [18]. Clearly, before microglia can be considered a viable therapeutic target for AD, their exact role in the different stages of the disease needs to be resolved. Overall, there is some consensus that moderate activation of glia might be beneficial, through an increase in Aβ clearance [19,20], whereas strong activation of microglial cells might slow down their ability to clear Aβ, increase production of detrimental pro-inflammatory cytokines and accelerate neuronal damage and cognitive decline [21] (Figure 1). A decreasing phagocytic ability of microglia is linked to cytokine release, which acts as a feedback mechanism, leading to a decrease in Aβ-binding scavenger receptors and Aβ-degrading enzymes in microglia [22]. Old APP/PS1 mice, for example, show a 2–5-fold decrease in levels of scavenging receptor A, CD36 and receptor for advanced glycosylation end-products and the proteolytic enzymes insulin, neprilysin and matrix metallopeinase-9, which coincides with an increase in IL-1 and TNF release [22]. Unfortunately the chronic nature of AD means that reduced microglial phagocytosis results in a worsening of AD pathology as microglia become overwhelmed and contribute to the Aβ deposition. The hypothesis that increased cytokine release contributes to AD progression fits well with observations that AD patients deteriorate faster after acute infections [23,24].

Recent studies with CX3CR1-deficient mice reveal further complexity with regard to the role of microglia in AD, in that opposing effects on Aβ and tau pathology are observed. CX3CR1 is the receptor for CX3CL1 (fractalkine) and is specifically expressed on microglia, allowing for neuronal–glial communication [25]. Transgenic mice expressing human tau protein crossed with other dementias, namely PSs (presenilins), APP (amyloid precursor protein) or tau protein [8]. Numerous strains of transgenic AD mice have been generated and the discussion of the relative merits and caveats of each of these is beyond the scope of the present review, but is dealt with in detail elsewhere [8,9]. Although transgenic mice show some features of human AD, they do not recapitulate all aspects of the disease, and in particular show only moderate, if any, neuronal loss. This is in stark contrast with the widespread neurodegeneration seen in human AD. Therefore one has to take findings from transgenic AD mice in context, especially since inflammation and cell death are closely linked in many neurodegenerative conditions [10]. It is possible that transgenic AD mice are more representative of asymptomatic AD [9].

More recently, transgenic rat models of AD that overexpress the mutated human proteins APP or tau protein have become available. As with transgenic mouse models of AD, the AD rats present with hallmark features of AD pathophysiology, including amyloid plaques or NFTs, microglial activation and behavioural deficit [11,12].

Therefore, although no single animal model perfectly reproduces all aspects of AD pathogenesis, they still present key features of the human disease and can be used to study underlying processes and possible treatments of AD.

Figure 1 | Schematic diagram illustrating the possible contribution of inflammation to AD pathophysiology

Right-pointing arrows indicate factors that worsen outcome, whereas the left-pointing arrow is beneficial. The overall outcome in AD is therefore a balance between these factors.

Peripheral inflammation

Neuroinflammation

Clearance of Aβ by gial cells

Aβ pathology

Healthy brain

AD Pathophysiology and cognitive deficit
CX3CR1-deficient mice show enhanced tau protein phosphorylation and behavioural impairments [26]. In contrast, reduced Aβ deposition is observed when CX3CR1-deficient mice are crossed with AD transgenics, an effect attributed to changes in the phagocytic capability of the microglia [27]. Such opposing effects of inflammation are supported by the finding that the widely used inflammatory stimulus, bacterial LPS (lipopolysaccharide), worsens tau protein pathology [28,29] while reducing Aβ load in transgenic AD mice [30]. However, contrasting effects of LPS on Aβ pathology have also been described in [31], and therefore one has to interpret studies with LPS cautiously, effects being dependent on the dose and route of administration.

Ultimately, the exact role of microglia in AD probably depends on their functional status, and further investigation is required into the precise phenotype of microglia at different stages of the disease [32].

Cytokines: good and bad?
The precise role of cytokines in AD remains to be fully determined. This is best illustrated by the pro-inflammatory cytokine IL-1, for which both detrimental and beneficial effects have been reported. Earlier clinical studies indirectly show that IL-1 may contribute to AD pathophysiology [33]. Similarly, deletion of the IL-1Ra (IL-1 receptor antagonist) gene in mice leads to increased susceptibility to intra-hippocampal injection of Aβ with increased neuroinflammation and loss of synaptic markers [34]. Conversely, transgenic overexpression of IL-1β chronically in mice reduces Aβ fibrillar plaques [35]. This dual role of IL-1 is mirrored by its effects on LTP (long-term potentiation), a process proposed as an underlying basis for learning and memory. At physiological levels, IL-1 is required for LTP, while at higher pathophysiological amounts, IL-1 is detrimental [36]. Therefore, although extensive data exist that show IL-1 as a key mediator of acute brain injury [37], in chronic neurodegenerative diseases such as AD, its contribution is more complex [38].

IFNγ is a well-studied cytokine in neuroinflammation, but unfortunately it is also one of the most ambiguous. IFNγ leads to the infiltration of T-cells and monocytes from the periphery into the cerebral space, without compromising the integrity of the BBB (blood–brain barrier) [39]. Monocyte invasion leads to a reduction in Aβ plaques but only if invasion occurs at an earlier stage than typically seen in untreated APP transgenic mice. Conversely, it has also been shown that IFNγ may contribute to Aβ plaque formation, deletion of the IFNγ receptor in APP transgenic mice leading to decreased plaque deposition and increased plaque degradation, an effect attributed to a decrease in BACE-1 (β-site amyloid precursor protein-cleaving enzyme 1; β-secretase-1) expression [40]. BACE-1 is an enzyme important in the processing of APP to Aβ42, which is neurotoxic, and low levels of BACE-1 are seen in APP/IFNγ receptor-deficient mice compared with the APP transgenic alone. IFNγ can enhance TNFα release from mixed glia, and both cytokines are able to enhance Aβ production [40].

Increased levels of TNFα (through adenovirus) in the triple transgenic AD mouse result in increased tau protein hyperphosphorylation, intra-neuronal Aβ deposits and also neuronal cell death [41]. The detrimental role of TNFα in AD is further supported by the fact that inhibition of TNF by various means reduces AD-like pathology and behaviour in transgenic AD mice [42,43]. TNFα also negatively feeds back on microglial clearance of Aβ plaques and has been implicated in the poor efficiency of microglia in late stage AD by down-regulation of receptors and enzymes on the microglia [22]. Interestingly, worsening of cognitive decline in AD patients is associated with high baseline levels of TNFα [23].

IL-6 is a cytokine that could play a dual role in AD: both protective and detrimental. Recent papers have indicated that IL-6 is associated with plaque clearance [44], while others have shown that IL-6 expression in APP transgenic mice leads to increased neurodegeneration and reduced learning ability [45]. The reasons for this discrepancy are unclear but they may be age-related since beneficial actions of IL-6 appear to occur early in the disease process, whereas negative consequences of IL-6 occur in aged animals. This highlights the importance of considering age when interpreting data from any AD transgenic study.

Immune cell infiltration
One of the most common effects of increased cytokine release is the infiltration of peripheral bone marrow-derived cells into sites of Aβ plaques. This immune cell invasion is due to the chemoattractant cytokines (chemokines) and increased permeability of the BBB [46]. Once at the site of inflammation, these infiltrating monocytes are more effective in phagocytosing Aβ plaque deposits than resident microglia, since they invade the plaque core and lead to decreased plaque sizes and levels [47]. Since resident microglia become ineffective at phagocytosis owing to cytokine feedback, it may be that the bone marrow-derived monocytes have not been exposed to this environment and thus maintain their phagocytic capability. Thus, for the treatment of AD, one attractive strategy might be to increase the number of blood-derived monocytes in the brain [48].

Concluding remarks
The conflicting reports on the role of neuroinflammatory mediators in AD can be explained in part by the differences in study design. A number of transgenic AD models are used, which show wide variation in the age of onset and severity of AD-like pathology and behavioural deficits. Therefore, since the age of animals and phenotype severity vary widely, this can complicate the interpretation of data and render comparisons between studies difficult.
Several risk factors for AD are associated with inflammation, and AD patients show increased cognitive decline with infection [23, 49]. Generally speaking, experimental studies in transgenic AD mice have not taken this into account. It is therefore possible that a better understanding of the contribution of inflammation to AD progression will be obtained from those experimental studies in transgenic AD mice that also include co-morbid factors that increase the systemic inflammatory drive, such as obesity, infection and atherosclerosis.

Ultimately, completely inhibiting inflammatory responses is probably not the best strategy in treating AD, since the studies described above clearly show that inflammation is a double-edged sword. In the early stages of AD, neuroinflammation appears to be beneficial in slowing disease progression and reducing severity; thus increasing the effectiveness at this stage could be an excellent preventive measure. Conversely, in the later stages of AD, chronic inflammation is detrimental to neuronal function and needs to be brought under control. Future strategies will require sensitive manipulation of the inflammatory process if indeed it is to be an effective target for AD treatment.

References


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