A state of delirium: Deciphering the effect of inflammation on tau pathology in Alzheimer's disease

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Abstract

Alzheimer’s disease (AD), the predominant form of dementia, is highly correlated with the abnormal hyperphosphorylation and aggregation of tau. Immune responses are key drivers of AD and how they contribute to tau pathology in human disease remains largely unknown. This review summarises current knowledge on the association between inflammatory processes and tau pathology. While, preclinical evidence suggests that inflammation can indeed induce tau hyperphosphorylation at both pre- and post-tangles epitopes, a better understanding of whether this develops into advanced pathological features such as neurofibrillary tangles is needed. Microglial cells, the immune phagocytes in the central nervous system, appear to play a key role in regulating tau pathology, but the underlying mechanisms are not fully understood. Their activation can be detrimental via the secretion of pro-inflammatory mediators, particularly interleukin-1β, but also potentially beneficial through phagocytosis of extracellular toxic tau oligomers. Nevertheless, anti-inflammatory treatments in animal models were found protective, but whether or not they affect microglial phagocytosis of tau species is unknown. However, one major challenge to our understanding of the role of inflammation in the progression of tau pathology is the preclinical models used to address this question. They mostly rely on the use of septic doses of lipopolysaccharide that do not reflect the inflammatory conditions experienced AD patients, questioning whether the impact of inflammation on tau pathology in these models is dose-dependent and relevant to the human disease. The use of more translational models of inflammation corroborated with verification in clinical investigations are necessary to progress our understanding of the interplay between inflammation and tau pathology.

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1. Introduction

Alzheimer’s disease (AD), the predominant form of dementia, is highly correlated with the abnormal hyperphosphorylation and aggregation of tau. Immune responses are key drivers of AD and how they contribute to tau pathology in human disease remains largely unknown. This review summarises current knowledge on the association between inflammatory processes and tau pathology. While, preclinical evidence suggests that inflammation can indeed induce tau hyperphosphorylation at both pre- and post-tangles epitopes, a better understanding of whether this develops into advanced pathological features such as neurofibrillary tangles is needed. Microglial cells, the immune phagocytes in the central nervous system, appear to play a key role in regulating tau pathology, but the underlying mechanisms are not fully understood. Their activation can be detrimental via the secretion of pro-inflammatory mediators, particularly interleukin-1β, but also potentially beneficial through phagocytosis of extracellular toxic tau oligomers. Nevertheless, anti-inflammatory treatments in animal models were found protective, but whether or not they affect microglial phagocytosis of tau species is unknown. However, one major challenge to our understanding of the role of inflammation in the progression of tau pathology is the preclinical models used to address this question. They mostly rely on the use of septic doses of lipopolysaccharide that do not reflect the inflammatory conditions experienced AD patients, questioning whether the impact of inflammation on tau pathology in these models is dose-dependent and relevant to the human disease. The use of more translational models of inflammation corroborated with verification in clinical investigations are necessary to progress our understanding of the interplay between inflammation and tau pathology.

Inflammation is considered a key mechanistic driver in AD where both Aβ plaques and NFT co-localize with microglia and astrocytes, the resident immune cells of the brain (Serrano-Pozo et al., 2011). Genome wide association studies (GWAS) suggest a strong association between AD and genes involved in the regulation of immunological function (Lambert et al., 2013), whereas epidemiological studies have revealed a reduced risk of developing the disease following long-term anti-inflammatory treatments (Vlad et al., 2008) and disease-exacerbating effects of infectious agents (Honjo et al., 2009). Corroborating these observations, pro-inflammatory stimuli have been shown to induce both amyloid and tau pathologies in animal models (Zilka et al., 2012).

To date, several randomised trials of anti-inflammatory agents have been conducted in AD. Despite the use of multiple types anti-inflammatory treatments, whether non-steroidal anti-inflammatory drugs (NSAID) or steroidal, all have failed to demonstrate clear clinical efficacy in AD patients (Jaturapatporn et al., 2012). Pathogenesis in AD develops years prior to symptom manifestation, and therefore, anti-inflammatory agents were suggested to be beneficial when administered.
2 Inflammation induces tau phosphorylation

Table 1 summarises the outcomes of studies assessing the effect of pro-inflammatory stimuli on tau pathology.

2.1. Systemic immune stimuli induce neuroinflammation

The majority of our understanding for the role played by inflammation on tau pathology relies on the use of systemic immune challenges, and particularly of the toll-like receptor 4 (TLR-4) agonist; lipopolysaccharide (LPS) which fails to cross the blood brain barrier (Banks and Robinson, 2010), thereby mimicking systemic infections. LPS nevertheless induces central inflammatory responses through a variety of including neural routes such as vagal afferents, humoral routes through circumventricular organs, infiltration of peripheral monocytes and through effects on brain endothelial cells (Miller and Raison, 2016; Pardon, 2015), and as such can affect tau pathology.

2.2. Inflammation induces tau phosphorylation in tau models

The first direct evidence for a role of inflammation in exacerbating tau pathology stemmed from in vitro studies with primary microglial cells stimulated with Aβ or LPS prior to being co-cultured with primary neocortical neurons (Li et al., 2003). This landmark study showed that secretion of the pro-inflammatory cytokine interleukin-1β (IL-1β) by microglial stimulation causes an increase in tau phosphorylation through activation of p38-mitogen-activated protein kinases (MAPK). This has been confirmed in vivo predominantly using the 3xTg model which exhibits both tau and amyloid pathologies (Oddo et al., 2003). A chronic treatment regimen with LPS (0.5 mg/kg twice a week for 6 weeks) triggered tau hyperphosphorylation at multiple phosphorylation sites associated with both pre- and post-tangle tau pathology in 3xTg mice, and at both early and advanced pathological stages (Kitazawa et al., 2005; Sy et al., 2011). Again, microglial activation and resulting secretion of IL-1β were implicated, via activation of either cyclin dependent kinase-5 (CDK-5) (Kitazawa et al., 2005) or glycogen synthase kinase-3β (GSK-3β) (Sy et al., 2011). The discrepancy in the kinases involved is likely due to differences in age and pathological conditions (Breitner et al., 2013) a recent updated systematic review still argues in favor of their use for prevention of AD (Wang et al., 2015).

The mechanisms underlying the involvement of immune responses in AD pathogenesis remain poorly understood because inflammation has both beneficial and detrimental effects which can be very context dependent (Heneka et al. 2016). Aβ pathology is exacerbated through the induction of pro-inflammatory mediators secreted from active immune cells such as microglia (Brugg et al., 1995) but conversely, activation of these cells can stimulate clearance of Aβ plaques via induction of phagocytosis (Fiala et al., 2007), demonstrating a dual role for inflammation on amyloid pathology. Less is known about the specific role of inflammatory processes on tau pathology, and to our knowledge, the effect of anti-inflammatory treatments on tau pathogenesis in humans is unknown. In preclinical models inflammation is generally seen as an exacerbating factor (Zilka et al., 2012) but recent data suggest that it may be beneficial as well (Majerova et al., 2014). Here, we will review the preclinical findings to shed light on the interplay between inflammation and tau pathology in AD.

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Bold indicates tau phosphorylation epitopes associated with post-tangle pathology (Augustinack et al., 2002).

Table 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Challenge</th>
<th>Time to cull</th>
<th>Effect on tau</th>
<th>Kinases implicated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary neuronal and microglia cultures</td>
<td>LPS (30 ng/ml)</td>
<td>n/a</td>
<td>Tau phosphorylation (epitope not specified)</td>
<td>↑ p38 MAPK</td>
<td>Li et al. (2003)</td>
</tr>
<tr>
<td>Primary neuronal and microglia cultures</td>
<td>IL-1β (30 ng/ml)</td>
<td>n/a</td>
<td>Tau phosphorylation (epitope not specified)</td>
<td>–</td>
<td>Li et al. (2003)</td>
</tr>
<tr>
<td>3xTg-AD (Amyloid + Tau)</td>
<td>LPS (6 weeks, twice per week, 0.5 mg/kg, i.p.)</td>
<td>24 h</td>
<td>↑pT231/pS235, ↑pS396/404</td>
<td>↑ CDK5, = GSK-3β, =JNK, = p38 MAPK</td>
<td>Kitazawa et al. (2005)</td>
</tr>
<tr>
<td>3xTg-AD (Amyloid + Tau)</td>
<td>LPS (6 weeks, twice per week, 0.5 mg/kg, i.p.)</td>
<td>48 h</td>
<td>↑Total tau, ↑pS202/pT205, ↑pT214, ↑pT212/pS214, ↑pT231/pS214, ↑pS396/404</td>
<td>↑ pS396/404 containing insoluble tau</td>
<td>Sy et al. (2011)</td>
</tr>
<tr>
<td>3xTg-AD (Amyloid + Tau)</td>
<td>MHV (i.p.)</td>
<td></td>
<td></td>
<td>↑ pS396/404</td>
<td>Li et al. (2003)</td>
</tr>
<tr>
<td>rTg4510 (Tau)</td>
<td>LPS (10 μg, i.c.v)</td>
<td>1 weeks</td>
<td>↑pS199/pS202, ↑pS396, ↓ Insoluble tau</td>
<td>↑ GSK-3β, ↑ p38 MAPK</td>
<td>Lee et al. (2010)</td>
</tr>
<tr>
<td>hTau (Tau)</td>
<td>LPS (1 mg/kg, i.p.)</td>
<td>24 h</td>
<td>↑pS202/pT205, ↑pT231</td>
<td>↑ CDK5, = JNK</td>
<td>Bhaskar et al. (2010)</td>
</tr>
<tr>
<td>C57BL/6 (WT)</td>
<td>LPS (10 mg/kg, i.p.)</td>
<td>24 h</td>
<td>↑pS202/pT205, ↑pT231, ↑pS396/404</td>
<td>↑ pS396/404</td>
<td>Bhaskar et al. (2010)</td>
</tr>
<tr>
<td>C57BL/6 (WT)</td>
<td>LPS (100 μg/kg, i.p.)</td>
<td>0–4 h</td>
<td>Transient: ↑pS396/404, ↓ Total tau, ↓ Insoluble tau</td>
<td>↑ GSK-3β, ↑ CDK5, = JNK, = p38 MAPK</td>
<td>Roe et al. (2010)</td>
</tr>
<tr>
<td>3xTg-AD (Amyloid + Tau)</td>
<td>R-flurbiprofen (10 mg/kg, daily, 2 m, Chow)</td>
<td>–</td>
<td>↑pS202/pT205, ↑pS396/404</td>
<td>↑ pS202, ↑pS396/404</td>
<td>Li et al. (2003)</td>
</tr>
<tr>
<td>3xTg-AD (Amyloid + Tau)</td>
<td>Ibuprofen (daily, 5 months, Chow)</td>
<td>–</td>
<td>–</td>
<td>↑ pS202, ↑pS396/404, ↓ Insoluble tau</td>
<td>Carreras et al. (2013)</td>
</tr>
<tr>
<td>hTau (Tau)</td>
<td>Minocycline (10 mg/kg daily, 14d, i.p.)</td>
<td>2 h</td>
<td>↑ pS396/404, ↓ Insoluble tau</td>
<td>–</td>
<td>McKee et al. (2008)</td>
</tr>
</tbody>
</table>

2.3. LPS induces tau phosphorylation in wild-type (WT) mice

While transgenic models provide relevance to pathogenesis of human tau, the use of wild-type (WT) mice has proved useful to examine LPS effects on non-pathogenic tau. Interestingly, a low 100 μg/kg dose of LPS was found to induce CDK5-dependent tau phosphorylation at post-tangle associated epitopes as early as 20 min post-injection, and which subsided within 4 h (Roe et al., 2011) indicating that low levels of inflammation are sufficient to transiently trigger tau phosphorylation. Conversely, Bhaskar et al., 2010 demonstrated that a septic dose of LPS...
(10 mg/kg) was not associated with changes in tau phosphorylation 24 h following administration in WT mice. The failure to observe tau hyperphosphorylation could be attributed to the use of a later time-point than Roe et al. (2011). However, when the mice were deficient of the fractalkine receptor, the same 10 mg/kg dose, an increase in microglial activation and tau phosphorylation was seen 24 h later at both pre- and post-tangle sites, and IL-1 receptor signalling was again found to be the underlying mechanism (Bhaskar et al., 2010). Taken together, these findings demonstrate that pro-inflammatory stimuli induce tau hyperphosphorylation at epitopes associated with both pre- and post-tangle pathology in both WT and transgenic models of AD.

3. What constitutes an alteration in tau pathogenesis?

3.1. Are changes in tau phosphorylation sufficient to draw conclusions on tau pathology?

Tau phosphorylation represents a mechanistic trigger for a pathological cascade which ultimately leads to the development of NFT. Both pre- and post-tangle associated phosphorylation epitopes were found to be induced following immune stimulation as summarized in Fig. 2, therefore the question arises as to the relative importance of specific phosphorylation epitopes. Indeed the association between tau phosphorylation and development of pathology is poorly understood, with increases in tau phosphorylation not always developing into tau aggregation. For instance, Lee et al. (2010) showed in the rTG510 model that while tau phosphorylation was increased following acute central LPS administration, tau aggregation remained unaffected. This indicates that assessing tau phosphorylation is not necessarily representative of an impact on tau pathology. In contrast, Sy et al. (2011) reported an increase in tau aggregation following a chronic LPS treatment regimen in the 3xTG model. Interestingly the same phosphorylation epitopes were affected in the two studies (Lee et al., 2010; Sy et al., 2011) possibly demonstrating a role of acute vs. chronic effects, although a role for amyloid in the latter study cannot be ruled out. In support of this hypothesis, a chronic reduction in basal inflammation induced through the tetracycline antibiotic: minocycline reduced levels of both phosphorylated and aggregated tau in the hTau model (Noble et al., 2009). Recent advancements have suggested tau oligomers as the tau species with the greatest pathogenic potential, with their accumulation at the synapses, rather than NFT, being thought to cause synaptic dysfunction (Guerrero-Muñoz et al., 2015). Tau oligomers have the unique ability to translocate across the synapse, propagating tau pathology into healthy neighbouring neurons in a process known as tau seeding (Guerrero-Muñoz et al., 2015). Assessing the effect of inflammation on extracellular tau species will enable greater insight into our understanding of the effects of inflammation on tau pathology.

3.2. Do inflammatory processes have a protective role in the development of tau pathology?

While the literature to date points towards a detrimental role of inflammation in exacerbating tau pathology, recent evidence suggests a novel beneficial role. As observed with amyloid pathology, this is attributed to microglial phagocytosis of tau species. Indeed, Majerova et al. found that both primary and immortalised microglial cells stimulated with LPS can phagocyte synthetic extracellular tau oligomers (Majerova et al., 2014). While this demonstrates that microglia have the propensity to phagocytose tau oligomers, caution is required as the concentration of the oligomers used was much greater than physiological in vivo concentrations (Majerova et al., 2014). C57BL/6 mice injected with both soluble and aggregated human tau likewise show microglial internalization of both species of tau (Bohos et al., 2015). Since the soluble tau injected included both monomeric and oligomeric tau, this provides indirect evidence that microglia have the potential to phagocytose toxic tau oligomers. Inflammation could therefore be providing a beneficial effect through inhibiting the spread of tau pathology by microglial phagocytosis of extracellular tau seeds as described in Fig. 1.

4. Is the inflammatory response modelled relevant to AD?

The data discussed above points towards dose-dependency in the effects of inflammation on tau pathology, questioning the relevance of the stimuli used to model AD pathogenesis. LPS doses higher than 1 mg/kg are considered to simulate sepsis rather than infection (Thomas et al., 2014), a condition less frequently observed in AD patients. Only one study used a dose below this threshold, 100 μg/kg, which is thought to reflect mild systemic infection (Murray et al., 2012) and only resulted in transient increases in tau phosphorylation (Roe et al., 2011). While chronic LPS treatment regimens, as used by Kitazawa et al. (2005), might be more relevant to AD pathogenesis, tolerance occurs with repeated LPS injections (Pardon, 2015), and this has to be taken into account when trying to identify which inflammatory processes contribute to the tau pathology. Furthermore, LPS models only bacterial infections, which is not representative of the range of inflammatory conditions experienced by AD patients.

4.1. Concluding remarks

Inflammation has been suggested to play a role in the development of tau pathology, but the underlying mechanisms remain poorly understood. While IL-1β signalling in microglia appears to be detrimental, the data discussed here questions the pertinence of using tau phosphorylation as a readout for tau pathology. Pro-inflammatory stimuli robustly...
induces tau phosphorylation in model systems, but whether or not this progresses into the pathological process of tau aggregation is largely unknown. Novel evidence also suggests a beneficial role for inflammation on tau pathology through induction of microglial phagocytosis of tau oligomers, with the potential to inhibit the spread of tau pathology. Thus, suppressing the immune system could prove paradoxical at pathological stages of AD by potentiating tau pathological seeding into healthy neurons (Heneka et al., 2016). Finally, the doses at which LPS were found to induce tau phosphorylation in mice are more representative of sepsis in humans, indicating that a substantial inflammatory response is needed to induce tau pathology in pre-clinical models. Through modelling mild and chronic infection akin to that seen in AD, we will be able to better understand the role played by inflammation in pathogenesis and treatment of AD.

Conflict of interests
Jane Garton and Peter Atkinson are employees of Eisai Ltd., and Eisai Inc., Lee Dawson is an employee of Astex Pharmaceuticals.

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