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Inflammation and Anti-Inflammatory Strategies for Alzheimer's Disease – A Mini-Review

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Abstract
Until recently, the central nervous system (CNS) has been thought to be an immune privileged organ. However, it is now understood that neuroinflammation is linked with the development of several CNS diseases including late-onset Alzheimer's disease (LOAD). The development of inflammation is a complex process involving a wide array of molecular interactions which in the CNS remains to be further characterized. The development of neuroinflammation may represent an important link between the early stages of LOAD and its pathological outcome. It is proposed that risks for LOAD, which include genetic, biological and environmental factors can each contribute to impairment of normal CNS regulation and function. The links between risk factors and the development of neuroinflammation are numerous and involve many complex interactions which contribute to vascular compromise, oxidative stress and ultimately neuroinflammation. Once this cascade of events is initiated, the process of neuroinflammation can become overactivated resulting in further cellular damage and loss of neuronal function. Additionally, neuroinflammation has been associated with the formation of amyloid plaques and neurofibrillary tangles, the pathological hallmarks of LOAD. Increased levels of inflammatory markers have been correlated with an advanced cognitive impairment. Based on this knowledge, new therapies aimed at limiting onset of neuroinflammation could arrest or even reverse the development of the disease.

Introduction

Late-onset Alzheimer's disease (LOAD) is characterized by progressive cognitive decline, impaired function and behavioural abnormalities. The pathological hallmarks are amyloid (Aβ) plaques and neurofibrillary tangles (NFT), but the exact mechanism by which these develop remains elusive. Evidence is accumulating that a common link exists between the seemingly divergent aetiological factors and the end-stage pathology; the development of neuroinflammation. Based on current understanding of neuroinflammation it is plausible to suggest that insidious development of LOAD may depend on gradual dysregulation of cerebral vasculature and normal cellular function within the brain. This would result in increased oxidative stress and the induction of a pro-inflammatory state that ultimately becomes manifest as LOAD. Acquiring a comprehensive knowledge of neuroinflammation in relation to LOAD pathogenesis will facilitate the development of novel therapeutics aimed to limit disease progression, or possibly reverse disease symptoms.

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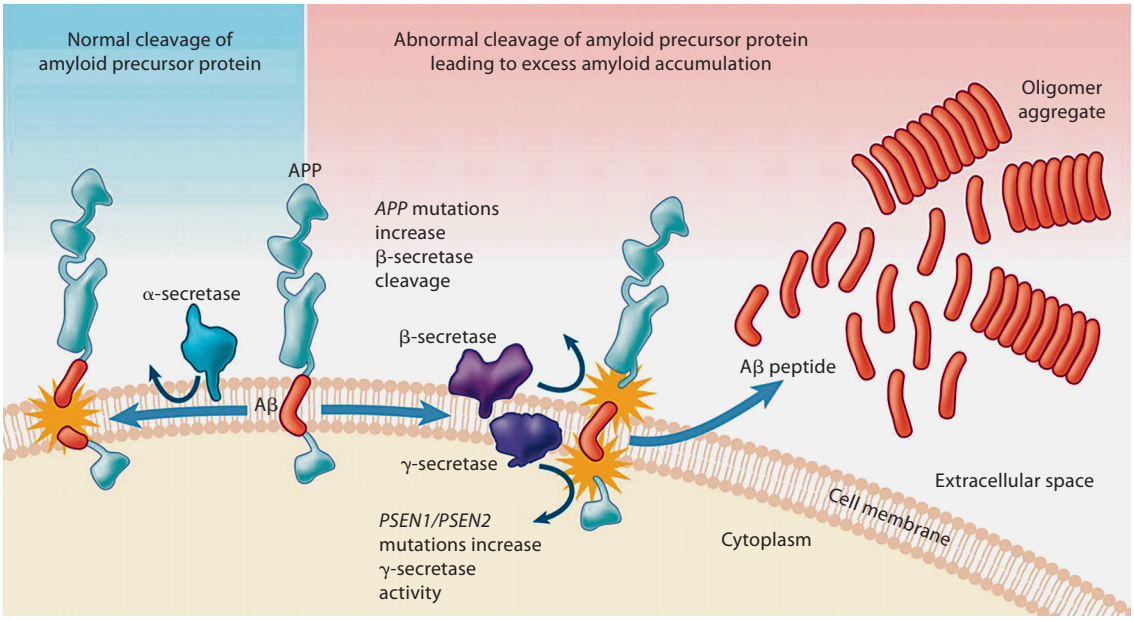

Fig. 1. Processing of APP and AB formation (from Patterson et al. [3]). Normal cleavage of APP (shown on left) involves the activity of α-secretase resulting in breakage of the AB segment. Abnormal cleavage of APP (shown on right) involves activity of β- and γ-secretase and leads to AB preservation and aggregation.

Pathological Hallmarks of LOAD

Amyloid Plaques

The amyloid hypothesis has been one of the prevailing theories explaining LOAD pathogenesis [1]. Details are summarized in figure 1.

Neurofibrillary Tangles

NFT develop as a result of τ hyperphosphorylation (fig. 2) [2]. These events are likely influenced by several indirect events including oxidative stress, development of inflammation and the increased burden of Aβ toxicity.

Risk Factors Associated with LOAD

Genetic Risk Factors

There is no known single genetic mutation that directly contributes to development of LOAD. Possession of an apolipoprotein E (APOE) ε4 allele has been shown to increase risk but as a susceptibility gene is neither necessary nor sufficient to cause LOAD. Rather it acts as a molecular chaperone that facilitates Aβ deposition. The risk of developing LOAD from a first-degree relative is low [3] suggesting pathogenesis is much more dependent on other aetiological factors.

Environmental Risk Factors

Epidemiological studies suggest a link between LOAD and vascular disease. A central factor is the potential for development of severe haemodynamic disturbances of the cerebral vasculature, leading to hypoperfusion [1]. Insufficient blood flow can contribute to increased oxidative stress leading to cellular damage and altered neuronal function. Increased oxidative stress is one of the earliest pathological changes in LOAD [3]. Cerebral hypoperfusion may ultimately contribute to Aβ plaque and NFT formation [2].

One of the main issues with understanding LOAD is the disparity between risk factors and disease pathogenesis. LOAD can be viewed, not as a single disease entity, but a cluster of various diseases with common outcome. Therefore, greater understanding of how various factors contribute to LOAD is required. A common link between risk factors and LOAD is oxidative stress and neuroinflammation. It is therefore necessary to explore how upstream events contribute to development of oxidative stress and understand the processes involved in neuroinflammation.

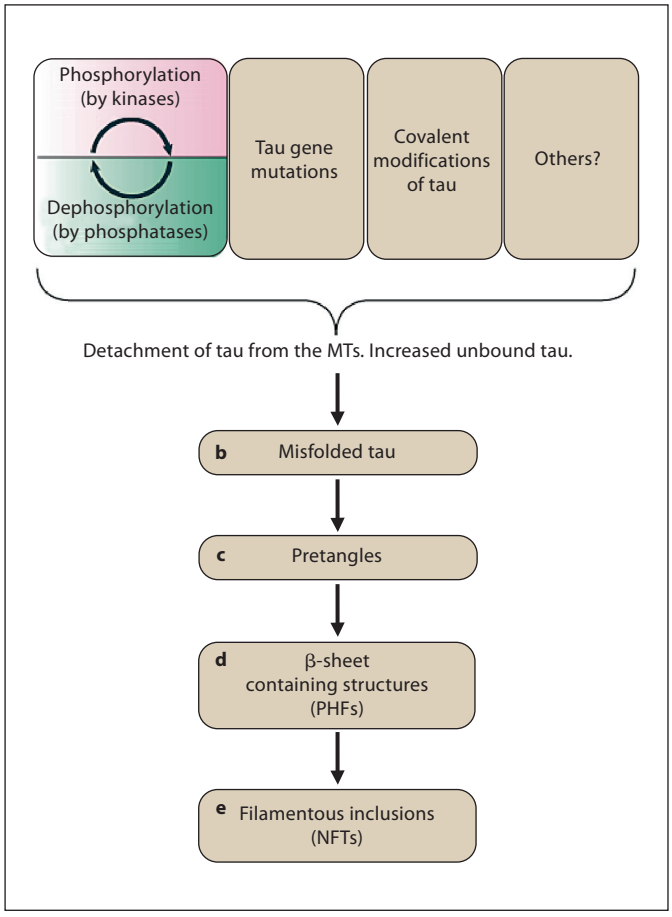

Fig. 2. Potential causes of τ aggregation (from Ballatore et al. [2]). There are several events that may lead to τ aggregation and the eventual formation of NFT. This likely includes upregulation of kinases/phosphatases coupling, genetic mutation, covalent modification and other uncharacterized events.

Mechanisms of Pathology

There is much to be discovered about specific precipitants of plaque and NFT formation, and their exact role in pathogenesis. Ongoing research has continually sought to link end-stage hallmarks of LOAD to a myriad of initial upstream events. Increasing evidence suggests an important unifying feature is development of oxidative stress leading to neuroinflammation. A mechanism is proposed in figure 3. It is likely that risk factors act through a common pathway that ultimately leads to Aβ plaque and NFT formation, manifesting as LOAD. This common pathway is likely initiated with cerebrovascular compromise.

Neurovascular Unit: Interplay between the Vasculature and the Central Nervous System

In ageing brain, insidious changes in blood flow and perfusion may contribute to initial stages of LOAD. Sufficient cerebral blood flow is normally ensured with tight coupling of neural activity [4]. This relies on integrity of the neurovascular unit (NVU), an axis formed by interaction of endothelium, pericytes, astrocytes, microglia and neurons (fig. 4) [4, 5]. The NVU is crucial for normal central nervous system (CNS) function by blood flow regulation, microvascular permeability, cell matrix interaction, neurotransmitter turnover, angiogenesis and neurogenesis [4]. In ageing brain, regulation of the NVU becomes progressively impaired, likely due to reduced cerebral perfusion. Depending on severity, cerebral blood flow reductions alter normal protein synthesis and regulation, impair ATP synthesis, alter electrolyte balance and may ultimately lead to neuronal death [4]. Overall breakdown of NVU integrity can affect cerebral function and contribute to increased oxidative stress.

Oxidative Stress: A Gateway to Neuroinflammation

Oxidative stress is created through unbalanced production of ROS and is one of the earliest pathological changes in LOAD [6]. Patients in early stages have reduced capacity to manage oxidative stress, evident through lower plasma antioxidant levels and altered antioxidant enzyme activity [7]. Development of vascular disease may lead to increased oxidative stress and subsequently to the metabolic defects noted in LOAD [6]. Vascular diseases, which become more common with age, can lead to critical levels of cerebral hypoperfusion and thus to breakdown of the NVU. Compared to normal brains, LOAD brains are typically hypoperfused and comparatively hypoxic [4]. Chronic hypoperfusion can trigger mitochondrial dysfunction, damage vascular cells and enhance production of ROS [6]. Increased oxidative stress can trigger microglial activation leading to the synthesis of pro-inflammatory mediators resulting in subsequent neuronal damage [5]. Oxidative stress can subsequently activate upregulation of cellular mediators that influence development of inflammation.

Neuroinflammation: The Pathological Beginning of LOAD

It was once thought that the CNS was 'immune privileged'; however, neuroinflammation has been actively related to onset of several neurodegenerative disorders, including LOAD [5, 8]. Neuroinflammation can be viewed as a part of a pathological triad of LOAD that includes Aβ

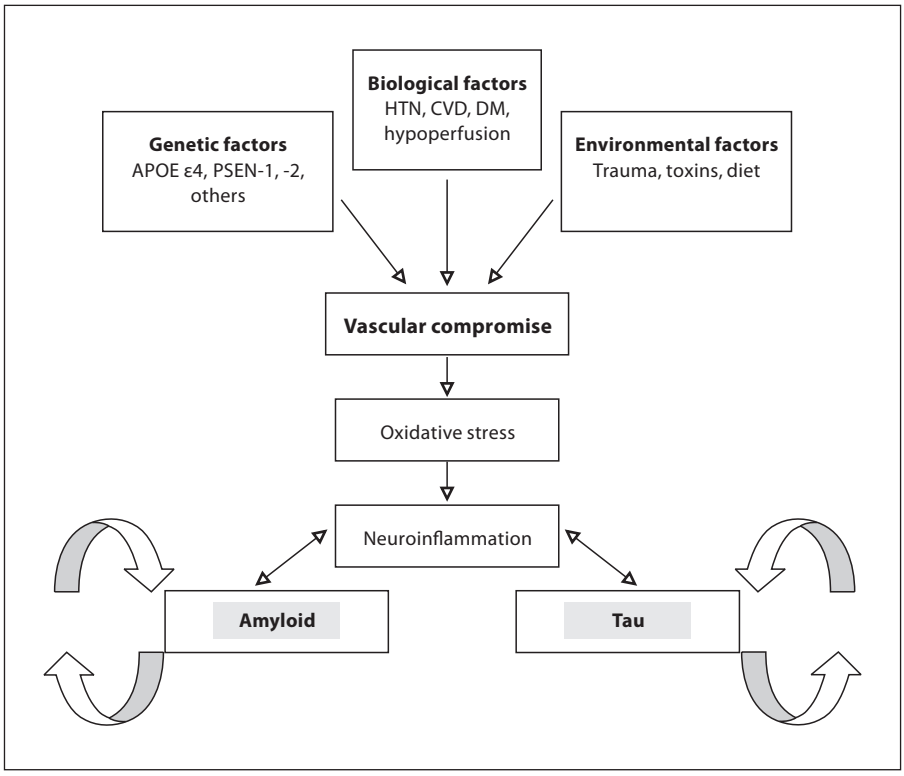

Fig. 3. Potential mechanisms of AD pathology. Several aetiological factors are thought to contribute to AD. These contribute to vascular compromise and result in cell dysregulation. Consequently this leads to an increased burden of oxidative stress and can activate an immune response. The resultant development of neuroinflammation can influence the amyloid processing/deposition and can likewise lead to the formation of τ aggregates and NFTs. Once formed, AB and NFT can further contribute to immune overactivation and thus their formation leads to the development of a positive feedback loop of a sustained neuroinflammatory response.

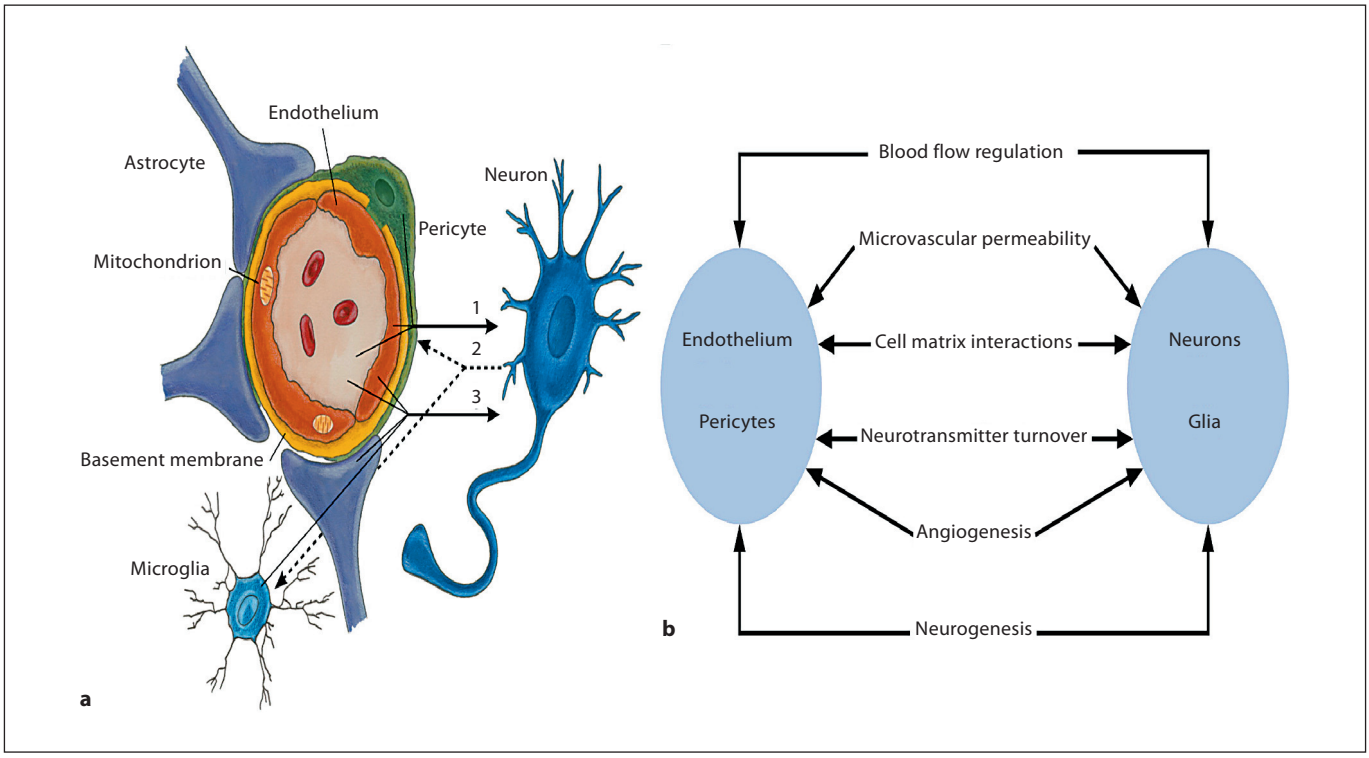

Fig. 4. Representation of the NVU (from Zlokovic [4]). **a** Cellular constituents of the NVU. **b** Regulation of normal homeostatic function in the CNS.

plaque and NFT formation [9]. Although it is not entirely clear whether neuroinflammation causes or results from neurodegeneration, it is more likely to arise as a consequence of vascular disturbance leading to oxidative stress. The neuroinflammatory response likely begins as a result of reduced cerebral blood flow, leading to development of cellular damage, oxidative stress and glial activation [10]. This can promote increased formation and aggregation of Aβ and NFT [11]. Increased Aβ plaque and NFT burden then exacerbates the immune response and adds to inflammatory stress. Once this cascade becomes established it likely initiates a positive feedback loop that rapidly and irreversibly progresses.

Peripheral Inflammation

While inflammation within the brain is thought to be important, there are also suggestions that links with inflammation in the periphery exist. Pro-inflammatory molecules from the periphery may enter the CNS and could increase cytokines or activate glial cells. This could produce an amplification of response. This could result from infection or inflammatory processes. Further evidence comes from finding that elevated C-reactive protein (an acute phase protein) is linked with risk of developing LOAD and acceleration of cognitive decline. It is interesting that risk factors for LOAD include hypertension, diabetes mellitus, hyperlipidaemia and obesity, all constituents of the metabolic syndrome. It appears that much of the damage associated with metabolic syndrome depends on the hyperglycaemic component, hyperinsulinaemia and insulin resistance. It is interesting that hyperinsulinaemia has been associated with increased inflammatory markers, providing a putative mechanism whereby these factors increase risk for LOAD.

Cellular Participants of Neuroinflammation

Endothelium

Endothelial cells are important regulators of cerebral perfusion and their integrity is crucial to the NVU. Endothelial cells play a crucial role in pathogenesis of several vascular diseases. In the brain they provide an important metabolic barrier, assisting in regulation of circulating cells and other macromolecules, acting in tandem with the physical barrier of the blood-brain barrier (BBB) [12]. The endothelium is very sensitive to oxidative stress. Chronic cerebral hypoperfusion contributes to oxidative stress resulting in mitochondrial dysfunction [10]. Long-term ischaemic-reperfusion events lead to disintegration of the mitochondrial ultrastructure, events which have been shown to occur before development of LOAD pathology [6]. Consequently, resultant cellular damage promotes an inflammatory response with glial activation and leukocyte recruitment leading to further inflammation and ultimately cognitive decline in LOAD [6].

Microglia

Microglia are critical cellular components of the NVU which support and protect neuronal function [4]. They orchestrate immune function and represent the first line of CNS defence [13]. Resting microglia are activated into roaming scavenging macrophages in the presence of pathogens, damaged cells or debris and mediate removal of unwanted material [8]. Microglia can also be recruited from circulating monocytes which cross the BBB. Activated microglia release a variety of pro-inflammatory mediators, including cytokines, chemokines, complement and ROS [13] that mediate important cellular function.

The role of microglia in LOAD is complex. Perturbations such as cerebral hypoperfusion can lead to microglial activation and neuroinflammation. Microglial activation has been linked to Aβ deposition, suggesting they can initiate and propagate neuroinflammatory damage. Microglia show increased chemotaxis toward Aβ deposits [13] and have been shown to phagocytose Aβ to facilitate removal. This results in upregulation of pro-inflammatory mediators and increased ROS burden, further contributing to oxidative stress and neuroinflammation [13]. Microglia likely play a role in both initial and chronic stages of LOAD. It is likely that microglia become overactivated and contribute to ongoing cognitive decline. Microglial activation combined with impaired ability to cope with increased oxidative stress is deleterious in the ageing brain [14].

Astrocytes

Astrocytes, the most abundant cells in human brain, maintain functional integrity of neuronal synapses. They act much like a scaffold in the CNS and communicate with each of the cells in the NVU. In LOAD, astrocytes have been shown to function in a similar manner to microglia by secreting a variety of pro-inflammatory cytokines and facilitating Aβ clearance and degradation. Astrocytes also overexpress a unique cytokine (S100B) [15] which is associated with development of dystrophic neurites in Aβ plaque-abundant brain regions. Furthermore, LOAD brains have been shown to have higher levels of

NOS-positive astrocytes indicating increased oxidative stress. Increased stimulation and overactivation of astrocytes can thus induce local CNS damage, attract additional microglia and further exacerbate oxidative stress. In LOAD upstream events such as cerebral hypoperfusion, in addition to formation of Aβ and NFT, can stimulate astrocytes. Overstimulation of astrocytes can act in tandem with microglia leading to development of a positive feedback loop contributing to neuronal damage [13], a likely scenario in LOAD. Complete understanding of the role of astrocytes in development and progress of LOAD requires further elucidation.

Neurons

Neurons produce a variety of pro-inflammatory mediators. This likely triggers further inflammation by recruiting additional microglia. Continuing evidence suggests that altered microglial-neuron interactions are early events leading to LOAD [5]. This is likely manifest through neuronal toxicity leading to apoptosis and gradual loss of neuronal function. Similar to other constituents of the NVU, this ultimately represents a positive feedback loop of uncontrolled production of inflammation [13].

Molecular Regulation of Neuroinflammation and LOAD Pathogenesis

Neuroinflammation is a choreographed process involving numerous cells and molecular mediators. The intended biological function is a protective mechanism against pathogens and abnormalities which naturally occur. However, if inflammation proceeds unregulated and normal control process are impaired, changes which occur can ultimately result in cellular death and destruction. In LOAD this process likely leads to, and facilitates Aβ plaque and NFT formation ultimately leading to clinical deterioration. Understanding the development of neuroinflammation however remains in its infancy. The section below summarizes some of the major molecular constituents influencing neuroinflammation.

Interleukin-1 (IL-1)

IL-1 is an important regulator of the inflammatory cascade. In the brain it is primarily released from activated microglia. It is found in two distinct isoforms (IL-1a and IL-1b), each encoded by separate homologous genes. The actions of both isoforms are regulated through a single receptor and elicit similar actions throughout the body [9]. In the brain, IL-1 levels become elevated in response to neuronal insult and overexpression leads to leukocyte recruitment [9]. In the development of LOAD, IL-1 is thought to be one of the most influential cytokines expressed during initial disease pathogenesis [9].

Cerebrospinal fluid levels of IL-1 are significantly elevated in LOAD compared to healthy controls [5]. Although the precise reasons for increased inflammatory markers require further clarification, there have been clear links to IL-1 overexpression and LOAD pathogenesis. IL-1 has been shown to upregulate expression of β-amyloid precursor protein (BAPP) in vitro. In human brain it has been suggested that following neuronal injury, increased IL-1 levels can influence rapid overexpression of BAPP [16]. In vitro experiments have shown that increased BAPP and Aβ can lead to upregulation of IL-1 [9]. These studies suggest that neuronal injury, whether arising from cerebral hypoperfusion, trauma or other secondary processes can profoundly influence IL-1 levels in the CNS. These increased levels contribute to Aβ burden in the CNS, which also contributes to IL-1 overexpression. Consequently, increased release of IL-1 can lead to development of a positive feedback loop resulting in a self-sustaining cycle of IL-1 production which can lead to cell injury and death [16].

Interleukin-6 (IL-6)

Similar to IL-1, IL-6 can exert a variety of effects within the human body and is released from microglia and astrocytes. IL-6 can both impede the inflammatory response by inhibiting tumour necrosis factor (TNF) and IL-1R, and promote the inflammatory response by influencing chemotaxis. This apparently conflicting role suggests that IL-6 may have a dual role in the development of inflammation and highlights the complexity of its involvement in the process of neuroinflammation. Unlike studies investigating IL-1, there has not been a direct correlation made between IL-6 activity and the upregulation of τ and Aβ synthesis [5]. Much remains to be understood about the role of IL-6 in the development of neuroinflammation and AD. Overall the complexity surrounding IL-6 activity marks a gap in current understanding of neuroinflammation.

Tumour Necrosis Factor (TNF)

TNF is one of the most important regulatory cytokines. Expression influences other important inflammatory cytokines including interleukins and granulocyte-monocyte colony-activating factor [17], which in turn can promote lymphocyte activation and acute phase protein synthesis. It exists in two main forms, TNF-α and

TNF-β. In normal brain both isoforms likely exist in a balanced state which limit and control local inflammatory processes. TNF-α is pro-inflammatory and most of its biological properties are mediated through TNF-RI and RII receptors. TNF-β has inhibitory effects on inflammatory and cellular responses; however, its complete role requires further elucidation.

TNF-α is thought to be the main regulator of the pro-inflammatory response in the brain [17]. Evidence suggests that TNF-α overproduction is likely one of the key events leading to LOAD pathogenesis. TNF-α is produced by microglia and overproduction has been linked with neuronal cell death. Cultured brain slices in vitro show TNF-α increases neuronal glutamate neurotoxicity and leads to cellular damage and death [18]. Animal models of LOAD have indicated that TNF-α upregulation can lead to increased Aβ and NFT formation. It is therefore interesting that spinal levels of TNF-α in AD patients are increased up to 25-fold and that levels of spinal TNF-α correlate with clinical deterioration of LOAD. Recently, therapeutic agents that inhibit TNF-α activity were shown to improve and reverse cognitive decline in some patients [19]. This exciting finding provides solid evidence for the role of TNF-α in the development of LOAD and strongly suggests that onset of neuroinflammation is central to LOAD onset.

Although TNF-α is produced in response to oxidative stress and the presence of Aβ [5], current gaps remain with regard to the precise sequence which leads to TNF-α overexpression. Furthermore, a better understanding of the normal balance of TNF-α and TNF-β and their receptor involvement is required. Although TNF has been suggested to play a protective role following acute brain insults [17], sustained or chronic perturbations of the brain, including impaired vascular function, may lead to manifestation of TNF induced damage.

TNF Superfamily Receptors

The actions of TNF-α are primarily mediated through two main receptors – TNF-RI and TNF-RII. These signalling pathways involve complex interplay of several proteins which can influence cell survival. TNF-RI is constitutively expressed on all nucleated cells at low levels and is associated with mediation of cellular apoptosis [17]. Animal studies have indicated that deletion of the TNF-RI gene prevents Aβ formation and diminishes plaque formation, suggesting an involvement in APP processing [20]. TNF-RII is primarily expressed on cells of haemopoietic origin as well as neurons, and it has a higher affinity for TNF compared to TNF-RI. TNF-RII has been shown to enhance apoptosis mediated by TNF-RI and enhance lymphocyte proliferation and activation. It has been reported that patients with LOAD had increased levels of TNF-RI and TNF-RII on T lymphocytes compared to controls, further suggesting pathogenesis is related to immune system activation [21].

Another important receptor included in this superfamily is TRAIL (TNF-related apoptosis inducing ligand), an integral membrane protein normally expressed on macrophages that participates in immune surveillance [22]. TRAIL associates with 5 receptors (DR1-DR5) of the TNF/NGF family where association with DR4 and DR5 lead to induction of cellular apoptosis following ligand binding [22]. Following Aβ exposure, expression of the DR5 receptor has been shown to be increased [22]. Furthermore, in LOAD patients TRAIL immunoreactivity is specifically localized to areas in close proximity to Aβ plaques. Conversely, non-LOAD patients do not display such immunoreactivity suggesting an important role for this molecule in LOAD pathogenesis. Inhibition of this signalling pathway can protect human neuronal cells from further Aβ toxicity [5].

The TNF family of receptors and their influence on the pathogenesis of LOAD represents many complex molecular relationships and interactions in the brain. Activation of the TNF-Rs in the brain seems to be at least partially responsible for influencing APP processing, Aβ deposition, immune activation and cell survival. Likewise, expression of TRAIL seems to form a direct link between LOAD and its pathogenesis and represents a promising avenue in development of future therapeutic targets.

Cyclooxygenase, Prostaglandins and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Prostaglandins (PGs) are ubiquitous signalling molecules with pleiotropic effects. Formation is regulated by cyclooxygenase (COX) activity which is present in the human body in both constitutive (COX-1) and inducible (COX-2, COX-3) forms. It is well established that COX activity and PG formation is inhibited by NSAIDs.

In the brain, PG formation is regulated by COX activity primarily in astrocytes and microglia [13]. In LOAD patients, COX-2 activity has been found to be elevated compared to controls [23]. Indeed, COX-2 activity has been linked to promotion of APP processing and cognitive decline in animal models [24]. Since increased COX activity is associated with LOAD, it is therefore expected that PGs contribute to LOAD pathology. Although there is not an abundance of clinical data regarding the action

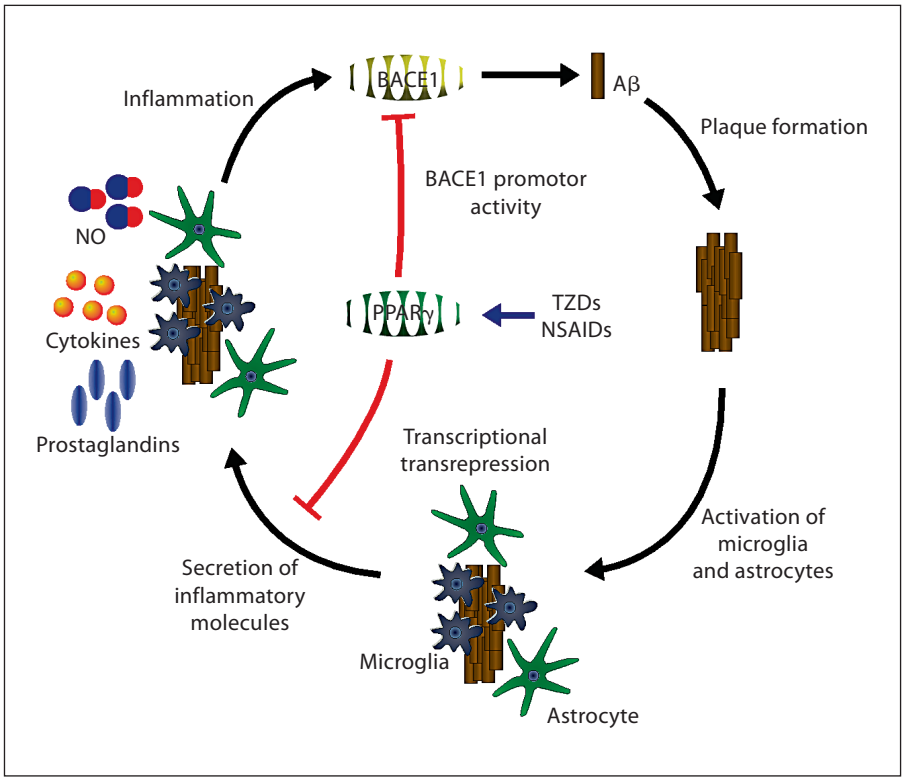

Fig. 5. Influence of PPARγ in AD (from Kummer and Heneka [33]). AB plaque formation is facilitated by the activity of BACE1 (β-secretase). Plaque deposition can increase the inflammatory burden in the CNS. PPARγ activity is influenced by TZDs and NSAIDs. Once activated, PPARγ can limit the secretion of pro-inflammatory molecules and decrease BACE1 activity.

of PGs in LOAD, animal evidence suggests that activity of PGE_2 has both pro-inflammatory and pro-amyloidogenic effects [5].

Based on involvement of COX/PGs in LOAD, it seems logical to assume NSAIDs would be therapeutically beneficial. Several retrospective studies previously indicated that chronic NSAID use reduces LOAD risk [5]. However, results from these studies did not provide a clear link between NSAIDs and AD prevention. This disappointing finding is likely due to several variables including: inadequate CNS drug penetration, inadequate doses and the variability of LOAD diagnosis and progression at the time of treatment [5]. Therefore, continued investigation of NSAID use in LOAD is warranted. Furthermore, NSAID activity has been shown to decrease levels of Aβ, a process that is likely mediated through separate pharmacological mechanisms than COX inhibition [25]. NSAIDs are known to influence peroxisome proliferator-activated receptor (PPAR) activity, and therefore their beneficial effects may be manifest through PPAR activation.

Peroxisome Proliferator-Activated Receptors

PPARs are ligand-activated nuclear receptors. There are three isoforms currently identified including PPARα, PPARβ/δ and PPARγ, each with splice variants. Natural ligands include eicosanoids and prostaglandin J_2, in addition to the synthetic thiazolidinedones, pioglitazone and rosiglitazone [26]. PPARs are involved in several processes including lipid and glucose metabolism, adipocyte differentiation and cholesterol homeostasis. In particular, PPARα and PPARγ can modulate oxidative stress, inhibit expression of inflammatory genes [27] and participate in age-related inflammatory changes. Although cell culture experiments indicate that PPARα agonists inhibit IL-6, TNF-α and COX-2 expression, evidence suggests that PPARγ plays a more prominent role in neuroinflammation.

PPARγ is expressed in microglia and astrocytes. It forms a heterodimer with the retinoid X receptor and following ligand activation can bind specific regions on target genes thus influencing transcription through both up- and downregulation [28]. PPARγ activity has been linked to Aβ clearance [29] and a reduction in BACE1 gene transcription [28] (fig. 5). Furthermore, recent evidence suggests involvement of the PPARγ gene in LOAD pathogenesis since PPARγ mRNA levels have been demonstrated to be elevated in LOAD patients [30]. Clinical work involving 500 LOAD patients showed that those

treated with rosiglitazone for 6 months showed significant improvement in cognition [31].

The mechanisms of PPARs action in the CNS remain to be further characterized. Of particular note is the idea that NSAIDs can modulate PPAR activity and even modulate the function of PPARγ. Evidence has also suggested that NSAIDs can influence γ-secretase activity and thus decrease Aβ production [32, 33]. This needs to be further substantiated; however, it does establish important links between NSAID activity and PPAR activation. Thus it raises the possibility that NSAID activity in LOAD is mediated through the PPAR pathway as opposed to COX inhibition. Future use of NSAIDs in LOAD will require establishment of optimum modes of delivery, a better understanding of effective doses needed to influence LOAD pathology, quantification of the effectiveness of NSAIDs used and the minimization of toxic side effects.

Apolipoprotein E

APOE is an important regulator of triglyceride and cholesterol metabolism and transport, thought to be involved in neuronal regulation and repair [34] since it has been shown that macrophages have the ability to secrete large quantities of APOE around damaged and regenerating nerve cells. In brain, APOE is mainly secreted by astrocytes; however, each cell displays capacity for its synthesis [34]. APOE is encoded by three different alleles and as such exists in three distinct isoforms including APOE ε2, ε3, and ε4. The presence of the APOE ε2 allele is correlated with the lowest risk of LOAD, whereas APOE ε4 is the best established risk factor for LOAD [35]. Indeed, each copy of APOE ε4 found in the genotype is associated with a greater risk of developing LOAD and is also correlated with earlier manifestation of the disease. APOE ε4 is not a causative factor for LOAD as individuals that carry 1–2 ε4 alleles may reach late old age without manifestations of dementia [36].

Although the precise links between APOE ε4 and LOAD remain to be elucidated, it is clear that APOE ε4 is associated with altered lipid metabolism. APOE ε4 expression is associated with vascular diseases. APOE ε4 poorly controls cholesterol homeostasis and is much less efficient at promoting cholesterol efflux from neurons and astrocytes [37]. It has been shown in vitro and in vivo that APOE ε4 inhibits clearance, facilitates production and promotes deposition of Aβ, ultimately leading to plaque formation [34]. The ability to form memories is reduced in APOE ε4 transgenic mice models when compared to wild-type mice [38]. The increased lipid burden within the brain and impaired lipid homeostasis may then contribute to increased oxidative stress and lead to cellular damage through lipid peroxidation, decreased vascular perfusion and development of an immune response.

Normal expression of APOE is necessary for the maintenance of neuronal integrity. Increased APOE ε4 expression results in impaired neuronal functioning, increasing the likelihood of Aβ formation and therefore can increase overall oxidative stress and neuroinflammation. Strategies to impair formation of APOE ε4, such as compounds that convert APOE ε4 to the ε3 form, block APOE ε4 processing or prevent associated mitochondrial dysfunction, may prove invaluable tools in diminishing the deleterious consequences of APOE ε4 expression [34]. Since APOE is implicated in LOAD, it is not surprising that links have been made between cholesterol metabolism and LOAD. Indeed, a body of evidence also links abnormal cholesterol metabolism in the brain to LOAD [39]; however, the link between peripheral cholesterol levels and LOAD remains controversial [36]. In the human body, about 25% of the total amount of cholesterol is synthesized by neuronal and glial cells [40]. These cholesterol molecules are embedded within the myelin, neuronal and glial membranes. Most cholesterol synthesized in the brain remains in the CNS, since most cholesterol is unable to cross the BBB [41]. This process is tightly regulated where cholesterol synthesis is balanced with excretion of 24S-hydroxycholestrol, which is able to cross the BBB [42].

Although systemic cholesterol synthesis and brain cholesterol synthesis are largely regulated independently, disturbances in systemic cholesterol homeostasis may influence brain cholesterol synthesis. Elevated levels of systemic cholesterol can lead to development of increased oxidative stress and may influence brain cholesterol biosynthesis by suppressing formation and increasing turnover [36]. This can lead to induction of cytoskeletal changes, NFT formation, and eventually cellular apoptosis [43]. Changes in lipid processing can also influence Aβ deposition in the brain since increased cholesterol turnover affects APP processing and can contribute to preferential β- and γ-secretase APP cleavage rather than normal α-secretase cleavage [44]. The notion that increased cholesterol turnover is present in brains of LOAD patients has been substantiated with the finding that those in early stages of the disease have increased levels of 24S-hydroxycholestrol [45]. This interesting finding suggests that lipid metabolism is altered in the development of LOAD pathogenesis. Overall it seems likely that cholesterol biosynthesis and Aβ formation are affected by APOE genotype.

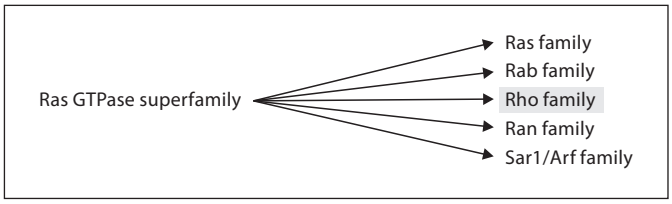

Fig. 6. Ras GTPase superfamily (adapted from Cole and Vassar [1]). The Ras GTPase superfamily is composed of 5 subfamilies. In turn, each of these subfamilies is also composed of several members.

Inhibition of cholesterol synthesis with the use of HMG-CoA reductase inhibitors (statins) has informed the complexities of cholesterol metabolism. Statins are pleiotropic molecules which have previously been implicated in reducing the risk of dementia [46]. Statins are believed to exert their effects by manipulating cholesterol and isoprenoid levels intracellularly leading to altered BAPP processing and membrane signalling [44]. The exact mechanism by which statins exert these effects requires further clarification; however, it has been suggested that statin-induced inhibition of isoprenylation may limit cell membrane raft interactions responsible for APP processing and Aβ formation [47].

Isoprenoids

Isoprenylation is an important cellular process that regulates protein localization and function [44]. It involves post-translational modification of a diverse range of proteins through the attachment of FPP (farnesylation) or GGPP (geranylgeranylation) to nuclear lamins, the subunits of trimeric G proteins, protein kinases and RAS and RAS-related GTPases [1]. It is a crucial process for normal protein function, localization and protein-protein interaction. Dysregulation of this process can lead to the development of several events consistent with LOAD pathogenesis including oxidative stress, inflammation, APP metabolism and τ phosphorylation [1].

In development of LOAD, evidence suggests that the GTPase superfamily may play an important role [48, 49]. The Ras GTPase superfamily is composed of 5 different subfamilies and each subfamily interacts with a subset of multiple effect molecules to exert a diverse array of effects (fig. 6). Within these subfamilies the Rho family is importantly linked to development of LOAD pathophysiology [1]. Specifically, Rho/ROCK and Rho/Rac1 interactions are thought to be important. Previous work has indicated that changes in levels and activity of isoprenoids and GTPases have been linked to increased Aβ42 secretion, alteration of normal APOE secretion, modulation of inflammatory pathways [50, 51] and increased τ phosphorylation [52]. (For a complete review, see Cole and Vassar [1].) Strategies to broaden the current understanding of signalling events associated with isoprenylation and the physiological processes it influences would greatly expand our knowledge. However, current understanding of isoprenoid activity remains limited and although in vitro work provides interesting links between isoprenoids and AD pathogenesis, further understanding of this relationship will require further in vivo and eventually clinical investigations [1]. The use of statins has facilitated the understanding of LOAD and has highlighted important roles for several isoprenoid moieties. It still remains to be determined if they will offer any therapeutic effect for AD pathology [1].

Therapeutics and the Future of LOAD Management

Events contributing to development of AD are numerous and complex. The pathogenesis involves a multitude of variables many of which remain to be quantified. Further knowledge of processes involved in development and progression of neuroinflammation will enhance current knowledge of LOAD. A better understanding of the interplay between cytokines, their receptor activity, second messengers, lipid metabolism and protein modification is necessary. Although there are many events which can lead to neuroinflammation, this process represents a common pathway of disease development (fig. 2). Through this the ability to develop novel therapeutic agents for AD will inevitably arise.

Despite this potential, the current pharmacological management of LOAD is limited to the use of cholinesterase inhibitors and memantine. Although there was initial promise with good epidemiological evidence suggesting that NSAIDs, oestrogen, statins and vitamin E would improve and prevent LOAD, randomized clinical trials have proved disappointing [53]. This likely represents the complex nature of LOAD and a need for multifactorial interventions. Other factors that contributed to negative results were duration of trials, problems with recruitment and retention, difficulties with power calculations and adherence to treatment regimens. The design of future therapies for LOAD will rely upon developing agents which arrest disease progression.

The role of dietary restriction (DR) in an inflammatory context is worth discussion. DR (reduced calorie in-

take with maintained nutrition) increases resistance of neurons to dysfunction and degeneration in experimental models of LOAD. Underlying the beneficial effects of DR is the attenuation of system-wide inflammatory processes including those occurring within the CNS which can produce concomitant beneficial effects on neurodegeneration and cognition.

Recent work highlighting the importance of neuroinflammation in LOAD has led to the prospect of exciting future therapeutic options. In particular, the recent trial with entanercept [19] has provided much insight into pathogenesis and possible future treatment options. Not only does this anti-inflammatory agent improve results of memory testing (MMSE) over a 6-month period, it also has been shown to improve verbal fluency and apraxia in patients [19]. Etanercept is a specific antagonist of TNF-α. Studies now suggest that TNF-α may also regulate synaptic transmission and mediate β-amyloid and β-amyloid oligomer disruption of memory mechanisms. The rapid onset of effect with etanercept suggests the synaptic effect is the most important. These results are exciting not only from the aspect of patient care, but also are strongly suggestive that neuroinflammation is integral to LOAD pathogenesis. Key to this idea is the involvement of neurovascular dysfunction and the activation of glia within the CNS. With use of anti-TNF agents as a tool to investigate pathogenic mechanisms of LOAD, future more direct targeting of components of the NVU including RAGE and LRP may be indicated. Currently we are on the cusp of new and exciting developments with regard to understanding LOAD pathogenesis and development of novel therapies specifically designed to limit pathological progression of the disease.

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