Neuroinflammation in Alzheimer's disease

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Increasing evidence suggests that Alzheimer's disease pathogenesis is not restricted to the neuronal compartment, but includes strong interactions with immunological mechanisms in the brain. Misfolded and aggregated proteins bind to pattern recognition receptors on microglia and astroglia, and trigger an innate immune response characterised by release of inflammatory mediators, which contribute to disease progression and severity. Genome-wide analysis suggests that several genes that increase the risk for sporadic Alzheimer's disease encode factors that regulate glial clearance of misfolded proteins and the inflammatory reaction. External factors, including systemic inflammation and obesity, are likely to interfere with immunological processes of the brain and further promote disease progression. Modulation of risk factors and targeting of these immune mechanisms could lead to future therapeutic or preventive strategies for Alzheimer's disease.

Introduction

At first glance, the specialties of immunology and neurobiology could not be more different. From a cellular perspective, the brain represents stasis, whereas the immune system represents motion. But these two perspectives have come together as efforts to understand the pathogenesis of neurodegenerative disease have borne fruit. Emerging evidence suggests that inflammation has a causal role in disease pathogenesis, and understanding and control of interactions between the immune system and the nervous system might be key to the prevention or delay of most late-onset CNS diseases. In Alzheimer's disease, neuroinflammation is not a passive system activated by emerging senile plaques and neurofibrillar tangles, but instead contributes as much (or more) to pathogenesis as do plaques and tangles themselves.[1] The important role of neuroinflammation is supported by findings that genes for immune receptors, including *TREM2*[2] and *CD33*,[3,4] are associated with Alzheimer's disease. Analysis of clinical manifestations that precede the dementia stage of Alzheimer's disease, such as mild cognitive impairment, further support an early and substantial involvement of inflammation in disease pathogenesis. In this Review we provide an overview of the neuroinflammatory landscape during Alzheimer's disease, including associated cell types and mediators, methods used to visualise neuroinflammation, and its clinical presentation and potential treatments.

Cellular players

Microglia

Microglia, the resident phagocytes of the CNS, are ubiquitously distributed in the brain. Microglia constantly use highly motile processes to survey their assigned brain regions for the presence of pathogens and cellular debris, and simultaneously provide factors that support tissue maintenance (figure 1).[5] At the same time, microglia are important players in the maintenance and plasticity of neuronal circuits, contributing to the protection and remodelling of synapses.[6] To some extent, this protective and remodelling action is mediated by release of trophic factors, including brain-derived neurotrophic factor, which contributes to memory formation.[7] Once activated by pathological triggers, such as neuronal death or protein aggregates, microglia extend their processes to the site of injury, and migrate to the lesion, where they initiate an innate immune response (figure 2). Detection of pathological triggers is mediated by receptors that recognise danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). In Alzheimer's disease, microglia are able to bind to soluble amyloid β (Aβ) oligomers and Aβ fibrils via cell-surface receptors, including SCARA1, CD36, CD14, α6β1 integrin, CD47, and Toll-like receptors (TLR2, TLR4, TLR6, and TLR9),[8–11] and this process is thought to be part of the inflammatory reaction in Alzheimer's disease. The Aβ peptide is derived from amyloid precursor protein (APP) by sequential cleavages by two membrane-bound proteases (figure 3).[12,13] The 42-aminoacid form of Aβ has a particularly strong tendency to form soluble oligomers and fibrils. Binding of Aβ with CD36, TLR4, and TLR6 results in activation of microglia, which start to produce proinflammatory cytokines and chemokines (figure 4).[10,14] In turn, genetic deletion of CD36, TLR4, or TLR6 in vitro reduces Aβ-induced cytokine production[10,14,15] and prevents intracellular amyloid accumulation and activation of multiprotein complexes known as inflammasomes.[15]

Microglial Aβ clearance mechanisms

In response to receptor ligation, microglia start to engulf Aβ fibrils by phagocytosis. As a result, these fibrils enter the endolysosomal pathway. By contrast with fibrillar Aβ, which is mostly resistant to enzymatic degradation, soluble Aβ can be degraded by various extracellular proteases.[16] In microglia, the proteases neprilysin and insulin-degrading enzyme (IDE) are of major importance.

In sporadic cases of Alzheimer's disease, inefficient clearance of Aβ has been identified as a major pathogenic pathway.[17] Increased cytokine concentrations, by downregulation of expression of Aβ phagocytosis receptors, are suggested to be responsible for insufficient microglial phagocytic capacity.[18] Further support for the hypothesis of compromised microglial function is provided by two studies[2,3] identifying rare mutations that convey an increased risk of Alzheimer's disease. A rare mutation in the extracellular domain of TREM2 increases risk of Alzheimer's disease to a similar extent to apolipoprotein E (ApoE) ε4.[2] TREM2 is highly expressed by microglia,[19,20] and mediates phagocytic clearance of neuronal debris.[21] Although a TREM2 ligand has not yet been discovered, TREM2 binding activity (putative TREM2 ligand expression) is detected on reactive astrocytes surrounding amyloid plaques and on damaged neurons and oligodendrocytes.[21] Likewise, a single-nucleotide polymorphism (SNP) in the gene encoding the microglial surface receptor CD33 reduces Aβ phagocytosis by peripheral macrophages isolated from heterozygous and homozygous mutation carriers. Additionally, increased Aβ deposition, as shown by Pittsburgh compound B (PiB)-PET, was detected in the brains of carriers of the rs3865444 allele in the CD33 Alzheimer's disease susceptibility locus.[3]

Microglial diversity

Microglia activation is a complex process that results in several phenotypes. Outside the CNS, activated macrophages have been categorised as those with a classic, proinflammatory (M1) phenotype associated with expression of cytotoxic genes,[22] and those with a non-inflammatory, alternative activation (M2) phenotype, associated with induction of specific proteins, including ARG1, FIZZ1, YM1, and IGF1.[23,24] Classic M1 activation is characterised by increased concentrations of pro-inflammatory cytokines, including TNFα, interleukin 1, interleukin 6, interleukin 12, and interleukin 18, and is accompanied by impaired phagocytic capacity.[25] The M2 state is characterised by secretion of the anti-inflammatory cytokines interleukin 4, interleukin 10, interleukin 13, and TGF-β, and increased phagocytic capacity without production of toxic nitric oxide.[26–28] A third phenotype might be a deactivated one associated with corticosteroids or TGF-β.[29,30] The M1 and M2 activation states represent the extremes of myeloid cell activation. Peripheral monocyte-derived macrophages exist in a diverse range of phenotypic states, particularly under conditions of chronic inflammation.[31] Microglia are also likely to exist in a range of phenotypic states during chronic inflammation: these cells have a wide range of phenotypes that are indicative of their response to the local environment, including physical interaction with other cells and their physiological activity in the brain. Importantly, the ability to isolate or image subsets of unperturbed microglia to characterise their gene expression and mode of action as discriminated by physiological markers is restricted at present.

Microglia priming

In the ageing CNS of mice, rats, and primates, microglia show enhanced sensitivity to inflammatory stimuli,[32] similar to that noted in microglia in brains with ongoing neurodegeneration. This phenomenon is termed priming. Priming might be caused by microglial senescence and might be associated with ageing. On the transcriptomic level, endogenous ligands are downregulated during ageing, whereas factors for host defence and neuroprotection are upregulated.[20] To what extent age-related microglia priming results from cell-autonomous cellular ageing, rather than prolonged exposure to the aged neural environment, is uncertain. In physiologically aged and senescence-accelerated mice, profound microglia priming was characterised by increased production of cytokines and reactive oxygen species, and enhanced phagocytic capacity. This model provided proof of principle that environmental effects, such as neuronal ageing, can drive microglia priming.

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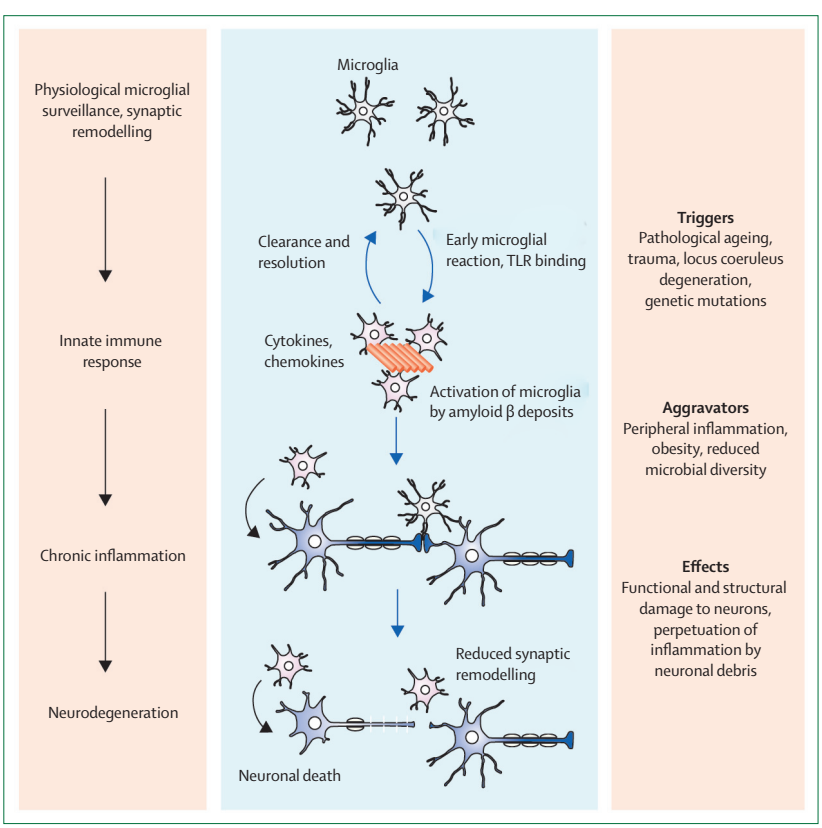

Figure 1: **Pathomechanistic sequelae of microglia activation**
Physiological functions of microglia, including tissue surveillance and synaptic remodelling, are compromised when microglia sense pathological amyloid β accumulations. Initially, the acute inflammatory response is thought to aid clearance and restore tissue homoeostasis. Triggers and aggravators promote sustained exposure and immune activation, which ultimately leads to chronic neuroinflammation. Perpetuation of microglia activation, persistent exposure to proinflammatory cytokines, and microglial process retraction cause functional and structural changes that result in neuronal degeneration. TLR=Toll-like receptor.

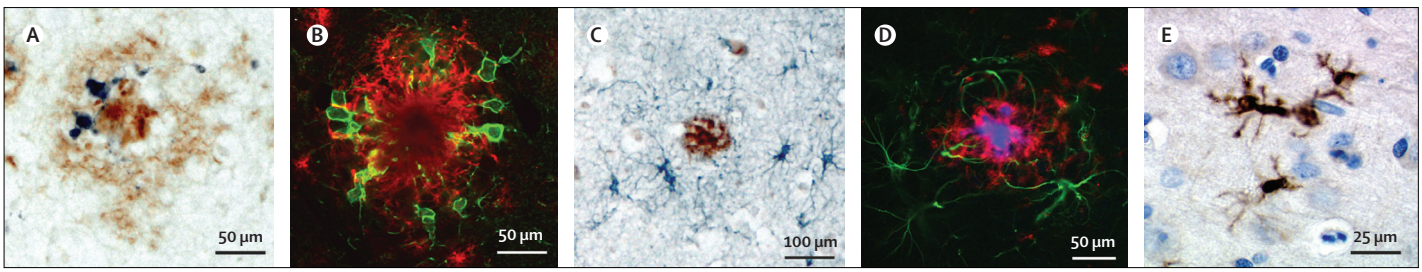

Figure 2: **Changes in microglia and astroglia in Alzheimer's disease**
Microglia and astroglia are key players in the inflammatory response: changes in microglia and astroglia are evident in the post-mortem brains of patients with Alzheimer's disease and in animal models of the disorder. (A) CD11b-positive microglia (blue) within an amyloid β (Aβ) deposit (brown) in the parietal cortex of a brain section from a patient with Alzheimer's disease. (B) Activated, IBA1-positive microglia (green) at an Aβ plaque site (red) in a brain section from an APP/PS1 transgenic mouse. (C) GFAP-positive astrocytes (blue) surround the site of Aβ deposition (brown) in the parietal cortex of a brain section from a patient with Alzheimer's disease. (D) GFAP-positive astrocytes (green) at an Aβ plaque site (red) in a brain section from an APP/PS1 transgenic mouse. (E) Interleukin-1β-positive microglia (brown) in the frontal cortex of a brain section from a patient with Alzheimer's disease.

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Weighted gene correlation network analysis revealed a characteristic pattern of gene expression for microglia priming, featuring increased pattern recognition and expression of interferon signalling genes. A similar gene expression network was reported in mouse models of age-related neurodegeneration, including APP/PS1 transgenic mice.[33] Microglia might also be primed by systemic inflammation in response to peripheral immune reaction.

Modulation of microglia

The emerging role of microglia activation in Alzheimer's disease pathogenesis makes these cells a legitimate therapeutic target. However, depending on the circumstances, microglia activation can have both beneficial and detrimental effects. Thus, microglia might have different roles and effects depending on the particular disease stage and which brain region is affected in each model. After exposure to a DAMP or PAMP, the acute microglial reaction aims to remove the recognised abnormality or pathological change. In the case of Alzheimer's disease, this type of inflammatory reaction is sterile because it involves the same receptors but no living pathogens. Under normal circumstances, such a reaction quickly resolves pathological changes with immediate benefit to the nearby environment. However, in Alzheimer's disease, several mechanisms, including ongoing formation of Aβ and positive-feedback loops between inflammation and APP processing, compromise cessation of inflammation. Instead, further accumulation of Aβ, neuronal debris, and, most probably, further activating factors establish chronic, non-resolving inflammation. Sustained exposure to Aβ, chemokines, cytokines, and other inflammatory mediators seems to be responsible for the persistent functional impairment of microglial cells seen at plaque sites.[40,41] As an intracellular regulator of microglial function, expression of the autophagy protein Beclin 1 is reduced in the brains of patients with Alzheimer's disease.[42] Beclin 1 has a role in retromer-mediated sorting of cellular components, including TREM2, APP, BACE1, and CD36, in the endolysosomal pathway. Reduction of Beclin 1 expression in vitro and in vivo interferes with efficient phagocytosis, resulting in decreased receptor recycling of CD36 and TREM2,[42] but more receptors might be affected.

Plasticity of the microglial phenotype is of fundamental importance, since resolution of inflammation clearly involves conversion to an alternative (ie, similar to M2) activation state associated with tissue repair, phagocytosis, and anti-inflammatory actions. Conversion of microglia from detrimental to beneficial players might be achieved by modulation of proinflammatory signalling pathways such as the NLRP3 inflammasome. Successful modification of these pathways, however, necessitates that they are exclusively restricted to microglia and do not have crucial functions in other cell types. Pharmacologically, transition to an alternative activation state could be achieved through the heterodimeric type II nuclear receptors PPARγ/RXR, PPARδ/RXR, and LXR/RXR. Agonists of these receptors are robustly anti-inflammatory and stimulate phagocytosis through induction of CD36, leading to increased microglial Aβ uptake.[43] Another target is the RXR itself, which might have a positive effect on both LXR-controlled and PPARγ-controlled genes. Agonism of RXR by bexarotene has been shown to cause rapid reduction of soluble Aβ, plaque load, and behavioural deficits by ApoE-dependent clearance of Aβ.[44] Nevertheless, results of this study could not be wholly reproduced by others.[45–48] Although aberrant and ineffective activation of microglia has been fairly well documented for prodromal Alzheimer's disease and moderate Alzheimer's disease, late-stage effects are less well understood. Some evidence exists of focal microglial senescence, especially surrounding neurofibrillary tangles.[40]

Blood-derived mononuclear cells

The precise contribution of blood-derived mononuclear cells infiltrating the CNS in Alzheimer's disease, such as innate immune responses of the brain, is so far unclear, and knowledge is restricted to animal studies. Results of

these animal studies have shown infiltration of peripheral mononuclear cells associated with amyloid plaques in mouse models.[34] Further, ablation of CD11b-positive cells in the APP/PS1 mouse model of Alzheimer's disease showed that peripheral mononuclear phagocytes have an important role to reduce the build-up of Aβ plaques.[34] Restriction of entry of blood-derived mononuclear cells into the brain, by deletion of the chemokine receptor CCR2 in the Tg2576 mouse model, led to increased plaque load,[35] although the mononuclear cell type was not specified. However, most of these studies used bone marrow irradiation and subsequent transplantation with fluorescent, and therefore traceable, cells. Irradiation of whole animals is likely to cause damage to the blood–brain barrier. A further study in which the brain was shielded, thereby limiting irradiation to the rest of the body, did not report any cerebral infiltration by peripheral macrophages, but concluded that perivascular macrophages, protected by shielding of the brain, were able to modulate Aβ deposition depending on the presence of CCR2.[36] Involvement of perivascular macrophages has also been shown for removal of Aβ in a mouse model of cerebral amyloid angiopathy.[37] Nevertheless, recruitment of bone-marrow-derived cells is almost absent in parabiosis mouse models, even 12 months after initiation.[38] Notably, in this context, ablation of microglia in APP/PS1 mice by the HSV thymidine kinase/ganciclovir system did not change the amyloid pathology, although 95% of microglia were lost and blood-derived monocytes were spared by use of bone-marrow-chimeric mice.[39] This result suggests that peripheral cells do not participate in phagocytosis of amyloid plaques, although the observation time was only 2–4 weeks. These results provide evidence against a substantial contribution of blood-derived monocytes, but support the idea that perivascular macrophages have some effect on removal of CNS Aβ depositions.

Astroglia

Pathological responses of astrocytes include reactive astrogliosis, a complex, multistage and pathology-specific reaction, whereas remodelling of astrocytes is generally aimed at neuroprotection and recovery of injured neural tissue.[49,50] Next to activated microglia, hypertrophic reactive astrocytes accumulate around senile plaques and are often seen in post-mortem human tissue from patients with Alzheimer's disease,[51] and in animal models of the disorder.[52] Glial cell activation might occur early in Alzheimer's disease, even before Aβ deposition.[53] Reactive astrocytes are characterised by increased expression of glial fibrillary acidic protein (GFAP) and signs of functional impairment;[54] however, astrocytes do not seem to lose their domain organisation, and no evidence of scar formation exists (figure 2). In animal models of Alzheimer's disease, the early response is marked by astroglial atrophy, which might have far-reaching effects on synaptic connectivity, because astrocytes are central to the maintenance of synaptic transmission, thereby contributing to cognitive deficits.[52,54–57] These signs of

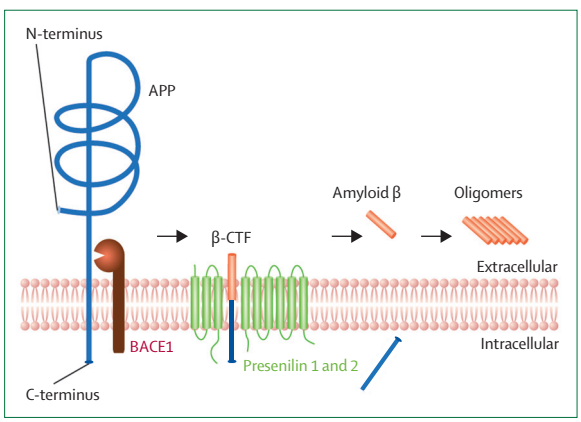

Figure 3: **Amyloidogenic processing of amyloid precursor protein**
Amyloid precursor protein (APP) is a type 1 transmembrane protein that is sequentially cleaved by two aspartate proteases. β-site APP cleaving enzyme 1 (the β-secretase BACE1) cleaves the protein to yield a C-terminal fragment (β-CTF) and secreted soluble peptide APPβ. β-CTF is then processed by presenilin 1 and 2 (part of the γ-secretase complex) to release the amyloid β peptide. The process results in differentially truncated C-termini, ranging from aminoacid 37 to 42. The 42-aminoacid form ($Aβ_{1-42}$) has a particularly strong tendency to form soluble oligomers and fibrils. These Aβ aggregates bind to cell-surface receptors on microglia, inducing an inflammatory activation that results in the secretion of proinflammatory cytokines, including TNFα and interleukin 1β. In this context, it has been shown that interleukin 1β aggragvates plaque formation by modulation of APP expression. Additionally, expression of BACE1 is upregulated by some cytokines, resulting in increased production of Aβ species.

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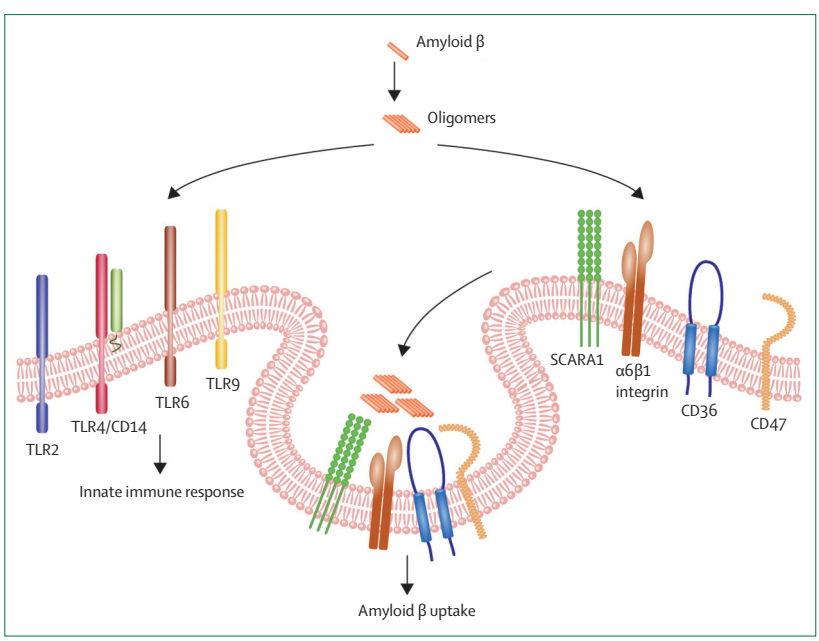

Figure 4: **Activation of microglia by amyloid β**
Amyloid β (Aβ) aggregates (oligomers) act on several Toll-like receptors on the microglial surface, triggering reactions of the innate immune system, including production of proinflammatory cytokines and chemokines. Aβ oligomers are internalised by microglia, aided by SCARA1, α6β1 integrins, CD36, and CD47.

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atrophy show clear spatiotemporal progression, appearing first in the entorhinal cortex, and affecting astrocytes located at a distance from senile plaques in the later stages of Alzheimer's disease. Like microglia, astrocytes release cytokines, interleukins, nitric oxide, and other potentially cytotoxic molecules after exposure to Aβ, thereby exacerbating the neuroinflammatory response. The importance of astroglial inflammation in Alzheimer's disease has been investigated by adeno-associated virus-driven suppression of the astrocytic reaction in APP/PS1 mice. Interference with the calcineurin/NFAT signalling pathway revealed improved cognition, reduced astrogliosis, and decreased Aβ concentrations.[58] Additionally, astrocytes have a potential role in internalisation and degradation of Aβ in vivo.[59] ApoE is needed for astrocyte-mediated clearance of Aβ,[60] and astrocyte-dependent lipidation of ApoE increases the capability of microglia to clear Aβ.[61,62] Furthermore, adult astrocytes upregulate expression of extracellular Aβ-degrading proteases,[63] such as neprilysin, IDE, ECE2, and ACE, after exposure to native Aβ deposits.[64] These proteases, and impaired function and atrophy of astrocytes, might contribute to reduced proteolytic clearance of Aβ. In addition to these clearing pathways, astrocytes have a role in clearance of soluble Aβ from the parenchyma by paravenous drainage.[65] This pathway depends on the astrocytic water channel aquaporin 4; deletion of this channel resulted in a substantial decrease in clearance via this pathway.

Mediators and modulators of neuroinflammation

Cytokines

Microglia and astrocytes are arguably the major source of cytokines in Alzheimer's disease. Cytokines contribute to nearly every aspect of neuroinflammation, including proinflammatory and anti-inflammatory processes, bystander neuronal injury, chemoattraction, and response of microglia to Aβ deposits. Microglia activation is both characterised by and modulated by cytokines. Increases in Aβ concentration in ageing TgAPPsw and PSAPP transgenic mice are associated with increased concentrations of proinflammatory cytokines, including TNFα, interleukin 6, interleukin 1α, and GM-CSF.[66] This observation suggests that pathological accumulation of Aβ is a key factor that drives neuroinflammatory responses in Alzheimer's disease. Additionally, exposure of microglia to pre-aggregated $A\beta_{1-42}$ increases production of pro-inflammatory cytokines (ie, pro-interleukin 1β, interleukin 6, and TNFα), MIP-1α, and M-CSF.[67] Furthermore, M-CSF concentrations in the plasma and CNS of patients at the dementia stage of Alzheimer's disease are substantially increased compared with age-matched healthy controls or patients with mild cognitive impairment.[67,68] Caspase 1 activation, which is needed for cleavage of interleukin 1β from its inactive proforms,[69] is similarly elevated in the brains of patients with mild cognitive impairment and Alzheimer's disease dementia.[70] As a result, high concentrations of the cardinal proinflammatory cytokine interleukin 1β are detected in microglial cells surrounding Aβ plaques in brains of patients with Alzheimer's disease (figure 2) and in CSF of patients. In vitro, interleukin 1β is released by activated microglia after stimulation with Aβ.[71] Interleukin 1β can, at least under some circumstances, favour Aβ deposition by modulation of APP expression and proteolysis.[72] Additionally, interleukin 12 and interleukin 23, which are known to be produced by leucocytes, are produced by microglia in mouse models of Alzheimer's disease,[73] and inhibition of these cytokines reduces Alzheimer's disease-like pathology,[73,74] although regulation of interleukin 12 in human CSF is debated.[73,75]

Evidence suggests that the proinflammatory environment present in the brains of patients with Alzheimer's disease and in transgenic mouse models of cerebral amyloidosis reaches damaging proportions. For example, risk for conversion from mild cognitive impairment to the dementia stage of Alzheimer's disease is increased in patients with elevated concentrations of the proinflammatory cytokine TNFα and decreased concentrations of anti-inflammatory TGF-β in the CSF.[76] Interleukin 1β, TNFα, and other cytokines might impair neuronal function even before leading to structural changes, as shown by suppression of long-term potentiation (LTP) of synaptic transmission. Several interactions, and increased expression of additional cytokines, chemokines, and innate immune receptors, favour an M1-like activation state in Alzheimer's disease. For example, in neuron–microglia co-cultures, the synergistic action of Aβ with either interferon γ or CD40 ligand triggers TNFα secretion and production of neurotoxic reactive oxygen species.[77–79] Additionally, the innate immune receptor TLR4 is responsible for increased concentrations of TNFα and MIP-1α in mouse models of Alzheimer's disease.[80]

Conversely, stimulation of some proinflammatory signalling pathways seems to be beneficial in mouse models of Alzheimer's disease. Transgenic expression of interleukin 1β in APP/PS1 mice led to robust neuroinflammation and reduction of amyloid plaque pathology.[81,82] These findings implicate interleukin 1β expression in activation of a beneficial form of neuroinflammation in APP/PS1 mice. In another study, AAV-mediated expression of interferon γ in the brains of the TgCRND8 mouse model showed the ability of this proinflammatory cytokine to enhance clearance of amyloid plaques, with a widespread increase in astrogliosis and microgliosis.[83] Additionally, these mice had decreased concentrations of soluble Aβ and Aβ plaque burden, without altered APP processing. Similar results were obtained using AAV-mediated expression of interleukin 6 and TNFα.[84,85] Conversely, expression of the anti-inflammatory cytokine interleukin 4 resulted in

exacerbation of Aβ deposition.[86] These results suggest that some beneficial forms of proinflammatory microglia activation potentially help to reduce Alzheimer's disease-like pathology in transgenic mouse models.

Chemokines

Chemokines have been suggested to regulate microglial migration to areas of neuroinflammation, thereby enhancing local inflammation in Alzheimer's disease.[87] In Alzheimer's disease, upregulation of CCL2, CCR3, and CCR5 in reactive microglia has been reported,[88,89] whereas CCL4 has been detected in reactive astrocytes near Aβ plaques.[88] In vitro, Aβ leads to generation of CXCL8 (also known as interleukin 8), CCL2, CCL3, and CCL4 in human macrophages and astrocytes,[90] and microglia cultured from autopsies of patients with Alzheimer's disease revealed increased expression of CXCL8, CCL2, and CCL3 after experimental exposure to Aβ.[91] In mouse models of Alzheimer's disease, modulation of neuronal survival,[92] plaque load,[93] and cognition[94] by the CX3CR1/CX3CL1 system has been shown. Furthermore, the receptors CCR5[95] and CCR2[35,96,97] can modulate the course of disease through effects on microglial position and function.

Caspases

Caspases are a family of intracellular proteases that are key mediators of apoptosis and inflammation. Of the inflammatory caspases, the catalytic activity of caspase 1 is tightly regulated by signal-dependent autoactivation within inflammasomes, which mediate caspase 1 autocatalytic activation and subsequent cleavage of precursors of interleukin 1β and interleukin 18 into bioactive cytokines.[98,99] Aβ fibrils can activate NRLP3 inflammasomes via lysosomal damage in mouse microglia.[100] Increased concentrations of active caspase 1 are detected in the brains of patients with Alzheimer's disease and APP/PS1 mice. Additionally, APP/PS1 mice deficient in NLRP3 or caspase 1 are mostly protected from spatial memory impairment, loss of hippocampal synaptic plasticity, associated behavioural disturbances, and other effects associated with Alzheimer's disease.[70] Deficiency of NLRP3 or caspase 1 in APP/PS1 mice seemed to shift microglial cells from a proinflammatory M1-like phenotype to a more neuroprotective M2-like phenotype.[70] Further, stimulation of microglia with various proinflammatory mediators led to orderly activation of apoptotic caspase 8 and caspase 3/7. Activated caspase 3 modulates NF-κB activation via PKCδ and increases production of neurotoxic proinflammatory mediators, such as interleukin 1β, TNFα, and nitric oxide. Inhibition of these caspases hindered microglia activation and neurotoxicity.[101] Incidentally, these caspases were activated in microglia in patients with Alzheimer's disease.[102] Pharmacological interventions with inhibitors of activated caspases have been reported to successfully exert neuroprotective effects in mouse models of Alzheimer's disease.[103,104]

Prostanoids and neuroprotectin D1

Prostanoids are derivatives of arachidonic acid synthesised by cyclooxygenase 1 and inducible cyclooxygenase 2, both of which are produced by microglia. In Alzheimer's disease, the suppressive effect of cyclooxygenase 1 inhibition on glia activation, amyloid deposition, and expression of inflammatory markers—switching microglia to an alternative phenotype—has been shown in a mouse model of Alzheimer's disease.[105] Additionally, concentrations of the proinflammatory prostaglandin PGE2, which binds to PTGER1–4 receptors, has proved to be elevated in patients with probable Alzheimer's disease.[106] PTGER1–3 receptors are expressed by microglia,[107] but are also expressed in other cells of the brain, particularly neurons. Microglial PTGER2 receptors inhibit Aβ phagocytosis and enhance neurotoxic activities of microglia in vitro.[108] This effect is complemented by findings that deletion of PTGER2 or PTGER3 receptors in mouse models of Alzheimer's disease decreased oxidative stress, neuroinflammation, Aβ burden, and BACE1 expression.[109–111]

Use of PTGER4 receptor agonist on microglia showed suppression of inflammation and increased uptake of synthetic Aβ, whereas deletion of PTGER4 receptor in the APP/PS1 mouse model of Alzheimer's disease increased plaque burden and production of proinflammatory cytokines such as interleukin 1β and CCL3.[112] Notably, expression of PTGER4 receptor was decreased in the cortex of patients with mild cognitive impairment and Alzheimer's disease,[112] suggesting that it might contribute to the inflammatory reaction in Alzheimer's disease. However, the role of PGE2 in neurodegeneration is probably complex owing to effects of PGE2 on other cell types such as neurons.

The neuroprotective docosahexaenoic acid derivative neuroprotectin D1 (also known as 10R,17S-DHA) is a major component of cell membranes,[113] and expression is decreased in early stages of Alzheimer's disease.[114] Neuroprotectin D1 is an autocrine/paracrine mediator of the resolution response during early stages of neuroinflammation, and downregulates amyloidogenic processing of APP, switches off proinflammatory gene expression, and promotes neural cell survival. Moreover, anti-amyloidogenic processing by neuroprotectin D1 targets α secretases and β secretases and PPARγ receptor activation. sAPPα, a peptide with neurotrophin-like activity, is an agonist for neuroprotectin D1 synthesis and is part of a cycle that sustains generation of the lipid mediator.[115]

Complement system

The complement system is a major constituent of the innate immune system, mainly involved in defence against pathogens. Activation of the proteolytic complement cascade results in opsonisation and, ultimately, in lysis of microorganisms. In the brain, the major cells that contribute to production of proteins of

the complement system are microglia and, to a lesser extent, astrocytes.[116] In Alzheimer's disease, activated factors of the complement system are associated with Aβ deposits.[117] Additionally, Aβ is able to activate the complement system in vitro via the so-called alternative pathway. The finding that variants of clusterin (apolipoprotein J), as a soluble inhibitor of the complement system, and the complement receptor CR1, involved in processing and clearance of opsonised immune complexes and a regulator of C3 convertase activity, are associated with Alzheimer's disease provides further evidence for the importance of the complement system in disease pathogenesis.[118,119]

Nitric oxide and reactive oxygen species

In addition to their direct actions via surface receptors, cytokines stimulate inducible nitric oxide synthase (iNOS) in microglia and astroglia, producing high concentrations of nitric oxide that can be toxic to neurons. iNOS is upregulated in brains of patients with Alzheimer's disease,[120] and genetic knockout of iNOS is protective in mouse models of Alzheimer's disease.[121] Likewise, NADPH oxidase (PHOX) is highly expressed by microglia, upregulated in Alzheimer's disease, and rapidly activated by inflammatory stimuli such as Aβ, resulting in production of hydrogen peroxide, which further promotes microglia activation.[122,123] Superoxide from PHOX reacts with iNOS-derived nitric oxide to form peroxynitrite.[124] Increased expression of iNOS in patients with Alzheimer's disease introduces post-translational modifications caused by nitric oxide,[125] which include nitration, S-nitrosylation, and dityrosine formation.[125] Nitration of the Aβ peptide at tyrosine 10 has been shown to increase the propensity of Aβ to aggregate and has been identified in the core of amyloid plaques.[126] More compelling, this modified peptide was able to initiate plaque formation in APP/PS1 mice, suggesting that it has a central role during the early phase of Alzheimer's disease. Nitrated Aβ suppressed hippocampal LTP more effectively than did non-nitrated Aβ, suggesting that this post-translational modification leads to functional and structural damage in the brains of patients with Alzheimer's disease. Evidence suggests that oxidative stress supports formation of this Aβ species.[127] Other nitric oxide-mediated modifications that might be relevant for Alzheimer's disease have already been reported,[128] and more are expected to follow.

Inflammatory changes of the neurovascular unit

Results of many epidemiological, clinical, and neuropathological studies have shown that vascular pathological change is an important risk factor for development of Alzheimer's disease. Moreover, Alzheimer's disease is associated with distinct inflammatory, functional, and morphological alterations of cerebral blood vessels and perivascular glia and neurons (the neurovascular unit). These early-onset and progressive changes, which are induced by combined effects of soluble Aβ oligomers and vascular Aβ deposits,[129,130] ultimately lead to decreased cerebral blood flow and impaired functional hyperaemia (ie, the ability of local blood flow to increase in response to neuronal activation).[131] Chronic cerebral hypoxia is further amplified by blood-borne factors such as platelets, which are chronically activated in models of, and patients with, Alzheimer's disease,[132] ultimately resulting in microinfarcts and neuronal injury. Moreover, the combination of mild hypoxia, inflammation of the neurovascular unit, and progressive Aβ accumulation in brain parenchyma, induces upregulation of AGER (also known as RAGE), which mediates Aβ transport into the brain across the blood–brain barrier.[133] Additionally, hypoxia directly induces amyloidogenic APP processing through several pathways involving β secretase, γ secretase, neprilysin, and others.[134] Taken together, chronic hypoxia in Alzheimer's disease directly induces neuronal injury, but also amplifies neurodegeneration by induction of amyloidogenic pathways and reduction of brain clearance of Aβ.

Factors that drive neuroinflammation

Aβ deposition alone might be sufficient to induce an inflammatory reaction that subsequently contributes to cognitive decline and development of Alzheimer's disease. In view of the possibility that Aβ deposition precedes cognitive deficits or clinical manifestation by decades, one might speculate that exogenous or endogenous factors can modify the innate immune response mounted by Aβ-exposed microglia. Thus, environmentally modifiable Alzheimer's disease risk factors, including systemic inflammation, obesity, and traumatic brain injury, might affect risk through sustained neuroinflammatory drive.

Systemic inflammation

Development of sickness behaviour[135] after a peripheral inflammatory challenge, such as an infection or an aseptic injury,[136] shows the communication between systemic inflammation and the brain. Sickness behaviour in response to an acute event is usually self-limited as a result of the presence of several regulatory mechanisms that dampen the central inflammatory response to peripheral challenge.[137] However, the inflammatory response to chronic, low-grade inflammation might be prolonged,[138] possibly because no anti-inflammatory response occurs.[139] Neuroinflammation in Alzheimer's disease is such a chronic reaction, because microglia might already be primed and are therefore highly responsive to further activation, causing a rapid switch to a damaging M1 phenotype.[23,140] This microglia priming is likely to result from various activators, such as chronic exposure to Aβ, neuronal debris, and chronic vascular changes, including cerebrovascular dysregulation and cerebral microinfarcts. This hypothesis is supported by results of animal studies

showing an exaggerated inflammatory and oxidative stress response to peripheral stimuli in aged mice,[141] increased concentrations of interleukin 1β in the CNS, and neuronal apoptosis in the ME7 prion mouse after peripheral challenge with lipopolysaccharide or polyinosinic-polycytidylic acid.[142–144] Other examples are the effects of osteoarthritis in APP/PS1/Col1-IL1βXAT mice, resulting in accelerated neuroinflammation and Aβ pathology.[145] Additionally, results of clinical studies of Alzheimer's disease show increased cognitive decline and exacerbation of sickness behaviour after acute and chronic systemic inflammation.[146,147]

Obesity

Obesity is defined as a medical disorder in which excess body fat has accumulated to the extent that it might have an adverse effect on health. Obesity increases a patient's propensity to acquire bacterial or viral infections, and thus directly increases the likelihood of systemic inflammation.[148,149] Moreover, white fat tissue has a high percentage of activated macrophages, which constantly secrete proinflammatory cytokines.[150] Notably, midlife obesity has been identified as a risk factor for Alzheimer's disease,[151] which is related to the fact that other Alzheimer's disease risk factors, such as high-cholesterol diet, reduced physical activity, and sedentary lifestyle, are associated with obesity.[152] As a possible result of obesity, type 2 diabetes accelerates memory dysfunction and neuroinflammation in a mouse model of Alzheimer's disease.[153] Obesity-associated reduced gut microbial diversity was associated with increased concentrations of proinflammatory markers in peripheral blood, and thus could be viewed as a factor contributing to risk for Alzheimer's disease.[154] Taken together, the evidence suggests that obesity increases the risk for Alzheimer's disease by the systemic and chronic presence of proinflammatory cytokines.

Traumatic brain injury

Several studies have established traumatic brain injury as a risk factor for development of Alzheimer's disease.[155] Experimentally, traumatic brain injury aggravates learning and memory deficits and deposition of Aβ in mouse models of Alzheimer's disease.[156,157] Results of animal and human studies have shown that microglia activation can persist for months or years after traumatic brain injury.[158,159] This inflammatory reaction might initially be important for phagocytic clearance of debris. However, sustained cerebral inflammation might either directly or indirectly promote development of Alzheimer's disease. Some cytokines implicated in traumatic brain injury can potentially increase BACE1 concentrations,[160] thereby shifting APP processing to amyloidogenic generation of Aβ (figure 3).[161] Additionally, chronic release of cytokines might decrease the capability of microglia to phagocytose and degrade Aβ, or might directly affect neuronal functions.

Locus coeruleus degeneration

Noradrenaline, in addition to its role as a neurotransmitter, has potent anti-inflammatory, anti-oxidative, neurotrophic, and neuroprotective actions.[162] The main source of noradrenaline in the brain is the locus coeruleus (LC), located at the dorsal part of the brain stem. The LC neurons project throughout the brain, although most terminals target the hippocampus and neocortex. Noradrenaline released from LC projections acts on adrenergic receptors expressed on neurons, microglia, and astrocytes.[162] The number of cells in the LC, and concentration of noradrenaline in the brain, decrease during normal ageing,[163] although more pronounced cell loss occurs in patients with Alzheimer's disease.[164] Experimental lesions of the LC in mouse models of Alzheimer's disease led to increased inflammation and neuronal damage, and an increase in Aβ plaque burden.[165,166] Thus, early degeneration of the LC and subsequent loss of noradrenaline-mediated innervation could substantially promote the inflammatory response to any stimulus, including Aβ. Experimental loss of noradrenaline compromised microglial migration and Aβ phagocytosis in vivo, suggesting that a loss of noradrenaline tone increases not only inflammation, but also Aβ deposition. Selective noradrenaline reuptake inhibitors,[167] α_2-adrenoceptor antagonists,[165] and the noradrenaline precursor L-threo-3,4-dihydroxyphenylserine,[168] which increase endogenous noradrenaline concentrations, can reduce neuroinflammation and partly rescue microglial functions.

Analysis of immune activation

In-vivo laser-scanning microscopy

During the past few decades, analysis of innate immunity of the brain was restricted to cell culture experiments and immunohistochemical detection of microglia. Little was known about the functional state of these cells in vivo. Methodological advances such as generation of transgenic mice with enhanced-GFP-labelled microglia, cranial window implantation, and Aβ plaque labelling with the fluorescent dye methoxy-XO4 have enabled longitudinal and live monitoring of the functional state of microglia in mouse models of disease. This technique enables analysis of microglial phenotypes over time and in relation to deposition of Aβ. Studies have shown that, in control and plaque-bearing mice, microglia migrate within the brain parenchyma with an average speed of 5–9 mm/month.[169] After formation of Aβ plaques, microglia rearrange their processes, becoming polarised, and move their somata towards the plaque, temporarily leaving the formerly surveyed area.[169] Individual microglia migrate towards new or pre-existing plaques within 1–2 days,[169,170] similar to observations made after acute laser-induced brain injury.[171] In addition to Aβ deposits, microglia are attracted to neurons that have undergone Alzheimer's disease-associated elimination.[92] Of note, microglial cells were recruited to neurons up to 7 days before their elimination, and neuronal loss could be

completely rescued by knockout of the receptor for chemokine fractalkine (CX3CR1).[92]

Imaging of inflammation in animals

Various molecular imaging techniques are used in laboratory and clinical settings to study the temporal and spatial relation of inflammatory changes associated with neurodegeneration. Because microglia respond quickly to lesions, these cells are good candidates for diagnostic markers of disease progression.[172] Therefore, several microglial cell-surface and mitochondrial receptors were used for development of in-vivo imaging ligands. One of these targets is the translocator protein TSPO,[173] a protein of the outer mitochondrial membrane that is increasingly expressed under conditions of neuroinflammation. Binding of the radiolabelled ligands ^{11}C-(R)-PK11195 and ^{18}F-DPA-714 to TSPO can be visualised using PET and SPECT.[174] In mouse models of Alzheimer's disease, progressive binding of PK11195 with age has been described.[175,176] This binding could be reliably detected by immunohistological means in APP/PS1 mice only at a late stage, when microglia and astroglia activation is already high.[177] Binding of both ^{3}H-(R)-PK11195 and ^{3}H-DPA-713 could be decreased by the PPARγ agonists pioglitazone and ciglitazone in the TASTPM mouse model,[176] and have anti-inflammatory and Aβ-lowering effects in mouse models of Alzheimer's disease.[178] Development of new TSPO ligands and other probes with improved bioavailability, decreased non-specific uptake, and increased specific binding[179,180] might enable detection of activated microglia at an even earlier disease stage.

Imaging of inflammation in human beings

Similar to results of studies in animals, increased microglia activation has been detected in patients with Alzheimer's disease by use of the TSPO ligand ^{11}C-PK11195. Binding potentials are reported to be increased up to 50% in association cortex.[181] Uptake of another TSPO ligand, ^{11}C-DAA1106, was reported to be increased by up to 33% in patients with Alzheimer's disease.[182] Cortical distribution of ^{11}C-PK11195 binding parallels that of amyloid deposition, as detected by the thioflavin analogue ^{11}C-PIB.[183] Concentrations of cortical ^{11}C-PK11195 signals in patients with Alzheimer's disease are likewise associated with cognitive impairment rated with the Mini-Mental State Examination,[184,185] suggesting that cortical microglia activation is detrimental to cognitive function. Additionally, a relation of neuroinflammation and the severity of Alzheimer's disease has been reported for the TSPO marker ^{11}C-PBR28.[186] In patients with mild cognitive impairment, ^{11}C-PK11195 PET detected inflammation in 40% of amnestic cases,[187] although another study with the same tracer failed to detect microglia activation. Seven patients with mild cognitive impairment showed a significant 27% mean increase in ^{11}C-DAA1106 uptake in the lateral temporal cortex, compared with controls (p=0·008).[182] Five of these seven patients with mild cognitive impairment, with ^{11}C-DAA1106 uptake more than 0·5 SDs above the mean in controls, progressed to dementia during a 2-year follow-up period.

Characterisation and monitoring of neuroinflammation in Alzheimer's disease

Although emerging evidence suggests that inflammation has a causal role in Alzheimer's disease pathogenesis, detection of inflammatory markers has not yet been established as a valuable method for diagnosis or monitoring of Alzheimer's disease. Nevertheless, novel data from a gene-expression analysis of post-mortem brains from patients with late-onset Alzheimer's disease highlighted an immune and microglia network dominated by genes implicated in phagocytosis.[188] These data, together with analysis of inflammation-related biomarkers in the CSF, peripheral blood, or directly in the brain by imaging, will be the focus of future studies. An important aspect of this search will be discovery of inflammatory biomarkers that can be used to identify prodromal stages of Alzheimer's disease (panel).

Detection of neuroinflammatory markers in CSF

During the past 25 years, several studies have investigated concentrations of proinflammatory and anti-inflammatory cytokines in the CSF of patients with mild cognitive impairment and Alzheimer's disease. Results of these studies are often debated;[189] however, the timepoint of sampling—that is, the stage of the disease—seems to be a crucial factor for investigation. Some studies highlight increased concentrations of cytokines in the CSF as risk factors for conversion of mild cognitive impairment to the dementia stage of Alzheimer's disease or as markers of the speed of cognitive decline and disease progression.[76,190] As with imaging consortia, to overcome inter-individual differences and to obtain a definite description of cytokine regulation and function in Alzheimer's disease, a high degree of method harmonisation and patient characterisation, together with longitudinal sampling over years, seems to be essential to progress beyond cross-sectional descriptions.

Systemic biomarkers

Results of a growing number of studies suggest that a sophisticated interaction occurs between the systemic environment and the brain. Thus, systemic immune cells and secreted signalling proteins communicate with the brain, and have been associated not only with neuroinflammation, but also with neurodegenerative processes in general.[191] Although some of these interactions might involve cells entering the nervous tissue, many more are likely to be mediated by soluble signalling molecules, such as blood-borne factors, present in the systemic environment. These factors can inhibit or promote adult neurogenesis in an age-

dependent manner or restore regeneration of the ageing brain in mice.[192,193] To identify such factors, scientists have tried to discover molecular or cellular changes in blood associated with neurodegenerative diseases.[194] Various proteomic methods have been used to identify blood-based biomarkers. Cytokines and trophic factors such as BDNF are typical biomarkers.[195–198] Using multiplex ELISA in plasma from controls and patients with mild dementia, mild cognitive impairment or Alzheimer's disease, protein signatures were described that might be specific to prodromal stages of the disease,[199] or that characterise patients who progress from a prodromal stage to the dementia stage of Alzheimer's disease.[200] Other signatures seem to correlate with ApoE[201] or with pathological changes such as Aβ and tau protein concentrations in CSF of patients with Alzheimer's disease.[202] About 200 communicome proteins were measured in plasma samples from patients participating in the Alzheimer's disease neuroimaging initiative, yielding protein signatures associated with patients who converted from mild cognitive impairment to the dementia stage of Alzheimer's disease.[203]

Clinical trials and epidemiological findings

Anti-inflammatory drugs

Non-steroidal anti-inflammatory drug (NSAID) epidemiology and clinical trial results (table)[204–219] have produced some healthy scepticism about apparent stage-dependent outcomes, but the disappointing results of these studies are perhaps not surprising when one considers that normal physiological cytokine regulation of glia activation and microglial phenotypes is highly context-dependent and stage-dependent.[5] Contextual factors modulating glial-activated phenotypes include immune-modulatory *APOE* genotype and newly identified Alzheimer's disease genes. Normal ageing is likewise associated with chronic activation of glia[220] and focal stage-dependent injury-induced factors. Context-dependent responses should be expected for NSAIDs that act as cyclooxygenase inhibitors to reduce concentrations of prostaglandin products—notably PGE2—which act through PTGER1–4 receptors to produce very different outcomes. For example, PTGER2 activation predominantly engages proinflammatory neurotoxic pathways and downregulates Aβ phagocytosis, whereas PTGER4 ligands can produce anti-inflammatory and neuroprotective effects.[221,222] Most importantly, these receptors have a role in promotion of resolution of chronic neuroinflammation.[223] Thus, conventional cyclooxygenase-inhibiting NSAIDs could block incipient inflammation-driven Alzheimer's disease pathogenesis at early stages. Additionally, these NSAIDS can have adverse effects in advanced disease, potentially by restriction of resolution and interference with phagocytic clearance of Aβ and extracellular tau aggregates.

***Panel:* Recommended steps to advance and harness understanding of neuroinflammation in Alzheimer's disease**

Develop new animal models
New models should recapitulate multiple facets of Alzheimer's disease and should not be restricted to transgenic expression of human mutations of familial Alzheimer's disease. In addition to amyloid β and tau pathology, models should include aspects of multiple neurotransmitter loss, disease spreading, and late onset of disease. Ideally, new models would show Alzheimer's disease-like vascular pathology, synaptic destruction, and neuronal loss. Future experiments with animal models should take into account disease modifiers such as systemic inflammation, insulin resistance, brain trauma, nutritional states, physical inactivity, and obesity.

Identify biomarkers of the inflammatory component
New biomarkers could be developed for disease diagnosis, and for monitoring of preventive and therapeutic strategies. Such biomarkers could be blood-based, CSF-based, or imaging-based, and enable discrimination of acute from chronic neuroinflammation.

Define pathologies and periods of neuroinflammation
Understanding is needed of the contributions of microglia, macrophages, astrocytes, neurons, and endothelial cells during the course of Alzheimer's disease. These insights could help to identify which inflammatory processes are protective, which are harmful, and which are relevant for disease pathogenesis at different stages of Alzheimer's disease.

Exploit effects of mutations, epigenetics, and the microbiome on neuroinflammation
Discoveries that suggest a direct immune-related modification of the onset, progress, and phenotype of Alzheimer's disease, including single-nucleotide polymorphisms (SNPs) in immune-associated genes, epigenetic immune regulation, and the effect of the microbiome on innate immunity, will be important to consider.

Design observational studies and preventive clinical trials to target immune response
Observational studies should closely monitor the clinical course of patients with SNPs in immune-related genes that have been associated with increased risk of Alzheimer's disease. Clinical trials could include preventive trials of strategies to inhibit detrimental aspects of neuroinflammation before any cognitive decline or to foster beneficial immunity, relevant to non-steroidal anti-inflammatory drug epidemiology.

Stage-dependent efficacy has likewise been suggested for anti-Aβ immunotherapy that stimulates microglial phagocytosis of Aβ, and potential benefits might be seen only with early intervention. A plausible argument is that ageing or Aβ-induced or injury-induced inflammation initiates tauopathy to drive neurodegeneration and downstream clinical decline. Thus, possible explanations for failure of immunotherapy or anti-inflammatory therapy to treat established dementia include an inability to halt the spread of fully established and seeded tauopathy and to rescue deficits driven by neuron loss. Whatever the explanations for past NSAID trial failures are, on the basis of compelling new genetic evidence for a causal role for innate immunity in Alzheimer's disease risk, new trials with both longer and earlier interventions and alternative approaches to favourably modulate neuroinflammation are warranted.

Interventional anti-inflammatory trials

Randomised trials with NSAIDs in patients with Alzheimer's disease show some evidence of success

	Drug	Participants	Treatment duration*	Primary endpoint	Finding
Rogers et al (1993)[204]	Indometacin 100–150 mg daily versus placebo	28 patients with AD dementia randomised 1:1	6 months	Cognitive trajectory on a battery of psychometric tests	Positive effects (after 36% attrition; p<0·003)
De Jong et al (2008)[205]	Indometacin 100 mg daily with omeprazole versus placebo	51 patients with mild-to-moderate AD randomised 1:1	1 year	Change in score on ADAS-Cog	Neutral-to-positive effects (after 25% attrition; not significant)
Aisen et al (2000)[206]	Prednisone (20 mg once daily tapered to 10 mg) versus placebo	138 patients with AD randomised 1:1	1 year	Change in score on ADAS-Cog	Neutral-to-negative effects (worsening of secondary endpoint behavioural measures; not significant)
Aisen et al (2003)[207]	Naproxen sodium 220 mg twice daily or rofecoxib 25 mg once daily versus placebo	351 patients with mild-to-moderate AD (MMSE score 13–26)	1 year	Change in score on ADAS-Cog	Neutral-to-negative effects, greater decline in rofecoxib group (p=0·09 after adjustment for multiple comparisons)
Aisen et al (2002)[208]	Nimesulide 100 mg twice daily versus placebo	40 patients with AD randomised 1:1	3 months	Composite of cognitive, behavioural, and functional outcomes	No apparent effect
Reines et al (2004)[209]	Rofecoxib 25 mg once daily versus placebo	692 patients with mild-to-moderate AD randomised 1:1	1 year	ADAS-Cog, CIBIC+	Trend towards negative effects (after 30% attrition)
Thal et al (2005)[210]	Rofecoxib 25 mg once daily versus placebo	1457 patients with MCI randomised 1:1	3·5 years	Change in status from MCI to AD dementia	Increased progression to AD dementia in rofecoxib group (p=0·011); no effects on secondary outcomes
Van Gool et al (2001)[211]	Hydroxychloroquine (200–400 mg once daily by body weight) versus placebo	168 patients with mild AD randomised 1:1	18 months	Functional status questionnaire, ADAS-Cog, behavioural symptoms	No apparent effect
ADAPT Research Group (2007 and 2008)[212,213]	Celecoxib 100 mg twice daily or naproxen sodium 220 mg twice daily versus placebo	2528 healthy individuals with family history of AD randomised 1:1:1.5	1–3 years	Onset of AD	Trend towards negative effects
ADAPT Research Group (2007 and 2008)[212,213]	Celecoxib 100 mg twice daily or naproxen sodium 220 mg twice daily versus placebo	2528 healthy individuals with family history of AD randomised 1:1:1.5	1–3 years	Cognitive decline on battery of neuropsychological tests	Trend towards negative effects
Simons et al (2002)[214]	Simvastatin up to 80 mg per day as tolerated versus placebo	44 patients with AD randomised 1:1	26 weeks	CSF biomarkers $A\beta_{1-40}$ and $A\beta_{1-42}$	No apparent effect
Sparks et al (2005)[215]	Atorvastatin 80 mg once daily versus placebo	67 patients with mild AD randomised 1:1	1 year	ADAS-Cog, CGI (co-primaries), LOCF analysis	Trend towards positive effects
Feldman et al (2010)[216]	Atorvastatin 80 mg once daily versus placebo	640 patients with mild-to-moderate AD (MMSE 13–25) randomised 1:1	72 weeks	ADAS-Cog, CGI (co-primaries)	No apparent effect
Harrington et al (2011)[217]	Rosiglitazone 2 mg or 8 mg daily	2981 patients with mild-to-moderate AD randomised 1:1	48 weeks	ADAS-Cog, CDR sum of boxes	No apparent effect
Breitner et al (2011)[218]	Celecoxib 100 mg twice daily or naproxen sodium 220 mg twice daily versus placebo	Follow-up of 2071 participants randomised in ADAPT	Follow-up 2–4 years after termination of treatments	Onset of AD, CSF tau, plasma tau, and CSF $A\beta_{1-42}$	No apparent effect for celecoxib, possible positive effects for naproxen (including reduced ratio of CSF tau to $A\beta_{1-42}$)
ADAPT Research Group (2013)[219]	Celecoxib 100 mg twice daily or naproxen sodium 220 mg twice daily versus placebo	Follow-up of 1537 participants randomised in ADAPT	Follow-up 5–7 years after termination of treatments	Onset of AD	No apparent effect

AD=Alzheimer's disease. ADAS-Cog=Alzheimer Disease Assessment Scale-cognitive portion. MMSE=Mini-Mental State Examination. CIBIC+=Clinician Interview-Based Impression of Change plus Caregiver Input. MCI=mild cognitive impairment. ADAPT= Alzheimer's Disease Anti-inflammatory Prevention Trial. Aβ=amyloid β. CGI=Clinician Global Impression. LOCF=last observation carried forward. CDR=Clinical Dementia Rating. *Duration of follow-up is given for participants followed after randomisation in the ADAPT trial.[218,219] We searched PubMed for publications up until 2014 using the search terms "anti-inflammatory", "Alzheimer" and "trial" for randomised controlled trials published in English. The table is not an exhaustive list of studies, but provides a list of trial results that have, in our view, had a notable effect on the direction of subsequent research. Priority was given to trials with sufficient power to give a meaningful result, definition of clinical outcomes, and specification of design method to enable firm conclusions to be drawn (including inference of uncertainty). Two trials[204,205] were included because of their effect on later work, despite the fact that they failed to meet the aforementioned criteria.

***Table*: Selected clinical trials of anti-inflammatory drugs in patients with Alzheimer's disease**

(table). Early trials with indometacin that suggested reduced cognitive decline[204] were not replicated,[205] and large-scale trials with other NSAIDs seemed to be unsuccessful.[207,209] Randomised trials with other anti-inflammatory drugs, including prednisone,[206] hydroxychloroquine,[211] simvastatin,[214] atorvastatin,[215,216] aspirin,[224] and rosiglitazone,[217] likewise showed no clinically significant changes in primary cognitive outcomes in patients with prodromal symptoms or Alzheimer's disease dementia. However, although a large randomised study of the NSAIDs naproxen and celecoxib initially reported a detrimental effect for both,[212] a longer-term follow-up of these patients suggested that timing and choice of specific NSAID might be key.[218] Thus, the early detrimental effects were mostly in a small group of patients with early cognitive impairment and, in keeping with epidemiological studies, naproxen seemed thereafter to be protective in patients who had been asymptomatic at baseline.[218]

Immunisation in patients and mouse models of Alzheimer's disease

In some (but not all) studies of effects of immunisation, preponderance of M1 and M2 phenotypes has been reported in response to specific conditions and cytokine exposures. With ageing, Aβ-depositing mice increase expression of alternative activation state genes and deactivation state genes at the expense of classic activation state genes.[225] However, Jimenez and colleagues[226] show age-associated increased expression of mRNA for TNFα, interleukin 1 and iNOS, but also interleukin 4, interleukin 10 and TGF-β, which they interpret as a shift from an alternative to classical activation state. When these mouse models are treated with antibodies, a shift in the activation state occurs over the course of treatment. Initial studies[227] noted reciprocal changes in markers such as kinases, with MAP kinase p38 declining and MAP kinase p44/42 increasing during antibody treatment. Subsequent work using mRNA markers to distinguish between the M1 and M2 phenotypes identified a transition from an M2 phenotype before treatment to an M1 phenotype after antibody treatment.[225] Similar shifts in microglia activation states seem to be associated with Alzheimer's disease in vaccination studies. Compared with tissues from non-vaccinated patients with Alzheimer's disease, tissue from vaccinated patients has reduced staining for several microglial markers, including the scavenger receptor SCARA1 and Fcγ receptor, and deposited Aβ.[228] Cases coming to autopsy within 2 years of the vaccination also showed increased levels of microhaemorrhage and vascular Aβ deposits, plus appearance of phagocytic microglia.[229] Similar outcomes were previously seen in aged mice treated with antibodies.[230] In the phase 2 and phase 3 trials with the antibody bapineuzumab, events referred to as Aβ-related imaging abnormalities were seen on MRI scans.[231] To some extent, these abnormalities in mouse models could be diminished by reduction of antibody affinity for Fcγ receptors via deglycosylation,[232] suggesting that the microglia activation caused by the antibody–antigen interaction might have a role in these vascular responses to immunotherapy.

Conclusions and future directions

Evidence exists that neuroinflammation might drive the pathogenic process in Alzheimer's disease. The brain can no longer be viewed as an immune-privileged organ, and advances in immunology need to be integrated into the known pathogenic pathways of diverse neurodegenerative disorders. The ligand–receptor interactions in the CNS microenvironment that keep microglia under tight control in the healthy brain are perturbed in chronic neurodegenerative disease, but when and how this occurs in Alzheimer's disease is unclear. Although the simple idea of activated microglia has been a useful one, it has no doubt hindered understanding and recognition of the diversity of microglial phenotypes and the extraordinary plasticity of these cells. An important goal of future studies will be to better understand the individual contributions of microglia and other cell types to the neuroinflammatory response during the course of Alzheimer's disease (panel). Other priorities include development of animal models that recapitulate several facets of the disease. The scarcity of methods to assay the different states of microglia activation in vivo adds to the difficulty of understanding the role of neuroinflammation in the human CNS at present. Improved ligands to target microglial activation for PET or other imaging modalities will be key to progress.

The innate immune cells of the brain respond rapidly to systemic events, and these responses are exaggerated in the ageing and diseased brain. In future studies, the effect of systemic comorbidities of Alzheimer's disease (such as diabetes and hypertension), associated systemic inflammation, and ageing as a major risk factor for Alzheimer's disease, should be considered in efforts to understand and exploit the immunological processes associated with the disease (panel). Recognition that modification of the immune system contributes to pathogenesis of chronic neurodegenerative diseases opens many potential routes to delay their onset and progression.

Search strategy and selection criteria

We searched PubMed for journal articles published in English between Jan 1, 2009, and Oct 31, 2014, for the terms "neuroinflammation" or "inflammation" and "Alzheimer", and included those papers judged to be most relevant to the focus of this Review. Additionally, we identified and included older papers with ground-breaking findings that led to recent research, using PubMed and by searches of the authors' own files and the reference lists of selected papers.

Contributors
All authors provided sections of text covering their area of expertise and participated in the proofreading and discussion. MTH and MPK wrote the manuscript and drafted the figures.

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