nature medicine

REVIEW

Inflammation in Alzheimer disease: driving force, bystander or beneficial response?

Tony Wyss-Coray

Alzheimer disease is a progressive dementia with unknown etiology that affects a growing number of the aging population. Increased expression of inflammatory mediators in postmortem brains of people with Alzheimer disease has been reported, and epidemiological studies link the use of anti-inflammatory drugs with reduced risk for the disorder. On the initial basis of this kind of evidence, inflammation has been proposed as a possible cause or driving force of Alzheimer disease. If true, this could have important implications for the development of new treatments. Alternatively, inflammation could simply be a byproduct of the disease process and may not substantially alter its course. Or components of the inflammatory response might even be beneficial and slow the disease. To address these possibilities, we need to determine whether inflammation in Alzheimer disease is an early event, whether it is genetically linked with the disease and whether manipulation of inflammatory pathways changes the course of the pathology. Although there is still little evidence that inflammation triggers or promotes Alzheimer disease, increasing evidence from mouse models suggests that certain inflammatory mediators are potent drivers of the disease. Related factors, on the other hand, elicit beneficial responses and can reduce disease.

Evidence for inflammation in Alzheimer disease

Alzheimer disease is an age-dependent neurodegenerative disorder that results in progressive loss of cognitive function. It is characterized by the accumulation of the amyloid-β (Aβ) peptide into amyloid plaques in the extracellular brain parenchyma[1] and the formation of tangles inside neurons as a result of abnormal phosphorylation of the microtubule-associated protein tau[2]. Rare autosomal dominant mutations in the gene for amyloid precursor protein (APP)[3] (which is processed into Aβ) and in presenilin genes[4] (which encode proteins involved in the processing of APP) have strongly implicated Aβ as a central player in Alzheimer disease. Amyloid deposits and tangles are accompanied by a marked loss of neurons in the neocortex and hippocampus.

Besides these hallmarks, prominent activation of inflammatory processes and the innate immune response are observed in the brains of people with Alzheimer disease (reviewed in refs. 5 and 6). In principle, this neuroinflammation (**Box 1**) may drive pathology or be the result of an ongoing disease process (**Fig. 1**). Activation of inflammatory pathways early or even preceding the disease would provide support for a causative role for inflammation, although inflammation could still be a driving force and accelerate disease if it is activated later on in the disease. It is also possible that inflammation induces beneficial immune responses that limit disease. Thus, broad inhibition of inflammation would not be desirable. Inflammation or beneficial responses may be linked to genetic polymorphisms or regulated by epigenetic factors such as the presence of other diseases (asthma, Crohn disease, arthritis, type I diabetes and others), microbial infections, traumatic injuries or drug use. Interestingly, a growing literature has linked polymorphisms in cytokines and other immune molecules with Alzheimer disease, and epidemiological studies indicate that use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the risk of Alzheimer disease (**Fig. 1**). Also, the role of immune and inflammatory processes may be more or less pronounced in different Alzheimer disease 'subtypes,' characterized by different levels of Aβ, tau and ubiquitin and the presence or absence of the apolipoprotein E (*APOE*) ε*4* allele[7].

Postmortem protein and gene expression studies. The development of specific antibodies in the 1980s made it possible to identify the presence of complement proteins and the expression of major histocompatibility complex (MHC) class II molecules on microglia surrounding amyloid plaques in the brains of people with Alzheimer disease[8,9]. Since then, many studies have confirmed the abnormal expression of cytokines, chemokines, complement proteins and other proteins typically associated with immune and inflammatory responses (reviewed in ref. 5).

Several of these studies not only used immunohistochemistry, but also validated changes in expression by western blot, ELISA or mRNA measurements. Unbiased gene expression analyses have confirmed some of these findings and identified additional proteins with roles in inflammation (reviewed in refs. 10 and 11). For example, in a comparison of genes differentially expressed in the CA1 region of individuals with mild Alzheimer disease and controls without dementia, interleukin (IL)-1α, IL-1β, cyclooxygenase (COX)-2, NF-κB1 and

Geriatric Research, Education and Clinical Center, Veterans Administration Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, California 94304, USA and Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Mail Code 5235, Stanford, California 94305, USA.
E-mail: twc@stanford.edu
Published online 7 September 2006; doi:10.1038/nm1484

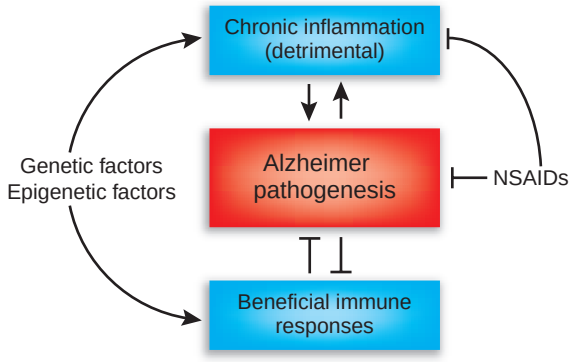

Figure 1 Possible roles of inflammation and NSAIDs in Alzheimer disease pathogenesis. Alzheimer disease is associated with chronic activation of inflammatory pathways that are likely to be detrimental and can in turn promote disease (↑↓). In contrast, beneficial immune responses, including phagocytosis or production of repair and trophic factors, may inhibit Alzheimer disease. It is also possible that the disease process inhibits these beneficial responses (straight inhibitory arrows). Both chronic inflammation and beneficial immune responses may be regulated genetically or by epigenetic factors including traumatic injury, infections or other diseases (curved arrows). They may promote disease or be a response to it. NSAIDs may exert beneficial effects on Alzheimer disease by directly inhibiting disease development, progression or both (for example, by reducing Aβ production) or by inhibiting inflammation (curved inhibitory arrows).

several other immune and inflammatory mediators were among the top 20 upregulated genes, whereas Smad3, a transcription factor of the transforming growth factor (TGF)-β pathway, was among the genes with the most substantially reduced expression[12]. In a thorough review studying microarray data in aging and Alzheimer disease, it was noted that genes associated with inflammation are increased in aging and that this is accentuated in Alzheimer disease[11]. Genes associated with inflammation reported to be increased in more than one study include NF-κB, the chemokine CCL20, IL-1α and tumor necrosis factor (TNF)-α–induced protein 2. Notable genes overlapping between individuals with Alzheimer disease and APP transgenic mice (a mouse model of the disease; see below) include complement components 4A and 4B, the microglial protein CD68 and the astrocyte activation marker glial fibrillary acidic protein (GFAP). However, there is overall disappointingly little overlap among the seven microarray studies on humans with Alzheimer disease, and there is even less overlap in reported changes in gene expression between humans and APP mouse models. This is less likely to be due to a lack of validity of individual expression measurements, and more likely to result from differences in experimental setups, interindividual differences and different statistical tools used to mine the data. In addition, the immune response of inbred mouse strains may be different from that of a genetically diverse human population, and the transgenic overproduction of mutant APP may result in additional activation of immune responses. In the end, the biological relevance of gene expression changes, whether detected by array or more conventional means, needs to be validated in different animal models and ultimately in humans.

A few studies have also used positron emission tomography (PET) to study inflammation in living individuals with Alzheimer disease by assessing binding of a synthetic ligand of the so-called peripheral benzodiazepine binding site to activated microglia. Although initial studies did not detect specific binding[13], the use of a refined method led to the observation of increased binding in the entorhinal, temporoparietal and cingulate cortex in those with mild to moderate Alzheimer disease[14]. A marked increase in binding was also observed in an additional study[15], but more data are needed to validate this approach and to determine whether microglia are activated at the earliest stages of Alzheimer disease.

These neuropathological and gene-expression studies show the activation of immune and inflammatory pathways in Alzheimer disease. But so far, they have failed to establish whether these changes occur early and may promote disease or are a compensatory reaction to a degenerative process. It is also critical to keep in mind that immune factors and inflammatory proteins are multifunctional (**Box 1**), and many have both pro- and anti-inflammatory effects. Therefore, their mere presence does

BOX 1 WHAT IS NEUROINFLAMMATION?

Inflammation is a specialized immune response of the organism to an invading pathogen, a traumatic event, or in general terms, an injurious agent. The agent may be foreign or self, as in a necrotic cell, and inflammation may be acute or chronic. Inflammation in the classical sense involves innate and adaptive immune responses. Innate immune responses involve typically macrophages, natural killer cells, the complement system and numerous cytokines, chemokines, acute phase proteins and arachidonate metabolites[5,112–114]. The adaptive immune response uses the same soluble mediators, as well as T and B lymphocytes and specific antibodies. The inflammatory reaction that characterizes most neurodegenerative diseases, including Alzheimer disease, is often called 'neuroinflammation' and consists mainly of elements of the innate immune response. Activated microglia—the resident macrophages of the brain—and astrocytes are the main cells that participate in this response. Adaptive immune responses and the infiltration of lymphocytes or polymorphonuclear cells are limited in Alzheimer disease.

Whereas an inflammatory response is necessary and crucial if the agent is a pathogen or dying cell, aberrant inflammatory responses are well-known causes of tissue damage and disease. Overly aggressive complement activation, for example, may kill invading bacteria but also destroy host cells. Persistent inflammatory responses can lead to permanent scarring and tissue damage. Many wrongly equate the term 'inflammation' with these detrimental outcomes only.

Inflammation is a biological process that recruits different cell types and molecules that may have many other functions outside inflammation. 'Inflammatory cell types' or, worse, 'pro- and anti-inflammatory' proteins do not exist, and these terms greatly complicate discussions about the role of inflammation in disease. They are only useful to describe the action of cells or proteins at a given time, not their inherent nature. Most cells are plastic enough that they can promote or inhibit an inflammatory response. This is even truer for cytokines or chemokines, which act in highly context-dependent ways. The 'proinflammatory' cytokine TNF-α, for example, may indeed promote an inflammatory response, but it may also kill cells, protect neurons or even modulate neurotransmission[118]. Conversely, the 'anti-inflammatory' protein TGF-β1 promotes inflammation and cellular infiltration early in an immune response, but is critical later in downregulating inflammation[119]. The same multifunctionality has been described for many other inflammatory mediators that have been more thoroughly investigated.

not predict whether they are beneficial or detrimental and whether they have a role in inflammation at all. Animal studies will be necessary to sort out this question.

Genetic linkage between inflammation and Alzheimer disease. Based on the observations that inflammatory pathways are activated in Alzheimer disease and that people with arthritis have a reduced risk for the disease, geneticists have been scanning the most obvious immune-regulatory genes for mutations or polymorphisms that might be linked with Alzheimer disease. Genes encoding many major cytokines, chemokines and acute-phase proteins have been surveyed and, although individual reports sometimes show statistically significant genetic linkages of single nucleotide polymorphisms or haplotypes in case-control studies, many of these findings have not been confirmed. Meta-analyses of multiple such association studies are therefore helpful in assessing an overall genetic effect. This has been done for more than a hundred genes, including several major immune mediators (see http://www.alzgene.org). These genes show only very modest effects on Alzheimer disease risk that are not statistically significant (**Table 1**). It is still possible that other polymorphisms in these or other genes associated with inflammation might show stronger linkage to Alzheimer disease in future studies or in selected groups of affected individuals.

Although polymorphisms in these immune-mediator genes do not currently support a strong genetic effect of immune responses on Alzheimer disease, it should be noted that ApoE may confer some of its risk for the disease by modulating immune responses. The *APOEε4* allele has a prevalence of 10–15% in most populations and is the major susceptibility allele for Alzheimer disease[16,17]. This genetic effect has been ascribed to the role of ApoE on lipid metabolism and Aβ generation, Aβ clearance or neuroprotection and regeneration[16]. Notably, lack of apoE expression in mice is associated with increased inflammation[16] and susceptibility to a number of pathogens, and mice with targeted replacement of the mouse *Apoe* with human *APOE4* show more production of TNF-α and IL-6 in response to lipopolysaccharide (LPS) than mice with human *APOE3* (ref. 18). How apoE exerts its effects on immune responses is still unclear, but a recent report showed that apoE is involved in the presentation of lipid antigens in the context of MHC class I–like CD1 molecules[19]. Lipid antigens have been implicated as autoantigens in mouse models of multiple sclerosis[20], and it is interesting in this context that *APOEε4* is associated with a more rapid progression and a more severe clinical course in humans with this autoimmune disorder[21]. Thus, genetic differences in *APOE* seem to modulate immune responses, and it will be interesting to determine whether this is relevant for Alzheimer disease.

Use of NSAIDs and Alzheimer disease risk. Support for early detrimental activation of inflammatory processes in Alzheimer disease came from epidemiological observations that found a lower incidence of the disease in those with arthritis[22]. Because most people with arthritis use NSAIDs, the most parsimonious conclusion is that these drugs reduce the risk of Alzheimer disease. Indeed, evidence from multiple case-control and population-based studies supported a roughly 50% reduction in Alzheimer disease risk in long-term users of NSAIDs and warranted their testing in clinical trials in people with Alzheimer disease. NSAIDs generally inhibit cyclooxygenase (COX)-1 and COX-2 (**Fig. 2**), which were identified as the main targets of the original NSAID acetylsalicylate (aspirin). Currently, there are dozens of NSAIDs on the market with a range of specificities for COX-1 and COX-2 or alternative targets (**Fig. 2**). Unfortunately, although a small trial of indomethacin in individuals with mild to moderate Alzheimer disease showed positive results[23], and a similar trial of diclofenac showed a trend[24], larger trials of rofecoxib and naproxen have failed (reviewed in ref. 25).

There are many reasons to explain the failure of these trials; the timing of treatment, dosing and the specificities of administered NSAIDs are most frequently cited. In addition, it is possible that genetic factors predisposing people to arthritis or other inflammatory diseases, rather than their use of NSAIDs, may reduce risk of Alzheimer disease, or that factors generally produced in the course of inflammatory diseases in the periphery are protective for Alzheimer disease. Although currently there is no experimental support for these possibilities, they should not be dismissed.

It is certainly intriguing that those with arthritis who used NSAIDs had a threefold reduction in the number of MHC class II–positive activated microglia in their brains at autopsy compared with normal controls without arthritis and no chronic use of NSAIDs[26]. This suggests that use of NSAIDs—or, again, having arthritis—reduces microglial activation. Of note, NSAID use and arthritis did not alter the number of amyloid plaques and tangles, which were found at low numbers in the absence of cognitive dysfunction or clinically diagnosed Alzheimer disease in this relatively large autopsy study (30 individuals per group).

That NSAID use may be responsible for reduced microglial MHC class II expression in these individuals is consistent with recent preclinical studies in animal models of Alzheimer disease. In the first such study,

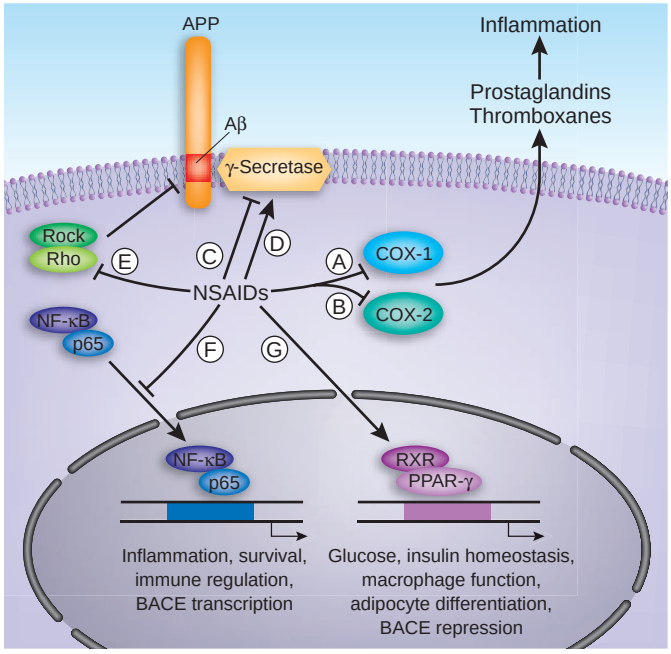

Figure 2 Reported effects of NSAIDs with respect to Alzheimer disease and APP processing. NSAIDs inhibit the production of prostaglandins and thromboxanes, key inducers of inflammation, by inhibiting their main targets COX-1 and COX-2 (arrows A, B). NSAIDs have many off-target effects, including the inhibition (C) or modulation (D) of γ-secretase, the inhibition of the small GTP-binding protein Rho and its associated kinase Rock (E) and the inhibition of translocation of the transcription factor NF-κB (F), which activates many genes that promote inflammation. It also activates BACE transcription. NSAIDs also stimulate activation of the nuclear receptor PPAR-γ (G), which, together with the retinoid X receptor (RXR), binds to DNA to increase macrophage function and repress BACE transcription. The following are examples of NSAIDs with the indicated effects: A: *S*-flurbiprofen, indomethacin, aspirin and ibuprofen (preferential COX-1 inhibitors[115]); B: rofecoxib, nimesulide, celecoxib and diclofenac (preferential COX-2 inhibitors[115]); C: sulindac sulfide, ibuprofen, indomethacin, flurbiprofen and meclofen[33,36–40]; D: celecoxib and sc-560[40,41]; E: sulindac sulfide, ibuprofen and indomethacin[31]; F: *R*-flurbiprofen, indomethacin[44]; G: indomethacin, ibuprofen, fenoprofen, flufenamic acid, naproxen, sulindac sulfide and HCT1026[116,117].

Table 1 Alzheimer disease risk associated with genetic polymorphisms in immune mediators.

Gene[a]	Odds ratio[b]	95% confidence interval	Allele; position in gene	Number of studies in meta-analysis
α1-ACT[c]	1.06	0.96–1.17	A17T	35
CCR2	0.85	0.58–1.26	V64I	3
CCR5	1.09	0.81–1.46	Δ32	3
MCP-1	1.15	0.85–1.54	G/A; –2518	5
Fas	1.06	0.87–1.28	G/A; –670	6
IL-1α	1.08	0.98–1.18	T/C; –889	22
IL-1β[c]	0.94	0.87–1.02	T/C; –511	15
IL-6	0.87	0.72–1.05	C/G; –174	13
IL-10[c]	0.86	0.63–1.18	G/A; –1082	6
ICAM-1	1.15	0.80–1.65	E469K	3
TGF-β1	1.01	0.91–1.13	T/C; –509	4
TNF-α[c]	0.96	0.75–1.24	A/G; –308	6

[a]CCR, chemokine CC motif receptor; MCP, monocyte chemotactic protein; ICAM, intercellular adhesion molecule. [b]All data were obtained from meta-analyses for the listed genes at AlzGene.org (Bertram, L., McQueen, M., Mullin, K., Blacker, D.& Tanzi, R. The AlzGene Database. *Alzheimer Research Forum* http://www.alzgene.org; accessed 11 April 2006). [c]More than one meta-analysis available on AlzGene; results in table are for polymorphisms with largest available number of studies. None of the other polymorphisms showed significant effects.

ibuprofen had a beneficial effect in APP transgenic mice[27]. Ibuprofen treatment for 6 months reduced amyloid plaque numbers, microglial activation around plaques and IL-1β abundance. This effect of ibuprofen and a few other NSAIDs on Aβ deposition in APP mice was confirmed in most subsequent studies (reviewed in ref. 28). However, there seems to be discordance between the abundance of classical COX-dependent inflammatory mediators and Aβ accumulation[29]: treatment of APP mice with indomethacin reduced cerebral prostaglandins by 90%, but reduced hippocampal Aβ only minimally and had no effect on cortical Aβ abundance. And other studies found no significant effects of NSAIDs at all in reducing Aβ deposition in APP mice[30]. The reasons for these discrepancies will have to be determined.

These and similar studies lead to the question of how NSAIDS reduce Aβ accumulation. Recent evidence suggests that certain NSAIDs may exert their effects independent of COX-1 or COX-2 (**Fig. 2**). At least two alternative targets of NSAIDs have received particular attention with respect to Alzheimer disease, the peroxisome proliferator-activated receptor-γ (PPAR-γ) and the presenilins. Several NSAIDs have also been reported to decrease the production of Aβ by inhibiting the small GTP-binding protein Rho and its associated kinase Rock (ref. 31). In addition, NSAIDs can reduce NF-κB translocation, inhibit MAP kinases or inhibit the cell cycle[32]. For these activities to be relevant *in vivo* and in Alzheimer disease, drug concentrations have to be within the range obtained in humans. Short-term treatments in mice suggest that the activities are indeed within the IC_{50} for COX-1 or COX-2 inhibition for specific NSAIDs[32,33].

Studies in cell culture and APP mice indicate that NSAIDs that activate PPAR-γ may reduce Aβ production by repressing the activity of the β-secretase cleaving enzyme 1 (*BACE1*) promoter, thus decreasing amyloidogenic processing of APP[34]. Notably, the abundance of PPAR-γ is reduced in APP mice and in Alzheimer disease postmortem cortical tissue compared with controls, whereas that of BACE1 is increased, and acute treatment with a PPAR-γ agonist reverses these differences and reduces the abundance of Aβ in APP mice[34]. In a different APP mouse model, chronic treatment with only half the dose of the PPAR-γ agonist did not reduce Aβ abundance significantly, although a strong trend was observed[35].

Numerous *in vitro* studies have also shown that certain NSAIDs can modulate γ-secretase and the production of Aβ[33,36–40]. For example, ibuprofen, indomethacin or sulindac sulfide reduce Aβ abundance in APP-expressing cells. Ibuprofen has this effect even in the absence of COX enzymes and also reduces Aβ after acute treatment in APP transgenic mice[36]. Notably, acute dosing of APP mice over 3 d produces drug levels in the low micromolar range in the brain, which, in the case of ibuprofen, are inversely correlated to Aβ levels[33]. Other NSAIDs, such as celecoxib, seem to selectively increase Aβ abundance in culture[40,41], although the relevance of these effects for Alzheimer disease pathogenesis in individuals taking such drugs is currently unclear[42].

Perhaps the most exciting finding from these studies on nonclassical targets of NSAIDs is the potent Aβ-reducing effect of flurbiprofen and its enantiomers. In particular, *R*-flurbiprofen, which does not inhibit COX activity and therefore has few COX-dependent side effects in humans, reduces Aβ abundance in APP mice at plasma levels below those achieved in humans with a therapeutic dose[33]. A recently concluded phase 2 trial of *R*-flurbiprofen (Flurizan) over 12 months showed a statistically significant slowing in decline of activities of daily living and global function in people with mild Alzheimer disease. In an optional follow-on study, *R*-flurbiprofen continued to slow functional and cognitive decline in individuals receiving up to 21 months of treatment (http://www.myriad.com/alzheimers/flurizan.php). A phase 3 trial is now underway and will hopefully confirm these encouraging preliminary results.

But as often with NSAIDs, there may be another twist to this story. *R*-Flurbiprofen at low doses was as efficient as its COX-inhibiting *S* enantiomer in limiting zymosan-induced hindpaw inflammation in rats[43]. This effect did not involve epimerization from the *R* to the *S* isomer, but seemed to be mediated by an inhibition of NF-κB translocation and consequent reduced COX-2 expression (**Fig. 2**). If confirmed, such a mechanism could support the inflammation hypothesis once again. In support of a COX-independent action of certain NSAIDs, indomethacin was also found to reduce NF-κB activity in APP mice, and this was accompanied by a >50% reduction in brain Aβ abundance[44].

So what are the lessons to be learned? Maybe that NSAIDs are not purely anti-inflammatory and that COX-2 may be the wrong target. It is striking that drugs with promising effects in humans and with Aβ-reducing properties in APP mice have targeted COX-1 and nonclassical pathways (such as PPAR-γ and γ-secretase), whereas unsuccessful clinical trials in humans have used mostly COX-2–specific inhibitors. Because most NSAIDs have multiple targets that do not involve classical inflammatory pathways, it seems difficult to definitively link their use to anti-inflammatory actions. More specific NSAIDs or new inhibitors of inflammation working through pathways not utilized by previous

Table 2 Genetic modulation of immune and inflammatory pathways in mouse models of Alzheimer disease.

Mouse model[a]	Manipulation[b]	Transgene targeting; function	Outcome (Alzheimer disease–like pathology in nervous system)	Reference
APP-PS1	NSE-COX2	Neuronal; prostaglandin synthesis	↑ Aβ accumulation, ↑ PGE_2	62
APP-PS1	PGE_2 receptor *EP2*$^{-/-}$	Knockout; PGE_2 signaling and microglial activation	↓ Aβ accumulation, ↓ β-CTF, ↓ lipid peroxidation	63
PDGF–APP-J20	Metallothionein-sCrry	Secreted; inhibits activation of complement C3 in brain and peripheral tissues	↑ Aβ accumulation, ↓ microglial activation (F4/80), accumulation of degenerating hippocampal neurons	67
Tg2576	*C1q*$^{-/-}$	Knockout; activation of classical pathway of complement	↔ Aβ, ↓ microglial activation (CD11b, F4/80, MHC-II), ↓ astrocyte activation (GFAP), rescue of synapto-dendritic degeneration	68
APP23	Thy1–IL-1ra	Secreted; binds IL-1 receptor and inhibits IL-1 signaling	↔ Aβ, ↑ MIP-1α and RANTES	c
APP23	GFAP–IL-6	Secreted; activates IL-6 signaling on CNS cells	↔ Aβ, same general↑ inflammation as seen in GFAP-IL6 mice (↑ cytokines, chemokines, acute-phase proteins)	c
PDGF–APP-J9	GFAP–TGF-β1	Secreted; activates TGF-β signaling on CNS cells	↓ Aβ accumulation overall, ↑ cerebrovascular Aβ, ↑ microglial activation (F4/80), ↑ astrocyte activation (GFAP)	69
APP-Tg2576	GFAP–MCP-1	Secreted; binds CCR2 on microglia, macrophages	↑ Aβ accumulation in diffuse deposits, ↑ number and clustering of microglia (Iba1), ↓ activation of microglia (MHC II, CD45)	75
APP-Tg2576	*CD40L*$^{-/-}$	Knockout; binds CD40 on microglia, neurons	↓ Aβ accumulation, ↓ microglial (CD11b) and astrocyte (GFAP) activation	76
PDGF–APP-J9	PDGF-RAGE	Neuronal; RAGE signaling	↑ Aβ accumulation, ↑ glial activation (CD11b, GFAP), ↑ NF-κB signaling, spatial learning and memory deficits, ↓ LTP, ↓ synaptic integrity	78
PDGF–APP-J9	PDGF–DN-RAGE	Neuronal; dominant negative RAGE inhibits RAGE signaling	↓ NF-κB signaling, improved spatial learning and memory, ↑ LTP	78
PDGF–APP-J9	GFAP–α1-ACT	Secreted; inhibits serine proteases	↑ Aβ accumulation	81
PDAPP	GFAP–α1-ACT	Secreted; inhibits serine proteases	↑ Aβ accumulation,	80

↔, no change; ↑, increased; ↓, decreased; NSE, neuron-specific enolase; β-CTF, APP C-terminal fragment; GFAP, glial fibrillary acidic protein; CCR, chemokine CC motif receptor; PDGF, platelet-derived growth factor; IL-1ra, IL-1 receptor antagonist. J9, J20, Tg2576 and 23 refer to the founder transgenic lines of the various mouse models used.

[a]Details on mouse models, including expression pattern and levels, the presence of mutations and so forth, are described in the cited papers. [b]Transgenic or targeted deletion of indicated factors. [c]M. Staufenbiel, personal communication.

drugs will probably be necessary to determine whether inflammation is driving Alzheimer disease.

Inflammation in animal models of Alzheimer disease

Whereas the human studies are essential to establish that inflammation is part of Alzheimer disease, animal models are necessary to manipulate inflammation *in vivo* and to efficiently test inflammation-based therapeutic strategies (**Box 2**)[45–52]. However, animal models will not be able to answer the question of the possible causal role of inflammation in Alzheimer disease. We also need to establish whether models with Alzheimer-like disease mimic the inflammatory processes observed in true Alzheimer disease.

Mouse models of Alzheimer disease show activation of immune and inflammatory pathways in the brain that include, at the minimum, microglial and astrocyte activation (reviewed in ref. 28). There is also increased production of cytokines, chemokines, complement proteins and acute-phase proteins[28]. Several groups have carried out unbiased gene expression microarray studies in APP and APP–presenilin-1 (PS1) mice and, as discussed above, some of the genes found to be expressed differentially between transgenic and control mice overlapped with genes identified in humans with Alzheimer disease[11]. A direct comparison of significant genes in different mouse models has not been done. This would be particularly interesting to do in young mice, preferably in different genetic backgrounds or outbred strains, to detect genes with the most dominant changes associated with disease progression. A study comparing different APP and APP-PS1 mice at young and old ages showed increased expression of complement genes (C1q and C4) and genes involved in phagocytosis (FcRI, CD14, TYRO-BP and CD68)

in old transgenic mice, but there were no striking increases in genes associated with inflammation in young mice[53]. Likewise, in a separate study, complement genes C1q and C4 and TYRO-BP were upregulated in 17- to 18-month-old APP-PS1 mice in regions where amyloid accumulates[54]. In 2-month-old APP Tg2576 mice (several months before amyloid pathology occurs in this model), the chemokine receptor CXCR4 was increased in an unbiased array experiment[55]. Whereas this receptor for the chemokine stromal-derived factor (SDF)-1 has clear roles in inflammation, it also has important functions in neuronal progenitor cell migration during development, in response to injury and in axonal pathfinding[56]. This example highlights again that we should be cautious when assigning labels to proteins (**Box 1**) and, in this case, about taking the increase in expression of the 'inflammatory protein' CXCR4 as an indication of early inflammation in APP mice.

Multiple transgenic mouse models overproducing human mutant tau isoforms in brain cells have been described[51], and most of these bear filaments composed of hyperphosphorylated tau and develop neurodegeneration. Mice overexpressing human tauP301S develop these pathological hallmarks accompanied by severe paraparesis, increased cerebral IL-1β and COX-2, and microglial activation[57]. It is not known whether these inflammatory changes precede tau pathology or simply reflect secondary responses to cell damage. In a single microarray study in 11-month-old P301L tau transgenic mice with tau pathology, there were no increases in inflammation-associated genes[58].

Thus, overproduction of mutant APP or tau does not seem to result in marked activation of microglial cells or astrocytes before Aβ accumulates or tangles form, and it does not result in the concerted and widespread activation of inflammatory signaling pathways. It is possible, however, that small soluble aggregates of Aβ trigger local activation of glial cells, as suggested by a recent study in young thymus cell antigen 1 (Thy1)-APP^{V717I} mice that had not yet developed Aβ deposits[59]. Intriguingly, neuronal BACE1 was increased within foci of activated microglia and astrocytes together with IL-1 and IL-6, suggesting that inflammatory processes may directly increase the local production of Aβ[59]. If this is the case, the small increases in Aβ production observed in people with familial forms of Alzheimer disease may be sufficient to cause inflammation and promote disease. At least one microarray study tried to address this issue in fibroblasts from over 20 individuals with different APP or presenilin Alzheimer disease–linked mutations[60]. Although the authors reported differences in gene expression between fibroblasts from affected and unaffected individuals, they did not disclose the identity of any markers[60]. In contrast to the absence of overt inflammatory changes in young APP or tau transgenic mice, glial cells and inflammatory pathways are clearly activated at later stages in the pathogenesis in these models.

BOX 2 TRANSGENIC MODELS OF ALZHEIMER DISEASE

Transgenic mouse models have greatly advanced our understanding of Alzheimer disease pathogenesis. They display Alzheimer disease–like pathology and have been invaluable in the development of preclinical testing of some of the most promising therapeutic approaches to the disease[45]. Transgenic mice overproducing human APP containing familial Alzheimer disease mutations show increased production of Aβ, which accumulates with age into diffuse or compact amyloid plaques[46]. The mice also show synaptic transmission deficits that often precede the formation of amyloid plaques[47], and they show neurodegeneration[48] and cognitive deficits[49]. In these models, APP containing familial Alzheimer disease mutations is typically overexpressed in neurons in the presence or absence of the PS1 transgene; PS1 further increases Aβ production and accelerates pathology. Mice overexpressing human tau protein mutants that are associated with familial forms of frontotemporal dementia—a dementia characterized by extensive tangle formation—develop neurofibrillary tangles similar to the ones observed in Alzheimer disease[50]. Additional models have been created, some of which develop cognitive deficits[51]. In addition, a mouse model has been described (3×Tg-AD mice) that harbors three mutant genes—tau^{P301L}, APP^{K670N,M671L} and PS1^{M146V}—and produces amyloid plaques and tangles and shows synaptic transmission deficits[52].

Manipulation of inflammation in Alzheimer disease models

Available mouse models for Alzheimer disease should be ideal to test whether specific immune and inflammatory mediators affect amyloid or tau pathology, neurodegeneration and functional outcomes. Indeed, a growing number of hypothesis-driven studies have shown that some mediators have prominent effects on Alzheimer-like disease in APP mice (**Fig. 3** and **Table 2**). No transgenic or knockout studies testing immune or inflammatory proteins in tau transgenic mice have been reported.

Genetic manipulations of inflammation. Mouse models have been instrumental in supporting a role for COX enzymes and their products—the prostaglandins—in Alzheimer disease. COX-2 overexpression in neurons induces neuronal apoptosis and cognitive deficits in transgenic mice[61]. A cross of these mice with APP-PS1 mice led to a doubling in Aβ plaque formation[62], supporting a detrimental role for COX-2 in neurodegeneration and a potential use of specific COX-2 inhibitors in the treatment of Alzheimer disease. Evidence for a role of prostaglandin E_2 (PGE_2) in Aβ accumulation comes from APP-PS1 mice in which the PGE_2 receptor EP2 has been removed[63]. Levels of F2-isoprostanes and F4-neuroprostanes, indicators of lipid peroxidation, were reduced by half in these mice compared with APP-PS1 mice, and this was associated with a 40–50% decrease in Aβ peptide and plaques. Because APP fragments cleaved by BACE were reduced only in old mice, not in young, the authors concluded that EP2 receptor signaling promotes BACE cleavage in an age-dependent way. In addition, primary microglia lacking EP2 were 2.5 times more efficient in phagocytosing Aβ and exerted significantly less neurotoxicity in cell culture[64]. These studies suggest that the EP2 receptor may be a valid target for inhibiting specific inflammatory processes involved in Alzheimer disease.

Because of its key role in initiating an inflammatory response[65], activation of the complement system in Alzheimer disease is of particular interest. Glial cells and neurons in the central nervous system (CNS) can produce most components of this complex cascade, and their production is increased in Alzheimer disease[5,66]. The function of the complement system in the CNS remains largely unknown. Overexpression of soluble complement receptor–related protein y (sCrry), which inhibits activation of the central component C3 of the complement cascade, results in increased Aβ load in the brains of APP transgenic mice and in the accumulation of degenerating neurons in the hippocampus[67], indicating that complement factors such as C3bi are involved in phagocytosis of amyloid and degenerating cells in the brain. In contrast, a different study showed no effect on Aβ accumulation in APP transgenic mice lacking C1q, the first component of the classical pathway of complement, but found that C1q deficiency ameliorates synaptic degeneration in APP mice[68]. It is possible that C3, which is inhibited by (sCrry), is involved in amyloid clearance, whereas C1q is not.

The IL-1 and IL-6 cytokine pathways have been implicated in Alzheimer disease on the basis of their increased activation in the brains of affected individuals and the proinflammatory effects of these

cytokines in culture[5]. Notably, astroglial overexpression of IL-6 or IL-1 receptor antagonists that bind to the IL-1 receptor and inhibit binding of IL-1α and IL-1β does not seem to affect amyloidosis in Thy1-APP751$^{K670N, M671L}$ line 23 (APP23) mice (M. Staufenbiel, Novartis Pharma, Basel, Switzerland; personal communication). In contrast, overexpression of TGF-β1 in APP mice results in a 70% reduction in parenchymal amyloid plaques and a 60–70% decrease in Aβ compared with APP transgenic littermate controls[69]. Like that in single transgenic TGF-β1 mice, increased astroglial TGF-β1 production in aged APP mice causes extensive microglial and astroglial activation in the hippocampus and cortex[69]. Both cell types are phagocytic, and activation of cultured microglia with TGF-β1 results in increased Aβ degradation[69]. In addition, primary adult astrocytes phagocytose Aβ bound to plastic or in brain sections from APP mice[70]. TGF-β1 also caused a marked shift in the site of Aβ accumulation. Whereas Aβ accumulates almost exclusively in parenchymal plaques in APP mice, most of the remaining Aβ is associated with vascular structures in APP–TGF-β1 mice, possibly owing to an increase in cerebrovascular basement membrane protein synthesis[71]. So, although fibrosis due to overexpression of TGF-β1 seems to promote the deposition of Aβ to vascular walls, TGF-β1–activated microglia, astrocytes or both can degrade Aβ and lower its overall concentration in the brain. In addition, TGF-β1 is neuroprotective in transgenic mice[72] and may reduce Aβ production by reducing neuronal stress. In people with Alzheimer disease, Aβ accumulation in parenchymal plaques seems to correlate inversely with Aβ in cerebral blood vessels[69,73,74], and it is tempting to speculate that TGF-β1 is involved in this process.

In a related model, monocyte chemotactic protein (MCP)-1, a potent chemoattractant of microglia and monocytes and macrophages, was overproduced from astrocytes in APP–MCP-1 double transgenic mice[75]. This leads to a twofold increase in the number of microglia, many in clusters around plaques, and a sharp increase in diffusely deposited Aβ[75]. Because the microglia do not express typical activation markers or ameboid shapes, the authors concluded that they may not be fully activated or phagocytic and that defective microglial clearance may be responsible for the overall Aβ increase. In another model, however, reduced microglial activation is associated with less, not more, Aβ deposition. In APP mice lacking CD40L, which binds to CD40 and provides an important costimulatory signal for immune cells including microglia[76], Aβ accumulation is strongly reduced[76]. In addition, neurons also express CD40, and CD40L treatment increases amyloidogenic processing of APP, suggesting that CD40L deficiency exerts its effects at least in part via neurons.

Inflammation may also be activated by binding of Aβ to specific receptors, thereby promoting the disease process as Aβ accumulates. The receptor for advanced glycation end products (RAGE) is one of several known receptors for Aβ and is particularly interesting in the context of inflammation because it activates the Jak-Stat and NF-κB signaling pathways[77]. An elegant set of experiments with RAGE transgenic and signaling-deficient mice showed a role for RAGE signaling in Aβ dependent neuronal perturbation that seems to involve NF-κB and MAPK signaling and results in increased synaptic transmission deficits and cognitive impairment[78]. Because these functional deficits preceded activation of glial cells, it is possible that the observed effects do not involve typical inflammatory responses in glia but perhaps instead neuronal dysregulation of NF-κB- and MAPK-dependent processes[78]. In addition, RAGE overexpression increased Aβ accumulation in APP mice (O. Arancio, Columbia University, New York; personal communication).

Other inflammatory mediators implicated in Alzheimer disease include acute-phase proteins such as α1-antichymotrypsin (α1-ACT). This protease inhibitor rapidly increases in abundance after injury and is associated with neurodegeneration[5]. In Alzheimer disease, mature amyloid deposits are decorated with α1-ACT (ref. 79), and increased expression of α1-ACT results in higher plaque burden independent of APP processing in APP transgenic mice[80,81], suggesting that α1-ACT either promotes aggregation or reduces clearance of Aβ.

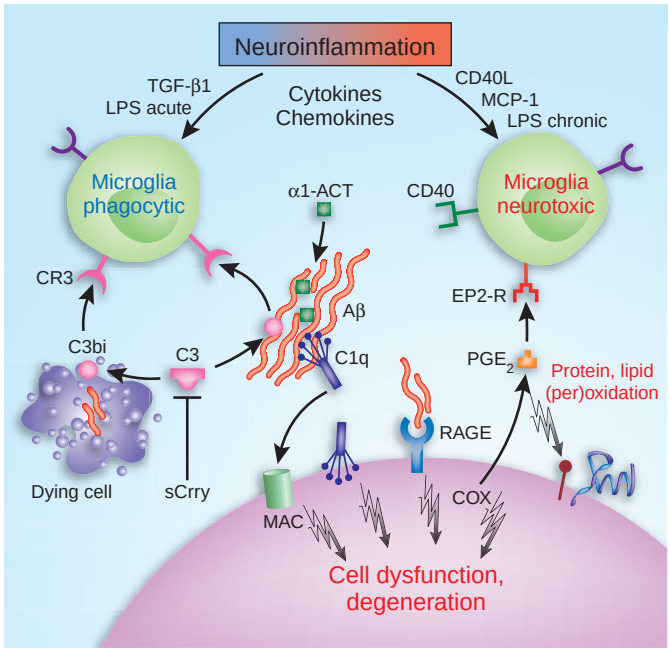

Figure 3 Transgenic and knockout studies in APP mice reveal beneficial and detrimental effects of immune and inflammatory factors. Inflammation may induce beneficial responses that include the activation of microglial cells to phagocytose dying cells or Aβ assemblies. TGF-β1 and acute LPS treatment seem to promote this phagocytic state. sCrry inhibits the activation of C3 and thus the deposition of C3bi onto dying cells or Aβ assemblies, which are possible targets for subsequent phagocytosis via complement receptor 3 (CR3). Inflammation can also induce detrimental responses and lead to the activation of microglia and the secretion of neurotoxic factors. CD40L, MCP-1 and chronic LPS treatment seem to favor such responses in APP mice. Acute-phase proteins such as α1-ACT can promote cerebral Aβ accumulation, whereas complement C1q may injure neurons directly or lead to activation of the lytic complement pathway and formation of the membrane attack complex (MAC). This transmembrane pore can disrupt cellular function. Binding of Aβ to RAGE can also lead to neuronal dysfunction. Lastly, COX can promote Aβ accumulation and neurodegeneration directly or through the production of PGE_2 and signaling via the EP-2 receptor on microglia.

In summary, genetic studies in mouse models show that there are multiple inflammatory mediators that have potent effects on Alzheimer disease–like pathogenesis (**Fig. 3**). The studies highlight the complexity in the function of immune and inflammatory mediators *in vivo* and in the CNS, in particular where these factors may have roles that are independent of inflammation. Note that negative results do not exclude a role for a tested factor in Alzheimer disease, as the models may not represent all aspects of the disease and the factors studied may have nonphysiological temporal and spatial expression patterns in transgenic or knockout mice. In addition, the functions or cellular distributions of immune molecules in mouse and humans may differ. The studies reviewed here also make it obvious that the field needs microglial markers that go beyond the loosely defined state of 'activation' and are instead associated with cellular functions. Despite these caveats, several new pathways have emerged as potential new therapeutic targets to reduce inflammatory processes associated with disease or to promote beneficial immune responses and reduce disease (**Fig. 3**).

Treatment of Alzheimer disease models with anti-inflammatory drugs

Manipulation with anti-inflammatory drugs or LPS. Pharmacological treatments provide an alternative to genetic approaches in studying the role of inflammation in Alzheimer disease. They have the advantage that time points of treatment and dosage can easily be varied. They have the disadvantage, however, that drugs may not be specific, and off-target effects are difficult to rule out. Treatments of APP mice with NSAIDs have been discussed above and are reviewed in more detail in reference 28. Besides NSAIDs, a new class of anti-inflammatory drugs—aminopyridazines—shows promise in reducing glial activation, cytokine production and neuronal damage in rats infused with synthetic Aβ[82]. It will be interesting to see whether these compounds are effective in reducing pathology or improving function in APP or tau transgenic mice and possibly in humans with Alzheimer disease.

To study the general effect of inflammatory processes on Alzheimer disease–like pathology directly, many groups have treated APP mice either acutely or chronically with LPS[28]. Although these reports initially seemed contradictory, a careful review shows that acute LPS treatment generally reduces (while chronic treatment increases) Aβ accumulation[28]. LPS induces a strong activation of innate and acquired immune responses and results in a prominent activation of microglial cells. These studies may help in dissociating microglial activation patterns and phenotypic markers associated with Aβ clearance from those seen in the chronic administration paradigms (**Fig. 3**).

Again, very few studies have been done in tau transgenic models, although there is substantial evidence that inflammatory processes may increase tau phosphorylation in other situations. For example, IL-1β is sufficient to induce tau phosphorylation in cell culture[83] and in rats implanted with pellets that slowly release IL-1β into the cortex[84]. Chronic administration of LPS, which induces IL-1 (among other cytokines), results in tau hyperphosphorylation in triply transgenic Alzheimer disease model mice[85] (see **Box 1**), an effect that is most likely dependent on cyclin-dependent kinase 5 (Cdk5) activity. In contrast to its action in APP transgenic mice (see above), LPS does not affect the abundance of cerebral Aβ or other APP cleavage products, suggesting that tau phosphorylation in this case is not dependent on increased Aβ production[85]. Whether specific inflammatory factors other than IL-1 are sufficient or even necessary to induce tau phosphorylation *in vivo* is unclear.

It is noteworthy in this context that hyperphosphorylation and aggregation of tau have been observed in experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis[86]. Several tau kinases are increased in the injured brainstem of EAE rats and associated with tau hyperphosphorylation. Anti-inflammatory treatment with prednisolone reduces both EAE and tau pathology. Notably, models of axonal damage or acute demyelination that do not involve inflammation, including deficiency of the oligodendroglial protein 2′,3′-cyclic nucleotide 3′- phosphodiesterase or treatment with cuprizone, do not cause tau hyperphosphorylation[86]. These results support the idea that inflammation may cause or accelerate tau phosphorylation, and the relevance of this process to tauopathies or Alzheimer disease needs to be explored.

Is there immune dysfunction in Alzheimer disease?

Increased numbers of activated microglia are present in most neurodegenerative diseases and, although they can secrete toxic factors under certain conditions, their primary function seems to be to protect the brain. In Alzheimer disease, microglia probably phagocytose and clear Aβ, at least under certain conditions, and because acute LPS treatment or traumatic brain injury can reduce Aβ deposition in APP mice[28], it has been hypothesized that activating immune responses may limit pathology (**Fig. 3**). Consistent with this idea and as reviewed extensively elsewhere[45], Aβ antibodies, either induced by active immunization with synthetic Aβ peptide or transferred passively, can reduce Aβ accumulation and functional deficits in APP mice, and this occurs at least in part through the action of microglial cells[45]. Conversely, defects in microglial function or other immune responses in the CNS or even in the periphery may promote Alzheimer disease. This may be the case in *op/op* mice, which lack macrophage colony-stimulating factor (M-CSF) and, as a consequence, have fewer macrophages and only around two-thirds the number of microglia found in wild-type mice[87]. These mice have been reported to develop Aβ immunoreactive deposits spontaneously in the brain parenchyma[88].

A limited number of microglia in the adult brain are continuously recruited from circulating monocytes to populate perivascular spaces or to differentiate into parenchymal microglia[89,90]. The number of these cells is increased after brain injury, and they can take on a resting or activated appearance[91]. Three recent studies asked whether these infiltrating blood-derived cells may have a role in Alzheimer disease. The authors transferred bone marrow cells from actin–green fluorescent protein (GFP) transgenic mice into irradiated young or old APP or wild-type mice and found more GFP-positive microglia in APP brains than in wild type[92–94]. In one study, this recruitment was found to be more pronounced if transplants were performed before Aβ had been deposited and was found to be further enhanced by intracranial injection of LPS[92]. Similarly, a 50–80% increase in GFP-positive cells was observed in APP23 mice that received transplants before onset of pathology, but it was also seen when plaques were already present[93]. Many of these cells had ameboid morphology and were likely to be microglia, although a surprisingly high number of T lymphocytes was also observed. Using electron microscopy, these authors concluded that microglia do not phagocytose Aβ[93], although this technique would probably not show digested material efficiently. To test the role of bone marrow–derived microglia in amyloid deposition, the authors of the third study depleted dividing macrophages in the brain using a thymidine kinase transgene expressed in myeloid cells in combination with the drug gancyclovir, and these authors showed that dividing, infiltrating myeloid cells are reducing plaque formation in APP mice[94]. One limitation of these three studies is that mice had to be lethally irradiated before bone marrow transplantation, and this is known to result in vascular inflammation and increased infiltration of immune cells into the brain[95]. In addition, the number of GFP-positive cells in the brain was overall quite small, and although some plaques were surrounded by several cells, on average there was no more than one cell detected per plaque[94]. Whether these few cells indeed have an active role in limiting Alzheimer disease will have to be addressed in additional studies.

Changes or defects in immune responses, notably in the blood, have also been reported in individuals with Alzheimer disease. For example, peripheral blood macrophages from affected individuals were found to be less effective in phagocytosis of Aβ, and monocytes were impaired in differentiating into macrophages[96]. Abnormalities in lymphocyte distribution in individuals with Alzheimer disease have been reported not only in the brain, where increased numbers of T lymphocytes, predominantly $CD8^+$ but also $CD4^+$, are present[97–99], but also in the blood. Thus, people with Alzheimer disease were reported to have fewer $CD8^+$ cells in the blood than people without[100,101], or fewer lymphocytes overall[102], although these findings were not always confirmed[103]. Other changes in immune function in individuals with Alzheimer disease include a reduction in peripheral T-cell activation by Aβ and other APP-derived peptides[104]. However, using a more sensitive assay with a strongly immunogenic Aβ peptide, people with Alzheimer disease were found to have

increased T lymphocyte reactivity against Aβ[105]. In addition, autoreactive B lymphocytes and autoantibodies against Aβ, which are found naturally in the serum of aging humans, are increased in Alzheimer disease and reported in different studies to either enhance Aβ toxicity[106], to correlate inversely with disease severity[107,108] or to be unchanged[109]. Autoantibodies against Aβ and RAGE are also several times higher in individuals with Alzheimer disease compared with controls, and antibody titers correlate inversely with cognitive function[110]. Of possible relevance to recent studies suggesting a key role for oligomeric forms of Aβ in Alzheimer disease, plasma autoantibodies against these Aβ assemblies were found to be lower in affected individuals than in controls without dementia, whereas titers for autoantibodies recognizing monomeric Aβ were similar in the two groups[111]. Lastly, dozens of reports show changes in levels of cytokines or other immune mediators in serum or plasma of individuals with Alzheimer disease compared with controls, but most studies have tested small samples that come from a single center and have not been confirmed by independent studies.

At this point, the significance for Alzheimer disease of lymphocytes, autoantibodies against Aβ and other molecules, or changes in immune mediators in the periphery are far from clear. If animal studies were to show prominent effects of these immune cells and molecules on Aβ or tau pathologies, however, it might be worth investigating their importance in large-scale human studies. This could also help in the development and evaluation of safe and efficient immunotherapies or in the diagnosis of Alzheimer disease.

Conclusion

The field has come a long way from the first descriptions of activated complement and microglia in Alzheimer brains to the sophisticated mouse models with genetic manipulations of inflammatory pathways we have today. Although there are still no convincing genetic or other data to support an early role of inflammation in Alzheimer disease, these mouse models indicate that inflammatory processes may be a driving force of pathology. Similarly, as the role of NSAIDs in Alzheimer disease remains controversial, the effects of NSAIDs in APP mice support, at least in part, a role for inflammation in the disease. In addition, a growing number of animal studies, apart from the Aβ immunization paradigm, support the idea that some inflammatory responses are beneficial and may be effective in preventing or treating the disease. In summary, the reviewed literature identifies immune and inflammatory pathways as potential modulators of Alzheimer disease and targets for therapeutic interventions.

ACKNOWLEDGMENTS

I would like to thank M. Buckwalter and M. Britschgi for comments on the manuscript, L. Bertram for advice on the use of the AlzGene genetic resource, and O. Arancio and M. Staufenbiel for communicating unpublished data. This work was supported by the John Douglas French Alzheimer's Foundation and the Veterans Administration Geriatric Research, Education and Clinical Center.

COMPETING INTERESTS STATEMENT

The author declares that he has no competing financial interests.

Published online at http://www.nature.com/naturemedicine
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Glenner, G.G. & Wong, C.W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**, 885–890 (1984).
2. Grundke-Iqbal, I. *et al.* Abnormal phosphorylation of the microtubule-associated protein τ (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. USA* **83**, 4913–4917 (1986).
3. Goate, A. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704–706 (1991).
4. Sherrington, R. *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760 (1995).
5. Akiyama, H. *et al.* Inflammation and Alzheimer's disease. *Neurobiol. Aging* **21**, 383–421 (2000).
6. Wyss-Coray, T. & Mucke, L. Inflammation in neurodegenerative disease: a double-edged sword. *Neuron* **35**, 419–432 (2002).
7. Iqbal, K. *et al.* Subgroups of Alzheimer's disease based on cerebrospinal fluid molecular markers. *Ann. Neurol.* **58**, 748–757 (2005).
8. Eikelenboom, P. & Stam, F.C. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. *Acta Neuropathol. (Berl.)* **57**, 239–242 (1982).
9. McGeer, P.L., Itagaki, S., Boyes, B.E. & McGeer, E.G. Reactive microglia are positive for HLA-DR in the *substantia nigra* of Parkinson's and Alzheimer's disease brains. *Neurology* **38**, 1285–1291 (1988).
10. Katsel, P.L., Davis, K.L. & Haroutunian, V. Large-scale microarray studies of gene expression in multiple regions of the brain in schizophrenia and Alzheimer's disease. *Int. Rev. Neurobiol.* **63**, 41–82 (2005).
11. Blalock, E.M. *et al.* Harnessing the power of gene microarrays for the study of brain aging and Alzheimer's disease: statistical reliability and functional correlation. *Ageing Res. Rev.* **4**, 481–512 (2005).
12. Colangelo, V. *et al.* Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J. Neurosci. Res.* **70**, 462–473 (2002).
13. Groom, G.N., Junck, L., Foster, N.L., Frey, K.A. & Kuhl, D.E. PET of peripheral benzodiazepine binding sites in the microgliosis of Alzheimer's disease. *J. Nucl. Med.* **36**, 2207–2210 (1995).
14. Cagnin, A. *et al.* *In-vivo* measurement of activated microglia in dementia. *Lancet* **358**, 461–467 (2001).
15. Versijpt, J.J. *et al.* Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study. *Eur. Neurol.* **50**, 39–47 (2003).
16. Mahley, R.W. & Rall, S.C., Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu. Rev. Genomics Hum. Genet.* **1**, 507–537 (2000).
17. Mahley, R. & Huang, Y. Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol.* **10**, 207–217 (1999).
18. Lynch, J.R. *et al.* APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J. Biol. Chem.* **278**, 48529–48533 (2003).
19. van den Elzen, P. *et al.* Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* **437**, 906–910 (2005).
20. Kanter, J.L. *et al.* Lipid microarrays identify key mediators of autoimmune brain inflammation. *Nat. Med.* **12**, 138–143 (2006).
21. Chapman, J. *et al.* *APOE* genotype is a major predictor of long-term progression of disability in MS. *Neurology* **56**, 312–316 (2001).
22. McGeer, P.L., McGeer, E., Rogers, J. & Sibley, J. Anti-inflammatory drugs and Alzheimer disease. *Lancet* **335**, 1037 (1990).
23. Rogers, J. *et al.* Clinical trial of indomethacin in Alzheimer's disease. *Neurology* **43**, 1609–1611 (1993).
24. Scharf, S., Mander, A., Ugoni, A., Vajda, F. & Christophidis, V.N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. *Neurology* **53**, 197–201 (1999).
25. Hoozemans, J.J. & O'Banion, M.K. The role of COX-1 and COX-2 in Alzheimer's disease pathology and the therapeutic potentials of non-steroidal anti-inflammatory drugs. *Curr. Drug Targets CNS Neurol. Disord.* **4**, 307–315 (2005).
26. Mackenzie, I.R.A. & Munoz, D.G. Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging. *Neurology* **50**, 986–990 (1998).
27. Lim, G.P. *et al.* Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *J. Neurosci.* **20**, 5709–5714 (2000).
28. Morgan, D., Gordon, M.N., Tan, J., Wilcock, D. & Rojiani, A.M. Dynamic complexity of the microglial activation response in transgenic models of amyloid deposition: implications for Alzheimer therapeutics. *J. Neuropathol. Exp. Neurol.* **64**, 743–753 (2005).
29. Quinn, J. *et al.* Inflammation and cerebral amyloidosis are disconnected in an animal model of Alzheimer's disease. *J. Neuroimmunol.* **137**, 32–41 (2003).
30. Lanz, T.A., Fici, G.J. & Merchant, K.M. Lack of specific amyloid-β(1–42) suppression by nonsteroidal anti-inflammatory drugs in young, plaque-free Tg2576 mice and in guinea pig neuronal cultures. *J. Pharmacol. Exp. Ther.* **312**, 399–406 (2005).
31. Zhou, Y. *et al.* Nonsteroidal anti-inflammatory drugs can lower amyloidogenic Aβ42 by inhibiting Rho. *Science* **302**, 1215–1217 (2003).
32. Tegeder, I., Pfeilschifter, J. & Geisslinger, G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J.* **15**, 2057–2072 (2001).
33. Eriksen, J.L. *et al.* NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aβ42 *in vivo*. *J. Clin. Invest.* **112**, 440–449 (2003).
34. Sastre, M. *et al.* Nonsteroidal anti-inflammatory drugs repress β-secretase gene promoter activity by the activation of PPARγ. *Proc. Natl. Acad. Sci. USA* **103**, 443–448 (2006).
35. Yan, Q. *et al.* Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. *J. Neurosci.* **23**, 7504–7509 (2003).
36. Weggen, S. *et al.* A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature* **414**, 212–216 (2001).
37. Takahashi, Y. *et al.* Sulindac sulfide is a noncompetitive γ-secretase inhibitor that

preferentially reduces Aβ42 generation. *J. Biol. Chem.* **278**, 18664–18670 (2003).
38. Lleo, A. *et al.* Nonsteroidal anti-inflammatory drugs lower Aβ42 and change presenilin 1 conformation. *Nat. Med.* **10**, 1065–1066 (2004).
39. Beher, D. *et al.* Selected non-steroidal anti-inflammatory drugs and their derivatives target γ-secretase at a novel site. Evidence for an allosteric mechanism. *J. Biol. Chem.* **279**, 43419–43426 (2004).
40. Gasparini, L., Rusconi, L., Xu, H., del Soldato, P. & Ongini, E. Modulation of beta-amyloid metabolism by non-steroidal anti-inflammatory drugs in neuronal cell cultures. *J. Neurochem.* **88**, 337–348 (2004).
41. Kukar, T. *et al.* Diverse compounds mimic Alzheimer disease–causing mutations by augmenting Aβ42 production. *Nat. Med.* **11**, 545–550 (2005).
42. Wyss-Coray, T. Killing pain, killing neurons? *Nat. Med.* **11**, 472–473 (2005).
43. Tegeder, I. *et al.* Inhibition of NF-κB and AP-1 activation by R- and S-flurbiprofen. *FASEB J.* **15**, 595–597 (2001).
44. Sung, S. *et al.* Modulation of nuclear factor-κB activity by indomethacin influences Aβ levels but not Aβ precursor protein metabolism in a model of Alzheimer's disease. *Am. J. Pathol.* **165**, 2197–2206 (2004).
45. Monsonego, A. & Weiner, H.L. Immunotherapeutic approaches to Alzheimer's disease. *Science* **302**, 834–838 (2003).
46. Games, D. *et al.* Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* **373**, 523–527 (1995).
47. Hsia, A. *et al.* Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. USA* **96**, 3228–3233 (1999).
48. Masliah, E. *et al.* Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. *J. Neurosci.* **16**, 5795–5811 (1996).
49. Hsiao, K. *et al.* Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102 (1996).
50. Lewis, J. *et al.* Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat. Genet.* **25**, 402–405 (2000).
51. McGowan, E., Eriksen, J. & Hutton, M. A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet.* **22**, 281–289 (2006).
52. Oddo, S. *et al.* Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* **39**, 409–421 (2003).
53. Wu, Z.L. *et al.* Comparative analysis of cortical gene expression in mouse models of Alzheimer's disease. *Neurobiol. Aging* **27**, 377–386 (2006).
54. Dickey, C.A. *et al.* Selectively reduced expression of synaptic plasticity-related genes in amyloid precursor protein + presenilin-1 transgenic mice. *J. Neurosci.* **23**, 5219–5226 (2003).
55. Reddy, P.H. *et al.* Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Hum. Mol. Genet.* **13**, 1225–1240 (2004).
56. Miller, R.J. & Tran, P.B. Chemokinetics. *Neuron* **47**, 621–623 (2005).
57. Bellucci, A. *et al.* Induction of inflammatory mediators and microglial activation in mice transgenic for mutant human P301S tau protein. *Am. J. Pathol.* **165**, 1643–1652 (2004).
58. Ho, L. *et al.* Gene expression profiling of the tau mutant (P301L) transgenic mouse brain. *Neurosci. Lett.* **310**, 1–4 (2001).
59. Heneka, M.T. *et al.* Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. *J. Neuroinflammation* [online] **2**, 22 (2005) (doi:10.1186/1742-2094-2-22).
60. Nagasaka, Y. *et al.* A unique gene expression signature discriminates familial Alzheimer's disease mutation carriers from their wild-type siblings. *Proc. Natl. Acad. Sci. USA* **102**, 14854–14859 (2005).
61. Andreasson, K.I. *et al.* Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. *J. Neurosci.* **21**, 8198–8209 (2001).
62. Xiang, Z. *et al.* Cyclooxygenase-2 promotes amyloid plaque deposition in a mouse model of Alzheimer's disease neuropathology. *Gene Expr.* **10**, 271–278 (2002).
63. Liang, X. *et al.* Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J. Neurosci.* **25**, 10180–10187 (2005).
64. Shie, F.S., Montine, K.S., Breyer, R.M. & Montine, T.J. Microglial EP2 as a new target to increase amyloid beta phagocytosis and decrease amyloid beta-induced damage to neurons. *Brain Pathol.* **15**, 134–138 (2005).
65. Carroll, M.C. The role of complement and complement receptors in induction and regulation of immunity. *Annu. Rev. Immunol.* **16**, 545–568 (1998).
66. Eikelenboom, P., Hack, C.E., Rozemuller, J.M. & Stam, F.C. Complement activation in amyloid plaques in Alzheimer's dementia. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **56**, 259–262 (1989).
67. Wyss-Coray, T. *et al.* Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc. Natl. Acad. Sci. USA* **99**, 10837–10842 (2002).
68. Fonseca, M.I., Zhou, J., Botto, M. & Tenner, A.J. Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. *J. Neurosci.* **24**, 6457–6465 (2004).
69. Wyss-Coray, T. *et al.* TGF-β1 promotes microglial amyloid-β clearance and reduces plaque burden in transgenic mice. *Nat. Med.* **7**, 612–618 (2001).
70. Wyss-Coray, T. *et al.* Adult mouse astrocytes degrade amyloid-β *in vitro* and *in situ*. *Nat. Med.* **9**, 453–457 (2003).
71. Wyss-Coray, T., Lin, C., Sanan, D., Mucke, L. & Masliah, E. Chronic overproduction of TGF-β1 in astrocytes promotes Alzheimer's disease-like microvascular degeneration in transgenic mice. *Am. J. Pathol.* **156**, 139–150 (2000).
72. Brionne, T.C., Tesseur, I., Masliah, E. & Wyss-Coray, T. Loss of TGF-β1 leads to increased neuronal cell death and microgliosis in mouse brain. *Neuron* **40**, 1133–1145 (2003).
73. Weller, R.O. *et al.* Cerebral amyloid angiopathy. Amyloid β accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am. J. Pathol.* **153**, 725–733 (1998).
74. Tian, J., Shi, J., Bailey, K. & Mann, D.M. Negative association between amyloid plaques and cerebral amyloid angiopathy in Alzheimer's disease. *Neurosci. Lett.* **352**, 137–140 (2003).
75. Yamamoto, M. *et al.* Overexpression of monocyte chemotactic protein-1/CCL2 in beta-amyloid precursor protein transgenic mice show accelerated diffuse beta-amyloid deposition. *Am. J. Pathol.* **166**, 1475–1485 (2005).
76. Tan, J. *et al.* Role of CD40 ligand in amyloidosis in transgenic Alzheimer's mice. *Nat. Neurosci.* **5**, 1288–1293 (2002).
77. Wyss-Coray, T. *et al.* Key signaling pathways regulate the biological activities and accumulation of amyloid-β. *Neurobiol. Aging* **22**, 967–973 (2001).
78. Arancio, O. *et al.* RAGE potentiates Aβ-induced perturbation of neuronal function in transgenic mice. *EMBO J.* **23**, 4096–4105 (2004).
79. Abraham, C.R., Shirahama, T. & Potter, H. Alpha 1-antichymotrypsin is associated solely with amyloid deposits containing the beta-protein. Amyloid and cell localization of alpha 1-antichymotrypsin. *Neurobiol. Aging* **11**, 123–129 (1990).
80. Nilsson, L.N. *et al.* α-1-Antichymotrypsin promotes β-sheet amyloid plaque deposition in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.* **21**, 1444–1451 (2001).
81. Mucke, L. *et al.* Astroglial expression of human α_1-antichymotrypsin enhances Alzheimer-like pathology in amyloid protein precursor transgenic mice. *Am. J. Pathol.* **157**, 2003–2010 (2000).
82. Craft, J.M., Watterson, D.M., Frautschy, S.A. & Van Eldik, L.J. Aminopyridazines inhibit beta-amyloid-induced glial activation and neuronal damage *in vivo*. *Neurobiol. Aging* **25**, 1283–1292 (2004).
83. Li, Y., Liu, L., Barger, S.W. & Griffin, W.S. Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. *J. Neurosci.* **23**, 1605–1611 (2003).
84. Sheng, J.G., Zhu, S.G., Jones, R.A., Griffin, W.S.T. & Mrak, R.E. Interleukin-1 promotes expression and phosphorylation of neurofilament and *tau* proteins *in vivo*. *Exp. Neurol.* **163**, 388–391 (2000).
85. Kitazawa, M., Oddo, S., Yamasaki, T.R., Green, K.N. & LaFerla, F.M. Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J. Neurosci.* **25**, 8843–8853 (2005).
86. Schneider, A. *et al.* Hyperphosphorylation and aggregation of tau in experimental autoimmune encephalomyelitis. *J. Biol. Chem.* **279**, 55833–55839 (2004).
87. Wegiel, J. *et al.* Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. *Brain Res.* **804**, 135–139 (1998).
88. Kaku, M. *et al.* Amyloid beta protein deposition and neuron loss in osteopetrotic (op/op) mice. *Brain Res. Brain Res. Protoc.* **12**, 104–108 (2003).
89. Hickey, W.F. Basic principles of immunological surveillance of the normal central nervous system. *Glia* **36**, 118–124 (2001).
90. Ono, K. *et al.* Migration of exogenous immature hematopoietic cells into adult mouse brain parenchyma under GFP-expressing bone marrow chimera. *Biochem. Biophys. Res. Commun.* **262**, 610–614 (1999).
91. Beck, H. *et al.* Participation of bone marrow-derived cells in long-term repair processes after experimental stroke. *J. Cereb. Blood Flow Metab.* **23**, 709–717 (2003).
92. Malm, T.M. *et al.* Bone-marrow-derived cells contribute to the recruitment of microglial cells in response to beta-amyloid deposition in APP/PS1 double transgenic Alzheimer mice. *Neurobiol. Dis.* **18**, 134–142 (2005).
93. Stalder, A.K. *et al.* Invasion of hematopoietic cells into the brain of amyloid precursor protein transgenic mice. *J. Neurosci.* **25**, 11125–11132 (2005).
94. Simard, A.R., Soulet, D., Gowing, G., Julien, J.P. & Rivest, S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* **49**, 489–502 (2006).
95. Chiang, C.S., McBride, W.H. & Withers, H.R. Radiation-induced astrocytic and microglial responses in mouse brain. *Radiother. Oncol.* **29**, 60–68 (1993).
96. Fiala, M., *et al.* Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *J. Alzheimers Dis.* **7**, 221–232; discussion 255–262 (2005).
97. Togo, T. *et al.* Occurrence of T cells in the brain of Alzheimer's disease and other neurological diseases. *J. Neuroimmunol.* **124**, 83–92 (2002).
98. Itagaki, S., McGeer, P.L. & Akiyama, H. Presence of T-cytotoxic suppressor and leucocyte common antigen positive cells in Alzheimer's disease brain tissue. *Neurosci. Lett.* **91**, 259–264 (1988).
99. Rogers, J., Luber-Narod, J., Styren, S.D. & Civin, W.H. Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Aging* **9**, 339–349 (1988).
100. Skias, D., Bania, M., Reder, A.T., Luchins, D. & Antel, J.P. Senile dementia of Alzheimer's type (SDAT): reduced T8+-cell-mediated suppressor activity. *Neurology* **35**, 1635–1638 (1985).
101. Pirttila, T., Mattinen, S. & Frey, H. The decrease of CD8-positive lymphocytes in

Alzheimer's disease. *J. Neurol. Sci.* **107**, 160–165 (1992).
102. Richartz-Salzburger, E., *et al.* Altered lymphocyte distribution in Alzheimer's disease. *J. Psychiatr. Res.* advance online publication, 3 March 2006 (doi:10.1016/j.jpsychires.2006.01.010).
103. Dysken, M.W. *et al.* Distribution of peripheral lymphocytes in Alzheimer patients and controls. *J. Psychiatr. Res.* **26**, 213–218 (1992).
104. Trieb, K., Ransmayr, G., Sgonc, R., Lassmann, H. & Grubeck-Loebenstein, B. APP peptides stimulate lymphocyte proliferation in normals, but not in patients with Alzheimer's disease. *Neurobiol. Aging* **17**, 541–547 (1996).
105. Monsonego, A. *et al.* Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimer disease. *J. Clin. Invest.* **112**, 415–422 (2003).
106. Nath, A. *et al.* Autoantibodies to amyloid beta-peptide (Aβ) are increased in Alzheimer's disease patients and Aβ antibodies can enhance Aβ neurotoxicity: implications for disease pathogenesis and vaccine development. *Neuromolecular Med.* **3**, 29–39 (2003).
107. Weksler, M.E. *et al.* Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp. Gerontol.* **37**, 943–948 (2002).
108. Du, Y. *et al.* Reduced levels of amyloid β-peptide antibody in Alzheimer's disease. *Neurology* **57**, 801–805 (2001).
109. Hyman, B.T. *et al.* Autoantibodies to amyloid-beta in Alzheimer's disease. *Ann. Neurol.* **49**, 808–810 (2001).
110. Mruthinti, S. *et al.* Autoimmunity in Alzheimer's disease: increased levels of circulating IgGs binding Aβ and RAGE peptides. *Neurobiol. Aging* **25**, 1023–1032 (2004).
111. Moir, R.D. *et al.* Autoantibodies to redox-modified oligomeric Aβ are attenuated in the plasma of Alzheimer's disease patients. *J. Biol. Chem.* **280**, 17458–17463 (2005).
112. Eddleston, M. & Mucke, L. Molecular profile of reactive astrocytes—implications for their role in neurologic disease. *Neuroscience* **54**, 15–36 (1993).
113. Mennicken, F., Maki, R., de Souza, E.B. & Quirion, R. Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning. *Trends Pharmacol. Sci.* **20**, 73–78 (1999).
114. Gasque, P., Dean, Y.D., McGreal, E.P., Beek, J.V. & Morgan, B.P. Complement components of the innate immune system in health and disease in the CNS. *Immunopharmacology* **49**, 171–186 (2000).
115. Warner, T.D. & Mitchell, J.A. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J.* **18**, 790–804 (2004).
116. Lehmann, J.M., Lenhard, J.M., Oliver, B.B., Ringold, G.M. & Kliewer, S.A. Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.* **272**, 3406–3410 (1997).
117. Bernardo, A., Ajmone-Cat, M.A., Gasparini, L., Ongini, E. & Minghetti, L. Nuclear receptor peroxisome proliferator-activated receptor-γ is activated in rat microglial cells by the anti-inflammatory drug HCT1026, a derivative of flurbiprofen. *J. Neurochem.* **92**, 895–903 (2005).
118. Stellwagen, D. & Malenka, R.C. Synaptic scaling mediated by glial TNF-α. *Nature* **440**, 1054–1059 (2006).
119. Wahl, S.M. Transforming growth factor beta (TGF-β) in inflammation: a cause and a cure. *J. Clin. Immunol.* **12**, 61–74 (1992).