J. Cell. Mol. Med. Vol 13, No 11-12, 2009 pp. 4329-4348

Guest Editor: B.O. Popescu

Neuronal death in Alzheimer's disease and therapeutic opportunities

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Received: May 20, 2009; Accepted: August 24, 2009

Abstract

Alzheimer's disease (AD) is an age-related neurodegenerative disease that affects approximately 24 million people worldwide. A number of different risk factors have been implicated in AD; however, neuritic (amyloid) plaques are considered as one of the defining risk factors and pathological hallmarks of the disease. In the past decade, enormous efforts have been devoted to understand the genetics and molecular pathogenesis leading to neuronal death in AD, which has been transferred into extensive experimental approaches aimed at reversing disease progression. Modern medicine is facing an increasing number of treatments available for vascular and neurodegenerative brain diseases, but no causal or neuroprotective treatment has yet been established. Almost all neurological conditions are characterized by progressive neuronal dysfunction, which, regardless of the pathogenetic mechanism, finally leads to neuronal death. The particular emphasis of this review is on risk factors and mechanisms resulting in neuronal loss in AD and current and prospective opportunities for therapeutic interventions. This review discusses these issues with a view to inspiring the development of new agents that could be useful for the treatment of AD.

Keywords: Alzheimer's disease • neuronal death • neurodegeneration • neurotoxicity • neuroprotection • amyloid plaques

Introduction

Alzheimer disease (AD) is a neurodegenerative disease that causes changes in brain function. AD usually affects people over the age of 65 years, with a plethora of devastating clinical symptoms such as progressive decline in memory, thinking, language and learning capacity. Age is the strongest predictor for the development and progression of AD and with the rapidly aging population, AD clearly poses a major health problem and socio-economic burden. An estimated 5–10% of the population

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doi:10.1111/j.1582-4934.2009.00889.x

aged 65 years and over, and 40% of the population greater than 85 years of age are likely to be affected [1]. The genetic predisposition accounts for only 5–10% and is associated with early onset [2, 3].

Given the aging population in Western countries, the grave suffering of the many individuals concerned and their families, and the increasing economic cost of AD in terms of both healthcare expenses and lost wages it makes it important to understand the disease mechanisms and propose effective treatment strategies. The clinical manifestation of AD is considered to be correlated with the degree of neuronal loss in the brain and more specifically in hippocampal and neocortical areas. While AD can be identified based on clinical symptoms and by excluding other conditions with similar phenomenology and psychopathology, a definite diagnosis is only possible post-mortem by histological analysis of the patient's brain. Extracellular senile (amyloid) plaques and intracellular neurofibrillary tangles (NFTs) are considered to be hallmarks of the disease [4]. Senile plaques contain extracellular deposits of amyloid-β protein (Aβ), α-synuclein, ubiquitin and apolipoprotein E. NFTs are intracellular and extracellular aggregates of hyperphosphorylated τ protein and apolipoprotein E [5]. Evidence that Aβ alone is not sufficient for initiating the pathophysiological cascade of AD came from experiments with transgenic mice, which suggested that sustained brain inflammation might be another essential factor in AD pathogenesis. Different inflammatory markers such as activated microglia and astrocytes, elevated levels of various cytokines and complement activation products are found in AD brains [6]. Complement proteins are reported to be integral components of amyloid plaques and cerebral vascular amyloid in AD. They are found at the earliest stages of amyloid deposition and their activation coincides with the progressive clinical manifestation of AD [7, 8].

Here we review the interrelationship between different risk factors for AD aetiology with particular emphasis on their role for neuronal death as well as different strategies for neuroprotection as a potentially promising therapeutic strategy.

Origin of the disease and mechanisms for neuronal death in AD

The pathogenesis of AD is not yet fully understood. Various hypotheses with respect to disease aetiology have been proposed and those that are most widely accepted on grounds of a solid experimental data base and clinical evidence are described below.

Inflammatory reaction

This hypothesis is based on observations that non-steroidal anti-inflammatory drugs (NSAIDs) can reduce neurotoxins in patients suffering from AD. In epidemiological and clinical studies, it was initially noted that patients on high doses of anti-inflammatory drugs exhibit a reduced incidence of AD and reduced the AD symptoms [9–14]. However, this hypothesis became even more attractive when it was demonstrated that the mediators and products of inflammatory reaction, such as cytokines [15, 16], complement proteins (reviewed in [17]), free radicals [18, 19], adhesion molecules [20, 21] and prostaglandins (reviewed in [22]), were neurotoxic in experiments with neural models. These products of inflammatory reactions may represent extracellular signals which initiate and promote neuronal degeneration in AD. For instance, long-time administration of NSAIDs to arthritic patients can reduce the risk of developing AD [7, 11]. Potent inflammatory molecules, such as cytokines, chemokines and complement factors, are present in both cerebrospinal fluid (CSF) and plaques from patients with AD [7]. Aggregated peptides, such as Aβ are shown to induce the production of pro-inflammatory cytokines like interleukin (IL)-1β, tumour necrosis factor (TNF)-α and chemokines (MIP-1α, MIP-1β, MCP-1) by microglial cells [23–25]. These inflammatory molecules were found to be toxic in neuronal models of AD and were implicated in extracellular signalling which initiated and promoted neuronal degeneration in AD. Several intracellular signals are candidates for mediating actions of inflammatory factors inside the cells such as Aβ [26, 27], ubiquitin [28, 29] and proteasome [30–32]. The observed protective effect of NSAIDs could be due to a reduction of microglial activation and/or decreased generation of Aβ [33, 34]. These observations are additionally confirmed by *in vitro* studies with human neuronal cells which seem to be protected from the toxic effects of Aβ by NSAIDs [35].

While epidemiological and experimental studies lend strong support for neuroinflammatory responses as drivers of AD pathogenesis, recent *in vivo* studies also support a beneficial role for such reactions (reviewed in [36]). A very strong support for the beneficial impact of neuroinflammation on neuronal survival and function came recently from a study with transgenic mice with brain-directed overexpression of human soluble IL-1 receptor antagonist [37]. Chronic blockade of IL-1 signalling in the brain of these animals was found associated with an atrophic phenotype of the brain and with modified levels of the amyloid precursor protein (APP) and presenilin 1 (PS1), a critical component of APP processing machinery (discussed below). A number of reports have provided evidence that activation of microglia and the subsequent degradation of amyloid plaques may underlie this phenomenon. These observations in animal models challenge earlier assumptions that IL-1 elevation and resulting neuroinflammatory processes play a purely detrimental role in AD, and prompt a need for new characterizations of IL-1 function.

Aβ-induced neurotoxicity

The extracellular Aβ deposition has attracted major attention as a cause of cytotoxicity in AD. The original 'amyloid hypothesis' argues that Aβ deposition is the initiator for AD pathogenesis, based on the following facts: Aβ is a major component of the amyloid plaques [38]; the deposition of Aβ occurs prior to other

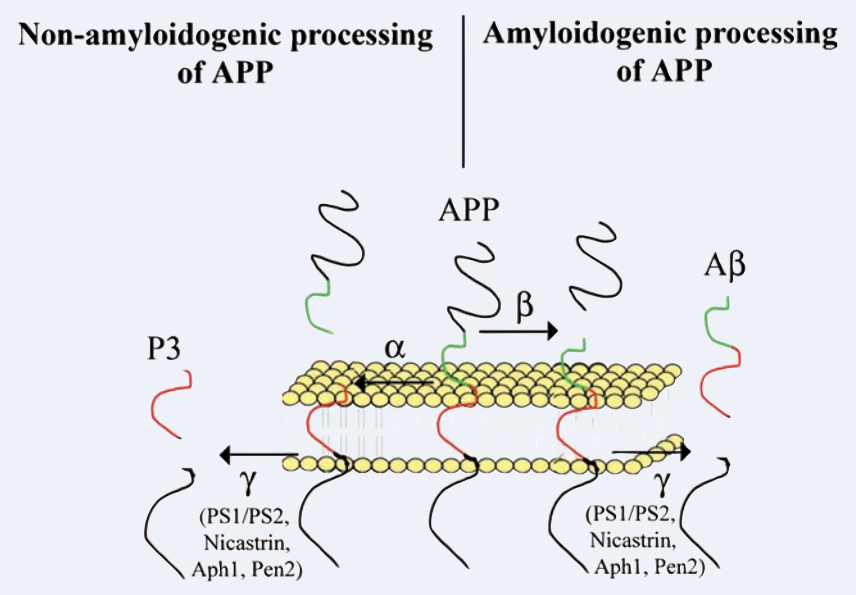

Fig. 1 Processing of APP. APP can be processed by different sets of enzymes: one pathway leads to amyloid plaque formation (amyloidogenic), while the other does not (non-amyloidogenic). In the non-amyloidogenic pathway, APP is cleaved first by α-secretase to yield a soluble N-terminal fragment and a C-terminal fragment integrated within the cellular membrane. The soluble protein may be involved in the enhancement of synaptogenesis, neurite outgrowth and neuronal survival, and is considered to be neuroprotective. The membrane fragment of the cleaved APP is acted upon by γ-secretase, a tetrameric complex comprised presenilins, nicastrin, Aph1 and Pen2, to yield a soluble N-terminal fragment (P3) and a membrane-bound C-terminal fragment. In the amyloidogenic pathway, APP is cleaved first by β-secretase, yielding a soluble N-terminal fragment and a membrane-bound C-terminal fragment. The membrane fragment is then cleaved by γ-secretase, yielding a membrane-bound C-terminal fragment and a soluble N-terminal fragment (Aβ). While Aβ is required for neuronal function, it can aggregate in the extracellular space of the brain to form amyloid plaques.

pathological events such as NFT formation and neuronal loss [39]; synthetic Aβ peptides, particularly Aβ1–42/43, induce neuronal death *in vitro* [40, 41]. Aβ is proteolytically derived from the β-APP by the action of β- and γ-secretases [42] (Fig. 1). These enzymes cleave at the N-terminus of the Aβ region of APP followed by cleavage at the C terminus producing peptides of a length of 39 to 43 amino acids. The two forms found predominantly in amyloid plaques and believed to be associated with the disease, are 40 and 42 amino acids long. Alternatively, the α-secretase cleaves APP within the Aβ domain yielding a non-toxic C terminal fragment, followed by γ-secretase cleavage resulting in a C terminal fragment-γ and a small peptide called P3 that is not considered amyloidogenic. Presenilins (PS1 and PS2) regulate the activity of γ-secretase and are involved in the processing of APP and in the generation of Aβ peptides [43] (Fig. 1). Presenilins were first identified as enzymes acting as γ-secretase [44]. More recently, it was discovered that presenilins are part of a tetrameric complex comprised the proteins nicastrin, Aph1 (anterior pharynx-defective phenotype) and Pen2 (presenilin enhancer) that altogether regulate the production of Aβ peptides [45, 46]. In AD patients carrying missense mutations on either of the genes for PS1 or PS2, it was reported that the overall levels of Aβ peptides were increased in the plasma, and that levels of the more amyloidogenic Aβ42 were increased in cultured fibroblasts of patients carrying inherited mutations on the genes for presenilins [47–49]. Experiments with animal models lacking expression of either PS1 or PS2 showed that between the two presenilins PS1 plays a major role regulating the activity of γ-secretase and thus Aβ formation [50, 51]. This new knowledge opens new hypotheses about the mechanism that orchestrates the regulated intra-membrane proteolysis in γ-secretase substrates and in APP in particular.

Interestingly, presenilins have been implicated not only in regulation of activity of γ-secretase but also in control of apoptotic machinery [52, 53]. AD associated presenilins showed to be substrates for proteolytic degradation. These investigations strongly suggest that alternative presenilin fragments could regulate cell survival. Furthermore, mutations in presenilin genes, which likely suppress proteolytic degradation of these proteins, sensitize neurons to apoptosis by different mechanisms (*e.g.* increased caspase-3 activation, production of oxyradicals, calcium signalling dysregulation). These data demonstrate the complex dual nature of regulation of neuronal death in AD by presenilins and suggest that any treatment targeting these proteins might be a double-edged sword and should be carefully considered.

Accumulated Aβ induces multiple cytotoxic effects, including oxidative stress, and alternation of ionic homeostasis in neurons [54, 55]. Aβ also alters the activities of various kinases, including GSK3β, cdk5, PKA and causes hyperphosphorylation of τ protein, leading to NFT formation [56–58]. These Aβ-initiated toxicities directly or indirectly induce neuronal cell death. Although this classical Aβ hypothesis does explain some of the mechanisms underlying the pathogenesis and progression of AD, there is also evidence against this hypothesis. For example, the quantity of Aβ deposits does not correlate with clinical features, as senile plaques are also found in brains of elderly subjects without dementia [59]. Accumulation of senile plaques does not necessarily correlate with the amount of synaptic loss [60, 61] and the severity of the clinical manifestation [62]. In addition, several lines of transgenic

mice with human familial AD mutant genes show considerable Aβ deposits in brain without exhibiting other AD-specific pathological features or behavioural abnormalities. Even though some evidence suggest that the Aβ deposition alone is not sufficient for the development of AD, formation of the senile plaques seems to be involved in triggering most of the subsequent pathogenetic phenomena.

Although neurotoxicity of Aβ has been initially attributed to its fibrillar forms, more recent studies showed that neurotoxins also comprise small diffusible Aβ oligomers called Aβ-derived diffusible ligands (ADDLs), which were found to kill mature neurons in organotypic central nervous system (CNS) cultures [63]. At cell surfaces, ADDLs bound to trypsin-sensitive sites and surface-derived tryptic peptides blocked binding and afforded neuroprotection. Remarkably, neurological dysfunction evoked by ADDLs occurred well in advance of cellular degeneration. Recently it has been demonstrated that non-fibrillar assemblies of Aβ possess electrophysiological activity, with the corollary that they may produce dementia by disrupting neuronal signalling prior to cell death [64]. Recent experiments have detected the presence of ADDLs in AD-afflicted brain tissue and in transgenic mice models of AD [65–67]. The presence of high affinity ADDL binding proteins in hippocampus and frontal cortex but not cerebellum parallels the regional specificity of AD pathology and suggests involvement of a toxin receptor-mediated mechanism. The properties of ADDLs and their presence in AD-distressed brain are consistent with their putative role even in the earliest stages of AD, including forms of mild cognitive impairment. Considering the central role of Aβ in AD pathology, Aβ peptides and mechanisms for their formation have been the primary target in a number of contemporary attempts to develop therapeutic agents against AD.

Complement-mediated neurodegeneration

Although viewed for years as a so-called immuno-privileged organ, the CNS contains and synthesizes many components of the immune system. Both glia and nerve cells in the brain can synthesize immunoglobulins [68] and complement components [69, 70]. Brain cells can express all of the complement components and should thus be considered as an important source of complement [71].

The two major fibrillar amyloid forms Aβ40 and Aβ42 directly activate the alternative and classical pathways of the complement system by binding to C3 and the globular B head of C1q resulting in the formation of pro-inflammatory molecules such as C3a, C5a and membrane attack complex (MAC). MAC permeabilizes the cell membrane, which can result in cell lysis (Fig. 2). Astrocytes and microglia in the CNS produce both soluble complement regulatory proteins (CReg) and membrane-bound CReg (CD59, CD55, CD46) [72], which ensures a good level of protection against complement lysis by inhibiting MAC formation. However, the same cannot be said for neurons. There is very little information regarding the expression of CReg in neuronal cells. Studies to date suggest that cultured primary neurons express only low levels of the membrane CReg (mCReg), CD59 and CD55 [73, 74]. Furthermore, in the frontal cortex and hippocampus of AD brains, a significant decrease in the expression of CD59 at both protein and mRNA levels was found when compared with brains of age-matched control individuals without dementia [75]. An interesting hypothesis suggests that at sublytic concentrations, MAC can alter the function of neurons by triggering various cellular signalling pathways [8]; however, this needs to be further explored and corroborated in further systematic investigations.

There is recent evidence that complement may also play a protective role in AD brain. The most convincing data for the protective role of components of the complement cascade demonstrate that production of C5a results in an activation of the neuroprotective mitogen activated protein kinases [76] (Fig. 2). Furthermore, animals genetically deficient in the complement component C5 were found to be more susceptible to hippocampal excitotoxic lesions [77]. These findings suggest a novel non-inflammatory role for C5a in modulating neuronal responses to excitotoxins. More recently, the protective role of the early activation stages of the complement cascade in AD was demonstrated in transgenic mice with human APP, in which complement was blocked by expression of soluble Crry (a rodent CReg with inhibitory activities similar to human CD46 and CD55) [78]. Results showed that mice expressing soluble Crry had a 2–3-fold increase in Aβ accumulation and neuronal degeneration compared to animals that did not express the inhibitor. However, Crry inhibits C3 activation, and thus prevents generation of C5a. It was suggested that the observed effect might be due to decreased C5a formation. Another recent study found that C1q protected cultured primary neurons against Aβ and SAP (serum amyloid P) induced neurotoxicity [79]. The exact mechanism of this protection has not yet been elucidated but the data suggest that C1q does not protect from Aβ induced apoptosis. However, considering the protective role of C5a, the C1q-mediated defence against senile plaques might be at least in part due to the generation of C5a. This speculation is supported by a recently published *in vivo* study on C3 deficient APP transgenic mice that demonstrated accelerated plaque deposition and neurodegeneration compared to C3 sufficient animals [80].

Although it is still disputable if complement system is a primary cause of neuronal degeneration or a secondary event in AD [81], the findings discussed above demonstrate that complement not only lyses neurons, but also generates inflammation resulting in an amplification of AD risk factors and neuronal death [17]. Thus, targeting the complement cascade seems an attractive candidate strategy for treating AD and other neurodegenerative diseases. However, complement seems to possess a dual, ambiguous role in the pathogenesis and progression of the AD which needs to be carefully considered when choosing targets for therapeutics to be developed.

Oxidative and nitrosylative damage

This hypothesis emphasizes the role of reactive oxygen and nitrogen species (ROS or RNS) in initiation and promotion of neurodegeneration in the brains of AD patients [18, 19]. Some of these radicals originate from normal brain function processes: it is

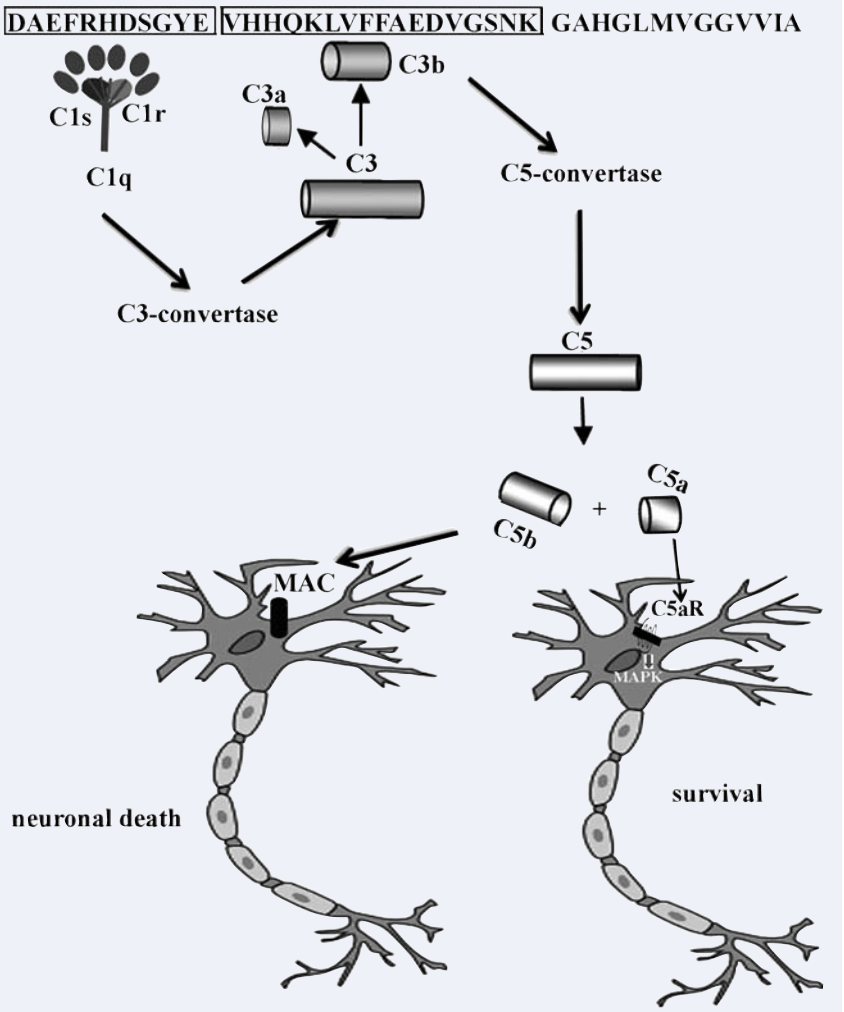

Fig. 2 Activation of complement system by the amyloid plaques and its dual role in neuronal death. C1q binds to the boxed sequence in aggregated amyloid peptides and activates classical pathway of complement system. This leads to cleavage of C3 to C3b which could bind to a different sequence from the amyloid peptide (boxed). C3b initiates generation of C5-convertase cleaving C5 into C5a and C5b. C5a interacts with the C5a receptor (C5aR) on the surface of neurons and activates the neuroprotective mitogen activated protein kinases. On the other hand, C5b initiates the terminal pathway of complement cascade which forms MAC which integrates into membrane of the weakly protected neurons resulting in their death.

known that the brain utilizes about 25% of respired oxygen and that about 2% of the oxygen consumed converts into ROS. Other free radicals are released during inflammatory reactions and by Aβ. This hypothesis for the AD aetiology is supported by the observation in some clinical trials that high doses of antioxidants might be beneficial to AD patients [82, 83].

Increased oxidative stress may enhance intracellular accumulation of Aβ in neurons [84]. In addition, studies show that membranes containing oxidatively damaged phospholipids accumulated Aβ faster than membranes containing only normal saturated phospholipids [85]. Based on this finding, it was proposed that one or some of the mechanisms of action in Aβ neurotoxicity might be free-radical mediated [86]. Further studies on protection of cultured neuronal cells against Aβ-induced toxicity by vitamin E supported this hypothesis [87]. Additional evidence of increased oxidative stress in AD include: AD patients have lower levels of vitamins A, E and β-carotene in their serum compared to control patients [88]; increased consumption of oxygen is found in AD patients [89]; activation of calcium-dependent neural proteinase (calpain), triggering formation of free radicals, was found in AD autopsy brain samples [90]; homogenates of frontal cortex from such autopsy samples revealed a 22% higher production of free radicals than those of age-matched controls [91]; increased neuronal nitric oxide synthase (nNOS) expression in reactive astrocytes correlated with apoptosis in hippocampal neurons of post-mortem brains from AD patients [92]; glutamine synthetase, a highly sensitive enzyme to oxidative stress, showed decreased activity in the AD autopsy brains [93]; increased levels of oxidized proteins are found in the blood of AD patients when compared with non-AD controls [93].

Proteasome inhibitor-induced neurotoxicity

Numerous studies now clearly demonstrate that inhibition of the proteasome is sufficient to induce cell death in both neuronal and

glial cells. These studies have shown that proteasome inhibitors can induce hallmarks of apoptosis, including caspase activation, cytochrome C release, elevated p53 expression, chromatin fragmentation and DNA laddering [94–99]. The induction of such a wide variety of cell death events by proteasome inhibitors suggests a critical role for proteasome activity in neuronal homeostasis. The proteasome is directly responsible for the degradation of a number of cell death factors and its inhibition is sufficient to increase their levels to a cytotoxic level. Furthermore, the proteasome prevents accumulation of proteins not directly related to cell death, but which can contribute to neuron death by conferring an increased half-life of neurotoxic proteins. For example, proteasome inhibition has been shown to increase the accumulation of cytotoxic Aβ [100, 101] and presenilin [102, 103]. It has been shown that heat shock proteins (HSPs) can enhance proteasome-mediated proteolysis [104, 105].

However, proteasome inhibition does not induce neuronal death in all neuron populations or experimental setups [106, 107]. It seems that proteasome inhibitor-induced toxicity is cell-type specific. It was demonstrated that proteasome inhibition could have very different effects on cell death based on the differential role of NF-κB – a downstream proteasome target which can have pro-apoptotic or anti-apoptotic effects depending on cell type [108, 109]. In transferring the knowledge obtained from *in vitro* studies to studies in the mature CNS, it is important to consider the fact that the majority of *in vitro* studies are conducted in cultures established from embryonic tissues or early postnatal brain. One thus must take into account the possibility that embryonic tissue may have a different dependence on proteasome activity than established neurons within the developed and mature, or, as in the case of AD patients, aged CNS.

Only recently, the importance of the proteasome in the physiology and pathology of the CNS has begun to emerge. It is likely that studies on this intriguing enzyme will yield further important information on how oxidative stress occurs in the CNS and how CNS protein turnover is regulated. Ultimately, this may lead to the design of useful new therapeutic strategies aimed at eliminating oxidative stress toxicity in the CNS, thereby contributing to the prevention and/or amelioration of clinical AD symptoms.

Cholesterol-induced neurotoxicity

All mammalian cells require cholesterol for the formation and maintenance of cell membrane permeability and fluidity. There is increasing evidence in support of the hypothesis that alterations in cholesterol levels influence the development of AD by affecting Aβ formation and distribution within cholesterol rich membranes. Proteolytic processing of APP is believed to occur at or in close proximity to the cholesterol-rich plasma membrane. When cultured human cells transfected with the APP cDNA are exposed to an inhibitor of the HMG-CoA reductase (the rate controlling enzyme of the metabolic pathway that produces cholesterol and other isoprenoids) β-secretase cleavage of newly synthesized APP is markedly reduced. Addition of solubilized cholesterol to these cells causes a four-fold increase in newly synthesized β-amyloidogenic products, while reduction of the cellular cholesterol level of hippocampal neurons inhibits formation of Aβ [110].

Above *in vitro* data correlate well with both *in vivo* experiments using AD animal models and *ex vivo* data obtained from post-mortem brains of AD patients. In a double transgenic mouse that overexpresses mutant APP and presenilin-1, a high cholesterol diet resulted in significantly increased levels of Aβ in brain tissue. The number and size of Aβ deposits were elevated. Levels of total Aβ were strongly correlated with the levels of both plasma and CNS cholesterol [111]. When these mice were treated with an inhibitor of HMG CoA reductase, a cholesterol-lowering drug, plasma cholesterol, brain Aβ peptidesand Aβ load decreased by more than two-fold [112]. Results in cholesterol-fed rabbit models are consistent with these results in murine transgenic models. Cholesterol-fed rabbits produce and accumulate Aβ in the brain and this accumulation can be reversed by removing cholesterol from the rabbits' diet [113, 114]. In post-mortem brains of AD patients, fluorometric staining has confirmed cholesterol accumulation in the core of mature senile plaques. Although it is not firmly established whether disruption of cholesterol balance at the cellular level is a primary cause of AD or rather a secondary effect of the disorder, the above evidence strongly support the view that membrane cholesterol is responsible for the binding and toxicity of Aβ.

Viral infections

Although the exact mechanisms of driving neuronal death by viral infection are not very clear, it was proposed that string inflammatory responses promoted by viral infections might be responsible for the increased risk of developing AD. A comparative examination of post-mortem brain tissue from 97 people with HIV/AIDS and 125 non-infected people of similar age established that Aβ plaques, indicative of AD, generally increased with age in both groups, but were more than twice as common in the HIV/AIDS group. Almost one in five of those who had died of AIDS in their 40s had the characteristic plaques but there was no evidence these plaques in the brain tissue of non-infected people of the same age. Two out of the ten HIV infected patients in their late 30s and early 40s who had died before developing AIDS also had the characteristic plaques. These findings strongly indicated that an inflammatory response in the brain, such as that caused by viral infection, may initiate and/or accelerate the pathological processes leading to AD [115].

Actually, a number of viral or bacterial pathogens (*Chlamydia Pneumoniae*, Herpes simplex type 1 (HSV-1) and type 2 (HSV-2), herpes virus 6 (HHV-6), cytomegalovirus (CMV) and *Helicobacter pylori* bacterium) have been implicated in AD [116–118]. As these pathogens are so common, however, they probably only contribute to AD if acting in concert with other factors, or under certain specific conditions. It was shown that the risk-promoting effects of Herpes simplex in AD are influenced by a number of molecular mechanisms such as possession of the ApoE4 allele [119], APP processing [120], and possible involvement of APP in the intraneuronal traffic of the

virus [121]. Herpes infection was shown to increase the production of Aβ in mouse brains [122]. Numerous other genes and proteins implicated in AD interact with the products of the Herpes simplex viral genome or play a role in its life cycle, suggesting a complex synergy between host and pathogen that may play an important role in the pathophysiology of AD (reviewed in [3]).

Interrelationship between different aetiopathogenic theories of AD

Many possible risk factors for AD have been investigated, with only very few showing positive associations and none defining the aetiology of the neurodegenerative disease on its own. Therefore, it is likely that interplay between at least some of these potential risk factors determines AD pathogenesis *via* a pathophysiological cascade.

Substantial evidence suggests that, apart from age, a history of head injury [123–127] and/or certain types of viral infections [128–131] increase the risk of AD. These two risk factors, however, are accompanied by a chronic inflammatory reaction leading to the release of a number of cytokines, including interferon (IFN)-γ and TNF-α. An inhibitory effect of IFN-γ on the translational machinery has been well-documented [132–134], leading to lower expression of proteins, including SC35 and hnRNPA1, whose downmodulation results in increased secretion of Aβ by cultured neurons [135]. A cytokine-dependent enhancement of Aβ production from APP-expressing astrocytes and cortical neurons in transgenic mice was also demonstrated [136]. Furthermore, HSV-1 protein ICP27 has been shown to inhibit host cell splicing [137–139] by altering phosphorylation of SC35 and other proteins [140], which we showed to yield in accumulation of Aβ [135]. Cholesterol and other 'raft' lipid components, such as sphingomyelin and galactosylceramide, induce aggregation of the secreted Aβ peptide [141, 142].The accumulated amyloid plaques on the other hand cause neuronal death by triggering a number of processes such as generation of ROS and RNS and inhibition of proteasomes, but one of the most important mechanisms is the activation of the complement system that not only lyses neurons, but also generates inflammation which in turn amplifies the AD risk factors. Due to the exceptionally complex events involved in AD neurodegeneration and their reciprocal relationship and interaction, it is difficult to determine what the primary cause of the disease is. It is likely that different primary risk factors synergize to initiate pathology. It is also possible that several different initial pathomechanisms ultimately result in a similar neuropathology (plaques, tangles) and clinical phenomenology (dementia), thus raising the question whether the diagnosis of AD really represents a single nosological entity.

Treatment approaches

AD is a very complex disease and its clinical management is highly challenging. Personality and behavioural changes, and the progressive difficulties with performing activities of daily living lead to dependence on external help and support. A cure for AD is still not available and clinicians and caregivers are challenged by an increasingly aging population with ever higher risk of dementia. The primary goals of treatment are to maximize the patient's ability to function in daily life, maintain quality of life, slow the progression of symptoms, treat depression and disruptive behaviour. However, at present all treatments for AD offer only modest symptomatic relief for periods of between six to eighteen months. Some of the currently used/researched agents are considered to delay or even prevent AD symptoms which could contribute to reducing the number of patient with dementia. However, until now complement proteins and CRegs have been overlooked as targets for the development of neuroprotective drugs for AD treatment. But accumulated data suggest that appropriate modulation of expression and/or activity of proteins controlling the complement cascade could be very beneficial for AD patients. Here we review the current, albeit still unsatisfactory state of this field. There are a number of different categories of drugs currently used in AD treatment (summarized in Table 1) and examples of each group are discussed below. Based on the ongoing research we also speculate on the future developments regarding AD therapeutics.

Drugs which modify AD symptoms

Cholinergic treatment

Acetylcholinesterase inhibitors are one of the most commonly used drugs to treat AD [143]. Their use stems from the observation that there is a deficiency in cholinergic neurotransmission in AD. According to one hypothesis, decreased cholinergic transmission plays a major role in the development of cognitive, functional and behavioural symptoms of AD [144, 145]. As a result of the pathological changes during AD there are decreases in biochemical indices of cholinergic functioning in neocortex and hippocampus that correlate with dementia severity [146–148]. The hypothesis is further supported by pharmacological studies using cholinergic agonists and antagonists and transgenic animal models that emphasize the close connection between cognition and cholinergic neurotransmission [149]. The cholinesterase inhibitors currently approved by the US Food and Drug Administration (FDA) are tacrine, donepezil, rivastigmine and galantamine. The mechanism of action of these drugs consists in increasing the availability of acetylcholine through an inhibition of the catabolic enzyme acetylcholinesterase.

Antiglutamic treatment

Another approach is to block glutamic neurotransmission. Glutamate is the main excitatory neurotransmitter in the brain and has been implicated in so-called excitotoxicity. One of its receptors – M-methyl-D-aspartate (NMDA), has been implicated in long-term potentiation, the neuronal mechanism responsible for learning and memory [150]. The low affinity non-competitive NMDA antagonist, Memantine, was approved by the FDA for AD treatment in

Table 1. Therapeutics in use or under research/development for treatment of AD [255–258]

Agents	Targets/mechanisms	Status
Disease-modifying therapies		
Donepezil (Aricepts)	Cholinesterase inhibitor	FDA approved
Rivastigmine (Exelon)	Cholinesterase inhibitor	FDA approved
Galantamine (Reminyl)	Cholinesterase inhibitor	FDA approved
Physostigmine Salicylate (Synapton)	Acetylcholinesterase inhibitor	Discontinued
Milameline (CI 979)	Partial muscarinic agonist; Increases central cholinergic activity	Discontinued
AF 102B (cevimeline HCL, Evoxac)	Blocks production of Aβ by increasing the activity of α-secretase and possibly by inhibiting γ-secretase	Discontinued
SB202026 (Memric, Sabcomeline)	Selective muscarinic M1 partial agonist	Discontinued
Tacrine (Cognex)	Acetylcholinesterase inhibitor	FDA approved
Metrifonate	Cholinesterase inhibitor	Discontinued
Memantine (Ebix, Namenda)	NMDA receptor antagonist	FDA approved
LY450139	γ-secretase inhibitor	Phase III
PF-04494700 (TTP488)	Inhibitor of Receptor for Advanced Glycation Endproducts (RAGE)	Phase II/IIa/IIb
NGX267 (AF267B)	M1 muscarinic agonist, α-secretase activator	Discontinued
AZD3480 (ispronicline, TC-1734)	Nicotinic agonist with high selectivity for neuronal α4β2 nicotinic (nAChR) receptors	Phase II/IIa/IIb
BMS-708163	γ-secretase inhibitor	Phase II/IIa/IIb
AL-108	Stabilizes microtubules, blocks Aβ aggregation	Phase II
MK 0752	γ-secretase inhibitor	Phase II
E2012	γ-secretase inhibitor	Phase I
AZD103 ATG-Z1	Inhibits Aβ fibrillization and disassembles preformed amyloid fibrils	Phase I Preclinical
OM99-2	BACE1 inhibitor	Investigational
KMI-429	Analogue for the aspartyle protease, BACE1 inhibitor	Investigational
GRL-8234	Analogue for the aspartyle protease, BACE1 inhibitor	Investigational
KNI-1027	BACE1 inhibitor	Investigational
GSK188909	BACE1 inhibitor	Investigational
	BACE1 inhibitor	
Symptom-management therapies		
Alpha-tocopherol (Vitamin E)		Phase III
Acetyl-l-carnitine HCl (ALCAR)	Destroys toxic free radicals Not exactly known (some hypotheses: mitochondrial/energy	Discontinued
Cerebrolysin	production; stabilizes membranes; decrease accumulations of toxic fatty acids)	FDA approved (outside USA)
Ibuprofen	Exerts nerve growth factor like activity on neurons from dorsal root ganglia; neurotrophic and neuroprotective agent	Phase III
Flurizan (MPC-7869, r-flurbiprofen)	Reduces prostaglandin activity by inhibiting prostaglandin	Discontinued
Huperzine (Cerebra capsule, Pharmassure)	synthetase; anti-inflammatory, analgesic ,and some antipyretic activity Selective amyloid-lowering agent	Phase II/IIa/IIb Discontinued
Naproxen (Aleve, Anaprox, Naprosyn) Estrogen (Premarin)	Selective inhibition of AChE; alterations in APP processing; reduction of neurotoxicity by Aβ; antioxidant effects; increase of NGF production	Phase III Phase III
Xaliproden	Anti-inflammatory	Phase III

Continued

Table 1 Continued

Agents	Targets/mechanisms	Status
Lecozotan	Thought to enhance growth of neurons in the basal forebrain; may have a direct effect on Aβ	Phase III
Dimebon	5-HT_{1a} receptor agonist	Phase II/IIa/IIb
PRX-03410	5-HT_{1a} receptor antagonist	Phase II/IIa/IIb
MEM 1003	Inhibitor of cholinesterase and NMDA receptors; Inhibits neuronal death, potentially by mitochondrial-mediated inhibition of apoptosis	Phase II/IIa/IIb
MEM 3454 (RO5313534)	Partial 5-HT4 receptor agonist	Phase II/IIa/IIb
CERE-110 (Nerve Growth Factor Gene Therapy)	Neuronal L-type calcium channel antagonist Selective nicotinic alpha-7 receptor partial agonist; 5-HT_3 receptor antagonist	
Inhibitors of complement system *Naturally occurring*	Trophic agent in the survival and maintenance of basal forebrain cholinergic neurons	
Polyphenolic flavonoids		Investigational
β-glycyrrhetinic acid steroid-like		Investigational
Polysaccharides (GR-2II, AGIIb-, BR-5I, AR-2IIa)		Investigational
Sulfated polysaccharide (fucan)	C3/C5-convertase inhibitors Inhibitor of Classical pathway	Investigational
Esters (rosmarinic acid)	*In vitro* inhibition of complement cascade at different stages	Investigational
Polyanionic carbohydrates		Investigational
Alkaloids Ca^{2+}-binding polymers	Inhibit the classical pathway by interfering with C1 activation or by inhibiting C3 cleavage	Investigational Investigational
Glycoproteins (CI, CVF)	C3/C5-convertase inhibitors	Investigational
Peptide-like related to glycopeptide antibiotics (complestatin)	Inhibitors of classical and alternative pathways Inhibitors of classical pathway	Investigational
Fungal metabolite K-76	Inhibitors of classical pathway	Investigational
Proteoglycans (decorin)	C3-convertase inhibitors	Investigational
Glycosaminoglycans (heparin)	Inhibitors of classical and alternative pathways	Investigational
Chondroitin sulphate proteoglycan (GCRF, CSPG)	Inhibitors of classical and alternative pathways	Investigational
C4-binding protein (C4bp)	C1q inhibitor	Investigational
Cyclic hexadepsipeptides	C3-convertase inhibitor C3bBb, factor B, C1q inhibitors	Investigational
Synthetic inhibitors		Investigational
Peptide analogues and derivatives	C4 inactivation *in vitro* and *in vivo*	
(C089, PR226, CBP2, compstatin, etc.)	C5aR antagonist *in vitro* and *in vivo*	Investigational
Diisopropyl fluorophosphates (DFP, BCX-1470)	C5aR or C3aR antagonists, bind to either C3, factor D, factor B or C1q	Investigational
K-76 analogues (TKIXc, K-76COOH)		Investigational
Nafamstat mesilate (FUT-175)		Investigational
Oligodeoxyribonucleotide containing phosphorothioate (PS-oligo)	Factor D binding	Investigational
Triterpenoid oleanolic acid derivatives	Inhibitors of classical and alternative pathways	Investigational
Inhibitory prodrug proteins (CD55- and CD59-prodrugs)	Inhibitors of classical and alternative pathways Inhibitors of classical and alternative pathways	
	C3-convertase inhibitor Inhibition of C3/C5-convertases or membrane attack complex (MAC)	

2004. Memantine acts by blocking the NMDA receptor, thus preventing calcium influx when neuronal firing rates are high, but leaves the calcium channel open for transmission at low stimulation rates [151]. Clinical studies demonstrate that the drug has rather symptomatic benefits for patients than a disease modifying effect [152, 153].

β- and γ-secretase inhibitors

β and γ secretases are the two enzymes critically responsible for the formation of amyloid plaques (Fig. 1). Therefore, based on the amyloid hypothesis, drugs that can prevent production, aggregation and deposition of Aβ are thought to be promising therapeutics for AD. In particular, the amyloidogenic pathway in neurons is initiated by β-secretase cleavage. The elucidation of the molecular identity of β-secretase, also called BACE1 (β-site APP cleaving enzyme 1) had been one of the key issues for the development of AD therapeutics. Animal experiments showed that mice lacking β-secretase are fertile, do not produce Aβ42 and have no obvious neurological deficits [154]. Clinical data support this approach since activity of β-secretase increases with age [155]. Development of β-secretase inhibitors (BSI), however, has proven to be challenging and to date none has been tested extensively in human beings [156]. The crystal structure of BACE1 shows that the active site is comprised a long cleft for substrate recognition [157]. The structural features of BACE1 are used for design of BSI. The first generation of BSI were designed as transition-state analogues for the aspartyle protease (*e.g.* OM99–2, KMI-429). The majority of commercially available potent BSI are peptide-based compounds containing a transition-state moiety (*e.g.* hydroxyethylene, statin, etc.). Promising recent data suggest that KMI-429, has great potential as it significantly reduced production and accumulation of senile plaques *in vivo* in brains of wild-type and transgenic mice [158]. A new generation of BSI was designed recently [159], containing hydroxyethylamine isostere and isophthalamide moiety (GRL-8234). This compound efficiently inhibits BACE1 activity not only *in vitro* but also *in vivo*. Another recently generated BSI containing a 2,6-pyridinedicarboxylyc, chelidamic or chelidonic residue at the P_2 position together with hydroxymethylcarbonyl isostere (KNI-1027) was also shown to inhibit efficiently BACE1 [160]. Considering the usage of BSI as drugs, making less peptidic compounds is mandatory to obtain sufficient oral absorption and penetration through the blood-brain barrier. These two issues have hampered progress in development of BSI for AD therapeutics, however, recently was generated the first orally bioactive non-peptidic BSI, GSK188909, able to reduce brain Aβ levels in APP transgenic mice [161].

Although γ-secretase has in many ways been an attractive target for AD therapeutics, interference with Notch processing and signalling may lead to toxicities that preclude clinical use of inhibitors of this protease. Knockout of Notch1 or PS1 is embryonic-lethal in mice [162, 163] but Notch signalling and γ-secretase activity are crucial in adulthood as well, because Notch plays a critical role in many cell differentiation events [164]. Indeed, treatment of mice with γ-secretase inhibitor (GSI) LY-411575 at 10 mg/kg/day for 15 days caused severe gastrointestinal toxicity and, at 10 mg/kg/day, also interfered with the maturation of B and T lymphocytes, effects that are due to inhibition of Notch processing and signalling [165, 166]. The related compound, benzolactam LY-450139, is two orders of magnitude less potent compared to LY-411575; however, this compound has moved into clinical trials, and so far it is the only GSI for which human trials have been reported. LY-450139 was chronically administered for 5 months to young APP transgenic mice, leading to reduced total brain Aβ and slower formation of Aβ plaques. In initial human trials in healthy volunteers [167] single doses of this GSI of up to 140 mg were apparently safe and reduced plasma Aβ levels by up to three quarters. However, steady-state Aβ in the CSF was not affected, and it is unclear if higher doses can lower brain Aβ without Notch-related side effects. Sulphonamide inhibitor BMS-299897 was developed to be selective for inhibiting the processing of APP over Notch [168, 169], although the use of different assays in this study does not allow simple comparisons. Single oral administration of this compound into APP transgenic mice gave an ED50 value of 30 mg/kg for lowering brain Aβ and 16 mg/kg for lower plasma Aβ at 3 hrs after dosing. Intraperitoneal administration in guinea pigs, which naturally produce high levels of Aβ that is identical in sequence to the human peptide, reduced brain, plasma and CSF Aβ with an ED50 of 30 mg/kg at 3 hrs after dosing.

The hope of using GSI for treatment of AD is tempered by the fact that γ-secretase cleaves numerous other type I membrane protein stubs that result from ectodomain shedding [170]. Agents selective for APP *versus* Notch may reveal new long-term toxicities due to blocking proteolysis of these other substrates, toxicities masked by the severe Notch-related effects with non-selective inhibitors. To address this important issue, the development of more potent agents that work by this mechanism will be critical. Further detailed analysis of design and therapeutic potential of BSI and GSI could be found in recently published reviews [171, 172] dedicated specifically to this issue.

Drugs which potentially prevent and modify AD symptoms

Vitamins and antioxidants

Growing evidence supporting the oxidative stress hypothesis has led to the concept of using antioxidants such as vitamin E (α-tocopherol) as a potential treatment approach in AD. However, vitamin E in combination with selegiline, a drug used for the treatment of early-stage Parkinson's disease, depression and senile dementia, showed no real benefits for the patients [82]. Other clinical trials also failed to show beneficial effects of vitamin E administration to AD patients [173–175]. These results together with recent observations that high doses of vitamin E (>400 IU/day) may increase mortality [176] suggest that vitamin E should not be given as an additive to other therapeutic approaches for AD treatment. Clinical results for selegeline treatment have been rather controversial suggesting either only a small benefit or no significant improvement in the cognition of AD patients [177].

The extract of the plant Ginkgo biloba was suggested to enhance cognition. In addition to putative antioxidant properties, the extract has been reported to reduce the aggregation of the Aβ peptide. However, in a randomized trial Ginkgo biloba did not significantly enhance memory in healthy elderly adults [178].

Non-steroidal anti-inflammatory drugs

Retrospective observational studies have shown that the use of NSAIDs may have protective properties regarding the development of AD [10, 179–182]. In epidemiological studies, the use of NSAIDs was associated with lower risk of developing AD and the benefits seem to be greater with long-term use [179]. However, NSAIDs only seem to offer protection if used before disease onset, treatment after the development of AD symptoms results in little or no benefit to the patients [183–187]. Therefore, currently NSAIDs are not used in the treatment of AD.

Oestrogen replacement therapy

Women have two- to three-fold higher age-specific prevalence rates of AD [188]. Women are oestrogen deficient after menopause, whereas men rather maintain hormone levels as testosterone undergoes aromatization to estradiol. Oestrogen is considered to have a number of beneficial features such as antioxidant and anti-inflammatory properties, interactions with neurotransmitters such as acetylcholine, and an ability to alter apolipoprotein which could lower the risk of developing AD. *In vivo* studies with low doses of conjugated oestrogen (Premarin) in a rat model showed that this treatment can prevent vascular deposition of Aβ, thus protecting the endothelial cell from toxic effects of the plaques [188, 189]. An anti-inflammatory effect of the Premarin has also been demonstrated.

Inhibitors of complement-mediated degeneration

Recently we reviewed in detail the important dual role of the complement system and its regulators in brain inflammation and neuronal damage in AD [17]. This dual role should be carefully considered when choosing therapeutic targets for AD treatment. Until now, complement proteins and CRegs have been largely overlooked as targets for developing AD drugs, but accumulated data suggest that appropriate modulation of expression and/or activity of proteins controlling the complement cascade could be of great clinical benefit for AD patients.

Cobra venom factor (CVF)

The earliest indication that inhibition of complement might be of therapeutic benefit came from analyses of the effects of decomplementation with CVF [190–194]. CVF binds mammalian fB in plasma resulting in cleavage of fB by fD and production of C3 convertase [195]. This process leads to consumption of all plasma C3 and lack of functional complement. However, after a week treatment with CVF, experimental animals develop neutralising antibodies against CVF which makes it ineffective for longer-term therapeutic applications, *e.g.* in AD. Nevertheless, CVF has provided a useful proof-of-concept for the theory that complement activation is of relevance in diverse neuropathologies.

Heparin and other polyionic agents

Heparin, a polyanionic glycosaminoglycan, has long been recognized as an *in vitro* inhibitor of complement [196]. Heparin affects complement activation at multiple levels, by binding and inactivating C1, blocking generation of the C3 convertases and interfering with the assembly of MAC [197–200]. Very few studies have addressed the potential use of heparin as a complement inhibitor *in vivo*. N-acetylated heparin with much reduced anticoagulant activity can inhibit CVF-induced complement activation in guinea pigs [201]. Further *in vitro* work on human samples demonstrated that heparins were much more efficient inhibitors in human than in guinea pig serum [202]. Interestingly, it was recently demonstrated that this drug can interfere with the metabolism of amyloid plaques and reduces the accumulation of Aβ [203]. Long-term treatment with enoxaparin, a low molecular weight heparin, in human APP751 transgenic mice can significantly reduce the deposition of amyloid plaques and Aβ aggregation [203]. Chronic treatment with enoxaparin is well tolerated and does not increase side effects such as haemorrhage in the brain. Although the exact mechanism is not yet clear, a reduced ability of Aβ to activate complement system and its classical pathway may play a role.

Numerous related and unrelated polyanionic molecules, including dextran sulphate, polyvinyl sulphate, polylysine and suramin, inhibit complement activation *in vitro* (reviewed in [204]). However, their applicability *in vivo* and particularly for treatment of AD has not been investigated.

Other small molecule inhibitors

Many synthetic and natural molecules have been shown to inhibit complement activation *in vitro*. A few natural inhibitors have shown efficient complement inhibition both *in vitro* and in animal models. The molecule termed K-76COOH, isolated from cultures of the fungus *Stachybotrys complementi*, inhibits complement at the C5 stage [205, 206]. Rosmarinic acid, extracted from the common shrub Rosemary, covalently binds activated C3b and prevents formation of the convertase [207, 208]. An extract of the Chinese medicinal herb, Ephedra, inhibits complement at multiple levels including the terminal pathway [209]. Among the synthetic complement inhibitors, the protease inhibitor FUT-175 is one of the best investigated. It inhibits C1r, C1s, fD and the C3/C5 convertases [210, 211]. FUT-175 is effective in numerous animal models of complement-mediated disease [212, 213], although it has not been studied in AD models. Some success has also been obtained using small molecules, such as peptides, that bind active sites of complement components or enzymes and prevent their participation in the cascade. Examples of such peptides are C089, PR226, CBP2 and compstatin. The use of the small molecule

inhibitors, however, is currently hampered by non-specific side effects or short half-life [214] and therefore, none of them are likely to be useful for therapy of chronic diseases such as AD unless these problems can be solved.

Recombinant protein inhibitors

In addition to the approaches described above, a plethora of soluble therapeutics has been developed which specifically inhibit the complement cascade [215]. Early agents were based on the naturally occurring membrane-associated CRegs, such as CD49, CD59 and Complement Receptor 1(CR1; CD35). Removal of membrane-anchoring domains using recombinant technology generated soluble molecules with the same regulatory functions as the 'parent' inhibitor which could be administered locally or systemically to inhibit complement. The best known of these is a soluble form of CR1 (sCR1), an agent developed nearly 30 years ago and used in the clinic, with limited success, for complement inhibition in myocardial infarction (MI) and cardiopulmonary bypass (CPB) [216–218]. These 'first-generation' agents have been engineered and modified over the years to improve characteristics such as half-life (by introducing antibody Fc domains) [219], membrane localisation (by using lipid 'tails') [220] and specific targeting of complement activation sites (by using fusion proteins comprising a domain that binds C3 activation fragments, thus localising to the site of C attack) [221]. Some success has also been obtained using small molecules, such as peptides, that bind active sites of complement components or enzymes and prevent their participation in the cascade. Their use, however, is hampered by non-specific side effects or short half-life [214]. Perhaps the most successful soluble therapeutic agents have been antibody-based agents, such as full-length immunoglobulin of single-chain Fv (scFv) fragments. Antibodies that bind C5, thereby preventing triggering of the terminal pathway and MAC formation, have been successfully used in the treatment of various pathologies exacerbated by complement activation. Anti-C5 in particular is used for treatment of paroxysmal nocturnal haemoglobinuria, MI and arthritis [222–224].

The problem however with many of these agents is that their action is systemic, totally blocking the complement cascade. In addition, if they are targeting the alternative, classical or MBL activation pathways, their long-term use can be detrimental, triggering immune complex disease and permitting bacterial infections as the key functions of complement in innate immunity are prevented. In the case of AD, it may be beneficial to selectively inhibit parts of the complement cascade. For example, inhibition of the terminal pathway using antibodies that allow C5a formation but prevent MAC formation may prevent neuronal damage but still permit the observed protective effects of C5a. Another agent, developed to selectively inhibit MAC formation, but which will still allow C5a generation, is a CD59-Ig fusion protein [225]. This has been tested in a murine model of laser-induced choroidal neovascularization and shown to have therapeutic effects. CD59-Ig, which is a long-lived agent, halts the complement cascade at the level of C8. Agents that specifically target complement inhibition to the vicinity of the amyloid plaques would avoid systemic inhibition of C, and provide enhanced protection to neurons. Previous *in vitro* studies have demonstrated that recombinant techniques can be used to fuse CReg to the carboxy-termini of antibodies or antibody fragments, thus locating the therapeutic action of the inhibitor to the site pre-determined by antibody specificity [226]. If recombinant antibodies to Aβ, or other antigens unique to the plaque, are developed, then specific targeting of CD59 or other substrates at sites of disease may be possible. It is clear that the exact role of each complement component in AD is yet to be understood (see *Complement-mediated neurodegeneration* section). Only then, specifically targeted anti-complement agents will be likely to be developed and to be of therapeutic benefit. Testing prototype agents in murine models of AD, or further investigation of the role of complement in the disease using component-deficient animals will help clarify the potential of anti-complement therapy in AD.

Anti-amyloid therapy ('vaccination')

The major aim of anti-amyloid therapy is to induce clearance of the amyloid plaques, one of the defining risk factors and pathological hallmarks of AD. A largely unexpected discovery showed that immunization with Aβ42 peptide prevents the appearance of amyloid pathology in a transgenic mouse model of AD [227]. Follow-up studies confirmed this finding in other models of the disease [228]. These experiments suggested that passive immunization with antibodies against human Aβ should decrease the neuritic plaques and this hypothesis was confirmed in transgenic mice, including improved performance in behavioural tests [229, 230]. Further to these promising experiments in animal-models without apparent side effects, clinical trials with a vaccine designated as AN1792 containing pre-aggregated Aβ42 and QS21 as an adjuvant were carried out. The design of this vaccine aimed to induce a strong cell-mediated immune response, since QS21 is a strong inducer of Th1 pro-inflammatory lymphocytes [231]. This initial trial involved 80 patients with mild to moderate AD [232] and was design to assess the antigenicity and toxicity of multiple immunisations with Aβ42 peptide containing QS21. Slightly more than half of the patients (53%) developed antibodies against Aβ. During the later stages of the phase I trial, the emulsifier polysorbate 80 was added to the vaccine, shifting the response from a predominantly Th2 biased to a pro-inflammatory Th1 [233]. In the subsequent phase II trial, 372 patients were enrolled with 300 receiving AN1792 in the polysorbate 80 formulation. Unfortunately, this trial had to be stopped prematurely after 6% of patients (18 out of 298 subjects) who received the vaccine developed acute meningoencephalitis [234]. Nevertheless, the trial and *ex vivo* studies yielded some important data. Striking Aβ clearance of parenchymal plaques had occurred, similar to what had been reported in the animal studies, confirming the validity of this approach for amyloid clearance in human beings [235–239]. Antibodies raised by this active immunisation were specific for senile plaques only and did not cross-react with the full-length

APP or other derivates [240]. Furthermore, neuropathological evaluation suggested involvement of activated microglia in plaque clearance [239, 241]. Another interesting observation suggested a mechanism for the observed Aβ plaque clearance, which actively involves complement system. C1q enhances the uptake of antibody-fibrillar Aβ complexes *in vitro* by microglia, possibly *via* C1qRp receptor [242]. Despite the striking Aβ clearance, several significant problems were apparent from the pathological and clinical data from these trials. Some of the pathologically examined cases showed a harmful T-cell reaction surrounding cerebral vessels, suggesting an excessive Th1 immune response. It appeared that the immune reaction triggered by the AN1792 vaccine was a double-edge sward, where the benefits of a humoural response against Aβ42 were surpassed in some individuals by a detrimental Th1-mediated inflammatory response [235, 243]. Furthermore, despite the apparent success in amyloid clearance, the clinical cognitive benefits from the vaccination were very modest compared to the placebo group [244, 245]. The above observations suggest a possibly promising future for passive or active immunisation against amyloid plaques. However, further studies on optimal time to begin immunisation, design of improved vaccines and pre-clinical models, determination of most appropriate target for vaccination (detail discussed in [246]) are mandatory. A combination of anti-amyloid therapy with anti-inflammatory therapeutics might be a solution to overcome currently existing problems such as devastating side effects (meningoencephalitis). However, whether such a combination would be beneficial for AD patients needs to be addressed in further empirical studies, after its safety has been confirmed.

Another interesting approach is to prevent cleavage of APP in the first place by blocking the cleavage site of β-secretases and thus formation of Aβ [247]. Antibodies that block APP cleavage by β-secretase were tested in an *in vitro* model in which cells expressed wild-type APP. These antibodies bound full-length APP, internalized it into the cells and inhibited both intra- and extracellular Aβ peptide formation. The same antibody has also been tested in a transgenic mouse model expressing human APP. The animals showed improved cognitive functions and a reduction in brain inflammation as well as in the incidence of microhaemorrhage. Interestingly, these beneficial effects were not associated with the levels of Aβ which were unaltered by antibody treatment [247]. Therefore, the exact mechanism by which this antibody induced these improvements in transgenic animals, remains to be elucidated.

Targeting proteasome inhibition

As proteasome inhibition may play a contributing role to neurodegeneration, it is important to determine mechanisms which may modulate that inhibition. One obvious target for proteasome inhibition could be an efficient up-regulation of the levels of HSPs within the CNS. Increasing the concentration of molecules of the HSP family should help to preserve proteasome activity and possibly result in delaying age-related proteasome inhibition. Alternatively, pharmacological interventions designed to stimulate proteasome activity within the CNS may provide some therapeutic benefit. Different studies have identified a number of pharmacological proteasome activators that may ultimately provide the basis for such treatments in the future [248–250]. Furthermore, a strategy increasing the expression of specific proteasome subunits might also be useful for the protection of neurons. A novel protein has been identified in yeast that is responsible for the transcription of a number of proteasome subunits [251]. Although no mammalian homologue has yet been found, these data raise the possibility of utilizing such a protein to elevate potentially beneficial levels of proteasome expression in the CNS of human beings.

The importance of the proteasome in CNS physiology and pathology is only beginning to emerge. It is likely that future studies on this intriguing enzyme will yield further important information, particularly on the regulation of protein turnover in the CNS. Ultimately, this may lead to designing of new and useful AD therapeutics.

Neuropeptide mixture (Cerebrolysin)

Cerebrolysin (CBL) is an anti-inflammatory mixture of neuropeptides (smaller than 10kDa) obtained from porcine brain tissue [252]. Due to its complexity, this therapeutic targets different mechanisms causing neuronal death. It exhibits unique neurotrophic and neuroprotective activity and reduces amyloid burden in animal models. It also improves neurodegenerative alterations in an APP model of AD [253]. CBL regulates the activity of cyclin-dependant kinase-5 and glycogen synthases kinase 3β, which phosphorylate APP, thus reducing levels of phosphorylated APP and accumulation of APP in neuritic processes [252]. This in turn reduces the level of available APP for cleavage by the secretases. Studies in patients with mild to moderate AD have shown that CBL improves cognitive performance [254]. In addition to the neuroprotective mechanisms described above, we have recently demonstrated that CBL can also protect of neurons from complement-mediated degeneration *via* increasing expression of CD59 and CD55 membrane-bound CReg, while expression of CD46 remains unaffected (unpublished data). Therefore, CBL has a multilevel effect on neurons possessing anti-inflammatory, anti-apoptotic, neurotrophic and neuroprotective properties, while at the same time inducing local inhibition of complement on neuronal surfaces. Further well-designed experiments are urgently needed to further assess the beneficial properties of CBL in AD, as well as its potential in other neuropsychiatric disorders.

Conclusions

Multiple approaches to understand the pathogenesis of AD have unravelled the mechanisms of neuronal death in AD. Aβ and inflammation risk factors are considered to be the principal players in AD pathogenesis. Inflammation, Aβ and other AD-related

pathological changes induce various types of toxic mechanisms that contribute to neuronal death. This indicates the complex nature of AD pathogenesis. Prevention and blockage of these mechanisms is critical for the palliative and curative therapy of AD. The advances in understanding the cellular, molecular and genetic mechanisms underlying the aetiopathogenesis of the disease lead to the identification of various potential therapeutic targets. The need for effective treatment is urgent, since current drugs for treating the cognitive impairments in AD are based on neurotransmitter replacement or modulation, which produce only mild symptomatic benefit with minimal impact on the disease process and causality. However, some very promising new strategies for fighting AD have been proposed including anti-amyloid treatment; and CBL, which protects neurons by simultaneously acting on a number of mechanisms involved in neuronal death (anti-amyloid, anti-inflammatory, anti-apoptotic, inhibition of complement system, neuroprotective, neurotrophic, etc). Due to the disease complexity, it is likely that therapeutics that aim against multiple targets will result in a more efficient management of AD and might also be effective in various forms of AD with different underlying pathophysiological mechanisms. Therefore, further extensive experimental studies, *in vitro* and *in vivo* are urgently needed.

Acknowledgements

We thank Claire Harris and B. Paul Morgan for helpful comments and discussions. R.D. was supported by the Medical Research Council, UK New Investigator Award Grant G0700102.

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