ABSTRACT

Alzheimer’s disease (AD) is the commonest neurodegenerative disorder of the elderly and results in progressive cognitive impairment. The main pathological features of the disease are the formation of extracellular amyloid plaques, intraneuronal neurofibrillary tangles and cerebral amyloid angiopathy. In addition, there is also an inflammatory response, in which activated microglial cells of the innate immune system appear to play a central role. This inflammation may help to protect neurons but an excessive or exaggerated response may cause neuronal damage or death through the release of potentially neurotoxic inflammatory mediators. Systemic infections may further activate the microglial cells in the central nervous system and so drive the neurodegenerative process. Long-term use of non-steroidal anti-inflammatory drugs protects against the development of AD. However, the mechanisms by which this occurs may be unrelated to cyclo-oxygenase inhibition and suppression of inflammation. Rather, these drugs may reduce the formation of amyloidogenic fragments from the amyloid precursor protein. Abnormalities of the peripheral immune system have been described in AD. Immunisation with anti-amyloid-beta antibodies, to stimulate the clearance of amyloid plaques in the brain, has been attempted in humans but further modifications of this form of therapy will be needed in the future to prevent undesirable side-effects.

INTRODUCTION

Alzheimer’s disease (AD) is a chronic, age-related, neurodegenerative disorder and the commonest cause of dementia in the elderly. It typically starts with the insidious onset of memory impairment and gradually progresses to affect multiple aspects of cognitive function. Behavioural and psychological symptoms occur frequently in the disease. In life, there are no specific diagnostic tests for AD. The diagnosis rests on the typical clinical picture and the exclusion of other possible causes of cognitive impairment.1,2

The neuropathological characteristics of the disease are the extracellular accumulation of amyloid plaques in areas of the brain such as the cortex and the hippocampus, the accumulation of amyloid-beta (Aβ) in blood vessel walls (cerebral amyloid angiopathy) and the intraneuronal formation of neurofibrillary tangles. The latter are composed of the hyperphosphorylated microtubular-associated protein, tau. There is associated neuronal loss, loss of synaptic connections and brain atrophy. While the pathological changes are only seen at autopsy, generalised brain atrophy, sometimes more prominent in the medial temporal lobe structures, can be seen on neuro-imaging.3

Most cases of AD are sporadic but rare familial forms of the disease do occur. Many of the latter are associated with point mutations in the gene encoding the amyloid precursor protein (APP), a transmembrane glycoprotein whose cleavage by β and γ-secretases results in the formation of Aβ peptides. When β-secretase (also called beta-amyloid cleavage enzyme-1 or BACE-1) cleaves APP, a 42-amino-acid-long Aβ fragment, Aβ42, is formed. This peptide has a particular propensity to aggregate and to form amyloid plaques. Cleavage of APP by α-secretase results in soluble, non-amyloidogenic fragments (Fig. 1).4 In the rare familial forms of AD as well as in trisomy 21-associated Down’s syndrome (where three copies of the APP gene located on chromosome 21 are present), there is an overproduction and, hence, accumulation of Aβ. However, in the more common, sporadic, late-onset AD, the problem appears to be primarily one of impaired elimination of Aβ.

![Fig. 1. Pathways of amyloid precursor protein (APP) degradation. Cleavage by β-secretase (beta-amyloid cleavage enzyme-1 or BACE-1) and γ-secretase yields the amyloidogenic amyloid-β (Aβ) fragment, shown on the left. C99 = a membrane-bound, 99-amino-acid-residue-long, C-terminal fragment; sAPPβ = a soluble extracellular fragment of APP; AICD = APP intracellular domain, a soluble cytosolic fragment. In the non-amyloidogenic pathway depicted on the right, α-secretase hydrolysis yields a soluble extracellular fragment of APP (sAPPα) and C83, a membrane-bound 83-amino-acid-residue fragment. The action of γ-secretase generates P3, a short peptide fragment, and AICD.](image-url)
accumulates in the cytoplasm of neurones to form Lewy bodies; in Creutzfeldt-Jakob disease (CJD) abnormal prion protein aggregates occur, and in Huntington’s disease the protein ‘huntingtin’ accumulates within the nuclei of neuronal cells in particular areas of the brain. Abnormal brain function thus appears to be a consequence of excessive accumulation or poor elimination of certain intra- or extraneuronal proteins.

INFLAMMATION IN AD

Another important pathological feature of AD is the presence of inflammatory processes often closely associated with the amyloid plaque and neurofibrillary tangle pathology. Inflammatory reactions are also seen in relation to the pathological features of Parkinson’s disease and other neurodegenerative disorders.

Microglial cells are the resident macrophages of the brain parenchyma derived from the monocyte/macrophage lineage. They form part of the body’s innate immune system, and typically surround the amyloid plaques. Reactive astrocytes are also found in the areas around the amyloid plaques (Fig. 2). These cells probably help to restrict or contain the disease process. Normally the microglial cells of the brain are present in a down-regulated or quiescent state. However, minor disturbances in the central nervous system (CNS) environment such as neuronal injury can trigger the transformation of these cells into an activated state. Activated microglia have a more highly branched morphological appearance than the resting cells and are capable of phagocytosis. The microglia surrounding the amyloid plaques probably phagocytose, or attempt to phagocytose, the Aβ in order to clear it from the extracellular spaces. While most of the resident microglia have migrated to the brain during embryogenesis, there is evidence that blood-derived monocytes are recruited into the brain in later life in both human and animal studies. Thus the APOE gene associated with familial AD has also demonstrated activation of inflammatory pathways in the brain as well as microglial and astrocytic activation. Gene expression studies using microarray assays have shown increased expression of genes related to inflammation in patients with AD. A number of studies have described weak associations between genetic polymorphisms in some immune mediators and the risk of AD.

Activated microglial cells have recently been visualised in the brains of living individuals using positron emission tomography (PET) scanning and the injection of a synthetic radioligand that binds to the peripheral benzodiazepine binding site on activated microglia. Apolipoprotein E (APOE) is a plasma protein involved in lipid transport. It has three common alleles: ε2, ε3 and ε4. The presence of one (heterozygous) or both (homozygous) ε4 alleles of the APOE gene has been shown to significantly increase the risk of AD. A meta-analysis by Farrer et al. in 1997 showed age-adjusted odds ratios for AD of 2.6 for ε2/ε4 heterozygotes, 3.2 for ε3/ε4 heterozygotes and 14.9 for ε4/ε4 homozygotes. Thus the APOE ε4 allele increases the risk for AD in a dose-dependent way. The mechanisms underlying this association are unknown but they may be linked to inflammation. Lynch et al. studied the inflammatory responses to bacterial lipopolysaccharide in mice carrying either the ε3 or ε4 human APOE alleles. They found significantly higher levels of both systemic and CNS pro-inflammatory cytokines in the ε4 compared with the ε3 mice. Thus APOE may have isoform-specific immunomodulatory properties, with ε4 increasing inflammatory responses.

Inflammation in the CNS therefore has a dual nature, as it has indeed in the rest of the body. A controlled and regulated inflammatory response is necessary to combat injury and infections, and to promote repair. However, an exaggerated response can lead to immune-mediated diseases such as rheumatoid arthritis and asthma in the periphery, and diseases such as multiple sclerosis and Alzheimer’s in the CNS. The balance between neuroprotection and neurotoxicity in AD is a dynamic one which may alter depending on the stage of the disease.

INTERACTIONS BETWEEN SYSTEMIC AND CNS INFLAMMATION

While microglia in the normal healthy brain are down-regulated, these cells may be partially activated or ‘primed’ in the brains of elderly individuals or in patients with the early pathological changes of AD. Systemic infections may serve as exogenous activators of these primed cells through a process of neuroimmune signalling from the periphery. Afferent impulses through the vagus nerve may mediate the signalling from the periphery to the CNS, especially from the abdominal cavity. Peripherally produced, circulating cytokines may also signal across the BBB by activating endothelial cells of the capillaries and perivascular macrophages to produce prostaglandin E2 (PGE2). The result of these processes is that primed brain microglia...
are further activated to secrete cytokines and other neurotoxic chemicals that damage neurones. Repeated systemic infections may, in fact, drive central neurodegeneration through repeated waves of neuronal damage. It is well known that systemic infections can cause delirium in the elderly and that patients with AD are at greater risk of developing delirium than cognitively normal elderly individuals. An episode of delirium caused by a systemic infection may occasionally be the harbinger of an incipient AD process. Activated microglia and increased brain levels of cytokines might well trigger, or underlie, these behavioural changes.

**NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND AD**

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclo-oxygenase (COX), the enzyme that catalyses the first step in the synthesis of prostanoids and thromboxanes. These chemicals are important mediators of inflammation. Cyclo-oxygenase-2 (COX-2), the inducible isoform of the enzyme, is normally expressed in populations of neuronal cells in the brain as well as in inflammatory processes. Epidemiological studies have shown that the long-term use of certain NSAIDs (taken before the advent of any cognitive changes) is associated with a reduced risk of developing AD. The hypothesis has therefore been that NSAIDs protect against AD by inhibiting inflammation.

However, clinical trials of NSAIDs (including COX-2 selective inhibitors) in established AD have failed to show any benefit or slowing in the progression of the disease. A longitudinal study of cerebrospinal fluid levels of a COX metabolite, PGE₂, showed that COX activity observed in mild cognitive impairment (MCI), a prodromal phase of AD, was increased. Furthermore, this increase was associated with a longer survival of patients. Postmortem studies have also shown that increased neuronal expression of COX is associated with early disease and that markers of microglial activation occur predominantly in the later stages. So early COX activity appears to be neuroprotective while the inflammation associated with microglial activation and neurotoxicity occurs later.

Why, then, do NSAIDs appear to protect against AD? A number of studies in transgenic mice carrying both the APP and presenilin-1 (PS1) mutant human genes associated with familial AD have shown that some NSAIDs such as indomethacin, ibuprofen, flurbiprofen and sulindac affect APP cleavage and Aβ metabolism, resulting in reduced Aβ deposition in the brain. These mechanisms of action are unrelated to COX inhibition and involve pathways such as activation of the peroxisome proliferator-activated receptor γ (PPAR-γ). The latter leads to reduced transcription of BACE-1, and hence reduced formation of the amyloidogenic fragments of APP. NSAIDs may also have an allosteric effect on γ-secretase, altering the cleavage site of APP in favour of the production of the shorter, more soluble and less fibrilogenic Aβ₄₂ fragment (as opposed to the amyloidogenic Aβ₄₀ peptide). The results of a phase 2 trial of the NSAID-derived compound, R-flurbiprofen (tarenflurbil), in mild AD, have recently been published. They showed a promising reduction in cognitive and functional decline in patients taking the drug compared with those taking a placebo.

**THE PERIPHERAL IMMUNE SYSTEM AND AD**

A number of studies have reported changes or defects in the immune responses in blood-derived cells of AD patients. Many of these studies, when compared, have shown contradictory results. Fiala et al. reported that peripheral blood monocytes from AD individuals were less effective in phagocytosing Aβ in vitro and that monocytes from AD patients were less able to differentiate into macrophages. Pirttilä et al. reported a reduction in CD8 positive lymphocytes in the peripheral blood of AD individuals compared with controls. Serum auto-antibodies against Aβ have been found to occur naturally in elderly individuals, and increased auto-antibodies have been reported in AD. Serum levels of cytokines and other inflammatory mediators have been compared in AD and age-matched controls in a number of studies but there are no consistent trends or conclusive findings.

**IMMUNISATION STRATEGIES FOR AD**

Modulation of the immune system through passive immunisation with anti-Aβ antibodies or active immunisation with the Aβ peptide has been studied in APP transgenic mouse models of AD and in human clinical trials. These have been comprehensively reviewed by Boche et al. Immunised animals showed clearance of the Aβ plaques if they were immunised after plaques were already present in the brain. If the immunisation was carried out before the onset of plaque formation, it prevented Aβ accumulation. Immunglobulin G (IgG) antibodies to Aβ were detected in the brains of these animals, indicating that the antibodies are capable of crossing the BBB. IgM antibodies that are produced early after immunisation are too large to cross the BBB but may nevertheless still contribute to plaque clearance by shifting the Aβ from the brain to the peripheral blood compartment.

Experimental evidence has suggested that the anti-Aβ antibodies change the activation state of the microglial cells and improve their ability to phagocytose the amyloid. One possible mechanism by which this may occur is through opsonisation with binding of the Fc portion of the anti-Aβ antibody to the Fc receptors on the microglia. Following immunisation, Aβ was found within the microglial cells and immunised mice were also reported to show improved ‘cognition’ compared with untreated animals.

In 2000 a pharmaceutical company began human trials of active Aβ₄₂ (Aβ₁₋₄₂) immunisation in 86 AD patients in Europe and the USA was started to test the efficacy of this treatment. The trial was, however, halted in 2002 after 18 of the 298 treated patients developed a subacute, aseptic, meningo-encephalic illness associated with neurological decline, a lymphocytosis in the cerebrospinal fluid and cerebral white matter changes on neuro-imaging. Subsequent histopathological examination of the brains of two of these patients showed a T-lymphocyte infiltration of the leptomeninges but also a remarkable clearance of the plaques in the neuronal cortex and a ‘moth-eaten’ appearance of some residual plaques. There was also a reduction in plaque-associated dystrophic neurites and evidence of Aβ phagocytosis by microglial cells. However, the cerebral amyloid angiopathy persisted or even increased and the neurofibillary tangle pathology appeared unaltered.

Sadly there was no significant change in cognitive function in these patients. Nevertheless, the idea that this form of immunotherapy may still have a future role in the treatment or prevention of AD has not been abandoned. Further modifications and refinements to the
immunisation schedule aimed at maximising the plaque-clearing effects and minimising the T-cell immune reaction are currently being undertaken in animal studies. It may be that this form of treatment administered before the onset of cognitive impairment, could prevent the clinical advent of AD.

Immunisation studies have also been performed in mouse models of prion disease and Parkinson’s disease.46,47 Novel forms of immunotherapy involving the administration of antibodies against the BACE-1 cleavage site of the APP are in the early stages of development. Arbel and Solomon48 reported a major reduction in intracellular Aβ levels following treatment of cell cultures with the antibodies. Other techniques aimed at enhancing microglial activation have included the use of a compound called NCI X2216. This is a nitric oxide-releasing derivative of the NSAID flurbiprofen. In a transgenic mouse model of AD it reduced the load of amyloid in the brain but also increased the activation of microglia around the plaques.49

CONCLUSION
Inflammation in the CNS is a complex process with both protective and potentially detrimental effects. While inflammatory processes may not be the primary cause of AD, an exaggerated inflammatory response may contribute to the pathology and drive the neurodegeneration. However, non-specific inhibition of inflammation is not desirable; targeted immunomodulatory therapy aimed at either inhibiting the neurotoxic components of inflammation or enhancing the neuroprotective function of microglial cells is likely to be more beneficial in the future.

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Declaration of conflict of interest
The authors declare no conflict of interest.

REFERENCES


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