Immunology and immunotherapy of Alzheimer's disease

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Abstract | Although Alzheimer's disease is considered to be a degenerative brain disease, it is clear that the immune system has an important role in the disease process. As discussed in this Review, immune-based therapies that are designed to remove amyloid-β peptide from the brain have produced positive results in animal models of the disease and are being tested in humans with Alzheimer's disease. Although immunotherapy holds great promise for the treatment of Alzheimer's disease, clinical trials of active amyloid-β vaccination of patients with Alzheimer's disease were discontinued after some patients developed meningoencephalitis. New immunotherapies using humoral and cell-based approaches are currently being investigated for the treatment and prevention of Alzheimer's disease.

Neurofibrillary tangles
Neurofibrillary tangles are pathological protein aggregates found in neurons of patients with Alzheimer's disease. Tangles are formed by hyperphosphorylation of a microtubule-associated protein known as Tau, causing it to aggregate in an insoluble form.

Association cortices
The neocortical regions that are not involved in primary sensory or motor processing. They include frontal areas subserving executive functions and temporoparietal areas supporting visuospatial processing.

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Alzheimer's disease is characterized by progressive memory deficits, cognitive impairment and personality changes. More than 20 million people are affected worldwide (BOX 1). The histopathological hallmarks are senile plaques and neurofibrillary tangles, which cause progressive synaptic dysfunction and, eventually, death of neurons, especially in the limbic and association cortices, which have roles in memory and navigation[1,2]. Senile plaques consist of a core of amyloid-β peptide deposits surrounded by degenerative presynaptic endings together with astrocytes and microglial cells. Neurofibrillary tangles are formed by neuronal intracellular deposition of Tau protein and result in the collapse of microtubules (FIG. 1). Although most cases of Alzheimer's disease are sporadic, a small fraction of cases have an autosomal dominant inheritance with onset before 65 years of age. Mutations that enhance the deposition of amyloid-β peptide are associated with familial forms of Alzheimer's disease. Currently, mutations in four genes are believed to have a role in this disease, presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), the ε4 allele of the apolipoprotein E (*APOE*) and amyloid precursor protein (*APP*), which are situated on chromosomes 1, 14, 19 and 21, respectively. Products of these genes are involved in the trafficking and proteolytic processing of APP[3].

Senile plaques containing fibrillar amyloid occur in the limbic system (especially the hippocampus) and association cortices in the brains of all patients with Alzheimer's disease[1]. Two types of extracellular amyloid deposit are present: neuritic and non-neuritic (diffuse) senile plaques. Although both types of plaque occur in the brains of non-demented old people, large quantities of neuritic plaques are found only in patients with Alzheimer's disease. The subunit of the amyloid fibrils found in patients with Alzheimer's disease is amyloid-β peptide, a 4-kDa peptide of 40 to 43 amino acids derived from proteolytic cleavage of APP, which is expressed in the heart, kidneys, lungs, spleen and intestines, as well as in the brain[3,4]. Amyloid-β peptide 1–42 (denoted amyloid-β_{1-42}) is mainly deposited in the brain parenchyma in patients with Alzheimer's disease, whereas amyloid-β_{1-40} is associated with cerebral amyloid angiopathy (CAA). Fragments of amyloid-β can also be deposited intracellularly[5]. The normal function of APP is not known, although it might function in the regulation of neurite outgrowth, in cell adhesion and in the migration of newly differentiated neurons in the developing cortex[6]. It is generally believed that accumulation of amyloid-β is the first event in the pathogenesis of Alzheimer's disease and this has led to the amyloid (or amyloid-β) hypothesis[4] (BOX 2). The probability that amyloid-β has a key role in Alzheimer's disease led to the development of *APP*-transgenic mouse models of Alzheimer's disease and, later, to immunotherapeutic approaches to decrease amyloid-β levels in the brain.

This article reviews the immune response that occurs secondary to amyloid deposition in the brain, resulting in activation of the complement cascade and microglial cells, and the recruitment of astrocytes. We then discuss immunotherapeutic strategies for the treatment of patients with Alzheimer's disease, which use both the innate and adaptive arms of the immune system. Immunotherapy of patients with Alzheimer's disease offers an important avenue for the treatment of this devastating disease, although initial clinical trials led to immune-related complications.

Box 1 | **Alzheimer's disease**

Alzheimer's disease is the most common cause of dementia. It usually begins after the age of 65 with the gradual development of forgetfulness accompanied by problems with other cognitive tasks, such as performing calculations, visuospatial orientation and language. There are usually no clinical signs apart from the dementia, and magnetic resonance imaging (MRI) shows nonspecific findings of atrophy and ventricular dilatation. Clinical dementia scales, such as the Mini-Mental State Examination, are used to rate the severity of mental impairment. On gross examination, the brains of patients with Alzheimer's disease show severe atrophy with a reduction in brain weight by, usually, more than 35%. The brain sulci (grooves) are widened, the gyri (ridges) are narrowed, and the third and lateral ventricles are dilated. There is a loss of both grey and white matter, specifically in the medial temporal lobes, which are severely affected. The pathological picture consists of neuronal-cell loss, deposition of amyloid plaques, neurofibrillary tangles and secondary inflammation. There are no treatments that prevent or halt the progression of this disease.

Amyloid
A general term for a variety of protein aggregates that accumulate as extracellular fibrils of 7–10 nm. They have common structural features, including a β-pleated sheet conformation and the ability to bind dyes such as Congo Red.

APP-transgenic mouse models

Many groups have created transgenic mice that express human wild-type APP, mutant APP, fragments of APP, and mutant forms of both human APP and presenilin 1 that are associated with Alzheimer's disease[7–12]. The location of the *APP* gene on human chromosome 21 indicates that a gene-dosage effect might explain the Alzheimer's disease-like pathology that develops in young patients with Down's syndrome (trisomy 21)[3]. At around 6–9 months of age, mice transgenic for the mutant human *APP* start to develop amyloid-β-rich plaques in the hippocampus, corpus callosum and cerebral cortex. With increasing age, the amyloid-β load increases and both types of plaque (the diffuse, mainly non-fibrillary, plaques and those with fibrillar amyloid cores) accumulate (FIG. 1).

There are differences in the biochemical and solubility characteristics of amyloid deposits in mice and humans[13]. As a result of post-translational processes, amyloid-β peptides in humans might be considerably more extensively modified and therefore more insoluble. Although, in general, *APP*-transgenic mice develop a high amyloid load, they do not develop all the neuropathological features that are characteristic of Alzheimer's disease, such as neurofibrillary tangles. Nevertheless, neurofibrillary tangles are not unique to Alzheimer's disease and can occur in other neurodegenerative diseases in the absence of amyloid-β deposits, such as fronto-temporal dementia and Alzheimer's disease with parkinsonism. Furthermore, *APP*-transgenic mice have cognitive impairments that roughly coincide with amyloid-β accumulation[14,15]. Amyloid plaques in humans and *APP*-transgenic mice are often closely surrounded by reactive astrocytes and dystrophic neurites, and neocortical regions of transgenic animals contain activated microglial cells, as seen in Alzheimer's disease. Therefore, despite limitations, *APP*-transgenic mice can be used as a model for the role of amyloid in Alzheimer's disease and have directly facilitated the investigation of immunotherapy for the treatment of this disease.

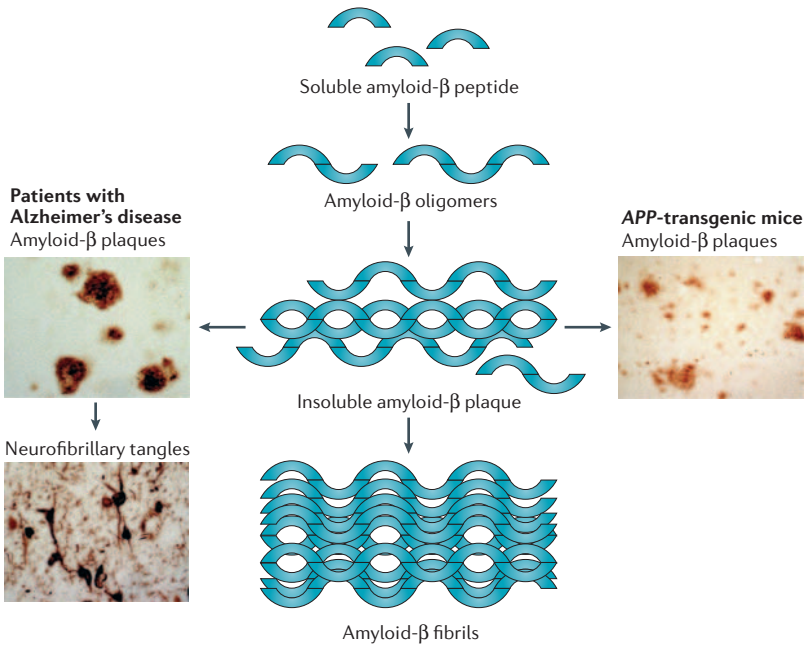

Figure 1 | **Amyloid-β formation and deposition in Alzheimer's disease and *APP*-transgenic mice.** Soluble amyloid-β peptide polymerizes to form oligomers, which fold to generate β-pleated sheet fibrils. Compact senile plaques comprised of amyloid-β fibrils are associated with pathological changes in the surrounding brain neurons, leading to their death. Recent studies have connected the amyloid-β plaques (see inset, upper left) with the formation of intracellular neurofibrillary tangles (see inset, lower left) comprised of hyperphosphorylated Tau protein. A mouse model of Alzheimer's disease has been generated in which animals overexpress a mutated form of human amyloid precursor protein (APP) in the brain. At 6–9 months of age, the *APP*-transgenic mice begin to develop senile plaques in the hippocampus, corpus callosum and cerebral cortex that can be stained with Congo Red (see inset, right). Images reproduced with permission from REF. 138 © (2006) Elsevier.

Innate immune responses to amyloid-β

Although inflammation had not been viewed as a hallmark of Alzheimer's disease, amyloid-β deposition activates a potentially pathological innate immune response in Alzheimer's disease [16]. Indeed, innate immune responses localize at sites of amyloid-β deposition and neurofibrillary tangle formation[16,17]. For example, senile (amyloid) plaques are often closely associated with activated microglial cells and surrounded by activated astrocytes that have abundant glial filaments known as ramified processes. Senile plaques also induce activation of the classical complement cascade[16,17] (FIG. 2). However, there is minimal (if any) evidence of T-cell infiltration in the area surrounding the amyloid plaques in patients with Alzheimer's disease[18].

Complement. Complement activation occurs in Alzheimer's disease and contributes to the local inflammatory response triggered by amyloid-β[19]. *In vitro*, both fibrillar amyloid-β and neurofibrillary tangles directly activate both the classical complement pathway and, in the absence of antibody, the alternative complement pathway. This might provide a mechanism for recruiting activated glial cells to plaques containing amyloid-β fibrils and lead to local inflammation, neuronal-cell dysfunction and ultimately degeneration[16]. Complement component 1q (C1q) is co-localized with most of the amyloid deposits in the brain of patients with Alzheimer's disease[20]. C1q is a hexamer of six identical subunits, such that the C1q A-chain-binding site is replicated at

Box 2 | **Amyloid hypothesis**

The primary cause of Alzheimer's disease is increased production and accumulation of an amyloid-β peptide of 42 amino acids (amyloid-β_{1-42}). In support of this, missense mutations in the genes encoding amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2*), which are involved in amyloid-β production, are found in patients with early onset Alzheimer's disease. Oligomerization of amyloid-β_{1-42} and deposition as diffuse plaques in the brain lead to microglial-cell and astrocyte activation. Amyloid-β oligomerization alters neuronal-cell ionic homeostasis and might enhance Tau phosphorylation, leading to the formation of neurofibrillary tangles. The end result of this process is widespread neuronal-cell dysfunction, with cell death and transmission deficits leading to dementia.

Astrocyte
A star-shaped glial cell of the central nervous system that forms a structural and functional interface between non-nervous tissues and neurons. Once activated, astrocytes express glial fibrillary acidic protein at the cell surface.

Microglial cell
A macrophage-lineage cell that is derived from bone marrow and is present in the central nervous system.

Tau
A neuronal protein that binds to microtubules, promoting their assembly and stability.

Amyloid precursor protein
A membrane glycoprotein component of fast axonal transport, from which amyloid-β is cleaved by proteolytic processing.

Limbic system
A collection of cortical and subcortical structures important for processing memory and emotional information. Prominent structures include the hippocampus and amygdala.

Amyloid fibrils
Structures formed by many disease-causing proteins when they aggregate. Amyloid fibrils share common biochemical characteristics such as detergent insolubility, high β-pleated sheet content and a cross β-structure, protease resistance and the ability to bind lipophilic dyes, such as Congo Red, Thioflavin S and Thioflavin T.

Cerebral amyloid angiopathy
A condition in which there is a deposition of amyloid-β_{1-40} in the walls of the arteries that supply the brain.

intervals six times on each complex. Therefore, by binding multiple amyloid-β molecules in positions that approximate those of the amyloid-β β-pleated sheet or by stabilizing already formed oligomers of amyloid-β, C1q could facilitate the formation of the amyloid-β fibrils that form the amyloid plaque[21,22]. C1q has been shown to bind to amyloid-β *in vitro* and to trigger the complement cascade, including the formation of the membrane attack complex, C5b–C9 (REF. 23). Nonetheless, complement activation might also have a beneficial effect by leading to the uptake of amyloid-β by microglial cells[24].

Microglial cells. Microglial cells represent 10% of the cells in the adult central nervous system (CNS) and are morphologically characterized by small somas and ramified processes. Following activation in response to infection, or during inflammation that occurs as part of the pathogenesis of diseases such as multiple sclerosis or as a result of CNS injury, local microglial cells undergo morphological changes that include shortening of cellular processes and enlargement of their somas (defined as an 'amoeboid' phenotype). Microglial cells also respond to 'foreign' material such as aggregated amyloid-β[16,17,25]. Parenchymal microglial cells are myeloid progenitor cells that can differentiate into macrophage-like or dendritic-like cells when stimulated with macrophage colony-stimulating factor (M-CSF) and therefore acquire antigen-presenting properties[26]. Activated microglial cells upregulate the expression of cell-surface proteins (MHC class II molecules, CD11b and scavenger receptors) and produce cytokines (tumour-necrosis factor (TNF), interleukin-6 (IL-6) and IL-1) and chemokines (CXC-chemokine ligand 8 (CXCL8) and CC-chemokine ligand 3 (CCL3)). In response to amyloid-β deposition in Alzheimer's disease, microglial cells express different cell-surface receptors and can differentiate into cells with varying properties. For example, they can gain phagocytic properties by expressing cell-surface scavenger receptor molecules or neurotoxic properties by increasing the production of reactive oxygen species (ROS).

In vitro, fibrillar amyloid-β, alone or with other activators, can stimulate the production of neurotoxic ROS by inducing the expression of NADPH oxidase and inducible nitric-oxide synthase (iNOS) by microglial cells and macrophages[27–31]. Amyloid-β can also indirectly stimulate iNOS expression by neuronal cells and subsequent nitric oxide (NO)-mediated neuronal-cell apoptosis in response to microglial-cell-secreted TNF[32,33]. Both lipopolysaccharide (LPS)- and amyloid-β-activated microglial cells cause neuronal-cell death in hippocampal sections[34]. Nevertheless, there is no clear evidence for microglial-cell neurotoxicity *in vivo*.

It has been proposed that the clinical symptoms that occur as part of Alzheimer's disease pathogenesis are due to a gradual increase of amyloid-β levels above a threshold that is no longer controlled by endogenous microglial-cell clearance. However, it is possible that dysfunction of microglial cells could also have a pathological role at early phases of the disease[35]. There are several studies of Alzheimer's disease showing that microglial-cell activation can lead to amyloid-β clearance[36,37], supporting the concept that an important immunotherapeutic avenue is through microglial-cell activation in a manner that leads to amyloid-β removal without toxicity. During and after phagocytosis of amyloid-β, microglial cells might express cell-surface MHC class II molecules, as has been observed for amyloid-β-fibril-associated microglial cells from patients with Alzheimer's disease[16,17]. Furthermore, compared with control brain tissue, microglial cells from postmortem samples taken from patients with Alzheimer's disease have increased expression of the pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-12 and TNF[16].

It seems that microglial-cell activation has a central role in both the innate immune response in Alzheimer's disease and in the adaptive immune responses that occur after therapeutic vaccination (discussed later)[16,17,38,39].

Astrocytes. Astrocytes support, nourish and protect neurons. Astrogliosis is an early pathological manifestation of Alzheimer's disease and might represent a response to the accumulation of amyloid-β and/or the increasing number of degenerating synapses and neurons[40]. Astrogliosis is characterized by increased expression of the astrocyte marker glial fibrillary acidic protein (GFAP), and it occurs mainly around amyloid-β deposits both in the brain parenchyma and the cerebral microvasculature[40,41]. Migration of astrocytes to amyloid-β plaques, as shown by *in vitro* studies, is promoted by the chemokines CCL2 and CCL3, which are released by activated microglial cells that surround the plaque[42]. Astrocytes that are recruited to amyloid-β plaques have the potential to both mediate neurotoxicity and participate in the clearance of amyloid-β. In brain sections from patients with Alzheimer's disease, activated astrocytes, as well as activated microglial cells, contain amyloid-β fragments[43]. Mouse astrocytes plated on amyloid-β-rich brain sections from *APP*-transgenic mice reduce the overall amyloid-β levels in these sections[44]. Clearance of amyloid-β by astrocytes has been suggested to depend on their expression of APOE[45], as astrocytes from *Apoe*$^{-/-}$ mice do not respond to or internalize amyloid-β deposits to the same extent as wild-type astrocytes[46].

Anti-inflammatory drugs in Alzheimer's disease

Epidemiological studies show a reduced prevalence of Alzheimer's disease among chronic users of non-steroidal anti-inflammatory drugs (NSAIDs)[42,47]. A recent Phase II clinical trial of the NSAID R-flurbiprofen (Flurizan™;

Myriad Genetics Inc.) in patients with mild Alzheimer's disease showed 62% less decline in activities of daily living compared with patients given the placebo[48]. The main target of NSAIDs are cyclooxygenase (COX) enzymes. COX enzymes mediate the conversion of arachidonic acid to prostaglandins, which are crucial components of the pro-inflammatory process. The expression of COX2, which is the inducible isoform found in neurons and other cells, is upregulated in the brains of patients with Alzheimer's disease[49], indicating that NSAIDs might be beneficial in Alzheimer's disease by reducing neurotoxic inflammation that occurs secondary to amyloid-β deposition. Mice overexpressing a transgene encoding COX2, together with expression of transgenes encoding Alzheimer's-disease-associated mutant forms of human APP (K670N, M671L) and presenilin 1 (A246E), showed increased amyloid-β load and plaque formation[50]. Inhibition of COX2 is thought to be neuroprotective in ischaemic and excitotoxic injury[51]. Classical NSAIDs, such as ibuprofen and indomethacin, through their non-selective inhibition of COX enzymes, can suppress the prostaglandin production by microglial cells that occurs in inflammatory reactions[52].

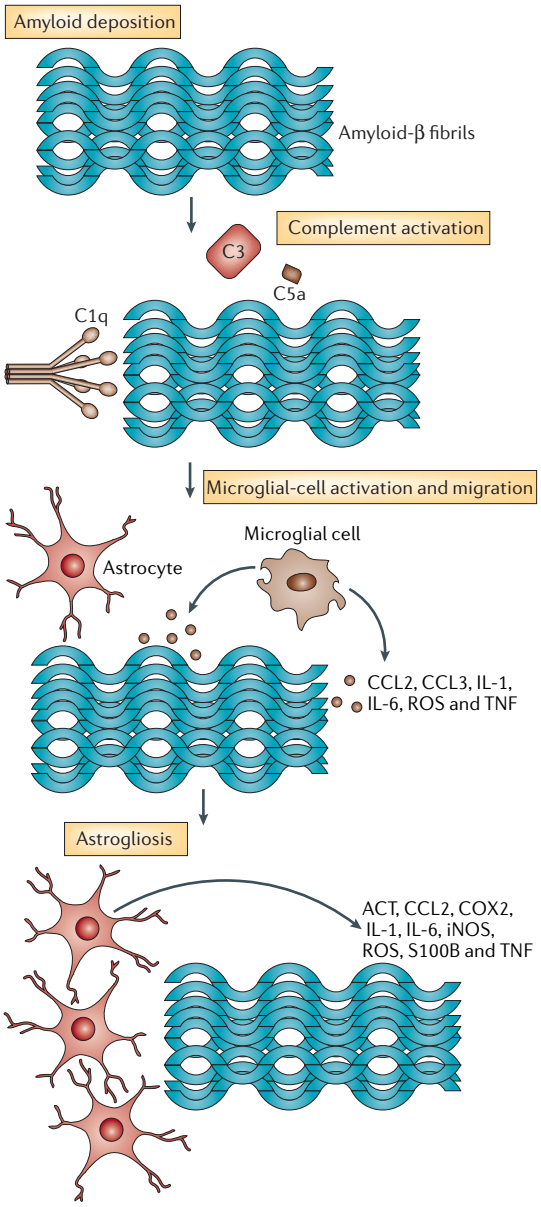

Figure 2 | **Innate immune response to amyloid-β in Alzheimer's disease.** Steps in the inflammatory process surrounding the amyloid plaques are shown. The production of complement components (C1q, C3 and C5) is the first stage in response to amyloid-β deposition, followed by migration of microglial cells to the brain. This is followed by astrogliosis, which is characterized by increased expression of the astrocyte marker glial fibrillary acidic protein (GFAP) in the brain. Both microglial cells and astrocytes secrete various pro-inflammatory mediators that exacerbate the process. ACT, α1-antichymotrypsin; CCL, CC-chemokine ligand; COX2, cyclooxygenase 2; IL, interleukin; iNOS, inducible nitric-oxide synthase; ROS, reactive oxygen species; TNF, tumour-necrosis factor.

However, a subset of NSAIDs lower amyloid-β_{1-42} levels independently of COX enzyme activity[53], apparently by modulating the activity of γ-secretase, which is a protease responsible for amyloid-β production. Similarly, amyloid-β-lowering NSAIDs were reported to reduce β- and γ-secretase activities that cleave APP at the amino- and carboxy-termini of the amyloid-β region, respectively[54]. Furthermore, independent of their anti-inflammatory activity, NSAIDs might affect amyloid-β cleavage from APP by altering multidrug resistance protein 4 (MRP4)[55]. MRP4 is expressed in several tissues, including the brain, and is involved in the transport into cells of several physiological substrates, such as cyclic nucleotides, steroid conjugates, folate and prostaglandins[55,56]. Some MRP4 substrates, such as cAMP or unconjugated oestradiol, regulate APP-amyloid-β metabolism by triggering the protein kinase A-dependent phosphorylation of a protein involved in APP maturation that occurs upstream to amyloid-β cleavage sites[57,58]. Early clinical trials of COX2 inhibitors have not succeeded in reducing cognitive or behavioural deficits in patients with Alzheimer's disease[59].

Several studies have shown that NSAIDs can reduce amyloid-β levels in mouse models (see Supplementary information S1, table), but the beneficial effects of NSAIDs reported in some epidemiological studies might not be due to anti-inflammatory activity but instead to effects on the protease that generates amyloid-β_{1-42} from APP. These findings support the view that decreasing the inflammatory response is not necessarily beneficial in Alzheimer's disease. In support of this, a study of an NO-releasing NSAID, NCX-2216, has demonstrated that microglial-cell activation can be beneficial[60]. This study showed that treating *APP*-transgenic mice with NCX-2216 for 5 months caused microglial-cell activation and a marked decrease in amyloid-β load, and this decrease was greater than that seen after treatment with ibuprofen (a COX1 and COX2 inhibitor) or the COX2 inhibitor celecoxib, which do not cause microglial-cell activation.

Amyloid-β-specific antibody

A body of work has shown that amyloid-β-specific antibodies clear amyloid assemblies *in vitro* and amyloid-β deposits in *APP*-transgenic mice[14,15,61–69] (TABLE 1).

Amyloid plaques
Sites of amyloid-β accumulation and dystrophic neurites in the brains of mouse models and patients with Alzheimer's disease.

Dystrophic neurites
Abnormal swelling that develops secondary to neuronal-cell stress in Alzheimer's disease and other neurodegenerative diseases.

Ramified process
Filamentous branches from the cell body that occur in association with activation of astrocytes and microglial cells.

Soma
The largest part of a cell, the cell body of a microglial cell or astrocyte.

Astrogliosis
An increase in the number of astrocytes owing to proliferation at sites of damage in the central nervous system.

Table 1 | **Amyloid-β-specific antibody as a mediator of amyloid clearance following active or passive immunization**

Model	Antibody or antigen	Route of immunization	Effect	Refs
Pheochromocytoma cells	Amyloid-β_{1-28}-specific antibody	*In vitro*	Inhibition and solubilization of fibrils of amyloid-β peptide through antibody recognition of the amino-terminal Glu-Phe-Arg-His epitope	61–64
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-42}	Subcutaneously with adjuvant	Reduced amyloid-β plaques, neuritic dystrophy and astrogliosis	65
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-42}	Nasally	Reduced cerebral amyloid burden	66
APP (K670N, M671L, V717F)-transgenic (CRND8) mice and *APP* (K670N, M671L), *PSEN1* (M146L) double-transgenic mice	Amyloid-β_{1-42}	Subcutaneously with adjuvant	Reduced behavioural impairment and amyloid-plaque deposition	14,15
APP (K670N, M671L)-transgenic (Tg2576) mice	A non-fibrillar amyloid-β_{1-30} homologous peptide	Subcutaneously with adjuvant	Reduced Alzheimer's-disease-associated pathology	70
APP (V717I)-transgenic mice	Filamentous phage displaying amyloid-β_{3-6} (Glu-Phe-Arg-His epitope)	Intraperitoneally	Reduced amyloid-β plaques and behavioural impairment	79,80
APP (K670N, M671L)-transgenic (Tg2576) mice	Recombinant adeno-associated virus vector expressing amyloid-β_{1-21}	Orally	Reduced amyloid-β plaques	77
APP (K670N, M671L)-transgenic (Tg2576) mice	Amyloid-β-encoding DNA vaccine	Intramuscularly	Decreased amyloid burden due to antibodies induced by DNA vaccination	76
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-6}- and amyloid-β_{3-6}-specific antibodies	Passive	Reduced amyloid-β plaques	67
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{13-28}-specific antibody	Passive	Reversion of memory deficits without reduction of brain amyloid-β burden	68,69
APP (K670N, M671L, V717F)-transgenic (CRND8) mice	Amyloid-β_{1-40}- and amyloid-β_{1-42}-specific antibodies	Passive	Attenuation of amyloid deposition	82
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{4-10}-specific antibody	Passive	Reduced amyloid-β plaques	83
APP (K670N, M671L)-transgenic (Tg2576) mice	Oligomeric amyloid-β_{1-40}-specific antibody	Passive	Reduced amyloid-β plaques, improvement in learning and memory	81

APP, amyloid precursor protein; CRND8, mice expressing double-mutant form of human APP 695 with K670N, M671L and V717F mutations; PDAPP, mice expressing a mutant form of human APP with V717F mutation; PSEN1, presenilin 1; Tg2576, mice expressing a mutant form of human APP 695 with K670N and M671L mutations.

The initial studies showed that antibodies specific for the N-terminal region of amyloid-β could prevent the formation of fibrillar amyloid-β *in vitro*[61,62]. Such antibodies bound to amyloid-β fibrils, restoring amyloid-β solubility and therefore preventing its neurotoxic effects on cell lines[61,62]. Using a phage-display library composed of filamentous phage displaying random combinatorial peptides, the Glu-Phe-Arg-His residues located at positions 3–6 of the N-terminus of the amyloid-β peptide were defined as the epitope of amyloid-β recognized by the antibodies that prevented amyloid aggregation. Binding of this epitope by the specific antibodies modulates the dynamics of aggregation as well as the resolubilization of pre-existing aggregates *in vitro*[63,64]. Therefore, amyloid-β-specific antibodies affected the structure and toxicity of amyloid-β.

Active amyloid-β immunization. Active immunization by parenteral immunization of *APP*-transgenic mice with synthetic amyloid-β in complete Freund's adjuvant (CFA), followed by boosting with amyloid-β in incomplete Freund's adjuvant, markedly decreased the number and density of amyloid-β deposits in mouse brain. This led to improvements in neuritic dystrophy and gliosis[65]. In addition, we observed amyloid clearance following repeated mucosal (intranasal) administration of amyloid-β peptide to *APP*-transgenic mice[66]. The reduction of amyloid-β roughly correlated with the titre of amyloid-β-specific antibody. Using the active immunization approaches, clearance of amyloid-β following immunization protected *APP*-transgenic mice from developing memory deficits[14,15]. This was achieved by immunizing *APP*-transgenic mice with the whole amyloid-β peptide (amyloid-β_{1-42}) or small fragments derived from it[70–77], such as the Glu-Phe-Arg-His peptide epitope (residues 3–6)[78–80].

Passive administration of amyloid-β-specific antibodies. Passive administration of monoclonal antibodies specific for synthetic peptide fragments of amyloid-β was effective in clearing amyloid-β and improving memory deficits in a mouse model of Alzheimer's disease[67,69].

Gliosis
The excess growth of neuroglial cells in the brain that usually follows neuronal-cell death and might lead to the formation of scar tissue.

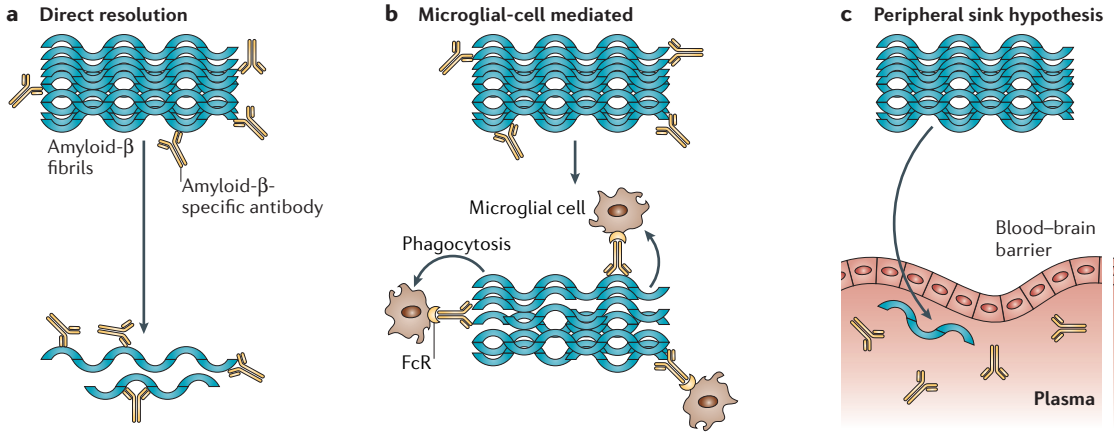

Figure 3 | **Mechanisms of amyloid-β clearance by amyloid-β-specific antibody.** There are three possible mechanisms by which amyloid-β-specific antibody might reduce brain amyloid-β deposition in animals. **a** | Dissolution of amyloid fibrils by binding to the amino (N)-terminus of amyloid-β. **b** | Fc receptor (FcR)-mediated phagocytosis of amyloid-β by microglial cells. **c** | Sequestration of circulating amyloid-β causing efflux of amyloid-β from the brain to the plasma (peripheral sink hypothesis).

The clearance of amyloid-β seemed to depend on the antibody entering the CNS and activating resident microglial cells. This effect also depended on antibody recognition of the N-terminus of the amyloid-β epitope (residues 1–6 and 3–6)[81–83]. Further studies showed that passive administration of amyloid-β-specific antibody could affect cognition without affecting insoluble amyloid-plaque burden in the brain, owing perhaps to the efflux of soluble amyloid-β from the brain into the plasma ('peripheral sink hypothesis', described later)[68]. Furthermore, intrahippocampal injection of amyloid-β-specific antibody in mice not only reduces extracellular amyloid-β plaques, but also reduces intracellular amyloid-β accumulation and neurofibrillary tangles in a triple-transgenic model, in which the mice have transgenes encoding presenilin 1, APP and Tau protein and develop both amyloid-β fibrils and neurofibrillary tangles[84].

Mechanism of amyloid-β clearance by amyloid-β-specific antibodies. There have been three mechanisms postulated to explain how antibodies reduce amyloid-β deposition in the brain[85] (FIG. 3). One possible mechanism is that there is a direct effect of antibody on amyloid-β, leading to dissolution of amyloid fibrils[62,64,86] or neutralization of amyloid-β oligomers[86]. In support of this, direct injection of antibody into the brain causes a decrease in amyloid-β and only antibodies against the N-terminus cause this effect, as has been shown *in vitro* in the presence of amyloid-β and antibody[62].

A second mechanism is that amyloid-β-specific antibody leads to Fc receptor (FcR)-mediated phagocytosis of amyloid-β by microglial cells[65,67,87]. Consistent with this, following peripheral administration of amyloid-β-specific antibody, activated microglial cells are found surrounding the amyloid-β plaques, and there is less clearance of amyloid-β following injection of amyloid-β-specific antibody in animals that have impaired microglial-cell function compared with wild-type animals[87].

The third mechanism, termed the peripheral sink hypothesis, postulates that administration of amyloid-β-specific antibody to the circulation results in a net efflux of amyloid-β from the brain to the plasma[69]. In support of this, investigators have observed rapid improvement in cognition in animals after intravenous injection of antibody and increased plasma concentrations of amyloid-β[68]. Moreover, a monoclonal antibody specific for the central domain of amyloid-β that does not bind to the amyloid plaques can still reduce cerebral amyloid-β burden[69]. Such findings indicate that intravenous administration of antibody against amyloid-β shifts the CNS–plasma amyloid-β equilibrium by acting as a binding protein in the periphery. Although other effects of amyloid-β-specific antibody have been observed, apart from direct injection of antibodies to the brain, it has been difficult to directly demonstrate co-localization of amyloid-β-specific antibody with amyloid plaques either after active immunization or passive peripheral administration.

Taken together, it is probable that all three of the mechanisms can occur, in part depending on the experimental system being studied.

Clinical trials of amyloid-β_{1-42} immunization

The finding that active vaccination with amyloid-β_{1-42} could markedly reduce quantities of total amyloid-β in an animal model[65], led to a randomized placebo-controlled Phase II clinical trial. An amyloid-β_{1-42} synthetic peptide (AN1792; Elan Pharmaceuticals Inc.) was administered with a previously tested T helper 1 (T_H1)-cell-inducing adjuvant (QS21) to individuals with mild to moderate Alzheimer's disease[88]. The Phase I clinical trial demonstrated apparent safety and tolerability of multiple injections of AN1792 plus QS21, which elicited antibody responses to amyloid-β_{1-42} in more than half of the elderly population studied[89]. In the Phase II clinical trial, 372 patients with mild to moderate Alzheimer's disease were randomized to receive intramuscular injections of AN1792 plus QS21 or a placebo on day 0

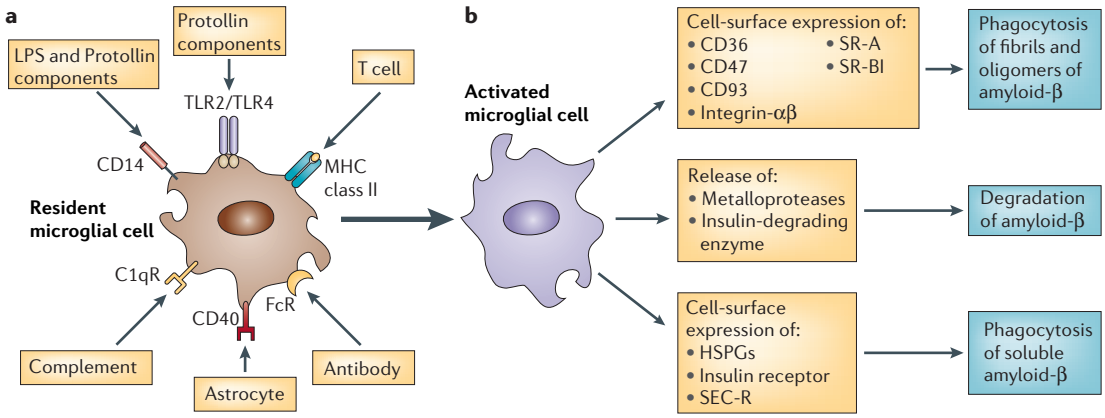

Figure 4 | **Mechanisms of clearance of amyloid-β. a** | Microglial cells can be activated by binding of various ligands to various cell-surface innate immune receptors: CD14 binds lipopolysaccharide (LPS) and components of Protollin; Toll-like receptor 2 (TLR2) and TLR4 bind Protollin components; MHC class II molecules interact with T-cell receptors; CD40 binds CD40 ligand expressed by T cells and astrocytes; complement receptors bind complement components such as C1q; and Fc receptors (FcRs) bind amyloid-β-specific antibodies. **b** | Activated microglial cells express various scavenger receptors (SRs) that mediate phagocytosis of amyloid-β, such as integrin-αβ, CD36, CD47, SR-A and SR-BI, the triggering of which leads to phagocytosis of amyloid-β fibrils and oligomers. Ligation of cell-surface heparan sulphate proteoglycans (HSPGs), insulin receptors and proteinase inhibitor (serpin)–enzyme complex receptor (SEC-R) on activated microglial cells leads to phagocytosis of soluble amyloid-β. Microglial cells can also degrade amyloid-β by releasing amyloid-β-degrading enzymes, such as metalloproteases, insulin-degrading enzyme and gelatinase A. Protollin, a proteosome-based adjuvant composed of purified outer membrane proteins of *Neisseria meningitidis* and lipopolysaccharide (GlaxoSmithKline Biologicals of North America).

and at months 1, 3, 6, 9 and 12 (REF. 90). Unfortunately, clinical symptoms and laboratory findings consistent with meningoencephalitis developed in 18 of 298 (6%) patients who were treated with AN1792 plus QS21 compared with 0 of 74 patients who received the placebo[90]. This led to abrupt discontinuation of the trial. Of the 18 patients who developed meningoencephalitis, 12 recovered to or close to baseline in weeks, whereas 6 remained with disabling cognitive or neurological sequelae[90]. All subjects were followed longitudinally after the cessation of the trial.

In the Phase II clinical trial, of those treated with AN1792 plus QS21, 59 (19.7%) patients developed the predetermined concentration of amyloid-β-specific antibody[88]. In a small subset of the clinical trial involving 30 patients, it was reported that those with plaque-reactive amyloid-β-specific antibodies in plasma had a significantly slower rate of cognitive decline than those patients that did not have detectable amyloid-β-specific antibodies[91]. However, in the clinical trial subjects as a whole, no significant differences were found between antibody-responder and placebo groups in performance on a series of cognitive tests, although analyses of composite scores across a series of neuropsychological tests showed significant differences favouring amyloid-β-specific antibody responders[88]. In another analysis of patients from this clinical trial, antibody responders had greater brain volume decreases than non-responders and this decrease in brain volume was associated with better cognitive performance[92]. Reasons for this include the possibility that the volume changes were due to amyloid removal and associated cerebral fluid shifts. Nevertheless, this effect was measured 12 months after the second (and last) immunization with amyloid-β. The question remains whether such brain volume changes in antibody responders compared with controls would differ over a longer time, particularly if no meningoencephalitis occurred. In the postmortem brains of certain subjects from this clinical trial, either with[36,93] or without[94] encephalitis, areas of apparent clearing of amyloid plaques in the cortex were observed, accompanied by abundant amyloid-β-immunoreactive microglial cells[94].

The cases of meningoencephalitis did not correlate with the presence or concentrations of antibody titres to amyloid-β[88,90], and it is now generally believed that the meningoencephalitis was due to a T-cell response against amyloid-β[38,39,95]. Cases of meningoencephalitis following AN1792 immunization had infiltrates of $CD4^+$ and $CD8^+$ T cells in the brain[93]. The belief that vaccination with amyloid-β in a T_H1-type adjuvant induced a T-cell response against amyloid-β is consistent with the observation that increased T-cell reactivity to amyloid-β exists in some older individuals and patients with Alzheimer's disease who were never exposed to antigen vaccines[95].

The autopsy of a patient who developed meningoencephalitis and later died of a pulmonary embolus showed apparent clearance of amyloid-β plaques from large areas of the neocortex, as well as a decrease in plaque-associated astrocytes and neuritic dystrophy[36]. Similar findings were described in a clinical trial subject without apparent meningoencephalitis [94]. In an unrelated autopsy of an Alzheimer's disease subject not in the Phase II clinical trial[37], a local reduction in senile plaques in a neocortical region affected by incomplete ischaemia owing to a stroke indicated that this finding, and those reported in cases of the Phase II clinical trial of AN1792 (REF. 36), could be due to phagocytosis of amyloid-β by activated microglial cells. Analysis of RNA expression patterns in blood samples, taken before and

Table 2 | **Effect of immune effector molecules on amyloid-β pathology**

Immune transgene or gene knockout in *APP*-transgenic mice	Effect	Reference
Tgfb transgene	• Reduced parenchymal amyloid-β plaques associated with microglial-cell activation • Increased production of pro-inflammatory mediators • Accumulation of substantial quantities of amyloid-β in cerebral blood vessels	104
Cox2 transgene	• Increased amyloid-β load and plaque formation	50
sCrry transgene	• Increased (2–3 fold) amyloid-β deposition at 12 months, accompanied by degeneration of neurons	100
Ifng transgene	• Encephalomyelitis and amyloid-β clearance following immunization with amyloid-β_{10-25}	135
Ccl2 transgene	• Increased amyloid-β deposition by reducing amyloid-β clearance through increased apolipoprotein E	
Cd40lg knockout	• Decreased astrogliosis and microgliosis associated with decreased amyloid-β levels and plaque load	137
C1q knockout	• No effect on total amyloid and fibrillar amyloid-β load in the frontal cortex and hippocampus • Reduced numbers of activated microglial cells surrounding the plaques • Decreased numbers of neuronal cells at 12 months	140
Fcgr knockout	• Attenuation of amyloid-β deposition following immunization with amyloid-β_{1-42}, equivalent to the reduction observed in immunized *APP*-transgenic mice	73
Igμ knockout (B-cell deficient)	• No effect on total amyloid and fibrillar amyloid-β load in the brain • No effect on EAE-induced amyloid clearance	

APP, amyloid precursor protein; *Cd40lg*, CD40 ligand; *Ccl2*, CC-chemokine ligand 2; *Cox2*, cyclooxygenase 2; *sCrry*, soluble complement receptor-related protein y; EAE, experimental autoimmune encephalomyelitis; *Fcrg*, Fc receptor for IgG; *Ifng*, interferon-γ; *Tgfb*, transforming growth factor-β.

after immunization of AN1792, showed that expression of genes involved in apoptosis and pro-inflammatory pathways (the TNF pathway, in particular) were risk factors for the development of meningoencephalitis[96]. However, expression of genes involved in protein synthesis, protein trafficking, DNA recombination, DNA repair and the cell cycle, were strongly associated with an antibody response to immunization[96]. Therefore, such biomarkers might be helpful in both identifying factors associated with risk of meningoencephalitis and factors associated with an immune response to therapy.

Although interrupted, the Phase II clinical trial of AN1792 provides further support for amyloid-β immunotherapy of Alzheimer's disease. Currently, there are two Phase I clinical trials of active immunization using ACC-001 (Elan Corporation Inc. and Wyeth), which contains amyloid-β_{1-7} derivatives[97], and CAD106 (Immunodrug™; Cytos Biotechnology AG and Novartis Pharma AG), which consists of an amyloid-β fragment coupled to a carrier.

Fluid percussion injury
Increase of fluid in the brain cranial cavity, leading to brain injury.

Passive administration of an amyloid-β-specific humanized monoclonal antibody is currently in a Phase II clinical trial in patients with Alzheimer's disease. In *APP*-transgenic mice, which have high levels of CAA, passive amyloid-β immunization caused some local microhaemorrhages associated with amyloid-bearing vessels[98]. As approximately 10% of people over 65 years of age and as many as 80% of patients with Alzheimer's disease exhibit some CAA, the success rate of amyloid-β immunotherapy might be improved by screening patients for the presence and severity of CAA (once this can be accomplished) before such therapies are undertaken[98].

Role of microglial cells in amyloid-β clearance

Microglial cells represent a natural mechanism by which protein aggregates and debris can be removed from the brain[25,36,37]. There are multiple routes by which resident microglial cells can be activated to promote the clearance of amyloid-β (FIG. 4). Examples in humans indicate that microglial-cell activation after immunization with amyloid-β or after a stroke might lead to amyloid-β clearance[36,37]. Furthermore, as discussed earlier, there is an increasing body of literature that links the activation of microglial cells with a reduction of amyloid deposition in the transgenic mouse models[99–102]. The clearance of amyloid-β from the brain following amyloid-β immunization was associated with enhanced microglial-cell activity around the remaining amyloid deposits[65]. Fluid percussion injury can activate microglial cells, which leads to the reduction of amyloid deposition[103]. Also, the crossing of *APP*-transgenic mice with mice that overexpress transforming growth factor-β (TGFβ) produced offspring with increased microglial-cell activation and reduced amyloid loads[104]. Moreover, blocking of complement activation diminished microglial-cell activity in *APP*-transgenic mice and led to increased amyloid loads[100] (TABLE 2). This supports the concept that in the mouse model of Alzheimer's disease, activation of microglial cells through C1q might have a beneficial role in decreasing amyloid load without causing significant neurotoxicity[24,105]. Therefore, cellular mechanisms that enhance microglial-cell phagocytosis of amyloid-β could have an important role in the immunotherapy of Alzheimer's disease.

Imaging of mouse amyloid deposits *in vivo* by two-photon confocal microscopy has shown that local clearance of amyloid plaques after direct application of an amyloid-β-specific antibody occurs in association with the local increase of activated microglial cells[106]. This indicates that amyloid clearance probably occurs by FcR-mediated phagocytosis of amyloid-β–antibody immune complexes by microglial cells. Moreover, it has been reported that increased cell-surface expression of the receptor for M-CSF by microglial cells accelerates phagocytosis of aggregated amyloid-β, by increasing microglial-cell expression of FcRs for IgG (FcγRs)[67,107]. Inhibition of amyloid-β-specific-antibody-induced microglial-cell activation by anti-inflammatory drugs, such as dexamethasone, inhibits the removal of fibrillar amyloid deposits[87]. Nevertheless, clearance of amyloid

has also been shown with amyloid-β-specific $F(ab')_2$ fragments, which do not bind FcRs, applied topically to the cortex of *APP*-transgenic mice through a craniotomy[108,87], and amyloid-β-specific $F(ab')_2$ fragments have also been shown to prevent amyloid neurotoxicity *in vitro*[109]. These results, together with marked reduction of amyloid-β load in the brains of FcγR-deficient *APP*-transgenic mice following immunization with amyloid-β[73], indicates an additional *in vivo* mechanism besides FcR-mediated clearance. The two mechanisms might occur independently or might operate in tandem, for example with microglial-cell removal of amyloid-β after antibody-mediated solubilization.

The innate immune response can include cellular activation following recognition of microbial components, such as LPS or other highly hydrophobic and aggregated structures. Toll-like receptor 4 (TLR4) and MD2 form a complex through which LPS and its receptor, CD14, can initiate an intracellular signal that induces pro-inflammatory responses[110]. In Alzheimer's disease, amyloid-β is highly hydrophobic and therefore could induce innate immune responses similar to those triggered by LPS. Furthermore, it has been shown that acute or chronic administration of LPS into the brain ventricles of rats can result in gliosis, cytokine production, increased APP concentrations and, in some cases, cognitive deficits[111,112]. Glial activation due to LPS administration has been thought to mimic some features of Alzheimer's disease[110,111]; indeed, other investigators have reported increased amyloid-β levels in response to LPS[113–115]. In these studies, LPS was given intravenously[113], intracerebroventricularly[114] or intraperitoneally[115]. By contrast, enhanced amyloid-β clearance was observed when LPS was injected intrahippocampally[99,116] or intracranially[117] to older *APP*-transgenic mice. Increased clearance following intrahippocampal injection was observed at 3, 7 and 14 days after injection but not at 28 days after the injection and correlated with the presence of activated microglial cells. Nasal administration of a proteosome-based adjuvant (Protollin; GlaxoSmithKline Biologicals of North America)[118] composed of purified outer membrane proteins of *Neisseria meningitidis* and LPS also reduces amyloid-β in an *APP*-transgenic mouse model in association with microglial-cell activation[102]. Protollin might function by stimulating microglial cells both by LPS through TLR4 and by porB, which makes up 70% of the proteosome protein and is known to activate antigen-presenting cells through TLR2.

Based on the aforementioned findings in animal models and human clinical trials, and as discussed later for the role of T cells in Alzheimer's disease immunotherapy, it is now clear that immune strategies that result in activation of microglial cells might be of significant benefit in the clearance of amyloid-β. We believe that this is true because once activated, microglial cells take on several properties that facilitate the clearance of amyloid-β (FIG. 4). First, activated microglial cells express several scavenger receptors (SRs), such as integrin-αβ[119], CD36 (REFS 25,120), CD47 (REF. 121), SR-A[122], SR-BI[122] and formyl peptide receptor 2 (REFS 123,124), the triggering of which leads to phagocytosis of amyloid-β fibrils or oligomers[125]. Second, soluble amyloid-β can be directly bound by microglial-cell receptors, including heparan sulphate proteoglycans (HSPGs)[126], insulin receptors[127] and proteinase inhibitor (serpin)–enzyme complex receptor (SEC-R)[128], resulting in phagocytosis of soluble amyloid-β. Last, activated microglial cells can degrade amyloid-β by releasing amyloid-β-degrading enzymes, such as metalloproteases[129], insulin-degrading enzyme[129] and gelatinase A[130]. So, there are multiple mechanisms by which microglial-cell activation can lead to enhanced clearance of amyloid-β from the brain, and the source of the microglial cells might be brain resident or bone-marrow-derived microglial cells[131].

Microglial cells do not have uniform properties and, as discussed previously, it is probable that there are different microglial-cell phenotypes that have the potential to be beneficial or harmful in Alzheimer's disease. For example, it has been proposed that those microglial cells that produce TNF have toxic properties for neuronal tissue, whereas microglial cells differentiated in the presence of IL-4 and interferon-γ (IFNγ) might be beneficial[132].

Role of T cells in immunotherapy

Although there is no clear T-cell response in the brains of patients with Alzheimer's disease, understanding T-cell responses and adaptive immunity has become important in the immunotherapy of Alzheimer's disease (TABLE 3). The meningoencephalitis observed following immunization of patients with Alzheimer's disease with amyloid-β_{1-42} in adjuvant is believed to be associated with a T-cell response to amyloid-β[38,39,95]. However, it is remarkable that no encephalitis was observed in the animal models, in which animals were immunized with amyloid-β in CFA. Although the reasons for this difference are unclear, it should be pointed out that the mouse immunization protocols[65], on which the human studies were based, involved immunization with amyloid-β and adjuvant but without *Bordetella pertussis* toxin. This toxin is required to induce experimental autoimmune encephalomyelitis (EAE) with myelin antigens, such as myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP). Furthermore, in the mouse model there is no neuronal-cell death, whereas in Alzheimer's disease in humans neuronal-cell death does occur, which could make humans more susceptible to encephalitis. Alternatively, the increased deposition of amyloid-β and microglial-cell activation in patients with Alzheimer's disease, compared with animals, could alter their susceptibility to encephalitis. Furthermore, intrinsic T-cell responses to amyloid-β are found in elderly humans[95], whereas in *APP*-transgenic models, expression of amyloid-β might lead to induction of a form of tolerance that limits the immune response to amyloid-β[133].

To address the question of whether amyloid-β immunization can lead to EAE, investigators have attempted to induce EAE in mice using amyloid-β. A type of EAE could be induced following immunization with amyloid-β plus CFA and large amounts of pertussis toxin[134]. We have been unable to repeat these observations by immunizing

Table 3 | **The role of T cells in immunotherapy of Alzheimer's disease**

Antigen	Model	Finding	Reference
Amyloid-β_{1-42} (AN1792) plus T_H1-cell-inducing adjuvant (QS21)	Humans	• Amyloid-β-specific antibodies clear amyloid-β • Induction of T_H1-cell response induces meningoencephalitis in 6% of cases	88
Amyloid-β_{1-42} (nasal immunization)	*APP* (V717F)-transgenic mice	• Amyloid-β-specific antibodies clear amyloid-β • Induction of T_H2/T_H3-cell response without induction of encephalitis	66
Amyloid-β_{10-25} plus CFA	*APP*, *Ifng* double-transgenic mice	• No induction of amyloid-β-specific antibodies • T_H1-cell response clears amyloid-β	135
MOG and PLP plus CFA	*APP*-transgenic, *Igμ*-knockout mice (B-cell deficient)	• No induction of amyloid-β-specific antibodies • Induction of EAE by T_H1-cell response • Activation of microglial cells clears amyloid-β	102
Glatiramer acetate plus Protollin (nasal immunization)	*APP*-transgenic mice	• No induction of amyloid-β-specific antibodies • Induction of T_H1- and T_H2-cell responses without induction of EAE • Activation of microglial cells	102

APP, amyloid precursor protein; CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalitis; *Ifng*, interferon-γ; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; Protollin, a proteosome-based adjuvant composed of purified outer membrane proteins of *Neisseria meningitidis* and lipopolysaccharide (GlaxoSmithKline Biologicals of North America); T_H, T helper.

wild-type or *APP*-transgenic mice with amyloid-β[102]. We have found, however, that immunization with amyloid-β in adjuvant induced encephalitis in *APP*-transgenic mice, in which a transgene encoding IFNγ was expressed under the control of the astrocyte-specific MBP promoter[135]. This indicates that the presence of the pro-inflammatory cytokine IFNγ might be necessary to facilitate the induction of the T_H1-cell response to amyloid-β in the brain. This, in part, might explain the occurrence of encephalitis in patients with Alzheimer's disease but not in the mouse models[135].

Because it is believed that a T_H1-cell response against amyloid-β caused the meningoencephalitis in AN1792-treated patients with Alzheimer's disease, shifting the balance away from T_H1-type responses might be important for preventing this complication in the future. We have found that nasal administration of amyloid-β induces amyloid-β-specific antibodies and the clearance of amyloid-β, without inducing a T_H1-type immune response, but rather generated T_H2 and/or T_H3 cells with no induction of encephalitis[136]. Strategies such as mucosal vaccination might, therefore, circumvent the problems observed with immunization of amyloid-β plus CFA or a T_H1-cell-inducing adjuvant such as QS21. Another approach currently under investigation (the Phase I clinical trial of CAD106) to avoid potential unwanted T_H1-cell responses is to link the B-cell epitope of amyloid-β to a carrier protein and therefore only obtain amyloid-β-specific antibody responses without a T-cell response. Epitopes presented by B cells leading to the production of amyloid-β-specific antibodies are located in residues 1–16 of amyloid-β_{1-42}, whereas residues 16–25 are recognized by T cells.

Nonetheless, it should be emphasized that T-cell responses might be beneficial in aiding the clearance of amyloid-β, most probably through the activation of microglial cells. It was reported that induction of EAE in *APP*-transgenic mice by immunization with myelin antigens (such as MOG and proteolipid protein) in CFA was associated with activation of microglial cells that co-localized with amyloid plaques and led to the clearance of amyloid-β[102]. This occurred independently of amyloid-β-specific antibody. Therefore, it might be postulated that myelin-reactive T_H1 cells that are activated in the periphery by immunization with myelin antigens migrate to the brain, where they release IFNγ and activate microglial cells. Mucosal (nasal or oral) vaccination with MBP mainly induces T_H2- and/or T_H3-cell responses, which are not associated with amyloid-β clearance in the *APP*-transgenic mouse model[66].

Despite their potential clinically beneficial role in patients with Alzheimer's disease by activating microglial cells, these myelin-reactive T_H1 cells cause encephalitis. For treatment of patients with Alzheimer's disease, a T-cell strategy would have to activate microglial cells through IFNγ production without causing encephalitis. This can be achieved in *APP*-transgenic mice by immunizing with glatiramer acetate (GA), which is a random amino-acid copolymer of alanine, lysine, glutamic acid and tyrosine used for the treatment of multiple sclerosis that induces both T_H1- and T_H2/T_H3-cell responses. Immunization of *APP*-transgenic mice with GA in CFA injected subcutaneously or GA in Protollin given nasally activates microglial cells without inducing encephalitis or neurotoxicity and leads to amyloid-β clearance[102].

Different types of T cell would be expected to have different effects on microglial-cell activation and therefore on amyloid clearance. T_H1 cells can promote microglial-cell activation through the secretion of IFNγ, which also upregulates the expression of MHC class II molecules by microglial cells, thereby enhancing the T-cell–microglial-cell interaction. Not all T-cell–microglial-cell interactions, however, lead to the clearance of amyloid-β. It has been shown that interaction of CD40 (expressed by microglial cells) with CD40 ligand (CD40L; expressed by T cells) mediates microglial-cell activation in response to amyloid-β[137] and *APP*-transgenic mice that are deficient in CD40L

showed reduced levels of amyloid-β and numbers of amyloid-β plaques[137]. This raises the possibility that microglial cells activated through CD40 might have a different phenotype to those that are activated by IFNγ or MHC class II molecules.

So, T cells might play a central role in non-antibody-mediated clearance of amyloid-β, most probably by stimulating microglial cells. The development of an immunotherapeutic approach for Alzheimer's disease might ultimately be most effective when the appropriate T-cell subset is induced together with protective microglial cells and amyloid-β-specific antibody responses.

Future perspectives

As described in this Review, immune-based therapy is proving increasingly important for the treatment Alzheimer's disease, but it must be approached with care. Given what has been learned using experimental systems and from clinical trials, both humoral and cellular immune responses can be effective in clearing amyloid-β. Humoral mechanisms involve binding of amyloid-β-specific antibodies to amyloid-β with neutralization of toxicity and/or activation of clearance mechanisms. Although the focus of amyloid-β-based immunotherapy has been on antibody-mediated clearance, antibody-independent approaches that target T cells or microglial cells are also effective in amyloid-β clearance. Generating T_H2/T_H3-type responses or inducing non-amyloid-β-specific T cells can avoid potentially pathogenic T_H1-cell responses. Direct stimulation of microglial cells through innate immune receptors might cause amyloid clearance independent of T- or B-cell responses. Emerging imaging techniques should provide an objective measure of whether the various immune strategies achieve amyloid-β clearance. Furthermore, although the amyloid hypothesis has been confirmed in preclinical research, the ultimate validation of this hypothesis will require treatments that reduce amyloid levels and cause concomitant neurological clinical improvement in patients with Alzheimer's disease. Furthermore, non-immune methods to reduce amyloid-β levels, such as those targeted to proteases that induce toxic amyloid-β peptides, might ultimately be used in combination with immune approaches. The attraction of immunotherapy of Alzheimer's disease lies in the ability to vaccinate large segments of the aging population to treat or prevent the devastating effects of this common neurological disorder.

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Competing interests statement
The authors declare no competing financial interests.

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ERRATUM

Immunology and immunotherapy of Alzheimer's disease

Howard L. Weiner and Dan Frenkel

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When published, the fifth and ninth rows of Table 2 had information missing. The corrected rows are shown below.

Immune transgene or gene knockout in *APP*-transgenic mice	Effect	Reference
Ccl2 transgene	• Increased amyloid-β deposition by reducing amyloid-β clearance through increased apolipoprotein E	139
Igμ knockout (B-cell deficient)	• No effect on total amyloid and fibrillar amyloid-β load in the brain • No effect on EAE-induced amyloid clearance	102