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SYMPOSIUM: Clearance of Aβ from the Brain in Alzheimer' Disease

The Role of the Immune System in Clearance of Aβ from the Brain

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Abstract

In Alzheimer's disease (AD), there is abnormal accumulation of Aβ and tau proteins in the brain. There is an associated immunological response, but it is still unclear whether this is beneficial or harmful. Inflammation in AD, specifically in the form of microglial activation, has, for many years, been considered to contribute to disease progression. However, two types of evidence suggest that it may be appropriate to revise this view: first, the disappointing results of prospective clinical trials of anti-inflammatory agents and, second, the observation that microglia can clear plaques in AD following Aβ immunization. Although Aβ immunization alters AD pathology, there is limited evidence so far of benefit to cognitive function. Immunization against microorganisms is almost always used as a method of disease prevention rather than to treat a disease process that has already started. In animal models, immunotherapy at an early age can protect against Aβ accumulation and it will be interesting to see if this can usefully be applied to humans to prevent AD.

INTRODUCTION

Alzheimer's disease (AD) is an age-related chronic neurodegenerative disease characterized by memory loss and severe cognitive decline. The key pathological features of AD relate to accumulation of Aβ protein and tau protein (72). Aβ accumulates in the form of plaques in the cortical grey matter and in the walls of cortical and leptomeningeal small arteries and arterioles in the form of cerebral amyloid angiopathy (CAA). Tau protein accumulates within neurons in cell bodies (tangles), fine calibre neuronal processes in the grey matter neuropil (neuropil threads) and in distorted and swollen neuronal processes in the region of Aβ plaques (dystrophic neurites). Detailed studies have also documented neuronal and synaptic loss (64, 114).

THE AMYLOID HYPOTHESIS

Evidence of a key role for Aβ peptide in AD pathogenesis gives rise to the "amyloid hypothesis" of AD (46). Aβ is a peptide predominantly 40 or 42 amino acids in length derived from the transmembrane protein amyloid precursor protein (APP) after cleavage by β and γ secretases (44, 67). Several lines of evidence from the human disease support a pivotal role for abnormalities of Aβ in the pathogenesis of AD, leading to neuronal dysfunction and cell death (99): (i) APP point mutations can cause familial AD; (ii) familial AD, whether because of point mutations of PSEN1, PSEN2 or APP, have in common increased production of Aβ42 which is particularly prone to aggregation; and (iii) Down's syndrome, which is usually due to trisomy 21 on which the APP gene is located, is associated with AD at an early age. It is worth noting that this evidence comes from the very rare genetically determined forms of AD, and whether the role of Aβ applies equally to the common sporadic form of AD is not yet known with certainty. A crucial test of the amyloid hypothesis would be whether prevention of Aβ aggregation prevents the neurodegenerative decline.

There is now good evidence that AD that has a genetic cause is due to an increase in the production of Aβ, resulting in Aβ accumulation. In the much more common sporadic AD, there is not good evidence for this and, instead, evidence has been emerging for an age-related impairment of elimination of Aβ as the cause of Aβ accumulation. It seems likely that there is a dynamic equilibrium between the production and elimination of Aβ protein in the human brain. Evidence for several potential mechanisms by which Aβ is normally eliminated from the brain has emerged, including (i) by cellular mechanisms involving microglia (85, 90); (ii) by enzymatic degradation (eg, neprilysin and insulin degrading enzyme) (54, 91); (iii) by transport across the blood–brain barrier mediated by the low density lipoprotein receptor-related protein-1 (LRP) receptor (101); and/or (iv) by bulk flow with interstitial fluid along the perivascular drainage pathway (97, 119).

Transgenic mice which over-express familial AD APP point mutations develop Aβ plaques as they age providing a valuable animal model (25, 36), although lacking tau pathology. Recent evidence in a triple transgenic animal model of AD, with point mutations in APP, PSEN1 and tau genes, indicates that Aβ accumulation precedes the tau pathology in a cascade of events that ultimately leads to the cognitive alterations (81).

Apolipoprotein E

Genetic studies in the early 1990s clearly identified polymorphism of the apolipoprotein E gene (*APOE*) as the major genetic risk factor for sporadic AD (110). *APOE* molecules are lipid carriers (15) involved in the redistribution of cholesterol within the brain to maintain the structural and functional integrity of membranes and synapses (63). *APOE* also acts as an Aβ-scavenging molecule that regulates Aβ concentration through internalization of *APOE* receptors by the endosomal/lysosomal pathway (89). In addition, recently, it has been revealed that Aβ has an essential physiological role in lipid homeostasis (43). This evidence suggests that clearance of Aβ is likely to be regulated by the *APOE*–Aβ interactions. Three different isoforms of *APOE* occur within the brain (*APOE* E2, E3 and E4), which differ in amino acids at position 112 and 158, according to the genotype of the individual (63). The increased risk of developing AD associated with *APOE* E4 may be due to its inability to internalize, and therefore clear, extracellular Aβ to endosomes/lysosomes. The evidence for this comes from a failure to develop plaques in transgenic mice that both over-express human APP and are *APOE* deficient (48). In addition, the biochemical difference of *APOE* E4 may induce the promotion of lipid rafts that have a suitable environment for the amyloidogenic processes (19). *APOE* genotype has also been associated with differences in microglia; both in the degree of microglial activation in AD brains (27) and in the microglial expression of inflammatory molecules (59, 62).

EVIDENCE FOR INVOLVEMENT OF THE IMMUNE SYSTEM IN AD

The immune system has evolved to protect the body against invasion by foreign microorganisms. The efficiency of the immune system is the result of two different but complementary forms of activation—innate and adaptive. Innate immunity is the first response to infection and plays a major role in controlling the infection during the gestation of adaptive immunity. The macrophage is a central component of innate immunity. If innate immunity is overcome by pathogens, adaptive immunity operating via dendritic cells, lymphocytes and antibodies, will build a specific response to the infection. The key property of adaptive immunity is to recognize pathogens specifically and to provide enhanced protection against re-infection.

A growing number of studies in AD have reported alterations in the immune system, including: the presence of circulating auto-antibodies; the presence in the brain of proteins from the complement system; abnormal production of cytokines; and changes in the distribution and activation of microglia. This implies that, in a sense, the immune system is capable of recognizing the proteins that aggregate in the brain in AD as abnormal or "foreign" proteins that should be disposed of. This raises the question as to whether this involvement of the immune system can help to ameliorate the progress of AD or simply adds to the damage.

Auto-antibodies

An increase in auto-antibodies, defined as antibodies to "self-tolerant" proteins, in the blood of healthy elderly humans has been observed (35, 45, 95). This led to two different hypotheses: (i) auto-antibodies contribute to the diseases associated with ageing, as occurs in autoimmune disease (116); or (ii) auto-antibodies play a role in eliminating senescent cells to maintain the integrity of the host (37). More recently, a number of studies have reported the presence of specific anti-Aβ antibodies in the blood and cerebrospinal fluid (CSF) of healthy humans and AD patients (26, 50, 73, 77, 118). More than 20 years ago, immunoglobulin (Ig)-G was seen by light and electron microscopy in the AD brain to be co-localized with neuritic plaques (28, 52, 53); however, the role of IgG in the AD process, including in plaque formation, remains unclear. Indeed, the findings in relation to antibodies in AD are somewhat inconsistent, with the level of anti-Aβ antibodies being either increased or decreased, possibly because of differences in the methodology employed. However, most of the studies have identified a decrease of anti-Aβ antibodies in AD patients compared with age-matched healthy controls (26, 73, 118), raising the possibility that some people are, in a sense, able to immunize themselves against Aβ and therefore protect themselves against AD.

Complement system

The complement system is a sophisticated system evolved to destroy pathogens and to assist in the phagocytosis of waste materials. Four main functions are carried out by complement: recognition, opsonization, activation of inflammation and killing of the pathogen. Fibrillar Aβ is a strong stimulator of the complement system (94) and can activate the classical (antibody-dependent) (2, 20, 57, 69, 117) and alternative (antibody-independent) (13, 111) pathways. Activation of complement by Aβ appears to be highly specific to fibrillar Aβ, as other peptides of similar size are unable to activate the complement system (13). Hyperphosphorylated tau contained in neurons can also activate the classical complement cascade (68, 100) as demonstrated by the staining of tau-positive neurons by anti-complement antibodies. Therefore, in AD, there is evidence that the complement system is strongly activated and could participate either in the exacerbation or amelioration of the pathology. In APP transgenic mice which have inhibition of the complement system at the level of C3 (the central component of complement), neurodegeneration is increased compared with non complement-inhibited controls (123). This finding suggests that the complement system may have a protective role in AD.

Cytokines

Four main cytokines have been investigated extensively in AD: interleukin 1 (IL-1), IL-6, tumor necrosis factor α (TNF-α) and transforming growth factor β1 (TGF-β1). Immunoreactivity for IL-1, IL-6 and TGF-β1 has been reported in association with Aβ plaques (41, 49, 115). Elevated TNF-α and TGF-β1 levels have been detected in the serum and CSF of AD patients (17, 18, 32, 113). It has been proposed that cytokines secreted by glia interact with neurons to form a positive feedback loop or "cytokine cycle" which, once initiated, leads to progressive neurodegeneration (42, 76). Initial studies found some evidence for associations between specific cytokine gene polymorphisms and AD, for example, in the IL-1 gene (78), but subsequent studies suggest these are not of major importance (92) with the possible exception of TNF gene

polymorphism (9). Overall, the role of the cytokines in AD, and particularly whether on balance they are beneficial or harmful, remains uncertain.

Microglia

A key component of the innate immune system in the brain is the microglial cell which is the representative of the monocyte/macrophage lineage. Microglia are activated in AD but, despite much interest in the subject, the role that microglia play and whether they are harmful or helpful, remains unclear. In non-neural tissues, the state of cell activation and the nature of the activating stimulus play a significant role in determining the spectrum of molecules that are secreted by a macrophage (1, 40). Furthermore, the initial state of activation of the macrophage is an important determinant of the magnitude of a particular response (40). Microglia are highly sensitive to disturbance of CNS homeostasis (58). They respond to signals which arise from injured neurons, and the presence of activated microglia seems to be limited spatially to those regions occupied by injured neurons and neuronal processes. However, the damage does not have to be lethal to the neurons in order to elicit a reactive microgliosis. This is illustrated by traumatic and ischemic lesions where peri-necrotic areas contain surviving neurons and reactive microglia (75, 107) or as observed following intra-hippocampal injection of bacterial component in a mouse brain (10). According to the type of injury or insult, activation of microglia can be either tolerated or, alternatively, harmful leading to a destructive profile of microglia associated with neuronal loss (88). A third phenotype of microglia has also been suggested in which microglia become dysfunctional with ageing, characterized by structural deterioration and increased apoptosis (108). As a result, microglia may lose their neuroprotective properties with advancing age (109) leading to chronic neurodegeneration such as AD.

It is important to consider the origin of the microglia that responds to brain pathology. There is now good evidence that two types of microglia coexist within the brain: first, resident microglia which are derived from the mesoderm and migrate to the brain during the embryogenesis (87) and, second, bone marrow-derived microglia which are characterized by recruitment from the blood during life in response to an appropriate stimulus from the brain (87, 103). Recent evidence from animal models suggests that the activated microglia that surround plaques have been recruited from the blood and are specifically attracted to the accumulated Aβ peptide (104). The authors postulated that blood-derived microglia are more efficient at presenting antigen and may be more efficient than resident microglia in phagocytosing Aβ (103). Study of mice lacking the chemokine receptor 2, a microglial cell-surface receptor that mediates recruitment of blood-derived microglia, supports the idea that bone marrow-derived macrophages infiltrate the brain and can clear Aβ from the brain (29). In human AD, despite the presence of abundant activated microglia, Aβ is rarely observed within microglia. When it is, the microglia seem unable to degrade Aβ as observed by electron microscopy (34, 85) unless, as demonstrated in an AD model, they express Toll-like receptors, receptors usually observed during a bacterial attack (39, 112). Nonetheless, an exception is observed when an infarct occurs in AD, the microglia are then able to phagocytose all of the tissue components, including Aβ plaques in the vicinity of the infarct (3).

Study of a prion mouse model, an analogous chronic neurodegenerative disease with accumulation of an amyloid protein, shows that (i) microglia may be activated by neurodegeneration rather than protein deposition (21, 122); and (ii) conversely, the microglia express TGF-β1 which down-regulates scavenger receptors hampering phagocytosis (11, 21). These events may also occur during the neurodegenerative process of AD, in which TGF-β1 is also expressed (65).

These observations highlight the point that, rather than simply demonstrating that microglia have been activated, it is the specific way in which microglia are activated that is important in determining the influence of microglia on neurodegeneration (88).

MANIPULATION OF THE IMMUNE SYSTEM AS A THERAPEUTIC TOOL

Anti-inflammatory therapy in Alzheimer's disease

Modulation of the immune system as a therapy for AD became of great interest in the 1990s. Early observations indicated that patients with rheumatoid disease who were on long-term anti-inflammatory therapy had a lower prevalence of AD than controls, raising the possibility that their medication may have been protective (71). The main mechanism postulated was that down-regulation of microglia would have a beneficial effect in preventing or slowing neurodegeneration in AD, with supporting evidence from animal models (60). However, subsequent prospective trials of anti-inflammatory agents have, to date, proved disappointing (51, 71). It has been suggested that key questions relating to this approach remain unanswered, for example (i) the type of anti-inflammatory agent to use; (ii) the dose at which use them; and (iii) the duration of the treatment (4).

Aβ immunization in animal models

Different approaches to immunization

Approaches have been developed using anti-Aβ antibodies, either injected directly (ie, passive immunization) or induced by active immunization with the Aβ peptide, in order to reduce Aβ formation or to facilitate clearance of Aβ from the brain. Initially, it was observed that aggregated Aβ *in vitro* was dissolved and its formation prevented in the presence of anti-Aβ antibodies (105, 106). Subsequently, in an APP transgenic mouse model of AD, active immunization with Aβ42 peptide demonstrated *in vivo* that it was possible to prevent or reverse Aβ accumulation in the brain (96). Studies of peripheral immunization with Aβ antibodies showed the presence of Aβ antibodies within the brain (6, 56). That antibodies could cross the blood–brain barrier was already known (93); however, the passive Aβ immunization experiments showed that the IgG antibodies to Aβ are directly involved in amyloid plaque removal (6). The specificity of the Aβ antibodies has been also investigated and it has been shown that only Aβ antibodies directed against the N-terminal part of the peptide were efficient to remove amyloid (7) and that the Fc part of the antibody was fundamental for the clearance of amyloid (7). IgM antibodies, produced by active vaccination before IgG antibodies, might also act in the clearance of Aβ despite their inability to cross the blood–brain

Table 1. Mechanisms of Aβ removal following Aβ immunization.

	Animal models	Human cases
Solubilization by anti-Aβ antibodies	✓ (6)	✓ (11, 86) (indirect evidence)
Phagocytosis by activated microglia	✓ (7, 120)	✓ (80)
Peripheral sink hypothesis	✓ (102)	Unknown

barrier because of their large size. Immunization of mice with a prototype vaccine containing a modified Aβ peptide that induced only IgM antibodies has shown a decrease of Aβ load in the brain (102). The IgM effect is known as the "sink hypothesis", defined by immunization modifying the balance between Aβ peptide and Aβ antibodies in the periphery and consequently attracting Aβ from the brain to the periphery (23, 24) (Table 1).

Intracranial injection of anti-Aβ antibodies induces two phases of Aβ removal: first, a rapid decrease starting 24 h after the administration and before cellular activation, and then after 3 days, a further decrease in Aβ associated with activation of microglia. This observation suggests that there are two different mechanisms involved in removal of Aβ, respectively, independent and dependent of microglial activation (120) (Table 1). Immunization appears to alter the activation state of microglia so that they are able to efficiently phagocytose the aggregated amyloid, although the precise mechanisms remain unclear. The Fc part of the antibody has been suggested to be essential to mediate the phagocytosis of amyloid through binding to the microglial Fc receptor (6, 7). On the other hand, Aβ42 immunization of APP transgenic mice crossed with FcRγ$^{-/-}$ mice and therefore lacking the possibility of activation of microglia by immune complexes also showed a decrease of amyloid plaques (22). Furthermore, by using *in vivo* multiphoton microscopy, intracranial application of F(ab')$_2$ fragments of anti-Aβ antibodies have been found sufficient to decrease amyloid load (5). These two experiments suggest the existence of an Fc-independent mechanism which results in phagocytosis of amyloid by microglia. It seems clear that the mechanisms by which the activated microglia are prompted to phagocytose amyloid plaques are not exclusive. The role of complement in the removal of Aβ has been explored relatively little, but it seems not to be essential for plaque clearance to occur (7). However, it has been suggested that these findings may reflect differences in complement activation, being relatively weak in the transgenic mouse models compared with AD patients (70).

In summary, the key pathological changes following Aβ immunization in animal models include: (i) prevention of Aβ deposits if the immunotherapy is administered before the onset of Aβ accumulation (96); (ii) clearance of Aβ plaques if the immunotherapy is started at an age at which plaques are already present in the brain (6, 96, 102); (iii) presence of Aβ in microglia (5); and (iv) removal of dystrophic neurites, defined in APP-transgenic mice as swollen axons and dendrites surrounded amyloid plaques which contain APP but not tau (14, 61, 96).

Immunization prevents neurodegeneration and functional decline

Removal of Aβ plaques in APP transgenic mice has been shown associated with an improvement of cognitive function in the treated animals by comparison with the untreated transgenic mice after active immunization (56, 74), passive immunization (121) or by using the "sink" mechanism (102). Interestingly, improvement of cognitive function in immunized APP-transgenic mice is observed with only an attenuated immune response (102) and does not need a complete clearance of Aβ plaques from the brain (56). Quantification of the synaptic density in immunized transgenic mice showed a prevention of the synaptic degeneration following active or passive procedures in association with the Aβ clearance (16).

Limitations of the animal models

Some caution is necessary in translating these impressive findings in animal models into expectations for the effects of immunotherapy in human AD. Since the first APP transgenic mouse model (36), numerous animal models have been designed based on the different APP point mutations that can cause familial AD. It is important to recognize that the APP transgenic mice are models of Aβ pathophysiology which lack some of the key features of AD, particularly tau pathology. Therefore, the state of chronic microglial activation in these animals may not simulate the inflammation in AD.

Immunization of the triple transgenic mice, characterized by the Aβ and tau accumulation, have shown: Aβ removal, clearance of the early but not late-hypersphosphorylated tau aggregates and improvement of cognitive function (82, 83). Investigation of inflammation in the triple transgenic mice has detected early inflammatory processes in relation with the Aβ and tau deposits (55); however, the consequences of immunization on the inflammatory compounds have still not been studied.

Aβ immunization in human AD

The first clinical trials of Aβ immunization

As a consequence of the finding that active Aβ42 immunization of APP transgenic mice results in plaque removal (96), a human clinical trial was initiated in 2000. This first trial was conducted in the UK involving 80 patients with mild to moderate AD (8) and was designed to assess the antigenicity and tolerability of multiple dose immunization with full length Aβ42 peptide with adjuvant (AN1792 + QS-21). No adverse events were identified and 53% of the immunized patients developed anti-Aβ antibodies at varying titers. A subsequent larger clinical trial (n = 372) designed to assess efficacy was halted when 18/298 (6%) of the patients developed a subacute neurological deterioration accompanied by cerebral white matter abnormalities on imaging and lymphocytes in the CSF (84).

Effects of Aβ immunization on the neuropathology of AD

Post-mortem neuropathology of patients who were immunized in these studies, and subsequently died for incidental reasons, has shown remarkable evidence of modification of the AD pathology (12, 31, 66, 79, 80) (Figure 1). Most importantly, there was evidence that the Aβ immunization had resulted in removal of Aβ plaques in a manner similar to that seen in the animal models, providing "proof of principle" for the rationale of the studies (Table 1). Comparison of the histological patterns of Aβ shows

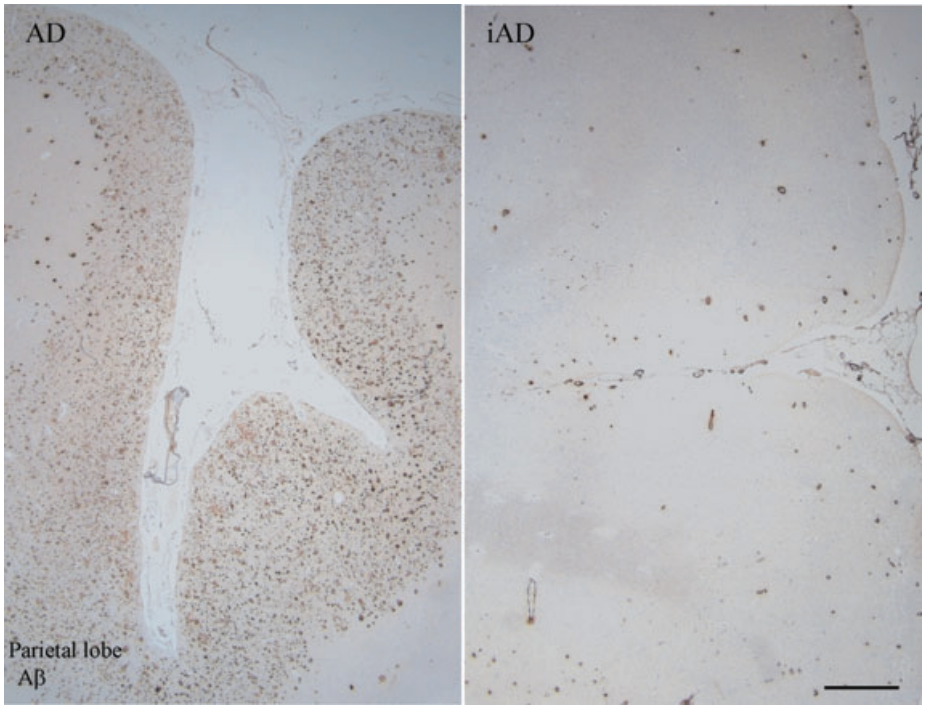

Figure 1. *Aβ immunohistochemistry in unimmunized Alzheimer's disease (AD) and after Aβ42 immunization*. Pattern of Aβ immunoreactivity (pan-Aβ antibody, residues 8–17) in the parietal lobe of an unimmunized AD case (AD) and an immunized AD case (iAD). The AD case reveals the presence of numerous Aβ plaques in the cerebral cortex, but whereas in the iAD case, the area is virtually devoid of plaques with a persistence of Aβ in the cerebral vasculature. Scale bar = 1 mm.

remarkably close modeling of the immunized AD patients by the APP transgenic mice (Table 2). The characteristic set of features shared by the immunized AD patients and the APP transgenic mice include: extensive areas of cerebral cortex cleared of plaques; a "moth-eaten" appearance of some of the residual plaques; the presence of "naked" dense plaque cores in otherwise plaque-free areas; persistence of CAA and association of Aβ with capillaries in plaque-free areas; and localization of Aβ in microglia (Figure 2), confirmed by confocal microscopy as representing Aβ phagocytosed by microglia (80). It is important to note that not all of these features were present in each of the cases examined, but together they can be considered as the defining alterations in the histological pattern of Aβ in the brain following active immunization with Aβ42 peptide.

Of particular interest, in view of the longstanding uncertainty of the causal inter-relationship between extracellular accumulation of Aβ and intracellular accumulation of tau protein, is the observation that in regions where plaques have been removed, tau-containing plaque-associated dystrophic neurites are absent. This implies that when plaques are removed, the dystrophic neurites are also removed, presumably either by resolution or phagocytosis. The APP transgenic mice do not have tau pathology but they do have

Table 2. Pathological features following Aβ42 immunization. Comparison between observations in the animal models and the human immunized AD cases. Abbreviations: AD = Alzheimer's disease; APP = amyloid precursor protein; CAA = cerebral amyloid angiopathy. N/A = not applicable; × = observed.

	Animal models	Immunized AD cases
Aβ pathology		
Removal of Aβ plaques	✓	✓
Aβ in microglia	✓	✓
Increased CAA	✓	✓
Tau pathology		
Removal of dystrophic neurites	✓ (APP-containing only)	✓
Persistence of tangles	N/A	✓
Persistence of neuropil threads	N/A	✓
Unexpected events		
T lymphocytes	×	✓ (some cases only)
Leucoencephalopathy	×	✓ (some cases only)

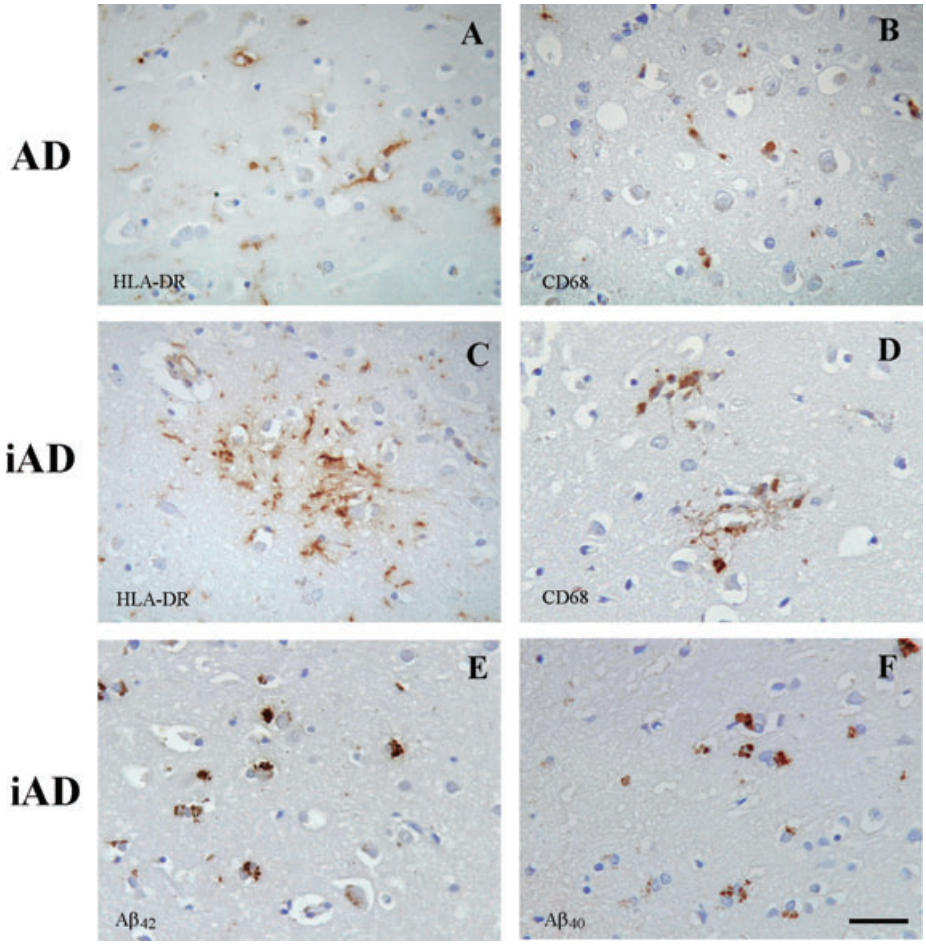

Figure 2. *Activated microglia in Alzheimer's disease (AD) vs. immunized AD (iAD).* Detection of HLA-DR (**A**) and CD68 (**B**) positive microglia in an unimmunized AD case. After Aβ42 immunization, HLA-DR (**C**) and CD68 (**D**) positive microglia are observed particularly clustered around residual plaques. Using isoform specific antibodies, Aβ42 (**E**) and Aβ40 (**F**) peptides are detected within microglia after Aβ42 immunization, rarely seen in unimmunized AD cases. Scale bar = 40 μm.

APP-immunoreactive plaque-associated dystrophic neurites and these resolve after plaque removal, again closely modeling the situation in human AD (14, 80). Studies in one case also provided evidence of local inhibition of the stress-activated protein kinase/c-Jun N-terminal kinase and p38 kinase, enzymes involved in tau phosphorylation, where Aβ had been removed (31). Nevertheless, quantification of tau immunoreactive tangles and neuropil threads does not show any clear evidence that they are reduced in regions of cortex where plaques have been removed (79, 80) (Table 2). The pathological changes observed in immunized AD are summarized in the Figure 3.

What pathophysiological events underlay the meningoencephalitis?

A striking difference between the animal models and the human studies was the development of "meningoencephalitis" in a small proportion of the patients which was not predicted in the preclinical studies (84). Two of the humans studied neuropathologically had this clinically defined side effect (31, 79); however, they both survived many months after this event, so study of the neuropathology can only provide limited information about the nature of the responsible pathological process. Nevertheless, important pathological correlates of this side effect are: (i) the presence of T lymphocytes in the leptomeninges, particularly near to blood vessels severely affect by CAA, but almost entirely absent from the cerebral cortex; and (ii) widespread changes in the deep cerebral white matter including rarefaction of myelinated fibers and abundant macrophages (Figure 4). To link these two features must be a matter of speculation but previous studies of severe CAA affecting cortical and leptomeningeal blood vessels have identified degenerative changes in the deep cerebral white matter as a common feature (98). Presumably, this unwanted side effect has potentially identifiable risk factors and AD patients with these factors could be deemed unsuitable for this form of therapy. Such risk factors might include, for example, a previously primed immune system, severe CAA and genetic variation, including possibly *APOE* genotype. A further risk factor for meningoencephalitis is likely to be the presence of Aβ in the brain, strengthening the argument for use of Aβ immunization as a preventative therapy. However, it is important to

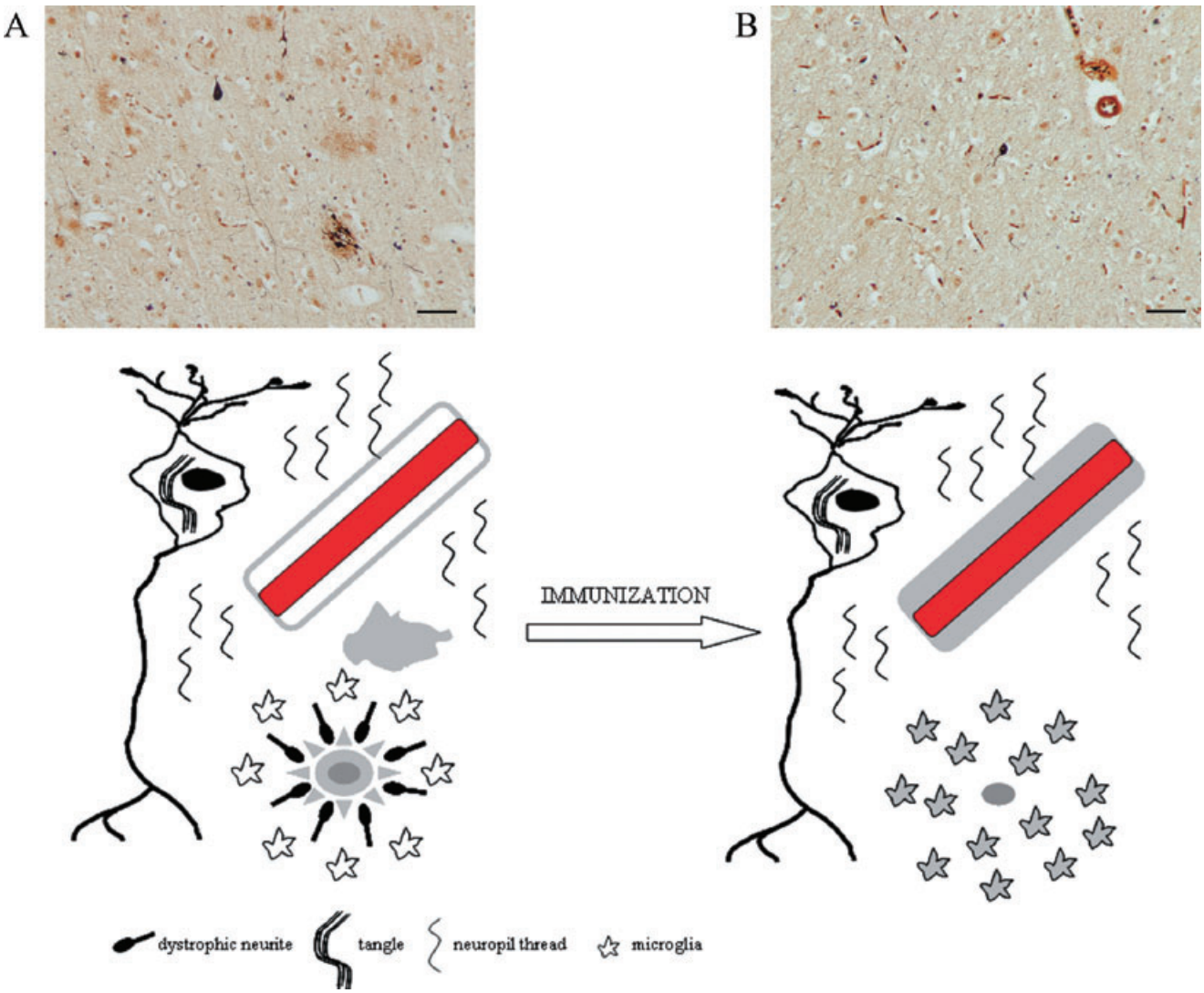

Figure 3. *Summary of the pathological changes observed in Alzheimer's disease (AD) after Aβ42 immunization.* AD pathology is characterized by the presence of Aβ plaques, dystrophic neurites, intraneuronal tangles and neuropil threads with activated microglia and cerebral amyloid angiopathy, as illustrated with modified Bielschowsky staining and in diagrammatic form (**A**). After Aβ42 immunization, the Aβ plaques and dystrophic neurites are removed, microglia have phagocytosed Aβ, Aβ is increased in the vasculature and the neuropil threads and tangles are still observed (**B**). Scale bar = 50 μm.

note that the meningoencephalitis is not required for the clearance of Aβ (66). The potentially beneficial and/or harmful effects of Aβ42 immunization are summarized in Table 3. Possible causes of the unwanted side effect in addition to the T lymphocyte infiltrate include an over-exuberant activation of microglia prompted by opsonization of Aβ and alterations in fluid balance in the brain triggered by interaction of antibody-Aβ immune complexes and the cerebral vasculature. Clear understanding of the pathophysiology of this phenomenon has important implications for future immunization trials as many of the current protocols have been specifically designed to avoid T lymphocyte activation. If T lymphocytes are responsible then it should not raise its head again as a problem, but if not then it may cloud the results of future immunization trials.

Intriguingly, the constellation of features identified in AD patients who have been immunized with Aβ as described above, and including an inflammatory infiltrate, has been identified in specific subgroups of unimmunized patients (30, 98). This raises the question as to whether Aβ pathology in natural disease may be in a dynamic state of flux, with episodic or progressive deposition and removal, possibly mediated at least in part by the immune system.

Effects on cognitive function

Overall, the data from the AD immunization trials so far do not seem to show evidence of a substantial effect on cognitive function, either in improving function or in preventing progressive decline

(8, 38) (Holmes, pers. comm.). However, it is important to bear in mind that the active immunization resulted in antibody production in only about half of the immunized patients and those patients produced antibodies at varying titers (8). Studies performed so far in subgroups of the initial trials, dividing patients into responders and non-responders, have suggested there may be some beneficial effect (38, 47). The results of passive immunization, in which the antibody levels available to the brain are controlled, are awaited with interest.

Intriguingly, and perhaps counter-intuitively, sequential *in vivo* imaging studies have shown evidence of accelerated cerebral atrophy in antibody responders compared with non-responders (33). The reasons for this reduction in brain volume are unclear, but might include a removal of plaque-associated proteins, a reduction in the glial cell reaction and fluid redistribution.

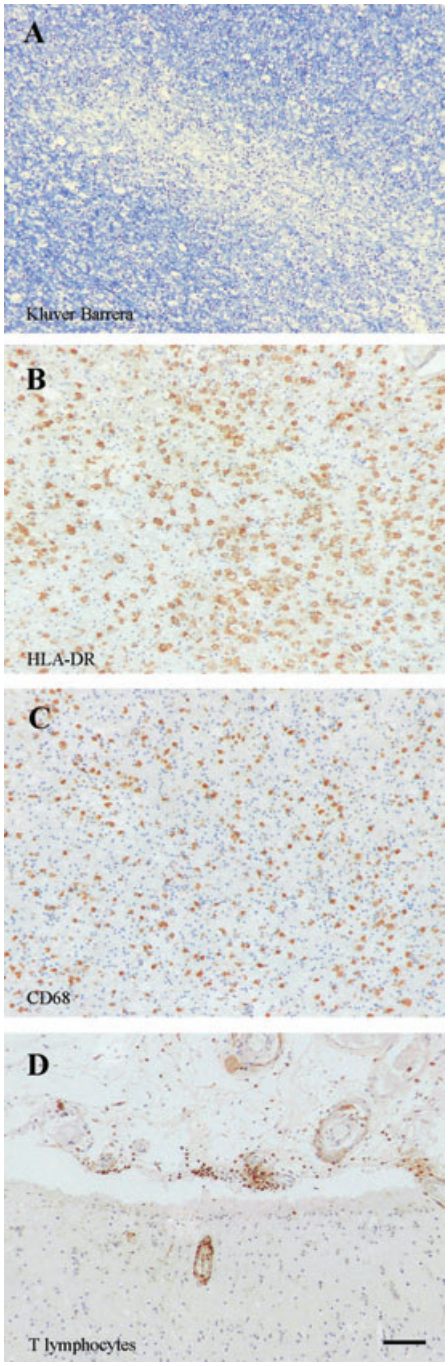

Figure 4. *Meningoencephalitis.* Features observed in one immunized patient with meningoencephalitis who came to autopsy more than 1 year later: rarefaction of white matter as detected by Kluver–Barrera staining (**A**), marked increase of HLA-DR (**B**) and CD68 (**C**) positive microglia/macrophages and the presence of T lymphocytes in the leptomeninges and in relation to a cerebral blood vessel (**D**, anti-CD45RO antibody). Scale bar = 50 μm.

Table 3. Effects of Aβ immunization on the neuropathology of AD. Abbreviations: AD = Alzheimer's disease; CAA = cerebral amyloid angiopathy.

Potentially beneficial effects	Potentially harmful effects
Removal of plaques	Increased soluble/oligomeric Aβ
Microglial activation	Microglial activation
Decreased CAA	Increased CAA
Resolution of dystrophic neurites	Presence of T lymphocytes
	Leucoencephalopathy

CONCLUSIONS

The amyloid hypothesis predicts that removal of Aβ from the brain, or prevention of its accumulation, will ameliorate the Alzheimer process. In transgenic animal models of Aβ accumulation, both passive and active Aβ immunization can result in removal of Aβ or prevent its accumulation, resulting in functional benefits. Studies of Aβ immunization in humans with AD have provided "proof of principle" that Aβ accumulation can be reversed. However, there is limited effect on some aspects of AD pathology, particularly tau accumulation, and limited evidence of functional benefit. Current concern with the Aβ immunization approach is centered on an unpredicted inflammatory complication that occurred in a minority of AD patients, and therefore, immunization protocols aimed to circumvent this problem are currently in clinical trials. The long-term consequences of activation of the immune system on cognitive function in AD may depend on the net balance of potentially beneficial and harmful effects on the complex array of pathological changes that are induced. Consequently, if it can be done safely, Aβ immunization at an earlier stage an preferably before irreversible AD-related brain damage has occurred will be of interest.

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