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Immunotherapy, vascular pathology, and microhemorrhages in transgenic mice

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Abstract

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that results in severe cognitive decline. Amyloid plaques are a principal pathology found in AD and are composed of aggregated amyloid-beta (A β) peptides. According to the amyloid hypothesis, A β peptides initiate the other pathologies characteristic for Alzheimer's disease including cognitive deficits. Immunotherapy against A β is a potential therapeutic for the treatment of humans with AD. While anti-A β immunotherapy has been shown to reduce amyloid burden in mouse models and now in humans, immunotherapy also exacerbates vascular pathologies. Cerebral amyloid angiopathy (CAA), that is, the accumulation of amyloid in the cerebrovasculature, is increased with immunotherapy in humans with AD and in mouse models of amyloid deposition. CAA persists in the brains of clinical trial patients that show removal of parenchymal amyloid. Mouse model studies also show that immunotherapy results in multiple small bleeds in the brain, termed microhemorrhages. The neurovascular unit is a term used to describe the cerebrovasculature and its associated cells – astrocytes, neurons, pericytes and microglia. CAA affects brain perfusion and there is now evidence that the neurovascular unit is affected in Alzheimer's disease when CAA is present. Understanding the type of damage to the neurovascular unit caused by CAA in AD and the underlying cause of microhemorrhage after immunotherapy is essential to the success of therapeutic vaccines as a treatment for Alzheimer's disease.

The neurovascular unit

The brain's high energy demands require a disproportionate blood supply. While the brain only composes 2% of the total body weight, it receives 15% of the cardiac output and consumes 20% of the oxygen utilized by the entire body. Maintenance and control of this blood supply requires communication between multiple components of the brain and cerebrovasculature. The cellular interface between the parenchyma of the brain and the circulating blood is composed of the blood vessel itself, perivascular neurons, pericytes, perivascular microglia and astrocytic end-feet and has been termed the neurovascular unit (NVU) ¹. The vascular component of the NVU includes the penetrating arteries that arise from the pial arteries on the surface of the brain, the arterioles and cerebral capillaries. Figure 1 summarizes the structures of the NVU. The larger arteries are composed of an endothelial layer, a smooth muscle layer and the adventitia composed of collagen, fibroblasts and perivascular nerves. Virchow-Robin spaces are CSF-filled spaces that separate the penetrating vessel from the brain. Astrocytic end-feet are located on the brain side of this space and impinge upon the pia mater that separates the fluid filled space from the adventitia of the blood vessel ². As the arteries branch and become smaller arterioles, the Virchow-Robin spaces shrink and disappear. At this point the astrocytic end-feet directly attach to the basement membrane of the vasculature ³. The arterioles lose

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their smooth muscle cell layer and, ultimately, become the cerebral capillaries. Capillaries are composed only of endothelial cells, pericytes and the capillary basal lamina, upon which the astrocytic end-feet append. The endothelial cells of capillaries form tight junctions that restrict movement across the brain-blood interface and thus form the blood-brain barrier.^{4, 5}

There are several levels of control over cerebral blood flow, all aimed at maintaining brain perfusion and matching the brain's energy supply and demand. The first is autoregulation, which is primarily at the level of the smaller arterioles⁶. The cerebrovasculature is capable of self-regulating vascular tone in response to changes in arterial CO₂ concentration⁷, blood pressure⁸, endothelial nitric oxide production⁹ or pH¹⁰. Another level of cerebrovascular control is neural, where sympathetic and parasympathetic inputs, as well as local interneurons, influence vascular tone. Numerous neurotransmitters have been identified in the neural control of cerebrovascular tone, however, NPY, NO, acetylcholine (ACh) and serotonin (5-HT) have emerged as the major transmitters¹¹. Finally, astrocytes are known to locally control cerebral blood flow^{12, 13} and participate in neurovascular coupling; the process of increasing blood flow to provide energy to active brain regions¹⁴.

While the neurovascular unit is responsible for controlling the cerebral blood flow, it also has several other essential functions. For example, potassium buffering is a key function of astrocytes within the neurovascular unit. During action potential firing there is an accumulation of potassium in the extracellular milieu. Failure to remove this potassium results in altered resting membrane potentials and, therefore, altered excitability¹⁵⁻¹⁸. This mechanism for regulation of extracellular potassium levels in the brain is thought to function by transferring potassium from the active neuronal area via uptake of the potassium into the astrocytes, followed by redistribution of the "excess" potassium to other areas of the brain or to the vasculature. Potassium is taken up by specific channels including the Kir4.1^{19, 20} and BK^{21, 22} channels localized to sites on the astrocyte membrane. It is passed through connexin-43 positive gap junctions^{23, 24} in the astrocytic syncytium to the astrocyte end feet located on blood vessels of the NVU. Astrocyte end feet also express a high density of Kir4.1 and BK channels as well as aquaporin channels that regulate water movement^{25, 26}. In this manner, the redistribution of potassium around the blood vessels is co-coordinated with water movement required to maintain osmotic balance^{27, 28}. Aquaporin 4 is the primary channel of the neurovascular unit and is highly polarized to the end-foot membrane²⁹.

Alzheimer's disease and its vascular pathologies

Alzheimer's disease (AD) is characterized by three pathologies necessary for a final diagnosis of AD; amyloid plaques, neurofibrillary tangles and neuron loss. Amyloid plaques are extracellular deposits of amyloid-beta protein (A β), derived from enzymatic cleavage of the membrane spanning amyloid precursor protein (APP), producing A β ₁₋₄₀ or the more insoluble A β ₁₋₄₂. Neurofibrillary tangles are intraneuronal aggregates of hyperphosphorylated tau protein. The amyloid hypothesis suggests that the production of A β and the deposition of amyloid is the initiating pathology that results in the abnormal phosphorylation of tau, which then results in neurodegeneration³⁰. Microglial and astrocytic –mediated neuroinflammation is an additional complex component of AD³¹, that remains poorly understood³². Microglia have been shown to phagocytose A β in vitro³³ suggesting microglia may participate in the removal of the abnormal protein aggregates in AD. Also, most recently, a live imaging study showed that microglia surround rapidly formed amyloid deposits, apparently preventing additional growth of the amyloid deposits, however, no active removal of amyloid was observed in this study³⁴.

Another accompanying pathology in AD is the accumulation of amyloid around cerebral blood vessels (termed Cerebral Amyloid Angiopathy- CAA). CAA occurs in 78 to 98% of

Alzheimer's patients³⁵ and is frequently considered to be a major pathological feature of AD. The CAA in AD is primarily observed in the arterioles with some capillary and artery involvement. The population-based Honolulu-Asia Aging Study recently demonstrated a significant correlation between cognition (tested by the CASI – Cognitive Abilities Screening Instrument score) and the presence of CAA. Men with AD in the absence of CAA showed a 16.6% lower CASI score than non-demented males while AD with CAA showed a 45.9% lower CASI score than non-demented males, which suggests that CAA contributes to the cognitive decline in Alzheimer's disease³⁶. Infiltrating inflammatory cells have been reported to be present in cases of severe CAA including T-cell infiltration and multinucleated giant-cell infiltration³⁷. CAA is also associated, in some cases, with the presence of bleeds in the brain. These bleeds can range from microhemorrhage (sometimes termed micro-aneurysm) to a full vessel rupture, termed an aneurysm. Reports suggest that approximately 30% of all AD cases include CAA-associated microhemorrhage; these are typically localized to the pial arteries, penetrating arteries and arterioles³⁸.

A group of patients suffering from hereditary cerebral amyloid angiopathy have been critical for understanding the effects of amyloid deposition in the vasculature. The most studied of these are the hereditary cerebral hemorrhage with amyloidosis–Dutch type (HCHWA-D)³⁹ and the Iowa-type hereditary cerebral amyloid angiopathy (ICAA). The Dutch patients typically have extensive CAA that ultimately results in recurrent lobar hemorrhages. Amyloid deposits in this population are localized to the leptomeningeal arteries and cortical arterioles and minimal parenchymal amyloid deposits are observed³⁹. The Iowa patients develop a dementia and have high levels of CAA in the meningeal and cortical arteries with complete occlusion of smaller arterioles and capillaries being reported. The patients develop small hemorrhages that coincide with regions of severe CAA and also show extensive neurofibrillary tangle pathology; sometimes in regions devoid of severe CAA⁴⁰. Hemorrhagic stroke has not been observed in these patients and their disease progression is more typical of AD⁴¹.

The mechanism by which amyloid accumulates at the blood vessels to form CAA in AD is largely unknown. A β can be transported across the blood-brain barrier where the receptor for advanced-glycation end-products (RAGE) transports A β from the blood to the brain and the low-density lipoprotein receptor-related protein (LRP) transports A β from the brain to the blood⁴². It has been suggested that faulty clearance of A β across the blood-brain barrier can increase brain A β concentrations and, thus, accelerate amyloid deposition contributing to disease⁴³. Recently LRP polymorphisms were shown to influence CAA levels in AD patients⁴⁴.

The neurovascular unit in AD remains a relatively understudied region and the cause(s) of the damage generated by amyloid deposition at brain blood vessels also remains unknown. Inflammation has been associated with vascular amyloid deposition and may play an important role. A recent study in APP transgenic mice with extensive vascular amyloid deposition (APPSwDI) showed that there is a microglial reaction to the vascular amyloid, and that inhibition of this microglial response by minocycline improves memory deficits despite no apparent change in CAA levels⁴⁵. In addition to the accumulation of CAA, other neurovascular unit changes are observed in AD and are likely linked to the presence of CAA. It has been shown in AD that there is a reduced number of cerebral microvessels and smooth muscle cell degeneration⁴⁶. Resting cerebral blood flow, and activity-induced increase in cerebral blood flow are both decreased in AD^{47, 48}. Most recently, we have found that CAA in AD results in loss of potassium and water channels known to be critical to potassium siphoning⁴⁹. Together, these data suggest a critical role for the neurovascular unit in the progression of AD.

Transgenic mouse models for AD and their vascular pathologies

Mouse models of amyloid deposition have been widely used by scientists studying therapeutic approaches based on the amyloid hypothesis in AD. These mouse models have been invaluable for studying rates of amyloid deposition, inflammation associated with this deposition, amyloid-dependent memory deficits and the impact of various therapies on these processes⁵⁰. APP transgenic mice also develop varying degrees of CAA and this generally occurs at more advanced ages when there is significant parenchymal amyloid already present⁵¹. It is important to acknowledge, however, that these animal models have several critical limitations. First, the mechanism for achieving amyloid deposition involves overexpression of mutated human genes. These genes are the amyloid precursor protein (APP) gene and the presenilin (PS) 1 and 2 genes. APP is cleaved to produce A β and mutations in the APP protein have been found to result in early onset familial-Alzheimer's disease (FAD). PS1 and PS2 are components of the γ -secretase complex; the enzyme responsible for the intra-membrane cleavage of APP to produce A β 40 and A β 42. Again, mutations in the PS genes result in FAD⁵². Together, these FAD mutations account for only approximately 5% of all AD cases worldwide, the remaining 95% are sporadic with no known cause. Second, the overexpression of human genes, and the resultant deposition of amyloid, does not produce the other pathologies characteristic of AD; neurofibrillary tangles and neuron loss. The mice have been shown to have some synaptic deficits and dystrophic neurites associated with the amyloid deposition, however, neuron counts have not shown significant or consistent neuronal loss in multiple areas of the brain⁵³⁻⁵⁵. Tau pathology is also very limited to those neurites immediately surrounding dense-cored amyloid plaques⁵⁶. Third, cognitive deficits in mouse may not completely mimic those observed in AD. These deficits are clearly amyloid dependent since they are present despite no neuron loss and are likely more reversible than those deficits in humans with AD⁵⁷. Despite all of these limitations, however, the APP mouse models have provided AD researchers with tools to study amyloid and therapeutic interventions in ways that were not previously possible.

Table 1 summarizes the transgenic mice discussed in this review with respect to the immunotherapy studies. The first APP transgenic shown to develop amyloid pathology was reported in 1995 by Games et al⁵⁸. This mouse carries an 18-fold overexpression of the V717F mutation in APP known to result in eFAD in humans. The first amyloid deposits are detected at 6 months of age and mild CAA is present by 18 months of age. The Tg2576 mouse is similar to the PDAPP mouse in time-course of pathology and carries a 5-fold overexpression of the K670M/M671L Swedish mutation in APP. The first amyloid deposits are detectable by 6 months and CAA is detectable by 18 months⁵⁹. The majority of immunotherapy studies have utilized either the PDAPP or Tg2576 transgenic mouse models. Additionally, the APP23 mouse carries a 7-fold overexpression of the Swedish mutation in APP but uses the neuron-directed Thy-1 promoter and develops CAA with spontaneous hemorrhage at advanced ages⁶⁰. This mouse has been shown to have some neuronal loss restricted to areas of plaques⁶¹. Finally, the APPSwDI mouse incorporates 3 APP mutations; Swedish K670N/M671L, Dutch E693Q and Iowa D694N. The Dutch and Iowa mutations are associated with human hereditary cerebral amyloid angiopathy^{39, 40}. This is a model of vascular amyloid deposition in addition to parenchymal amyloid deposition⁶². One caveat to this mouse is that the amyloid deposition in the vasculature is primarily in capillaries. This is in contrast to the CAA in human AD which is primarily in the arterioles with some capillary involvement. Recently, capillary CAA levels was shown to be closely correlated to Alzheimer's pathology which suggests a critical role for amyloid accumulation in the capillaries⁶³.

Incorporation of presenilin mutations into APP transgenic mice results in acceleration of amyloid pathology. Two APP+PS1 transgenic mice have been used to examine immunotherapy and its vascular outcomes. The first, described in 1998, crossed the Tg2576 APP transgenic mouse⁵⁹ to the M146L PS1 transgenic mouse⁶⁴ to produce APP+PS1 transgenic mice which

were heterozygous for both genes⁶⁵. This mouse shows significant amyloid pathology as early as 6 months and shows cognitive deficits at 12 months of age⁶⁶. The second APP+PS1 mouse incorporated the APPSw mutations K594M/N595L (which typically do not develop amyloid deposits⁶⁷) with the exon 9-deleted variant of human PS1. The mice were generated by co-injection of the chimeric mouse/human APPSw and human PS1-dE9 vectors and the two transgenes co-integrated as a single locus⁶⁸. Both the APP+PS1 transgenic mice do not show extensive vascular amyloid pathology despite the extremely high levels of amyloid deposition.

Beyond the accumulation of CAA, changes in the neurovascular unit have not been examined in detail in any of the APP or the APP + PS1 transgenic mouse models of AD. Recently, Takano et al⁶⁹ showed that Tg2576 mice and APPSwDI mice both had impaired vascular function at early ages prior to significant accumulation of amyloid. Takano also found altered astrocytic Ca²⁺ responses in astrocytes that could lead to an early change in the neurovascular coupling⁶⁹. Our recent studies clearly demonstrate CAA-associated structural changes in astrocytes at the neurovascular unit in two mouse models of AD. These changes include loss of potassium and water channels known to be critical to potassium siphoning and loss of astrocytic end-feet from the cerebrovasculature⁴⁹.

Immunotherapy overview

Anti-A β immunotherapy was first described by Dale Schenk and colleagues in 1999⁷⁰. However, the first suggestion that anti-A β antibodies may interfere with amyloid formation was made by Beka Solomon and colleagues in 1997⁷¹. Schenk et al showed that vaccination of PDAPP mice with fibrillar A β 1–42 emulsified in an immune adjuvant stimulated the production of anti-A β antibodies. Most surprisingly, this approach lowered amyloid deposits in the brains of older PDAPP mice and prevented formation of amyloid deposits in younger mice. Following this initial report there were two back-to-back reports from different laboratories reporting that A β vaccination improved cognition in the APP+PS1^{65, 72} and the TgCRND8^{73, 74} mice.

There are two different types of anti-A β immunotherapy that have been studied. The first is active immunization. Active immunization describes the administration of A β in an immune adjuvant and results in stimulation of the individual's immune system to generate antibodies against the immunogen. The disadvantage of this method is that it relies on the subjects' own immune system for the generation of anti-A β antibodies. The second type of immunotherapy is passive immunization. Passive immunization bypasses the requirement for an immune response by directly infusing anti-A β antibodies into the subject. Passive immunization has the clear benefit of ability to withdraw the drug in the event of an adverse reaction.

The mechanisms of action of anti-A β antibodies in the removal of A β from the brain have been studied by several groups. Data suggest three potential mechanisms of action; each of which likely contribute to the removal of A β . Microglial-mediated removal of amyloid plaques (discussed in this special issue by D Morgan) via Fc γ -receptor activation was first suggested as a mechanism in the initial immunotherapy report by Schenk et al⁷⁰. Later studies showed that antibody in the brain, introduced by application onto the brain⁷⁵ or intrahippocampal injection⁷⁶, activated microglia and reduced brain amyloid plaque load. In addition, non-Fc dependent mechanisms were identified by intrahippocampal injections of F(ab')₂ fragments⁷⁷ or application of F(ab')₂ fragments onto the brain surface⁷⁸. Both studies showed reduction of brain A β levels in the absence of Fc-receptor signaling. These non-Fc mediated mechanisms may involve a catalytic disaggregation of amyloid plaques by the antibody. This mechanism suggests that the anti-A β antibody binds to the A β in amyloid deposits, disrupting the tertiary structure of the plaque and so disaggregating the A β peptides. This mechanism was shown in a series of experiments by Solomon and colleagues^{71, 79, 80}.

Finally, anti-A β antibodies have been shown to act through a “peripheral sink” mechanism. This mechanism suggests that anti-A β antibodies in the plasma bind circulating A β that disrupts the brain-blood equilibrium of A β and results in an efflux of A β from the brain. The peripheral sink mechanism was first proposed by DeMattos and colleagues⁸¹ who showed data that following a single intravenous injection of a monoclonal anti-A β antibody a rapid increase in circulating A β levels was observed. This phenomenon has been observed by several other groups studying immunotherapy in transgenic mice^{82, 83} and rhesus monkeys⁸⁴. A β appears to enter the perivascular drainage route at the level of the capillary and drains along the walls of cortical arteries and leptomeningeal arteries. This movement of interstitial fluid occurs in the opposite direction of blood flow⁸⁵. The peripheral sink mechanism relies, at least in part, on this drainage pathway for A β to exit the brain into the circulating blood. If this is the case then any impairment in this drainage pathway could exacerbate CAA accumulation. Thus, the presence of CAA could indicate a failure of this drainage mechanism⁸⁶.

Vascular effects of immunotherapy

The majority of published data on anti-A β immunotherapy has been extremely positive and supportive of this therapeutic approach in AD. However, a report in 2001 provided some reason for caution. Pfeifer et al⁸⁷ showed in a brief report that anti-A β antibody injection in a standard systemic passive immunization protocol resulted in microhemorrhages in APP23 mice. Microhemorrhages are small, microscopic bleeds which are typically detected by Prussian blue staining. Prussian blue detects small granules of hemosiderin, the degradation product of hemoglobin that is found in microglia associated with the vasculature. Approximately 30% of all AD cases include CAA-associated microhemorrhage, these are typically localized to the pial arteries, penetrating arteries and arterioles. Microhemorrhages are not typically observed in AD with an absence of CAA³⁸.

To date, eight separate studies on passive immunization to reduce brain A β levels using different transgenic mouse models, both modes of immunotherapy (passive or active) and different antibodies against A β have demonstrated increased incidence of microhemorrhage. These studies are summarized in table 2. Immunotherapy studies have generally been split between disease preventative studies and disease reversal studies. Preventative studies typically begin treatment at early ages prior to the onset of amyloid deposition and continue treatment until the mice reach an age when extensive amyloid pathology is normally observed. The aim is to prevent the formation of amyloid deposition and the majority of these studies have not reported specifically examining CAA and microhemorrhage. Reversal studies, on the other hand, wait until the mice have significant amyloid pathology and then begin treatment to examine removal of A β and inhibition or reversal of damage. The only preventative study examining vascular effects of immunotherapy was reported recently by Shroeter and colleagues.⁸⁸ This study began anti-A β antibody treatment of PDAPP mice at aged 12 months, when there is some parenchymal amyloid pathology but minimal vascular amyloid deposition⁸⁸. The authors observed a dose-dependent reduction in CAA accumulation after 6 months of treatment. However, there was a dose-dependent increase in microhemorrhage occurrence. Seven of the eight studies reporting microhemorrhage have examined predominantly reversal effects. Six reversal studies initiated treatment of APP transgenic mice aged 19 months or older; an age when extensive amyloid deposition is present and CAA is known to be relatively abundant^{87, 89-93}. Of these reports all observed an increase in microhemorrhage severity. Three of the six studies showed a significant increase in CAA accumulation⁸⁹⁻⁹¹. Of the remaining 3 reversal studies, one states that CAA levels were unchanged⁸⁷ and the others did not report examination of CAA levels^{92, 93}. Finally, a reversal study in the double transgenic APP+PS1 mice showed microhemorrhage and increased CAA levels following active vaccination initiated at 10 months of age⁹⁴. Thus, combined data from mouse models strongly

suggest that microhemorrhage occurs as a result of anti-A β immunotherapy, frequently accompanied by an accumulation of CAA.

The mechanism by which immunotherapy causes increased occurrence of microhemorrhage remains to be discovered. One hypothesis is that the clearance of amyloid from the brain results in the accumulation of CAA and this CAA results in overall weakening of vasculature causing leakage and, therefore, microhemorrhage. While several studies have shown that immunotherapy, itself, leads to increased CAA⁸⁹⁻⁹¹ other studies have not shown this effect^{87, 92}. However, these negative studies did not specifically measure CAA levels. The most recent passive immunization study by Schroeter and colleagues⁸⁸ shows increased incidence of microhemorrhage despite low levels of CAA in PDAPP mice at 12 months of age suggesting no direct association between CAA levels and microhemorrhage. Since PDAPP mice do not accumulate significant amounts of CAA until 18 months of age, the decreased CAA observed in the vaccinated mice likely represents prevention of CAA accumulation by the passive A β immunotherapy.

A study by Burbach and colleagues examined the vascular ultrastructure of APP23 mice that developed microhemorrhage following immunotherapy and found no changes in vascular structure by electron microscopy as a result of passive immunization⁹³. In both bleeding and non-bleeding vessels the endothelium remained intact and no significant difference in the amount of amyloid deposited within the vessel was observed. In addition, the number of perivascular macrophages were unchanged following immunization suggesting a limited role for perivascular inflammation. Because only macrophage number was measured, however, it is not possible to eliminate altered inflammatory responses following immunization as a factor in the microhemorrhage.

Immunotherapy has been shown to stimulate microglial activation resulting in partial clearance of parenchymal amyloid by microglia⁸². It was thought that microglia may deposit the phagocytosed amyloid in the vasculature. It was also possible that antibody-opsonized A β in the form of CAA could cause a perivascular inflammatory response resulting in microhemorrhage. In 2006 it was shown that a deglycosylated anti-A β antibody (2H6 anti-A β 33–40 IgG2b) results in decreased parenchymal amyloid and improved cognition. Deglycosylation of an antibody significantly impairs Fc-receptor binding and activation, thus reducing immune activation of macrophages including microglia⁹⁵. Importantly, deglycosylated anti-A β antibodies attenuated but did not completely prevent CAA accumulation. Microhemorrhage incidence was reduced compared to the intact antibody but still increased compared to untreated controls⁸⁹. These data from the deglycosylated antibody study suggest that antibody/Fc-receptor binding to microglia and subsequent immune activation are not the sole events in A β -antibody mediated microhemorrhage. Thus, blocking the Fc interaction is unlikely to completely prevent the vascular adverse effects of anti-A β immunotherapy.

Given the limited data from humans and mice regarding the impact of CAA accumulation on brain perfusion⁴⁶, neurovascular coupling⁴⁸ and astrocyte damage⁴⁹ it is possible that CAA accumulation as a result of immunotherapy either generates or exacerbates neurovascular changes. To date, however, these experiments have not been performed and the question still remains. Furthermore, the functional consequences of microhemorrhage remain unclear. Despite the presence of microhemorrhages, significant cognitive benefit of A β immunotherapy has been reported⁸⁹⁻⁹¹. It is doubtful that these small bleeds lack any functional consequences. In the vascular dementia literature it has been shown that small subcortical bleeds, termed lacunes, have distinct behavioral outcomes. In humans thalamic lacunes have been shown to cause a decline in memory performance and nonthalamic lacunes cause a decline in psychomotor speed⁹⁶. Also, lacunes occurring in the mamillothalamic tract have been

associated with decline in episodic memory and executive function⁹⁷. In cognitively normal patients the presence of lacunes was associated with subtle but significant changes in visual memory and executive function⁹⁸. Overall, these data indicate that microbleeds do contribute to declining cognitive function, however, the location of the bleed clearly determines which component of memory and behavior is affected. Microhemorrhages resulting from immunotherapy in mouse models of AD are found in multiple brain regions⁸⁷⁻⁹⁴. Thus, it may be difficult to measure consistent functional changes in brain hemodynamics or in animal behavior. This large variation in location may explain, at least in part, the failure to detect distinct functional consequences of microhemorrhage.

Clinical implications

Active immunization against A β advanced to clinical trials in patients with AD. The trial was called AN1792 and involved up to five immunizations over a 36-week period. The clinical trial was suspended due to an occurrence of subacute meningoencephalitis (inflammation of the brain and meninges) in approximately 6% of patients⁹⁹. Despite these adverse events, reports from the trial or from subsequent analyses were mainly positive. Hock et al.⁸⁴ reported that vaccination slowed cognitive decline in a cohort from the trial after one year. Several case study autopsy reports on individual trial participants have now been published. Most of these reports have described reduced A β plaque density^{100, 101} while neurofibrillary tangle numbers appeared to be unchanged in this limited number of patients.¹⁰⁰⁻¹⁰² Most recently, a study reporting on 8 trial patients followed clinically since cessation of the trial and examined at autopsy provided some intriguing data. This study showed that the longer the survival, the less amyloid present in the brain, however, cognitive decline was almost identical to control AD patients and tau pathology was largely unaffected¹⁰³. While this report did not specifically examine the vascular effects of the immunization, the authors did report using CAA severity and A β accumulation around capillaries as indicators of active amyloid removal. The presence of bleeding in this study was not reported. These findings echo the observations of several other autopsy reports from the AN1792 trial which described the persistence of severe CAA¹⁰⁰⁻¹⁰². One of these reports specifically looked for bleeding and showed the presence of multiple microhemorrhages associated with CAA¹⁰².

Elan Corporation currently has their A β -specific antibody, bapineuzumab, in a phase 3 clinical trial (AAB-001). The clinical trial findings to date were recently presented at the ICAD conference (Chicago, 2008)¹⁰⁴. The data presented were promising and showed good safety and tolerability. In those patients who completed the trial a trend was observed toward significant cognitive improvement. Removal of ApoE4 carriers from the analyzed population produced statistically significant improvement in cognition for the remainder of the study. While no explanation was provided for the reduced response in ApoE4 carriers, it was found that 12 patients developed vasogenic edema and 10 of these were ApoE4 carriers. Vasogenic edema is generated by fluid leakage from the blood vessels into the brain parenchyma via a damaged blood-brain barrier. Since ApoE4 carriers have higher levels of CAA than non-APOE4 carriers¹⁰⁵, this difference may be critical to the increased degree of vascular edema and the reduced responsiveness to bapineuzumab treatment in APOE4 carriers. Also, ApoE4 carriers develop an altered inflammatory response that may contribute to the enhanced susceptibility¹⁰⁶⁻¹⁰⁸. Other clinical trials in phase 1 and 2 have reported good safety outcomes but no cognitive outcomes have been reported. Importantly, all trials are excluding patients with cerebrovascular disease or MRI vascular abnormalities.

Summary

The neurovascular unit is a complex system that is critical to the control of cerebral blood flow and maintenance of the brain's unique environment. Perturbations in neurovascular unit

function are likely to have significant consequences. Data is now emerging that the presence of amyloid deposits at the cerebrovasculature significantly impacts cerebral blood flow and promotes changes in the neurovascular unit. While immunotherapy remains an exciting therapeutic approach for the treatment of Alzheimer's disease, accumulation and persistence of CAA and microhemorrhage following immunotherapy suggests caution for these clinical trials. It is clear that a much better understanding of the cerebrovasculature damage caused by CAA is required. If this can be achieved, immunotherapy may continue to be one of the most promising therapeutic approaches for Alzheimer's disease.

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Abbreviations

A β , Amyloid-beta; CAA, cerebral amyloid angiopathy; AD, Alzheimer's disease; NVU, neurovascular unit; CSF, cerebrospinal fluid; NPY, neuropeptide Y; APP, amyloid precursor protein.

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Figure 1.
Schematic showing the progression of blood vessels in the neurovascular unit and their surrounding cells.

Table 1

Overview of mouse models discussed in this review.

Transgenic mouse model	Mutation(s)	Expression level of transgene	Rate of amyloid deposition	Tau Pathology	Neuron loss	CAA severity	Primary Ref
<u>PDAPP</u>	Human APP V717F	18-fold	Moderate	+/-	-	+	58
<u>Tg2576</u>	Human APP K670M/M671L	5-fold	Moderate	+/-	-	+	59
<u>APP23</u>	Human APP K670N/M671L	7-fold	Moderate	+/-	+	++	60
<u>APPSwDI</u>	Human APP K670N/M671L, E693Q, D694N	0.5-fold	Rapid	+/-	-	+++	62
<u>APP+PS1 Tg2576 x M146L</u>	Human APP KM670/671NL; PS1 M146L	5-fold (APP)	Rapid	+/-	-	+	65
<u>APP+PS1 APPSwe x PS1dE9</u>	Human APP K594M/N595L PS1-dE9	3-fold (APP)	Rapid	+/-	-	+	68

Summary of experiments describing microhemorrhage in transgenic mouse studies of anti-amyloid immunotherapy.

Table 2

Mouse model	Type of immunotherapy	Isotype and epitope	Dose and interval	Age / mo start-end	CAA	Reference
APP23	Passive	Aβ3-6 IgG1	0.5mg weekly	21-26	Unchanged	87
Tg2576	Passive	Aβ28-40 IgG1	10mg/kg weekly	19-22 and 23-28	Increased	90
PDAPP	Passive	Aβ1-5 IgG1	50mg/kg weekly	22-23.5	Increased	92
Tg2576	Passive	Aβ33-40 IgG2b Intact and deglycosylated	10mg/kg weekly	20-24	Not tested	89
APP23	Passive	Aβ3-6 IgG2a	0.5mg weekly	20-25	Increased	93
APP+PS1 Tg2576 × M146L	Active	various	100mg Aβ + Freund's adjuvant	10-20	Not tested	94
Tg2576	Passive	Aβ33-40 IgG2b deglycosylated	30mg/kg	19-22	Increased	91
			10mg/kg		Increased	
			3mg/kg weekly		Increased	
PDAPP	Passive	Aβ1-5 IgG2a	3mg/kg	12-18	Decreased	88
			0.3mg/kg weekly		Decreased	