NEURODEGENERATION AND THE BRAIN BARRIERS

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Introduction

Intense research efforts in the past two decades to understand the pathogenesis of neurodegenerative diseases and design effective therapeutics have been focused mainly on neurons. Although this so-called “neuro-centric” view has contributed to our understanding of neuronal dysfunction, death pathways, and accumulation of proteinaceous aggregates during chronic neurodegenerative processes, this approach has not resulted in disease-modifying therapeutics. This suggests that the pathogenesis of neurodegenerative disorders is more complex than previously thought, and that the lack of success of neurocentric-based therapies may be due to the participation of non-neuronal cells in the disease process.

Although Alzheimer’s disease (AD) has been traditionally classified as a neurodegenerative dementia without cerebrovascular changes, current epidemiological, pathological, experimental, and imaging studies suggest that such classification is no longer tenable. Many vascular risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, obesity, homocysteinemia, apoE4 genotype have recently been shown to increase the risk of AD. Growing evidence suggests that silent brain infarcts and cerebral hypoperfusion can increase risk for dementia and cognitive impairment in the elderly. Considerable data suggest that there are important pathogenic mechanisms such as inflammation, oxidative stress, and apoE4 expression common to both AD and cardiovascular disease. Endothelial cells are widely recognized as key players in the development of cardiovascular disease and increasing evidence implicates dysfunction of brain endothelial cells in the pathogenesis of neurodegenerative diseases. Indeed, vascular dysfunction has been linked to a growing number of neurodegenerative disorders including, AD, Parkinson’s disease (PD), ALS, spinal cord injury and Huntington’s disease.

In the clinical arena of neurodegenerative disorders, the blood-brain barrier (BBB) is traditionally envisioned as irrelevant or as simply a barrier to treatment. However, recent studies have raised the possibility that the BBB and blood-CSF barrier (BCSFB) may play important roles in pathology and progression in a broad spectrum of CNS disorders including AD, PD, ALS, and multiple sclerosis. From
both a clinical and basic science perspective, the barrier systems of the brain are the keys to brain homeostasis and the preservation of neuronal integrity. It has become clearer since 2000 that there are alterations of the cerebral microcirculation/BBB in neurodegenerative disease that need to be explored and may provide possible new targets for therapeutic development.

**State of science in brain barriers research**

Key areas of emphasis from the basic science perspective:

- **BBB and A transport in and out of the brain: Role of RAGE and LRP-1**
- Changes in BBB permeability: ischemia, age and apoE
- Cerebral microcirculation as both a source and a target of inflammatory factors
- Oxidative stress and the BBB: reactive oxygen species and nitric oxide
- Aberrant angiogenesis in neurodegenerative diseases

**BBB and A transport in and out of the brain: Role of RAGE and LRP-1:** The receptor for advanced glycation end products (RAGE) is thought to be a primary transporter of A from systemic circulation across the BBB and into brain, while the low-density lipoprotein receptor-related protein-1 (LRP-1) mediates transport of A in the other direction, that is, out of the brain. AD is associated with changes in relative distribution of RAGE and LRP-1 receptors in human hippocampus and cortex, suggesting AD may develop as a clearance storage disorder with the resulting accumulation of A and neurodegeneration² [Figure 1]. Based on the BBB transport-clearance hypothesis, various novel immune and non-immune anti-A clearance treatments for AD are currently under development. Among others these include inhibitors of RAGE-A interaction at the BBB which results in reduced A transport into the brain, reduced neuroinflammation and increased responses of cerebral blood flow to brain activation.³
**Changes in BBB permeability: ischemia, age and apoE:** In the past, the absence of a gross disruption of BBB function, as measured by current technologies, has led to the assumption that the BBB is unaltered in neurodegenerative disease. However, more recent data have suggested that a focally dysfunctional BBB is unable to regulate the influx/efflux of neurotoxic amyloid peptides and could participate in the pathogenesis of AD lesions. Furthermore, there are regional (focal) differences in the presence of AD lesions and there appears to be a pathogenic relationship among development of AD lesions, Aβ-positive vessels, and impaired BBB function. Indeed, both increased BBB permeability to Aβ and reduced elimination of Aβ have been shown to precede senile plaque formation in an AD model. In the Tg2576 AD mice, vascular CBF dysfunction and dysfunction in BBB transport were evident in animals as young as 4 months; implying that structural changes to the BBB caused by elevated Aβ could play a central role in AD development and might define an early point of intervention for designing effective therapy against the disease.

Another area with emerging interest is the role of apoE in regulating BBB integrity. It is well documented that inheritance of the apoE allele ε4 increases the risk of developing cardiovascular disease and AD. It has been recently shown that both blood- and tissue-derived apoE are important for BBB function and integrity. Furthermore, there is an age-dependent defect in BBB function that is exacerbated in apoE-/-mice. ApoE4 has also been associated with increased loss of agrin, a major basal lamina associated heparin-sulfate proteoglycan, from the cerebral capillary basement membrane. Since vascular dysfunction is found in patients with age-related neurodegenerative diseases, such as AD, age-related BBB-dysfunction could underlie these defects and may thus be an important contributor to the cumulative neuronal damage characteristic of these diseases. Finally, CNS hypoperfusion may be an underlying defect/contributor in several neurodegenerative diseases. Cerebral ischemia induces an increase in BBB permeability, upregulates expression of amyloid precursor protein, and aggravates ALS functional deficits and pathology.

**Cerebral microcirculation as both a source and a target of inflammatory factors:** A substantial literature demonstrates activation of inflammatory processes in pathologically vulnerable regions of the AD brain.
and documents the presence of a large number of inflammatory molecules (13). Also, in both human and animal studies anti-inflammatory drugs appear to reduce the risk of AD pathology and in some cases enhance cognitive performance.\textsuperscript{14,15} Microvascular endothelial cells are a rich source of both cytokines and chemokines and release inflammatory factors in response to a wide variety of stimuli.\textsuperscript{16} Isolated brain microvessels from AD patients have high levels of both cell-associated and soluble cytokines and chemokines including IL-1, IL-6, IL-8, TNF, TGF-\textgreek{a} and monocyte chemoattractant protein-1 compared to vessels from age-matched non-AD controls.\textsuperscript{17-19} Since 2000 a large body of work has demonstrated that the cerebral microcirculation is in an “activated pro-inflammatory” state in neurodegenerative diseases such as AD, MS, ALS and others.

**Oxidative stress and the BBB: Reactive oxygen species and nitric oxide:** Considerable evidence points to oxidative stress as an important trigger in the complex chain of events leading to neurodegenerative diseases such as AD and PD.\textsuperscript{20,21} Postmortem analysis of AD brains show elevated markers of oxidative stress including protein nitrotyrosine, carbonyls in proteins, lipid oxidation products, and oxidized DNA bases.\textsuperscript{22,23} Despite widespread consensus that oxidative stress is an important feature of the injured CNS, the source of radical species as well as the factors that regulate their release/synthesis are not well defined. Recently, a role for the microcirculation as a source of reactive oxygen species (ROS) in neurodegenerative disease has been investigated. In this regard, both oxidized and nitrated proteins are found in the vessel walls in AD.\textsuperscript{24,25} Also, it has been shown that brain microvessels when injured by anoxia/reoxygenation (an in vitro model of ischemia/reperfusion injury) release oxygen free radicals (specifically hydroxyl radicals).\textsuperscript{26} Homocysteine is thought to promote cardiovascular disease through endothelial dysfunction and oxidative stress. Homocysteine has been implicated in the pathogenesis of AD by data showing that serum homocysteine levels are significantly higher in AD patients than in controls.\textsuperscript{27}

In the past decade, nitric oxide has emerged as important neuromodulator in the brain as well as a neurotoxin, at high concentrations.\textsuperscript{28} In the cerebral microcirculation a significant increase in nitric oxide release has been demonstrated in the AD brain. In these AD vessels the levels of both endothelial nitric
oxide synthase and inducible nitric oxide synthase are high. High levels of inducible nitric oxide synthase are consistent with the pro-inflammatory state of the microcirculation in AD. Elevated vascular production of nitric oxide, a potentially neurotoxic mediator in the CNS, may contribute to the susceptibility of neurons to injury and cell death in neurodegenerative disease.

**Aberrant angiogenesis in neurodegenerative diseases:** Recent work has discovered the role of the mesenchyme homeobox gene 2 (MEOX2) in neurovascular dysfunction in AD. MEOX2-gene is vascularly restricted gene expressed exclusively in the vascular system in brain and particularly at the BBB. Its downregulation in AD leads to aberrant brain capillary morphogenesis, abnormal responses to angiogenic growth factors, improper barrierogenesis and downregulation of A clearance receptors associated with reduced brain capillary flow. Moreover, low levels of MEOX2 gene in AD endothelium abort the angiogenesis at an early stage of brain capillary morphogenesis resulting in formation on non-patent capillaries and reduced capillary network. Since in the presence of low MEOX2 and A, VEGF FGF and other pro-angiogenic factors fail to restore normal vascular remodeling, search for new therapies to regulate angiogenesis in AD is warranted.

Factors and processes characteristic of vascular activation and angiogenic stimulation have been documented in the AD brain. Genome-wide expression profiling in the AD brain has identified a marked upregulation of genes that promote angiogenesis. Expression of angiogenic factors may result from chronic hypoxia which is known to stimulate angiogenesis as well as contribute to the clinical and pathological manifestations of AD. It has been shown that in AD brain microvessels express or release inflammatory proteins, including thrombin, vascular endothelial growth factor, angiopoietin-2, tumor necrosis factor-α, transforming growth factor-β, interleukin (IL) IL-1α, IL-6, IL-8, monocyte chemoattractant protein-1, hypoxia inducible factor-1α, matrix metalloproteinases, and integrins (V V V V V V s), all of which have been implicated in endothelial activation and regulation of angiogenesis. Despite increases in several pro-angiogenic factors in the AD brain, evidence for increased vascularity in AD is lacking. On the contrary, the present evidence suggests that the angiogenic process and vascular remodeling are aberrant and/or impaired in aged tissues, with several studies showing decreased
microvascular density and aberrant capillary tube formation in the AD brain.\textsuperscript{33-36} In this regard, it has been also demonstrated that \textsuperscript{A} peptides have strong anti-angiogenic properties in several models of angiogenesis in vivo and in vitro.\textsuperscript{37-39}

A recently proposed model suggests that in response to a persistent stimulus, such as cerebral hypoperfusion, one of the major clinical features in AD, brain endothelial cells become activated or acquire a senescent, pro-inflammatory state. Senescent endothelial cells overexpress a number of adhesion proteins and may produce a surge of inflammatory cytokines/chemokines depending on the type. Despite the continued presence of the stimulus, an imbalance of pro- and anti-angiogenic factors and aborted angiogenic signaling prevents new vessel growth. Therefore, in the absence of feedback signals to shut off vascular activation, endothelial cells remain activated and elaborate a large number of proteases, inflammatory proteins and other gene products with biologic activity that could injure or kill neurons\textsuperscript{32} [Figure 2]. This phenotypic modulation of brain endothelial cells is important given an increasing recognition for the role of the neurovascular unit and neurovascular dysregulation/dysfunction in the development of cognitive decline in AD. Identification of “vascular activation” as a target in AD would stimulate translational investigations in this newly defined area and may lead to novel therapeutic approaches for the treatment of this devastating disease.

Key areas of emphasis from the \textit{clinical perspective}:

- BBB function and etiology of neurodegeneration
- BBB and rate of progression of neurodegeneration
- Circumvention of the BBB for treatment

\textbf{BBB and etiology of neurodegenerative disorders}: The etiology of the vast majority of PD is presumed to be due to a combination of environmental and genetic factors. Environmental factors are most commonly thought to be exposure to endogenous or exogenous toxins that gain access to the central nervous system. Genetic factors are envisioned as multiple genes that positively or negatively influence the
probability of developing PD by affecting the absorption or metabolism of these putative toxins. Variability in transport of putative toxins into and out of the brain is another obvious potential mechanism for sensitivity to toxins.

Evidence for variability of BBB transport processes contributing to PD comes from studies of polymorphisms of the multiple drug resistance (MDR1) gene in Chinese populations that suggest that some polymorphisms reduce the risk of developing PD. Further, a direct measure of the MDR1 function in PD and controls by examining the retention of a positron labeled verapamil (a substrate for MDR1) by PET scanning found greater retention in PD subjects than in normal controls. This observation suggests that MDR1 activity was less in PD and could lead to increased retention of a putative toxin in the PD brain. Interestingly, the increased retention of verapamil was primarily in the midbrain and not throughout the brain, suggesting that there might be regional differences in transporters within the brain. Paraquat, a widely used herbicide, can produce a parkinsonism model in rodents. The effect of paraquat in this model can be blocked by competitive inhibition of the BBB large neutral amino acid transporter giving further credence to the importance of access of putative toxins to the brain.

**BBB and progression of neurodegeneration:** Alterations in the BBB and B-CSF-B could alter the rate of deterioration with neurodegenerative disorders. Slowing the rate of progression could make a large impact on disability in neurodegenerative disorders.

There is evidence of alteration of the BBB in AD as discussed above that may potentially exist before AD becomes clinically apparent. Clinical evidence for increased BBB permeability in AD is an increased concentration of the prothrombin in the brain parenchyma in advanced AD. Alterations of BBB or BCSFB permeability are also implicated by an elevation of CSF albumin in the CSF of a subset of AD which is associated with more rapid progression of AD. This does not appear to be a nonspecific finding associated with neurodegeneration; it is not present in PD. These effects could be related to the mild inflammatory response seen in neurodegenerative disorders although this explanation would not explain why an elevated albumin index is not seen in PD where there are also indications of an
inflammatory response. It will be important to define whether these changes are a response to neurodegeneration or are independent contributors to disease progression.

In rats made hemiparkinsonian by unilateral injections of 6-hydroxydopamine, a catecholamine neurotoxin, and treated with levodopa, there is evidence of endothelial proliferation and increased permeability to levodopa. The endothelial proliferation and changes in levodopa entry were present only in the basal ganglia, subventricular zone and hippocampal dentate gyrus and not elsewhere, suggesting regional regulation of BBB transport. The changes in regional capillary endothelial cells have been correlated with the development of dyskinesia, a troublesome adverse effect of long-term levodopa treatment in PD. Thus, disease treatments may alter the BBB and thereby the course of the disease and response to therapy.

A final example of change in BBB and BCSFB that may alter rate of progression of neurodegeneration is CSF production, clearance and composition. CSF production is reduced in AD but not PD. The reduction in CSF formation reduces CSF turnover which has important implications if the CSF acts as a sink for toxic molecules such as Aβ. A clinical observation that bolsters the suspicion that CSF turnover may be important is the fact that the clinical triad of dementia, gait disorder and urinary incontinence of normal pressure hydrocephalus (NPH) is often associated with pathological evidence of AD, subcortical microvascular disease and possibly other neurodegenerative disorders. Further, patients with NPH and these other disorders may respond, at least for many months, to ventricular shunting.

**Circumvention of the BBB:** One of the great successes in neurodegenerative disorders was the discovery of depletion of dopamine in PD and the recognition that the precursor of dopamine, L-DOPA, was transported across the BBB and provided symptomatic benefit to patients. This therapy was further refined by preventing the catabolism of L-DOPA in the periphery by DOPA decarboxylase with carbidopa, a compound that inhibited DOPA decarboxylase but did not cross the BBB to inhibit the central DOPA decarboxylase which is essential to L-DOPA’s conversion to dopamine.
However, other therapies for PD have been thwarted by the BBB. A good example is the saga with glial derived neurotrophic factor (GDNF). GDNF is a potent neurotrophin that will revive damaged dopaminergic nerve terminals in animal models of PD.\textsuperscript{50} This peptide will not cross the BBB and alternative methods of administration have been attempted. In PD subjects, intracerebroventricular administration by implanted reservoirs and ventricular catheters proved that GDNF was biologically active but was without clinical efficacy.\textsuperscript{51} A postmortem on a patient dying of other causes found no evidence that intraventricular GDNF affected dopaminergic nerve terminals in the putamen which lies centimeters from the lateral ventricle. A subsequent study infused GDNF directly into the putamen with implanted catheters producing PET evidence of enhanced dopaminergic function in the immediate vicinity of the catheter tip but no clinical improvement.\textsuperscript{52} A clinical trial that is just beginning will use gene therapy with an adenovirus carrying the gene for neurturin, another neurotrophin from the GDNF family. This treatment will require four needle passes and eight injections into each putamen. Another strategy being pursued includes programming stem cells to produce GDNF and implanting them into the putamen. Another alternative under consideration is methods to enhance the synthesis and release of endogenous GDNF with smaller molecules that will cross the BBB, as has been done with brain-derived neurotrophic factor (BDNF) in animal models.\textsuperscript{53} Even activity may influence neurotrophin activity in brain.\textsuperscript{54} The development of less invasive methods to deliver therapeutic molecules such as peptides, small RNAs and genes to specific regions of the CNS is clearly very necessary.

We need to consider other routes into the CNS. One novel route under investigation is intranasal delivery of peptides. Novel routes to the brain may also be linked to the etiology of PD. Braak has shaken up the PD scientific community by presenting evidence that PD begins in the dorsal motor nucleus of the vagus nerve (in the medulla) and not in the midbrain dopaminergic neurons as has been generally assumed for the past 4 decades.\textsuperscript{55} Further, since this site is connected to the periphery by the vagus nerve, he has proposed that some toxic factor enters the CNS via the vagus nerve and the pathological process then progresses up the neuroaxis. Blood-nerve barriers may be critical to this hypothesis.

**Barriers to progress in the field**
For transport studies: The normal clearance pathways for most metabolites that are not degraded in situ are the barriers of the CNS: the capillary endothelium, the choroid plexus epithelium and the CSF circulation. A better understanding of the continuum between normal aging and the age-related dementias requires that we explore the changes in the barrier structure, receptors and their expression, that underlie the transport of metabolites into and out of the CNS at all of the barrier sites.

Good standardized in vitro and in vivo models of the BBB and BCSFB are critical. Models to study transport dynamics and equilibrium at the BBB are not widely available and can be found in a small number of highly specialized laboratories. And how do we model the BBB and altered neurovascular signaling that occurs in various diseases? Can we make the BBB and its broader neurovascular signals a much larger part of the CNS disorders consciousness? Right now, we are still way too focused on the “neuobiology” of disease rather than the integrative biology of the entire brain which should include an in-depth understanding of the BBB functions. Comparative transport studies of the BBB and BCSFB within a given model will furnish more insight on the complex interplay between the cerebral interstitial fluid and the large-cavity CSF. Such interaction of barrier systems is a key factor in understanding the homeostatic mechanisms that assure the proper extracellular environment of neurons.

For permeability studies: There has been a lack of emphasis on developing comorbidity models which include vascular factor models (such as ischemia/reperfusion injury) and models of neurodegenerative disorders, as well as lack of well-defined models of the age-related dementias. With the increased aging of the population such research will become imperative. Also, improvement of neuroimaging techniques that will allow identification/resolution of subtle, even transient, changes in BBB permeability will be extremely important.

For inflammatory and neurovascular unit cross-talk studies: Challenges for the future include understanding the cross-talk between non-neuronal cell types (glia, microglia) and cells of the vessel wall. Identifying how these cells respond to, process, and/or synthesize inflammatory mediators is critical to
understanding how they regulate the neuronal microenvironment. Despite a large body of data implicating inflammation in the pathogenesis of neurodegenerative disease and epidemiological studies showing that sustained use of anti-inflammatory drugs may prevent or slow down the progression of neurodegenerative diseases the small number of clinical trials carried out so far using anti-inflammatory drugs, were minimal and equivocal in their outcome. Potential reasons for these mixed results include timing of drug administration, nonselective inhibition of cyclooxynagenase (COX), inappropriate use of particular anti-inflammatory drugs for a given disease or disease progression/ severity, sub-optimal dose in target site, or limited penetration to the brain through the BBB. Therefore, design of anti-inflammatory drugs for the treatment of neurodegenerative diseases based upon better BBB penetration, and with minimal adverse events, would be appropriate. In addition, relevant genetic differences among patients should be considered in planning new anti-inflammatory drugs, for improved efficacy. Furthermore, due to the possible co-involvement of oxidative stress and excitotoxicity in the pathogenesis of these diseases, combination therapy with antioxidants or glutamate antagonists or a multi-potent drug might be much more effective in successfully treating neurodegenerative diseases.

**For oxidative stress studies:** New technologies and approaches to measuring short-lived ROS in vivo in real time could significantly improve our understanding of how oxidants contribute to brain injury. It is also important to develop new generation of antioxidant agents. Because ROS as well as nitric oxide have important functions in cellular signaling and in the regulation of gene expression, separate from their toxic effects, development of site or level specific antioxidants will be needed to effectively block the toxic effects of these molecules.

**For aberrant angiogenesis:** Future work to test angiogenic capabilities should assess the temporal or causal association between acquisition of the activated-angiogenic phenotype and the onset of disease. A causal link between the angiogenic phenotype and disease progression could be evaluated using antiangiogenic drugs. Administration of these drugs to animals prior to the onset of behavioral changes and AD pathology will determine whether inhibiting the activated-angiogenic phenotype affects the course of disease. Development of new treatments to curb aberrant angiogenesis caused by A\(^+\), MEOX2,
senescent endothelial phenotype, and injured or activated endothelial cells deserve special attention in future studies. On the other hand, VEGF2R anti-angiogenic therapy has been shown to aggravate the disease process and enhance disease onset in models of ALS and cautions use of this approach for other neurodegenerative disorders.

For etiology of neurodegeneration: A general lack of knowledge is the major barrier to developing this area. There needs to be an exploration of transport mechanisms for substances of interest in neurodegenerative disorders. Transport of proteins that accumulate in the disorders, analogous to beta amyloid, are candidates. Alpha synuclein, a major component of the Lewy body in PD, TAU, a component of neurofibrillar tangles, paraquat, other insecticides and other potential toxins are candidates. Exploring polymorphisms in these transporters to look for genetic susceptibility to neurodegenerative disorders is a priority. If transporters are contributors to susceptibility or progression of neurodegeneration, manipulation of transport processes may offer neuroprotective and treatment strategies.

For progression of neurodegeneration: The question of whether the heterogeneity of the BBB contributes to the selective vulnerability in neurodegeneration is less important in PD since Braak emphasized that PD began in the medulla and appeared to progress up the neuroaxis. However, the heterogeneity of BBB could be very important in targeting therapies for neurodegeneration to particular brain areas. For example the efforts to get genes, small RNAs and peptides into the putamen would be much more effective if these agents could be targeted to the putamen or striatum selectively by utilizing transport mechanisms unique to the striatum. Mapping the heterogeneity of the BBB should be a high priority.

For circumvention of BBB: The critical need for this area is a noninvasive method to evaluate CSF turnover and preferentially be able to separate problems with production and problems with clearance. Molecules that would selectively be secreted by the choroid plexus and could be imaged by some means would perhaps allow a better assessment of CSF dynamics.
Delivery of therapeutic agents across the BBB is critical but is being covered in another section of this report. Systemic administration that could be targeted for specific areas of the brain, such as the striatum for parkinsonism, would be ideal. However, research is also needed to explore methods to employ delivery from the ventricular CSF to brain and the safe use of convection enhanced delivery of agents directly into parenchyma.

**Recommendations to advance the field and resources required**

Increase the number of scientists/clinicians interested in BBB/cerebrovasculature.

Fund and promote inter-institutional, multidisciplinary teams.

Develop interactive, web-based database of BBB scientists/clinicians.

Develop studies that support cause and effect rather than just associations.

Explore the genome of the neurovascular unit and choroid plexus to:
- Examine polymorphisms linked to various neurodegenerative disorders.
- Create conditional knockouts of genes important to microvasculature structure (tight junctions, basement membrane, etc).
- Spin off new transport systems for drug delivery.

Develop new vascular-based clearance strategies.

Design strategies to control aberrant brain angiogenesis

Develop in vivo imaging of brain microcirculation in models of human neurodegenerative disorders to follow the kinetics of the disease process.

**Summary and single most important issue to advance the field**
Because ageing is the most important risk factor for neurodegeneration, we hypothesize changes in the normal neuronal milieu caused by alterations of the cerebral microvasculature, CSF circulation and disruption of the neurovascular unit that occur with aging predisposes the CNS to neurodegeneration. We propose examining the changes in the neurovascular unit and CSF turnover that occur with aging. Specifically, we need studies of: 1. Secretion of endothelial toxins and inflammatory factors, 2. Alterations in other structural components of the neurovascular unit (tight junctions, basal lamina, pericytes, astrocytic foot processes, etc), 3. CSF production, clearance and composition, 4/ Amyloid clearance and clearance of other proteinaceous aggregates which accumulate in brain interstitial fluid or cells, 5. Role of non-neuronal cells in the pathogenesis of neurodegenerative disorders and 6. Aberrant brain angiogenesis.
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Figure 1. Schematic of blood–brain barrier and blood–CSF barrier (inset) transport routes for A. A efflux across the BBB can predict brain amyloid burden in AD models and the development of plaques shifts the A transport equilibrium. Double deletion of the genes encoding apoJ and apoE accelerates A pathology in APP-overexpressing mice, raising a possibility that these apolipoproteins affect A clearance and/or metabolism. Gp330/megalin/LRP-2, an apoJ receptor at the BBB and the choroid epithelium, could participate in clearance of A–apoJ complexes from the brain across the BBB, and from CSF across the choroid epithelium of the blood–CSF barrier (inset) to maintain the sink action of CSF. P-glycoprotein at the luminal side of the BBB could reduce brain endothelial A by promoting its efflux into blood. In addition to transport, interaction of A with RAGE amplifies neurovascular stress and inflammation. LRP, an endocytotic and signaling receptor that has ligands including A, apoE, a2M and APP, is linked genetically to AD and influences APP processing and A clearance. APP-overexpressing mice overexpressing the LRP mini-receptor in neurons, however, accumulate soluble A in brain. By contrast, LRP on brain capillary endothelium clears A to blood with affinity inversely related to the content of -sheets in A, and lipoprotein receptors on astrocytes promote apoE-dependent degradation of A deposits. Whether LRP on brain endothelium can also clear oligomeric A, and whether chaperone proteins can assist clearance of aggregated A across the BBB, is not known. (Reprinted with permission from Elsevier)

Figure 2. Proposed scheme for endothelial activation in Alzheimer's disease.

Under normal conditions, endothelial cells in response to a stimulus such as hypoxia or IL-1 elaborate a large number of gene products with biological activity including inflammatory cytokines and proteases. Endothelial cells then migrate, proliferate and form new blood vessels, which through a negative feedback loop turn off the process. In AD, endothelial cell activation in response to stimuli occurs, but no migration, proliferation or new blood vessels develop. Thus, in AD endothelial cells continue to release inflammatory cytokines and proteases, with deleterious consequences for neurons.
Figure 2

EC migration and proliferation
New blood vessel formation

EC migration and proliferation
No new blood vessel formation

EC
AD pathology

EC
AD pathology

Normal