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Blood will out: vascular contributions to Alzheimer's disease

Sidney Strickland

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Review

The fundamental pathology in Alzheimer's disease (AD) is neuronal dysfunction leading to cognitive impairment. The amyloid-β peptide (Aβ), derived from amyloid precursor protein, is one driver of AD, but how it leads to neuronal dysfunction is not established. In this Review, I discuss the complexity of AD and possible cause-and-effect relationships between Aβ and the vascular and hemostatic systems. AD can be considered a multifactorial syndrome with various contributing pathological mechanisms. Therefore, as is routinely done with cancer, it will be important to classify patients with respect to their disease signature so that specific pathologies, including vascular pathways, can be therapeutically targeted.

Blood will out: vascular contributions to Alzheimer's disease

Sidney Strickland

Patricia and John Rosenwald Laboratory of Neurobiology and Genetics, The Rockefeller University, New York, New York, USA.

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Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia, affecting approximately 5.5 million Americans. With an aging population worldwide, it is predicted to be a public health crisis in the coming decades. In spite of extensive preclinical and clinical efforts, little progress has been made in preventing or reversing this disease. A better understanding of AD requires reconceptualizing the disorder as an amalgam of dysfunctions rather than one pathology. An analogous evolution has occurred in cancer biology; decades ago, the search for a "magic bullet" for cancer treatment suggested that cancer was one disease and that it would be possible to identify a single drug to cure all forms. With today's advanced knowledge of the many mechanisms that lead to cancer, that original concept is very dated. Yet, researchers today still discuss "a" cure for AD, implying that a single pathological pathway is responsible for all cases. For example, the tag line on a 2016 cover of *Time* magazine read, "The Alzheimer's Pill."

It is becoming obvious that AD, like cancer, is a complex disease with multiple pathogenic mechanisms. One pathway that has been largely overlooked is vascular dysregulation. For example, a relatively recent and highly cited hypothetical temporal ordering of AD pathologies did not include vascular pathology in the possible contributors to the disease (1). Accumulating evidence, detailed in this review, indicates that vascular dysregulation plays a major role in cognitive decline. Undoubtedly, attacking the disease successfully will involve identifying and targeting various mechanisms, as has been done in the revolution in cancer treatment.

Multiple mechanisms can contribute to AD pathology

In cancer, the fundamental pathology is unregulated cell division. However, this aspect by itself is not necessarily lethal. Other mechanisms — for example, immune system evasion and metastasis — can compound too much cell division and exacerbate the morbidity of the disease. Cancer treatments have been developed to counteract these ancillary pathways, and these treatments can improve patient outcome. One can envisage a similar situation for AD. The fundamental pathology is neuronal dysfunction causing cognitive decline, and this pathway can be accelerated by many other abnormalities such as defects in autophagy (2), synaptic toxicity (3), and oxidative stress/mitochondrial dysfunction (4). Of all the many possible contributors, inflammation (5) and vascular abnormalities (6, 7) appear to be especially significant (Figure 1 and see below).

Could vascular mechanisms benefit AD diagnosis or treatment?

If ancillary mechanisms can contribute to AD, one consideration is how to determine the relevance of these pathways in individual patients, especially if the mechanisms are not specific to AD. Again, cancer provides a useful analogy. Estrogen and progesterone receptor expression is not specific to breast cancer, and also occurs in normal tissues and other cancers. Nevertheless, in patients with breast cancer, hormone receptor expression predicts response to antiestrogen therapy such as tamoxifen and such therapy improves patient outcome. Likewise, patients with cognitive impairment could be tested for vascular abnormalities and inflammation. Those that exhibit these ancillary pathologies could then be treated for those conditions. As with cancer, it seems likely that this approach would benefit this subset of patients.

Is there a connection between vascular pathology and AD?

The classical pathological hallmarks of AD are amyloid-β peptide (Aβ) plaques, tau tangles, neuroinflammation, and neuronal loss (8). The Aβ deposition can be in the brain parenchyma or in and around cerebral blood vessels, a condition known as cerebral amyloid angiopathy (CAA), discussed below (9). Less discussed is that AD is very often associated with cerebrovascular abnormalities (6, 10–13). These cerebral pathologies include microinfarcts, hemorrhage, decreased cerebral blood flow, small vessel disease, and white matter abnormalities (14, 15). Three recent studies of

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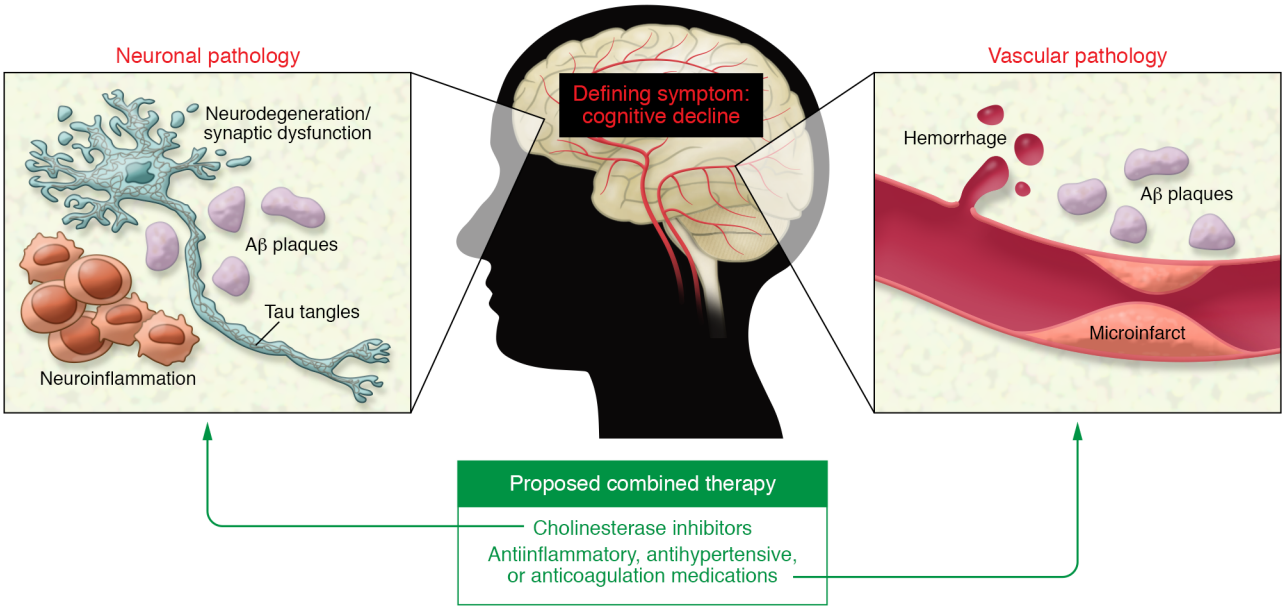

Figure 1. Multiple pathogenic pathways contribute to and provide therapeutic targets for AD. While neuronal dysfunction is the defining pathology underlying cognitive decline in AD, this disease is likely the product of multiple pathogenic mechanisms and might benefit from combination therapy (128–130). Increasing evidence implicates vascular dysfunction in AD pathogenesis. Aβ plaques and tau tangles drive neuronal dysfunction and neuroinflammation through mechanisms that are not fully established. Aβ is also implicated in vascular pathology, having been shown to interact with fibrin(ogen) and the contact system. The prevalence of vascular dysfunction in patients exhibiting cognitive decline suggests that combining existing treatment strategies targeting neuronal dysfunction (e.g., cholinesterase inhibitors) and vascular dysfunction (e.g., antiinflammatory, antihypertensive, or anticoagulant medications) may advance AD treatment efficacy.

vascular disease in AD autopsy samples showed that concurrent vascular disease is very common in AD and strongly correlates with cognitive dysfunction (16–18).

Reinforcing the concept that the vascular system influences AD, multiple studies have reported that exercise, which improves cerebrovascular health, can decrease the risk and/or delay progression of dementia. Benefits of increased physical activity include improved memory performance and reduced hippocampal atrophy (19–21), increased gray matter volume and production of neurotrophic factors (22), lower risk of mortality (23, 24), and reduced risk of AD (25). However, this association is complex (26), as exercise could also have nonvascular benefits, and other studies have found no improvement in risk of dementia with exercise (27). Mechanisms by which exercise could influence AD are discussed below.

Another link between AD and the vascular system is CAA, the deposition of Aβ in and around blood vessels of the brain (9), which affects 80% to 95% of AD patients (15, 16). CAA can cause blood vessel occlusion, microinfarcts, ischemia, microbleeds, and inflammation, conditions that can weaken the blood vessel wall and cause life-threatening hemorrhage (8, 15, 28). All of these conditions could contribute to neuronal death that is associated with AD progression.

AD and vascular dysfunction: independent or causal pathologies?

It is clear that vascular dysfunction can itself lead to cognitive decline. However, the coexistence of AD and cerebrovascular pathology prompts questions of whether these conditions are always independent comorbidities, as they are both more prevalent in the aged population. If so, there could be synergistic effects on brain function. Alternatively, there could be a mechanistic link between AD and vascular pathology (29, 30).

Early vascular pathologies in late-onset AD patients

A recent study used a multifactorial, data-driven analysis to examine various pathologies in 1,171 healthy and late-onset AD (LOAD) subjects and assign a temporal ordering of disease progression (31). The conclusion of this study was that vascular dysregulation, measured by arterial spin labeling of cerebral blood flow (CBF), is the earliest and strongest brain pathology associated with LOAD. A tentative ordering of abnormalities was (a) initial vascular dysregulation, (b) Aβ deposition, (c) metabolic dysfunction, (d) functional impairment, and (e) gray matter atrophy. Given thresholds for detection, the exact order of the pathologies is not certain, but these analyses strongly "suggest that intra-brain vascular dysregulation is an early pathological event during disease development" (31).

Vascular pathologies appear early in early-onset AD patients

An analysis of early-onset AD (EOAD) patients who harbor autosomal-dominant mutations provides strong evidence for a connection between AD and vascular pathology (32). EOAD-associated mutations occur in amyloid precursor protein (APP), from which the Aβ peptide is derived, or in γ-secretase, which helps excise Aβ from APP (33). These mutations are virtually fully penetrant, and by analyzing DNA sequences, one can iden-

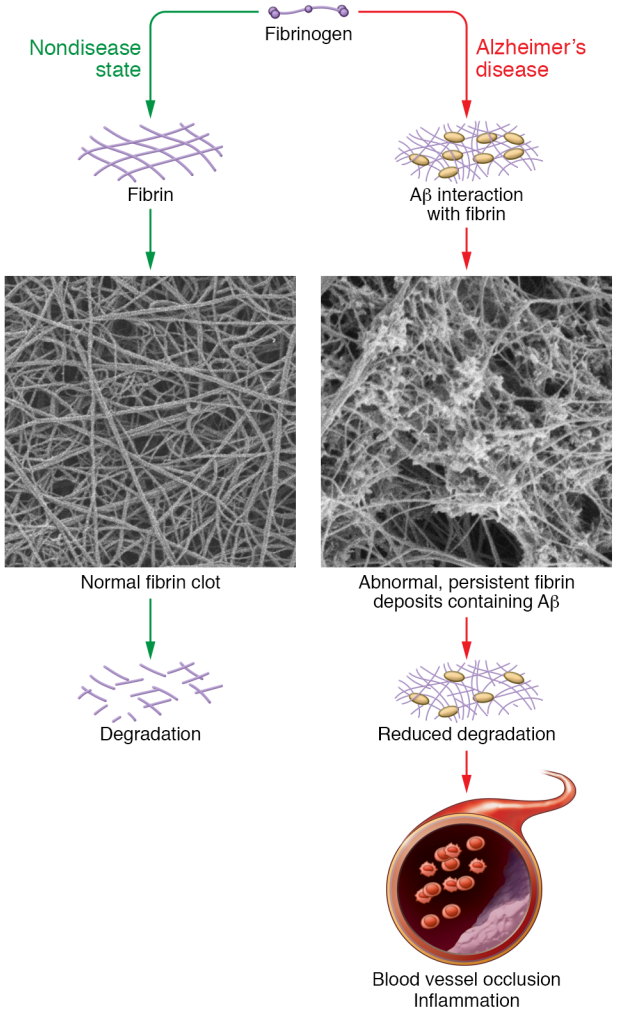

Figure 2. Possible influence of Aβ on fibrin deposition and AD pathology. The interaction of Aβ with fibrinogen leads to increased formation of structurally abnormal fibrin clots that are resistant to degradation. This persistent fibrin and resulting predisposition towards blood vessel occlusion and inflammation could contribute to the neurodegeneration observed in AD. Images reproduced with permission from Cortes-Canteli et al. (53).

tify at the time of birth which patients will develop EOAD at a predictable age decades later. A comparison of mutation carriers and noncarriers reveals that the first abnormalities detected in these EOAD patients are alterations in Aβ levels, which occur approximately 30 years before the onset of symptoms (estimated year of onset, EYO). The next pathology to emerge is white matter hyperintensities (WMHs), which are abnormalities observed during brain imaging. In carrier brains, WMH abnormalities were evident in general 6.6 years before EYO and in the occipital lobes as early as 22 years before EYO (32). This study did not measure CBF, so it cannot be compared to the results for LOAD patients discussed above.

WMHs are part of the spectrum of small vessel cerebrovascular disease (34, 35), including ischemic and hemorrhagic stroke, microbleeds, brain atrophy (36), chronic hypoperfusion (37), and an increase in blood-brain barrier (BBB) permeability, which causes fluid to leak into the surrounding brain tissue (38). What is notable is that these lesions predict the clinical outcome of AD as well as or more reliably than established markers such as hippocampal volume and atrophy (39, 40). In the EOAD study (32), mutation carriers and noncarriers were relatively young, virtually identical demographically, and at equal risk for inheriting an autosomal-dominant mutation. Thus, these findings provide strong evidence that vascular dysfunction does not reflect comorbidity or an independent pathophysiology, but rather that vascular dysfunction and Aβ pathology are causally related (32). In recent years, there has been evidence that WMHs are also associated with LOAD (41–43). In particular, WMH volume and amyloid pathology are linked in LOAD (42–44). However, it should be noted that one study found no association between WMHs and AD pathology (45).

Another link between AD and cerebrovascular disease stems from pathological outcomes in patients with autosomal-dominant mutations. Mutations in the Aβ sequence at residues 21–23 (for example, Flemish, Dutch, Iowa, and Italian mutations) are associated with massive CAA, leading to weakening of the vessel wall and frequent infarcts and hemorrhage (46, 47). Mutations in presenilin-1, a protein component of γ-secretase that helps release Aβ from APP, can also show severe cerebrovascular effects (48). All of this evidence indicates a connection between vascular pathology and AD.

Mechanistic links between AD and vascular pathology

Aβ interaction with fibrin. Fibrin is the major protein component of blood clots and is critical for normal hemostasis (49). It is well established that in addition to its beneficial functions, persistent fibrin can lead to or exacerbate many pathological conditions, including atherosclerosis, rheumatoid arthritis, stroke, spinal cord injury, multiple sclerosis, muscular dystrophy, peripheral nerve regeneration, and even bacterial infection (50–52).

Postmortem AD patient brains have been analyzed with antibodies that do not distinguish between soluble fibrinogen and insoluble fibrin. These studies showed extensive fibrinogen/fibrin deposition in the brain (53–60), which in some cases was attributed to a leaky BBB. Further studies using extraction procedures that remove all soluble fibrinogen and staining with fibrin-specific antibodies showed that insoluble fibrin is greatly increased in AD patient brains compared with nondemented controls, with differences in some regions reaching 100-fold (61). The fibrin deposition often colocalized with parenchymal Aβ deposits and lysosome-associated membrane protein 1 (LAMP-1), which is upregulated in the human AD brain (62) and cerebrospinal fluid (CSF) (63) and enriched in dystrophic areas surrounding amyloid plaques in AD mice (64, 65) and human AD patient brains (62, 66). Fibrin is also found codeposited with Aβ in areas of CAA (53, 67).

High levels of fibrinogen in plasma increase the risk for dementia (68, 69), and fibrinogen in CSF (70–72) and plasma (73, 74) can serve as a useful biomarker to identify AD progression. In addition, fibrinogen has been proposed to be one of the few blood-based biomarkers that is specific for AD and does not apply to other brain disorders (75).

The association of brain fibrin and AD prompts the question of whether this deposition is a result of AD, or whether it contributes to the pathology. AD has been linked to the APP-derived Aβ peptide, a known driver of AD pathology (76–78). Aβ binds to fibrinogen and fibrin (79, 80), leading to blood clots that are structurally

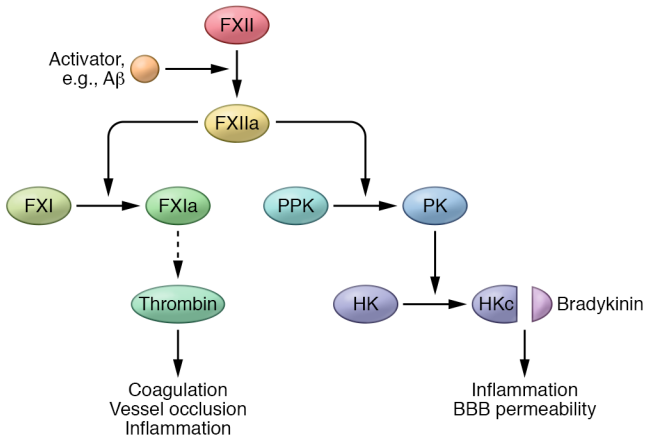

Figure 3. The contact activation system. Aβ-mediated dysregulation or overactivation of the contact system could contribute to the coagulopathy and inflammation observed in AD. Aβ can trigger activation of factor XII (FXII) into FXIIa. FXIIa's activation of FXI initiates the intrinsic coagulation system and fibrin formation, whereas its activation of plasma prekallikrein leads to inflammation. PPK, plasma prekallikrein; PK, plasma kallikrein; HK, high molecular weight kininogen; HKc, cleaved HK. a = activated form.

abnormal and harder to degrade in vitro and in vivo than normal clots (53, 80). Thus, fibrin clots formed in AD patients and mice might be persistent and cause vessel occlusion and neuroinflammation, which could contribute to neuronal death and other disease pathologies (Figure 2).

Data in humans and mice indicate that fibrin deposition in AD brains is not simply a comorbidity due to the aging population at risk, but is instead a driving factor of the disease. Studies in atrial fibrillation patients with and without warfarin treatment showed that anticoagulants can protect against dementia (81). Earlier work with dementia patients reached the same conclusion (82). In AD mice, anticoagulant treatment is protective (83–85) and injection of fibrinogen into the brain induces demyelination (86), reminiscent of the white matter abnormalities observed in AD patients discussed above. More specifically, in AD mice treatment with a small molecule that blocks the Aβ-fibrin(ogen) interaction significantly improves the course of disease (87) and reducing fibrinogen levels results in reduced pathology and better cognitive ability (53, 60, 61). Both of these studies substantiate a role for fibrin.

The mechanism by which fibrin accelerates neuronal degeneration remains unknown. There are two likely possibilities: (a) occlusion — fibrin clots are deposited in the vascular and perivascular space, resulting in reduced blood flow, increased Aβ accumulation due to binding to clots, and neuronal damage due to deprivation of oxygen and nutrients; and (b) inflammation — fibrin deposits drive a chronic inflammatory state that leads to cellular damage (50).

AD is associated with inflammation, which in some cases can be a beneficial response but in other cases can be toxic to cells (88, 89). AD patient brains have increased inflammation, mutations in immune-related molecules lead to an increased risk of AD, and nonsteroidal antiinflammatory drugs show some effects at reducing AD risk (5). Furthermore, AD mice that are incapable of mounting a proinflammatory response show improvement in pathology and cognition (90, 91). Since fibrin is a driver of inflammation, it could therefore contribute to the initiation of the inflammation observed in AD (88).

The contact system, intrinsic blood coagulation, and Aβ. The contact system is initiated by activation of the serine protease factor XII (FXII). Once converted to its active serine protease form, FXIIa can launch both prothrombotic and proinflammatory pathways (92). Regarding the prothrombotic arm, FXIIa can activate factor XI (FXI), which leads to thrombin generation and fibrin formation via the intrinsic blood coagulation pathway (93). In the proinflammatory arm, FXII can activate plasma prekallikrein (PPK), which leads to the release of bradykinin via cleavage of high molecular weight kininogen (HK). Bradykinin is a potent nonapeptide vasodilator that can activate inflammatory processes (Figure 3 and ref. 94). Thus, the contact system can initiate vascular pathology and inflammation (92, 95), both of which have been implicated in AD, and could contribute to disease pathology.

Many connections between FXII and AD have been reported, suggesting that this system may play a role in disease development. Aβ plaques contain FXII (96), the AD brain parenchyma exhibits higher plasma kallikrein (PK) activity (97), and AD patients have increased HK cleavage in their CSF (98). Consistent with a role for the contact system, AD patients have higher plasma levels of FXIIa and increased HK cleavage compared with nondemented individuals (99). Experiments with a mouse model of AD also show increased contact system activation (99, 100).

The contact system can be triggered by Aβ, which can activate FXII (101–104). This increase in FXIIa leads to elevated thrombin generation, kallikrein activity, and HK cleavage in AD patient plasma (100). Increased contact system activation is also observed in AD mouse model plasma and in plasma from wild-type mice injected intravenously with Aβ42. These results demonstrate that Aβ42-mediated contact system activation can occur in the AD circulation and suggest new pathogenic mechanisms, diagnostic tests, and therapies for AD.

Experiments in mice suggest that the contact system is a direct effector of AD pathology. Depletion of FXII using antisense oligonucleotide treatment ameliorates pathology in AD mice in early-stage disease (100). These results indicate that dysregulated contact system activation contributes to AD pathology.

Contact system activation could be used in conjunction with other diagnostic procedures such as PET and MRI imaging, CSF analysis, and/or cognitive testing to help stratify patients by their pathological profile and help guide therapy. Furthermore, a link between FXII activation and the pathogenesis of AD provides a possible novel approach to treatment since the contact system is an attractive target for therapy (105). Humans deficient in FXII and mice with knockout of the *FXII*, *FXI*, or *Kng1* (encoding HK) gene all have normal hemostasis (95). However, deficiencies in the contact system protect mice from clotting after arterial injury and experimental cerebral ischemia (92, 106).

Based on these considerations, a promising therapeutic approach to slow disease progression without affecting normal hemostasis would be to block activation of the contact system. This approach would also block bradykinin release from HK and reduce inflammation. Thus, the contact system may reveal new targets to suppress both thrombotic and inflammatory contribu-

tions to AD progression. Positive results could be applied to AD patients rapidly; a small molecule inhibitor of PK, ecallantide, is already FDA approved for treatment of hereditary angioedema, a condition that results from excess contact system activation (107). Furthermore, an antibody inhibitor of PK (108) is slated for a phase 3 trial and possible FDA approval by 2018. Some of these reagents might be useful for the treatment of AD in the future.

Could vascular pathology trigger AD?

The above discussion concentrates on mechanisms by which Aβ can drive vascular pathology. The reverse scenario is also a possibility, i.e., that vascular pathology could accelerate AD.

$$\text{AD} \rightleftarrows \text{Vascular Dysfunction}$$

Clearance of Aβ is critical to keep its concentration low in the brain and the cerebrovascular circulation, and decreased clearance may be a major cause of increased Aβ deposition in the AD brain (109, 110). Mechanisms that remove Aβ from the brain include BBB transport and movement from the CSF and parenchyma into the blood (111–114). If the cerebrovascular system were compromised, it could impede removal of Aβ and lead to increased concentration in the brain. Thus, one could envisage a vicious cycle whereby Aβ negatively affects the circulation, which in turn reduces clearance of Aβ and increases its toxic effects.

Another possible contribution of vascular pathology leading to AD could be BBB breakdown. Patients with mild cognitive impairment have been shown to have a compromised BBB (115). This condition could allow plasma proteins, including fibrinogen, to gain access to the brain parenchyma (116) where they could contribute to inflammation and promote neurodegeneration.

Finally, decreases in blood flow can lead to hypoxic tissues and the induction of the hypoxia-inducible factor HIF-1α. This transcription factor can activate γ-secretase, which could lead to increased Aβ production (117, 118). Thus, one can envisage a vicious cycle whereby Aβ causes vascular insufficiency, which in turn leads to increased Aβ.

Diagnostic possibilities stemming from vascular contributions to AD

As mentioned, if vascular dysregulation is a significant factor in some AD cases, it opens a therapeutic window to treat one aspect of the pathology. In addition, vascular involvement offers a possibility of blood-based biomarkers that could help identify contributing pathologies. Analysis of plasma has shown that AD patients and AD mice have increased contact system activation, as evidenced by increased FXII activation and HK cleavage (99, 100). To accurately assess contact activation in blood requires careful attention to many variables, including blood collection, anticoagulation, plasma preparation, sample storage, and analytical procedures (119). Nevertheless, it is possible to standardize procedures to maximize reproducibility. Having a biomarker in blood as an early diagnostic to help guide treatment could lead to a significant benefit for some patients.

Genetics of vascular involvement in AD

This review has concentrated on vascular and inflammatory drivers of AD. In genetic studies, inflammatory genes have emerged as risk factors for AD (120, 121). If vascular dysfunction is also a contributing factor in AD pathogenesis, why haven't genes associated with clotting and hemostasis turned up in screens for risk factors? One possible reason is that genes that inhibit clotting might prevent or delay AD, so they would not be observed in significant numbers in AD populations, but rather, in individuals at low risk for AD. One would need to look for protective genes; such a study has been done, and it uncovered an APP mutation that confers protection against AD (122). However, mutations that induce changes in clotting may also carry a risk of bleeding, so these patients might be present in low numbers in the aged population. It is clear that several genes identified as risk factors for AD, including Apo E4 (123) and PICALM (124, 125), can affect cerebrovascular function via the BBB (126, 127).

Conclusions

Multiple contributing pathologies affect the risk of developing AD. Just as treating multiple pathways in cancer has improved outcomes, a similar evolution of therapy can be envisaged for AD. The development of assays to classify patients and treat their specific constellation of pathologies will be required to make progress in treatment. Although reversal of cognitive decline would be the ideal treatment, and next to that a complete inhibition of progression, simply slowing the rate of progression of symptoms by 50% would be a very significant advance. Accomplishing this goal is going to require, in general, a wider range of analysis and treatment options than are currently employed, and specifically, more thorough investigations into correlations between AD and vascular dysfunction.

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Address correspondence to: Sidney Strickland, Laboratory of Neurobiology and Genetics, 1230 York Avenue, New York, New York 10021, USA. Phone: 212.327.8705; Email: strickland@rockefeller.edu.

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