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Alzheimer disease makes new blood contacts

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In this issue of *Blood*, Chen and colleagues demonstrate an unanticipated role for the contact system in a murine model of Alzheimer disease (AD). The study bolsters the key role of vascular dysfunction in AD by identifying factor XII (FXII) activation as a new molecular player in AD pathogenesis through mechanisms linked to cerebral fibrin deposition, neuroinflammation, and neuronal damage.¹

Cerebrovascular pathology and neuroinflammation are central to AD pathogenesis.² Accumulating evidence overwhelmingly points to the coagulation cascade as a mechanism linking these two temporally closely related pathologic processes in AD. Activation of coagulation has been implicated in driving detrimental vessel wall pathology and neuroinflammatory cascades in AD and other neurodegenerative disorders. Fibrin derived from the thrombin-mediated conversion of fibrinogen is the end product of the coagulation cascade. Multiple studies in humans and experimental animal models have shown that fibrin is causally linked with neuroinflammation.³ Primarily because of its unique binding to specific integrin receptors on inflammatory cells, fibrin mediates, controls, and sometimes triggers neuroinflammation.^{4,5} Fibrin is, therefore, implicated in the pathogenesis of various neurological diseases with an inflammatory component, including

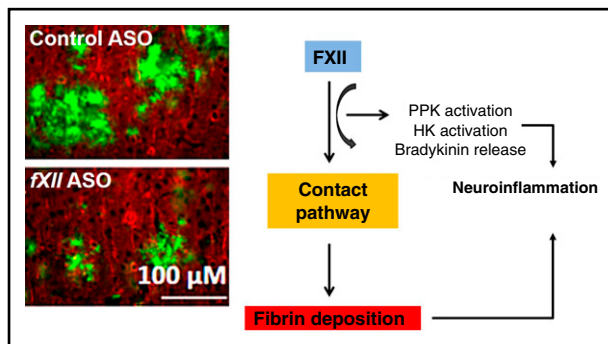
vasculitis, stroke, brain trauma, and multiple sclerosis.³ Importantly, fibrin has a central role in AD pathogenesis by promoting neurodegeneration and neuroinflammation and impairing cognitive function.⁶⁻⁸ Indeed, inhibiting the interaction between amyloid beta (A β) and fibrinogen rescues cerebrovascular occlusion and cognitive impairment in vivo.⁶ It remains, however, unclear how the upstream components of the coagulation cascade affect neuroinflammatory disease pathogenesis.

In their study, Chen et al further elucidated the role of FXII in AD pathogenesis. FXII drives the contact system, which initiates procoagulant and proinflammatory pathways by activating FXI and plasma prekallikrein (PPK), respectively. Previous elegant studies by the authors showed that the FXII-mediated contact pathway is activated in AD patients by A β , thereby stimulating the conversion of fibrinogen to the inflammatory fibrin.⁷ Given the dual procoagulant and proinflammatory

roles of FXII, the authors argued that this system could be activated at early AD stages. Using the TgCRND8 AD mouse model harboring three mutations in the amyloid precursor protein pertinent to A β pathology, the authors found a temporal correlation between activation of the inflammatory branch of the contact pathway and the onset of neuroinflammation, as indicated by increased cleavage of high-molecular-weight plasma kininogen (HK). Strikingly, by depleting FXII, the authors showed that plasma HK cleavage, neuroinflammation, cerebral fibrin deposition, and neurodegeneration were reduced and cognitive function was improved in the mice studied. These results provide further evidence for the role of the coagulation cascade in AD and suggest that individual members of the cascade, including FXII, may be potential therapeutic AD targets.

Given the synergy between neuroinflammation and vascular pathology in AD pathogenesis, this study raises the important question how they are linked mechanistically. The authors asked first whether a temporal correlation exists between HK cleavage/contact system activation and AD progression. By measuring plasma intact HK (HKi) levels in TgCRND8 mice at different disease stages, they found that these were already decreased in 3-month-old mice, indicating increased HK cleavage starting at early A β pathology. These actions were accompanied by increased astrogliosis and microgliosis, although the latter became significant only at 6 months of age. Whether this differential temporal regulation between the HK and microgliosis data indicates that the temporal correlation between changes in HK cleavage and onset of microglia activation is actually less pronounced than suggested by the authors or is due to the authors' use of Iba-1 instead of the more sensitive CD11b as a marker of microglia activation in these specific experiments is unclear. Interestingly, by depleting plasma FXII with antisense oligonucleotide (ASO)-mediated messenger RNA knockdown, levels of both HKi and PPK in the treated transgenic mice were similar to those in the wild-type controls. These findings indicate that FXII activation indeed mediates the proinflammatory branch of the contact system in an AD-like mouse model, thus promoting neuroinflammatory responses.

In a subsequent set of experiments, the authors treated 4-month-old TgCRND8 mice



Coagulation FXII has an essential role in inflammation-mediated neuronal damage and cognitive impairment in an AD mouse model. Similar to depletion of fibrinogen, depletion of FXII decreases neuroinflammatory responses and subsequent brain pathology. Histological images have been adapted from Figure 6 in the article by Chen et al that begins on page 2547.

with FXII ASO for 2 months. This treatment paradigm reduced cerebral inflammation and fibrin deposition but, importantly, also decreased neuronal damage and improved cognitive performance in the Barnes maze and contextual fear-conditioning paradigm. These results underline the strong links that exist among the coagulation cascade, neuroinflammation, and neuronal damage (see figure).³ Nevertheless, the nature of the fibrin deposits found in this study (ie, whether they are intravascular clots, perivascular deposits, and/or actual parenchymal deposits associated with A β) cannot be evaluated from the data. Future studies are also warranted to determine the exact temporal effects of FXII and the downstream members of the coagulation cascade on the specific components of the neuronal circuitry.

Given that FXII binds to endothelial cells via the gC1q receptor, could FXII extravasate into the brain and bind to central nervous system (CNS) proteins?⁹ Do the different A β species in the brain activate FXII to a different extent than those in the circulation? These are some pertinent questions raised by the exciting findings of this study. As the authors suggest, activation of FXII ultimately leads to release of bradykinin, which may increase blood–brain barrier permeability. Therefore, entry of FXII into the CNS, similar to that which is well established for fibrinogen, is likely. Determining the presence and fate of FXII in the CNS may thus reveal unexpected and highly interesting novel binding interactions between FXII and CNS proteins. As for the latter question, the group previously showed in vitro that oligomeric A β ₄₂ in plasma activates FXII more potently than that observed for its monomeric and fibrillar forms.⁷ However, it is not known how the aggregation state of A β ₄₂ and possibly of other A β species determines the activation of FXII in vivo, especially in the critical brain milieu.

This exciting study by Chen and colleagues positions FXII among the key players of the coagulation cascade that contribute to AD pathogenesis by instigating neuroinflammatory responses. It shows encouraging promise for FXII and other members of the coagulation cascade as potential, highly needed novel therapeutic targets in neurodegenerative diseases.

Conflict-of-interest disclosure: K.A. served as a consultant for Lundbeck and is a named inventor on patents and patent applications related to

fibrin. M.M. declares no competing financial interests. ■

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Comment on Du et al, page 2570

Pirfenidone: a breath of fresh air for cGVHD

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In this issue of *Blood*, Du et al¹ show that pirfenidone has remarkable therapeutic efficacy in bronchiolitis obliterans (BO) models of chronic graft-versus-host disease (cGVHD). Their data support the use of pirfenidone as a new agent in early-phase clinical trials for cGVHD treatment.

CGVHD is a frequent and lethal complication arising in patients that receive allogeneic hematopoietic stem cell transplants (HSCTs). Earlier it was widely thought that graft-versus-host disease (GVHD) that presented within 100 days posttransplant was acute GVHD (aGVHD), whereas GVHD that arose past the “100-day” mark was cGVHD. This arbitrary cutoff is now accepted to be an oversimplification, with the knowledge that symptoms of cGVHD can overlap and coexist in time with aGVHD. A number of studies in the recent past have shown that cGVHD is initiated by T cells, predominantly the T-helper 17 (Th17)/IL-17–secreting CD8+ T (Tc17) cells with involvement of the follicular T-helper cells that foster aberrant and elevated germinal center reactions ultimately promoting both allo- and auto-antibody production.²

Clinically, cGVHD is characterized mainly by scleroderma, BO, and oral lichen planus–like lesions.^{2–4} There is significant morbidity and mortality associated with cGVHD; in particular, patients with BO have a 5-year mortality rate of 41%. These poor results underscore the need to develop effective anti-cGVHD therapies.⁵

Pirfenidone is an oral antifibrotic therapy that was recently approved by the US Food and Drug Administration for the treatment of idiopathic pulmonary fibrosis, where it has been shown that pirfenidone reduces disease progression and increases lung function in humans.⁶ Several publications have also shown the protective effect of pirfenidone in murine lung allotransplant/pulmonary fibrosis models.⁷ Given these complementary findings, it makes perfect sense to investigate whether pirfenidone would have any therapeutic benefit