Microglial activation in Alzheimer’s disease (AD): In 1907, Dr. Alois Alzheimer, a Bavarian-born German psychiatrist and neuropathologist, published an article describing the clinical and neuropathological features of an unclassified psychiatric disorder. The disorder was later named AD and is currently the most common brain disorder (Takata et al., 2021). AD involves the accumulation of amyloid-β (Aβ) and hyperphosphorylated tau proteins in the brain, which are associated with senile plaques and neurofibrillary tangles, respectively (Vergara et al., 2019; Takata et al., 2021). AD is characterized by cognitive impairment and memory loss with hippocampal neurodegeneration (Kim et al., 2021).

The accumulation of neurotoxic forms of Aβ and hyperphosphorylated tau are now well-known pathophysiological hallmarks of AD (Vergara et al., 2019; Kim et al., 2021; Takata et al., 2021). However, clinical trials conducted with pharmaceutical agents that target Aβ or tau have failed to demonstrate efficacy in treating AD. These trials have shown only modest and temporary relief from symptoms but no cure for this disease (Kim et al., 2021). Thus, alternative therapeutic targets to prevent and treat AD must be investigated.

In 1919, the enigmatic phagocytic cells detected in the brain under neurodegenerative conditions were identified as microglia by Dr. Pío del Río-Hortega (Sierra et al., 2016; Takata et al., 2021). Since then, many researchers have been trying to understand the relationship between microglial function and human brain disorders. Many are studying whether control of microglial activation can be used to treat neurotoxic inflammation associated with neurodegenerative diseases. Moreover, although the etiology of AD remains unclear, accumulating evidence suggests that systemic inflammation and related vascular dysfunction play important etiological roles in AD and precede its clinical manifestation (Choi et al., 2005; Chung et al., 2020; Kim et al., 2010, 2021; Takata et al., 2021). When stimulated by activators, microglial cells undergo phagocytic morphological changes indicated by enlarged cell bodies and short processes. Invading pathogens promote the activation of microglial cells, producing pro-inflammatory mediators including various cytokines, chemokines, inducible nitric oxide synthase, and cyclooxygenase-2. This ultimately leads to neurotoxicity and neurodegenerative disease progression (Choi et al., 2005; Kim et al., 2010, 2012; Chung et al., 2020; Takata et al., 2021). Furthermore, activated microglial cells can produce reactive oxygen species, such as O₂⁻ and O₂⁻-derived oxidants, by activating nicotinamide adenine dinucleotide phosphate oxidase. Reactive oxygen species are also involved in neuroinflammatory processes causing neurodegeneration (Choi et al., 2005; Leem et al., 2016; Chung et al., 2020; Takata et al., 2021) and cooperatively drive the pathology observed in the AD brain. Altogether, it is essential to examine the endogenous molecules and pathogenic mechanisms associated with microglial activation that cause neurotoxic brain inflammation. Further understanding is crucial for developing a useful treatment strategy for AD.

Prothrombin kringle-2 (pKr-2) and microglial activation in the adult brain: pKr-2, a domain of prothrombin, is generated by active thrombin originating from the cleavage of prothrombin (Kim et al., 2010, 2021). The actions of pKr-2 are well-established in reference to angiogenesis and clotting activity mediated by thrombin (Dasgupta and Thiagarajan, 2007; Kim et al., 2010; Leem et al., 2016). Although there are few reports on the role of pKr-2 in the CNS, studies have reported elevated levels of both prothrombin and thrombin in the postmortem brains of patients with neurodegenerative diseases (Choi et al., 2005; Leem et al., 2016). Increased levels of prothrombin and thrombin may be caused by blood-brain barrier leakage. This suggests that pKr-2 levels could be upregulated in the adult brain with neurodegenerative diseases (Kim et al., 2010; Leem et al., 2016). Recently, we reported that pKr-2 was upregulated in the postmortem substantia nigra (SN) of patients with PD. We also found that its upregulation induced microglial activation, resulting in neurodegeneration in the SN of murine brains (Kim et al., 2010; Shin et al., 2015; Leem et al., 2016). In previous studies, pKr-2 administration triggered microglial activation in the SN of the murine brain and increased the expression levels of neurotoxic cytokines including interleukin-1β, tumor necrosis factor-α, and interleukin-6, inflammatory mediators such as inducible nitric oxide synthase and cyclooxygenase-2, and toll-like receptor 4, initiating the activation of the innate immune response by recognizing pathogens as a pattern-recognition receptor (Kim et al., 2010; Shin et al., 2015; Leem et al., 2016). Furthermore, pKr-2-activated microglia produced O₂⁻ and O₂⁻-derived oxidants, resulting in neurodegeneration in the murine cortex through the activation of nicotinamide adenine dinucleotide phosphate oxidase (Leem et al., 2009). Contrarily, control of microglial activation in toll-like receptor 4-deficient mice contributed to protecting the postsynaptic dopaminergic system from pKr-2 administration in vivo (Shin et al., 2015; Leem et al., 2016). The previous results suggest that pKr-2 expression can be increased in the lesioned brain, and its upregulation may be associated with neurotoxic effects following microglial activation in the adult brain with AD.

BBB disruption and neurodegeneration due to pKr-2 upregulation in the hippocampus of the adult brain in AD: Cerebrovascular alteration leading to BBB disruption is one of the pathological hallmarks in the hippocampi of patients with early AD (Merlini et al., 2019; Kim et al., 2021). BBB leakage results in the infiltration of circulating substances such as thrombin and fibrinogen into the brain parenchyma (Merlini et al., 2019; Kim et al., 2021). The upregulation of blood proteins in the hippocampus could contribute to microglial activation and neurodegeneration with cognitive decline in the adult murine brain (Merlini et al., 2019; Kim et al., 2021). This suggests that efforts to develop therapeutic and preventive agents for neurodegenerative diseases such as AD and PD can be aimed at suppressing microglial activation and its pathogenic mechanisms. It is, therefore, essential to examine the endogenous molecules and pathogenic mechanisms associated with microglial activation that leak through the BBB as well as the mechanisms causing neurotoxic events in the adult brain. We previously reported that the administration of pKr-2 could induce microglial activation and neurodegeneration in the nigrostriatal dopaminergic system of the murine brain. We observed a significant increase in pKr-2 expression in the SN of patients with PD compared with that in age-matched controls (Kim et al., 2010; Shin et al., 2015; Leem et al., 2016). However, the primary source and cause of pKr-2 upregulation in the adult brain remain to be clarified, and so far, no reports have described the role of pKr-2 in neuroinflammatory mechanisms causing hippocampal neurodegeneration in AD.

In a recent study, we measured the protein levels of pKr-2 in postmortem hippocampal tissues of patients with AD and compared these with those of age-matched controls. We also examined microglial activation and neurodegeneration after pKr-2 administration in the hippocampus of the mouse brain in vivo. In addition, we investigated whether pKr-2 upregulation could be caused by BBB disruption in five familial AD (5 x FAD) mice and whether the
Inhibition of its upregulation diminishes the pathological processes (Kim et al., 2021). Experimental results demonstrated a significant increase in pKr-2 expression. Levels of neuroinflammatory factors were measured in the postmortem hippocampi of patients with AD compared to those in age-matched controls. In addition, increases in neuroinflammatory molecules induced by microglial activation following pKr-2 upregulation contributed to hippocampal neurodegeneration and object cognitive impairments in adult mice (Kim et al., 2021). Caffeine supply or treatment with rivaroxaban inhibits pKr-2 production as an inhibitor of factor Xa associated with thrombin production. Interestingly, BBB reinforcement afterward inhibited pKr-2 upregulation and neurotoxic symptoms, such as neuroinflammation, neurodegeneration, and object cognitive impairments, in 5 × FAD mice with no effect on the levels of Aβ in the hippocampi (Kim et al., 2021). In addition to the upregulation of pKr-2 expression in patients with AD, these experimental results demonstrated that pKr-2 is an important pathogenic factor causing neurotoxic inflammation in the adult brain. Its upregulation could be induced by BBB disruption in neurodegenerative diseases. It is worth noting that pKr-2 did not affect Aβ oligomerization and accumulation in the hippocampi of either wild-type or 5 × FAD mice (Kim et al., 2021).

**Conclusion:** Although pKr-2 levels increased in neurodegenerative diseases, the major source and cause of pKr-2 increase in the adult brain remain unclear. We recently demonstrated that the penetration of prothrombin and thrombin into the hippocampus following BBB disruption could cause pKr-2 upregulation in the brain of 5 × FAD mice in vivo. This upregulation did not affect Aβ oligomerization and accumulation, but it did contribute to loss of neuronal nuclei expression and elevated neuroinflammation by activating microglia. Moreover, neuroinflammation caused by pKr-2 upregulation resulted in hippocampal neurodegeneration and object recognition decline in 5 × FAD mice (Kim et al., 2021). This is depicted in the schematic of pKr-2-induced neurodegeneration in the hippocampus of the AD brain (Figure 1). Aβ accumulation and other endogenous factors, such as thrombin and fibrinogen, certainly contribute to neurotoxicity through the induction of neuroinflammation. However, we assert that microglial activation by pKr-2 upregulation resulting from BBB disruption is a critical neurotoxic mechanism in the hippocampus of the adult brain. This contributes to neurodegeneration and object cognitive impairments via neuroinflammatory responses. Furthermore, the control of pKr-2 upregulation, which could involve BBB reinforcement, can enhance the treatment of AD and related conditions.

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This work was supported by the National Research Foundation of Korea (NRF-2020R1A2C2007954) and the Korea Healthcare Technology R&D (HI21C1795) grants funded by the Korean government.

**Figure 1** Schematic of prothrombin kringle-2 (pKr-2) upregulation and neurodegeneration by blood-brain barrier (BBB) disruption in the hippocampus with Alzheimer’s disease (AD). As demonstrated in five familial AD mice (Kim et al., 2021), pKr-2 upregulation can be caused by the penetration of prothrombin and thrombin after BBB disruption in AD brain, and its upregulation contributes to microglial activation and neurodegeneration in the hippocampus with AD, suggesting that the control of pKr-2 upregulation can be useful for preventing and treating AD and related conditions. ROS: Reactive oxygen species.