Dysfunction of TGF-β signaling in Alzheimer's disease

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Accumulation of β-amyloid peptide (Aβ) in the brain is believed to trigger a complex and poorly understood pathologic reaction that results in the development of Alzheimer's disease (AD). Despite intensive study, there is no consensus as to how Aβ accumulation causes neurodegeneration in AD. In this issue of the *JCI*, Tesseur et al. report that the expression of TGF-β type II receptor (TβRII) by neurons is reduced very early in the course of AD and that reduced TGF-β signaling increased Aβ deposition and neurodegeneration in a mouse model of AD (see the related article beginning on page 3060). Intriguingly, reduced TGF-β signaling in neuroblastoma cells resulted in neuritic dystrophy and increased levels of secreted Aβ. Collectively, these data suggest that dysfunction of the TGF-β/TβRII signaling axis in the AD brain may accelerate Aβ deposition and neurodegeneration.

Alzheimer's disease (AD) is the most common cause of dementia occurring in the elderly. It is characterized pathologically by the deposition of β-amyloid peptide (Aβ) in plaques, the development of neurofibrillary tangles (NFTs), and loss of synapses and neurons. Although age is strongly associated with nonautosomal forms of AD, it is not clear whether Aβ accumulation is promoted by changes in the aging brain or whether this is simply a stochastic process associated with aging. Aβ aggregation may cause neurodegeneration through multiple pathways. It has been hypothesized that neurodegeneration results from a chronic inflammatory response to deposited amyloid (1, 2). Alternatively, the various forms of Aβ aggregates may be directly neurotoxic (3, 4). Indeed, a great deal of recent research has focused on small, soluble aggregates of Aβ peptides, termed *Aβ oligomers*, which can directly alter synaptic function (5, 6). Others have postulated that intracellular deposits of Aβ contribute to AD neurodegeneration (7).

One of the major obstacles limiting our understanding of Aβ-induced pathologies has been the failure to recapitulate a complete AD phenotype in mice in which brain Aβ deposits are observed. Although they recapitulate many features of AD, including the plaque-associated reactive gliosis and neuritic alterations, in the absence of additional manipulations they do not show robust neurodegeneration, irreversible memory loss, or NFT formation. Some AD researchers have used such data to argue that Aβ accumulation does not cause AD. However, many in the field believe that physiologic differences between humans and mice might underlie the lack of a complete AD phenotype in mice in which brain Aβ deposits are observed.

Reduced TGF-β signaling in AD

The study by Tesseur et al. (8) in this issue of the *JCI* provides an elegant example of how identification of molecular changes in the brains of AD patients can be used to guide modeling studies and thereby provide insight into factors that may contribute to neurodegeneration and Aβ accumulation in AD. These studies, which focused on the TGF-β/TGF-β type II receptor (TGF-β/TβRII) signaling pathway, demonstrate a role for decreased neuronal TGF-β signaling in age-dependent neurodegeneration and Aβ deposition both in human AD and in AD mouse models. TβRII is a high affinity serine/threonine receptor for TGF-β that signals as part of a complex with activin-like kinase 5 (ALK5; also known as TGF-β type I receptor). The authors performed a detailed analysis of TβRII levels in AD brain tissue and showed that the levels of TβRII are reduced early in the course of the disease. Reduced TβRII levels were found exclusively in AD brain tissue, not in brain tissue affected by any of the several other neurodegenerative conditions analyzed. TGF-β signaling in the brain confers neuroprotection in part by regulating levels of neurotrophins (9); thus, reduced TβRII levels indicate a likely dysfunction in TGF-β–mediated neuroprotective signaling events in the AD brain. Reduced TGF-β signaling, therefore, may lead to neurotrophic factor deficiencies and thus neuronal dysfunction.

Modeling TGF-β signaling in AD mouse models

To further explore the role of TGF-β signaling dysfunction in AD, the authors examined the effects of reducing TβRII signaling by inducibly expressing a kinase-deficient TβRII transgene (TβRIIΔk) in the brains of mice or transiently expressing this kinase-dead receptor in neuroblastoma cells (8). TβRIIΔk expression in the brains of mice resulted in age-dependent neurodegeneration including synaptic loss, dendritic alterations, and neuronal loss. Moreover, expression of TβRIIΔk in human amyloid precursor protein (hAPP) mice significantly enhanced Aβ deposition at 20 months of age but not earlier (i.e., in mice aged 14 months or less). Increases in Aβ deposition did not appear to be attributable to increased production of hAPP, changes in Aβ-degrading enzymes, changes in apoE expression or involvement of microglia, or increased production of Aβ in the young mice. However, TβRIIΔk expression in neuroblastoma cells resulted in beading of neurites, neurite retraction, and rounding of cell bodies — all characteristic features of neurodegeneration — and also increased Aβ production. The increase in Aβ level was associated with increased levels of β-secretase–derived, APP–processing intermediates, suggesting that the TβRIIΔk-induced increase in Aβ level is attributable to enhanced amyloido-

Nonstandard abbreviations used: Aβ, β-amyloid peptide; AD, Alzheimer's disease; ALK5, activin-like kinase 5; hAPP, human amyloid precursor protein; TβRII, TGF-β type II receptor; TβRIIΔk, kinase-deficient TβRII.

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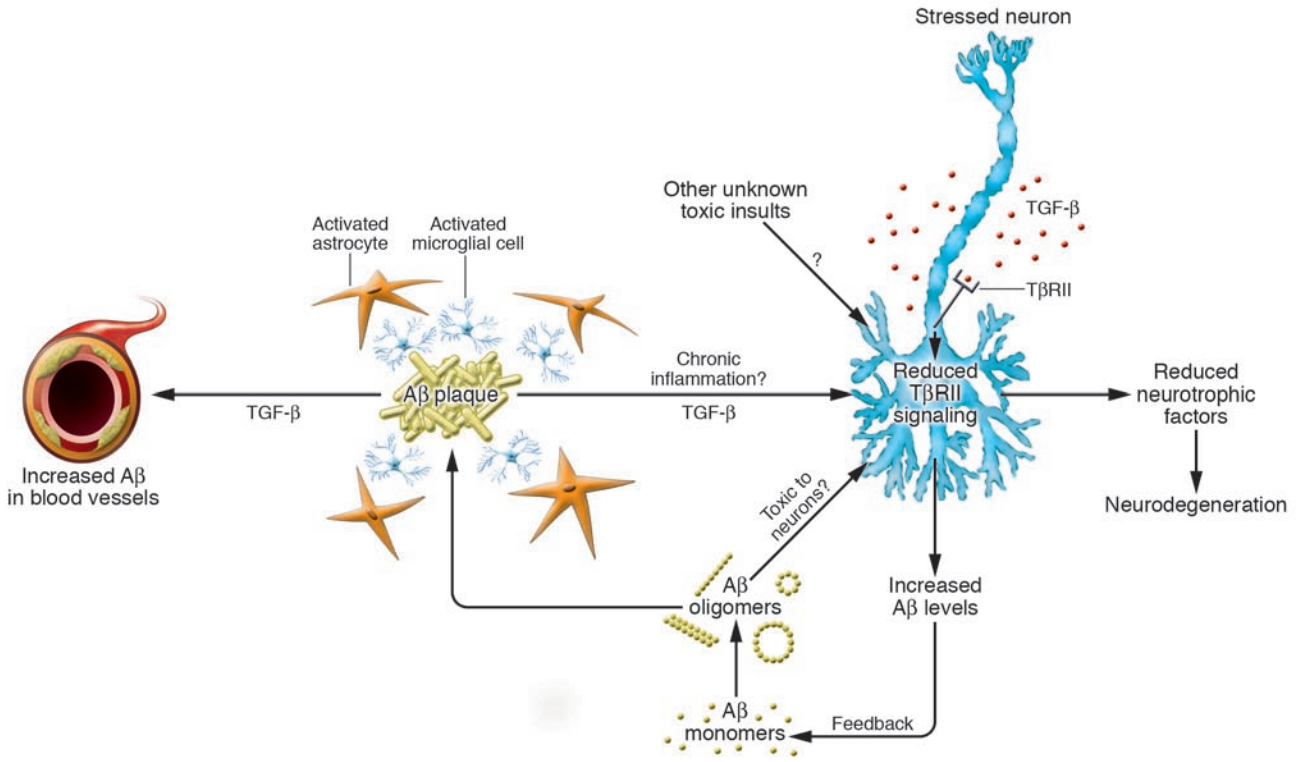

Figure 1
TGF-β signaling in AD. Aβ accumulates in the AD brain as amyloid within senile plaques and other smaller, soluble aggregates (oligomers). Aβ plaques are associated with a chronic inflammatory state, including reactive astro- and microgliosis and increased production of numerous inflammatory proteins. Chronic inflammation is hypothesized to induce a complex set of changes in neurons that may ultimately lead to neurodegeneration. The neuroprotective cytokine TGF-β is increased in AD and may reduce deposition of Aβ as plaques while enhancing deposition in the cerebral vessels. In this issue of the *JCI*, Tesseur et al. (8) show that there is reduced TGF-β signaling in the AD brain, which in turn may promote neuronal degeneration by suppression of neurotrophic factor expression or dysfunction of other unknown neuroprotective pathways. Decreased TGF-β signaling in neurons may also increase Aβ levels, enhancing amyloid deposition. Thus, the inflammatory response to Aβ deposits may initiate a positive feedback loop that exacerbates, rather than ameliorates, AD pathology.

genic processing of hAPP. Collectively, these studies suggest that defects in TGF-β signaling may contribute to AD pathogenesis by promoting neurodegeneration and initiating a feedback loop in which the degenerating cell produces more Aβ, thereby enhancing amyloid deposition (Figure 1). At present it is not clear what causes the downregulation of TβRII signaling in AD. Levels of TGF-β and other cytokines are known to be elevated in the AD brain. It is possible that TβRII levels may be downregulated in AD neurons either directly, in response to increased TGF-β levels, or indirectly, in response to other cytokines and/or factors. TβRII downregulation has been observed in a mouse model of focal ischemia (10) and certain cancer cells (11), and one study demonstrated TGF-β–dependent downregulation of TβRII levels (12). It is also not clear precisely how reduced TGF-β signaling alters Aβ processing or whether the observed increase in Aβ production accounts for the increase in deposition in the 20-month-old TβRIIΔk/hAPP mice.

Although there are extensive examples in the literature of the deleterious effects of TβRII signaling loss in cells (e.g., TβRII knockout in T cells results in uncontrolled T cell proliferation and autoimmune disease; ref. 13), to our knowledge this is the first report to show neurodegeneration due to loss of TGF-β signaling (8). TGF-β expressed by neurons can protect neurons from CNS inflammation and injury (14) and also play a pivotal role in regulating neuronal development and survival (15). Thus, the results of the present study together with the previous finding of this group that TGF-β can modulate amyloid deposition (16) indicate that reestablishment of TGF-β signaling may be a novel therapeutic approach to AD, simultaneously targeting a neurodegenerative pathway and preventing Aβ deposition.

On a more general level, these studies highlight the growing recognition that proteins regulating immune function can have significant roles in both the normal and the diseased brain. For example, it was recently reported that an MHC class I receptor plays a critical role in neuronal plasticity (17) and that complement inhibition can enhance plaque deposition and neurodegeneration in mice (18). Future studies examining the role of immune molecules that affect normal aging and disease processes in the CNS are likely to yield novel insights into their functions and roles in CNS diseases.

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Microglia: a cellular vehicle for CNS gene therapy

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by deficiency of the enzyme arylsulfatase A (ARSA). MLD is characterized by progressive demyelination and neurological deficits. Treatment of MLD is still a challenge due to the fact that the blood-brain barrier is a major obstacle for most therapeutic substances. In this issue of the *JCI*, Biffi et al. report that genetically modified hematopoietic precursor cells transduced to overexpress ARSA and transplanted into mice with a targeted disruption of the murine *Arsa* gene (*Arsa*$^{-/-}$ mice) migrated into the CNS and cross-corrected brain ARSA deficiency (see the related article beginning on page 3070). Microglia served as a cellular vehicle to effectively deliver the enzyme to other brain cells while hepatocytes overexpressing ARSA increased plasma ARSA levels but failed to deliver ARSA into the CNS.

The blood-brain barrier: an obstacle for CNS therapy

Innate and adaptive immune responses are strongly reduced in the CNS, leading to a status of immune privilege. This is a highly regulated and adapted process in which those immune responses that would induce collateral injury to "innocent bystander" tissues are avoided while certain features of the immune response are allowed (1). This special type of immune compromise protects CNS tissue, particularly neurons and their connections, from immune-mediated damage that would threaten the survival of the host.

The blood-brain barrier (BBB) strongly contributes to the immune-privileged status of the CNS. The BBB is a unique membranous barrier that tightly segregates the brain from the circulating blood. The capillaries of the CNS form a permeability barrier and are structurally different from other tissues (Figure 1). The blood capillaries of the vertebrate brain and spinal cord are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight junctions. The tight junctions between endothelial cells result in a very high transendothelial electrical resistance of 1500–2000 Ω cm^2 in the CNS compared with 3–33 Ω cm^2 in other tissues (2, 3). Furthermore, astrocyte foot processes encapsulate the capillaries, forming a second barrier of the BBB. Therefore, only lipid-soluble molecules that can freely diffuse through the capillary endothelial membrane may passively cross the BBB. This barrier makes the CNS practically inaccessible to lipid-insoluble compounds such as small polar molecules or any larger protein.

Consequently, the BBB is an obstacle for CNS therapy and a serious bottleneck in drug development for CNS diseases. Theoretically, the amount of randomly selected drugs having bioavailability in the CNS is less than 2% of small molecules and practically 0% of large molecules (2, 3). These numbers are also reflected by the drugs currently available for CNS diseases. Of over 7,000 potential drugs in the comprehensive medicinal chemistry database, only 5% of all drugs treat the CNS (4). Interestingly, the typical CNS-active drug is very small in size with an average molecular mass of 0.36 kDa and is used for treatment of a very limited number of diseases, such as affective disorders, schizophrenia, epilepsy, and chronic pain (2, 3). Furthermore, some drugs are obtainable that utilize the active transport system of the BBB. For example, L-dihydroxyphenylalanine (L-DOPA), a dopamine-replacement therapy for Parkinson disease patients, is a substrate for the BBB transporter L–amino acid transporter-1 (5). Once it crosses the BBB, L-DOPA is decarboxylated back to dopamine via aromatic amino acid decarboxylase. Thus, L-DOPA is an example of a prodrug that traverses the biological membranes of the BBB, not via lipid mediation, but via a carrier transport system.

Nonstandard abbreviations used: ARSA, arylsulfatase A; BBB, blood-brain barrier; LSD, lysosomal storage disorder; MLD, metachromatic leukodystrophy.

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