Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect?

Dennis W. Dickson


Commentary

Increasing evidence suggests that selective neuronal loss in neurodegenerative diseases involves activation of cysteine aspartyl proteases (caspases), which initiate and execute apoptosis. In Alzheimer disease both extracellular amyloid deposits and intracellular amyloid \( \beta \) protein may activate caspases, leading to cleavage of nuclear and cytoskeletal proteins, including tau protein. Proteolysis of tau may be critical to neurofibrillary degeneration, which correlates with dementia.

Find the latest version:

http://jci.me/22317
Increasing evidence suggests that selective neuronal loss in neurodegenerative diseases involves activation of cysteine aspartyl proteases (caspases), which initiate and execute apoptosis. In Alzheimer disease both extracellular amyloid deposits and intracellular amyloid β protein may activate caspases, leading to cleavage of nuclear and cytoskeletal proteins, including tau protein. Proteolysis of tau may be critical to neurofibrillary degeneration, which correlates with dementia (see the related article beginning on page 121).

Alzheimer disease (AD), the most common cause of dementia in the elderly, is associated with senile plaques and neurofibrillary tangles (NFTs), but the relationship between these two neuropathologic lesions has been difficult to discover. Senile plaques are heterogeneous lesions composed of extracellular amyloid β protein (Aβ), dystrophic neuronal processes, and reactive glia (1), while NFTs are intracellular lesions composed of filamentous aggregates of the microtubule-associated protein tau (2). Genetic factors have implicated Aβ in the pathogenesis of AD since mutations are found in the Aβ precursor (APP) as well as in enzymes involved in the production of Aβ (reviewed in ref. 3). Moreover, genes implicated in AD by linkage studies encode proteins that degrade Aβ, such as insulin-degrading enzyme. The major genetic risk factor for late-onset AD, apolipoprotein E, promotes Aβ aggregation and colocalizes with Aβ in senile plaques.

The conundrum that has plagued research on AD is that clinicopathologic studies have not shown strong correlations between cognitive impairment and Aβ. In particular, the degree of cognitive impairment in AD is not as closely tied to the amount of amyloid deposited in the brain as it is to the amount of abnormal tau protein in the brain and the density and distribution of NFTs (4, 5). This is not entirely surprising since NFTs are composed of proteins that are part of the neuronal cytoskeleton, which supports vital structural and dynamic neuronal functions. Amyloid, on the other hand, is a relatively innocuous extracellular deposit. It has been suggested that cytoskeletal disruption may act as the proximate cause of progressive synaptic and neuronal loss by interfering with axoplasmic and dendritic transport, which starves the cell of trophic support (6). This dysfunction and eventual death of neurons manifests clinically as cognitive impairment.

A major challenge of the amyloid cascade hypothesis for AD (Figure 1), which posits that amyloid formation leads to neuronal loss and dementia (7), is determining the link between Aβ, the protein most clearly linked to the cause of AD, and tau, the protein that is most clearly associated with clinical manifestations of AD. The studies by Rissman and coworkers in this issue of the JCI (8) suggest that apoptotic mechanisms may be the missing link.

Apoptosis in AD

Apoptosis has been the focus of intense research in the last several decades as a means of controlling cell populations in normal development and inflammation through programmed cell death. Failure to control cell numbers through apoptosis is common in cancer, while excessive apoptosis is viewed to play a role in a number of neurologic disorders in addition to AD, including stroke and Parkinson disease (PD) (9).

comments

Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect?

Dennis W. Dickson

Departments of Pathology and Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA.

Increasing evidence suggests that selective neuronal loss in neurodegenerative diseases involves activation of cysteine aspartyl proteases (caspases), which initiate and execute apoptosis. In Alzheimer disease both extracellular amyloid deposits and intracellular amyloid β protein may activate caspases, leading to cleavage of nuclear and cytoskeletal proteins, including tau protein. Proteolysis of tau may be critical to neurofibrillary degeneration, which correlates with dementia (see the related article beginning on page 121).

Alzheimer disease (AD), the most common cause of dementia in the elderly, is associated with senile plaques and neurofibrillary tangles (NFTs), but the relationship between these two neuropathologic lesions has been difficult to discover. Senile plaques are heterogeneous lesions composed of extracellular amyloid β protein (Aβ), dystrophic neuronal processes, and reactive glia (1), while NFTs are intracellular lesions composed of filamentous aggregates of the microtubule-associated protein tau (2). Genetic factors have implicated Aβ in the pathogenesis of AD since mutations are found in the Aβ precursor (APP) as well as in enzymes involved in the production of Aβ (reviewed in ref. 3). Moreover, genes implicated in AD by linkage studies encode proteins that degrade Aβ, such as insulin-degrading enzyme. The major genetic risk factor for late-onset AD, apolipoprotein E, promotes Aβ aggregation and colocalizes with Aβ in senile plaques.

The conundrum that has plagued research on AD is that clinicopathologic studies have not shown strong correlations between cognitive impairment and Aβ. In particular, the degree of cognitive impairment in AD is not as closely tied to the amount of amyloid deposited in the brain as it is to the amount of abnormal tau protein in the brain and the density and distribution of NFTs (4, 5). This is not entirely surprising since NFTs are composed of proteins that are part of the neuronal cytoskeleton, which supports vital structural and dynamic neuronal functions. Amyloid, on the other hand, is a relatively innocuous extracellular deposit. It has been suggested that cytoskeletal disruption may act as the proximate cause of progressive synaptic and neuronal loss by interfering with axoplasmic and dendritic transport, which starves the cell of trophic support (6). This dysfunction and eventual death of neurons manifests clinically as cognitive impairment.

A major challenge of the amyloid cascade hypothesis for AD (Figure 1), which posits that amyloid formation leads to neuronal loss and dementia (7), is determining the link between Aβ, the protein most clearly linked to the cause of AD, and tau, the protein that is most clearly associated with clinical manifestations of AD. The studies by Rissman and coworkers in this issue of the JCI (8) suggest that apoptotic mechanisms may be the missing link.

Apoptosis in AD

Apoptosis has been the focus of intense research in the last several decades as a means of controlling cell populations in normal development and inflammation through programmed cell death. Failure to control cell numbers through apoptosis is common in cancer, while excessive apoptosis is viewed to play a role in a number of neurologic disorders in addition to AD, including stroke and Parkinson disease (PD) (9).

Increasing evidence suggests that selective neuronal loss in neurodegenerative diseases involves activation of cysteine aspartyl proteases (caspases), which initiate and execute apoptosis. In Alzheimer disease both extracellular amyloid deposits and intracellular amyloid β protein may activate caspases, leading to cleavage of nuclear and cytoskeletal proteins, including tau protein. Proteolysis of tau may be critical to neurofibrillary degeneration, which correlates with dementia (see the related article beginning on page 121).

Alzheimer disease (AD), the most common cause of dementia in the elderly, is associated with senile plaques and neurofibrillary tangles (NFTs), but the relationship between these two neuropathologic lesions has been difficult to discover. Senile plaques are heterogeneous lesions composed of extracellular amyloid β protein (Aβ), dystrophic neuronal processes, and reactive glia (1), while NFTs are intracellular lesions composed of filamentous aggregates of the microtubule-associated protein tau (2). Genetic factors have implicated Aβ in the pathogenesis of AD since mutations are found in the Aβ precursor (APP) as well as in enzymes involved in the production of Aβ (reviewed in ref. 3). Moreover, genes implicated in AD by linkage studies encode proteins that degrade Aβ, such as insulin-degrading enzyme. The major genetic risk factor for late-onset AD, apolipoprotein E, promotes Aβ aggregation and colocalizes with Aβ in senile plaques.

The conundrum that has plagued research on AD is that clinicopathologic studies have not shown strong correlations between cognitive impairment and Aβ. In particular, the degree of cognitive impairment in AD is not as closely tied to the amount of amyloid deposited in the brain as it is to the amount of abnormal tau protein in the brain and the density and distribution of NFTs (4, 5). This is not entirely surprising since NFTs are composed of proteins that are part of the neuronal cytoskeleton, which supports vital structural and dynamic neuronal functions. Amyloid, on the other hand, is a relatively innocuous extracellular deposit. It has been suggested that cytoskeletal disruption may act as the proximate cause of progressive synaptic and neuronal loss by interfering with axoplasmic and dendritic transport, which starves the cell of trophic support (6). This dysfunction and eventual death of neurons manifests clinically as cognitive impairment.

A major challenge of the amyloid cascade hypothesis for AD (Figure 1), which posits that amyloid formation leads to neuronal loss and dementia (7), is determining the link between Aβ, the protein most clearly linked to the cause of AD, and tau, the protein that is most clearly associated with clinical manifestations of AD. The studies by Rissman and coworkers in this issue of the JCI (8) suggest that apoptotic mechanisms may be the missing link.

Apoptosis in AD

Apoptosis has been the focus of intense research in the last several decades as a means of controlling cell populations in normal development and inflammation through programmed cell death. Failure to control cell numbers through apoptosis is common in cancer, while excessive apoptosis is viewed to play a role in a number of neurologic disorders in addition to AD, including stroke and Parkinson disease (PD) (9).
Enthusiasm for apoptosis, however, as a mechanism for neuronal death in AD has been tempered in recent years. The initial evidence for apoptosis in AD came from cell culture experiments that were not always physiologically relevant, including exposure of cells to very high concentrations of Aβ or to Aβ peptides that do not exist in nature. Evidence for frank cellular apoptosis in AD is controversial, but there is growing recognition that apoptotic mechanisms may play a role in disease pathogenesis in the absence of overt apoptosis (10). Apoptosis is an attractive mechanism for neuronal death in neurodegenerative diseases for several reasons. Neuronal death in degenerative diseases is selective at the individual cell level and not associated with inflammation. In AD, neuronal loss is prominent in the cerebral cortex and the limbic lobe, while different neuronal populations are vulnerable in other neurodegenerative diseases. For example, in PD, brainstem monoaminergic neurons are affected. Cellular death via apoptosis is not associated with disruption of the cell membrane, and clearance of cellular debris is by facultative tissue phagocytes rather than professional macrophages of the reticuloendothelial system. Apoptosis also has a number of characteristic morphologic hallmarks, such as nuclear condensation and fragmentation, which have been very difficult to identify in AD brains.

Another feature of apoptosis is intranucleosomal cleavage that produces DNA nicks that can be labeled by specific techniques, such as TUNEL. Initial reports of extensive neuronal TUNEL labeling in AD have been difficult to confirm, and much of the labeling appears to be related to damage to DNA that occurs as a postmortem event.
artifact (11). Another approach to studying apoptosis is to examine enzymes that are involved in mediating programmed cell death. About a dozen such proteases have been identified; they share the property of being cysteine aspartyl proteases and are referred to as caspases. Caspases participate in apoptosis through initiation of intracellular cascades and in executing the final outcome, including proteolytic cleavage of cytoskeletal proteins and proteins of the nuclear scaffold. Both membrane (e.g., spectrin) and cytosolic (e.g., intermediate filament) cytoskeletal proteins are targets of caspase cleavage. Apoptosis is initiated through intracellular mechanisms that often involve alterations in mitochondria or endoplasmic reticulum and by signaling through cell membrane death receptors—the so-called intrinsic and extrinsic apoptotic pathways (12) (Figure 2).

Activation of caspases has been reported in AD using antibodies that are specific to the activated forms of the enzymes, which themselves are activated by proteolysis (13). Increasingly, apoptotic mechanisms are viewed to play a role in neurodegeneration. The two major pathways to cellular apoptosis are intrinsic and extrinsic. The extrinsic pathway involves signaling through cell surface death receptors, such as the TNF receptor, which are regulated by decoy receptors and Fas-associated death domain–like interleukin-1β–converting enzyme inhibitory proteins (FLIPs). Direct binding of Aβ or Aβ oligomers to death receptors remains to be shown, but the pattern of activation of downstream caspases (e.g., caspases 2 and 8) supports involvement of the extrinsic pathway in Aβ-mediated apoptotic processes. Alternatively, intracellular Aβ produced in the ER may lead to ER stress, or binding of Aβ to a mitochondrial alcohol dehydrogenase may lead to mitochondrial stress. Both entries into the intrinsic pathway may activate downstream apoptotic mechanisms. While details of the upstream mechanisms and mediators remain to be defined, activated executioner caspases 3 and 7 are capable of cleaving tau protein, which may favor formation of NFTs. Asterisk indicates possible sites of action of Aβ. Caspases 1 and 5 are involved in cytokine activation.

**Table 1**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Proteases</th>
<th>Proteolytic products</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>α-secretase, β-secretase</td>
<td>sAPPα, sAPPβ</td>
<td>Alzheimer disease and aging</td>
</tr>
<tr>
<td></td>
<td>α- and γ-secretases, Caspase 3</td>
<td>Aβ1-40/42 (P3), Aβ1-40/42, Aβ11-40/42 C31</td>
<td></td>
</tr>
<tr>
<td>Tau</td>
<td>Caspases 3 and 7, Calpain</td>
<td>N-terminal fragment (Δtau), Multiple truncated species</td>
<td>Alzheimer disease and taudopathies</td>
</tr>
<tr>
<td>α-Synuclein</td>
<td>Unknown</td>
<td>N- and C-terminal truncation</td>
<td>Parkinson disease and Lewy body dementia</td>
</tr>
</tbody>
</table>

APP is cleaved by a number of different proteases to generate a range of peptide fragments that are found in the brain in AD and pathological aging. APP is cleaved by α-secretase to generate secreted APPα (sAPPα) and a carboxy-terminal fragment. Further cleavage of this fragment by γ-secretase, generates Aβ1-40/42, which is also referred to as P3. Secreted forms of APP have neurotrophic and neuroprotective properties. Similarly, β-secretase generates secreted APPβ (sAPPβ) and a carboxy-terminal fragment that upon further cleavage by γ-secretase generates Aβ. Heterogeneity of cleavage by both β-secretase and γ-secretase generates a family of peptides starting at residues 11 and 11 and ending at residues 40 and 42. APP is a target for caspase cleavage, which produces a carboxy terminal 31 amino acid product as well. Tau cleavage by caspases 3 and 7 generates a protein truncated at residue Asp421 in the carboxy half of the molecule, which has been referred to as Δtau. Calpains also cleave tau at a number of sites, depending on the enzyme concentration and the extent of proteolysis. Tau aggregates are characteristic not only of AD, but also of a family of neurodegenerative disorders referred to as the taudopathies. The protein that accumulates in Lewy bodies in Parkinson disease and dementia with Lewy bodies, α-synuclein, is also subject to cleavage; however, the responsible proteases remain to be determined.

**Figure 2**

The interface of the two major molecules implicated in AD pathogenesis with molecular mechanisms of apoptosis. The two major pathways to cellular apoptosis are intrinsic and extrinsic. The extrinsic pathway involves signaling through cell surface death receptors, such as the TNF receptor, which are regulated by decoy receptors and Fas-associated death domain–like interleukin-1β–converting enzyme inhibitory proteins (FLIPs). Direct binding of Aβ or Aβ oligomers to death receptors remains to be shown, but the pattern of activation of downstream caspases (e.g., caspases 2 and 8) supports involvement of the extrinsic pathway in Aβ-mediated apoptotic processes. Alternatively, intracellular Aβ produced in the ER may lead to ER stress, or binding of Aβ to a mitochondrial alcohol dehydrogenase may lead to mitochondrial stress. Both entries into the intrinsic pathway may activate downstream apoptotic mechanisms. While details of the upstream mechanisms and mediators remain to be defined, activated executioner caspases 3 and 7 are capable of cleaving tau protein, which may favor formation of NFTs. Asterisk indicates possible sites of action of Aβ. Caspases 1 and 5 are involved in cytokine activation.
be more relevant. Recent studies have drawn attention to the possible role of intracellular Aβ in neurodegeneration (16, 17). Accumulation of Aβ in endoplasmic reticulum or endosomes, where it may be synthesized, may activate apoptotic mechanisms through the unfolded protein response or endoplasmic reticulum stress. Alternatively, intracellular Aβ may bind to alcohol dehydrogenase within mitochondria and activate apoptosis through mitochondrial stress (18).

One of the consequences of caspase activation as discussed by Rissman and coworkers (8), as well as in the previous studies by Gamblin and others (19), is cleavage of tau protein. This is a significant finding because, in order for tau to form fibrils similar to those in NFTs, a number of conditions must be met (20). Fragments of tau, particularly those that contain the microtubule-binding domain, which is critical for self-interaction, more readily aggregate into fibrils than full-length tau. Other substances contribute to efficient fibril formation, particularly polyansions such as heparan sulfate proteoglycans, RNA, or arachidonic acid.

**Tau proteolysis and neurofibrillary degeneration**

The role of conformational changes in tau is also considered by some to be critical (21). Since tau is a natively unfolded molecule, its crystal structure is unknown and most current ideas about conformational changes in tau are based upon speculation rather than any hard structural data. Conformational changes in tau are inferred from antibodies that react with tau in solution-based assays or with pathologi- cal tau in immunohistochemistry, but not with denatured tau in Western blots. The epitopes of these putative conformational antibodies may be discontinuous, and this is the case for the epitope recognized by MC1 (21, 22), which is the monoclonal antibody used by Rissman and coworkers (8) to detect abnormal forms of tau protein. The recognition of this tau epitope by MC1 is dependent on specific amino- and carboxyl-terminal truncated forms of α-synuclein and tau are subject to proteolysis in AD, since amino- and carboxyl-terminal truncated forms of α-synuclein are enriched in diseased brains (28). As with AD, it remains to be determined with certainty if the proteolytic events are necessary and early events that lead to neurodegeneration. Alternatively, they may be secondary processes in degenerating neurons subjected to activation of apoptotic mechanisms or flooding of intracellular compartments by calcium due to other undetermined pathogenic processes.

Address correspondence to: Dennis W. Dickson, Department of Pathology (Neuropathology), Mayo Clinic, 4500 San Pablo Road, Jacksonville, Florida 32224, USA. Phone: (904) 953-7137; Fax: (904) 953-7117; E-mail: dickson.dennis@mayo.edu.