Mechanisms of Neurodegenerative Disorders

Part 1: Protein Aggregates

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Despite a vast array of causes of neurodegenerative diseases, research has identified common pathways through which the neurodegeneration proceeds. Part 1 of this neurological review article will address a confluence of research suggesting that neurodegeneration is often linked with the accumulation of insoluble protein aggregates; part 2, the mechanisms through which neurodegeneration occurs—apoptosis, necrosis, and excitotoxicity. Along with the common mechanisms for inducing neurodegeneration, we will also review common defense mechanisms that protect against toxic insults. Each of these pathways offers potential targets for pharmaceutical intervention.

ACCUMULATION OF PROTEIN AGGREGATES AS A PRIMARY DISEASE-INITIATING EVENT

One of the striking findings of neurodegeneration research is the observation that most of the proteins implicated in disease have a strong propensity to aggregate. For instance, neuritic senile plaques and neurofibrillary tangles are the hallmarks of Alzheimer disease (AD), Lewy bodies accumulate in Parkinson disease, and Pick bodies are prevalent in Pick disease. Even in diseases in which inclusions were not initially recognized as a major part of the illness, recent use of immunochemical techniques has identified inclusions. Nuclear aggregates appear in Huntington disease, modified prion protein accumulates in Creutzfeld-Jacob disease, and superoxide dismutase accumulates in familial amyotrophic lateral sclerosis. In each of these cases, the inclusion is composed of a protein that is intimately connected with the cause of the illness. We know that the proteins that accumulate in inclusions are capable of causing neurodegeneration because in each case particular mutations in these proteins cause familial forms of the disease. This shows that alterations in the biology of the protein are sufficient to drive the disease process. Although in some cases the formation of large aggregates of the particular protein might not actually be causative, it is clear that in all cases aggregates of relevant proteins are associated with disease. Thus, aggregation is a central aspect of the biology of many neurodegenerative diseases, but the role of aggregates in neurodegeneration is continuing to be elucidated.

MECHANISMS OF AGGREGATION: INSIGHTS FROM β-AMYLOID (Aβ)

The kinetics of aggregation are analogous to the kinetics of crystalization. In both aggregation and crystalization, the rate determining step is forming the seed for the aggregate. The significance of seeding in aggregation is seen to be analogous to the kinetics of the crystalization. Seeding is so important that supersaturated solutions can exist stably in absence of a nucleus; however, once seed crystals are added, crystallization rapidly ensues. Once the seeding process occurs, concentration determines the subsequent rate and amount of crystalization. Similar kinetics seem to apply to aggregation in neurodegenerative illness. The role of aggregation in disease is best understood for AD, where aggregation of Aβ seems to drive the illness. The predominant species of Aβ is 40 amino acids long (Aβ40) and has a moderate tendency to aggregate. Although Aβ aggregates, the in vitro process requires several days to occur, even for a concentrated solution of Aβ40. Any factor that speeds this aggregation process will ultimately
speed the illness. The first critical factor is seeding. Just as crystalization can be stimulated by seeding, so can Aβ aggregation. About 5% of Aβ is produced as a 42- to 43-residues-long peptide, termed “Aβ42/3.” The salient feature of Aβ42/3 is that it rapidly aggregates and often provides the seed for further Aβ aggregation. Thus, in AD the nidus for aggregation, and the core of neuritic senile plaques, is made up of Aβ42/3, while the periphery is made up of Aβ40. A second important factor is concentration. Any process that increases the concentration of Aβ will increase the rate of aggregation and the progression of the disease. Thus, most, if not all, of the early-onset cases of familial AD are caused by mutations in amyloid precursor protein (APP) or presenilins that increase the production of Aβ42/3 or total Aβ. The increased production speeds up the kinetics of aggregation and leads to early-onset disease. β-Amyloid levels also increase with aging. The increase in Aβ might be an important factor stimulating Aβ aggregation and neuritic senile plaque formation. Once neuritic senile plaque formation is established, newly produced Aβ likely aggregates rapidly. Because of this, the concentration of soluble Aβ actually decreases in patients with AD.

Because the kinetics of aggregation is so critical, any factor that alters it affects the progression of AD. The 2 proteins that have been identified as genetic risk factors for AD, α₂-macroglobulin and the cholesterol transport protein apolipoprotein E4 (APOE4), both affect Aβ aggregation and levels. Controversy exists over the exact mechanism of action of APOE4. Initial studies on pure APOE4 suggested that APOE4 increases aggregation. This hypothesis is supported by the observation that APOE knockout mice that overexpress human APP develop fewer neuritic senile plaques than their counterparts expressing APOE. However, APOE is a lipoprotein and studies of APOE/lipid complexes, which more closely resemble the physiological state of APOE, suggest that APOE normally promotes removal of Aβ via the low-density lipoprotein–related receptor protein. In this system, APOE4 is less efficient at removing Aβ, which suggests that it might promote senile plaque formation by the removal of Aβ. Recent research using transgenic mice has demonstrated the actions of APOE on both Aβ deposition and removal. In mice overexpressing APP, APOE seems to reduce Aβ deposition initially (9 months), but then promotes its accumulation later (15 months). Work on α₂-macroglobulin parallels the latter model for APOE4. α₂-Macroglobulin is a cofactor facilitating Aβ removal, like APOE, and it is also a genetic risk factor for AD, like APOE4. Thus, the 2 putative risk factors for AD, APOE4 and α₂-macroglobulin both seem to increase the risk of AD by affecting the aggregation of Aβ.

OTHER PROTEIN AGGREGATES

Protein aggregation also seems to contribute to other neurodegenerative diseases. α-Synuclein, tau protein, prion protein, Huntington, and superoxide dismutase have all been shown to aggregate. In most cases, unless a seed is added, aggregation of these proteins is a slow process. α-Synuclein has perhaps the greatest tendency to aggregate under physiological conditions, but the aggregation process can be accelerated by adding Aβ. Our work suggests that α-synuclein is a homologue of the 14-3-3 protein, which is a protein chaperone that normally exists as a dimer. The biophysical behavior of α-synuclein is similar to that of Aβ. Both Aβ and α-synuclein rapidly aggregate on exposure to metals (B.W. and O. Stroja, MS, M. Farrer, PhD, N. Mehta, PhD, and J. Hardy, PhD, unpublished data, December 1990). Iron stimulates the aggregation of α-synuclein, which then causes iron to accumulate in cells as α-synuclein aggregates (B.W. and O. Stroja, MS, M. Farrer, PhD, N. Mehta, PhD, and J. Hardy, PhD, unpublished data, December 1990). The ability of α-synuclein to sequester iron in neurons might actually be an essential aspect of its role in neurodegeneration. Accumulation of iron would promote the Fenton reaction, which results in the formation of highly reactive hydroxyl radicals, and oxidative stress-related neurodegeneration.

Mutations in tau protein that are associated with frontotemporal dementia with parkinsonism-17 also appear to increase the tendency of the protein to aggregate, in some cases by directly stimulating aggregation and in other cases by increasing production of a 4-repeat isoform that has an increased tendency to aggregate. The aggregates of tau proteins form distinctive filaments. Unfortunately, the mechanism of aggregation is unknown for the most prevalent disease with showing Tau aggregates, AD, but the recent discovery of the frontotemporal dementia with parkinsonism-17 mutations might shed light on the mechanism of tau aggregation in AD. Native prion proteins do not aggregate, but a conformation change in the protein induces a conformational change, yielding a protein termed “PrPSc,” and initiates a process analogous to aggregation. PrPSc acts as a template for further conformational changes of native prion proteins. These PrPsc proteins then accumulate in the brain much like aggregates. In Huntington disease, a polyglutamine expansion creates a mutant Huntingtin protein that has a high tendency to aggregate. Recent studies have shown that polyglutamine aggregates accumulate in the brains of patients with Huntington disease both as intranuclear aggregates and as cytoplasmic aggregates. Because polyglutamine expansions cause several other neurodegenerative disorders, it is possible that aggregation of these proteins containing these expansions might underlie these illnesses. However, recent evidence suggests that these aggregations of Huntingtin might not be the actual trigger for cell death in Huntington disease. In amyotrophic lateral sclerosis, mutant superoxide dismutases tend to aggregate, although once again, the aggregation does not seem to drive the illness. The tendency to aggregate for each of these proteins implicated in neurodegenerative disorders emphasizes the importance of aggregation in neurodegeneration, even if we do not fully understand how aggregation leads to neurodegeneration.

THE IMPLICATIONS OF AGGREGATION FOR MOLECULAR GENETICS

The importance of aggregation creates an interesting twist on our typical understanding of molecular genetics. Many of the genetic mutations that produce neurodegenerative disease have a genetically dominant phenotype. There is
an old maxim in molecular genetics that an autosomal dominant disorder implies gain of function, while an autosomal recessive disorder implies loss of function. In the case of enzymes, mutations that cause constitutive activation lead to a genetically dominant phenotype, because the enzyme can now continuously stimulate the cell or organism. Conversely, mutations that render a protein inactive are recessive because genes in both alleles must be mutated to fully eliminate enzyme activity. It is important to realize, though, that in neurodegenerative diseases gain of biological activity, such as constitutive activation of an enzyme, is not required to produce a dominant phenotype.

This is because the new element introduced by the mutation is the tendency to aggregate, rather than a gain of a function related to the function of the native protein. The aggregates that form from these mutations might be related to pathological accumulations of misfolded proteins, termed “aggresomes.” For instance, mutated Huntington protein tends to aggregate. The aggregate is unrelated to the original function of the protein, but certainly introduces into the neuron a new element that is toxic. Thus, although the mutation in Huntington might not change its biological activity, the mutation still introduces something new into the cell—an aggregate. For these reasons, it is more accurate to refer to the mutations that cause many neurodegenerative diseases as “gain of aggregation (GAG) disease” rather than “gain of function diseases.”

TRIGGERS OF NEURODEGENERATION AND KNOWN MECHANISMS OF NERVE CELL DEATH

Although most neurodegenerative events are of multifactorial origin, the mechanisms by which aggregates produce neurotoxicity are being elucidated. The biochemical pathways leading to neurotoxicity are understood best for Aβ. Free radicals are thought to play a critical role in Aβ-mediated neurotoxicity. The formation of Aβ as aggregates and its deposition in senile plaques are believed to be a central step in the pathogenesis of AD. β-Amyloid has neurotoxic activities in vitro and under special conditions also in vivo. This neurotoxicity is mediated via the generation of oxygen free radicals and the accumulation of hydrogen peroxide (H2O2) with its fatal oxidative consequences.

β-Amyloid seems capable of inducing free radical production through multiple pathways (Figure). One pathway is by binding metals, such as zinc, copper, and iron. Binding to metals seems to stimulate aggregation of Aβ and metal chelators have been shown to prevent Aβ aggregation. Metals are important because they catalyze the conversion of H2O2 to hydroxyl radicals (•OH) through the Fenton reaction and the Haber-Weiss reaction:

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot \text{OH} + \cdot \text{OH} + \text{Fe}^{3+} \]  
(Fenton Reaction)

\[ \text{O}_{2} + \cdot \text{H}_2\text{O}_2 \rightarrow \text{O}_2^- + \cdot \text{OH} + \cdot \text{OH} \]  
(Haber-Weiss Reaction)

The ability of Aβ has important consequences, as Aβ accumulates so do metals. Thus, as Aβ and metals accumulate in neuritic senile plaques, OH production increases. Other amyloidogenic peptides including the human amylin, which is found as aggregates in Langerhans islet cell tissue, the Parkinson disease-associated α-synuclein protein, and the Creutzfeld-Jacob disease-associated prion proteins might all bind metals and induce neurodegeneration through a similar oxidative mechanisms. For instance, α-synuclein aggregates on exposure to iron or copper, and the prion protein has been shown to bind copper, and metals have been shown to stimulate aggregation of each of these proteins. If Fenton chemistry is a common pathway for multiple neurodegenerative diseases, then inhibiting free radical production might be an important pharmaceutical strategy. Potential approaches are discussed in part 2 of these neurological review articles.

Aggregates can also stimulate free radical production via receptor-mediated pathways (Figure). Binding of Aβ to specific receptors, such as RAGE (receptor for advanced glycation end products) or type 2 scavenger receptors, can induce free radical production by stimulating the activity of the reduced form of nicotinamide adenine dinucleotide oxidases. The accumulation of excitatory amino acids such as l-glutamate can induce nerve cell death by overactivation of specific glutamate receptors or by the induction of oxidative events. The latter are initiated by the competition of glutamate for the neuronal cysteine-antporter system that in a first step leads to a depletion of intracellular glutathione and in a second step to an accumulation of H2O2. The consequences are the peroxidation of membrane lipids as oxidative chain reaction and the lysis of the cells.

One final target of Aβ that is important in AD is the immune system. The Aβ40 present in neuritic senile plaques apparently activates microglia, possibly by binding type 2 scavenger receptors. Activated microglia produce large amounts of free radicals and so might contribute significantly to the free radical burden in the AD-affected brain. Clinical studies highlight the potential importance of inflammation in AD. Patients who had taken at least 1 year of high-dose anti-inflammatory
therapy have a reduced prevalence of AD. Although anti-inflammatory therapy often has major side effects, a new generation of pharmaceuticals that inhibit cyclooxygenase 2 with few side effects are being introduced. These medicines, such as celecoxib (Celebrin; Monsanto Co, St Louis, Mo), have high blood-brain barrier permeability. If inflammation does contribute significantly to AD abnormality, then use of cyclooxygenase 2 inhibitors might revolutionize the treatment of AD.

THERAPEUTIC APPROACHES TO INHIBITING THE ACCUMULATION OF AGGREGATES

Recognition of the importance of aggregated Aβ in neurodegenerative diseases has spawned extensive research into the mechanisms to prevent the accumulation of protein aggregates. The most dramatic success in this field has been achieved using immunizations against aggregated Aβ. To examine whether the immune system could be stimulated to remove neuritic senile plaques, a team of researchers, lead by Dale Schenk, PhD, at Elan Pharmaceuticals Research Corp, South San Francisco, Calif (formerly Athena Neurosciences Inc) used mice that develop neuritic senile plaques because they overexpress a mutant form of APP that generates high levels of Aβ. They then immunized these mice monthly for 11 months with aggregated Aβ. The mice that had been immunized had virtually no abnormality compared with an amyloid burden of 2.65% in the controls. Particularly striking was that even the animal with the lowest titer, less than one-tenth of that of the rest of the group, still had virtually no abnormality. The immunization protocol was also able to reverse amyloid deposition, and prevent further amyloid deposition, in older mice that already had neuritic senile plaques. Because vaccines require less preclinical testing than medicinal pharmaceuticals, this vaccine might be tested on humans in the near future.

Even if the amyloid vaccine is not useful for humans, there are other approaches that also seem promising. Investigators have identified several compounds that can inhibit production of Aβ. These compounds are often referred to as β- and γ-secretase inhibitors, based on the particular cleavage of APP that the compounds inhibit. Although none of these inhibitors have entered clinical trials, many promising candidate pharmaceuticals have been presented at meetings. The quality of the inhibitors is likely to improve rapidly with the cloning of a putative β-secretase, which is an aspartyl protease, termed BACE. Many investigators have claimed to identify a secretase previously, but BACE seems to be a true APP secretase because its pharmacological profile replicates the known profile of β-secretase; and inhibiting transcription of BACE messenger RNA inhibits β-secretase activity. Interestingly, BACE seems to be a member of a family of homologous proteins, because other laboratories have now identified homologues of BACE. Identification of BACE and its homologues will allow more precise design of β-secretase inhibitors using rational drug design.

One other promising approach for preventing the accumulation of protein aggregates is the use of compounds that bind to Aβ and prevent its aggregation. Soto et al have shown that homologues of Aβ that have pro-line substitutions can prevent Aβ from aggregating. This is because the kink put into protein structures by prolines prevents formation of β-pleated sheet aggregates. However, delivery of such artificial peptides into the brain remains a challenge. On the other hand, melatonin has the capacity to intercalate into β-pleated sheet structures and break them up. Doses of melatonin in micromoles per liter seem capable of breaking up Aβ aggregates. Since melatonin is also an antioxidant, melatonin might help patients with AD through 2 separate mechanisms. A clinical trial of melatonin is in progress, so the hypothesized benefit of melatonin in AD is being tested directly. Thus, identification of the importance of Aβ aggregation in AD has led to new treatment strategies that are either being tested or soon to be tested in the clinic. Insights into the role of protein aggregation in other diseases might lead to novel treatment approaches for these disorders as well.

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REFERENCES