Therapeutics in Alzheimer’s and Prion Diseases
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Abstract
There is increasing recognition that numerous neurodegenerative conditions have the same underlying pathogenetic mechanism, namely a change in protein conformation, where the β-sheet content is increased. In Alzheimer’s disease, amyloid deposition in the form of neuritic plaques and congophilic angiopathy is driven by the conversion of normal soluble amyloid-β peptide (sAβ) to Aβ plaques; while in the prionoses the critical event is the conversion of normal prion protein, PrP0, to the disease-associated form, PrPSc. This common theme in the pathogenesis of these disorders and the extracellular localization of the accumulating abnormal protein make them highly amenable to therapeutic approaches based on experimental manipulation of protein conformation and clearance. A number of different approaches under current development include drugs which affect the processing of the precursor proteins drugs the clearance of the amyloidogenic protein, and which inhibit or prevent the conformation change and immunological approaches. Particularly interesting are compounds termed ‘β-sheet breakers’ that directly target the abnormal conformational change both for Aβ- and PrPSc-related deposits. In addition, immune system activation can serve as β-sheet breakers and/or to increase the clearance of the disease-associated proteins. These conformation-based approaches appear to hold the best promise for therapies for this devastating group of disorders.

Introduction
As the average age in Western populations rises, neurodegenerative disorders are becoming increasingly common and an ever greater healthcare burden. In the U.S.A. there were approx. 4.8 million Alzheimer’s disease (AD) cases in 2000. By 2050 that figure is expected to increase to 14.5 million, a trend mirrored in the population of many other countries. Prion diseases represent another neurodegenerative category, which currently is much more rare than AD. However, the recent emergence of new-variant Creutzfeldt-Jakob disease has raised the spectre of a large population at risk for this illness, as well as causing great concern regarding the safety of blood bank supplies. The underlying pathogenesis of both AD and prion disease, as well as many other neurodegenerative conditions, is the conversion of a soluble normal prion protein into an insoluble, aggregated, β-sheet-rich form that is toxic [1]. In AD a critical event is the conversion of the normal soluble amyloid-β peptide (sAβ) into fibrillar Aβ, within neuritic plaques and congophilic angiopathy [2]. Similarly, in the prionoses the central event is the conversion of the normal prion protein, PrP0, to its disease-associated form, PrPSc [3]. An increased β-sheet content characterizes both Aβ deposits and PrPSc. With the acquisition of this increased β-sheet structure the proteins become highly toxic and have a tendency to aggregate. Sometimes this aggregation is associated with amyloid fibril formation. In AD, Aβ
deposits are part of the definition of its pathology; however, in sporadic Creutzfeldt–Jakob disease PrPsc amyloid deposits are found in only about 10% of cases. Current evidence suggests that these misfolded proteins are most toxic when in soluble, low-order oligomeric aggregates [4,5]. Over 20 unrelated proteins are known to aggregate in association with human amyloid disease [1,5,6]. Indeed, many proteins can under certain experimental conditions be induced to form toxic oligomeric aggregates [7]. The recognition of the importance of abnormal conformation as a common thread in many human diseases has lead to the development of both anti-β-sheet compounds as well as immunological approaches that affect the conversion of the normal precursor protein into the pathological conformer. Both AD and prion diseases are particularly amenable to these types of approach, since the β-sheet-rich pathological conformers in these illness accumulate in extracellular spaces where they are more easily accessible.

**Therapy for AD**

The major neuropathological features of AD are the deposition of Aβ in the form of neuritic plaques and congophilic angiopathy, where it is fibrillar and has a high β-sheet content. The Aβ also exists as a normal peptide in biological fluids, where it is called sAβ and is thought to have a more random-coil and/or α-helical secondary structure. Hence, a critical event in the pathogenesis of AD is the conformational change of sAβ to Aβ [2]. The suggestion that Aβ plays this primal role in AD is referred to as the amyloid cascade hypothesis. Evidence that amyloid plays such a critical role in the early pathogenesis of AD, with neurofibrillary tangle formation occurring as a later reactive change, comes from various studies, as follows. (a) Genetic analyses of families with early-onset autosomal dominant AD have revealed mutations in chromosome 21, near or within the Aβ sequence, in addition to mutations within the presenilin 1 and 2 genes. Most of these mutations lead to an increased production of Aβ1–42 and/or total Aβ, whereas some enhance the fibrillogenicity of sAβ peptides [2,8,9]. (b) Down's syndrome patients have three copies of the amyloid-β precursor protein (APP) gene and develop AD neuropathology at an early age [2,9]. (c) Aβ oligomers/fibrils are toxic in neuronal culture [10] and to some extent when injected into animal brains, whereas the monomeric, soluble form appears to be less toxic [4,11–14]. (d) Inheritance of the apoE4 allele increases the risk of AD and correlates with increased cerebral amyloid deposition [15,16].

Currently, the only available therapies for AD are cholinergic drugs and the use of vitamin E as an antioxidant; both of these approaches have very limited effect. Other potential therapies include cholesterol-lowering drugs, anti-inflammatory compounds, hormonal therapies and other antioxidants. These types of approach are under clinical trial currently; however, it is likely that they will not have a dramatic effect. Production of sAβ from its precursor protein, called APP, requires cleavage at both its N- and C-termini by β- and γ-secretase, respectively. Inhibitors of these secretases are also potential therapeutic approaches for AD [17]. However, a major limitation of secretase inhibitors is that these enzymes appear to have many other important functions, besides cleavage of APP. This is particularly clear in the case of γ-secretase, where recent evidence indicates that this enzyme also cleaves E-cadherin, which influences the important Wnt signalling pathway [18]. Another potentially exciting approach is the inhibition of the conformational change between normal sAβ and Aβ, by ‘β-sheet breakers’. We and others have designed Aβ-homologous peptides, with proline residues that inhibit a β-sheet conformation. Using an *in vitro* assay of Aβ fibrillogenesis, a five-residue peptide, iAβ5, was found to have the greatest activity [19]. In a rat brain model of Aβ amyloidosis, iAβ5 inhibited fibril formation when administered at the same time as an intra-cerebral inoculation of Aβ1–42. In rats where the Aβ1–42 was injected without iAβ5, large Congo Red-positive fibrillar deposits formed [20]. These results indicated that peptides such as iAβ5 could be used to prevent the development of Aβ deposits. We have also used the rat model of AD to show that iAβ5 can reverse existing deposits of Aβ1–42 [21]. In this experiment Aβ1–42 was first stereotactically injected into the amygdala of the rat and allowed to form fibrillar deposits over a period of 8 days. At that point the rats were injected with iAβ5, control peptides or vehicle, followed by histological examination 8 days later. The rats injected with the iAβ5 showed evidence of disassembly of the Aβ fibrils that had formed *in vivo* [21]. Several other compounds have also been shown to disassemble Aβ fibrils, using *in vitro* assays, such as melatonin [22,23], nicotine [24], apolipoprotein J [25], anthracycline [26], rifampicin [27,28], hexadecyl-N-methylpiperidinium...
and Congo Red. It remains to be determined if any of these \(\beta\)-sheet breakers will be active in vivo. A number of laboratories are currently working on improving the pharmacokinetics of these compounds.

The immune response as a therapy in the prionoses and AD

It has recently been shown that immunization of transgenic mice with AD-related neuropathology, using fibrillar A\(\beta\)1-42 as an antigen, reduces or prevents cerebral A\(\beta\) amyloid deposits. These mice were immunized before the onset of AD-type neuropathology (at 6 weeks of age), or when some amyloid deposition had occurred (at 11 months of age). At 13 months, the former group had virtually no amyloid plaques or associated histopathology. In the latter group, amyloid burden, neuritic dystrophy and astrogliosis were also significantly reduced in the A\(\beta\)1-42-treated group following 4 and 7 months treatment. An antibody-mediated response is likely to be critical for a therapeutic response, since similar results have been obtained with passive immunization. Significantly, this treatment approach is associated with cognitive benefits in the mice. Although these promising behavioral studies have diminished safety concerns regarding the use of A\(\beta\)1-42 for immunization in humans, this approach may still be inappropriate for human use. First, A\(\beta\)1-42 forms toxic fibrils, and it has been demonstrated by others and us that A\(\beta\)1-40/42 can cross the blood-brain barrier in experimental animals. Also, even in small quantities A\(\beta\)1-42 may seed fibril formation. Therefore, in humans it is possible that aggregated/fibrillar A\(\beta\)1-42, which was used for immunization by Schenk et al., can cross the blood-brain barrier and co-deposit on existing amyloid plaques, leading to increased toxicity, and may actually promote plaque formation. This would be difficult to detect in short-term clinical studies because the toxicity of A\(\beta\)1-42 is likely to be chronic. AD is commonly thought to progress for decades before cognitive impairments are seen. Unfortunately, findings in the mouse cannot always be extrapolated to humans. Toxicity has not been an issue in the Tg mouse models for AD because human A\(\beta\)1-42 is less toxic for the mouse; even with massive deposition of human A\(\beta\)1-42, none of the Tg mice show significant neuronal loss. Therefore, therapeutic findings in these mouse models must be cautiously interpreted, and may not fully apply to the human condition.

Because of these potential safety concerns, we have designed a synthetic non-amyloidogenic peptide homologous to A\(\beta\) that has a reduced ability to adopt a \(\beta\)-sheet conformation and which therefore confers a much lower risk of toxicity in humans. This peptide was designed to have reduced fibrillogenic potential and enhanced immunogenicity while maintaining the major immunogenic sites of A\(\beta\) peptides, which are residues 1-11 and 22-28. Accordingly, the peptide contains the first 30 amino acid residues of A\(\beta\) with six lysine residues

**Figure 1**

Prolongation of the incubation time of I39A-strain PrP\(^{Sc}\) inoculated intraperitoneally in wild-type CD-I mice that were subject to vaccination with recombinant mouse PrP (recPrP)

Prophylactic vaccination with recPrP delays clinical disease onset in mice exposed to both the 10-fold (\(P = 0.002\)) and 1000-fold (\(P = 0.04\)) inoculations of PrP\(^{Sc}\), with day 0 being the first day an animal scored positive for disease. Group 1 mice were controls receiving PrP\(^{Sc}\) inoculum at a 10-fold dilution, while group 2 was inoculated at the same dilution but also received recPrP vaccination. Group 3 mice were controls inoculated with PrP\(^{Sc}\) inoculum at a 1000-fold dilution, while group 4 received the same dilution of inoculum along with recPrP vaccination. The two control groups received adjuvant and vehicle injections.

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<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>control: 10 fold PrP(^{Sc}) dilution</td>
</tr>
<tr>
<td>2</td>
<td>vaccinated: 10 fold PrP(^{Sc}) dilution</td>
</tr>
<tr>
<td>3</td>
<td>control: 1000 fold PrP(^{Sc}) dilution</td>
</tr>
<tr>
<td>4</td>
<td>vaccinated: 1000 fold PrP(^{Sc}) dilution</td>
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Two-way ANOVA shows a significant effect for vaccination (\(P < 0.0005\))
at the N-terminus. Poly-L-lysine is known to increase immunogenicity and previous in vitro studies have shown that the addition of lysine residues reduces the fibrillogenecity of Aβ peptides [45,46]. Our immunization in AD model Tg mice for 7 months with K6Aβ1-30-NH₂, a non-amyloidogenic, non-toxic Aβ-homologous peptide, reduced cortical and hippocampal brain amyloid burden by 89 % and 81 %, respectively [47]. Concurrently, brain levels of soluble Aβ1-42 were reduced by 57 %. These promising findings suggest that immunization with non-amyloidogenic Aβ derivatives represents a potentially safer therapeutic approach to reducing amyloid burden in AD, instead of using toxic Aβ fibrils.

We have recently used a similar approach for the treatment of prion disease [48]. All prion diseases are fatal with currently no effective treatment. A number of medications have been shown in experimental systems to be effective at preventing development of scrapie. These include Congo Red [49,50], anthracelines [51], amphotericin B [52,53] and sulphated polyanions [54]. Some of these have been shown to prolong the incubation time of animals infected with scrapie, but these agents have limitations in terms of toxic effects and/or unfavourable pharmacokinetic properties. We have used a mouse model of prion infection, where wild-type CD-1 mice are intraperitoneally exposed to 139A strain PrP⁰. We found that mice vaccinated with recombinant mouse prion protein (recPrP) had a delay in the onset of prion disease (see Figure 1) [48]. Vaccination was performed both prior to and after peripheral prion exposure. A delay in disease onset was seen in both groups, but was more prolonged in animals immunized prior to exposure. The increase in the incubation period closely correlated with the anti-PrP antibody titre. Our preliminary studies using anti-mouse PrP monoclonal antibodies for passive immunization in the same animal system confirm that the therapeutic response is dependent primarily on humoral immunity. These promising findings suggest that a similar approach may work in humans or other mammalian species at risk from prion disease.

Summary

There is growing evidence that numerous neurodegenerative conditions have the same underlying pathogenetic mechanism, namely a change in protein conformation, where the β-sheet content is increased. In AD, amyloid deposition in the form of neuritic plaques and congophilic angiopathy is driven by the conversion of sAβ into Aβ, while in the prionoses the critical event is the conversion of PrP⁰ into PrP⁴⁰. Several reports have shown that immunization with Aβ peptides is very effective at reducing the amyloid burden in Tg mouse models of AD. Passive immunization studies in these models indicate that antibody-mediated clearance of Aβ peptides is paramount for a therapeutic response. Our recent studies [48] extend this immunological approach to prion disease and suggest that this strategy may be applied to all types of conformational disorder. Of critical importance to extending these active immunization approaches to humans is designing immunogens that have no potential innate toxicity; hence, in our AD vaccination studies we used a non-fibrillar, non-toxic Aβ-homologous peptide instead of the native sequence peptide. In our future approaches for prion vaccination we will also use the same strategy to limit potential toxicity. Our growing understanding of the basic mechanisms underlying the conformational disorders raises the hope that therapeutic approaches based directly on preventing and reversing these pathological protein conformers will be a reality in the future.

This work was supported by National Institutes of Health grants AG15408, AG20197 and AG20245.

References