REVIEW ARTICLE

Alzheimer’s centennial legacy: prospects for rational therapeutic intervention targeting the Aβ amyloid pathway

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It is now 100 years since the nosological definition of Alzheimer’s disease emerged. In the first 80 years, very little progress was made in understanding the mechanisms that caused the brain to degenerate in a remarkably specific fashion (amyloid accumulation with neurofibrillary changes). Over the past 20 years, there has been an explosion of knowledge which continues today at an exponential rate. The molecular pathways underlying the synaptic dysfunction in Alzheimer’s disease have delivered many validated therapeutic and diagnostic targets. A variety of therapeutic strategies aimed at disease modification are now in clinical development.

Keywords: Aβ amyloid pathway; Alzheimer’s disease; amyloid beta precursor protein

Abbreviations: Aβ = amyloid β peptide; AChE = acetylcholinesterase; AChE-I = AChE inhibitors; APP = amyloid beta (A4) precursor protein; MPAC = metal–protein attenuating compounds; NMDA = N-methyl-D-aspartate


Introduction

Although Alzheimer is now credited with the elucidation of a distinct disease entity, the record clearly shows that others (including Bonfiglio, Fischer, Kraepelin and Perusini) contributed substantially to the original nosological demarcation of ‘pre-senile dementia’. Nevertheless, we recognize today Alzheimer’s case description exactly 100 years ago as the first step in a circuitous pathway leading to our current theories of pathogenesis based around the molecular and genetic discoveries of the past 20 years. As a tribute to the early pioneers who painstakingly described the pathognomonic changes of Alzheimer’s disease. It is worthwhile to reconsider and appreciate the images generated of the plaques and tangles with which they worked (Fig. 1). It has long been appreciated that the plaques, tangles, neuritic change and reactive gliosis hold the key to understanding this disease process. In the 80 years following Alzheimer’s description, little progress was made in unravelling the molecular and genetic basis of the disease. Fortunately, major advances have occurred in the last 20 years.

Background

In this review, we summarize our current thinking about the biogenesis of the amyloid β peptide (Aβ) from the amyloid beta (A4) precursor protein (APP), and how this pathway presents therapeutic and diagnostic targets that are now in clinical development. The discoveries that have underpinned our current theories are summarized in Table 1, and reflect our particular interests—other researchers may see things very differently. The pathways leading to the discovery and characterization of the Aβ amyloid peptide have been set out in detail recently (Masters and Beyreuther, 2006). We commenced our project to isolate and characterize the amyloid plaques in the human brain in the late 1970s (Masters et al., 1981; Merz et al., 1983). After the initial sequencing of the N-terminus of Aβ derived from cerebral leptomeninges (Glenner and Wong, 1984a, b), we were able to correct and extend the sequence of plaque-derived amyloid, and determine the likely C-terminus (Masters et al., 1985a,b; Kang et al., 1987). Through the discovery of the N- and C-termini, it became apparent that the Aβ peptide was derived by proteolytic action on the APP (Kang et al.,...
1987), and that inhibition of the biogenesis of Aβ could be achieved by targeting these proteolytic events. The current state of clinical development of this approach is discussed below.

Very early in our research collaboration (Masters et al., 1985b), we recognized that charge effects would have a major contribution to aggregation of Aβ, and that protonation of the two histidine residues at position 13 and 14 could affect dimerization of Aβ (which at the time we referred to as Aβ) into a stable subunit, which could in turn form the building blocks of oligomers of Aβ. These oligomers have now become the focus of an intense research effort (Table 2), as they must represent an intermediate species that is capable of folding into a fibril-generating pathway leading to amorphous or compact amyloid plaques (Davies et al., 1988). Our analysis of the amyloid plaque cores also revealed non-proteinaceous components, including metal ions. This was at a time when the aluminium theory of Alzheimer’s disease was still quite strong, and we speculated on the role of aluminium silicates in the amyloid plaques. Subsequent work has turned our attention to other metal ions (zinc and copper, in particular).

Independent of our work on amyloid plaques, a separate line of enquiry revealed that the Alzheimer’s disease brain was under oxidative stress (Martins et al., 1986). Although we did not make the connection at the time, it now seems clear that the toxic oligomers of Aβ may be the principal source of this oxidative damage. The first clue for this came from the studies of Dyrks et al. (1992a,b, 1993), which showed that aggregation/oligomerization of Aβ was mediated by metal-catalysed oxidation through the histidine, tyrosine and methionine residues in Aβ. Oxidative modification of these residues also led to cross-link formation, and the aggregation/oligomerization of Aβ was reversible with antioxidants. Around the same time, we identified metal (Zn++, Cu++) binding sites on the ectodomain of APP (Bush et al., 1993; Hesse et al., 1994; Multhaup et al., 1994a,b, 1995) and in the Aβ region of APP (Bush et al., 1994). Redox-active metal ions, such as Cu++, were found to be involved in redox chemistry (Cu++ reduced to Cu+) when in contact with APP and Aβ (Multhaup et al., 1996), and capable of generating H2O2 and other reactive oxygen species (ROS) (Huang et al., 1994; Multhaup et al., 1998).

Why is there now so much interest in the APP/Aβ pathway? In 2005 alone, >780 papers appeared on APP/Aβ, many of which deal with the pre-clinical and clinical therapeutic strategies directed at the APP/Aβ pathway (Fig. 2). The theory that underlies this pathway as the principal and proximal causal mechanism in Alzheimer’s disease is pinned to two critical series of observations: first, mutations in the gene encoding APP [and the presenilin (PS) genes as components of the γ-secretase machinery] are causally linked to early onset familial Alzheimer’s disease (Levy et al., 1990; van Broeckhoven et al., 1990; Chartier-Harlin et al., 1991; Sherrington et al., 1995); second, genetically engineered mice with these mutations (Quon et al.,

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**Fig. 1** (A) Figure 42 from Spielmeyer (1922). Silver impregnations showing the neurofibrillary changes in the cytoplasm of neurons. (B) Figure 201 from Spielmeyer (1922). Two senile plaques, using Bielschowsky silver impregnations, showing the central amyloid core and associated glial cell reactions.
The Alzheimer’s disease brain is under oxidative stress (Martins et al., 1990; van Broeckhoven et al., 1999). These proteases (γ- and β-secretases) have become prime therapeutic targets.

Stable dimers of Aβ (Aβ40) may represent the soluble form of Aβ; oligomers [tetramers (A41) or hexadecamers (A62)] may form the basic amyloid subunit (Masters et al., 1985b).

Non-proteinaceous components of plaques include metals and other ions (silicon, aluminium, calcium) (Masters et al., 1985b).

The Alzheimer’s disease brain is under oxidative stress (Martins et al., 1986).

Amorphous plaques may represent earlier aggregates of more soluble Aβ species (Davies et al., 1988; Rumble et al., 1989).

Aβ is confirmed in its predicted transmembrane orientation (Dyrks et al., 1988; Weidemann et al., 1989), and shedding of APP into biological fluids occurs (Weidemann et al., 1989; Rumble et al., 1989).

Identification of pathogenic mutations in APP gene conclusively demonstrate that the primary pathway of Alzheimer’s disease involves APP/Aβ (Levy et al., 1990; van Broeckhoven et al., 1990; Chartier-Harlin et al., 1991).

Aβ in solution has a β-turn (Sorimachi et al., 1990) and has a high propensity for aggregation driven by hydrophobic residues (Hilbich et al., 1991, 1992).

Soluble Aβ may be produced and released by cultured cells during normal metabolism (Haas et al., 1992; Shoji et al., 1992).

Aβ in membrane lipid does not aggregate or form oligomers (Dyrks et al., 1992b).

Aggregation of Aβ is mediated by metal-catalysed oxidation (Fe²⁺/H₂O₂); requires oxidative modifications of histidine, tyrosine and methionine, and protein cross-linkage; and is reversed with anti-oxidants (Dyrks et al., 1992a,b, 1993).

APP axonal trafficking has a major effect on APP processing, and can be modulated by factors such as cholesterol (Koo et al., 1990; Tiernari et al., 1996).

APP and Aβ bind Zn²⁺, Cu²⁺ metal ions (Bush et al., 1993, 1994; Hesse et al., 1994; Multhaup et al., 1994, 1996). Redox-active metals lead to redox chemistry (Multhaup et al., 1996). Aβ aggregation (dimer, oligomer) and ROS generation (Huang et al., 1999; Opazo et al., 2002).

Aβ may occur in intracellular compartments (Fuller et al., 1995; Hartmann et al., 1997; Tiernari et al., 1997).

Cells with APP knockout phenotype are relatively resistant to Aβ toxicity (White et al., 1998).

Aggregated oligomerized Aβ can be solubilized with small molecular weight compounds that have relatively low affinities for metal ions (copper, zinc) (Cherny et al., 1999). These MPAC have the capacity to ameliorate the Aβ burden and toxicity in transgenic Alzheimer’s disease mouse models (Cherny et al., 2001) and possibly in humans with Alzheimer’s disease (Ritchie et al., 2003).

1991; Games et al., 1995) recapitulate the human disease in that plaques, perivascular amyloid, neurofibrillary changes and behavioural impairments are induced. More recently, a very tight association between the mean age at onset of pedigrees with PS mutation-related familial Alzheimer’s disease and the ratio of secreted Aβ to total Aβ was found (Duering et al., 2005; Kumar-Singh et al., 2006). This, together with the development of a robust Aβ-neuroimaging ligand (a thioflavin T analogue), which as a biomarker clearly differentiates Alzheimer’s disease and mild cognitive impairment from normal controls and other neurodegenerative diseases (Klunk et al., 2004; Mathis et al., 2005; Fagan et al., 2006), adds much more strength to the Aβ theory (Fig. 3).

But the single most important challenge to test the theory remains as the demonstration that a drug targeting the APP/Aβ pathway actually modifies the natural history of the disease. To this end, the criteria set out by Cummings (2006), and listed in Table 3, have clarified the standards to be met when we come to assessing this test of the Aβ theory of Alzheimer’s disease. The first criterion (a plausible mechanism of action in a validated model) has been achieved by many of the therapeutic strategies reviewed below. But no drug has yet met any of the other four criteria, although we remain optimistic that the current pace of activity will deliver a result in the not too distant future.

Upstream events in the APP/Aβ pathway

The targets derived from the APP/Aβ pathway outlined in Fig. 2 are listed in more detail in Table 4. While it is not a comprehensive or exhaustive listing, it does present a novel and logical way of classifying the wide range of current research activity being undertaken in this area.

Age and environmental factors

Of all the external variables that determine risk of getting Alzheimer’s disease, age and the environment stand out...
as factors that demand explanations. Yet, for all their obviousness, no reasonable explanations have been forthcoming. While many of the biochemical events listed in the APP/Aβ pathway are known to be developmentally regulated, very little information is yet available on what happens under normal ageing conditions. Partial loss of function of a critical biochemical reaction would seem to be a good starting point for investigation, either as an upstream or as a downstream event; or a ‘double hit’ phenomenon could be invoked, as seen in the early development of ideas on oncogenesis. Whichever, the incontrovertible link between ageing and Alzheimer’s disease remains obscure in mechanistic terms.

Similarly, the interactions between the environment and the risk for Alzheimer’s disease have attracted many

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<th>Table 2</th>
<th>Aβ comes in many flavours, including soluble (toxic) Aβ species: synonyms</th>
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<td>Aβ soluble dimers (Aβ), tetramers (A14), etc., forming oligomers (Masters et al., 1985a)</td>
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<td>Amorphous aggregates (Davies et al., 1988; Huang et al., 2000)</td>
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<td>Aβ-derived diffusible ligands (ADDLs) (Lambert et al., 1998)</td>
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<td>Procofibril (Harper et al., 1997; Walsh et al., 1997)</td>
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<td>Spherical particles (Gorman et al., 2003)</td>
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<td>Spherical prefibrillar aggregates (Frost et al., 2003)</td>
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<td>Toxic Aβ soluble species (McLean et al., 1999)</td>
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Fig. 2 Schematic outline of the upstream and downstream events that surround the central APP/Aβ pathway.
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**Table 3** Alzheimer’s disease modification by drug intervention: criteria

(i) Plausible mechanism of action in a validated model
(ii) Clinical trial evidence based on the Lerber staggered start design
(iii) Difference in survival to a meaningful clinical outcome
(iv) Change in rate (slope) of decline
(v) Demonstrable drug-placebo difference on an accepted biomarker of disease progression


epidemiological studies. Diet and exercise remain as the two most interesting variables. General caloric restriction has often been associated with longevity in rodent models of ageing, and recent studies in transgenic models (Wang et al., 2005) suggest an effect on Aβ plaque load or α-secretase processing of APP. The effects of exercise (Adlard et al., 2005) and environmental enrichment (Jankowsky et al., 2005a) have also been examined in transgenic models with encouraging results. Lazarov et al. (2005) found a change in a downstream event, an increase in the enzymatic activity of neprilysin (NPE), an Aβ-degrading protease, in response to environmental enrichment. These downstream events are discussed in more detail below.

Specific dietary intakes, especially naturally occurring anti-oxidants or metal ions remain largely under-investigated as risk factors. As methods for diagnosis and population-based screening improve (using plasma biomarkers or specific ligands of Aβ for neuroimaging), it will become more feasible to analytically examine the dietary risk profiles of discrete populations, overcoming current limitations on sensitivity and specificity of case ascertainment. A surprising study has already pre-empted dietary modulation of Alzheimer’s disease through the consumption of transgenic Aβ-expressing potatoes (Youm et al., 2005)! The proposed mechanism involves low-level immune-mediated clearance of Aβ deposits.

**The effect of modulation of neurotransmitter systems on APP processing**

Acetylcholinesterase (AChE) was discovered to be present in Alzheimer’s disease amyloid plaques 40 years ago, and the activity of choline acetyltransferase (CAT) was found to be decreased in the Alzheimer’s disease brain 30 years ago. From these observations the cholinergic hypothesis/theory of the disease arose, which led to the development of AChE inhibitors (AChE-I) as a therapeutic strategy, with apparent success, despite the lack of any plausible explanation for the presence of AChE in plaques and the underlying loss of CAT. A paradox then emerged: subjects treated with AChE-I responded with a compensatory increase in AChE levels. This might have been expected to negate the intended effect of the AChE-I on the availability of ACh for cholinergic transmission. At the same time, clinical trials of AChE-Is and their meta-analyses continued to show favourable, albeit mild, effects on cognitive parameters, at least during the first 6–12 months of treatment. Against this background, basic and clinical investigators have recently turned their attention towards other possible mechanisms of action of the AChE-Is, especially on the APP/Aβ pathway, and have begun to ask whether these drugs might have any disease-modifying effects (Caccamo et al., 2006).

Various aspects of AChE-I actions on the upstream and downstream APP/Aβ pathway have been reported: attenuating the effects of Aβ-induced neuronal cytotoxicity (Kimura et al., 2005), promoting α-secretase or decreasing β-secretase activity (Caccamo et al., 2006; Zimmerman et al., 2005), inhibiting Aβ aggregation or inhibiting GSK 3β activity and tau phosphorylation (Caccamo et al., 2006). One group found no effect on Aβ amyloid plaque load while still improving behavioural deficits in a transgenic mouse model (Dong et al., 2005), while another group found that inhibitors of butyrylcholinesterase had a lowering effect on cellular APP and Aβ and brain Aβ in transgenic mice (Greig et al., 2005).

The modulation of glutamatergic transmission in Alzheimer’s disease has also received increasing attention with the results of the memantine clinical trials aimed at blocking (non-competitively) the action of N-methyl-D-aspartate (NMDA) receptors. With the growing awareness that the toxic soluble oligomers of Aβ may inhibit LTP at the pre-synaptic level and that Aβ promotes the endocytosis of the NMDA receptor [mediated in part through α-7 nicotinic receptor, protein phosphatase PPP2B and tyrosine phosphatase STEP (Snyder et al., 2005)], the findings that memantine has beneficial behavioural effects in both Aβ toxicity models (Yamada et al., 2005) and...
APP transgenic mouse models (Van Dam et al., 2005) requires further work that might tie all these observations together. NMDA receptor activation may promote Aβ production (Lesné et al., 2005) and Aβ may also modulate α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid [AMPA] receptors (Chang et al., 2006; Shemer et al., 2006), further contributing to the impairment of synaptic function.

Other cerebral or general systemic factors

One suspects that there will be many other upstream factors that play into the APP/Aβ pathway, but few have been identified to date. A particularly contentious area has been the role of the vascular supply to the brain and the effects of ischaemia (atherosclerosis) and hypertension. Historically, this has deep roots, going back to the days when ‘arteriosclerosis’ was thought to cause all forms of dementia. Similarly, head trauma has been considered as a risk factor for Alzheimer’s disease, and APP has been identified as a sensitive marker of axonal damage following traumatic brain injury. But neither hypoxia nor trauma has yet been shown to be a major risk factor, and neither has been shown to promote the long-term amyloidogenic processing of APP.

Central steps in the APP/Aβ pathway

Targeting the APP gene or genes with products interacting directly with APP

With the advent of RNA interference (RNAi) silencing, it is to be expected that attempts at direct APP gene regulation will emerge. As a forerunner to this, models in which the overexpressed human APP transgene in mice can be downregulated with doxycycline provide a proof of principle that rapid control over Aβ expression and deposition can be obtained without gross adverse side-effects (Jankowsky et al., 2005). Unexpectedly, Aβ deposits formed before the onset of downregulation seemed to be remarkably stable, indicating that any treatment of this type in isolation might have to be administered early in the natural history of Alzheimer’s disease. Using RNAi techniques in transfected cell lines (Xie et al., 2005), targeting the X11 gene (APAB) successfully increased APP C-terminal fragments and lowered Aβ levels; X11 is a known interactor with the cytoplasmic...
domain of APP, and presents a novel method of possibly modulating γ-secretase cleavage.

**APP-interacting systems**

As a presumptive cell surface receptor, APP probably has ligands and effector mechanisms for signal transduction. Nearly 200 proteins have been reported as having direct interactions with APP. Suspected ligands in the extracellular domain include growth factors [nerve growth factor (NGF) in particular], heparin-containing extracellular matrix, metals (through the extracellular Cu/Zn binding domain) and APP itself through hetero- and homo-dimerization. Small compounds such as propentofylline (Chauhan et al., 2005) can affect NGF release, and through this modulate the amyloidogenic pathway. Other small compounds may bind directly to APP (Espeseth et al., 2005) and affect its processing.

A controversial area involves the effects of hormones (oestrogens and testosterone especially) and how they may affect APP metabolism. Conflicting results in experimental models have appeared, in which oestrogen deficiency exacerbates Aβ in the APP23 transgenic model (Yue et al., 2005) and neither oestrogen deprivation nor replacement affected Aβ deposition in the PDAPP [platelet-derived growth factor (pdgf)-β chain promoter with Indiana mutation-V717F in APP] transgenic model (Green et al., 2005). Further studies are clearly required for unravelling this important area where there is an epidemiological impression that females have a higher incidence of Alzheimer’s disease than males (this impression does not appear to have ever been subjected to a prospective analytical epidemiological study). The mechanisms through which oestrogen/testosterone might act remain obscure, but include oestrogen-dependent regulation of metal homeostasis in the brain through the expression of the neuronal zinc transporter, ZnT3.

Cholesterol and inhibitors of cholesterol synthesis (statins) have been shown to significantly alter APP processing in vitro, with a reduction in β-secretase cleavage and lessened Aβ production. Cholesterol-dependent aggregation/oligomerization has also been reported. While some early phase clinical trials with statins have shown encouraging results (Massé et al., 2005), others have not (Höglund et al., 2005). Cholesterol-independent effects have also been noted for statins acting on isoprenyl intermediates in the cholesterol biosynthetic pathways, with a putative anti-inflammatory effect induced by reactive microglia (Cole et al., 2005; Cordle et al., 2005). This might conflict with the current theory that microglia are involved in the beneficial process of clearing Aβ deposits.

If eventually cholesterol does prove to be a risk factor, then the observations (Papassotriopoulos et al., 2005) of an association between the disease and the expression levels and haplotypes of the 5′ region of the cholesterol 25-hydroxylase (CH25H) gene on chromosome 10 may provide a plausible explanation: one in which cerebral cholesterol metabolism (as distinct from systemic cholesterol and its association with atherosclerosis) directly plays into the APP processing and transport pathways.

**APP proteolytic processing**

As outlined in the Background section above, the biogenesis of Aβ has been the prime validated drug target for Alzheimer’s disease since the discovery of the proteolytic processing of APP in 1987. Molecular details of the C-terminal cleavage (γ-secretase) were the first to emerge (Sherrington et al., 1995), followed by the α- and β-cleavage mechanisms (Sinha et al., 1999). Subsequent elucidation of δ-, ε- and ζ-cleavages has added another layer of complexity. Drug discovery programmes reflect this sequence of events: many large pharmaceutical companies have γ-secretase inhibitors or modulators in clinical development, while the β-secretase inhibitors are several years behind, largely in pre-clinical discovery.

**γ-Secretase inhibitors and modulators**

During 2004, the first publications of in vivo γ-secretase inhibition/modulation of Aβ42 biogenesis appeared. One of the first inhibitors [DAPT (N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester) was shown to be effective in acute experiments in behavioural tests (contextual fear conditioning) in the Tg 2576 Alzheimer’s disease mouse model (Comery et al., 2005). Modifications to the chemical structure of DAPT have now improved its delivery to the brain (Quéléver et al., 2005), as with other compounds (Laras et al., 2005), in the hope of achieving lower effective dosages minimizing the risk of adverse peripheral effects. Many diverse classes of inhibitors and modulators are showing very favourable acute pharmacokinetics, with rapid lowering of plasma and CSF Aβ levels (Anderson et al., 2005; Barten et al., 2005; Lanz et al., 2004, 2005; Grimwood et al., 2005; Peretto et al., 2005; Best et al., 2006). Importantly, there is now strong evidence linking plasma and CSF Aβ levels, indicating that the brain/CSF pool of Aβ is at least in part a significant proportion of the plasma Aβ pool. There are still methodological issues in measuring Aβ, using either ELISA or western blotting techniques (which soluble oligomeric species are being measured, and what forms of Aβ: total, Aβ40, Aβ42?). Nevertheless, these preliminary data offer some hope that plasma Aβ species may eventually prove to be a reliable marker of cerebral Aβ turnover. Further explorations of the properties of γ-secretase inhibitors are revealing unanticipated effects on synaptic function (Dash et al., 2005). New classes of γ-secretase inhibitors/modulators continue to be disclosed, as part of the effort to develop compounds devoid of side-effects (Sparéy et al., 2005). The major concern is the inhibition of signalling in the Notch pathway, which affects cellular differentiation (Curry et al., 2005; van Es et al., 2005). Ironically, γ-secretase inhibitor compounds originally developed for Alzheimer’s disease are now being trialled...

The first in-human Phase I results to be published (Siemers et al., 2005, 2006) have shown that the Lilly compound LY450139 achieved a significant lowering of plasma Aβ, but not CSF Aβ, in normal volunteers (up to 50 mg/day for 14 days) or subjects with Alzheimer’s disease (up to 40 mg/day for 6 weeks). The drug was well tolerated. Higher dosages may be required to achieve a reduction in CSF levels. The results of Phase II studies with read-outs on cognitive variables are eagerly awaited. In the meantime, further research on the mechanistic operations of the γ-secretase complex (Sato et al., 2005; Fukumori et al., 2006; Kakuda et al., 2006; Morohashi et al., 2006; Yagishita et al., 2006) may lead to new paths of drug discovery, as might gene targeting of presenilin, PEN-2, APH-1, nicastrin and TMP21 lead to selective regulation of γ-secretase activity (Xie et al., 2005a; Chen et al., 2006).

**β-Secretase (BACE) inhibitors**

Although ~5 years behind the development of the γ-secretase inhibitors, much progress has been made in the discovery and design of compounds that target the active site of BACE-1. Improved assays and structural-based *in silico* designs have added to the existing pipeline of drugs in early pre-clinical development (Kimura et al., 2005; Kornacker et al., 2005; Lefranc-Jullien et al., 2005; Huang et al., 2006) or early discovery programmes. Other proteins interacting with BACE-1 may become drug targets, and gene targeting of BACE-1 mRNA using siRNA is also producing encouraging preliminary results (Singer et al., 2005). As with γ-secretase, unanticipated side-effects on other BACE-1 substrates or downstream consequences of BACE-1 inhibition may prove difficult to circumvent.

**Drugs targeting Aβ and its varied conformations**

**Monomers (A₄), dimers (A₈) and trimers (A₁₂)**

In contrast to the inhibition of Aβ biogenesis, therapeutic strategies that directly target Aβ itself should inherently have a lower risk of throwing up unanticipated side-effects, as the accumulated Aβ molecule is restricted to Alzheimer’s disease. If the Aβ fragment (or its domain within APP) does, however, subserve some critical normal function, then targeting Aβ itself might interfere with this function and thereby lead to adverse side-effects, but to date, a normal function for Aβ has not been identified. APP knockout mice are viable and healthy, providing some support for this idea.

Current models of the physical state of Aβ are evolving. Whilst resident in the membrane, Aβ is assumed to be in an α-helical conformation. Following sequential β- and γ-cleavages, Aβ as a monomer (A₄), dimer (A₈) [or perhaps even as a trimer (A₁₂)] is translocated into the extracellular space, and may transition there into a β-strand-enriched structure. These structures may then progress towards oligomers/protofibrils through to polymers/fibrils of amyloid filaments.

The mechanisms through which Aβ causes damage to neurons (‘the toxic gain-of-function’) are slowly emerging. There are many theories: the two most favoured include the ability of Aβ to generate oxidative stress and the hydrophobic interaction of Aβ with lipid membranes, particularly the synaptic plasma membrane. Our current working model (Table 5) incorporates both theories: we have defined a metal binding domain near the N-terminus of Aβ that is capable of binding Zn²⁺ (which causes Aβ to precipitate) or redox-active Cu²⁺. When Cu²⁺ binds Aβ, it not only causes a significant increase in insolubility but also induces a series of electron transfers that result in histidine bridge formation, tyrosine 10 radicalization, di-tyrosine cross-linking and oxidation of methionine 35. Ultimately, in the presence of reductants, this results in the production of H₂O₂ and hydroxyl radicals, capable of inflicting short-range oxidative damage to proteins, lipids, sterols, nucleic acids, etc.

**Table 5 The toxic Aβ oligomer pathway: the current theory**

| Aβ-Cu²⁺ facilitates intermolecular histidine bridge formation (Curtain et al., 2001; Tickler et al., 2005; Smith et al., 2006) |
| Membrane association of Aβ monomer/dimer occurs (Curtain et al., 2003; Lau et al., 2006; G.D. Ciccotosto, unpublished data) |
| Exposed Met35 required to promote redox chemistry in conjunction with metal binding site (Barnham et al., 2003; Curtain et al., 2001, 2003; Ciccotosto et al., 2004) |
| Tyr10 radical forms and Met35 is oxidized; one consequence is di-tyrosine cross-linkage (Barnham et al., 2004; Smith et al., 2006), and copper induces formation of soluble oligomers (Barnham et al., 2004) |
| Soluble species of Aβ, not plaques, are the principal determinants of neurodegeneration (McLean et al., 1999) |
| ROS (H₂O₂) production may result in flipping of phosphatidyl serines from cytoplasmic face of membrane to the exterior (White et al., 2001), which will promote further binding of Aβ to the membrane |
| Dimeric/oligomeric soluble Aβ species associate with negatively charged head groups of phospholipids on outer surface of plasma/synaptic membrane (Smith et al., 2006; Lau et al., 2006) |
| Oxidation of sterols (cholestenone), lipids (4-hydroxy-2-nonenal) and proteins (carbonyls) (Smith et al., 2006) |
| Perturbation of plasma/synaptic membranes/proteins leads to chronic dysfunction and disease |
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and so forth (Tabner et al., 2005). Our studies show that toxicity to neurons in culture is associated with the ability of Aβ to associate with the lipid head group on the outer surface of the plasma membrane (Lau et al., 2006).

If this schema is only partially correct, then it is clear that any therapeutic strategy targeting Aβ directly might have multiple routes, many intersecting and overlapping. Thus, targeting the metal binding site of Aβ might relate to Aβ in one or more of its varied conformations (α-helix, β-strand, β-sheet; A₄, A₈, A₁₆ versus higher-order oligomers versus polymerized fibril) or whilst interacting with other proteins or lipids.

In consideration of targeting the metal binding site on Aβ, we have developed the concept of an MPAC—a metal–protein attenuating compound—in distinction to the more widely known term of metal chelator. The MPAC has relatively weak binding constants for metals, and is able to compete with the target site for the metal ion. As a consequence, an MPAC should not alter the general homeostasis of metal ions in the whole animal. In contrast, a metal chelator has high, effectively irreversible, binding constants for metal ions. A chelator might affect the metal binding to Aβ through deletion of the total pool of bioavailable metal, but is not expected necessarily to interact with the Aβ metal binding site itself.

The study of MPACs in Alzheimer’s disease has been initiated with studies of clioquinol, an 8-OH quinoline, with encouraging pre-clinical (Cherry et al., 2001) and early Phase II clinical results (Ritchie et al., 2003). The next-generation MPAC has progressed to a new chemical entity based around the 8-OH quinoline structure. This compound (PBT2-Prana Biotechnology) has passed Phase I and will soon commence Phase II clinical development.

Additional binding sites on Aβ, such as the glycosaminoglycan (GAG) site [HHQK (13–16)], have been targeted with compounds such as 3-aminopropanesulphonic acid [3-APS (Alzheimed); Neurochem]. The results of early clinical trials have been released by the company, with some effects seen on CSF Aβ₄₂, but none on ADAS-cog or Mini-Mental State Examination (MMSE). A large Phase III study is under way, coupled to an open-label extension study. The double-blind study results are expected in January 2007.

We have identified other structural changes or mechanisms of toxicity for Aβ that include the oxidative modifications of Tyr10 and Met55, the interaction of Aβ with the polar head groups of the lipid bilayer or the interaction of Aβ with other proteins. These areas remain very much in the early discovery phase and may deliver LPACs—lipid–protein attenuating compounds, or PPACs—protein–protein attenuating compounds.

β-Oligomers/protofibrils and polymers/fibrils of Aβ

The pharmaceutical industry has for a long time interrogated its libraries for compounds that are anti-aggregants and/or anti-fibrillogenic. Many hits with compounds that look similar to Congo Red have never been developed. Similarly, compounds capable of disaggregating or defibrillating Aβ have been sought, but not with the intensity of the search for anti-aggregants. While many peptidyl/protein-like designs have been examined, other small molecules have been discovered that hold some promise (Kanapathipillai et al., 2005; Lin et al., 2005; Wang et al., 2005; McLaurin et al., 2006). Most interesting, however, is the development of assays specifically designed to examine the effects of soluble β-oligomers of Aβ (possibly the trimeric form A₁₂) and to use these assays in a discovery process of small compounds capable of inhibiting β-oligomer formation (Walsh et al., 2005).

Targeting the downstream effects of Aβ

There are many productive lines of enquiry being applied to the downstream effects of Aβ, beginning with the direct consequences of Aβ toxicity and oxidative damage through to the promotion of Aβ clearance/degradation. Big questions remain on the role of the innate immune system and the value of targeting neurofibrillary tangle formation.

Ameliorating the toxic gain-of-function of Aβ: anti-oxidants, neuroprotectants and other products of natural origin

Existing knowledge and screens of natural product libraries have thrown up a wide variety of anti-oxidants and ‘neuroprotectants’ that have an effect on the actions of Aβ in experimental assays of its toxicity. Many of these assays are difficult to control, and there is little agreement in the field as to their validity. Nevertheless, an increasing number of papers are appearing reporting efficacy of compounds derived from plants [ferulic acid (Sultana et al., 2005), green tea extracts (Rezaei-Zadeh et al., 2005), curcumin (Yang et al., 2005) and resveratol (Marambaud et al., 2005)], and other natural products [docosahexaenoic acid (Lim et al., 2005), vitamin E (Quintanilla et al., 2005), oestrogens (Coma et al., 2005) and glutathione (Woltjer et al., 2005)]. From these investigations, a common theme emerges: that a wide variety of anti-oxidants can ameliorate the toxic gain of function of Aβ. This is consistent with our argument that Aβ itself is the principal pro-oxidant in Alzheimer’s disease. Other lines of evidence are emerging that contribute to an understanding of the oxidative stress (Nathan et al., 2005) or form a feed-forward mechanism (Tong et al., 2005) to account for the progressive nature of the disease.

Suppressing brain ‘inflammation’

There is considerable controversy around the concept that the Alzheimer’s disease brain is undergoing inflammation. As usually understood, inflammatory changes are not visible. What Alzheimer, Cajal (1928) and their contemporaries
recognized was that microglia were increased in number, activated and, together with astrocytes, were reacting to some underlying factor, possibly the amyloid within the plaque. They also recognized that the dystrophic neurites and drusge Entartung associated with the perivascular amyloid deposits could represent the reactive and regenerative response of neurons to the same injurious process. It is surprising, therefore, in recent times for the idea of ‘inflammation’ in Alzheimer’s disease to have gained such ground. In this scenario, the microglia are seen as inflammatory invaders causing damage through their release of cytokines and other powerful destructive molecules designed to respond to injury. This innate immune reaction would therefore exacerbate the clinical expression of Alzheimer’s disease and lead to its progression towards neuronal dysfunction and death. From this, trials of anti-inflammatory have been conducted, and considerable research efforts undertaken to examine the effects of anti-inflammatory in a variety of experimental models. These include the non-steroidal anti-inflammatory (Morihara et al., 2005), peroxisome proliferator-activated receptor-γ agonists (Echeverria et al., 2005; Heneka et al., 2005; Sastre et al., 2006) and cannabinoids (Ramírez et al., 2005). To date, no prospective clinical trial with an anti-inflammatory has shown a convincing beneficial outcome. In the light of the data emerging around the immunization/immunomodulation strategies against Aβ (see below), the counter-hypothesis that microglia are actually beneficial could prove to be correct.

Targeting tau aggregation in the Aβ pathway

While Aβ has captured the imagination of most Alzheimer’s disease researchers, studies of the neurofibrillary tangle and its constituent, the tau microtubule-associated protein, have progressed to a point where clear therapeutic strategies are emerging. The exact form of tau that causes neuronal degeneration is now being re-examined (Duff and Planal, 2005), with data emerging that the soluble aggregated species, akin to soluble β-oligomers of Aβ, might represent the best target. The binding sites on tau (Mukrasch et al., 2005) for a variety of interactors are potential targets. Downregulation of expression of the tau gene (Santacruz et al., 2005) or altering the alternative splicing (Rodriguez-Martin et al., 2005) also offer some new strategies.

As the molecular basis for the accumulation of tau in the diseased brain becomes clearer, so will the precise therapeutic target. If tau accumulation is closely linked to Aβ toxicity, then oxidative modifications of tau become understandable (Santa-Maria et al., 2005; Zhang et al., 2005; Reynolds et al., 2005a, b, 2006) and subject to antioxidative classes of drugs. Looking at the normal function and processing of tau has raised the possibility of using microtubule-stabilizing agents such as paclitaxel (Taxol) (Michaelis et al., 2005). Great controversy still persists on the role of normal and abnormal phosphorylation of tau in its passage from a highly soluble cytoskeletal-associated protein into an aggregated neurofibrillary tangle. If phosphorylation of specific amino acids by specific kinases such as c-Abl, Cdk5, GSK-3, ERK2 or MAPK proves to be pathogenic, then specific kinase inhibitors [including novel compounds (le Corre et al., 2006) or well-recognized drugs such as lithium (Noble et al., 2005)] might be developed for Alzheimer’s disease—indeed, a trial with lithium is currently in progress in the United Kingdom. However, if phosphorylation proves to be a secondary event, following aggregation and accumulation of intracellular tau, then this approach would not be expected to be useful. Other post-translational modifications including proteolytic cleavages have been proposed (Cotman et al., 2005)—all amenable to therapeutic drug discoveries. As with Aβ, small compounds capable of inhibiting aggregation and fibrillization of tau are now being examined in vitro (Necula et al., 2005; Taniguchi et al., 2005), but require much more work in animal models.

How does ApoE fit within the Aβ pathway?

As the major (if not the sole) genetic risk factor for determining the age at onset, it is surprising that we still do not have a definitive explanation on its mechanism of action. Targeting the ApoE gene directly, or aiming for the delivery of the protective ApoE isofrom (Dodart et al., 2005), offers some prospect of therapeutic intervention. However, understanding the precise interaction between ApoE and the processing of APP/Aβ is likely to yield more amenable therapeutic strategies. At this time, it appears most likely that ApoE acts through the clearance mechanisms governing Aβ metabolism.

Using immunization and immunomodulation of Aβ to promote clearance and inhibit toxicity (neutralization)

Since 1999, increasing evidence has accumulated to make a compelling antibody-mediated Aβ clearance/neutralization strategy. Experiments in mouse models continue to demonstrate efficacy (Banks et al., 2005; Brendza et al., 2005; Buttini et al., 2005; Klyubin et al., 2005; Rowan et al., 2005; Bales et al., 2006; Levites et al., 2006; Ma et al., 2006; Maier et al., 2006). The aborted clinical trial with the Elan Aβ12 antigen (AN1792) has provided a wealth of clinical information (Gilman et al., 2005; Lee et al., 2005a), which will assist further development of strategies designed to avoid the auto-immune adverse events (Lee et al., 2005b; Racke et al., 2005). Chief among these will be avoidance of T-cell-mediated responses and the development of passive immunization protocols (Hartman et al., 2005). The results of the current clinical trials by Elan using passive immunization are awaited with great interest (see below). In the meantime, novel methods of antigen presentation
Alteration (Okura et al., 2006; Qu et al., 2006) and the use of neo-epitopes (Arbel et al., 2005) are under investigation. Neo-epitopes generated post-transationally by modification of Ab (through oxidative mechanisms, as discussed above) should have inherently less potential to generate an auto-immune adverse reaction (Lee et al., 2006).

A startling process of lateral thinking has emerged with the report (Alvarez et al., 2006) of the use of Cerebrolysin in a successful Phase II study of Alzheimer’s disease in Spain and Romania. The product is a proteolytic extract of pig brain and is administered by multiple intravenous infusions over an 8 week period. Putting aside the possibility of transmitting a porcine form of prion disease, the method raises interesting regulatory and religious issues.

Modulating the Ab degradation pathway
The re-uptake, clearance and degradation of Ab is still subject to considerable uncertainties. If sporadic Alzheimer’s disease is the result of a low-level shift (<10%, for example) in the efficiency in any of these mechanisms, then a therapeutic strategy aimed at restoring or by-passing this faulty mechanism could be very useful. Each of the different pools of Ab probably has slightly different mechanisms of elimination, varying with the cellular compartment in which Ab resides over the course of its catabolic cycle. Several pieces of evidence point towards the enzymes NPE and insulin degrading enzyme as key players (Farris et al., 2005; Saito et al., 2005; Saido et al., 2006), but the highly sought evidence from gene linkage studies remains elusive (Eckman and Eckman, 2005). A new candidate, angiotensin-converting enzyme (ACE), has emerged (Hemming and Selkoe, 2005), and it will be of great interest to learn whether the ACE inhibitors could be having an adverse influence over the natural history of the disease.

The future
The clinical development of drugs directly targeting the Ab pathway is at an early stage of evolution. In Table 6 we list the publicly disclosed trials that are in progress or which have completed/discontinued with drugs that have been developed specifically to target the Ab pathway. The γ-secretase inhibitors trials are of immense theoretical interest, as they are likely to provide the most compelling support for the Ab theory of Alzheimer’s disease. The trials around the Ab metal binding or CAG binding sites also have the potential to address this aspect. Early clinical development of Ab aggregation inhibitors has been reported (McLaurin et al., 2006). Immunization/immunomodulation of Ab holds great promise for elucidating the Ab clearance/neutralization strategies in which there is currently a dearth of information. A variety of prospective statin-mediated approaches will also test the hypothesis that cholesterol has an important role in the biogenesis of Alzheimer’s disease. The anti-oxidant trials have the disadvantage of lacking specificity for Ab, but nonetheless will continue to provide much needed guidance for the general theory of the diseased brain being under oxidative stress.

It is extremely unlikely that a single class of compound or targeting a single mechanism of action will be sufficient to treat this illness. For this complex disease, it is far more
likely that a combination of drugs targeting various aspects of the greater APP/Aβ pathway will evolve into some form of rational therapy. Trials now in progress should represent the very beginning of the enlightenments required to find the right combinations—all predicated on the assumption that the APP/Aβ pathway underlies the cause of the disease.

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