Alzheimer's Disease: Targets For Drug Development

A.J. Larner*

Consultant Neurologist, Cognitive Function Clinic, Walton Centre for Neurology and Neurosurgery, Lower Lane, Fazakerley, LIVERPOOL, L9 7LJ, U.K.

Abstract: The numerous advances in the understanding of the neurobiology of Alzheimer's disease in the past 15 years have suggested many new potential targets for therapeutic intervention. This article gives a broad overview of the spectrum of targets for AD treatment, with particular emphasis on amyloid β-peptides and tau protein.

1. INTRODUCTION: THE NEED FOR NEW DRUGS IN ALZHEIMER'S DISEASE

The introduction of cholinesterase inhibitors (ChEI) for the treatment of Alzheimer's disease (AD) has helped to transform the field from a province of therapeutic nihilism into one of hope [1-3]. Nonetheless, the shortcomings of ChEIs must be acknowledged: the clinical response is hetereogeneous, and the effects essentially palliative rather than disease-modifying, presumably because these drugs address a consequence of the disease process (acetylcholine deficiency) rather than one of the early steps in disease pathogenesis.

With the ageing of the world's population, bringing with it increasing numbers at risk of developing AD, there is an urgent need to develop better pharmacotherapies. A number of strategies may be envisaged, which may overlap as well:

- Delaying onset, or reducing risk, of AD ("primary prevention");
- Modifying the disease process, either before or after clinical features become apparent ("disease modification");
- Symptomatic therapy for those already afflicted.

With the identification of modifiable risk factors, such as raised blood pressure and serum cholesterol [4], prevention of some cases may be feasible [5]. However, it would seem that more efficacious drug therapies must address processes involved in the pathogenesis of AD in order to effect disease modification. This article argues that the best hope for such advances comes from an understanding of the molecular pathology of AD, and from intervention early in the course of the disease. Increasing evidence for the possibility of identifying AD at an early stage ("preclinical AD" or "minimal cognitive impairment"), based on genetic testing or, of more relevance to the commoner sporadic form of AD, by means of subtle neuropsychological deficits insufficient to mandate the diagnosis of dementia according to widely accepted diagnostic criteria [6,7], opens up opportunities for earlier case ascertainment, and hopefully disease-modifying treatment.

The AD brain is characterised by a number of neuropathological findings: extracellular amyloid deposits composed principally of amyloid β-peptides, intracellular neurofibrillary tangles and neuropil threads composed principally of tau protein, neuronal and synaptic loss, and up-regulation of markers of inflammation. Although none of these changes is absolutely specific to AD, occurring in other neurodegenerative and metabolic conditions and even in normal ageing as well, nonetheless the characterisation of these lesions at the ultrastructural and molecular level has provided the impetus for new therapeutic approaches. The study of AD has proceeded apace over the past 15 years and; the literature on the neurobiology of AD is huge, such that only certain aspects relevant to the targeting of new drugs can be discussed here. Hence, amyloid β-peptides and tau protein form the principal focus of this article.

2.1 Amyloid Plaques: Physiology and Pathology of Amyloid β-Peptides

Amyloid β-peptides (Aβ) were first characterised from vascular amyloid deposits in AD brain by Glenner & Wong [8] and subsequently from plaque amyloid by Masters *et al*. [9]. The peptides show both C- and N-terminal heterogeneity, ranging in length from 39-43 amino acids. Aβ are derived from the amyloid precursor protein (APP), a membrane spanning glycoprotein of uncertain function, encoded on chromosome 21. Much has been learned of the metabolism of APP and Aβ, and of the effects on Aβ in model systems [10,11].

The discovery of a variety of mutations within the APP gene in a small number of families with autosomal dominant familial AD clearly established the critical importance of Aβ in the pathogenesis of AD, as enunciated in the (so-called) amyloid hypothesis [12]. Mutations within the presenilin genes (PS-1 and PS-2), the commonest identified genetic mutations deterministic for familial AD [13,14], also result

*Address correspondence to this author at the Consultant Neurologist, Cognitive Function Clinic, Walton Centre for Neurology and Neurosurgery, Lower Lane, Fazakerley, LIVERPOOL, L9 7LJ, U.K.; FAX: (44) 151 529 5512; e-mail: larner-a@wcnn-tr.nwest.nhs.uk

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in increased synthesis of Aβ [15]. Although doubts about the amyloid hypothesis as originally stated have been voiced and alternatives proposed [16-19], the critical role of Aβ (as opposed to amyloid *per se*) in the pathogenesis of AD remains accepted by most researchers. Amyloid plaque pathology correlates rather poorly with cognitive status in AD (*cf.* neurofibrillary pathology, *vide infra*); it may be that soluble Aβ rather than plaque deposition is the primary pathological trigger [20]. If the amyloid hypothesis is substantially correct, its implication is that hindering Aβ production and/or deposition should ameliorate all the downstream steps of the pathogenetic cascade, including neuropathological and clinical changes.

Testing of the "amyloid hypothesis" and of potential AD therapies, may be facilitated by the use of transgenic mice which carry mutant human APP genes: these animals reproduce the amyloid plaque pathology of AD, but show little in the way of neuronal death or neurofibrillary tangle and neuritic thread pathology [21-25]. The rodent brain may be intrinsically less susceptible to Aβ toxicity than the primate brain, and the latter shows increasing susceptibility with increasing age [26]. Despite their shortcomings, transgenic mice remain the best animal models of AD currently available [27].

It is apparent that APP holoprotein may be constitutively processed by an enzyme named α-secretase to form a soluble N-terminus (sAPPα) and a membrane spanning C-terminus. Since α-secretase cuts within the Aβ sequence, it precludes Aβ formation; hence this pathway is termed non-amyloidogenic (Fig. (**1**)). Production of Aβ via the amyloidogenic pathway requires the sequential action of two other secretases, named β and γ, acting sequentially to generate the N- and C-termini of Aβ respectively (Fig. (**1**)). The identification of β- and γ-secretases has been a key priority in AD research, since these synthetic enzymes are of potential importance as therapeutic targets. β-secretase has been characterised as a novel aspartyl protease [28-31], also known as β-site APP cleaving enzyme (BACE) or Asp2, the gene for which is linked to chromosome 11q23-24. The exact identity of γ-secretase remains uncertain but it is closely related to presenilin function. Neuronal cultures from PS-1 knockout mice showed marked suppression of γ-secretase activity, but α- and β-secretase cleavage of APP was unaffected [32], suggesting that presenilins mediate the production of Aβ1-42, and indeed may even be the γ-secretase. Mutation of either of two transmembrane aspartate residues in PS-1 results in loss of γ-secretase activity, suggesting that PS-1 is either a diaspartyl cofactor for γ-secretase, or is itself γ-secretase [33].

A critical experimental observation was the finding that long Aβ (Aβ1-42) has a greater propensity to self-aggregate into β-sheet conformations than the more common species Aβ1-40 (short Aβ), and is more neurotoxic [34,35]. Moreover, Aβ1-42 is the first species to be deposited in the brains of individuals harbouring APP mutations [36], and in individuals with Down syndrome who invariably develop AD neuropathology in their 40s and 50s [37]. Transgenic mutant APP mice also deposit Aβ1-42 first [38]. Moreover presenilin gene mutations (both PS-1 and PS-2) result in increased synthesis of long Aβ [15,39] although transgenic mice bearing these mutations do not reproduce AD plaque pathology [39].

2.2 Amyloid β-Peptides: Drug Targets

From our understanding of the physiology and pathology of Aβ, a number of possible targets for drug development emerge:

- Blockade of Aβ synthesis from APP, for example by inhibition of β- and/or γ-secretases;
- Blockade of the neurotoxic properties of Aβ, for example by blocking Aβ aggregation or fibrillization, since this is thought to be crucial for Aβ neurotoxicity, or possibly by blockade of cellular receptors for Aβ.

Many agents have already been claimed as modifiers of Aβ metabolism, most related to either post-translational processing of APP or to the neurotoxic properties of Aβ [40], but none has yet reached the clinical arena.

2.2.1 Blockade of Aβ Synthesis

Blockade of Aβ synthesis from APP by inhibition of β- and/or γ-secretase is an attractive therapeutic option, and one which is increasingly feasible. As a clinical approach it does

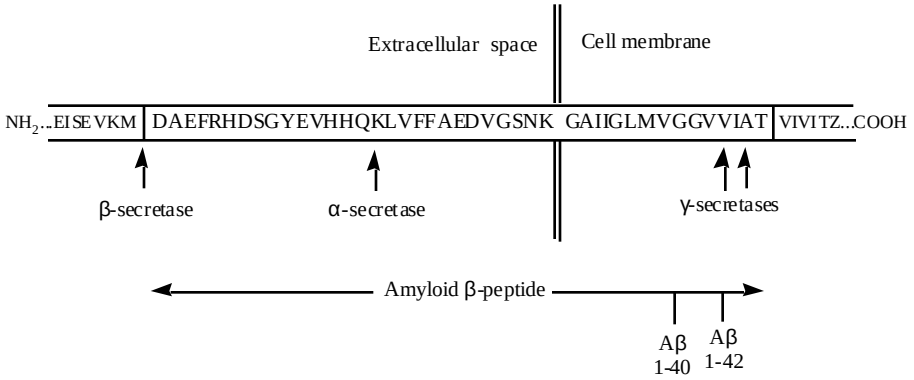

Fig. (1). Schematic of APP, showing sites of action of secretase enzymes (Reprinted with permission of the editor from *Expert Opinion on Therapeutic Patents,* **2001**, 11(6), 1047-50).

presuppose that Aβ has no critical physiological activities, but this is not certainly established; low concentrations of Aβ are found in body fluids of normal individuals [41]. Specific β- and γ-secretase inhibitors might also permit a definitive test of the amyloid hypothesis. Elimination of presenilin would also reduce Aβ production.

Because of the importance of C-terminal heterogeneity for the neurotoxic properties of Aβ, the search for γ-secretase inhibitors attracted most attention initially. Inhibition of Aβ production through the γ-secretase pathway has been reported with peptide aldehyde inhibitors of serine/cysteine proteinases, and boronic acid inhibitors of serine proteinases [42-44]. Also described are difluoroketone peptidomimetics which inhibit γ-secretase [45]. The functional γ-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenyl-glycine *t*-butyl ester has been shown to reduce brain Aβ levels in transgenic mice in a dose-dependent manner [46].

Following the characterisation of β-secretase [28-31], statine tetrapeptides have been claimed as inhibitors of the aspartyl protease BACE [47]. A report of a tripeptide aldehyde, designed on the basis of the structure of the β-secretase cleavage site, which inhibits Aβ formation (IC_{50} = 700nM), has also appeared [48].

The effects of β- and γ-secretase inhibitors in animal models of AD have yet to be fully reported. Considering their possible development for clinical use, much is already known about the *in vivo* chemistry and side-effects of protease inhibitors since they are already an integral part of the treatment of HIV disease.

2.2.2 Blockade of Aβ Neurotoxicity

Aβ neurotoxicity seems to be largely dependent on the aggregation of molecules, but there may be a number of mechanism(s) by which aggregated Aβ exerts deleterious effects: increased oxidative stress, for example at the cell membrane; destabilised intracellular [Ca^{2+}]; and cross-linking of cell surface receptors [10,11,49]. Some of these activities may prove to be therapeutic targets. For instance, a clinical trial of vitamin E and α-tocopherol, known antioxidants, has shown a delay in specific endpoints in AD patients such as institutionalisation [50]. An increasing number of antioxidants are claimed for use in neurodegenerative disease [51].

Aβ molecules within amyloid plaques are in a dynamic steady state equilibrium of aggregation and disaggregation [52], suggesting that agents capable of shifting the equilibrium in favour of disaggregation might lead to the dissolution of plaques or prevent plaque formation. Short peptides which block formation of β-pleated sheet structure of Aβ, and hence the formation of Aβ fibrils, so-called "breaker peptides", have been shown to disassemble preformed plaques *in vitro* and prevent Aβ deposition in animal models [53,54]. Peptides *per se* present certain problems as therapeutic agents, particularly their susceptibility to degradation by naturally occurring enzymes and poor blood-brain barrier permeability, but this might be obviated by using small peptide like molecules [55]. Small molecule anionic sulphonates or sulphates have also been reported to arrest amyloidosis [56].

Perhaps the most dramatic example of reducing Aβ plaque burden was observed by injecting PDAPP transgenic mice [21] with $Aβ_{42}$ as a "vaccine": in young mice, destined to develop amyloid plaques, very few developed, whereas in older mice the plaque burden was greatly reduced [57]. Vaccinated animals produced high titres of anti-Aβ antibodies. Peripherally administered antibodies to Aβ also reduced pathology in PDAPP mice, antibody triggering plaque clearance by microglia through Fc receptor-mediated phagocytosis and subsequent peptide degradation [58]. Immunotherapeutic clearance of plaques may also be directly visualised with multiphoton microscopy imaging techniques using fluorophores [59]. Some forms of learning and memory (but not all) do decline with age and Aβ accumulation in PDAPP mice, suggesting that these animals are suitable for behavioural assays of relevance to clinical AD [60]. In two other transgenic animal models of AD, reports of vaccination ameliorating cognitive deficits have appeared, along with a reduction in Aβ plaques [61,62]. Although the precise mechanism of action of vaccination is not certain, it seems likely that it enhances Aβ clearance from the brain. Phase I clinical studies of the vaccine AN-1792 (Elan) in 100 patients with mild to moderate AD have apparently shown no adverse effects or evidence that it does evoke an immune response [63]. Clinical trials are planned for 2002 and the results are keenly anticipated.

When Aβ biology was first investigated, the homology of Aβ with tachykinins, such as substance P, was remarked upon and its was suggested that Aβ might effect its cellular actions through tachykinin receptors [64], although this was subsequently disproved [65]. Although Aβ formation and action may occur entirely intracellularly [66], the possibility that its effects are mediated through the cell membrane remains, and other potential cellular receptors for Aβ have been described. Yan *et al*. [67] showed that Aβ binds with high affinity to the receptor for advanced glycation end-products (RAGE), and that RAGE receptor expression is increased in neurones and microglia in the AD brain, suggesting sustained receptor activation with deleterious effects on neurones [68]. Hence, blockade of the RAGE receptor might conceivably prevent the deleterious effects of Aβ.

Yan *et al*. [68] claimed that the work on the RAGE receptor was the first to propose cellular signal transduction receptors as mediators of Aβ toxicity since the suggested involvement of tachykinin receptors, but this claim was perhaps disingenuous since others had thought about the issue, and indeed a potential cellular receptor for Aβ had been proposed, *viz*. APP in its transmembrane orientation [69]. The proposal (the "reciprocity hypothesis") was that APP (receptor) and Aβ (ligand) interacted at the cellular membrane as part of a paracrine negative feedback signal transduction system which balanced the opposing actions of sAPPα (neurotrophic) and Aβ (neuroinhibitory) on neuronal growth and survival [69]. Recently experimental evidence suggesting that APP is a cellular receptor for Aβ has been forthcoming: APP-null neurones are less susceptible to Aβ neurotoxicity [70]. An implication of the reciprocity

hypothesis is that α- and β-secretases compete for APP, which is a substrate for both enzymes, and evidence for this has emerged [28,71]. Hence, up-regulating α-secretase activity, as much as inhibiting β-secretase, might detour more APP molecules through the non-amyloidogenic pathway and reduce Aβ production from the amyloidogenic pathway. AIT-082, a purine hypoxanthine derivative which has been trialled in AD, may act through enhancing α-secretase pathway activity [72]. The characterisation of the α-secretase enzyme as a member of the metalloproteinase family [73,74] might facilitate this approach.

All neurotrophins bind to the cellular receptor p75, specificity of neurotrophin effect being determined through interaction with tyrosine receptor kinases (Trk). p75 may also be a receptor for Aβ [75], perhaps explaining the selective vulnerability of cholinergic forebrain neurones which are dependent on NGF for their survival.

An endoplasmic reticulum-associated Aβ-binding protein (ERAB) has been characterised as an hydroxysteroid dehydrogenase enzyme, also known as amyloid β peptide binding protein alcohol dehydrogenase (ABAD; [68,76]). ABAD appears to be an important intracellular co-factor in Aβ toxicity, and hence a possible therapeutic target. Inhibitors of ABAD might block intracellular Aβ toxicity [68,76].

Blockade of Aβ neurotoxicity by proteolytic cleavage of Aβ, for example with cathepsins, has been suggested. However, this seems inadvisable as a therapeutic strategy since N-terminal truncated Aβ (NTTAβ) may themselves exert neurotoxic effects [18,19]. Russo *et al*. [77] observed increased accumulation of NTTAβ in AD patients with PS-1 mutations compared to sporadic AD, which correlated with earlier onset and shorter duration of disease.

3.1 Neurofibrillary Tangles: Physiology and Pathology of Tau Protein

Neurofibrillary tangles (NFTs) are argyrophilic intracellular structures composed at the ultrastructural level of paired helical filaments (PHFs). At the molecular level PHFs are composed principally of the microtubule-associated protein tau (τ; [78]). The physiological functions of tau relate to cytoskeletal integrity, stabilizing microtubules for axonal transport processes and facilitating tubulin polymerisation for neurite elongation.

Tau immunohistochemistry labels NFTs and also dystrophic neurites, which are found both surrounding neuritic ("mature") amyloid plaques and scatterred throughout the neuropil ("neuropil threads"; NTs). These latter structures may indicate aberrant neurite growth in AD brain [79]. NFTs and NTs show a stereotyped spatial and temporal progression in the AD brain which permits staging of the disease [80,81]. The cortical density of NFTs, especially in temporal neocortex and parahippocampal gyrus, correlates with the severity of cognitive impairment in AD better than senile plaque density [82,83]. Tau positive filamentous lesions are also evident in a number of other neurodegenerative conditions, including Pick's disease, corticobasal degeneration, and frontotemporal dementias with parkinsonism linked to chromosome 17 (FTDP-17). Mutations in the tau gene have recently been identified in FTDP-17, establishing the importance of tau in the neurodegenerative process [84,85].

PHF-tau isolated from AD brain is both hyperphosphorylated and abnormally phosphorylated [86]. In this form, tau is less able to bind to microtubules and this may lead to dysfunction of the cytoskeleton, for example impaired axonal transport, leading to dystrophic neurite formation, neuronal disconnection and eventually neuronal death [17]. Aβ may act as a stimulus to these cytoskeletal changes [87,88], possibly through the induction of tau phosphorylation [89]. However, debate has arisen as to the precise timing of tau hyperphosphorylation with respect to the disease pathogenesis, with Wischik and his colleagues producing evidence that hyperphosphorylation is a late epiphenomenon rather than an early event of pathogenetic importance (*e.g.* [90]). Rather, they argue that a conformational change in tau, akin to that for other "amyloidogenic" proteins such as prion protein, favours tau-tau aggregation into fibrils, a process which is self-propagating [90]. Resolution of the arguments regarding the pathogenetic significance of tau phosphorylation and aggregation has crucial implications for the appropriate therapeutic approach to neurofibrillary change.

3.2 Tau Protein: Drug Targets

From our understanding of the physiology and pathology of tau protein, a number of possible targets for drug development emerge:

- Blockade of tau hyperphosphorylation;
- Blockade of tau aggregation.

The critical physiological role of tau suggesting blockade of tau production *per se* is probably not desirable. A variety of agents have been claimed to modify tau metabolism and hence be of use in the treatment of AD [91,92] but none has yet reached the clinical arena.

3.2.1 Tau Phosphorylation Inhibitors

Many enzymes capable of phosphorylating tau have been identified, mostly on the basis of *in vitro* studies, hence raising questions as to relevance *in vivo*. Only glycogen synthase kinase 3β (GSK-3β) has been shown to phosphorylate tau in an AD-like pattern in intact cells [93]. This observation may be of particular significance in view of the finding that PS-1 binds to both GSK-3β and tau, a physical association which may promote tau phosphorylation [94].

GSK-3β is inhibited by lithium chloride [95,96], a drug which has been used for many years in the treatment of bipolar affective disorders. Other possible GSK-3β inhibitors have been claimed (see [92] for further details) including substituted purine derivatives, maleimide derivatives, indolocarbazole derivatives, hydroxyflavones, pyrimidones,

and hymenialdisine. Other compounds that claimed to inhibit the formation of abnormally phosphorylated PHF include propanones and other indolocarbazoles (reviewed in [91,92]). Activation of protein phosphatases, to dephosphorylate tau, has also been suggested as a therapeutic approach [97].

Clearly the therapeutic efficacy of inhibitors of tau phosphorylation, and/or activators of tau dephosphorylation, hinges on the thorny question of whether abnormal tau phosphorylation is relevant to the disease pathogenesis and whether experimental observations translate into clinically meaningful activities remains to be seen. The poverty of neurofibrillary change in animal models of AD means testing such possibilities *in vivo* is currently problematic. Tau transgenic animals may facilitate such studies.

3.2.2 Tau Aggregation Blockers

Using a tau-tau binding assay, diaminophenothiazines such as methylene blue have been shown to inhibit tau aggregation [98]. Similar claims have been made for thioxanthenes and certain polyanions (reviewed in [91]). Both phenothiazines and thioxanthenes have been used for many years for the treatment of psychiatric illness, and phenothiazines have also been claimed to reduce tau phosphorylation. Microtubule stabilisers such as paclitaxel may also prevent tau aggregation. No studies of these agents in animal models of AD are yet reported.

4. NEURONAL LOSS: APOPTOSIS; NEUROTROPHINS

Loss of neurones and synapses is one of the characteristics of AD brain pathology, for example the basal forebrain cholinergic neurones which are the principal cholinergic input to the cortex. However, it is not known by what mechanism(s) neurones are lost: there are many possibilities, including excitotoxicity, oxidative stress, destabilised intracellular calcium ion homeostasis, direct toxic effects of Aβ, and apoptosis [99].

In view of the absence of an overt inflammatory response within the AD brain, the possibility that apoptosis, or programmed cell death, might account for neuronal loss has seemed an attractive explanation. Apoptosis is a crucial mechanism in neural development, but it has been less easy to demonstrate a role in pathological states. Nevertheless, circumstantial evidence for neuronal apoptosis in AD and other neurodegenerative diseases has accrued [100,101]. Aβ has been shown to induce apoptosis *in vitro* [102] and neurones deficient for certain caspases (proteinase enzymes involved in the initiator and effector stages of apoptosis) are less vulnerable to Aβ toxicity [101]. Caspases have been shown to cleave the C-terminus of APP to produce a peptide, C31, which is a potent inducer of apoptosis [103]. Pro-apoptotic roles for the presenilins have been suggested [104,105] although this remains controversial [14]. Nonetheless, a number of agents have been claimed for therapeutic use in AD (and other neurodegenerative diseases) as modulators of apoptosis (reviewed in [100]), and some drugs already in use may act in this way [106].

Since apoptosis is a multistep pathway, its modulation for therapeutic purposes might occur at a number of points [100,101]. Important players which may be amenable to therapeutic intervention include various caspases [107] and poly(ADP-ribose) polymerase (PARP) [108]. Inhibitors for these enzymes have been described (*e.g.* [109]) but testing in animal models of AD is awaited: this may prove difficult because of the poverty of cell loss in such models [23,24].

The cholinergic basal forebrain neurones which die early in the course of AD [110], contributing to the cholinergic deficiency in AD cortex, are dependent for their survival on retrograde transport of nerve growth factor (NGF), the prototypical neurotrophic molecule, from their cellular targets [111,112]. The possibility that AD might result from NGF deficiency was first postulated twenty years ago [113]. Treatment of AD with exogenous NGF, for example by means of intracerebroventricular infusion, has been attempted, without conspicuous success. Very recently, neuronal transplantation of cells genetically engineered to secrete NGF has been performed, but the clinical outcome has yet to be reported. Although a similar approach has produced benefit in the age-associated clinical and biochemical deficits in experimental animals [114] it is unclear whether it will be clinically successful, since the AD brain is not deficient in NGF *per se*, rather there is a problem with transportation of NGF to appropriate targets, perhaps consequent on the cellular functional changes following neurofibrillary disruption. Other neurotrophins which might be relevant as therapeutic targets in AD include brain-derived neurotrophic factor (BDNF), which is deficient in AD brain [115], and small molecule and protein-based neurotrophic ligands, for example directed to p75 receptor on cholinergic neurones [55]. p75 has been suggested as a cellular receptor for Aβ [75], and Aβ can activate immediate early genes similar to those induced by trophic factor withdrawal.

5. INFLAMMATORY RESPONSE; GLIAL CELLS

Although there is no overt inflammatory response evident when observing the AD brain with standard histochemical reagents, immunohistochemical studies have made it abundantly clear that various cellular and molecular elements of the inflammatory process are involved in AD, including microglia, astrocytes, complement, acute phase proteins (*e.g.* α_1-antichymotrypsin, C-reactive protein), prostaglandins, cytokines (*e.g.* interleukins 1α, 6) and reactive oxygen species [116]. Many of these elements, all of which have the potential to interact with one another, are to be found within amyloid plaques. A generalised glial cell reaction, as judged by up-regulation of glial fibrillary acidic protein (GFAP), is found throughout the AD brain [117].

Aβ may be the key player in activating this inflammatory response. Microglia may be activated by Aβ [118], possibly through binding to RAGE receptors [67], and may produce further Aβ [119]. Expression of inflammatory proteins by activated microglia, such as macrophage-colony stimulating factor (M-CSF), attracts more microglia, thus setting up a vicious cycle [67,68]. Using an *in vivo* positron emission tomograhy marker for activated glia ([^{11}C](*R*)-PK11195),

imaging evidence for early microglial activation in AD has been reported [120]. Targeting of microglia may therefore be a valid approach. The xanthine derivative propentofylline may, amongst other actions, inhibit cytotoxic functions of activated microglia ("glial modulation"), and antibodies directed against Aβ may stimulate plaque clearance by microglia [58]. Aβ vaccine may act by a similar mechanism [57]. Other methods for activating microglia may be used, such as the cytokine transforming growth factor-β1 [121].

Perhaps the most tantalizing evidence related to the significance of inflammatory mechanisms in AD has emerged from retrospective epidemiological studies showing that use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of developing AD [122]. However, to date only one small prospective study of a NSAID, indomethacin, has reported benefit [123]. NSAID use may reduce microglial activation in the brain [124] and may also inhibit AGE activity. New trials and strategies aimed at the inflammatory process in AD are in hand, for example using inhibitors of cyclo-oxygenases (COX) 1 and 2 [116]. Since selective COX-2 inhibitors have a better side effect profile, trials with agents such as meloxicam, celecoxib and rofecoxib are keenly awaited. However, a recent trial of hydroxychloroquine, an anti-inflammatory drug already used in conditions such as rheumatoid arthritis, showed no slowing in the rate of decline in mild to moderate AD [125]. Maybe such interventions will need to be made earlier to be clinically meaningful.

6. CONCLUSIONS

Although enormous strides in understanding the molecular and cellular biology of AD have been made in the last 15 years, this has currently had little impact on the pharmacological treatment of the condition, the options for which remain largely limited to ChEIs [1]. However, the new information does indicate areas where therapeutic drug design would be appropriate. New treatments for AD would inevitably require comparison with ChEIs before their widespread introduction, but nonetheless some speculations about how we might approach the treatment of AD in the future seem permissible. Some of these therapies might be applicable not only to AD but also to other neurodegenerative disorders such as frontotemporal dementias, dementia with Lewy bodies, and prion disease, for which no treatments are currently available.

Undoubtedly a greater role for the primary prevention of AD must be given prominence, for example through control of blood pressure and serum cholesterol [4,5], avoidance of head injuries (possibly through targeting genetically susceptible individuals, *e.g.* those carrying Â4 ApoE alleles; [126]), and possibly through the prescription of oestrogen replacement therapy to postmenopausal women [127]. Perhaps individuals deemed at risk on the grounds of unmodifiable risk factors, such as family or personal history, or genetic markers (*e.g.* apolipoprotein E genotype), might be given regular anti-inflammatory and/or antioxidant medications with a good side-effect profile as prophylaxis.

If one accepts the amyloid hypothesis, then targeting Aβ synthesis and deposition would seem logical while designing new drugs for AD. The availability of the Aβ vaccine, and of β- and γ-secretase inhibitors, will allow a rigorous test of the amyloid hypothesis, and clinical results with such agents are keenly anticipated. However, preventing or reversing Aβ deposition in transgenic mice poses different problems from doing so in humans, and whether neuropathological change would translate into meaningful neuropsychological improvement, especially in advanced AD cases, is moot. For the small number of individuals identified as carrying deterministic APP mutations, the Aβ vaccine may be the treatment of choice. Whether it may also be appropriate for those carrying PS-1 or PS-2 mutations, or those deemed at risk of sporadic AD (*e.g.* ApoE Â4/Â4 carrier, sustaining serious head injury), or even all members of the ageing population, remains to be seen.

In the future, cocktails of drugs aimed at multiple targets in AD pathogenesis might be used, akin to multiple chemotherapeutic drug regimes used for neoplastic disease. For example, assuming the validity of the amyloid hypothesis, using a combination of β- and γ-secretase inhibitors (possibly with α-secretase stimulators) to block Aβ synthesis, breaker peptides or analogues thereof to block Aβ aggregation, antioxidants to block Aβ neurotoxicity, and antagonists to Aβ receptors (RAGE, APP, p75), would not be illogical, aiming to tackle the pathogenetic cascade at several points. However, such an approach would necessitate great care to avoid drug interactions and side-effects, particularly in elderly patients who are more susceptible to such effects, and if given to at risk but asymptomatic individuals. Such cocktails might be more acceptable for those with established disease, but might pose problems of compliance and perhaps be less likely to effect benefit. Whether NGF or small molecule neurotrophin agonists [55,127] might also have a role in established disease, perhaps alongside cholinesterase inhibitors, remains to be seen, likewise enhancers of microglial activation whose pro-inflammatory effects may need to be balanced by using anti-inflammatory medications and antioxidants.

For the future, some of the molecular players in AD pathogenesis may be susceptible to modification by antisense oligonucloetide strategies, which offer exquisite specificity [128]. Various strategies to circumvent the blood-brain barrier to optimize drug delivery are also being investigated, which may be applicable in AD [129].

Much work will be required to translate the discoveries of the last few years into clinical therapies. The time lag from the first characterisation of the cholinergic deficit in AD brain to the introduction of ChEIs was of the order of 20 years. With the increasing pace of research, the focus on specific molecules, and the availability of animal models, new therapies may emerge more quickly, although carefully planned and appropriately powered clinical trials assessing specific outcome measures [130] will be required before any agent can be licensed for widespread clinical usage. However, a fundamental clinical issue may still limit drug efficacy, namely early and reliable identification of AD patients. It remains the case that even for those experienced in the clinical diagnosis of AD, clinical-pathological agreement occurs in only around 70-80% of cases. Neuropsychological, neuroimaging, and neurochemical markers, perhaps in

combination, which permit early and reliable identification of AD will also be required to permit early and efficacious therapeutic intervention.

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