Challenging views of Alzheimer's disease

Cincinnati, Ohio, USA 27–29 July 2001

Meeting organizers: Keith A Crutcher, Stephen R Robinson and Mark A Smith

Meeting report by: J Wesson Ashford[†]

[†]*Author for correspondence: Sanders-Brown Center on Aging, 101 Sanders-Brown Building, University of Kentucky, Lexington, KY 40502-0230, USA*
Tel.: +1 859 281 4931 Fax: +1 859 281 4803 washford@uky.edu

The impetus for organizing this meeting came from a concern that numerous biases were limiting the study of Alzheimer's disease (AD). Many investigators have been alarmed that standard meeting formats, the review of submitted articles and national funding decisions favor particular views, to the detriment of progress in the field. This innovative approach of applying a debate format to scientific interchange allowed a head-to-head analysis of conflicting positions on key biological issues in AD. The debates were designed as a fair forum for presentation of 'heretical' hypotheses, close scrutiny of 'entrenched' ideas and improvement of communication. Both sides of all issues had strong points needing assimilation to further the understanding of AD and move the field forward.

Program

The specific areas of point-counterpoint were:

- Does amyloid or its precursor cause familial AD?
- ApoE4: is it the absence of good or the presence of bad?
- Is nonfamilial AD inherited?
- Fire! Are oxidation & inflammation the culprits in AD?
- Is AD a vascular or a metabolic disorder?
- Were the tauists right all along?
- Cyclin towards, or away from, dementia?
- Summation

Does amyloid or its precursor cause familial AD?

Moderator: S Snyder

β-amyloid deposition is essential to AD neuropathology
S Estus, B Vassar, M Kindy, D Borchelt

Dysfunction of APP is essential to AD neuropathology
R Neve, C Atwood, S Robinson

The first issue to be challenged was the role of β-amyloid (Aβ) in AD, in its safest arena of presumed causation, familial AD. The earliest onset cases of AD have been attributed to mutations in the amyloid-precursor protein (APP) gene and the presenilin 1 and 2 genes, which are thought to play a role in cleavage of APP as γ-secretases. It was first argued that by including the presence of Aβ plaques in the pathological definition of AD, with no clear relation between plaques and disease progression, that a tautology was created. Therefore, the definition automatically excludes those cases without deposition. However, since Aβ depositions are commonly present in the elderly brain and do not by themselves constitute AD, while many cases of dementia have neurofibrillary changes without Aβ deposition, this tautology may have misdirected the field to considering Aβ deposition to be a critical factor in development of AD. A case was made for APP expression being important in neuroplasticity, with Aβ potentially playing an important role in the formation of new synapses. It was considered that Aβ formation could be a normal neuronal response since deposition is increased when stress, such as traumatic injury or ischemia, has occurred. Aβ production may also be a normal brain response to help neurons regenerate, with Aβ deposition occurring only in extreme circumstances or when the genetic predisposition favors excessive response.

There was considerable discussion about the role of Aβ immunization, a procedure currently under study as a treatment for AD. In mouse models, Aβ immunization decreases the development of Aβ burden and prevents behavioral deterioration. Alternatively, the immunization does not eliminate Aβ deposits once they have formed and it does not improve behavior after it has deteriorated. While interesting, there was considerable concern that no model accurately reflects the human condition of AD and conclusions from such model interventions must be interpreted cautiously.

The general conclusion was that APP expression and Aβ production are probably both involved in neuroplastic mechanisms and their dysfunction is important in the development of AD. However, there was progressive acknowledgement that the amyloid story is only one component of AD, though there remains hope that Aβ vaccination could eliminate AD.

ApoE4: is it the absence of good or the presence of bad?

Moderator: JW Ashford

Absence of ApoE3 or ApoE2 contributes to AD pathology
MJ LaDu, W Rebeck, M Kindy

Presence of ApoE4 contributes to AD pathology
K Crutcher, B Nathan, J Raber, B Teter

Debates 2 and 3 focused on the role of the apolipoprotein E (ApoE) gene in AD. Debate 2 considered where the ε2 and ε3 alleles were more protective against AD than the ε4 allele or whether

 ISSN 1473-7175

the ε4 allele contributed specifically to the development of AD. This debate began with a presentation of evidence that the sprouting component of neuroplasticity is dependent on ApoE and that the allegedly abnormal effect of the E4 protein is to promote sprouting. Numerous abnormalities were related to the E4 protein, including defects in microtubule polymerization, cholesterol trafficking and oxidation, as well as neurotoxicity. Mice genetically modified to have ε4/4 genotype have less synaptophysin than ApoE-knockout mice and E4 protein kills more neurons in culture than E3. However, the opponents pointed-out that E3 protein binds Aβ, improving clearance and preventing deposition of fibrillar Aβ better than E4, while E4 protein does not have adverse effects in other neurodegenerative diseases, such as Parkinson's or stroke. Also, the ApoE knockout mice show impaired neuroplasticity. Further, ApoE protein alleles protect against oxidative stress, with E2 > E3 > E4.

The important context is that the ε4 allele is the ancestral form, with ε3 appearing about 300,000 years ago and relatively rapidly becoming the predominant form. ε2 has been in existence for about 200,000 years, though proliferating less extensively so far. Therefore, the key question remains to delineate the specific adaptive advantages of ε2 and ε3 over ε4. Further, each allele has additional subpolymorphisms that could explain additional variations in the effects of different alleles. There are also polymorphisms in the ApoE promoter gene that could be linked to variations in expression and in turn affect AD development.

Is nonfamilial AD inherited?

Moderator: Robert B Petersen

Nonfamilial AD is mainly due to genetic factors
J Mortimer, JW Ashford

Nonfamilial AD is mainly due to environmental factors
A Campbell, W Grant, R Itzhaki, J Savory

The third debate addressed the issue of nonfamilial AD, which constitutes at least 95% of all cases. The issue was the classic nature-nurture question. The aluminum theory, the first serious causal theory of AD, was presented but there were also arguments against the hypothesis that AD is caused by environmental aluminum. The second environmental factor presented was dietary cholesterol. There are highly significant correlations between dietary fat and cholesterol intake and the prevalence of AD across many countries. However, this finding has methodological concerns, such as cultural issues that may account for the relationship. Controlled comparisons are needed to elucidate the precise role of diet in AD causation. An interesting conundrum is that dietary habits established early in life could mimic genetic influences and genetic factors could influence dietary preferences. Recent evidence of a link between cholesterol lowering drugs and AD prevention provides attractive evidence to direct interest to this theory. Herpes-simplex virus Type 1 (HSV1) was presented and data is being collected, which links this virus to AD in patients with an ApoE-ε4 allele. Numerous other environmental agents, such as homocysteine and traumatic brain injury, might also contribute to AD development.

At this time, the role of genetic factors in AD is well established and ApoE is the most important. In the US the ApoE-ε4 allele occurs in 22% of the whole population and 60% of AD patients in clinics, by itself responsible for 50% of the AD cases. The ε4/4 genotype carries 24 times the AD risk of the ε2/3 genotype. The ε4 allele has been referred to as a 'susceptibility' gene, but no ε4/4 carrier has been shown to reach age 90 without having AD. Other familial factors may also play roles in AD. Twin studies of AD show high concordance in monozygotic twins and suggest that AD heritability exceeds 70% above age 70 – though a question was raised as to whether diet affected this result. Evidence from the 'Nun Study' has indicated that AD predisposition may be established by late adolescence, diminishing the role of later environmental variations and suggesting that causative factors establish their impact early. Education, often viewed as an environmental factor protecting against AD, may be a function of earlier genetic or environmental influences related to later vulnerability to AD.

Nature and nurture must be seen as interacting. For example, the sickle-cell gene is harmless at sea level, deadly at high elevations and protective in regions where malaria is endemic. Until we know how to modify or prevent the impact of genetic factors in AD, we must watch our diets and prevent traumatic brain injury. At this time, genotyping for diagnosis or risk-estimation is not accepted as standard medical practice, in spite of the important information that it provides. However, many patients and family members are regularly told their ApoE genotype. This information should be given freely along with genetic counseling to those requesting it.

Fire! Are oxidation & inflammation the culprits in AD?

Moderator: H Ghanbari

Oxidative stress & inflammation are essential to AD pathogenesis
A Butterfield, S Griffin, G Munch, GM Pasinetti

Oxidative stress & inflammation are secondary to AD pathogenesis
C Atwood, S Robinson, MA Smith

In this debate, the central issues of oxidation and inflammation were presented. Oxidative stress and free-radical-related pathology have been theorized to be central factors in the aging process. A considerable amount of circumstantial evidence has linked oxidative processes to AD as well. Part of the supporting evidence is that Aβ induces oxidative processes and certain heavy metal ions, including iron and copper, which may be elevated in the AD brain, possibly attached to Aβ, also cause oxidative stress. However, it is unclear if the presence of these factors is primary or secondary in AD. Further, the role of antioxidants as a treatment for AD has only been weakly supported.

Inflammation is clearly occurring in AD and many pathways associated with the development of this inflammatory response were presented, including specific segments

of the inflammatory cascade. Aβ provokes a neurotoxic response by microglia. But, again, it was unclear if these factors were an early, primary part of the AD pathological process, or a late response to other factors. In this case too, whether anti-inflammatory drugs are protective or slow the rate of disease progression has still not been clarified. Techniques are needed to allow for the assessment of Aβ deposition, inflammation and oxidation in the living brain.

Is AD a vascular or metabolic disorder?

Moderator: CH Phelps

AD is primarily due to vascular pathology
P Grammas, M Yamada, B Zlokovic

AD is primarily due to a disorder of brain metaboliam
J Blass, G Gibson, S Hoyer

This heated discussion pitted those arguing that AD is caused by intrinsic disorders of brain metabolism against those that considered that AD is related to vascular factors.

The case for intrinsic metabolic factors highlighted fundamental metabolic pathways that are disrupted in AD and linked these pathways directly to the well-known drops in glucose utilization by the AD brain. However, the drop in glucose metabolism could also be secondary to primary loss of neuronal volume, regardless of the cause. An important new point made was that insulin, which is also produced in the hypothalamus, could control the enzyme glycogen synthase kinase (GSK) 3β. This enzyme may play a central role in the hyper-phosphorylation of tau, the presumed critical precursor event to neurofilament formation and neurofibrillary tangle deposition and loss of control of this enzyme could lead to AD.

From the counter-point, evidence was presented that vascular Aβ may be more closely associated with tau pathology than the distribution of diffuse or neuritic plaque Aβ. Further, the blood-brain-barrier endothelial cells may play the critical role in regulating the neuronal microenvironment and the failure of these cells could lead to the critical changes that precipitate AD pathology.

While both sides presented important aspects of AD, neither was able to convince the audience that they had established the foundation for AD causation.

Were the tauists right all along?

Moderator: D Morgan

Neurofibrillary pathology is central to AD
S Binder, K Iqbal

Synaptic dysfunction precedes cytoskeletal pathology
D Borchelt, B Honer, P Coleman, H Geerts

This dispute pitted two fundamental AD camps against each other: those believing that pathology of the microtubule-associated protein-tau is central to the development of AD (Tauists) *versus* those that consider tau changes to be secondary to Aβ changes (βAptists) or related to other factors. The Tauist team laid out the fundamental cascade of changes thought to lead to AD pathology, starting with a variety of causative factors leading to excessive phosphorylation of tau and in turn to neurofilament build-up resulting in slow neuronal degeneration. In this line, the increased molar concentration of phosphorylated-tau relates to loss of neuronal processes but cell death is an inconsequential late-stage event. The neurofibrillary tangles and resulting synapse loss are the factors that are most closely related to dementia severity in AD patients.

The opposing team presented evidence that synaptic dysfunction precedes cytoskeletal pathology. Aβ secretion influences neuronal activity and modulates synaptic turnover, putting Aβ in the critical position for causing AD pathology. Further, in early AD there seems to be an increase of synaptophysin and other synaptic proteins before neurofibrillary tangles develop. This team raised concern about whether phosphorylation of tau by GSK-3β was a central event, especially since numerous other brain kinases are also available to phosphorylate tau. Also, no tau mutations are known to cause AD, while all of the genetic mutations that cause familial AD are related to APP metabolism.

By way of rapprochement, it was acknowledged by both sides that mouse models were not satisfactorily analogous to AD and autopsy studies most often examined pathology that was very late in a long process and not necessarily reflective of primary changes. Clearly, there is still not enough information available to understand the AD process.

There was a residual impression from the debate developed by audience questions that aberrant tau phosphorylation is a central aspect of AD, though its cause is still undetermined. It is tau hyperphosphorylation that leads to neurofibrillary changes, perhaps most importantly in the form of neuropil threads. The disruption of the cytoskeletal structure and particularly dendritic flow, impedes communication between the cell body and the synapse, leading to a disturbance of neuroplastic changes. However, many of the functions presented in these debates have been shown to disrupt neuroplasticity, particularly those functions associated with new synapse formation. Therefore, there was some consensus that the central attack of the AD pathological process is on neuroplastic mechanisms and disruption of these neuroplastic mechanisms leads to all of the sequelae of AD. Reference was made to the initial proposal of this theory [1] and recent papers that have augmented and further developed this concept [2–5].

Cyclin towards or away from dementia?

Moderator: JP Blass

Re-expression of cell cycle proteins induces neuronal cell death during AD
K Herrup, T Arendt

Re-expression of cell cycle proteins is a response to neuronal injury in AD
MA Smith, R Bowser

The final debate focused on a controversial issue as to whether cell cycle proteins are reactivated in neurons during the development of AD neuropathology. The issues about whether neurons affected by AD pathology were re-entering cell cycles or whether cell death was even relevant to AD remained in dispute. Numerous cell cycle proteins are expressed in neurons and glia in several areas of the brains of AD patients where neuropathology is abundant. But the problem remained that only sequential activation would signify cell cycle entry,

while the disorganized activation of these proteins could reflect the aberrant responses of cell bodies that are losing contact with their synapses. Thus, it was not clear whether the neuroplastic changes attacked by the AD process are related to basic processes in the affected cells or has a relation to the cell cycle.

Summation

Moderator: C Kircher

In the end, the moderators were allowed to voice their impressions about the debates and speculate about future directions in AD. There was unanimous support for the debate format as a means to improve communication in this field. In reviewing each of the debates, the emphasis was not on whether there was a winning side but rather that there were important new ways to view problems that took into account the issues on both sides of the debates. There was a perception that researchers need to understand the disease process relative to the afflicted patients, not in terms of cell culture or mouse models. There was a clear request for better diagnostic techniques. Dr. Creighton Phelps, the Director of the Alzheimer's disease Centers Program at the National Institute on Aging, referred specifically to new brain imaging techniques by Drs. Nicholas Fox and Scott Small that could improve diagnosis and measure brain atrophy over a period as short as 2 months. Clinical trials are also being organized with a PET ligand, DDNP, which was developed by Dr. Gary Small at UCLA which tags plaques and tangles in the brain. Understanding of cholinergic mechanisms in AD in the late 70s and early 80s has led to the recently successful application of anticholinesterase treatments of AD, which have provided tremendous economic benefits. Basic information that is under development at this time and being fostered by improved communication, such as this conference, will lead to even better treatments and hopefully prevention of AD in the future.

The consensus was that the debate format at this conference was excellent and should be repeated in 2003 and extended to the clinical arena of AD diagnosis and treatment. There was an exuberant level of appreciation for the innovators and organizers of the conference, Keith A Crutcher, Stephen R Robinson and Mark A Smith.

References

Program highlights are posted at:
www.worldeventsforum.com/alzheimer.html

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