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Review

IMMUNOLOGICAL ASPECTS AND ANTI-AMYLOID STRATEGY FOR ALZHEIMER'S DEMENTIA

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Alzheimer's dementia (AD) is the most common form of dementia among the elderly, accounting for at least two-thirds of all dementia cases. It represents a costly burden, since its global prevalence is estimated at 24 million cases. Amyloid beta or Aβ plaques and neurofibrillary tangles define AD pathologically but do not fully explain it, because dementia may also be caused by inflammation resulting in neuronal, axonal synaptic loss and dysfunction. An important component of AD pathophysiology are amyloid plaques surrounded by activated microglia, cytokines, and complement components, suggesting inflammation. In the diagnosis of AD, cerebrospinal fluid markers, especially *in vivo* amyloid measurements, contribute to an accurate assessment of AD pathology and differential diagnosis. Aβ levels are a very good marker for the presence of amyloid deposits in the brain, while total tau and phosphorylated tau are useful for the detection of neurodegeneration. The implementation of anti-amyloid therapy and other disease-modifying interventions may have immense clinical impact if initiated at an early or presymptomatic stage of AD, before significant brain damage occurs. This paper briefly reviews the abovementioned topics and provides recommendations for future studies.

KEY WORDS: *Alzheimer's disease, amyloid-β (Aβ) plaques, anti-amyloid therapy, inflammation, microglia*

Alzheimer's dementia (AD) is a progressive degenerative disease of the brain and the most common cause of dementia among elderly persons, accounting for at least two-thirds of all dementia cases (1, 2). The global prevalence of dementia is estimated to be as high as 24 million cases and is predicted to double every 20 years by 2040, which will undoubtedly lead to an even higher and more costly burden than at present (3). AD is generally defined as a progressive decline in cognitive functions which typically begins with memory impairment and a characteristic change in personality and executive functions. Before death, individuals usually become dependent on caregivers. There is no available cure.

The neuropathological hallmarks of an AD brain include diffuse and neuritic extracellular plaques composed of amyloid beta (Aβ) peptides (Figure 1), which are frequently surrounded by dystrophic neurites and intraneuronal neurofibrillary tangles (NFT), which are in turn composed of paired helical filaments or phosphorylated tau proteins (4). These hallmark pathologies are often accompanied by reactive microgliosis and neuron and synapse loss. The aetiological mechanisms underlying the neuropathological changes in AD still remain unclear but are probably affected by environmental, genetic, and neuroinflammatory factors (3).

Up to 3 % of AD cases are caused by an autosomal dominant mutation with three thus far identified genes: the amyloid precursor protein (*APP*), presenilin 1 (*PSEN 1)*, and presenilin 2 *(PSEN 2)* (5). The Aβ peptide is cleaved from the APP by the sequential

activities of β-secretase and γ-secretase enzymes. Aβ occurs in multiple forms, including those ranging from 37 to 43 amino acids in length. Among these, $Aß_{42}$ seems to be essential for initiating Aβ aggregation and is considered central to the amyloid cascade hypothesis of AD (6, 7). The APOE ε4 allele is the most important genetic risk factor for sporadic AD (8).

Inflammation mediated by activated microglia is an important component of AD pathophysiology (9) and neuritic plaques are the foci of the local inflammatory response (10). Aβ neuritic plaques are surrounded by activated microglia, cytokines, and complement components, which are called „inflammatory foci". In contrast, diffuse plaques without reactive microglia are considered clinically benign Aβ deposits.

These objectively measured AD biomarkers are useful for the diagnosis, longitudinal assessment, and evaluation of the subsequent therapeutic response. Aβ, one of the most frequently used biomarkers, is produced in neurons and secreted into the brain's extracellular space. Measurements of cerebrospinal fluid (CSF) amyloid peptide levels can indicate the extent of peptide generation and clearance in the brain, particularly in radio-labelled amino acid infusion studies (11). The mean concentration of $Aβ_{42}$, a major component of Aß, in the CSF was reduced by as much as 50 % in AD subjects relative to age-matched controls (12), as the result of Aβ deposition in amyloid plaques, which prevented its transit from the brain into the CSF. In support of this hypothesis, all individuals with Aß deposits showed low concentrations of Aß in the CSF regardless of their cognitive status (13-15). However, CSF Aβ does not correlate well with disease duration or severity (6), which is consistent with ^{11}C-labeled Pittsburgh Compound B (^{11}C-PIB) results from a study that showed that amyloid retention does not change significantly during the symptomatic stages of AD (16), and further supports the finding that amyloid pathology occurs very early in the process of this disease (6). Thus, CSF Aß can serve as a diagnostic and surrogate biomarker for Aß deposition in the brain. The decrease in CSF Aß appears to precede amyloid retention as detected by amyloid imaging using ^{11}C-PIB, signifying what is perhaps the first indication of AD pathology in cognitively normal individuals (13, 15, 17).

Tau is a major protein component of, at least initially, intraneuronal NFT and is elevated in the CSF of most AD patients. In addition, it has been shown that total tau (T-tau) levels in the CSF can rapidly

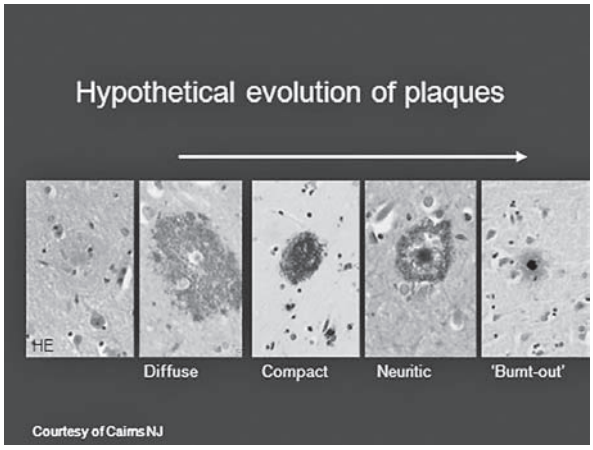

Figure 1 *The evolution of plaques in Alzheimer's disease. Diffuse plaques, also called benign plaques, occur much earlier than neuritic plaques, thus supporting the idea of a therapeutic intervention in the early stage of the disease. (Courtesy of Professor Nigel J Cairns, PhD, Washington University in St Louis, MO, USA)*

increase following neuronal injury, indicating the severity of the underlying neurodegeneration (15). T-tau, as a biomarker of neuronal injury, can transiently increase after any acute brain injury, such as stroke or physical trauma (16). The abnormal phosphorylated tau (p-tau) (17) has increased specificity for discriminating AD from other dementias [e.g., fronto-temporal dementia or dementia with Lewy body (DLB) (2, 18) and non-demented controls (11)]. Distinguishing DLB from AD is a major clinical challenge due to different optimal management, allowing for the initiation of effective pharmacotherapy and avoiding neuroleptic sensitivity. The deposition of Aβ peptides drives cerebral neuroinflammation by activating microglia. Indeed, Aβ activation of the NLRP3 inflammasome in microglia is fundamental for interleukin-1β (IL-1B) maturation and subsequent inflammatory events. However, it remains unknown whether the NLRP3 inflammasome, which leads to the production of the pro-inflammatory IL-1B, thus indicating an inflammatory process, plays a role in the progression of AD (22). On an animal model, behavioural and cognitive functions were improved by reducing the signalling from this particular inflammasome, making inflammasomes a promising therapeutic target for AD therapy (22). Chronic inflammation coupled with neuronal ageing induces cellular stress and concomitant impairments in basic neuronal functions. The beneficial side of the

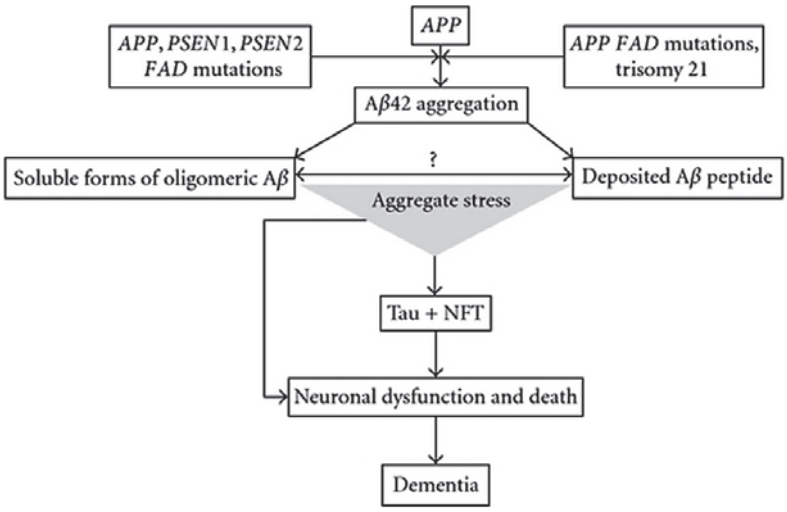

Figure 2 *The Amyloid Cascade Hypothesis (from Reitz C; Int J Alzheimers Dis 2012, 2012:369808)*

inflammation immune system against amyloids is the reduction of Aβ and the immunization against Aβ.

Today's anti-amyloid strategies include immune therapies and vaccines for amyloid clearance, plaque busters, and amyloid clearance enhancers. These drugs are designed as an antibody to bind Aβ. The stimulation of Aβ clearance from the brain of AD patients via the administration of Aβ antigens (active vaccination) or anti-Aβ monoclonal amyloid antibodies (passive vaccination) has already been applied. In 2001, the first clinical trial with the Aβ peptide active vaccine (AN1792, consisting of preaggregate Aβ and an immune adjuvant, QS-21) was initiated. After the second dose, however, meningoencephalitis occurred in 5 % to 6 % of immunized patients, and the trial was stopped. The clearance of Aβ from the human brain, however, was successful (23), suggesting that the immune response generated against the peptide elicited the clearance of Aβ plaques. Passive immunotherapy with anti-amyloid substances is currently in development.

Bapineuzumab *(β-amyloid peptide (i) neutralizing (u) monoclonal antibody),* was designed to bind to Aβ in patients with mild-to-moderate forms of AD, but all Phase III clinical trials of bapineuzumab have been halted in 2012, because the drug did not meet the endpoints for cognition and global function (24). Also, the occurrence of vasogenic oedema after bapineuzumab, and more rarely brain microhaemorrhages (especially in Apo E ε4 carriers), has raised concerns about the safety of these antibodies directed against the N-terminus of Aβ. Recently, solanezumab (*soluble amyloid neutralizing (u) monoclonal antibody*), a humanized anti-Aβ monoclonal antibody directed against the Aβ peptide, was shown to neutralize soluble Aβ species, thus becoming the first therapeutic drug to be evaluated in the anti-amyloid treatment in asymptomatic AD (25). This was the first application of an Aβ clearing drug in older people thought to be in the presymptomatic stage of AD with evidence of amyloid in their brains shown by ^{11}C-labeled PIB imaging (26), but with no clinical symptoms of the disease yet. The clearance of plaques, however, has not yet been shown to reverse memory in clinical trials, even though many scientists in the field feel that immunotherapy holds promise. The failure of bapineuzumab to produce benefits has aroused some scepticism about the amyloid cascade hypothesis (3), which holds that toxic amyloid proteins cleaved from the APP initiate AD (Figure 2).

Recently, a coding mutation (A673T) found in the *APP* gene conferred strong protection against AD even in people who carried the APOE4 gene, therefore demonstrating the importance of keeping Aβ levels in the brain low (1). The discovery of the protective APP mutation in approx. 1 % of Icelanders suggested that lowering amyloids in the brain, either with anti-amyloid therapy or some other treatment, must begin long before the symptoms of AD set in. As a result, the levels of Aβ in the CSF began to decline 25 years before the expected onset of symptoms, which is a signal that the protein is being sequestered in the brain

as insoluble plaque, as shown in a group of 128 participants from the prospective and longitudinal Dominantly Inherited Alzheimer Network (DIAN) study on early onset AD (27). Several grants for Alzheimer's prevention studies were recently awarded by the US National Institutes of Health (NIH) in order to test anti-amyloid drugs on members of a Colombian family, the largest extended family in the world with a gene mutation that causes them to develop AD early, showing cognitive impairment by around age 45, and full dementia several years later. In this trial, family members as young as 30 will receive another anti-amyloid monoclonal antibody called crenezumab (25). So far, three investigational monoclonal antibodies against amyloids have been selected for clinical trials that will try to prevent dementia in people who are on the path to AD due to an inherited autosomal-dominant mutation (28).

In the majority of cases (97 %) involving late-onset AD caused by a number of risk-factor genes, identifying these genes will be the greatest advancement towards effective treatment. Based on the new diagnostic criteria for AD (26, 29) and recent experience with major failures of anti-Aβ drugs in mild-to-moderate AD patients, one could argue that clinical trials on potential disease-modifying drugs, including immunological approaches, should be performed in the early or preclinical stages of AD, years before the first symptoms occur (30).

In summary, despite tremendous investments in basic and clinical research, no cure or preventive treatment for Alzheimer's dementia has been yielded. The amyloid-beta (Aβ) peptide has become a major therapeutic target in AD. The genetic mutations cause increased Aβ levels, followed by amyloidosis, tauopathy, brain atrophy, and decreased metabolism. Inflammatory processes are strongly correlated with the onset and progression of AD in humans, and could have a pivotal role in AD aetiology. Based on the new diagnostic criteria and recent experiences with anti-Aβ drugs in early-stage AD patients, one could argue that the treatment for AD patients should start years before the first onset of clinical symptoms.

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Sažetak

IMUNOLOŠKI ASPEKTI I ANTIAMILOIDNE STRATEGIJE ZA ALZHEIMEROVU DEMENCIJU

Alzheimerova bolest (AB) najučestaliji je oblik demencije u starijih: najmanje dvije trećine sveukupnih slučajeva demencija upravo je AB. Prevalencija bolesti u svijetu procjenjuje se na 24 milijuna slučajeva, što je značajno financijsko opterećenje. Amiloidni beta (Aβ) plakovi (eng. *amyloid plaques*) i neurofibrilarni snopići (eng. *neurofibrillary tangles*) glavne su patološke karakteristike AB-a, jer uključuju i upalu, neuronalnu leziju te gubitak odnosno disfunkciju aksona i sinapsa. Amiloidni plakovi okružuju aktiviranu mikrogliju, citokine i komponente komplementa, što je pokazatelj upale, koja je važna karika u patofiziologiji AB-a. Prilikom dijagnosticiranja AB-a važnu ulogu imaju markeri likvora i *in vivo* detekcija amiloida u mozgu, koji pridonose optimalnoj procjeni patologije ove bolesti i njene diferencijalne dijagnoze. Za detekciju amiloida u mozgu koristi se Aβ u cerebrospinalnom likvoru, a za detekciju neurodegenerativnih promjena iznimno su važni total-tau (T-tau) i fosforilirani-tau (p-tau) markeri. Uvođenje antiamiloidne i ostalih terapija moglo bi imati značajne pozitivne kliničke učinke ako bi se s terapijom započelo rano i/ili u presimptomatskoj fazi bolesti, prije nego dođe do značajnog gubitka neurona. U ovom radu dan je kratak prikaz spomenutih tema, uz osvrt na aktualna i buduća istraživanja i kliničke studije primjenom monoklonalnih protutijela protiv amiloida kao mogućih uzročnika AB-a u početnoj i/ili ranoj fazi bolesti.

KLJUČNE RIJEČI: *Alzheimerova bolest, amiloid-beta (Aβ) plak, antiamiloidna terapija, mikroglija, upala*

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