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Review

Alzheimer's disease and gut microbiota modifications: The long way between preclinical studies and clinical evidence

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ABSTRACT

Recent studies have suggested the role of an infectious component in the pathogenesis of Alzheimer's disease (AD). In light of this, research has focused on some bacteria constituting the intestinal microbial flora which can produce amyloid. Once generated, the latter hypothetically triggers a systemic inflammatory response which compromises complex brain functions, such as learning and memory. Clinical studies have shown that, in cognitively impaired elderly patients with brain amyloidosis, there is lower abundance in the gut of *E. rectale* and *B. fragilis*, two bacterial species which have an anti-inflammatory activity, versus a greater amount of pro-inflammatory *genera* such as *Escherichia/Shigella*. According to these findings, some clinical studies have demonstrated that supplementation with *Lactobacilli*- and *Bifidobacteria*- based probiotics has improved cognitive, sensory and emotional functions in subjects with AD. Moreover, certain herbal products, in particular dietetic polyphenols, have proved capable of restoring dysbiosis and, therefore, their prebiotic role could be effective in counteracting the onset of AD regardless of their activity of free radical scavenging or enhancement of the cell stress response. One of the recent greatest novelties in the field of neurodegenerative diseases is the chance to prevent or slow down AD progression with agents, such as probiotics and prebiotics, acting outside the central nervous system.

Contents

1. Background

Alzheimer's disease (AD) is a chronic, rapidly progressive neurodegenerative disease, characterized by memory loss, inability to carry out normal daily life activities and behavioral changes. At present, AD is considered the most common form of dementia in the elderly and the first cause of hospitalization or admission to medical nursing homes [1].

The Alzheimer's Association has recently provided accurate epidemiological data on the incidence and prevalence of AD in the

Abbreviations: Aβ, amyloid-β-peptide; ACh, acetylcholine; Apo, apolipoprotein; APP, amyloid precursor protein; CYP, cytochrome P-450; DYRK1, dual specificity tyrosine-phosphorylation-regulated kinase 1A; GABA, γ-aminobutyric acid; GSK, glycogen synthase- kinase; HSV-1, *herpes simplex* virus type I; IBS, irritable bowel syndrome; IL, interleukin; LRP1, Low density lipoprotein receptor-related protein 1; MMSE, mini-mental state examination; NFkB, nuclear factor kB; NFT, neurofibrillary tangles; NP, neuritic plaques; PS, presenilin; TREM2, triggering receptor expressed on myeloid cells 2; UGT, UDP-glucuronosyltransferase.

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United States. In particular, it was estimated that 5.5 million Americans were affected by AD, including 5.3 million people aged 65 years or over, with only 200,000 below the age of 65, the latter affected by the so-called younger-onset AD. Alzheimer's disease increases with age, reaching 32% in subjects aged 85 years or older [1]. Women are more affected than men (two-thirds vs. one-third, respectively), and the reason mainly lies in the fact that women have a higher survival rate than men and age is, as previously mentioned, the most important risk factor in developing AD [1]. Further interesting data concern ethnicity: African-American and Hispanic elderly people have about a twofold AD prevalence than the white age peers, and this difference does not seem to be due to genetic factors, but rather to lifestyle and particular diseases (e.g., diabetes and cardiovascular diseases) affecting Afro-Americans and Hispanics [1]. It has been estimated that approximately 480,000 individuals over 65 years of age will be affected by AD in 2017, once again the most affected groups ranging between 75 and 84 years of age and >85 years of age (12 new cases per 1000 people vs. 37 new cases per 1000 people, respectively) [1]. The situation will also worsen in the near future as, in 2050, the number of people over 65 with AD will increase from the current 5.3 million to about 13.8 million subjects [1]. According to the data provided by the Center for Disease Control and Prevention until 2014, the number of subjects dying principally of AD was 93541, although this figure may be underestimated considering that subjects with AD could also have died of severe comorbidities and, therefore, the diagnosis of AD is not evident from the death certificate [1].

Unfortunately, such accurate and updated data on AD epidemiology are not available for Europe. In a recent *meta*-analysis, Niu et al. [2] have reported that the prevalence of AD increases with age reaching about 23% in subjects aged over 86. This study has also confirmed that women are more affected than men (7.13% vs. 3.31%). As far as the incidence is concerned, once again the groups mainly affected are those aged between 75 and 84 years and >85 years (14 cases per 1000 people vs. 36 cases per 1000 people respectively) [2].

2. Pathogenesis of AD

From a histopathological viewpoint, AD is characterized by two main hallmarks, i.e. cerebral deposits of neuritic plaques (NP), consisting of assembled and insoluble forms of amyloid-β-peptide (Aβ), and by neurofibrillary tangles (NFT) composed of hyperphosphorylated microtubule-associated tau protein [3].

Amyloid-β-peptide is produced through a proteolytic process involving the substrate amyloid precursor protein (APP), a type-I transmembrane protein highly expressed in neurons, and two aspartyl proteases, such as β- and γ- secretases [4–6]. The combined action of these enzymes on APP leads to the production of both an extracellular soluble form of APP (sAPPβ) and C-terminal fragments (CTF 99 and CTF 89) and the release of Aβ peptides of various length, ranging from 38 to 43 amino acids [7,8]. $A\beta_{1\text{-}40}$ is the most abundant, but less toxic form found in the cerebrospinal fluid, whereas the $A\beta_{1\text{-}42}$ form is more hydrophobic, prone to self-aggregation at low concentrations and whose accumulation has been reported in NP [7,8]. Once formed, Aβ activates specific kinases, such as glycogen synthase-kinase (GSK)-3β and DYRK1A which, in turn, trigger the hyperphosphorylation and further aggregation of tau protein into NFT [9,10]. Although several scientific papers supported the traditional amyloid hypothesis linking Aβ deposition to AD progression, a close correlation between insoluble plaque density and cognitive deficits in AD patients has not been demonstrated [11–13]. Over the last decade, new scientific evidence from preclinical and clinical studies has suggested that an imbalance between the production and clearance of soluble forms of Aβ peptides might be an early and initiating pathological feature responsible for the synaptic dysfunction and cognitive decline in AD [14].

Several molecular mechanisms of neurodegeneration in AD linking neuronal toxicity to Aβ and tau protein have so far been hypothesized, including neuroinflammation, oxidative stress, impaired cell stress response, mitochondrial dysfunction, lipid metabolism, apoptosis, disruption of Ca^{2+} homeostasis, reduced cytoskeletal integrity, alterations of Notch signaling, enzymatic deregulation (phosphatases, kinases, proteases), epigenetic changes, and, most importantly, the failure of neurotransmitter pathways [15–21]. However, none of the aforementioned mechanisms per se elucidates all the histopathological and multifactorial molecular changes described in AD.

In addition to this traditional theory that attributes a major pathogenic role to brain-born Aβ, further data have lately hypothesized the involvement of infectious agents [22–24]. In a recent paper, Itzhaki et al. [25] provided an extensive overview on the contribution of *herpes simplex* virus type I (HSV-1), *Chlamidophila pneumoniae*, spirochetes and fungi as initiators of AD. In particular, HSV-1 has been shown to promote the intraneuronal accumulation of Aβ and tau hyperphosphorylation through the activation of GSK-3β, which, in turn, are responsible for the depression of synaptic activity [26,27]. A central topic of this article is the contribution of bacteria to the pathogenesis of AD through multiple mechanisms. Indeed, *Bacillus subtilis*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* produce functional extracellular amyloid fibers which prime the innate immune system strengthening the inflammatory response to cerebral Aβ [28–31]. Furthermore, *E.coli* lipopolysaccharide has been shown to promote the formation of fibrillar Aβ and, thus, it might be directly involved in the pathogenesis of AD [30,32].

In addition to being the etiologic agents of diseases, many forms of bacteria are present in the organism as commensals as they carry out important physiological functions. In this regard, it is worth considering commensal lactobacilli that provide acid pH to the vagina, preventing infections from other infectious agents (e.g., mycetes), as well as the microbial flora that colonizes both the oral cavity and intestine.

3. Gut microbiota

A high number of bacteria colonize the human colon. Recent studies have identified about 1000 bacterial species and 7000 bacterial strains for a total of 10^{13}-10^{14} microrganisms in the gut [33,34]. Among the most popular *phyla* are the *Firmicutes* and *Bacteroidetes*, which make up 51% and 48% of the whole microbiota [33,35]. To the *phylum Firmicutes*, including both Gram-positive and Gram- negative bacteria, belong the *genera Lactobacillus* (Gram-positive), *Eubacterium* (Gram-positive) and *Clostridium* (Gram-positive), although the latter in a minor proportion; on the other hand, the *genera Bacteroides* and *Prevotella* belong to the *phylum Bacteroidetes*, formed by Gram-negative bacteria [33,35,36]. The remainder 1% is formed by other *phyla*, such as *Proteobacteria* (Gram-negative, in particular the *genus Escherichia*), *Actinobacteria* (Gram-positive, particularly the *genus Bifidobacterium*), *Fusobacteria* (Gram- negative), *Spirochaetes* (Gram-negative), *Verrucomicrobia* (Gram-negative) and *Lentispherae* (Gram-negative) [33,37].

To date, it was thought that the microbial gut was only involved in colon-specific activities, such as the fermentation of carbohydrates, vitamin synthesis (e.g. vitamins B and K) and the metabolism of xenobiotics; furthermore, the gut microflora worked as a functional barrier to prevent pathogenic bacteria from invading the gastrointestinal tract [36,38,39]. In recent years, microbial gut

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