Expert Review of Anti-infective Therapy

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Is Alzheimer's disease a polymicrobial host microbiome dysbiosis?

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1. Introduction

The question of whether Alzheimer's disease (AD) is an infectious condition has been proposed previously but, received little support. This appears mainly due to an inability of being able to satisfy Koch's postulates in the context of chronic neurodegenerative diseases. The clinical signs of cognitive deficit and the neuropathological markers of amyloid-beta (Aβ) plaques and phosphorylated tau neurofibrillary tangles (p-TauNFTs) define AD. Clinical trials based on the concept that Aβ removal may successfully reverse memory loss as a plausible therapy have failed; thus negating the theory of a causal relationship. We address the question of AD being a non-transmittable infectious disease from the perspective of microbial dysbiosis of the host's microbiome.

The Human Microbiome Project consortium (2012) estimated that the human gastrointestinal tract, of which, the oral and nasal cavities are a part, contains around 1014 microorganisms, out-numbering the cells of the host by 100 to 1.[1,2] At a genetic level, microbes contribute to 150-fold more genes over the total number of genes in an individual, implying both bacteria and the host employ host/bacterial genes for their harmonious relationship during health. The nasal/oral/gut symbiotic microbiome, therefore, acts as a "surrogate human organ".[3] What, then, is the impact on a genetically vulnerable elderly individual when the bacterial surrogate human organ becomes dysbiotic?[4]

It is becoming clear that the polymorphic *Apolipoprotein* gene (E4) allele (*APOE ϵ4*) susceptibility gene of AD induces a dysregulated innate immune inflammatory response via cytokine liberation by deregulating C1q to keep the classical complement pathway activated in the brain.[5] Hence these individuals possess an inflammatory phenotype at the outset. *APOE ϵ4* genetic susceptibility in AD is also associated with atherosclerosis, and other cerebro/cardiovascular conditions implicating the role of co-morbid states in the onset of this neurodegenerative condition. Of recommendation is the review by Fulop et al.[6] The apolipoprotein E null mice, demonstrate susceptibility to infection,[7] suggesting microbes will feature in AD subjects due to altered *APOE ϵ4* gene function. In this context, common microbial infectious agents, especially *Porphyromonas gingivalis*, may be associated with the AD brain via apparent shared common disease pathways of the innate immune system acting to enhance and perpetuate the inflammatory burden.[8] Inflammatory mediators can erode the proteins that preserve the full integrity of the blood-brain barrier (BBB) within the brain, as shown previously.[9] Nation et al. have shown that the clinical impact of a BBB breach is cognitive impairment[10]. An alternative mechanism for cognitive impairment is via inflammation, whereby microglia induce excessive pruning (loss) of synapses.[11]

The argument on whether spirochetes are "dementia important" appears to be a historic one, originating from the Dr. Alzheimer, Dr. Fisher and Dr. Gaetano era who allegedly examined the same demented brain tissue specimens without detecting spirochetes; leading to scientists 'agreeing to disagree'. One would expect with the improvements in methodologies now available to scientists, that the debate could be concluded accepting the outstanding efforts of Miklossy who has detected *Borrelia burgdorferi* in AD brains implicating their role in dementia.[12,13]

The reports supporting a fungal association within AD brains is also unravelling. *Actinomyces* species have been detected in post-mortem AD brains by next generation high throughput sequencing methodologies.[14,15] *Actinomyces* species are at the interface of bacteria and fungi as they show up with Gram-positive characteristics (bacteria) and with Grocott's silver impregnation (fungi). Interestingly, *P. gingivalis* has some synergy with *Actinomyces* in AD brains as cases that were positive for *P. gingivalis* lipopolysaccharide were also positive for *Actinomyces* species when analysed by next generation sequencing.[15,16]

1.1 Blood-brain barrier and netrophil defects

The dominant microbes detected consistently from AD brains are select species of spirochaetes; herpes simplex type 1 virus (HSV1), *Chlamydia pneumoniae*, *P. gingivalis*, and select fungi.[16-20] These microbes appear adept at altering the opsonophagocytic activity of neutrophil function. They manipulate monocytes to become defective and to act as 'Trojan horses'; meaning the monocyte has lost its legitimate function and the pathogen, for example, *C. Pneumoniae*, can use it as a vector for its survival and a place to multiply and a means of spread to the brain. A permeable BBB enables pathogens within defective monocytes to directly access the brain. *P. gingivalis* uses several pathways including the vascular route, via daily bacteraemias caused by gingival bleeding after toothbrushing or chewing food on periodontally involved teeth; and via a permeable BBB through aging and with the onset of AD.[21,22]

The olfactory pathway includes the nose, which contains neurosensory cells and olfactory glands for smelling odours. Several nerve fibres from these cells pass through cribiform plate foramina of the ethmoid bone, which partitions the nose from the brain. The porous barrier between the nasal passages allows neurosensory cell fibres to enter the brain in the entorhinal region, which connects with the hippocampus, as previously described.[23] This appears the pathway of choice for *C. Pneumoniae* and HSV1 to gain access into the brain.[6]

1.2 Inflammation in the context of an infection

The existence of pathogens in AD brains signifies inflammation, that always follows an infectious episode in the body. If not resolved early, this results in neuronal loss and glial cell cytokine secretion, which poses a risk to individuals with inherited polymorphic *APOE ϵ4*[24] because their glial cells are already primed for immediate activation. Microglia are the resident macrophages of the brain with a primary innate immune function.[25] They become activated following an immune challenge leading to secretion of cytokines, chemokines, prostaglandins, nitric oxide and reactive oxygen species.[26] Intracerebrally, these cytokines can erode proteins that normally preserve the full integrity of the BBB. Conversely, patients with periodontal disease have elevated levels of the same cytokines in their blood, suggesting an extracerebral source of the BBB breach.

1.3 AD Hallmark proteins and polymicrobial infections

If we were to consider the neuropathological lesions, plaques and p-tauNFTs, of AD as being end stage phenomenon, then it may be possible to trace their origins from previous infections. Based on the current literature, the antimicrobial protection hypothesis of AD provides a convincing argument for plausible causal links of Aβ[27]. Research from the Moir and Tanzi laboratories has convincingly demonstrated that the Aβ plaques of AD represent antimicrobial peptides that combat "polymicrobial" infections in the brain.[27-30] This concept strongly links the Aβ lesion to microbes (bacteria, viruses and fungi). Furthermore, inflammation resulting as the consequence of Aβ is in line with its antimicrobial peptide properties. In support of this, Illievski et al.[31] confirmed that Aβ plaques arise in mice brains following *P. gingivalis* (serotype 1) oral infection, and this suggests an overall contribution of this bacterium, and others including HSV1 and fungi, to Aβ hallmark lesions in the brain. If $A\beta_{1\text{-}40/42}$ plaques are metabolites of the human *amyloid precursor protein* (*APP*) gene in AD brains, then how can prokayote proteins mix with eukaryote proteins to form the same lesion? One explantion is that the Aβ refers largely to a conformational state of a truncated protein (β pleated sheet structure of fragmented APP). Bacterial and some other proteins in nature can undergo conformational changes to form β pleated sheet structures under appropriate conditions.[32] Therefore, it is plausible to suggest that the insoluble $A\beta_{1\text{-}40/42}$ plaques may be remants of an extracellular polymeric substance scaffold from a former miniature biofilm consortium as described by Dueholm and Nielsen[32], and supported by Miklossy.[13] This would require evidence of the brain harbourings a biofilm prior to clinical AD, and, to date, remains the missing link cementing this theory.

The NFTs represent destabilized microtubules. Dominy et al.[19] have provided some clues towards why tau-binding microtubules may be succumbing to disease in AD. The pathological microbial link with both hallmark proteins links back to lipopolysaccharide and "gingipains", a

protease secreted by *P. gingivalis*, that can be found in its outer membrane vesicles, with potential to cause AD in some individuals.[19,33] However, a stronger argument for the role of pathogenic tau in AD development is evidence of tau to be a substrate for gingipains.[19] Some of the fragments generated from tau appear to be neurotoxic and may contribute to the severity and progression of AD. Alternatively, gingipains, following their release by *P. gingivalis*, enter the cytoplasm for detoxification. This, in turn, may lead to release of tau fragments into the brain parenchyma. Small extracellular fragments of tau may subsequently be taken up by neurons facilitating their spread in a phenomenon known as 'tau spreading'.

2. Conclusions

The sporadic form of AD has a multitude of pathways for its expression and the microbial contribution from dysbiotic host microbiomes can be involved from comorbid states. In this case, periodontal disease and its association with multiple other diseases, especially arteriosclerotic vascular disease,[34] are strong candidates for perpetuating inflammation. If AD was to be regarded as an infectious disease, it would be a polymicrobial non-transmissible infection of the brain resulting from a dysbiotic host microbiome (an environmental factor, acting in concert with *APOE ϵ4* susceptibility). Adult periodontal disease of 10 years and longer duration double the risk of developing AD.[35,36] Warren and colleagues found that poor oral hygiene was more likely to contribute to the severity of dementia, and that these patients suffered silently from tooth related pain, which may be reflected in their difficult clinical behaviour.[37] We are of the opinion that the pathogen load (poor oral hygiene) is the likely risk for AD at any age[38] and the general public have their own perception of adequate oral hygiene. This behavioral perception and often painless progression of periodontal disease, masking the need to seek dental treatment, makes it difficult to engage with people to enforce the idea that their oral hygiene on daily basis is subjective, and as such, carries the risk of developing dementia.

The oral pathogen *P. gingivalis* hypothesis for AD has provided the basis for current drug testing which targets its toxic proteases to reduce the risk of AD development.[19] This novel treatment is undergoing phase III clinical trials (GAIN Trial: Phase 2/3 Study of COR388 in subjects with AD. ClinicalTrials.gov Identifier: NCT03823404). If successful, this will give greater credence to the hypothesis that a subgroup of sporadic AD results from a polymicrobial host microbiome dysbiosis. As periodontal disease is not transmissible *per se*, the same analogy applies to AD if the dysbiotic microbiome pathogens have a causative role. This will further enforce the vital importance of

modifiable risk factors in preventing and/or delaying AD onset and challenges the WHO to accept poor oral hygiene as a robust risk factor for AD.

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