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The Essential Role of Biofilms in Alzheimer's Disease

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ABSTRACT

Biofilms are made by microbes and are exceedingly common in nature. On examination of pathological specimens from the hippocampi in Alzheimer's disease (AD) brains, biofilms have been observed both intra and extra-cellularly. It is highly probable that the microbes that create the biofilms in AD are spirochetes of either Lyme or dental origin. Borrelia burgdorferi of Lyme disease and T. denticola (representative of the dental organisms) have been found by PCR analysis, and Borrelia burgdorferi has been cultured from AD brains. Simultaneously with making biofilms in vitro, these cultivated Borrelia have been shown to make beta amyloid precursor protein (ABPP) and amyloid beta (Aβ) in pure culture. Comparatively, in the intracellular location in vivo, the Aβ (formed by the spirochetes while making biofilm), when meshing with tau protein, causes tau to be phosphorylated by a known interaction. When tau is hyperphosphorylated tau (p-tau), it no longer functions to stabilize neuronal dendrites, and those dendrites disintegrate. Extracellular biofilms are coated with Aβ (which is antimicrobial). Further, those biofilms attract Toll-like receptor 2 from the innate immune system; this molecule attempts to kill the spirochetes, but is ineffective, because it is unable to penetrate the biofilm. NFkB, one of the intermediates in the MyD88 pathway generated by TLR2, catalyzes beta amyloid converting enzyme which, in turn, catalyzes beta and gamma secretase that cleave ABPP to Aβ. Consequently, in the formation of biofilm, Aβ is created; and, in the TLR2/MyD88 response to the "spirochete-coated" biofilm, Aβ is also created. Finally, p-tau, the other major element of the pathology, is directly related to the creation of the biofilms. Biofilms are thus integral to the pathology of AD.

Keywords

Biofilms, Spirochetes, Toll-like receptor 2, Beta amyloid, Hyperphosphorylated tau, Tangles, Senile plaques, Alzheimer's disease, General paresis.

Abbreviations

AD: Alzheimer's Disease; PCR: Polymerase Chain Reaction; AβPP: Amyloid Beta Precursor Protein; Aβ: Amyloid Beta; p: tau-hyperphosphorylated tau; NFkB: Nuclear Factor Kappa B; TLR2: Toll-like receptor 2, MyD88: Myeloid Differentiation Pathway 88; PAS: Periodic Acid Schiff; CR: Congo Red; CP: Chlamydia Pneumoniae; HSV: Herpes Simplex Virus; HTLV1: Human T-cell Virus Type 1; TNFα: Tumor Necrosis Factor Alpha.

Biofilm pathology and microbiology in Alzheimer's disease

Biofilms are undeniably present in Alzheimer's disease (AD). First, on pathological examination of hippocampal specimens from post mortem brains, biofilms have been seen with routine staining with periodic acid Schiff (PAS) which stains the polysaccharides that form the bulk of the biomass [1]. Second, in the same specimens, they are also visualized on staining with Congo red which stains the amyloid that forms the infrastructure and is the major proteinaceous component of the biofilms [1]. In vitro biofilms, formed by Borrelial spirochetes cultured from AD brains, and, in vivo biofilms, noted in those same brains, have also been seen on gross examination and with fluorescent staining with Thioflavin S [2]. Further, these same biofilms have been highlighted by immunopathology that stained for bacterial peptidoglycan (which also recognizes the polysaccharide matrix like PAS) [2]. Last, their presence has been noted in vitro and in vivo on fluorescent in situ hybridization (FISH analysis) by Miklossy and Macdonald [2,3].

When biofilms are present, it is indicative of the presence of microbes that made them. In nature, 90% or more of microbes reside in biofilms, so it is not an uncommon occurrence [4]. Most frequently, biofilms form by quorum sensing, which is a population sensing modality the microbes contain with ten microbes in any

direction being the lowest number of organisms needed to form a biofilm [5]. This is important because the size of the biofilm produced has to fit within the cell cytoplasm in intracellular biofilms. Inside the neuron, the spirochetal biofilm easily fits in the cytoplasm. Microbes have many genes for quorum sensing [6]. Thus, biofilm formation is dependent on how rapidly the organisms divide (to develop the necessary quorum, and this varies widely: minutes for staphylococci and months for spirochetes [7].

When stressed, as with osmotic shock for instance, microbes make biofilms even more rapidly while bypassing the quorum sensing mechanism (This was demonstrated in vitro with the Borrelial spirochetes) [2]. All the preceding leads to the rationale for microbes to make biofilms: the biofilm coating (slime) protects the microbes from environmental stresses and, in humans, from the immune system and from antibiotics. Planktonic (non-agglutinated) microbes are sensitive to antibiotics, whereas those in biofilms are nearly all resistant.

Biofilms eventually reach a size where some (exporter) cells are released from the matrix, and these are capable of forming new biofilms at proximal or distal sites [8]. Certain chemicals such as iron and homocysteine cause biofilm dispersion, and this eventually causes the formation of new biofilms, just as the natural process of exporter cells does [9]. Medications, such as rifampin, citalopram, and others also cause biofilm dispersion; the mechanism that rifampin employs (poles holes in the film) has been identified; the mechanism(s) for the others is/are not currently known [10].

Biofilm pathophysiology in Alzheimer's disease

Miklossy showed AD to be an infectious disease (albeit a chronic infectious disease), first by pathologically visualizing spirochetes in the tissue and next by culturing Borrelia burgdorferi from fresh post mortem brains, confirming the prior observations of Macdonald [2,11]. Next came analysis by the Koch/Hill postulates which showed AD to be infectious and then a comparison of syphilitic dementia and AD. Syphilitic dementia (general paresis [GP]) and AD had similar clinical and pathological findings: specifically, on pathology, spirochetes, senile plaques, Aβ, tau protein, neurofibrillary tangles, and pronounced atrophy were all noted on side-by-side pathology examinations of GP and AD [12,13]. With all the similarities, GP serves as an excellent prototype for AD.

Previously, polymerase chain reaction (PCR) observations have identified 25% Borrelia and 75% dental spirochetes in AD [14]. There were multiple species of dental spirochetes found in the affected brains which are also known to be part of the oral flora "pathobiome". Both Borrelia and dental spirochetes are known to create biofilms, so their presence in a setting (AD brains) shown to contain biofilms would not be surprising.

The biofilms have been found inside neurons as well as extracellularly in the surrounding tissue in AD [15]. Intracellular biofilms have previously been noted in such diverse chronic diseases as urinary tract infections and psoriasis [16,17]. The extracellular biofilms in AD co-localize with Aβ which is perhaps not surprising because of Aβ's anti-bacterial properties [3,18].

It has been shown that biofilms have attachment sites for other organisms, and this is a possible mechanism for other organisms such as C. pneumoniae (CP), Herpes simplex virus (HSV), and P. acnes (among others) to be found in AD [5]. Consequently, they would be co-inhabitants of the spirochetal biofilm; incidentally, neither CP and HSV has been shown to make biofilms. Even if that were possible, the biofilms produced would only be intracellular and not extracellular. This concept is advanced because CP is an obligate intracellular bacterium and thus would appear incapable of initiating an extracellular biofilm. HSV, because of its viral nature, would require the DNA of a host cell which it would "hi-jack" and use to form biofilm [5]. HSV would be very unlikely to initiate extracellular biofilms either. Further, to date, only two viruses, HTLV1 and molluscum contagiosum, have been shown to produce biofilms, and those biofilms are both found intracellularly [1,20].

In vitro, Borrelia spirochetes have been shown to produce biofilms, ABPP, and Aβ [2]. This pure culture forms in the absence of cells. In vivo, the biofilms have been seen intracellularly as has been Aβ [15]. It is important to note again that the process of biofilm formation by spirochetes is of long duration: it takes up to two years to form a single biofilm because spirochetes divide so slowly.

Once in place, spirochetal biofilms initiate pathological processes. When the biofilms and Aβ are intracellular, the Aβ acts on tau protein, and, by known pathways, induces the formation of hyperphosphorylated tau (p-tau) [15,21]. This Aβ /tau interaction is perhaps the most critical of all the pathological processes in AD because it eventually leads to the formation of neurofibrillary tangles and the disintegration of neuronal dendrites. When this occurs, the neuron is no longer functional, and the synapses so necessary to mentation and memory are lost. Moreover, as the dendrites, which are stabilized by tau protein, disintegrate because the p-tau is non-functional, the p-tau, Aβ, ABPP, spirochetal biofilms, DNA, neurofibrillary tangles, and other intracellular organelles are emptied into the surrounding extracellular space. This supplies a nidus for the development of extracellular biofilms.

Extracellular biofilms are an essential component of senile plaques which are an essential component of AD pathology [13]. In the extracellular location, the biofilms attract the innate immune system, especially Toll-like receptor (TLR2), the presence of which has recently been identified.1 Biofilms, no matter whether they are formed by gram positive or gram-negative organisms, attract TLR2 [21]. The "curli" fibers in the biofilm have receptor sites for this molecule [22]. Upregulated TLR2 utilizes the myeloid differentiation 88 (MyD88) pathway to inactivate microbes [5]. To accomplish this task, this pathway generates NFkB and TNFa; however, those lethal molecules cannot penetrate the biofilm, and they destroy the surrounding tissue instead [5].

Further, the NFkB that is generated from TLR2 and the MyD88

pathway catalyzes beta amyloid converting enzyme which catalyzes beta and gamma secretase that leads to the formation of Aβ from ABPP. Consequently, Aβ is formed by this pathway as well as being formed simultaneously during the intracellular creation of biofilms. It is somewhat ironic that, where Aβ is antimicrobial, it is produced simultaneously when the spirochetes create biofilms. It is not ironic that it is produced by the interaction of TLR2 and the MyD88 pathway because that pathway is inherently antimicrobial. Aβ may then be considered part of the innate immune system.

Conclusion

Biofilms are made by microbes and, by definition, whenever and wherever biofilms are present, microbes are there also. In AD, biofilms contribute to the entire pathology. The birth of these biofilms arises from the spirochetes (both Lyme and dental) that have both been shown to be present. The overarching pathway is: spirochetes create biofilms that directly or indirectly create p-tau, tangles, Aβ, neuron destruction, and inflammation in AD. The various factors that worsen the disease have recently been outlined and their presence and influence in the above pathway have been summarized. Fewer factors lead to the improvement in the disease, but the factor that is most logical is the administration of an antibiotic to kill the spirochetes before they make biofilms or before they even arrive at the brain and begin the process.

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