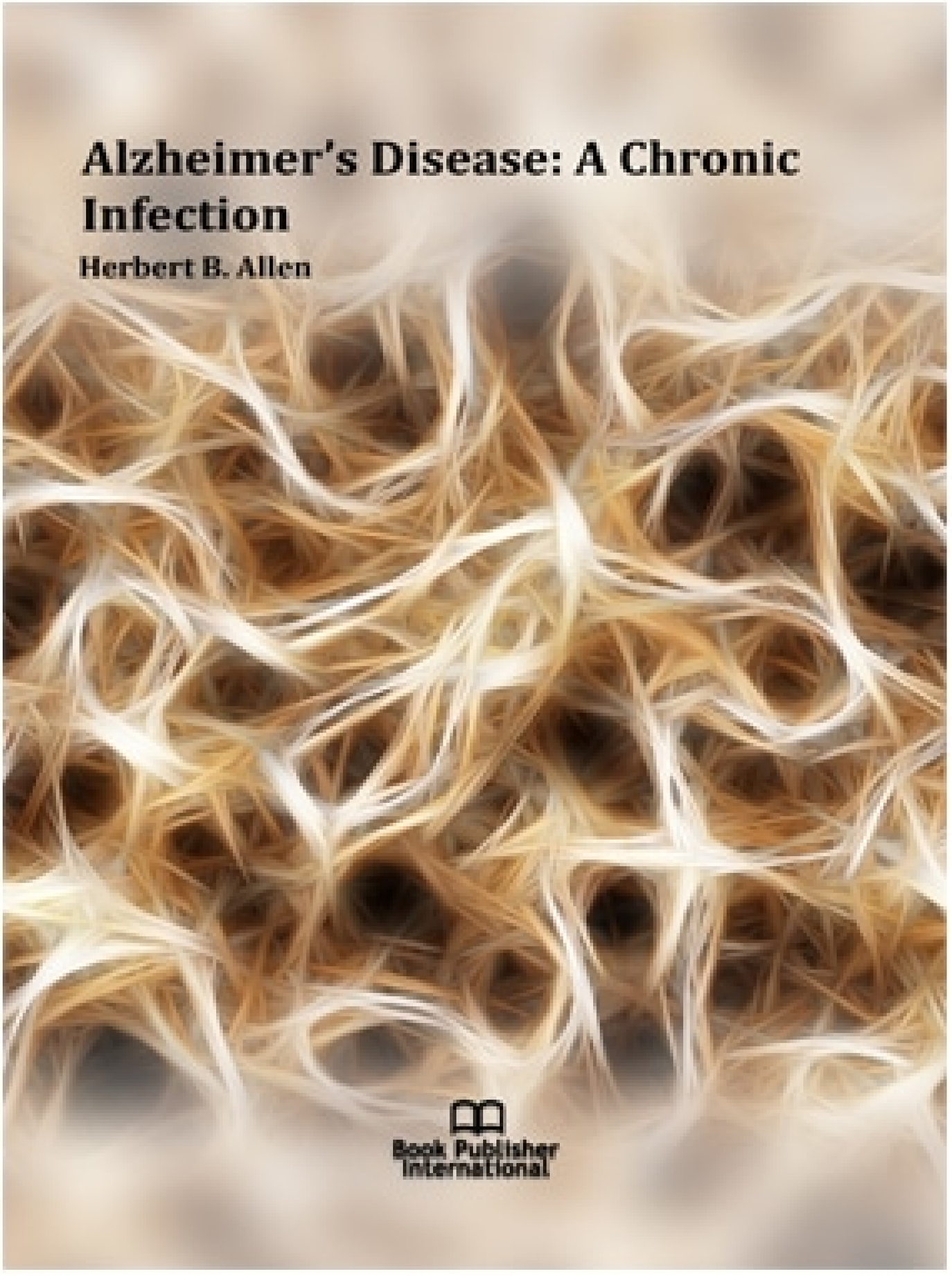
Alzheimer's Disease: A Chronic Infection

Herbert B. Allen



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Contents

PREFACE

This book deals with Alzheimer's Disease. The book also covers key areas in spirochetes, beta amyloid (Aß), biofilms, essential role, spirochete/biofilm hypothesis, amyloid, immune system, worsening, cerebrovascular accident, stroke, chronic diseases, Penicillin, Streptococcus, arthritides, upregulation, antibiotics, hyperphosphorylated tau (p-tau), Borrelia burgdorferi and dental treponemes, Treatment of Alzheimer's Disease, nuclear factor kappa B, myeloid differentiation pathway 88. This book contains various materials suitable for students, researchers and academicians of this area.

DEDICATION

For Laraine, my wife of over half a century, my prime editor, and my one true love.

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ABSTRACT

During the past few years, Alzheimer's disease (AD) has been shown to be a chronic infection originating with a spirochete. These spirochetes form biofilms like most other microbes; moreover, in large measure, the biofilms contribute to both the chronicity and the pathogenesis of the disease. Once in a biofilm, the microbes become undetectable and resistant to the immune system and to antibiotics. Stroke, diabetes, nicotine, haloperidol, diet soft drinks, and others have all been shown to cause worsening of Alzheimer's disease (AD) by their impact on biofilms. Penicillin, administered before the spirochetes form biofilms, would very likely prevent the disease.

Keywords: Alzheimer's disease; cause; treatment.

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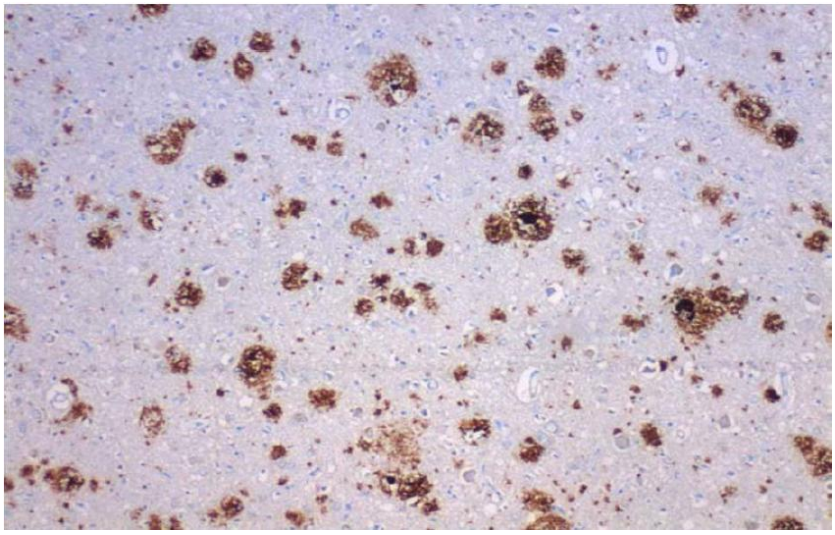
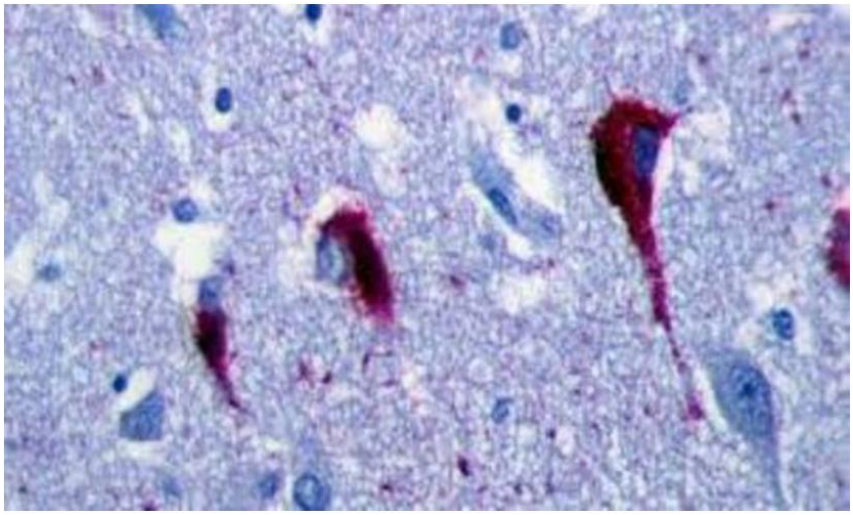
INTRODUCTION

For the past 25 years, the beta amyloid (Aβ) hypothesis has prevailed as the cause of Alzheimer's disease. One reason for the predominance of this concept is the exceedingly large amount of Aβ in the affected brains (Fig. 1). The huge quantity of this molecule may have blinded many to other potential causes. One other etiologic factor is the presence of neurofibrillary tangles (NFT), also known as hyperphosphorylated tau (p-tau), which has gained support recently (Fig. 2). Not considered in the discussion of etiology has been microbial causation.



Aβ immunostain 5X

Fig. 1. Beta amyloid in Alzheimer's brain tissue
A large amount of beta amyloid (staining brown-black) is present in this hippocampal specimen from an Alzheimer's disease patient



PAS stain 40X

Fig. 2. Neurofibrillary tangles
Neurofibrillary tangles (staining red-purple) in a hippocampal section from an Alzheimer's disease patient

Oskar Fischer, who reported on the largest number of demented patients in the early 1900s, thought the disease was infectious and pathologically looked like "actinomycosis" (There is a similarity in the pathology of "sulfur granules" seen in actinomycosis and the senile plaques of Alzheimer's disease.) However, this concept was lost when Kraepelin promoted early dementia as Alzheimer's disease, even though Fischer had 16 cases versus Alzheimer with one.

From a dermatology and dermatopathology perspective, my interest in Alzheimer's disease (AD) began when I read Miklossy's work where she cultured *Borrelia* from AD brains. Lyme disease is caused by *Borrelia burgdorferi* and is a dermatological disease first beginning with the Erythema migrans (or bullseye) lesion. Further interest was sparked when she reported that the pathology of syphilis and AD were similar. Syphilis, in its primary and secondary forms, is also a dermatological disease; tertiary syphilis was once in the purview of dermatology and was recognized by the American Board of Dermatology and Syphilology until the mid-1960s. Tertiary syphilis separates into 3 large divisions: 1) dermatologic with gumma formation, 2) cardiovascular, and 3) neurologic with tabes dorsalis and general paresis (GP) of the insane (syphilitic dementia). Inasmuch as the two diseases (AD and syphilis) were linked pathologically and clinically (especially in the tertiary form) and where they were linked by the presence of the microbial spirochetes, it seemed of interest to study this disease with pathological, immunopathological, and microbiological methods similar to those used in our recent evaluation of other chronic cutaneous diseases such as atopic dermatitis and psoriasis. These diseases themselves were thought to be immunological and genetic and were not considered microbial in origin, but we showed that they were.

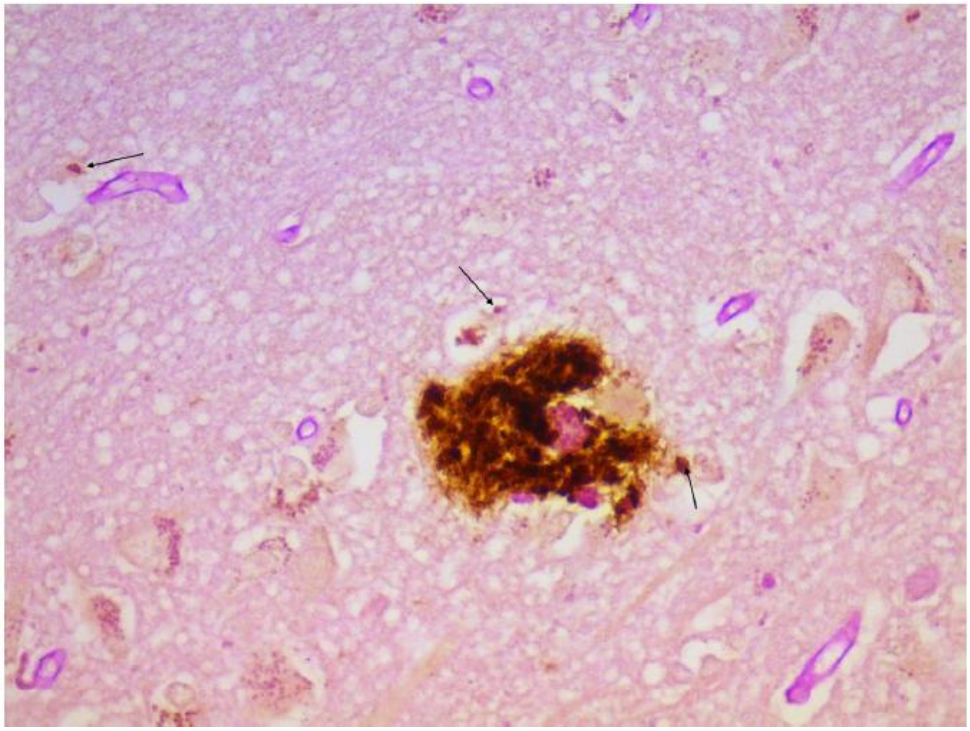
In both atopic dermatitis and psoriasis, the use of these pathological and microbiological methods revealed the presence of microbial biofilms; these were in the eccrine sweat ducts in atopic dermatitis and in the tonsils in psoriasis. In atopic dermatitis, the biofilms were formed by normal flora *staphylococci*; and, in psoriasis, they were made by *streptococci.* The biofilms were extracellular in both locations; additionally, they were intracellular in psoriasis.

The biofilms activated the innate immune system molecule (Toll-like receptor 2 [TLR2]) which was present surrounding the occluded eccrine ducts in the stratum corneum in atopic dermatitis and in the dermal capillaries in psoriasis. Additionally, El-Rachkidy found an anti-streptococcal specific immunoglobulin G (IgG) in the serum of psoriasis patients. This represented activation of the adaptive immune system. Consequently, both arms of the immune system were involved in psoriasis.

TLR2 utilizes the myeloid differentiation 88 (MyD88) pathway which generates NFκB and TNFα to inactivate bacteria. However, if it encounters biofilms, TNFα is unable to penetrate through the slime and kill the bacteria within. IgG has a greater repertoire for killing bacteria (complement, alternate complement, killer T cells, and many cytokines), but it is also unable to penetrate the biofilms. Thus, any activity generated is likely to harm the surrounding tissue as an "innocent bystander".

I have shown biofilms to be present in AD just as in the aforementioned skin diseases; these have been extracellular as in both psoriasis and atopic dermatitis, and intracellular as in psoriasis. TLR2 was also present in the brain tissue as it was in both the cutaneous diseases. IgG was not found inasmuch as it cannot pass through the blood brain barrier. Only after stroke or traumatic brain injury is IgG found in the brain.

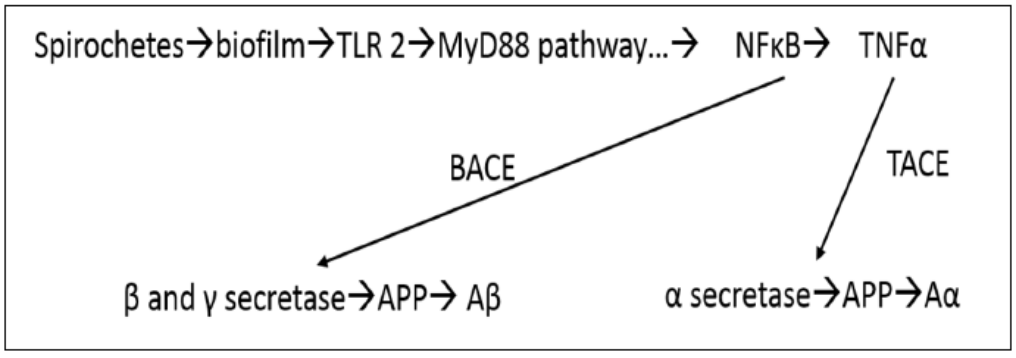
Of interest, Aβ co-localized with the extracellular biofilms (Fig. 3); Aβ has been shown by Soscia et al to be antimicrobial and, by its positioning, was likely attempting (unsuccessfully) to penetrate the biofilm. Miklossy showed that *Borrelia* grown in pure culture from AD brains created biofilms *in vitro*. Simultaneously (and surprisingly) the microbes also created AβPP and Aβ. Further, I demonstrated pathologically the presence of Aβ both extracellularly and intracellularly *in vivo.*



Aβ immuno and PAS, stains combined 40X

Fig. 3. Beta amyloid and spirochetal biofilm co-localize
Biofilm, represented as the dark pink stain in the midst of the brown-black beta amyloid, co-localizes with the beta amyloid. Intracellular Aβ is also noted (arrows)

Moreover, TLR2, in utilizing the MyD88 pathway, generates NFκB which is known to catalyze beta amyloid converting enzyme (BACE); this enzyme then catalyzes both beta and gamma secretase that cleave amyloid beta precursor protein into Aβ (Fig. 4).



BACE is beta amyloid precursor protein converting enzyme

APP is amyloid precursor protein

Fig. 4. Pathway to Aβ from TLR2/MyD 88 pathway
From J Alz Dis. 2016;53:1271-1276

Consequently, the spirochetes and biofilm are largely responsible for the presence of Aβ because Aβ is produced both by the microbes themselves at the same time they make biofilms (Miklossy), and, additionally, by the innate immune system response to those biofilms as well. It must be stated that *Borrelia* are not the only spirochetes in the brain to make biofilms; it has been shown that dental spirochetes make up 75% or more of these microbes.

The intracellular biofilms and Aβ are perhaps more important than the extracellular ones. This relates to the fact that Iqbal has shown, when Aβ joins with ordinary tau protein, it creates hyperphosphorylated tau (p-tau). Ordinary tau stabilizes neuronal dendrites, but p-tau causes disintegration of the dendrites (Fig. 5). This leads ultimately to loss of transmission of impulses from one neuron to another and to neuronal cell death. With this, the ingredients inside the neuron are now extruded into the extracellular space. Consequently, NFT, Aβ, and biofilms then contribute to the extracellular material available to TLR2 for further immunogenic activity.

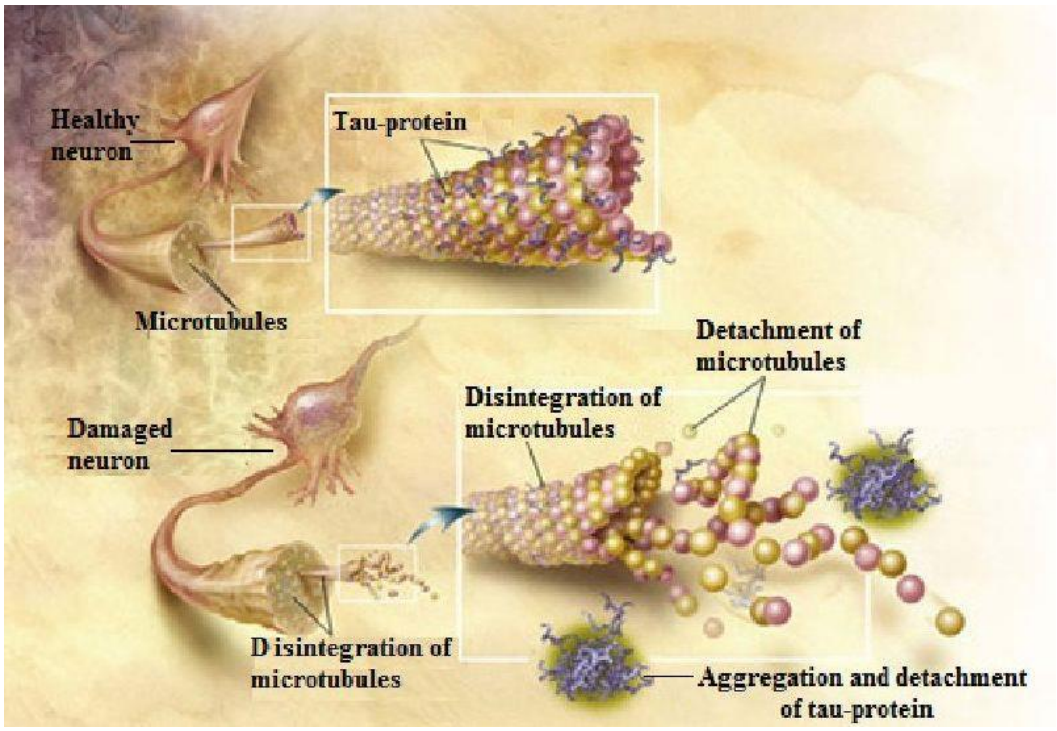


Fig. 5. P-tau leading to disintegration of neuronal dendrites
Tau protein when phosphorylated causes disruption of the neuronal dendrite (from ADEAR/Wikimedia Commons)

The loss of the neuronal function by the formation of the NFTs and subsequent cell death would lead eventually to shrinkage of the tissue and the atrophy notable in AD. Thus, the diminished neural transmission, the presence of p-tau, the presence of Aβ, and the presence of the immune system can be related to spirochetes and their biofilms. The biofilms contribute to making this infectious disease chronic. Spirochetes, or other organisms (such as meningitis B) acting individually and not in a community, would cause an acute encephalitis or meningoencephalitis and a very short, and often deadly, disease course.

As concerns other organisms: *herpes simplex* virus (HSV) and *Chlamydia pneumoniae* (CP) and more recently *porphyromonas* have been found in AD brains and have been promoted as possible etiologic agents of the disease. Several factors mitigate against this: foremost is the pathology. It is "helical" and not coccoid or viral. Next is the production of biofilms; neither HSV nor CP have ever been shown to make biofilms. *Porphyromonas*, as a dental organism, has made biofilms; but, again,

the pathology of porphyromonas would be coccoid, not helical. Biofilms have attachment sites for other organisms, and this is likely the process whereby these other organisms are found in the brains (Fig. 6). The biofilm has been considered a "hotel rather than a single-family home." Also, syphilis, the prototypical disease for AD clinically and pathologically, is spirochetal.

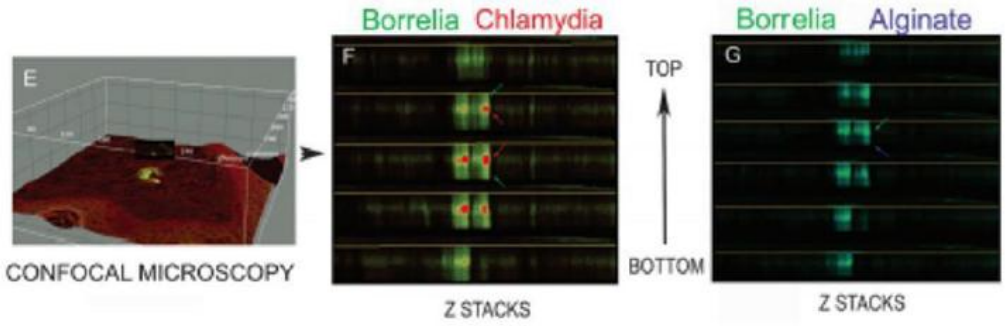


Fig. 6. Borrelial biofilm incorporating Chlamydia pneumoniae
Chlamydia (red} is clearly seen inside the borrelial biofilm (green) in the central section
From Sapi, Eur J Microbiol Immunol (Bp). 2019 Apr 11;9(2):46-55

In discussing biofilms, it is also important to consider things that contribute to their formation or to their destruction. In AD, I have shown that "making" them or "breaking" them contributes to worsening of the disease. Examples of this would include diabetes causing them to be made because the hyperosmolality inherent in that disease causes organisms to make biofilms to protect themselves. Nicotine, as a biofilm disperser, is an example of the biofilm "buster" which ultimately leads to more biofilms. More biofilms means more disease.

Lastly, as regards treatment that focused primarily on the Aβ hypothesis, billions of dollars have been spent in the USA in trying to find something that would be beneficial. In over 200 trials, nothing has been found to be effective. Where syphilis is its prototype, I firmly believe it is important to treat AD preventively (just as for the prevention of tertiary syphilis). Treatment after the disease has begun would not and could not reverse the disease course. It may be possible to arrest the downwardly spiraling course of the disease or slow its progression. Practically speaking, because of the intracellular biofilms, even that may prove difficult. Thus, periodic treatment with a full course of a bactericidal antibiotic that crosses the blood brain barrier and the cell membrane would be advisable. This should prove to be as effective as treating syphilis any time before the onset of the tertiary disease. A yearly course might be advisable because of the seeding of the brain from dental organisms.

Other chronic neurologic diseases, in fact, other chronic diseases in general, may be similarly produced by microbes that make biofilms and upregulate the immune system. Discovering their source may prove very enlightening.

A compilation of my works will form the bulk of this e book. What follows are discussions of:

1) The leading role of spirochetes in AD,
2) The essential role of biofilms in AD,
3) Pathways within the pathway to AD,
4) The (crucial) impact of intracellular biofilms,
5) The presence of microbes, biofilms, and the immunologic response in AD,
6) The variable factors influencing the disease,
7) A comparison of AD and other chronic diseases in which biofilms play a role,
8) The potential treatment for prevention and/or amelioration of the disease,
9) The bioethics related to the many factors in the disease course.
10) Future efforts to combat the disease

Woven into the discussion will be novel findings in AD of intracellular Aβ, co-localization of biofilms and Aβ, the upregulation of innate immune system molecule TLR2, linkage to production in vivo of Aβ by spirochetes and the immune reaction to the spirochetal biofilm, mechanisms of factors known to worsen the disease in regard to the spirochete/hypothesis, and mechanisms for things that would improve and likely prevent the disease. A listing of future efforts to thwart the disease ends the discourse.

Chapter 1
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Discussion on the Leading Role of Spirochetes in Alzheimer's Disease

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ABSTRACT

For 25 years, the beta amyloid (Aβ) theory has been dominant as the cause of Alzheimer's disease (AD); and, recently, hyperphosphorylated tau (p-tau) has assumed a larger role. In consideration of the recent pathological and microbiological findings, it seems appropriate and important to elevate the pathogenic microbe theory and highlight the spirochete as the etiologic agent of this dreaded disease. The evidence for spirochetes causing AD is considerable and cogent. Foremost in the discussion is the microscopic pathology of general paresis (GP), a known spirochetal disease, and AD. The pathology is identical; in both, there are senile plaques, p-tau, neurofibrillary tangles, a massive amount of AB, and the presence of spirochetes. Spirochetes have been cultivated from AD brains; this has never been demonstrated in GP or any other stage of syphilis. Further, polymerase chain reaction (PCR) has identified the presence of the spirochetes (25% Lyme, and 75% dental). The debility of AD urges the beginning of therapeutic interventions against these spirochetes before they arrive in the brain or before they begin to make biofilms.

Keywords: Alzheimer's disease; spirochetes; beta amyloid (Aβ).

1. INTRODUCTION

Once in the brain, the spirochetes form biofilms (as do 90% of microbes in nature); this occurs both intra and extracellularly. The intracellular biofilms lead to the production of p-tau because the spirochetes make Aβ simultaneously while creating biofilm. Aβ induces the formation of p-tau from ordinary tau; this ultimately leads to destruction of the neuron. The biofilms that are extracellular attract the innate system molecule Toll-like receptor 2 which together with known pathways leads both to tissue destruction and to increased Aβ production. Thus, inside the cell, the spirochetes themselves make Aβ while making the biofilm; and, outside the cell, they ultimately are responsible for the creation of Aβ. This activity is the same, yet different, from the microbes/biofilms in other chronic diseases like atopic dermatitis, psoriasis, leprosy, tinea versicolor, arteriosclerosis, arthritis, and others.

Consequently, the spirochetes most assuredly are present in AD brains, and they are responsible for all the observed pathological changes found in those brains. Further, the dramatic changes in pathology are almost certainly related to the devastating clinical changes that occur. As a proof of concept, we show how items known to make AD worse (or better) confirm the spirochetal pathogenesis.

This will be a defense of the microbial pathogen theory of Alzheimer's disease (AD), especially with regard to dental and Lyme spirochetes. One of the most cogent concepts in this defense is in the pathology. The pathology of AD is identical to the pathology of general paresis (GP) which is the dementia seen in tertiary syphilis [1]. AD and GP have the same helical pathology, the same senile plaques, the same neurofibrillary tangles, the same hyperphosphorylated tau (p-tau), the same massive deposition of beta amyloid (Aβ), and the same late-stage cerebral atrophy [1]. (Figs. 1.1, 1.2)

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Where the clinical findings of GP and AD are similar, it might logically follow that a similar microbe is involved in the pathogenesis of each disease [2]. It is our contention that the evidence for spirochetal origin is even stronger for AD than it is for GP.

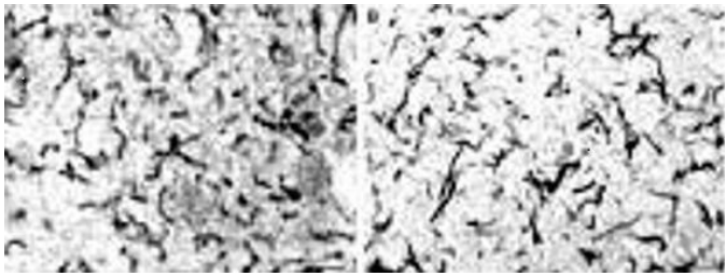

Fig. 1.1. Helical nature of both syphilis and AD
Spirochetes in Syphilis (left) and AD (right) from Miklossy Ref. [1]

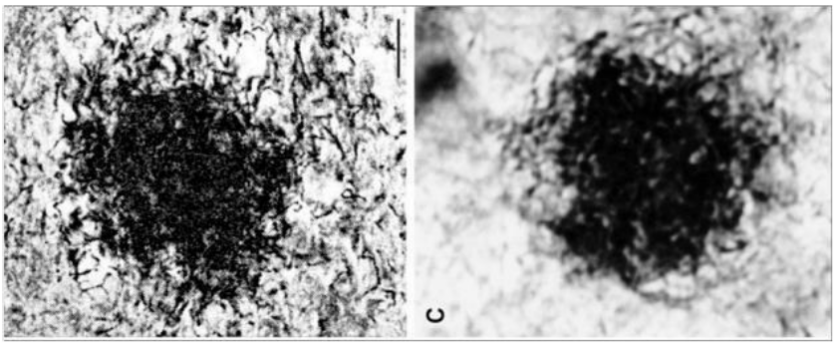

Fig. 1.2. Senile plaques in syphilis (left) and AD (right) from Miklossy Ref. [1]

Lyme spirochetes have been cultured from AD brains, first by Macdonald in 1986 and 1988, and, more recently, by Miklossy in 2016 [3-5]. To date, dental spirochetes have not been cultured, just as *T. pallidum* in GP, has not been cultivated. Lyme and dental organisms have been shown by polymerase chain reaction (PCR) of AD brains, which is also strong evidence for their presence [6,7]. (GP was eradicated before PCR techniques became widely available.) MacDonald and Miranda [8] first reported the presence of *Borrelia burgdorferi* in the brain of a patient suffering from AD in 1987, which was later confirmed by the same and other authors [9]. The spirochetes have been identified microscopically in AD brains just as in GP: noted were the typical corkscrew forms as well as the much less frequently seen circular cystic and granulovacuolar forms [10]. Epidemiologic studies linking periodontal disease with AD have identified a strong association of that oral disease with AD [11]. Spirochetes accumulate in gray matter areas of the brain, particularly in the cerebral cortex [12,13,9].

Once inside the brain, the spirochetes make biofilms, albeit slowly, both intra and extracellularly [14]. When stressed, the cultured spirochetes formed biofilms, amyloid beta precursor protein (AßPP), and Aß [15]. The biofilms are likely foremost in the pathogenesis of AD as they are in many other chronic diseases such as atopic dermatitis, psoriasis, leprosy, tinea versicolor, molluscum contagiosum, arthritis, and arteriosclerosis [16,17,18,19]. Allen et al have shown that biofilms are present inside the neurons [14]. Biofilms, made by spirochetes, in this location are doubly protected from the immune system, from antibiotics, and from most other stressors [14].

Miklossy, confirming the findings of Macdonald, has shown that the Lyme spirochetes cultured from post mortem AD brains make biofilms [5]. In the process of creating the "slime" coating, the spirochetes also make both AβPP and Aβ [5]. Interestingly, this production of Aβ is not exclusive to spirochetes: staphylococci that create biofilms in eccrine sweat ducts in atopic dermatitis have been

shown to produce Aβ also. (Fig. 1.3) Thus, the presence of the biofilms and Aβ has been shown histologically *in vivo*, as well as visually and microscopically *in vitro* [5,20].

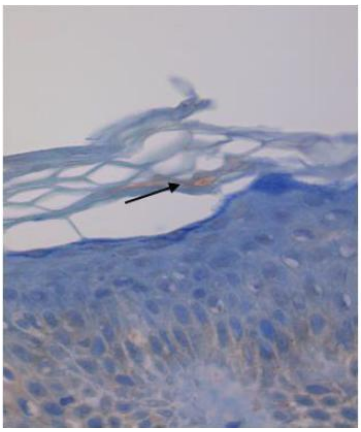

Fig. 1.3. Aβ present in occluded duct in eczema (occlusion from staphylococcal biofilm)
Immunostain for ABeta; positive (brown) staining is present in the eccrine duct within the stratum corneum (arrow). Ductal occlusion is the hallmark of eczema and results from biofilm made by normal flora staphylococci

Tau protein is an essential component of neurons where it functions to stabilize the neuronal dendrites. When Aβ meets tau protein, it leads to the production of hyperphosphorylated tau (p-tau) [21,22]. During this process intracellular masses (tangles) are formed, and this leads to the disintegration of the dendrites and neuronal destruction with emptying of the intracellular contents into the surrounding tissue (see Fig. 5 in Introduction). Consequently, with this process, neurofibrillary tangles, Aβ, AβPP, biofilms and cell organelles can now be found in the extracellular space surrounding the moribund neuronal cell. Moreover, Miklossy has found spirochetes in the neurofibrillary tangles [1].

The volumes of the biofilms that form in the extracellular space compared to the intracellular ones are much larger (100-700X); thus, they will have many receptor sites for the innate system molecule Toll-like receptor 2 (TLR2) [23] (Fig. 1.4). TLR2 has been shown to be present in the extracellular space as well [24]; upregulated TLR2 as a first responder activates the Myeloid differentiation 88 pathway (MyD88) which produces NFκB and TNFα [25]. These molecules are produced to inactivate or kill bacteria or other organisms. Inasmuch as these organisms are hidden in biofilms, the "killer" molecules cannot penetrate the protective "slime" and, instead, kill the surrounding tissue [26]. Upregulated TLR2 and the activated MyD88 pathway also catalyze the formation of β and γ secretase that cleave AβPP to form Aβ [2]. Ergo, Aβ is generated from 2 sources: one is from the microbes (spirochetes) themselves in the process of forming biofilms; the other is generated from the activation of TLR2 [2].

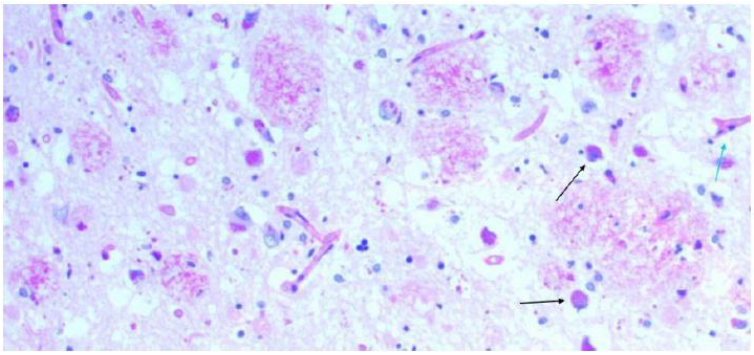

Fig. 1.4. Extracellular Senile plaques (biofilm) and intracellular biofilms
Large pink deposits (senile plaques); intracellular biofilms (black arrows); NFT (blue arrow). PAS stain 10X

When the neurons are disrupted and the contents of the cell are emptied into the extracellular space, abundant AβPP is released. This becomes a source for Aβ in addition to that which is generated during the formation of the larger, extracellular biofilms (plaques).Aβ is anti-microbial [27] yet it cannot impact the spirochetes because of the film surrounding them. Macdonald and Allen have shown this histopathologically and immunomicroscopically [24,28]. The large quantities of Aβ have a "space occupying" presence that interrupts and disrupts the neurocircuitry.

Biofilms have many attachment sites for other organisms [29]. This may account for other organisms such as C. pneumoniae, P. acnes, H. simplex and others being found in the AD brains [30,31,32,33] (see Fig. 6 in Introduction). These same organisms have been found in arteriosclerotic plaques (which also contain biomass) [19,34]. Herpes simplex virus has not been shown to make biofilms in vivo: to date, HTLV1 and molluscum contagiosum have been shown to make biofilms [18,35]. The viruses in these two skin diseases likely hijack the cell's DNA and use this to create the biofilms that have been identified. It is possible that HSV may do this in its ordinary facial or genital locations; but, to date this has not been shown to occur. In those settings, this could possibly relate to the chronicity and recurrences noted. HSV, as a co-inhabitant of the AD biofilms, would seemingly not have a primary role in the disease.

Biofilms in chronic diseases differ in many ways: the most obvious is they are made by different organisms [16]. Next, they may not be situated in the organs involved; e.g., streptococcal biofilms in the tonsils (and not in the skin) in psoriasis and M. leprae biofilms in the liver, kidneys, and spleen in leprosy [16,17]. Nearly all activate the innate immune system (TLR2), but some engage the adaptive immune system as well. Tinea versicolor, a biofilm associated disease activates neither arm of the immune system and thus is not associated with any symptoms [16]. All the organisms make Aα which makes up the infrastructure of the biofilms; the spirochetes in AD, while creating biofilms, make Aβ [5]. The staphylococci in eczema have been shown to do the same. Many biofilms form the environmental part of the double hit phenomenon; e.g. eczema where the biofilms in the occluded ducts form the environmental part of the double hit phenomenon and the filaggrin (or other) gene forms the genetic hit. The environmental hit in psoriasis is streptococcal biofilms in the tonsils and the PSORS genes form the environmental hit [16]. Biofilms differ as regards interaction with other pathways, namely PAR2 in eczema and NFkB-BACE in AD [16,20]. Most biofilms are part of an inflammatory cascade, but recent findings have shown them to be associated with proliferative diseases (molluscum contagiosum [benign] and HPV in squamous cell carcinoma in situ [18].

Biofilms inside neurons are likely produced in accordance with the microbe's quorum sensing mechanism [36]. Outside the neuron, biofilms are subject to many other factors, such as hyperosmolality (in diabetes) and many dispersers such as drugs and chemicals [37]. All the external factors that make AD worse can be explained by the pathways emanating from spirochetes and the production of biofilms [38].

From the above, we believe that spirochetes are the microbes active in the pathogenesis of AD. Their presence in the affected brains has been documented as has their subsequent activity, namely making biofilms. Aβ has been shown to result from the formation of these biofilms intra and extracellularly. Also, Aβ has been shown to be produced by known pathways in which the biofilms interact with TLR2. Neuronal destruction resulting from intracellular Aβ interacting with tau protein to produce p-tau has also been outlined. All the pathogenic processes in AD appear to be generated from the spirochetes. The debility of AD urges the beginning of therapeutic interventions against these spirochetes before they arrive in the brain or before they begin to make biofilms.

2. CONCLUSION

Spirochetes, both Lyme and dental, have been found in AD brains similar to *T. pallidum* in syphilitic dementia (GP). Where the pathology of AD and GP is identical and where biofilms have been found in both diseases, it is reasonable to conclude that biofilms in both diseases are produced by the spirochetes. The pathology in each disease is helical, not coccoid, rod like, or viral. Spirochetes cultured from AD brains have actually been shown to make biofilms *in vitro* and simultaneously make Aβ and ABPP. When this process occurs intracellularly, the Aβ upregulates the production of p-tau.

This leads to the formation of NFT and disintegration of neuronal dendrites. Extracellular biofilms are considerably larger and upregulate the innate immune slate the innate immune system (TLR2) which leads to further development of Aβ and ABPP. Thus, the presence of NFT and Aβ are directly related to spirochetes.

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Chapter 2
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Focusing on 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. *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. 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.

Keywords: Alzheimer's disease; biofilms; essential role.

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

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1. BIOFILM PATHOLOGY AND MICROBIOLOGY IN ALZHEIMER'S DISEASE

Biofilms are undeniably present in Alzheimer's disease (AD). Most microorganisms have the ability to form biofilms. Bacteria in biofilm are covered by a "slime"- layer, which protects them from stressful environmental conditions [1,2,3], therefore, the cultivation and eradication of microorganisms in biofilms is more difficult [4,5]. 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 [6]. (Fig. 2.1) 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 [6]. *In vitro* biofilms, formed by Borrelial spirochetes cultured from AD brains, and, *in vivo* biofilms, noted in those same brains (Fig. 2.2), have also been seen on gross examination and with fluorescent staining with Thioflavin S [7]. Further, these same biofilms have been highlighted by immunopathology that stained for bacterial peptidoglycan (which also recognizes the polysaccharide matrix like PAS) [7]. Last, their presence has been noted *in vitro* and *in vivo* on fluorescent in situ hybridization (FISH analysis) by Miklossy and Macdonald [7,8].

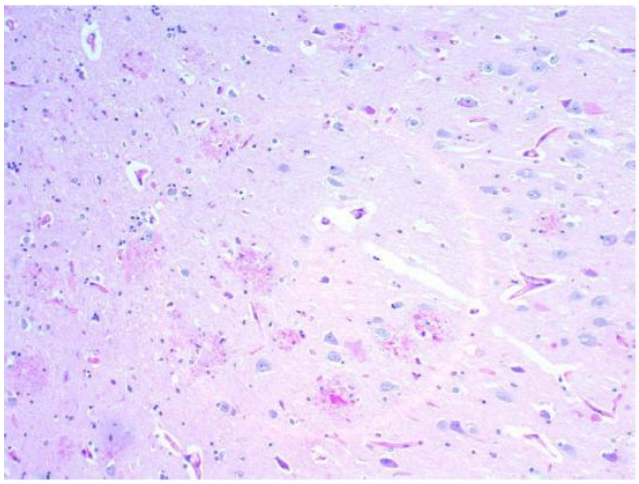

Fig. 2.1. Alzheimer's disease hippocampus (senile plaques)
Plaques composed of polysaccharides stain with PAS 10X

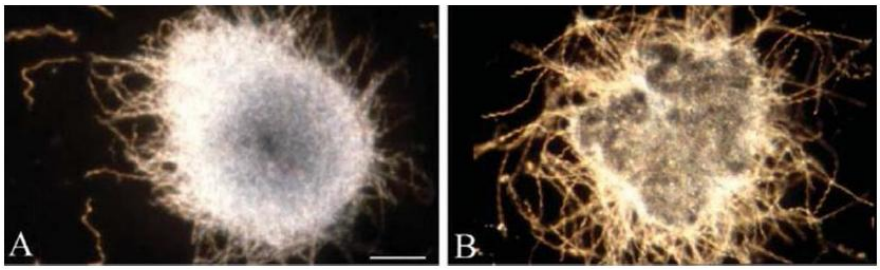

Fig. 2.2. Biofilm from cultured Borrelia
Biofilm formed in vitro; image B shows water channels (from Miklossy J Alz Dis 2016)

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 (Fig. 2.3), so it is not an uncommon occurrence [9]. 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 [10]. 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 [11]. Many bacteria have been found to regulate diverse physiological processes and group activities through a mechanism called quorum sensing, in which bacterial cells produce, detect and respond to small diffusible signal molecule [12,13,14]. 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 [15].

Fig. 2.3. Biofilms made by other organisms: Candida (L), penicillium (R)

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 in Fig. 2.2) [7]. 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 [16]. 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 [17]. 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 [18].

2. 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 [7,19]. Next came analysis by Koch/Hill postulates which showed AD to be infectious and, finally, 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 pathological examinations of GP and AD [20,21]. 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 [22]. 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 [23,24]. The extracellular biofilms in AD co- localize with Aβ which is perhaps not surprising because of Aβ's anti-bacterial properties [8,25].

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 [10]. 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 [10]. 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 [26,27].

In vitro, Borrelia spirochetes have been shown to produce biofilms, ABPP, and Aβ [7]. This pure culture forms *in vitro* in the absence of cells. *In vivo,* the biofilms have been seen intracellularly as has been Aβ [28]. 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. it has been shown *in vitro* that Aβ and ABPP are created when the spirochetes form biofilms [7]. The intracellular presence of biofilms and Aβ has also recently been shown *in vivo* [28]. When it is intracellular, the Aβ acts on tau protein and, by known pathways, induces the formation of hyperphosphorylated tau (p-tau) [29]. 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. Also, as the dendrites disintegrate, 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 [21]. In the extracellular location, the biofilms attract the innate immune system, especially Toll-like receptor (TLR2), the presence of which has recently been identified [6]. Biofilms, no matter whether they are formed by gram positive or gram negative organisms, attract TLR2 [29]. The "curli" fibers in the biofilm have receptor sites for this molecule [30]. Upregulated TLR2 utilizes the myeloid differentiation 88 (MyD88) pathway to inactivate microbes [10]. 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 [10].

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, along with the TLR2.

3. CONCLUSIONS

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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Discussing Alzheimer's Disease: Parsing the Pathways Leading to the Disease Based on the Spirochete/Biofilm Hypothesis

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ABSTRACT

A pathway which highlighted spirochetes (*Borrelia burgdorferi* and dental treponemes) that made biofilms which led to Alzheimer's disease has recently been promulgated. All the Alzheimer's disease, on which this pathway was based, had been specifically confirmed both clinically and pathologically. This current work will show putative and plausible individual pathways within that overall pathway that were studied. First and foremost, intracellular spirochetes make biofilms and concurrently make beta amyloid; this has been shown *in vitro* in pure culture and *in vivo*. The beta amyloid together with tau protein leads to hyperphosphorylated tau that leads to neurofibrillary tangles and dendrite disintegration. Many drugs and environmental states interact with that pathway and generally lead to further disease progression. (These drugs such as haloperidol, and environmental states such as hyperosmolality have been known to cause worsening of the disease.) Few things lead to reversal of the pathway, though L-serine stands out among them. All these pathways would not even exist or be activated were the spirochetes not present. The above hypothesis is based on observed findings from patients with Alzheimer's disease and from pathways known to be generated from those findings.

Keywords: Alzheimer's disease; spirochete/biofilm hypothesis; biofilms.

1. DEFINITION: BIOFILM

A biofilm is a community of microbial organisms encased in a slime coating that arises in response to environmental or antimicrobial stressors. The biofilm consists of extracellular polysaccharides and a proteinaceous infrastructure of amyloid. The polysaccharides stain histologically with periodic acid Schiff, and the amyloid stains with Congo red. Other components of biofilms include smaller amounts of DNA, RNA, water, lipids, and exporter cells. Biofilms generally form by quorum sensing, and the organisms have many genes for this for this to occur. They also preferentially attach to other substrates such as catheters. Biofilms have attachment sites for Toll-like receptor 2 which, in turn, utilizes the Myeloid Differentiation 88 pathway to attempt to inactivate the microbes. (Chronic) diseases result when that activity attacks surrounding tissue and generates deleterious activity; further, biofilms are often polymicrobial. Extracellular biofilms lead to activation of the innate immune system (similar to other chronic diseases), and this together with the innate immune system's major pathway (MyD88) leads to the production of beta amyloid.

A pathway to Alzheimer's disease (AD) has recently been delineated that, in essence, described spirochetes making biofilms that subsequently led to AD [1] (Fig. 3.1). The human body harbors various types of spirochetes. More than 60 different *Treponema* species are found within the oral cavity [2,3], which are present in a large part of the population [4]. This hypothesis was based on a template that had shown AD to be similar to general paresis (GP) of the insane of tertiary syphilis in regard to both the clinical and pathological presentations [5]. This similarity showed the major

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pathological components (spirochetes, senile plaques, neurofibrillary tangles, and beta amyloid [Abeta]) were present in both AD and GP. Also noted, in both, were granulovacuolar degeneration, neuronal loss, and tissue atrophy [5,6].

Fig. 3.1. Overall pathway

The spirochetal biofilms have been shown to be present both intra and extra-cellularly where they produce dramatically different effects that have been demonstrated both pathologically and microbiologically [7]. The biofilms have been shown to be made by spirochetes; the primary evidence for this is the growth in culture of *Borrelia burgdorferi* from the brains of AD patients. These cultured organisms, in turn, made biofilms when put under environmental stress. Also, produced by these organisms was β amyloid precursor protein and smaller amounts of Aβ [8,9]. During the formation of the biofilms by the spirochetes, the spirochetes have also been shown, in pure culture, to make beta amyloid precursor protein (ABPP) and Abeta simultaneously with the biofilm [8]. Abeta has been demonstrated pathologically to be both intracellular and extracellular [7]. Abeta interacts with tau protein; and, this leads to tau hyperphosphorylation (p-tau), localized Ca 2+ elevation, tau missorting into dendrites, and destruction of microtubules and spines [10,11]. One isoform of p-tau has actually been shown to be protective of dendrites [12]. The toxic p-tau leads to disintegration of the neuronal dendrites (because they are no longer stabilized by ordinary tau protein), and it also leads to the formation of neurofibrillary tangles (NFT) [5]. Spirochetes have been found in NFT [5]. This is a very important pathway because of the neuronal destruction that eventuates. (Figs. 3.2, 3.3) This was foreshadowed when transgenic mice had their cognitive decline ameliorated by reduction of Abeta and tau, but not Abeta alone [13].

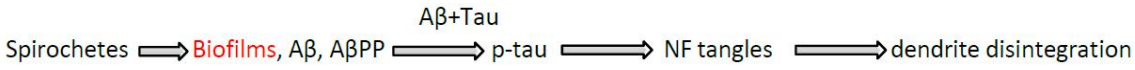

Fig. 3.2. Intracellular pathway

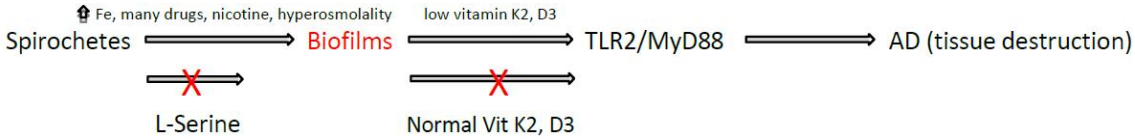

Fig. 3.3. Extracellular pathway

Continuing with the various pathways leading to dementia, intracellular biofilms within the hippocampal neurons likely form by "quorum sensing" [14]. The quorum requires 10 microbes in any direction to begin forming a biofilm [15]. The community formed in this manner "fits" in the cytoplasm of the neuron [9,16]. If the space is not large enough, the organisms remain planktonic without a biofilm developing. It is unlikely that biofilm dispersers such as nicotine have much impact on intracellular biofilms because only 30% (at most) crosses the cell membrane [17,18]. It is possible that hypermosmolality impacts intracellular biofilms because of the osmosis of fluid from inside to outside the cell would stress the biofilm [19]. A neurotoxin, beta methyl amino alanine (BMAA), may enter into the intracellular space and impact biofilms because it triggers formation of neurofibrillary tangles and Abeta deposits in the brains of Vervet monkeys [20].

Extracellular biofilms in the brain behave similarly to extracellular biofilms in other diseases [21]. Namely, they upregulate Toll-like receptor 2 (TLR2) of the innate immune system, and they are also subject to both biofilm dispersers and aggregators as well as environmental compounds and states [22]. Many drugs are biofilm dispersers (furans, piperidines, pyrroles, thiophenes, and rifampin) and thus cause disruption and subsequent new formation of more biofilms [22]. This is similar to the extrusion of exporter cells as a natural occurrence in a biofilm. An example of a pharmacological

biofilm disperser causing severe difficulty is Haloperidol (a piperidine) which leads to a 200% increase in death when administered to AD patients [23].

TLR2 that is upregulated by extracellular biofilms, even those that are created by gram negative organisms, utilizes the myeloid differentiation 88 pathway (MyD88) to inactivate microbes, but is unable to penetrate the biofilm, so the spirochetes remain safely ensconced inside [24,25].

The TLR2/MyD88 generates NFkB and TNFa that utilize another pathway whereby NFkB catalyzes beta amyloid converting enzyme that, in turn, catalyzes β and γ secretase that cleaves off the terminal portions of ABPP to form Abeta [24]. Thus, extracellular Abeta is generated by this sequence as well as that which arises when the p-tau neurons degenerate. This latter leads to the equivalent of exporter cells being extruded from a mature biofilm (see Fig. 5.5 in Chapter 5).

Biofilms form more readily when the surrounding serum contains low vitamin E and elevated serum iron [26]. The opposite occurs in a serum rich with L-serine which inhibits quorum sensing [20]. Caffeine may also be a mild quorum sensing inhibitor [27]. Vitamins K2 and D3, if low, cause upregulation of TLR2 leading to consequences already discussed; normal to slightly elevated K2 and D3 do the opposite and lead to a lower impact of TLR2 (Fig. 3.3) [28,29].

These various pathways would not exist if the spirochetes initiating them were killed prior to the formation of biofilms. The Borrelial and dental spirochetes are susceptible to penicillin, and penicillin derivatives, and a once yearly course of that antibiotic would seem sufficient to carry this out. Azithromycin would be an alternative for the penicillin allergic. The course derives from the treatment of syphilis, the absolute prototype for AD. Treatment with penicillin anytime prior to tertiary is curative. The yearly course could be considered like a “vaccine”, necessary because of the constant seeding of dental spirochetes in the brain. This would likely reduce resistance as well because, once organisms are in a biofilm, they pass resistance genes horizontally. The dental seeding is unlike syphilis or Lyme disease where the exposure is likely to be a one-time event. Such as approach seems rational until such time as a serological test (or other) is developed which can predict AD, just as the RPR predicts tertiary syphilis [30]. The serologic test has been foremost in the disappearance of tertiary syphilis. Another chronic biofilm disease, leprosy, has nearly disappeared with the administration of Dapsone and rifampicin (a biofilm disperser) [31]. Such a protocol would be unsuccessful in AD because it would be rendered too late in the course of the disease [32].

Other efforts have presented these pathways with a singular focus [6,8,14,18,20]. This work attempts to align each of them into an overall pathway to the disease. It seems apparent that the intracellular occurrences are more important inasmuch as they lead to destruction of the neurons. All this is shown to be a result of a chronic infection which spirochetes and their biofilms play a leading role. This conforms to other chronic infections in which microbes and their biofilms cause the diseases [21].

2. CONCLUSION

The overarching pathway in AD is spirochetes make biofilms that create AD. This pathway has two main branches: intracellular (likely the more important) and extracellular. In the process of making biofilms, the spirochetes also make Aβ and ABPP. The Aβ together with ordinary tau protein make p-tau, and this leads to neuronal dendrite disintegration. The extracellular pathway upregulates TLR2, and this, by a known pathway (catalyzed by BACE) creates more Aβ. Thus, all the hallmark pathological features are created by the spirochetes and their biofilms. This has all been demonstrated both *in vitro* and *in vivo*.

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Chapter 4
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The Novel Finding of Intracellular Biofilms in Alzheimer's Disease

Herbert B. Allen[1,2,3*]

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ABSTRACT

We previously have found biofilms in the hippocampi of Alzheimer's disease (AD) post mortem brain specimens. We had seen them in an extracellular location and noted them to be present in the areas of pathological plaque formation. Other investigators have found the presence of spirochetes (Lyme and dental) in affected (AD) brains, and these have been correlated with Treponema pallidum. In a recent historical comparison of the pathology of syphilis, the histological findings of syphilis and AD were shown to be exactly the same. Further, spirochetes have been cultured from the affected brains and have been found to make biofilms and beta amyloid precursor protein. Utilizing the same pathological methods as in our prior study, we have found biofilms in an intracellular location. The similarity of this finding to other diseases has been presented; and, the impact of the "intra" versus the "extra" cellular location is discussed. In the future, we will show this phenomenon of intracellular biofilms is not unique to the organs mentioned, but is also present in such diverse diseases as leprosy and psoriasis. Consequently, it is another example of nature behaving similarly as regards the pathogenesis of many diseases.

Keywords: Alzheimer's disease; biofilms; beta amyloid.

1. INTRODUCTION

We, and others, have found biofilms in the brains of Alzheimer's disease (AD) patients [1,2]. These biofilms have been located primarily in the pathological plaques of that disease. As such, they are in an extracellular location, and the amyloidogenic "curli" fibers of the biofilm activate Toll- like receptor 2 (TLR2) of the innate immune system [3]. The major pathway utilized by TLR2, in its role of inactivating invading pathogens, is the MyD88 pathway which eventuates in NFκB and TNFα [4].

NFκB, together with β amyloid converting enzyme (BACE), catylyzes β secretase which cleaves off the terminal portion of β amyloid precursor protein to form beta amyloid (Aβ) [4]. Fibrillar and oligomeric forms of Aβ appear neurotoxic in vitro and in vivo. Importantly, in specific transgenic (Tg) mouse models of AD the lack of Aβ correlates with the absence of neuronal loss and improved cognitive function [5,6,7,8]. Aβ is antimicrobial [9] and it surrounds the plaques of AD but cannot penetrate the biofilm [1,2]. The TNFα (produced by the innate immune system) cannot penetrate the biofilms either, so it has been postulated to kill the surrounding tissue instead [10]. Further, TNFα together with TNFα converting enzyme catalyzes α secretase which eventuates in α amyloid.

The biofilms have been shown to be made by spirochetes; the primary evidence for this is the growth in culture of Borrelia burgdorferi from the brains of AD patients. These cultured organisms, in turn, made biofilms when put under environmental stress. Also produced by these organisms was β amyloid precursor protein and smaller amounts of Aβ [11]. Consequently, the paradigm for this process would be:

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Microbes (spirochetes)→biofilms→innate immune system--TNFα →tissue destruction

Microbes→biofilms→innate immune system --NFκB→βACE→β secretase→Aβ→tissue destruction

Spirochetes are the primary organisms involved because of the evidence both from the cultures [11,12], and from the PCR evidence implicating Borrelia and dental spirochetes in a 25%/75% ratio [13,14]. The dental organisms have also been strongly linked epidemiologically to AD [15].

Our current investigation involves examination of AD brains for intracellular (not extracellular) biofilms. Using the same staining patterns as in our previous pathological examinations, we have found "intra" cellular biofilms in all the AD brains, and none of the controls.

2. METHODS

We re-examined hippocampal specimens with the techniques described previously [1]. Seven hippocampal specimens from patients who had previously been confirmed both clinically and pathologically (post mortem) to have Alzheimer's disease were re-examined by five pathologists. Ten control hippocampal specimens, from age and sex matched patients who died of unrelated, non-cerebral diseases and/or causes were included for study. All specimens were stained with hematoxylin and eosin (H+E), periodic acid Schiff (PAS), Congo red routine stains; all specimens were also stained with treponema pallidum (TPI), β amyloid, CD 282 (TLR 2) and CD 284 (TLR 4) immunostains. The technique for these stains was as previously published. PAS and β amyloid stains were applied sequentially to the specimens and were examined. Routine light microscopy was employed. The specimens were not "blinded" because by gross examination alone, the AD specimens could be distinguished from the controls.

3. RESULTS

In all the hippocampal specimens from AD patients, we found positive intracellular staining with both PAS and Congo red. The positive PAS indicates the presence of polysaccharides, and the positive Congo red indicates the presence of amyloid (Figs. 4.1, 4.2). The other results that had been previously presented were re-affirmed [1].

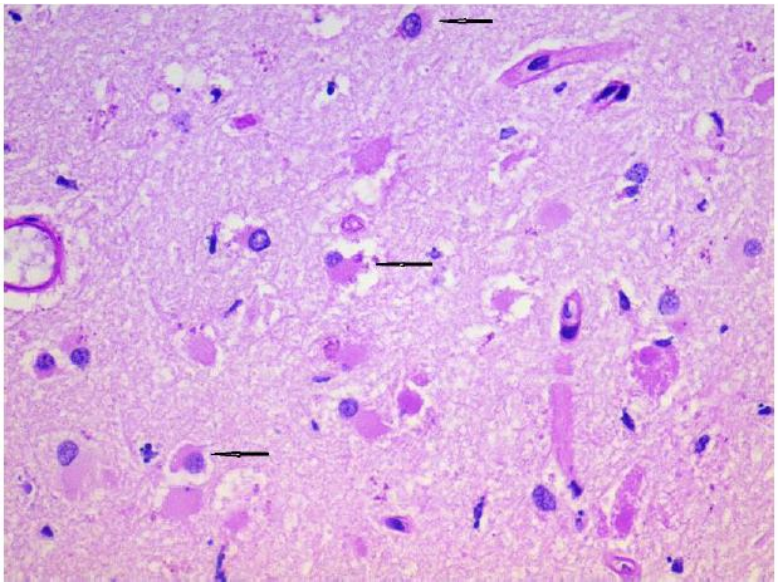

Fig. 4.1. PAS stain of AD hippocampus (40X)
Positive staining in cytoplasm represents the polysaccharides that make the bulk of the biofilm.

Arrows show some of the neurons with positive cytoplasmic staining

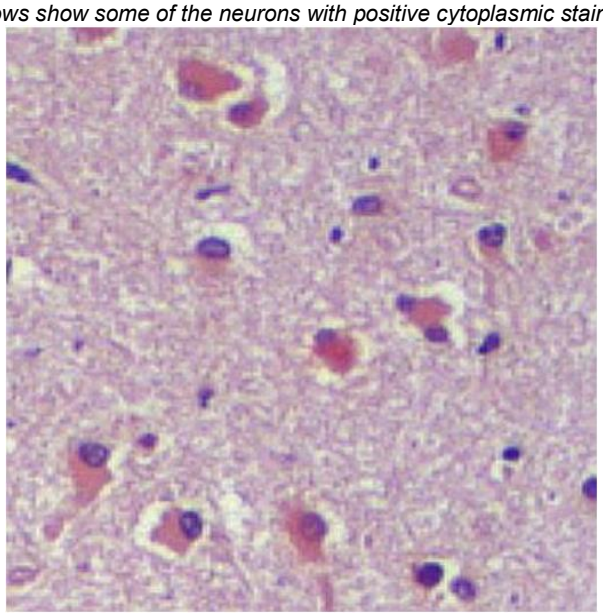

Fig. 4.2. Congo red staining of AD hippocampus 40X
Red staining in the cytoplasm represents amyloid which is the infrastructure of biofilms

4. DISCUSSION

Intracellular biofilms have not been noted previously in AD; however, they have been found in urinary tract disease and in pulmonary disease [16,17]. The intracellular biofilms in cystitis have been referred to as "pods", and these "pods" when extruded into the surrounding tissue are fully capable of creating new, and larger, biofilms.

One possible impact of intracellular biofilms in AD is shown in the diagram (Fig. 4.3). The intracellular biofilms could be extruded from the neurons as portrayed and become neurofibrillary tangles. These tangles have been shown to be present in tertiary syphilis as in AD [18]. They have also been shown, in the same historical comparison of the two diseases, to contain spirochetes (T pallidum in syphilis and Lyme/dental in AD.

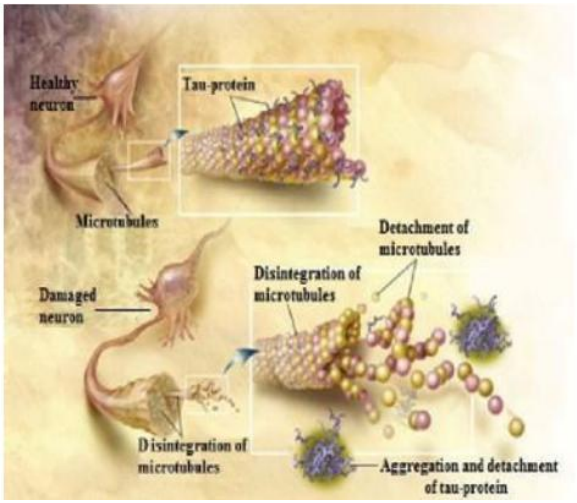

Fig. 4.3.

Tau protein when phosphorylated causes disruption of the neuronal dendrite. (from ADEAR/Wikimedia Commons)

Biofilms are present inside the epithelial cells lining the bladder. Because of the intracellular location in the bladder, lungs and brain, the organisms have another level of protection in addition to the extracellular polysaccharide, "slime" coating. Situated inside the cell walls, they do not appear to be recognized by the immune system (as they are not in the urinary intracellular location). Thus, they remain a "nidus" of infection that contributes to the chronicity of the diseases. As "pods", the biofilms retain all the capabilities of extracellular biofilms: immune system avoidance, gene transfer, and reduced antibiotic diffusion [19]. Further, although it is not employed, they retain the ability to down regulate the immune response [20]. In the future, we will show this phenomenon of intracellular biofilms is not unique to the organs mentioned, but is also present in such diverse diseases as leprosy and psoriasis. Consequently, it is another example of nature behaving similarly as regards the pathogenesis of many diseases.

5. CONCLUSION

This chapter shows *in vivo* what Miklossy showed *in vitro*; namely that spirochetal cultures (from postmortem brains of AD patients) make biofilms. This finding mimics other diseases where different microbes make biofilms and create both internal and cutaneous diseases. In subsequent observations (noted in previous chapters), the spirochetes make Aβ and ABPP when making biofilms. The formation of Aβ intracellularly, together with tau protein that is already there, creates p-tau, a crucial element in AD.

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Chapter 5
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Assessing the Role of Spirochetes, Biofilms, the Immune System, and Amyloid with Regard to Potential Treatment and Prevention of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is an infectious disease caused by spirochetes, and these spirochetes form biofilms, which attract the innate immune system. The innate immune system first responder, Toll-like receptor 2, generates both NFκB and TNFα which try to kill the spirochetes in the biofilm, but cannot penetrate the "slime". NFκB is also responsible for the generation of amyloid beta (β) which itself is anti-microbial. Aβ cannot penetrate the biofilm either, and its accumulation leads to destruction of the cerebral neurocircuitry. Treatment with penicillin (as in tertiary syphilis, the comparator to AD) is outlined; a biofilm dispersing agent may need to be added to the protocol. Treatment with a bactericidal antibiotic with a concomitant biofilm disperser seems most reasonable; but, as has been stated previously, any neurologic damage is irreversible. It is therefore of the utmost importance to treat early in the course of this disease.

Keywords: Alzheimer's disease; amyloid; immune system; spirochetes; biofilms.

1. INTRODUCTION

Where spirochetes have been found in the brains of Alzheimer's disease (AD), it may be considered an infectious disease; this is the first and most important consideration [1,2]. It is also a chronic disease, a biofilm-associated disease, [3] and an autoimmune disease [4]. Further, it is a debilitating disease, a socially destructive disease, an exceedingly expensive disease, and, lastly, a deadly disease [5]. Early-onset AD is uncommon, accounting for less than 1% of all AD cases, and is caused primarily by autosomal dominant mutations in either the amyloid precursor protein (APP) or the presenilin (presenilin-1 or presenilin-2) genes [6,7,8,9,10,11]. This review will focus on the biofilm portion of the disorder as well as the autoimmune response. It will also touch on some rational therapeutic concepts for this most irrational of diseases.

The infectious nature of AD was revealed when spirochetes (both dental and Lyme) were shown to be present in the brains of affected patients [1]. The dental microbes travel from the oral cavity during times of disruption of the dental plaque and subsequent bacteremia following dental procedures; i.e., any time blood is seen. The hippocampus (which is the initial site of cerebral involvement in AD) is approximately 4 cm from the posterior pharynx. Lyme borrelia travel to the brain via the blood stream during the secondary stage of that disease following the erythema migrans lesion [12]. This secondary stage is characterized by fever, myalgias, arthralgias, and other systemic symptoms. The spirochetes have an affinity for neural tissue and pass through the blood-brain barrier easily [13].

Once the spirochetes are in the brain, they attach, divide (albeit very, very slowly) [14], and multiply. When they reach a quorum, they begin to spin out a biofilm (Fig. 5.1) [15]. This represents

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approximately 150 spirochetal cells which are 0.3 microns in diameter (10 cells are necessary on a two-dimensional culture plate for a quorum to begin). Because of the exceedingly slow division, it takes approximately 2 years to accumulate sufficient organisms to make one biofilm. The biofilm is protective and is a response of the organisms to ensure their survival, inasmuch as it encases them in "slime" (Fig. 5.2).

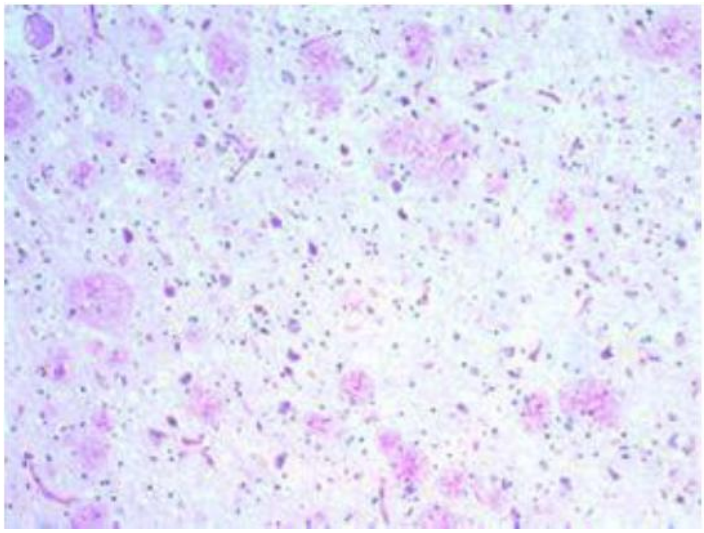


Fig. 5.1. Hippocampus AD
PAS stains polysaccharides (pink masses) that make up the bulk of the biofilms 10X

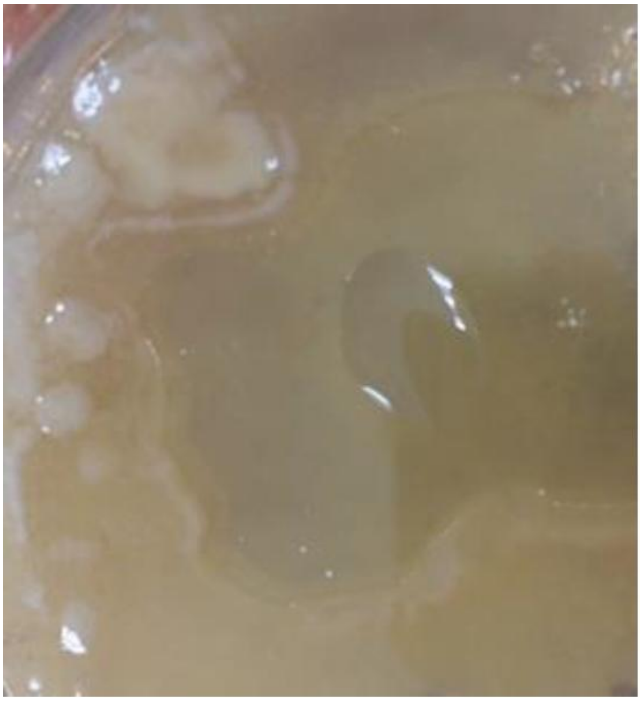


Fig. 5.2. Biofilm (slime)
Typical biofilm (as produced by yeasts in tinea versicolor) from ref. [14]

Quorum sensing is one triggering mechanism for the production of biofilms; other organisms in other diseases may form biofilms when subjected to different stimuli. These stimuli include salt and water,

as seen in eczema and tinea versicolor [16,17]. Low dose antibiotics and quorum sensing are seen in psoriasis [18] and arthritis [4]. Further, elevated temperatures and exposure to alcohol and other chemicals promote biofilms [19].

At some point after attachment and formation of the biofilms, the innate immune system becomes activated and attempts to destroy them [14]. Even though the spirochetes are weakly gram negative, Toll-like receptor 2 (TLR 2) has been shown to be the first responder to the organisms incorporated in the extracellular polysaccharide slime (Fig. 5.3) [14]. TLR 2 itself has recently been shown to be attracted to the "curli" fibers produced by the organisms within the biofilm [20]. These fibers are the major component of the proteinaceous portion of the biomass and are not only immunogenic but are also important in the attachment of the biofilms. Ordinarily, Toll-like receptor 4, rather than TLR 2, responds to gram-negative organisms.

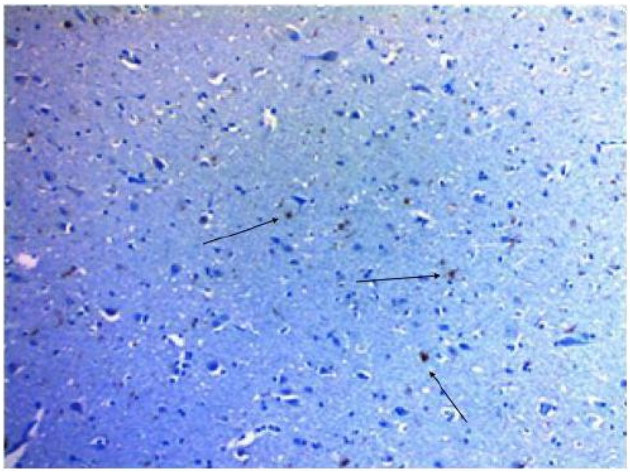


Fig. 5.3. TLR 2 in AD hippocampus
CD 282 (TLR 2) showing black deposits in the tissue. (arrows) From ref. [14]

TLR 2 kills primarily by means of tumor necrosis factor alpha (TNF α) generated by the myeloid differentiation pathway D88 (MyD88). TLR 2 coats the microbes (Fig. 5.4) and generates both nuclear factor- B (NFkB) and TNFα. This is the process utilized for killing when the organisms are planktonic (free floating) and not in a biofilm. Neither TLR 2 nor TNFα can penetrate biofilm; consequently, it has been theorized that the TNFα destroys the surrounding neural tissue instead [14].

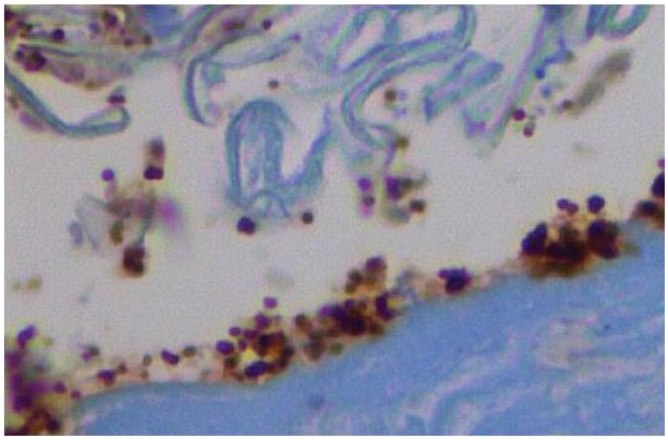


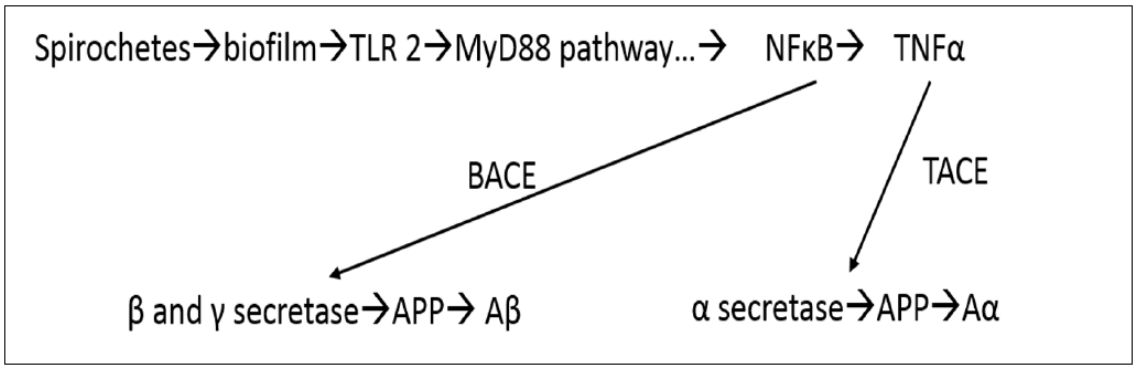
Fig. 5.4. Candidiasis–TLR 2 coats the yeasts
Activated TLR 2 coats yeasts in the stratum corneum in candidiasis; control location of TLR 2 is in epidermal basal layer (CD 282) 40X. From ref. [14]

Almost all organisms make biofilms, and, as has been previously stated, these biofilms protect the microbes dwelling within from noxious agents whether chemical, immunologic, or other. The bulk of a biofilm is made up of extracellular polysaccharides. Inside and out there are curli fibers; other amyloid fibers may be within and their purpose is to serve as an infrastructure for the polysaccharides. There are also DNA and water channels, as well as the microbes themselves within the biofilm [21,22]. None of the commonly used antibiotics penetrate biofilms; and, none of the immunologic molecules from either arm of the immune system, whether innate or adaptive, are able to penetrate either.

Ordinarily, the adaptive immune system including B cells, immunoglobulins, and T cells with their cytokines are excluded from the brain by the blood-brain barrier. That is until traumatic brain injury disrupts that barrier: at that point, B lymphocytes and IgG flood the cerebrum [23]. These immunogens kill by complement, alternate complement, killer T cells, cytokines (including TNFα and others). The killing of brain tissue around the plaques of AD is much more rapid and much more destructive with the adaptive immune system. This is without doubt the reason that AD occurs within 3 years after a cerebrovascular accident; ordinarily, it takes 30-50 years to develop. Further, it is most probably the reason that chronic traumatic encephalopathy (CTE) is so rapidly progressive after many concussions [14]. A concussion may pictorially and practically be considered an ecchymosis, and, as such, is comparable to a hemorrhagic cerebrovascular accident. CTE is currently the scourge of the National Football League where head trauma is a frequent occurrence.

Elucidation of the role of amyloid-β (Aβ) has been challenging: Aβ is a constant in AD and, in fact, it has been thought to be pathogenic by many. It, however, has been shown recently to be antimicrobial [24], and, even more recently, the pathway to its formation has been made apparent [25,26] This pathway (Fig. 5.5) derives from the MyD88 pathway activated by TLR 2. TNF-α, generated by TLR 2, in conjugation with TNF-α converting enzyme (TACE) becomes alpha secretase and splits amyloid precursor protein (APP) to make amyloid alpha. The NF-kB generated by the same MyD88 pathway, together with Aβ converting enzyme (BACE), activates beta and gamma secretases that cleave the APP. The APP then becomes Aβ and attacks the biofilm but cannot penetrate it. Consequently, it encompasses the biofilm and its buildup destroys the neurocircuitry of the brain (Fig. 5.6).

This is the very essence of autoimmunity, namely the body attacking itself; this occurs when the body's own innate immune system produces TNF-α or Aβ and attacks the biofilm encasing the spirochetes. In the process of doing this, the surrounding tissue is destroyed instead. Such is the case with the biofilm produced by staphylococcus in eczema and streptococcus in psoriasis; these biofilms call forth the innate immune system and the whole process of tissue destruction is set in motion [4]. The consequences of AD are much more dire however, because they lead to total destruction of the mind.



BACE is beta amyloid precursor protein converting enzyme

APP is amyloid precursor protein

Fig. 5.5. Pathway to Aβ from TLR2/MyD 88 pathway
From J Alz Dis 2016; 53: 1271-1276

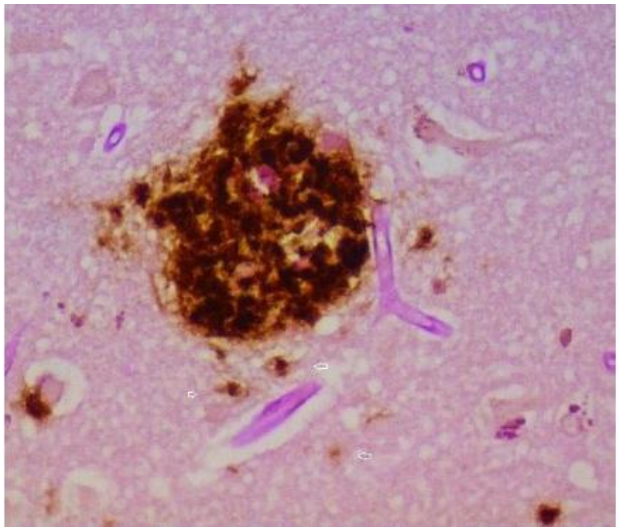


Fig. 5.6. Abeta coating biofilm
Abeta co-localizes with PAS (biofilm) in AD plaque. Combined PAS stain and Abeta immunostain; shows co-localization of biofilm and A_beta 40X. From ref. [14]

Any treatment of AD must take into consideration these biofilms. The pathway toward such treatment has previously been set by the treatment of syphilis. Syphilis, in its tertiary form (general paresis of the insane), has been shown to have exactly the same pathology as AD. The same plaques, neurofibrillary tangles, Aβ, and tau protein are present in both.

Where the pathology is the same and where both diseases are caused by spirochetes, and where spirochetes are sensitive to penicillin, a reasonable approach would be to follow the same treatment schedule as syphilis [27]. With that treatment, penicillin administered at any time prior to the onset of tertiary syphilis is curative. The same can reasonably be said for AD; penicillin administered any time prior to the onset of tertiary disease would also be curative. Lyme disease is most closely aligned with syphilis with erythema migrans equivalent to the chancre. In most cases, it is one tick bite compared to one chancre, so the treatment could be reasonably the same [13]. With dental organisms, exposure is ongoing; thus, the treatment would need to be tailored to the patient's dental health. One could imagine penicillin administered once or twice yearly (or perhaps more frequently) in certain situations (CTE?). The same could be said for the 5% of AD "pre"sufferers who have the APOE 4 gene for AD. CTE mimics the genetic disease. It must be stated that any neural damage is irreversible; thus, the importance and urgency of treating early in this disease course.

Treatment for patients in the early stages of dementia would need more than penicillin; they would also need an agent to disperse the biofilm [28]. Fortunately, there are such agents, and many are already being employed in AD patients. These agents include furans (citalopram), [29] thiophenes (olanzapine), [30] piperidines (donepezil), [31] pyrroles (azoles), [32] and rifampin [33]. Donepazil, for example, may be an anticholinesterase inhibitor, but it is also a biofilm disperser, so it may be helpful for a short time, but be harmful long term. The dispersal effect would potentially create many more plaques. The same may be said for haloperidol whose use in AD is already shunned.

Specifically, for early dementia, penicillin may be administered as IV or IM injections (IM would be 1.2 mu biweekly for 3 doses), probenecid 500 mg bid (to increase the serum concentration of penicillin by decreasing excretion, citalopram 20 mg daily, and rifampin 300 mg bid. These may be adjusted with the use of other medications. None of this is codified; but the current treatment is most likely harmful with the biofilms being dispersed without the spirochetes being killed. This would conceivably lead to many more biofilms, because all the spirochetes within the previous biofilm are capable of making new biofilms. The other major consideration is to treat in the "latent" stage for AD with penicillin by itself. Presumably, this would be similar to the treatment of latent syphilis. Also, it would be important

to treat prior to any dental surgery just as is being done for joint implants. Consequently, the organisms would be treated before they reached the brain in the case of dental surgery and before they did damage (made biofilms) in latent disease. Syphilis, in truth, is different because its presence is revealed by a serology. However, until a serologic test is available for AD, treatment, as has been proposed herein, seems rational. It is also relatively inexpensive, both as to medical costs and the cost of ongoing care of dementia patients. The story of AD is then one of spirochetes that make biofilms that activate the innate immune system. The first responder is TLR 2 and TLR 2 generates NF-kB and TNF-α that not only damage tissue in an attempt to kill the biofilm-encased spirochetes, but also lead to the production of Aβ. All of the foregoing leads to dementia. Treatment with a bactericidal antibiotic with a concomitant biofilm disperser seems most reasonable; but, as has been stated previously, any neurologic damage is irreversible. It is therefore of the utmost importance to treat early in the course of this disease.

2. CONCLUSION

This chapter outlines the role of extracellular biofilms in AD by creating Aβ and upregulating the innate immune system. Early treatment with a bactericidal antibiotic along with a biofilm disperser is emphasized; this will likely stop the progression of the disease. Early administration of the antibiotic would very likely prevent the disease, and prevention is critical.

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Chapter 6
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Considering Alzheimer's Disease: Possible Mechanisms for Worsening of the Disease

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ABSTRACT

Stroke, diabetes, nicotine, haloperidol, diet soft drinks, and others have all been shown to cause worsening of Alzheimer's disease (AD). In the following, we outline a possible mechanism for each of these entities to cause worsening by impacting a pathway to AD that we have developed based on our observations and those of others. That pathway includes microbes that make biofilms which activate the innate immune system; this ultimately leads to tissue destruction. The leading candidates for the microbes are pathogenic periodontal spirochetes and Lyme spirochetes which we believe are the driving forces in the formation of biofilms. We show how diabetes and its inherent hyperosmolality causes worsening of AD because the microbes make more biofilms in the presence of the hyperosmolar stress. More biofilms lead to more activation of the innate immune system (biofilms have receptor sites for Toll-like receptor 2 [TLR2]). Also outlined is how the dispersal of biofilms via nicotine and other commonly ingested/inhaled chemicals and medications leads to more severe disease.

Consequently, either "making" them as with diabetes, or "breaking" them as with nicotine, results in more biofilms and more activation of TLR2. Low serum levels of vitamins K2 or D3 lead to upregulation of TLR2 again causing worsening of the disease from increased innate immune system activation. Involvement of the adaptive arm of the immune system, in conjunction with biofilms, also leads to neurologic sequelae. Cerebrovascular accident (CVA), stroke, is the most disastrous malefactor of all because it is accompanied by activation of the adaptive immune system (lymphocytes and IgG) after disruption of the blood brain barrier. This creates massive tissue damage very rapidly. There are many fewer things that make Alzheimer's disease better when compared to worsening it. These are briefly mentioned. Early treatment would help prevent not only Abeta, as been outlined, but also the development of hyperphosphorylated tau. Thus, both the major pathological findings, Abeta and tau, would be addressed.

Keywords: Alzheimer's disease; worsening; cerebrovascular accident; stroke.

1. INTRODUCTION

Previous work by Macdonald, Riviere, and Miklossy has shown Alzheimer's disease (AD) to be microbial in nature [1,2,3]. Macdonald, in fact, was able to culture Borrelia burgdorferi spirochetes from an AD brain [1]. This finding was completely disregarded. Subsequently, Riviere and Miklossy by polymerase chain reaction (PCR) were able to identify Borrelia burgdorferi spirochetes (25%) and dental spirochetes (75%) in AD brains. Further, Miklossy has been able to cultivate Borrelia burgdorferi from AD brains [4]. This has substantiated Macdonald's observations [1]. In an extensive and comprehensive work, Miklossy has recently shown AD to be similar pathologically when compared to general paresis (GP) [5]. GP, tertiary neurosyphilis, is also caused by a spirochete (*T. pallidum*) and is the classic disease associated with dementia [5]. Syphilis prevalence is decreasing, it is yet to be eradicated [6]. Psychiatric manifestations appear late in the course of the disease and can range from subtle changes in personality, affective, and psychotic symptoms to

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cognitive decline like delirium and dementia [7,8]. In GP and AD, both the clinical features (dementia, in particular) and the pathological features (neurofibrillary tangles and plaques) are the same [5]. Spirochetes are clearly visible in the brains of both AD and syphilitic dementia [5].

Allen has recently shown that senile plaques (that are a signature pathologic finding of AD) are composed of biofilms made by the spirochetes [9]. This confirms the work of Macdonald [10]. Biofilm formation is a predictable occurrence because bacteria prefer to live in communities rather than in the planktonic state [11]. In fact, biofilm living is the way most organisms exist in nature [11,12]. Biofilm is an organized aggregate of microorganisms livingwithin an extracellular polymeric matrix that they produce andirreversibly attached to fetish or living surface which will notremove unless rinse quickly [13,14,15]. The biofilms are a protective barrier for the microbes against environmental changes and against the immune system and/or antibiotics. Biofilms made by one organism contain attachment sites for other organisms [16]; this is clearly demonstrated in the biofilms causing dental plaque [17]. In dental plaque, streptococcus mutans is the organism generally responsible for attachment of the biofilm; it is then joined by porphyromonas and pathogenic spirochetes to form the community [18]. This finding may account for various organisms such as C. Pneumoniae [19] and Herpes simplex in the brains of AD patients [20].

The microbes (spirochetes) in the affected brains make the biofilms, most likely through quorum sensing, a population sensing mechanism they possess, rather than by formation due to environmental stress [21]. The spirochetes divide so slowly that it takes considerable time (up to two years) to develop sufficient numbers to form a single plaque [21]. In addition to making the biofilms, the spirochetes create beta amyloid precursor protein (BAPP) as well as beta amyloid (Abeta) itself [4]. Further, the presence of biofilms causes activation of the innate immune system in the form of Toll-like receptor 2 (TLR2) because there are receptor sites on biofilms for that molecule [22].

Supporting evidence for this comes from the fact that TLR2 has been shown to be present in the areas of senile plaques, as well as throughout the tissue [21] In its main mode of response, TLR2, via the myeloid differentiation 88 pathway (MyD88), generates nuclear factor kappa b and tumor necrosis factor alpha (TNFa) in an attempt to kill the microbes inside the biofilm. TNFa cannot penetrate the biofilm, so it has been thought to attack the surrounding neural tissue instead (the "innocent bystander" concept) [9]. The pathway that characterizes this concept is as follows: microbes lead to biofilms which activate the innate immune system and cause tissue destruction. Given the lengthy time for AD to develop, this is a possible mechanism for the observed tissue destruction to occur because it not only takes an extended period of time for the biofilms to form, it also takes a long time for the TLR2 to work.

Additionally, TLR2 also leads to the production of Abeta, by activating the MyD88 pathway which generates NFkB. NFkB, acting in conjunction with beta amyloid converting enzyme (BACE), catalyzes beta and gamma secretase which cleave off the terminal portions of the BAPP to form Abeta [21,23]. Moreover, Abeta has been found to be an antimicrobial peptide [24]. Abeta also attempts to kill the biofilm-forming spirochetes, but it is unable to do so because it is unable to penetrate the slime. Its buildup further impairs the neurocircuitry [9]. The foregoing, based on observations, is the proposed pathogenesis of AD from spirochetes to Abeta (Fig. 6.1). To be presented in the following are various things that are known to make AD worse. We will show how each causes worsening based on the pathogenesis outlined above.

2. TRAUMATIC BRAIN INJURY

AD is well known to occur much more rapidly and be much more devastating after a cerebrovascular accident (stroke) [25]. Ordinarily, AD takes three decades or more to develop; after a stroke, it is reduced to 1-3 years. The blood brain barrier is disrupted in the area of the stroke, and this is rapidly followed by an influx of lymphocytes followed by a massive buildup of immunoglobulin G (IgG) [25]. With this influx of lymphocytes and IgG, the adaptive immune system is in play and is armed with far greater destructive power (classical and alternate complement systems, killer T cells and cytokines) than the innate system (NFkB and TNFa). This allows for the rapid destruction of the neural tissue

because, even with all that "killing" apparatus, the biofilms remain impenetrable. The surrounding tissue is killed instead (again the "innocent bystander" theory) [9].

Chronic traumatic encephalopathy (CTE) has many of the same pathological features as AD and GP (senile plaques, neurofibrillary tangles, Aβ and hyperphosphorylated tau protein) [26]. The repeated concussions and traumatic brain injury associated with CTE would cause breaks in the blood brain barrier (BBB) and allow for the adaptive immune system to work similarly to its action in stroke. Again, this would occur much more rapidly and destructively leading to the profound clinical and pathological AD-type changes. CTE has been found not only in American football players where it was first described by Omalu [26], but also in boxers, soccer players and others. Boxers have a 90% occurrence of concussions and champions have succumbed to the disease [27]. At the 2014 World Cup, 81 concussions occurred and all but three players returned to the pitch [28].

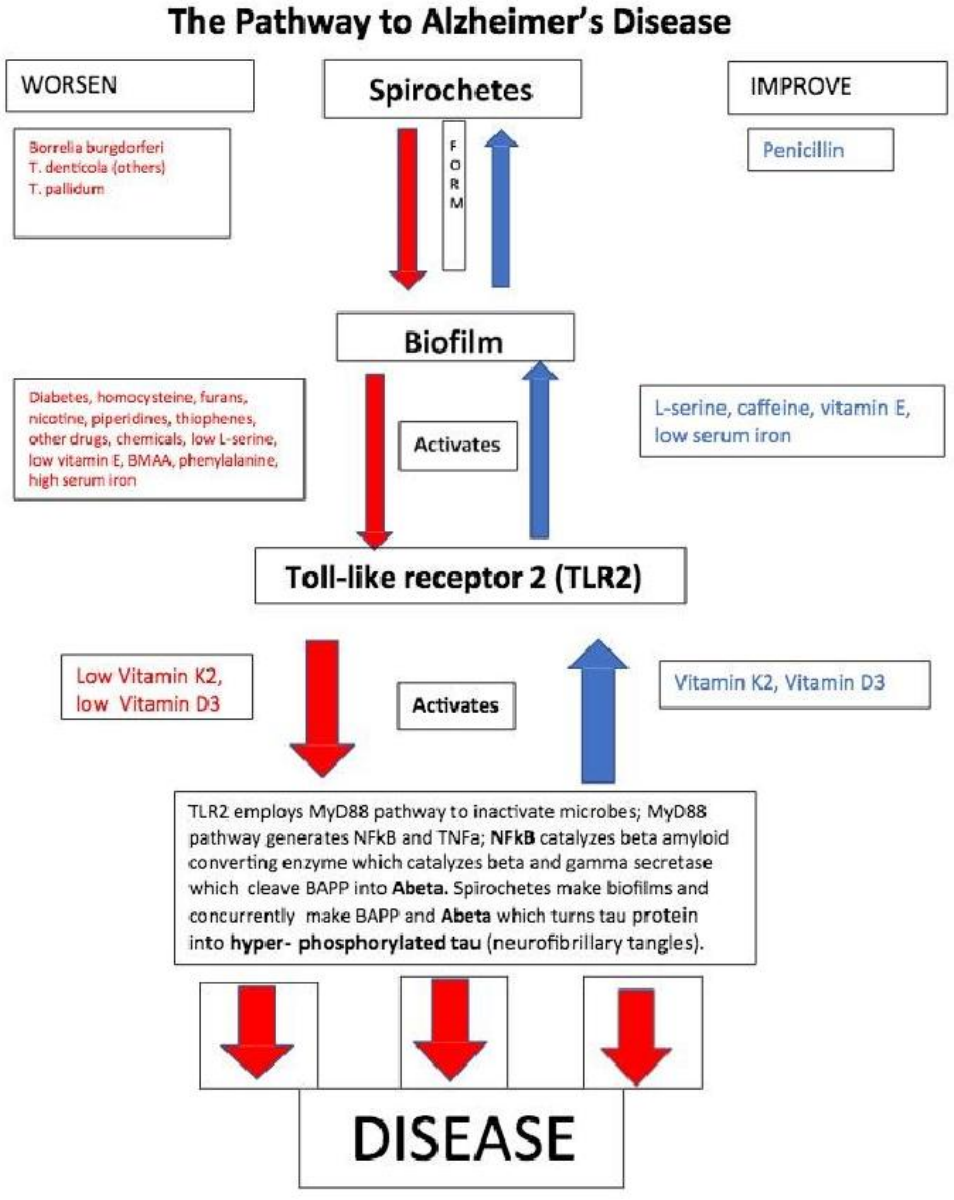

Fig. 6.1. The pathway to Alzheimer's disease

3. BIOFILM PRODUCTION

The next disorder to make AD worse is diabetes [29]. Recently, this has conceptually been shown to occur because of the increased serum osmolality that is present in diabetes (Fig. 6.1). Hyper osmolality has been shown to be a strong stimulus for biofilm formation [30]. Because of this, the organisms make more biofilms more rapidly, without waiting for a "quorum" to be reached [31]. Similarly, homocysteine has been observed to encourage organisms to make biofilms; consequently, it may be compared to hyperglycemia [32] (Fig. 6.1). Biofilms form more rapidly in the presence of salt and water which have recently been shown to be the provocative factors in eczema. In this disease, the biofilms form in the sweat ducts and trigger its onset [33]. Sub- therapeutic levels of antibiotics, lowered pH, and many other factors that induce stress (for the organisms) also trigger biofilm production [34].

Hyper osmolality increases biofilm production; increased biofilm production induces greater TLR2 which results in greater amounts of TNFa which results in greater tissue destruction. Also, this is possibly the pathway for diabetes to make arteriosclerosis worse: biofilms and activated TLR2 have recently been found in the arterial plaques in that disease [35]. Further, this may possibly be the pathway for many other chronic diseases including arthritis [36]. The effect of hyperosmolality, the effect of salt, water, subtherapeutic levels of antibiotics and lowered pH cause similar behavior of spirochetes in vitro, namely to form agglomerations and biofilm formation in these unfavorable conditions [37].

4. BIOFILM DISPERSION

Cigarette smoking is known to make AD worse, and the role of nicotine in that process has recently been outlined [38] [Fig. 6.1]. Nicotine is a biofilm disperser; conceptually, once a biofilm is dispersed (in effect creating "exporter cells") and there are no bactericidal antibiotics present, a whole crop of new biofilms gets sown, seeded by the dispersed organisms. With TLR2 activated, new levels of destruction are created, and the AD gets worse.

Biofilm dispersion may also be caused by many drugs: [Fig. 6.1] one of these is rifampin which "pokes holes" in biofilms [39]. This drug has recently been shown to have been the key element in one of the greatest advances in medicine, namely the disappearance of leprosy [40]. With the addition of rifampin to the regimen, Dapsone was now able to penetrate the biofilms (which were in the skin and internal organs) and kill the mycobacteria inside. The incidence of leprosy worldwide plummeted from 12 million in 1985 to less than a million in 2015 [41]. However, the addition of rifampin to any regimen in AD would very likely make the disease worse because the spirochetes within the biofilm need to be killed. And, in AD, even if they are killed by penicillin, as M. leprae have been killed by Dapsone, the resulting debris (dispersed biofilm, spirochetes, Abeta, and more) in the brain is likely to overwhelm the microglia; and, they would likely be unable to clear the considerable detritus. The blood brain barrier helps keep things out of the brain, but it works both ways when it does not allow large amounts of debris to be removed from inside the brain [21].

Many other medications are biofilm dispersers, and these also are capable of worsening AD. They belong to several different categories of chemical compounds such as piperidines, pyrroles, thiophenes, and furans [42]. For discussion, haloperidol, a piperidine will be considered [43]. Use of this medication has been shown not only to cause worsening in AD, but also cause a 200% increase in mortality of AD patients given the drug [44]. As stated, haloperidol is a piperidine, and those compounds cause biofilm dispersion creating many new foci of biofilms and "exporter" cells capable of forming new biofilms. Each new focus of biofilm attracts TLR2 which creates TNFa and greater tissue destruction ensues. Consequently, if biofilms are "made" as in diabetes, [29] or "broken", as with haloperidol, the result is the same: more biofilms, more activation of TLR2 and more tissue destruction [Fig. 6.1].

Another chemical impacts AD unfavorably: Beta methyl amino alanine (BMAA) [45]. This substance has been shown epidemiologically to create many neurofibrillary tangle diseases multiple sclerosis, Parkinson's disease, and Alzheimer's disease and others) on Guam, and this has been further documented in primates (vervet monkeys) [45]. BMAA is a biofilm disperser, thus it behaves in the same way as the piperidines and furans and creates catastrophic worsening of the diseases [44,45].

AD has a signature pathologic finding of neurofibrillary tangles; and these tangles have been associated with *T. pallidum* in syphilis. The neurofibrillary tangles in AD have been shown to contain spirochetes [5].

Recently, diet soft drinks have been shown to triple the incidence of AD and stroke [46]. Phenylalanine, a constituent of aspartame (the major sweetener in those drinks) is a congener of BMAA. It has been shown both to be a biofilm disperser and a biofilm growth (size) limiter [47,48]. Thus, biofilms that arise in this setting would be more numerous because of the "dispersion" and, also, more numerous because fewer organisms would be required to fill the mature biofilm. This, consequently, would give many more targets for activation of TLR2 which then would directly lead to increased tissue destruction. The increase in arteriosclerotic stroke is related to the same mechanism: as stated previously, biofilms are present in the plaques of carotid artery endarterectomy specimens [49]. Disruption of these and similar plaques would be (and has been shown to be) disastrous [50,51].

5. INNATE IMMUNE SYSTEM

The presence of low amounts of vitamin K2 adversely affects AD: a low concentration of K2 upregulates TLR2 [52] leading to increased TNFa and greater tissue destruction [Fig. 6.1]. Adequate amounts of K2 lead to downregulation of TLR2 giving the opposite effect. Moreover, similar effects are noted with inadequate vitamin D3 [53]. Vitamin A and magnesium might also have similar effects via similar mechanisms [54]. All four of these compounds (vitamin K2, vitamin D3, magnesium, vitamin A) appear to work in tandem.

6. GENETIC FACTORS

The above are known factors that lead to worsening of AD. The disease itself appears to be a "double hit" phenomenon with the "environmental" hit being the microbes and their biofilms which has been the major thrust of this commentary. The "genetic" hit seems apparent from twin studies (80% concordance in monozygotic twins) [55,56] and from other treatises [57]. As with other "double hit" chronic diseases such as atopic dermatitis and psoriasis where filaggrin and PSORS2 appear to be the major genes involved [58], there are many more genes that have a role in AD. In atopic dermatitis, representative other genes include steroid sulfatase and transglutaminase-1 among others; and, in psoriasis, it is PSORS1,3,4 that are potentially involved [58].

In AD, the APOε4 gene appears to be the equivalent to filaggrin in atopic dermatitis, inasmuch as it is the most commonly found gene in the most common presentation of the disease [59]. The genes in early onset AD patients could be considered in the light of "making AD worse", and they are the gene for BAPP, and the gene at the AD3 locus [59]. The latter (AD3) is responsible for 70% of early onset (age 30-60) AD. Although aggressive, early onset AD represents only 5% of the total AD population [59].

7. NECESSITY OF EARLY TREATMENT

The final thing that makes AD worse is perhaps the most obvious of all: namely, the disease would not even exist if Lyme spirochetes were treated effectively at the earliest stage of the disease, and if the bacteremia surrounding dental procedures and other oral manipulations was treated effectively [60]. Consequently, in the paradigm (microbes creating biofilms which activate the innate immune system and cause tissue destruction), we have outlined things that cause worsening of AD at each step. One, lack of effective treatment directed (early) at the microbes is probably the most important. Two, "making" or "breaking" biofilms has been proven injurious. Three, substances that impact the immune system, such as vitamin K2, have been shown to have a profound effect. Moreover, it is the immune system that is responsible for considerable tissue damage leading to this dreaded disorder [60].

8. PUTATIVE PREVENTION AND EFFICIENT THERAPY IN AD

Things that make AD better are many fewer in number. Prevention is the first and most important factor in making AD better: prevent the spirochetes from reaching the brain, or prevent them from

making biofilms [21]. In syphilis, treatment with penicillin in the primary, secondary, early or late latent stages prevents tertiary syphilis; penicillin should be similarly effective in other spirochetal diseases. What is lacking is a similar serologic test (to the RPR) and the will to discard the primacy of the beta amyloid hypothesis that is now 25 years old. Recently, a microarray test tested 100% positive for early AD; perhaps this can be adjusted to find the disease before it begins [61]. Until that time, we are left with treating with penicillin for Lyme disease and for pre-dental exposures. As discussed in prior works, dental work introduces spirochetes and other microbes into the circulation leading to hematogenous and other modes of dissemination, such as via lymphatics. The patient is transiently bacteremic. Given the affinity of spirochetes for neurons, the brain will be affected. Consequently, the presence of a bactericidal antibiotic in the serum at the time of dental work would kill the microbes and prevent them from "taking up residence" in the brain [21]. Aggressive periodontal work (covered by penicillin) would lessen the spirochetal burden as well [62].

Compounds that inhibit the growth of biofilms help prevent AD. The current "best" candidate for this is L-serine [45]. It inhibits quorum sensing which is the main initiator of biofilms [63]. Microbes have genes for sensing population density and "spin out" biofilm whenever a critical density is reached [21]. Another quorum sensing inhibitor is caffeine [64]. Whether it is as effective as L-serine is debatable. Caffeine also reduces biofilm attachment which is necessary for a biofilm to be functional (in microbiologic terms). Caffeine was also shown recently to have an ameliorating impact on AD, and other chronic diseases [65]. Vitamin C, ascorbic acid, is another anti-attachment compound for biofilms [66]. Though it has not been evaluated for efficacy, vitamin C seems weaker than either L-serine or caffeine. Iron acts inversely to L- serine: low iron levels act as growth inhibitors of biofilms while high levels encourage the formation of biofilms and the subsequent immune activity [67]. Lowering serum iron seems a useful mechanism for decreasing the incidence of AD [68].

TLR2 has been shown to be inversely related to vitamin K2; low K2 results in larger amounts of TLR2 and vice versa [52]. Thus the addition of K2 to the diet should help attenuate the immune system activity generated in AD by TLR2. Vitamin D3, vitamin A, and magnesium have similar effects towards limiting tissue destruction by the immune system [53,54].

Lastly, limiting biofilm production would also limit Abeta deposition in the extracellular space because the microbes are responsible for the production of beta amyloid precursor protein as recently demonstrated [4]. The process is nearly self-contained in that the activity of TLR2 invoking the MyD88 pathway that generates NFkB actually catalyzes the formation of both beta and gamma secretase. Thus, if there are no organisms there would be no biofilms and, most probably, no Abeta accumulation.

9. END NOTE

Recent work of Miklossy showed Lyme spirochetes cultured from AD brains could be forced *in vitro* to make biofilms [4]. In so doing, the organisms not only made biofilms, but also made BAPP and Abeta. We have recently observed intracellular biofilms in AD brains and these showed Abeta as well [69,70]. The significance of this is intracellular Abeta leads to hyperphosphorylated tau protein and ultimately to dendritic disintegration [71]. Consequently, early treatment would help prevent not only Abeta, as been outlined, but also the development of hyperphosphorylated tau. Thus, both the major pathological findings, Abeta and tau, would be addressed.

10. CONCLUSION

The reasons why stroke, diabetes, haloperidol, and others cause worsening of AD are explored. They include "breaking" biofilms (haloperidol) or "making" biofilms (diabetes). They also include upregulating TLR2 (low Vitamin D3). The most important factor that worsens (or causes) AD is infection with spirochetes, Lyme or dental. Periodic treatment with penicillin or azithromycin, would likely prevent the entire cascade of events from spirochetes to biofilm, to TLR2, and inexorably to disease.

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Chapter 7
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Emphasizing the Impact of Biofilms in Alzheimer's Disease Compared to Other Diseases in Which They Play a Role

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ABSTRACT

Herein we present the findings related to biofilms in Alzheimer's disease and compare them to known findings related to biofilms in other chronic diseases. Similarities include microbes making the biofilms both intra and extracellularly, the interaction of the innate immune system in many instances, the devastating impact of the adaptive immune system, and the devastating impact resulting from the various genes involved. Differences include location, the production of beta amyloid, neurofibrillary tangles, and hyperphosphorylated tau protein. The diseases compared include atopic dermatitis, psoriasis, tinea versicolor, leprosy, gout, rheumatoid arthritis and other arthritides. It is obvious that Alzheimer's disease differs from the other chronic diseases in the production of Abeta, p-tau, and neurofibrillary tangles. It does not differ with regard to the adaptive immune system creating more destruction than the innate (cf stroke), and it does not differ in regard to the impact of a gene (cf AD 7) also creating destruction.

Keywords: Alzheimer's disease; biofilms; chronic diseases.

1. INTRODUCTION

We have recently shown that biofilms created by Lyme and dental spirochetes play an etiologic role in Alzheimer's disease [1]. In affected brains, spirochetes have been seen with bright field microscopy and identified by culture and/or polymerase chain reaction [2,3]. Spirochetes form plaque-like cortical masses or colonies [4,5,6]. In pure culture, Borrelia burgdorferi from the affected brains have been shown to make beta amyloid (Abeta) and beta amyloid precursor protein at the same time they create biofilms [7]. This has been demonstrated in vivo as well [8]. It is tempting to speculate that the implication of these results is that Aβ acts as a trigger for a degenerative process that continues even if it is removed [9,10]. The in vivo observation was made with biofilms found both intracellularly and extracellularly.

Further, it has been shown that biofilms and Abeta found intracellularly lead to neurofibrillary tangles because Abeta together with ordinary tau protein leads to the production of hyperphosphorylated tau (p-tau) [11]. The p-tau is unable to stabilize dendrites as does ordinary tau [12]. The affected dendrites subsequently degenerate, and this leads to neurofibrillary tangles and neuronal cell death [13]. It is important to note, however, that synaptic and dendritic spine pathology is common amongst several neurodegenerative diseases and may represent the same pathogenic mechanisms across them all [14,15]. The extracellular biofilms are present in the senile plaques which are made up of biofilms that are coated with Abeta [16]. These extracellular biofilms are made not only by spirochetes that are in the extracellular space, but also by those that are extruded when the neuronal dendrites disintegrate, and the intracellular organisms are now found extracellularly.

Consequently, the extracellular Abeta derives not only from that which was once intracellular, but also from the impact of the innate immune system molecule Toll-like receptor 2 (TLR2) that has been

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shown to be upregulated by biofilm [17]. Utilizing known pathways, TLR 2 leads to nuclear factor kappa B (NFkB) which catalyzes Abeta converting enzyme that, in turn, catalyzes beta and gamma secretase that convert beta amyloid precursor protein to Abeta [17]. Thus, the Abeta is made by the microbes when they make biofilms and by the reaction of the immune system to the biofilm.

The same pathological findings found in Alzheimer's disease are also present in syphilitic dementia, general paresis (GP) [18]. Thus, the two diseases, Alzheimer's disease and GP, are similar both clinically and pathologically. The only difference is different spirochetes in each disease. Presumably, Alzheimer's disease would respond similarly to the administration of penicillin as in GP if given before the onset of tertiary spirochetosis [19].

By contrast, the chronic skin disease tinea versicolor caused by Malassezia furfur/ovale is a biofilm disease that creates no symptoms, only color change and skin peeling [20]. This is because the biofilms form in the stratum corneum of the epidermis that is devoid of live cells, and consequently cannot generate an immune response. Tinea versicolor becomes a superb control as a biofilm that has no immune interaction.

Atopic dermatitis (eczema), on the other hand, is a chronic skin disease in which extracellular biofilms made by normal flora staphylococci form in the eccrine sweat glands, upregulate TLR 2, activate PAR 2 (a potent pruritogen), and create the typical rash [21]. Eczema has been termed "the itch that rashes." This disease is a double-hit phenomenon with the gene being filaggrin (or similar) and the environmental component being the staphylococcal biofilms occluding the sweat ducts [22]. Without the genetic component, the patients get "miliaria" from the sweat duct occlusion. All the various forms of eczema (flexural, facial-extensor, dyshidrotic, etc.) show the same pathological and microbiological findings [22]. Diseases thought to have an eczema component such as Doucas Kapetanakis disease and Meyerson's nevus also have similar pathology [22].

Certain skin diseases not thought to be eczema, such as seborrheic dermatitis, granular parakeratosis, and tinea pedis also have the same pathology and the same microbiology (occluded sweat ducts filled with staphylococcal created biofilms). In these disorders, the derangement of the stratum corneum from Malassezia yeasts in seborrheic dermatitis, and minute granules in granular parakeratosis, and fungal hyphae in tinea pedis causes similar changes in the stratum corneum as does the filaggrin gene that creates a faulty outer layer of the integument [23].

The innate immune response leading to the intense itching is followed by the adaptive response once the basement membrane is breached. This is similar to the situation in Alzheimer's disease when the blood brain barrier is breached as in stroke, and the adaptive immune system floods the brain [24]. This leads to dementia in 1-3 years as opposed to the 20-30 years with the ordinary progression of Alzheimer's disease [24].

Psoriasis differs from eczema in that the biofilms are made by Streptococci and are located in the tonsils and not the skin.19 The biofilms are found both intra and extracellularly. Further, both arms of the immune system (innate and adaptive) are involved [25,26]. Psoriasis is also a double-hit disease with the genetic factor being one of the PSORS genes (or others) and the environmental component the streptococcal-created biofilms [27]. Without the gene, patients can have an elevated circulating anti streptococcal IgG and no psoriatic lesions unlike their counterparts with plaque psoriasis (cf controls in El Rachkidy's monumental work).

An important comparison with psoriasis and Alzheimer's disease is the comparison of treatment with biologics such as adalimumab with the various molecules that attempted to limit the production of Abeta. The adalimumab inhibits tumor necrosis factor alpha (TNFa), a late appearing cytokine in the pathway generated by the immune system; this biologic is extraordinarily effective in eliminating the symptoms and signs of psoriasis. However, the skin can restore and regenerate itself whereas the molecules attempting to diminish Abeta, by acting similarly late in the cascade of events, are "doomed" to failure because the neurons cannot regenerate or be restored. Further, only the extracellular material could be addressed and no intracellular biofilms or Abeta could be targeted. This

is problematic because the intracellular biofilms and Abeta likely play the leading role in Alzheimer's disease [28].

Leprosy is a chronic cutaneous biofilm disease caused by Mycobacterium leprae; the biofilms are situated extracellularly in the lever, spleen, and kidneys and not in the skin until late in lepromatous leprosy. At this stage, very large histiocytes (globi) are present in the dermis and are filled with acid fast microbes and biofilms [29]. The immune system is activated and leads to much of the damage to much of the peripheral nerve system damage noted in this biblical disease [30].

Leprosy has nearly disappeared over the past 40 years (incidence of 15 million in 1975 vs 800,000 in 2015). This is largely due to the addition of rifampin, a biofilm disperser ("buster"), to the therapeutic regimen. It was added because the organisms were becoming resistant to Dapsone, and, consequenty, this was added simply as another antibiotic. This was a fortuitous choice because rifampin clearly offered more; but, only recently, has its true nature been revealed [31].

Similar treatment (penicillin/rifampin) for Alzheimer's disease would be ineffective because, as with the biologics mentioned previously, it is too late in the course of the disease to be helpful. However, preventive treatment periodically with penicillin has been suggested as a rational measure. Penicillin crosses the blood brain barrier, travels intracellularly, and is bactericidal to sensitive microbes [32]. All spirochetes are such microbes, and where the comparator disease (GP) has been eliminated by penicillin, it seems both rational and ethical to consider similar treatment. Whether rifampin and penicillin might have a useful impact in very early disease would require a clinical trial with the penicillin/rifampin compared to one of the anticholinesterase drugs currently in use.

The best known and studied biofilms are dental biofilms, and these generally have multiple organisms in the agglomerations. The main ones joining the dental spirochetes are Streptococcus mutans and porphyromonas [33]. The skin biofilms, in which there is definitive, microbiology show them to be monomorphous. It is likely that the biofilms in the Alzheimer's brains are more similar to the dental biofilms rather than those in the skin. Chlamydia pneumoniae, herpes simplex, and porphyromonas gingivalis have all been shown to be present in Alzheimer's brains along with the spirochetes [34,35,36].

It is likely that the spirochetes play a dominant role in these brains for many reasons. Among them are the pathology which is "helical" and not coccoid, rodlike, or viral [18]. Next, *C. pneumonia*e and HSV have never been shown to make biofilms and that is an important consideration because biofilms play an etiologic role in the disease. Third, *C. pneumonia*e and HSV are obligate intracellular microbes so they would not be able to form the extracellular biofilms that make up the senile plaques. Further, biofilms of one microbe have attachment sites for other organisms; this has been shown with 3-D confocal microscopy to be the case in Borrelial biofilms which encase *C. pneumonia* [37]. Further, with the pathology of GP and Alzheimer's disease being the same, and with the brains teeming with spirochetes, it is likely that GP is an exceedingly good prototype for Alzheimer's, and inasmuch as GP has been eradicated by penicillin, it is a very strong possibility that pre Alzheimer's disease would respond similarly. Whether the multi-organism biofilm renders the process even more difficult to treat is unknown.

As mentioned previously HSV has not been shown to make biofilms, even though it is very likely it does. Other viruses have; Molluscum contagiosum virus and oncogenic human papilloma virus (HPV) have been shown to make biofilms in their skin lesions [38,39]. These are intracellular and the biofilm is present in the epidermal cells. Because of their intracellular location, they do not activate either arm of the immune system; this is corroborated by the lack of symptoms in the lesions of either disease.

The HPV biofilms have been found in organ transplant patients of color in non-sun exposed skin. The lesions were pathologically determined to be squamous cell carcinoma in situ, and 6/9 of the lesions stained positive immunohistochemically for HPV 16,18 in the epidermal cells. No extracellular biofilm was noted in either disease. Consequently, in considering brain tissue, HSV is likely a co-conspirator at most in Alzheimer's disease because it is unable to create extracellular biofilms. In the skin, Molluscum virus and HPV are thought to "hijack" the epidermal cell's DNA to create biofilms [39].

Arthritis, in all its forms, has been shown to be a biofilm disease [40]. We recently have found biofilms in cutaneous gouty tophi and in rheumatoid arthritis nodules [41,42]. These biofilms were different in their nature because they had a more acidic biomass [41,42]. This milieu favors gram negative organisms, but the microbiology has not yet been done. The lesions clinically were asymptomatic which likely accounted for their not activating the innate immune system.

What we have attempted to show in this work is that Alzheimer's disease is similar to other chronic diseases relating to the impact of biofilms on disease progression. The innate immune system is upregulated in Alzheimer's disease and eczema; the adaptive immune system is activated in psoriasis and leprosy and in Alzheimer's disease after stroke. Intracellular biofilms have been seen in MC, psoriasis, squamous cell carcinoma in situ, and Alzheimer's disease. Extracellular biofilms have been seen in eczema, psoriasis, leprosy, tinea versicolor, rheumatoid arthritis nodules, gouty tophi and Alzheimer's disease. The diseases evoked by the different organisms are different even though the microbes all make biofilms. Much of the difference is related to location, the organs involved, and which arm of the immune system is activated. Another factor is the presence (or lack) of a gene, especially in a double-hit disease.

It is obvious that Alzheimer's disease differs from the other chronic diseases in the production of Abeta, p-tau, and neurofibrillary tangles. It does not differ with regard to the adaptive immune system creating more destruction than the innate (cf stroke), and it does not differ in regard to the impact of a gene (cf AD 7) also creating destruction.

2. CONCLUSION

AD differs from other chronic diseases by the production and impact of Aβ, p-tau, and neurofibrillary tangles. AD is similar to psoriasis as concerns both intra and extracellular biofilms. AD does not differ with regard to the adaptive immune system causing more tissue damage than the innate, nor does it differ in regard to a gene causing destruction in psoriatic arthritis (*TYK*2 and *TRAF*31*P*2) and AD (*AD*7).

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Chapter 8
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An Overview of Penicillin: The Old/New Wonder Drug

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ABSTRACT

Penicillin (PCN) has been shown to treat psoriasis effectively and be curative in many cases. Streptococcus is the organism responsible for beginning the process and has previously escaped detection by moving intracellularly or by forming biofilms. The treatment is low dose for many months and thus is similar to rheumatic fever. Arthritis has been shown to be caused by biofilm-forming dental and Lyme spirochetes, and these organisms, like the streptococcus in psoriasis, have escaped detection. Penicillin, plus a biofilm-dispersing agent is effective in treating arthritis in which tissue destruction has not already occurred. Alzheimer's disease has been shown to be caused by those same spirochetes involved in arthritis, and, is in every way, similar to the dementia of neurosyphilis caused by *Treponema pallidum*. These organisms make biofilms that induce B amyloid and a Toll-like receptor 2 response leading to tissue destruction. Penicillin given prior to the organisms' arrival in the brain (or before they create biofilms) would effectively prevent dementia in Alzheimer's as it does in syphilis.

We have shown that biofilm-forming staphylococci are integral to the etiology of atopic dermatitis. Along with standard corticosteroid therapy, antibacterial treatment, as opposed *to* antibiotics, appears to be a better treatment in AD because all the organisms are multi-drug resistant and 60% are MRSA or MSRE. Treatment with PCN in psoriasis, arthritis, and syphilis, has thus far not led to resistance and may actually prevent resistance by killing organisms before they make biofilms and share resistance genes. Due to its efficacy and affordability, much effort needs to be put into investigation on the therapeutic role of penicillin in psoriasis, arthritides (including rheumatoid arthritis and osteoarthritis), Lyme disease and Alzheimer's disease. The association of streptococci and spirochetes with the corresponding diseases like psoriasis, Lyme disease, arthritides, and Alzheimer's disease suggests treatment with penicillin can be just as miraculous as when it was first introduced.

Keywords: Penicillin; Streptococcus; biofilm; arthritides.

1. INTRODUCTION

From its very first use in the USA in 1942, where it completely reversed a downward spiraling case of streptococcal puerperal fever, penicillin has claimed status as a "miracle" drug. After treatment and convalescence, this most fortunate patient lived another 57 years and died in 1999, at the advanced age of 90. Although it was discovered in 1928 by Alexander Fleming, a microbiologist at St. Mary's Hospital in London, penicillin languished for more than a decade before its importance was noticed. As its antimicrobial properties became more apparent, large enough quantities were produced for clinical trials through the efforts of Florey et al. [1]. Then came World War II, and the U.S. government became intensely interested in penicillin because, in previous wars, soldiers were more likely to die from wound infections than from the wounds themselves. The government was anxious for anything that would reduce American casualties, and it made penicillin production a priority. More than 20 companies were encouraged to join the effort to produce sufficient quantities of penicillin for the military. Production ramped up so much that by the invasion of Normandy in June 1944, companies

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were producing 100 billion units of penicillin per month. Since then, the penicillin has been used in a wide spectrum of diseases caused by Beta hemolytic *Streptococcus pyogenes* (includingstreptococcal pharyngitis, rheumatic fever and scarlet fever amongst many others), *Diplococcus pneumoniae, Nisseria gonorrhea* and meningitidis, syphilis and gonorrhea [2]. The antibiotic, which contains a β-lactam group, is now known to wield its antibiotic power by preventing the formation of peptidoglycan cross-links in the bacterial cell wall. A number of semisynthetic penicillin derivatives improving on the properties of penicillin have been developed since penicillin was first commercialized. Ampicillin, patented by Beecham in 1961, improved the oral absorption of penicillin. Amoxicillin, also patented by Beecham in 1964, further improved oral absorption. These compounds and other penicillin derivatives share the b-lactam nucleus but have different side chains.

Penicillin's efficacy toward various microbes, its wide distribution in the human body, and its low systemic toxicity has given it a significant impact in the field of infectious disease. Although resistance to penicillin has emerged, it remains a very common antimicrobial treatment [2]. Penicillin, like other components of the beta-lactam antibiotics, contains a four-membered beta-lactam ring, which is responsible for the inhibition of transpeptidase [3,4]. In addition to its current uses, this article will explore other potential indications for penicillin, some of which have already been discussed more than 5 decades ago. Further, because of the recent recognition that microbes may form biofilms or internalize within cell cytoplasm, and thus not be available to either the immune system or antimicrobial antibiotics, the impact of antibiotics, as currently utilized, has been drastically diminished.

2. OLD APPLICATIONS

2.1 Syphilis

For centuries, syphilis stood as one of the most devastating diseases facing society. Sir William Osler's adage "he who knows syphilis, knows medicine" finds its veracity in the recognition that syphilis has the potential to cause pathology in most organ systems. Patients faced with the severe complications of tertiary syphilis were initially vainly treated with permutations of mercury and arsenicals. Prior to the advent of penicillin, it is estimated that the incidence of primary and secondary syphilis was 66.4 cases per 100,000 in the United States [5]. The introduction of penicillin in 1943 led to a rapid decline of 3.9 cases per 100,000 by 1956 [5]. The publication on the treatment of four syphilitic patients with penicillin by Mahoney et al was soon quickly followed by a case report of the successful resolution of tertiary cutaneous syphilis with penicillin [6]. While the earliest trials of penicillin often failed in hindsight due to incorrect dosing or impurities, the burgeoning success of penicillin in treating tertiary syphilis originally with 320,000 U of penicillin over an 8 day time period lent credence that the cure to syphilis would lay in the newfound drug [7]. Old treatments quickly died out and penicillin abruptly became the mainstay. After declining to a historic low in the year 2000, the number of syphilis cases in the United States has been increasing and now exceeds 55 000 new cases each year [8]. Penicillin has been the treatment of choice for more than half a century, but questions regarding the appropriate therapeutic regimen for various stages of syphilis still exist [9].

Even today, penicillin continues to be the gold standard of therapy for syphilis. The dosage and duration of treatment vary per stage, with oral penicillin often not achieving adequate blood levels. First line therapy for early syphilis remains benzathine penicillin. Probenecid has also been used to amplify drug levels in the serum and CSF [5]. Late latent stages of syphilis have been difficult to ascertain proper levels and dosing of penicillin with the current accepted recommendations being a three dose regimen [10]. Cardiovascular syphilis necessitates three doses of benzathine penicillin, 2.4 mU, at weekly intervals [11]. Neurosyphilis, as one of the most devastating sequelae to syphilis infection, relies strongest on the prevention of further damage and/or progression to tabes dorsalis and general paresis. While the damage caused cannot be reversed, a high resolution rate is found with crystalline penicillin G, 12-24 mU daily for 10 to 14 days or procaine penicillin 2.4 mU daily with probenecid, 500 mg p.o. four times a day, both for 10 to 14 days. Benzathine penicillin cannot achieve adequate levels within the CSF [5]. Tertiary syphilis has been virtually eliminated by treatment of earlier stages of syphilis; this has been immensely aided by a serologic test that is exceedingly

sensitive. However, the fact remains that treatment early in the course of this spirochetosis has prevented tertiary syphilis from occurring.

2.2 Streptococcal Infections

The most common use for penicillin in the present day is for the treatment of streptococcal infections. *Streptococcal pneumoniae* continues to pose a lethal disease course even in the post-vaccination era, presenting in the pediatric population particularly in the form of otitis media or in the general population as pneumonia and meningitis. Despite resistance rates, *S. pneumoniae* continues to have an adequate response to penicillin, particularly in the situations of pneumonia or meningitis. Guidelines continue to dictate that patients with or at risk for splenic dysfunction such as sickle cell anaemia are suggested to begin penicillin prophylaxis in children upon diagnosis or at least by two months of age [12]. The PROPS study determined dosing with sickle cell disease children younger than five recommended to take penicillin V potassium 125 mg twice daily, and children over five penicillin V potassium 250 mg twice daily [13]. Prophylaxis with penicillin may generally be discontinued upon five years of age unless the patient has suffered a previous severe pneumococcal infection or has functional asplenia [12].

Mild soft tissue, middle ear, and skin infections along with pharyngitis are the acute illnesses associated with group A streptococcal infection with delayed complications being scarlet fever, rheumatic fever, post-streptococcal glomerulonephritis, and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). While it is still uncertain whether penicillin may impact glomerulonephritis or PANDAS once pathology has already set in, treatment is critical for cessation of primary disease progression and prevention of rheumatic fever, which will be discussed later [14]. Scarlet fever is one of the most diagnostic cutaneous presentations of GAS infections with the distinctive papular erythematous "sandpaper"rash [15]. The cutaneous manifestation typically accompanies the pharyngeal infection with the rash emerging due to erythrogenic toxins produced by the bacterium [14]. Treatment remains a ten-day course of oral penicillin VK or erythromycin, or a single intramuscular injection of penicillin G benzathine. If administered within 1 week of onset of acute pharyngitis, acute renal failure may be prevented [16].

2.3 Rheumatic Fever

In a similar fashion, penicillin has equally revolutionized the treatment of rheumatic fever and the subsequent cardiac complications. Acute rheumatic fever occurs 2-3 weeks following infection of the pharynx by Group A streptococcus (GAS) [17]. Manifestations of acute rheumatic fever include arthritis, chorea, erythema marginatum and most importantly, carditis. Reinfection by GAS notably leads to valvular destruction and eventual heart failure [18]. While the reason exactly why infection by GAS causes rheumatic fever has yet to be determined, current hypotheses stipulate a relationship between the M protein of the bacterium, biofilm formation, and molecular mimicry between antibodies against bacterial proteins and cardiac membranes. Work by Catanzaro et al. noted that the development of rheumatic fever required living streptococci throughout the convalescent period, making penicillin treatment and prophylaxis essential [19]. Treatment of acute rheumatic fever involves penicillin in alternate roles.

Prevention of acute rheumatic fever is essential and relies on quick diagnosis and treatment of streptococcus infection with penicillin or penicillin derivatives. The advent of penicillin and rapid antibiotic treatment of streptococcus has greatly contributed to the decline of rheumatic fever in the developed world. For patients with acute rheumatic fever, therapy relies on secondary prevention. Benzathine penicillin G administered intramuscularly over 4 weeks is the preferred choice. Continued administration of penicillin for prolonged periods of time, depending on age of infection, is warranted. Generally a minimum of five to ten years of prophylaxis is recommended, significant valvular damage necessitates lifelong prophylaxis [20]. Penicillin is ideally provided intramuscularly as oral prophylaxis falls prey to patient adherence and even with optimal adherence has a higher risk of recurrence [21]. As in syphilis, early administration of penicillin has led to near disappearance of rheumatic fever (except for occasional outbreaks) [22].

2.4 New Applications

While penicillin has made a significant impact on the above diseases, multiple studies have begun to shed light on the potential use of penicillin in other diseases. These include psoriasis, Lyme disease, multiple arthritides, and Alzheimer's disease, to name a few. Except for psoriasis, penicillin for these diseases is largely theoretical and conceptual, but the considerations for its usage are cogent. These considerations take into account the presence and impact of biofilm formation by the various organisms and the effect on the immune system (both innate and adaptive) that is generated.

2.5 Psoriasis

There are many lines of evidence leading to streptococcus as the antigen in psoriasis. The first is guttate psoriasis which has been shown to follow streptococcal pharyngitis. In plaque psoriasis, the "streptococcus as antigen" story is not as clear, and the reason is the organism can neither be cultured, nor does it generate any serologic evidence of its presence. This is due to internalization of the of the streptococcus into (tonsillar) cell cytoplasm and/or the production of biofilms [23]. Both of these phenomena lead to negative cultures and negative serologies. There is recent immunologic evidence of streptococcus and plaque psoriasis: a streptococcal extract activates T-cells; and, further, there is a markedly elevated streptococcal specific IgG in the serum of plaque psoriasis patients represents humoral immunity [24]. Thus both arms of the adaptive immune system have been shown to be involved. The innate immune system has recently been shown to be involved also. Toll-like receptor 2 (TLR 2) has been found to be activated on the blood monocytes in psoriatic arthritis [25] and serum TLR 2 has been found in the upper dermal capillaries [26].

There is epidemiologic evidence as well: if there is no streptococcus in the environment, there is no psoriasis. Northernmost Europe and certain Pacific islands (including Australia among others) have demonstrated this [27]. If streptococcus is the antigen, then penicillin and other anti-streptococcal antibiotics should be beneficial. There is solid evidence for this, first from case reports and small series [28]; and second from 2 larger series, the first employing IM benzathine penicillin in which the results were spectacular (PASI 90-near total clearing) [29]. The second utilized oral azithromycin (with pulse dosing) again with remarkable improvement (PASI 75- marked clearing) [30]. These studies were conducted with the treatment rendered over a long period of time, which appears to be very important with this type of therapy, just as in rheumatic fever. The lengthy administration is likely necessary due to the aforementioned internalization of the organism or the presence of the biofilms. The penicillin would be present and bactericidal when the organism externalized or emerged from the biofilm. Moreover, the serum antibody needs to decay; this very likely also contributes to the prolongation of the treatment.

The administration of the penicillin may be similar to that of Saxena, with IM bicillin, or may be similar to rheumatic fever with 250 mg oral penicillin daily. The oral dose may be adjusted as well; and instead of a "pulse" of azithromycin 500 mg daily followed by 10 days off, administration of 500 mg on each weekend day seems more practicable and may lead to better compliance. If the patients are not cured, as a percentage was not, a biofilm disperser could be considered for co-administration. In that regard, psoriasis straddles the old and the new penicillin where it is effective alone in the "old" cures; and, where in the "new" cures, it requires an additional agent to "break through" biofilms to kill the streptococci hidden within (Table 8.1). These agents, considered from a dermatological point of view, work either topically or systemically. An example of the topical use would be silver sulfadiazine which is widely used in burns. The systemic use of various agents, especially rifampin, is postulated for co-administration with penicillin (and/or other antibiotics). One agent that is not listed in Table 8.1 that has emerged as an important biofilm disperser is L-serine. L-serine has a role in the prevention and treatment of Alzheimer's disease, as will be mentioned below. The next diseases to be discussed all require such co-administration.

Surgery is another way to remove the streptococcus and tonsillectomy has been shown to have a beneficial effect [31,32]. There are other foci other than the tonsils where streptococcus can be found; there have been reports on perianal streptococcus and guttate psoriasis. However, cutaneous streptococcus has been more aligned with glomerulonephritis than to psoriasis [33].

Table 8.1. Biofilm dispersers and inhibitors

Topical	Systemic
Gold [34]	Niacinamide [35]
Silver [36,37,38,39,40,41]	Furans/Furan Precursors: [42,43,44,45]
Platinum [46]	- Nitrofurantoin
Selenium [47,48,49]	- Citalopram
Cinnamates [50,51,52]	- Pregabalin
Tannic acid [53,54]	Hydroxychloroquine [55,56]
Curcumin [57]	Rifampin [58,59]
Honey [60]	Ascorbic Acid [61,62]
Hyaluronidase [63]	Quinolones [64,65]
L-tryptophan [66]	Piperidines [67] (donepezil, haloperidol))
Flavanoids [68]	Pyrroles [69] (resperidine, celecoxib)
Cysteine [70]	Thiophenes [71] (olanzapine)

2.6 Lyme Disease

Where Lyme spirochetes (Borrelia) have been found in the brains of Alzheimer's disease, this makes Lyme disease similar to syphilis caused by the spirochete T. pallidum. In fact, it appears to follow a similar course with primary (Erythema migrans), secondary (generalized systemic symptoms), and tertiary (brain, heart, joints) stages [72,73]. Further, the pathology of syphilis has recently been shown to be the same as Alzheimer's disease.70 We have shown the plaques in Alzheimer's disease represent biofilms71. These most assuredly are made by the organisms; in the instance of General Paresis, they would be made by T. pallidum, and in the case of AD, they would be caused by dental spirochetes (75%) and Lyme spirochetes (25%) [74]. All these organisms are sensitive to penicillin in their planktonic state; thus it seems most reasonable to treat them just like syphilis (before they arrive in the brain or before they do damage) [75].

In addition, syphilis that is untreated progresses to tertiary in only 35% of patients; it seems no coincidence that Lyme disease treated with doxycycline shows tertiary findings in 35% of the patients [76,77,78]. It is almost as if treating the disease with doxycycline may lead to resolution of erythema migrans, but otherwise is like no treatment at all.

2.7 Arthritides

Dental and Borrelia spirochetes have been associated with arthritis [79,80]. In turn, these organisms have both been shown to cause biofilms that lead to arthritis. In a recent work regarding arthritis, seemingly sterile joints were found to contain biofilm [81]. The microbes are relatively slow in causing symptoms and joint destruction and often take many years. Frequently, the disease they create is termed "wear and tear arthritis." [81]. In light of the other spirochetal biofilms and the destruction they are associated with, it is probable that the innate immune system is involved [82]. Where these organisms are generating biofilms, they are unrecoverable except by PCR. With rheumatoid arthritis, the destruction is much more severe and much more rapid [83]. This is likely due to the adaptive immune system being activated [84].

With this as background, a small pilot study was undertaken to see if penicillin and a biofilm disperser would ameliorate the arthritis. 7/10 patients taking this protocol showed relief of symptoms; the 3/10, who did not have symptom relief, needed joint replacement within 3 months [85]. These preliminary results indicate that penicillin given with a biofilm disperser is most beneficial for osteoarthritis. For rheumatoid arthritis, where the adaptive immune system is at work and it carries all the destructive capacity of immunoglobulins, complement, alternate pathway, and T cells, it seems that early intervention with penicillin and a biofilm-dispersing agent would be most prudent. If the subsequent destruction were to be prevented by this approach, then penicillin would once again be included in the 'wonder drug" category.

2.8 General Paresis of the Insane and Alzheimer's Disease

General paresis of the insane (GPI) (tertiary syphilis) was the most common type of dementia through the first half of the twentieth century [86]. It was first thought that GPI was caused by chronic inflammation in the arachnoid lining of the brain until Esmarch and Jessen in 1857 raised the hypothesis of causal relationship between syphilis and GPI. Once T. pallidum was discovered and once the efficacy of penicillin became evident, any treatment with penicillin prior to tertiary syphilis was curative. However, GPI patients treated with penicillin were not able to gain memories that were already lost. With this as background, it was proposed that early treatment and prevention with penicillin would be necessary to prevent the progression of the disease certainly prior to the cognitive and behavioral signs of GPI [86]. The efficacy of penicillin in treating GPI shaped the approach to controlling Alzheimer's disease, which was only recognized in 1960s to be the most frequent dementia, previously known as senile dementia, seen in elderly individuals [86]. Further reviews and studies confirmed that chronic infection with spirochetal infections can lead to dementia and produce the clinical and pathological hallmarks of AD with oral treponemes comprising 75% and Lyme treponemes 25% [74,87]. There have not been any studies on the treatment and control of AD using penicillin. However, in a recent historical review of the pathology of neurosyphilis, it was shown to be completely the same as Alzheimer's disease with plaques and tangles and severe neuronal loss [74].

Given the same pathology in Alzheimer's disease as syphilis, and given similar spirochetal organisms sensitive to penicillin, treatment with penicillin before those spirochetes travel to the brain or before they create damage, would conceivably be curative just as it is in syphilis [75]. Succinctly, AD appears to be similar to syphilis except it is caused by a different spirochete. That similarity also extends to the primary, secondary, latent, and tertiary staging so familiar in syphilis. As in syphilis, treatment prior to tertiary is exceedingly important. Where it recently has been shown that the plaques in AD are formed by biofilms, the co-administration of a biofilm-dispersing agent along with the penicillin would be unlikely to reverse pathological changes, but may be able to help prevent further progression of the disease.

The importance of biofilms and biofilm-dispersing agents in the prevention of Alzheimer's disease, particularly L-serine, is eloquently illustrated in the observations of Dr. Paul Alan Cox and colleagues. As an ethnobiologist, Dr. Cox was particularly fascinated with the markedly increased incidence of Lou Gehrig's, Parkinson's, and Alzheimer's diseases among the Chamorro people of Guam [88]. This was attributed to a unique diet high in beta-Methylamino-L- alanine (BMAA), a neurotoxic substance [88]. Meanwhile, distant Ogimi villagers on the Japanese island of Okinawa who ate a diet of tofu and seaweed that contained large quantities of L-serine did not have these diseases, nor did they have high rates of arthritis [86]. Dr. Cox's observations further corroborate the mechanism of action that we propose for the pathology and prevention of Alzheimer's disease: that a biofilm inducer, in this case BMAA activates the immune system and thereby causes tissue damage and disease; while a biofilm inhibitor via quorum-sensing inhibition, in this case L-serine does not induce an immune system activation, preventing tissue damage and disease.

In the diseases above, (psoriasis, Lyme disease, various arthritides, and Alzheimer's disease), penicillin promises to be very effective, especially when co-administered with a biofilm "buster". All the organisms are capable of making biofilms and do so mostly through the "quorum sensing" mechanism they contain. The arthritides, linked epidemiologically to dental spirochetes, are the only diseases in the discussion where the penicillin sensitive microbes have not been identified in the tissue.

3. CONCLUSION

Few innovations have made the tremendous impact on the medical field as penicillin. Diseases today considered to be innocuous and quickly treated were once a death sentence for millions. Penicillin is well known throughout the medical community as the therapy for streptococcal infection, syphilis, and acute rheumatic fever. Due to its efficacy and affordability, much effort needs to be put into investigation on the therapeutic role of penicillin in psoriasis, arthritides (including rheumatoid arthritis and osteoarthritis), Lyme disease and Alzheimer's disease. The association of streptococci and

spirochetes with the corresponding diseases like psoriasis, Lyme disease, arthritides, and Alzheimer's disease suggests treatment with penicillin can be just as miraculous as when it was first introduced.

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Chapter 9
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Discussion on the Bioethical Challenges Arising from the Microbiology and Pathology of Alzheimer's Disease

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ABSTRACT

We have presented evidence based on our work and the work of others that Alzheimer's disease is caused by spirochetes that make biofilms both inside and outside of neurons. The extracellular biofilms have been shown to cause upregulation of the innate immune system molecule Toll-like receptor 2 (TLR2). The TLR2, by known pathways, eventuates in nuclear factor kappa B (NFκB) and tumor necrosis factor alpha (TNFα) and these molecules lead to beta amyloid and tissue destruction respectively. This well-documented concept of microbial pathogenicity has been largely disregarded in favor of the beta amyloid hypothesis which has been in place for the past twenty-five years. These factors comprise the first ethical challenge. The second challenge is treatment and research efforts are being utilized at, or near, the end of the pathogenic cascade and not at the beginning of the process at which time the spirochetes are easily treatable. Last is the markedly expensive effort to develop new therapeutic agents (none of which has been curative) which are not and have not been aimed at the true pathogen. All these together could lead to a large ethical challenge in the new millennium. Given all the above and considering that millions of patients have been/and are involved, ignoring the likely microbial pathogenesis in AD could possibly become one of history's greatest ethical calamities.

Keywords: Alzheimer's disease; spirochetes; biofilms; upregulation.

1. INTRODUCTION

We have recently written about how the ethics in Lyme disease and psoriasis are challenged [1,2]. This paper will focus on the bioethics of Alzheimer's disease in terms of the microbial pathogen hypothesis. Since Lyme spirochetes (*Borrelia burgdorferi*) were cultured by Macdonald in 1986 and again in 1988 from Alzheimer's disease brains [3,4] and, more recently, by Miklossy [5] similar bioethical inferences can be drawn from the seemingly disparate diseases. In Lyme disease, the continued presence of the spirochete has been disregarded. Yet, when the Lyme spirochetes can be cultured from affected brains (tertiary Lyme disease), the denial of their presence seems spurious. Further as concerns psoriasis, the evidence for group A streptococcus as an etiology in psoriasis is not miniscule but has been considered as such [2]. The same may be said for Alzheimer's disease where the prevailing hypothesis for the past 25 years has been centered on β amyloid, while the microbial pathogenesis has received little support [6]. Additionally, Lyme organisms make up 25% while dental spirochetes make up 75% of the microbes in Alzheimer's cases [7]. Kennedy argues that a "compassionate suspension of judgment" when diagnoses are difficult can only serve to further research, respect patients, and recognize that those findings that are not immediately understood will not be simply disregarded [8,9].

We have also reported on how the organisms make biofilms and upregulate the innate immune system molecule Toll-like receptor 2 (TLR2) [10]. Nearly all bacteria make biofilms, so Borrelia-

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derived biofilms would not be unusual. Bacterial biofilms, even those biofilms associated with gram negative organisms such as spirochetes, have external receptor sites for TLR2, which ordinarily responds to gram positive organisms [11]. The TLR2 that has been identified by immunopathological staining has been observed throughout the sections examined and did not appear to be localized to microglia or to the amyloid plaques [10]. TLR2, by known pathways, generates NFκB and TNFα in an effort to kill the offending pathogens. However, the biofilm protects the organisms and the TLR2 is unable to penetrate and kill the microbes; this process is largely responsible for the destruction of the cerebral neurocircuitry because the neural tissue is "in killed in the line of fire" as an innocent bystander [12]. Moreover, NFκB, through known pathways, generates β amyloid from β amyloid precursor protein (AβPP) by catalyzing β amyloid converting enzyme [13]. This precursor (AβPP) has definitively been shown to be made by the microbes [6]. The biofilms also have receptor sites for other organisms [14]; it has been compared to a "hotel" rather than a "single family home." This may be a possible explanation for multiple organisms (such as *C. pneumoniae* and *herpes simplex*) being found in analysis of AD brains [15,16].

Tau protein ordinarily stabilizes neuronal dendrites; however, when it is hyperphosphorylated, it loses its functionality and allows for disintegration of those dendrites into neurofibrillary tangles. The pathologic finding that these tangles contain spirochetes [17], and the recent pathologic finding of intracellular biofilms may bring the discussion of microbial pathogenesis into sharper focus [17]. The spirochetes have been noted to be widely distributed in many areas including, among others the plaques where there is a co-aggregation of biofilm and Aβ. [10,17]. In a forthcoming treatise, we will show Aβ in an intracellular location, corresponding to the biofilms already observed there [17]. With Miklossy's findings that the cultured spirochetes made Aβ along with the biofilms, and with and did not appear to be localized to microglia or to the amyloid plaques [10]. TLR2, by known pathways, generates NFκB and TNFα in an effort to kill the offending pathogens. However, the biofilm protects the organisms and the TLR2 is unable to penetrate and kill the microbes; this process is largely responsible for the destruction of the cerebral neurocircuitry because the neural tissue is "in killed in the line of fire" as an innocent bystander [12]. Moreover, NFκB, through known pathways, generates β amyloid from β amyloid precursor protein (AβPP) by catalyzing β amyloid converting enzyme [13]. This precursor (AβPP) has definitively been shown to be made by the microbes [6]. The biofilms also have receptor sites for other organisms [14]; it has been compared to a "hotel" rather than a "single family home." This may be a possible explanation for multiple organisms (such as *C. pneumoniae* and *herpes simplex*) being found in analysis of AD brains [15,16]. Tau protein ordinarily stabilizes neuronal dendrites; however, when it is hyperphosphorylated, it loses its functionality and allows for disintegration of those dendrites into neurofibrillary tangles. The pathologic finding that these tangles contain spirochetes [17], and the recent pathologic finding of intracellular biofilms may bring the discussion of microbial pathogenesis into sharper focus [17]. The spirochetes have been noted to be widely distributed in many areas including, among others the plaques where there is a co-aggregation of biofilm and Aβ. [10,17]. In a forthcoming treatise, we will show Aβ in an intracellular location, corresponding to the biofilms already observed there [17]. With Miklossy's findings that the cultured spirochetes made Aβ along with the biofilms, and with the findings that Aβ contributes to hyperphosphorylation of Tau which ultimately leads to disintegration of the dendrites, the factors influencing tangles appear to be in place [18,19,20].

Seemingly, all the essential elements in AD (beta amyloid, AβPP, plaques, tangles, Tau protein, and neurodestruction) can be explained by the following: spirochetes enter the brain from the circulation or by other pathways, and these spirochetes form biofilms both intra and extracellulary. During the formation of the biofilms, Aβ is formed intracellularly and this induces hyperphosphorylation of Tau and leads to the formation of tangles and neuronal disintegration. The biofilms in the extracellular space upregulate the innate immune system (TLR2); by known pathways, this leads to production of extracellular Aβ. The surrounding tissue is subsequently destroyed. There are thus two ways for the Aβ to be formed: one by the microbes directly during the formation of biofilms, and the other by the action of the innate immune system.

Alzheimer's disease has been compared to general paresis of the insane (tertiary syphilis), and the neuropathology has been found to be exactly the same in both diseases [17]. Tertiary syphilis has been eradicated by treatment in the early stages of syphilis [21]. One would expect similar results with

treatment of Alzheimer's disease rendered before the organisms arrive at the brain or before they do damage. The spirochetes are all sensitive to penicillin (no resistance has been noted to date); and, if this antibiotic is given before dental procedures and for early Lyme disease, similar results as in syphilis should be achievable [7]. Why not treat early in the cycle rather than later? Treating early in the cycle would also include aggressive treatment for periodontal disease [22]. It seems apparent that spirochetes have the leading role in the production of Alzheimer's disease; why not kill them?

In the comparison of bioethics of Lyme disease, psoriasis, and AD, different parameters are invoked. In Lyme disease, the current treatment at all stages of the disease is ineffective. In psoriasis, the current treatments are effective but are not aimed at events early in the pathogenesis of the disease. Consequently, they must be continued perpetually. In AD, there are no effective treatments, but the current clinical trials and research (based mostly on combatting beta amyloid) are all aimed at events occurring late in the pathogenic cascade. This has cost the pharmaceutical industry and society billions without a single effective agent being developed. This raises the ethical challenge of ignoring the microbial "pathogen" theory of this disease.

By overlooking or disregarding the evidence that has been marshalled over the past three decades, an opportunity to prevent Alzheimer's disease has been lost. In syphilis which serves as a prototype, one can administer the effective antibiotics in the primary, secondary, and latent stages of the disease and predictably achieve the expected response (cure/prevention). This spirochetal disease (syphilis) responds to penicillin at all stages except for tertiary. All that is necessary for consideration of this treatment in Alzheimer's disease, and, indeed this concept, is epistemic humility [23]. This is very similar to the situation in psoriasis where Evidence Based Medicine (EBM) carries a "stipulative definition" because there is no reason to justify limiting the evidence for use of antibiotics for psoriasis while giving much more credence to "mainstream research" that focuses on symptomatic therapeutic options and not on causative therapies [2]. "Stipulative" in Alzheimer's disease would then refer to the evidence for microbial pathogenicity being "stipulated" as non-contributory to the disease. The word 'evidence' reveals its ambiguous nature in the context of research and the precarious position for patients when that "evidence" is used to justify this approach to clinical practice [24,25].

While recognizing costs to the pharmaceutical industry and society, the other ethical challenge is the continued research aimed at limiting or preventing beta amyloid accumulation with treatment without considering alternatives. The costs have been staggering to the healthcare system with the cost to bring one of these new drugs to market of 2.6 billion (or more) dollars [26]. Continuation of this (? misdirected?) research results in a sort of "rational inconsistency" which overlooks the problems because of the single focus on β amyloid [27]. Treatment in the other diseases in which biologics are employed may be shown (in the future) to have similar ethical challenges. This would be true if those other diseases also had microbial pathogens as their source. Alzheimer's disease is one such disease where monoclonal antibody trials are being undertaken; where the source likely is a microbe [27]. Limiting the body's reaction to that microbe, without treating the offending agent, not only seems unethical but senseless [23].

Given all the above and considering that millions of patients have been/and are involved, ignoring the likely microbial pathogenesis in AD could possibly become one of history's greatest ethical calamities. The original observation of Borrelia in the brains of AD patients by Macdonald [3] might be discounted, but the PCR observations of spirochetes by Riviere [28] subsequently confirmed by Miklossy [7] cannot be ignored. This could dwarf the ethical situation in Tuskegee and Oslo (both of which involved untreated syphilis) with numbers of patients, the impact on families, and the colossal expenditures made in pursuing a faulty theory [29,30].

2. CONCLUSION

Like atopic dermatitis and psoriasis, AD is a chronic infectious disease in which microbes (spirochetes) make biofilms that are responsible for the disease. Also, like atopic dermatitis and psoriasis, the bioethics centered around AD are deeply flawed. The chronicity of the infection is engendered by the biofilms because, in that state, the microbes elude detection. For 25 years, the amyloid hypothesis has been dominant as the cause of the disease, and all efforts at therapy have

been aimed at that molecule, and p-tau, have failed. We have shown that, by disregarding the evidence of a microbial pathogen, the opportunity to prevent the disease has been lost. The cost in human suffering and monetary pursuit of worthless therapies has resulted in "rational inconsistency." Further, the consideration of organisms that do not make biofilms is similarly challenged.

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Chapter 10
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Focusing on the Future Efforts to Thwart Memory Loss in Alzheimer's Disease

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ABSTRACT

During the past few years, Alzheimer's disease (AD) has been shown to be a chronic infection. Spirochetes, Lyme Borrelia and dental treponemes, are the etiologic agents; these spirochetes make biofilms, and, in so doing, are responsible for the formation of beta amyloid and hyperphosphorylated tau protein. The pathways for all these components of AD has been outlined. The following are possible future efforts based on the above information. These include attempting to culture the dental spirochetes as well as attempting to develop a serologic test for pre-AD. A trial for early AD is proposed, as is a possible way of preventing AD with periodic courses of effective antibiotics. This regimen would likely need to be continued for many months. A trial prior to the onset of the disease, would hardly be ethical, because the control arm would get AD if the microbes were unopposed.

Keywords: Alzheimer's disease; chronic infection; spirochetes; antibiotics.

ABBREVIATIONS

AD : Alzheimer's Disease;
PCR : Polymerase Chain Reaction;
GP : General Paresis;
ABPP : Abeta Precursor Protein

1. INTRODUCTION

It has been shown recently that Alzheimer's disease (AD) is a chronic infectious disease with the causative microbe being a spirochete. Lyme (Borrelial) spirochetes have been cultured from affected brains, and, they and dental spirochetes (various treponemata) have been found by polymerase chain reaction (PCR) [1-3]. Further, the infection has been shown to satisfy Koch- Hill postulates relating to, and firmly establishing, its infectious nature [2]. The spirochetes have been visualized pathologically and have been shown to create pathological changes similar, in every way, to those seen in another spirochetal disease, syphilitic dementia, which is termed general paresis (GP) [4]. Further, no coccobacillary forms and no viral changes have been noted in the pathology specimens examined. The pathology of AD is strictly "helical". Current pharmacological choices available to clinicians treating AD include cognitive enhancers for the treatment of the cognitive deficit [5] and mood stabilizers, antipsychotics, antidepressants, and hypnotics for the treatment of behavioral disturbance [6,7].

The spirochetes form biofilms like most other microbes; and, in large measure, the biofilms contribute to the chronicity of the disease [8]. Once in a biofilm, the microbes become resistant to antibiotics and to both arms of the immune system, innate and adaptive. In the extracellular space, the biofilms attract Toll-like receptor 2 (they have receptor sites for this molecule), and, by known pathways (MyD88 and NFkB), this interaction leads to the production of beta amyloid (Abeta) [9,10]. The Abeta,

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which is also anti-microbial, cannot penetrate the biofilm, just like antibiotics, and it has been shown that it encases the biofilm without engaging the spirochetes within [10]. The spirochetes also make biofilms intracellularly, and somewhat surprisingly, make Abeta precursor protein (ABPP) and Abeta simultaneously [11,12]. This process has been demonstrated both in vitro and in vivo. It has also been discovered recently that Abeta in contact with tau protein causes the tau protein to become phosphorylated, and it no longer functions as a dendrite stabilizer leading to dendrite and cell collapse [13,14]. The neurofibrillary tangles in the different diseases have some distinctive morphological features and may exhibit a distinct composition of tau isoforms that differs from AD [15,16]. With that, the neuron is no longer functional. With this as background, the contemplated future actions related to this hypothesis, are at least five in number:

a. Popularize the concept of a bacterial origin for AD.
b. Culture the dental spirochetes.
c. Develop a serologic test that would identify AD before it is clinically apparent similar to the RPR in GP (tertiary syphilis).
d. Treat with penicillin or another bactericidal antibiotic for three weeks once yearly.
e. Initiate a clinical trial in mild cognitive impairment (MCI) to discern whether the disease progression can be stopped or slowed. Each of these 5 will be discussed further in the following.

For the past twenty-five years, the beta amyloid causation of AD has been predominant. This is understandable because this molecule is abundant in affected brains. However, as it has been shown, the microbes make Abeta while they are in the process of making biofilms [12]; further, the response of the innate immune system to the biofilms also creates Abeta. Thus, its creation is largely dependent on the microbes making biofilms. P-tau has recently been implicated in causing AD. As has been previously outlined, this is also directly related to the microbes making biofilms. The Abeta, which is produced at the same time as the biofilms, interacts with tau protein and converts it to p-tau [13,14]. Thus, p-tau is also largely dependent on the microbes making biofilms. When Lister made his monumental discovery about microbes causing the incredible suppuration rates after surgery and a way to prevent it, it took 20 years before the concept became widely known and practiced. His method for disseminating the information was by teaching students (he taught hundreds) and by writing about it. Fortunately, the British Medical Journal was receptive to his findings and became the beacon for the discovery [17]. Once the current concept gains traction, there are many more ways of disseminating information than were available to Lister. AD is such a catastrophe in human and financial terms that all the modalities for transmission of microbial nature of this dreaded disease should be employed. Culturing the oral spirochetes would be a useful undertaking. Treponema, in general and *T. pallidum* (syphilis) in particular, have evaded being cultivated. However, very recently, *T. pallidum* has apparently been cultured [18].

With similar techniques, it may be possible to culture the many dental spirochetes. *T. pallidum* takes considerable time to divide (as much as 3 months) and this may be one of the difficulties in getting it into culture. The serologic test for syphilis, together with penicillin treatment for positive tests, has relegated tertiary syphilis, including GP, to history. That disease has been eradicated. Currently, there is a microarray test for MCI, or early AD [19]. Perhaps this test can be modified to include pre-AD; such an alteration would lead to treatment before the disease begins, which is crucial. Treatment after the disease begins is fraught with difficulty, as is documented by 200 failed clinical trials in disease that has already begun. This fits with the pathology showing nearly all the neurons in the AD patients filled with biofilms. Until a serologic test is available, it is reasonable to consider treating with yearly penicillin (ex. Amoxicillin 500mg tid x 3 weeks) which would very likely prevent the disease. Penicillin crosses the blood brain barrier and the neuronal cell membrane and is bactericidal to sensitive microbes. All spirochetes are sensitive to penicillin. Consequently, a yearly course of penicillin would most likely kill the spirochetes prior to their making biofilms. This correlates with its treatment in GP; if penicillin is given anytime prior to the onset of dementia, this disease would be prevented, just as GP has been prevented. Again, where GP and AD have the same pathology, one would expect these results with similar treatment.

The reason for yearly treatment is the constant seeding of dental microbes, as opposed to one time exposure to *T. pallidum* in syphilis. As to resistance, it is likely that less resistance would be

forthcoming with this regimen because microbes inside biofilms trade resistance genes horizontally which increases resistance. It is apparent that spirochetes by themselves (planktonic) do not cause the disease; it is when they form communities that they assume pathogenicity. Alternative therapies include Penicillin VK 500mg qid, or for penicillin allergic, Azithromycin 500mg bid x 1 week then 500mg per day x 2 weeks. For penicillin regimens, adding probenecid 500mg tid doubles the serum concentration because it decreases renal excretion. When, and if, benzathine penicillin becomes more available, it is a better option because it, plus or minus probenecid, does not require patient compliance with the treatment regimen. It is unclear whether any treatment will stall or prevent progression of the disease. This is a place where a clinical trial would potentially be helpful. The arms of the trial would be any current regimen, such as memantine vs amoxicillin/azithromycin plus rifampin 300mg daily. The active arm of the trial includes both the antibiotic and a known biofilm disperser (rifampin) [20]. This regimen would likely need to be continued for many months. A trial prior to the onset of the disease, would hardly be ethical, because the control arm would get AD if the microbes were unopposed.

2. CONCLUSION

In the future, to help prevent AD, it will be useful to be able to culture dental spirochetes as has been done with Lyme spirochetes. It would also be useful to develop a blood test for pre-AD, similar to the RPR in syphilis. To prevent the disease, periodic administration (yearly) of effective antibiotics should be considered, especially for those with a genetic proclivity for the disease, and for those who have sustained traumatic brain injury. Such antibiotics would be penicillin or azithromycin. A clinical trial in patients with mild cognitive impairment with either of these antibiotics, together with a biofilm disperser for perhaps 3 months or more, would be most useful. Last, and probably most important, popularizing the microbial hypothesis for this disease, seems paramount.

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He is an MD graduate of Johns Hopkins. Internship at Johns Hopkins was followed by residency in dermatology and dermatopathology at the Naval Hospital in Philadelphia. He is board certified in dermatology and dermatopathology. Next, he did partnership practice in dermatology in NJ for 25 years during which time he was a volunteer attending at U Pennsylvania and Hahnemann. He has been Professor and Chair (emeritus recently) of dermatology at Drexel University. In microbiology, he revolutionized the treatment of tinea capitis and kerion. More recently, he has been working with biofilms and the immune system. He has uncovered the origin of various chronic skin diseases, such as atopic dermatitis and psoriasis, and various chronic internal diseases such as rheumatoid arthritis and arteriosclerosis. When Miklossy cultured Lyme Borrelia from the brains of Alzheimer's patients (confirming the much earlier work of Macdonald), and, when she showed the pathology of syphilitic dementia and Alzheimer's disease were the same, he applied the same microbiologic, pathologic, and immunologic techniques used previously to study this tragic disorder. Like the skin diseases, the microbes (Borrelial and dental spirochetes) in Alzheimer's disease made biofilms which is a slime coating encasing the community. These biofilms were intracellular, as in psoriasis; in that location, they activated the formation of hyperphosphorylated tau protein leading to destruction of the neurons. In the extracellular space, the biofilms activated the innate immune system leading to tissue destruction and beta amyloid formation. Consequently, the pathology and the tissue damage are largely caused by the biofilms that are created by the spirochetes. He has hypothesized that the Alzheimer's disease can be prevented by a yearly course of penicillin and has presented this work in the USA and Europe. He is a graduate of Juilliard (cello) and owns the historic Molly Stark House (1759).