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RESEARCH ARTICLE

The Presence and Impact of Bacteria and Biofilms in Chronic Skin and Systemic Diseases

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ABSTRACT

This will be an overview of our studies on biofilms and the diseases with which they are associated. Where possible, it will include the microbes that create these biofilms, their locations in the body, and their impact on the innate and adaptive immune systems. It will include all the diseases which we have recently classified as eczema in addition to those previously considered as such. It will also include psoriasis, tinea versicolor, leprosy, tertiary Lyme disease and Alzheimer's disease, the deposition diseases (arteriosclerosis, gout pretibial myxedema), the necrobiotic granulomas (granuloma annulare, rheumatoid nodules, and necrobiosis lipoidica), molluscum contagiosum and squamous cell carcinoma in situ in pigmented transplant patients. Consequently, many chronic skin and systemic diseases have been shown to be biofilm diseases. This implies that these disorders have a large, often determinative microbiological component. Where known, the mechanism whereby these biofilms interact with the immune system will be discussed.

Stipulations re Biofilms

1. Biofilms are common in nature; more than 90% of microbes in nature live in biofilms.
2. All organisms, bacteria, mycobacteria, viruses, fungi and others can and do form biofilms.
3. Biofilms are made of extracellular polysaccharides that make up the bulk of the biofilm. They also contain amyloid (ihat forms the infrastructure of the biofilm), microbial cells, DNA, and water channels.
4. 10 microbial cells in every direction are necessary to begin biofilm formation.
5. The size of the biofilm depends on the size of the microbe making it. For example, Malassezia furfur/ovale (3.0μ) yeasts form a biofilm that is too large to fit into an eccrine duct (30μ), thus it cannot cause eczema. The ultimate size of the spirochetal biofilm needs to fit inside the neuronal cytoplasm to cause Alzheimer's disease; also, the streptococcal biofilm needs to fit into the cytoplasm of the ectodermal cells lining the tonsils in psoriasis.
6. In the tonsil, the extracellular polysaccharides (slime) are "outside" the microbial cells.
7. Biofilms have attachment sites for other organisms, so the biofilms may be polymicrobial. The best example of this is dental plaque: streptococcus mutans initiates the biofilm and attaches to the tooth; next porphyromonas gingivalis joins in; and last; T. denticola (representing all dental spirochetes) joins. The biofilm is conceptually 3 layered.
8. The biofilm has attachment sites for Toll-like receptor 2 molecules of the innate immune system. These ordinarily are involved with gram-positive bacteria, but they attach to gram-negative biofilms as well.
9. Exporter cells are extruded from the biofilm and can leave and establish a new biofilm elsewhere.
10. Biofilms are multidrug resistant; they pass drug resistant genes horizontally between cells inside the biofilm.
11. Many topical and systemic biofilm dispersing agents are available. The best systemic agent studied has been rifampin which "pokes holes" in the biofilm. This must be taken with an antibiotic which will kill the organisms, otherwise the cells in the biofilm become exporter cells and will make new biofilms. Nicotine is an example of this.

Disease- Atopic Dermatitis/Eczema

The first disease to be discussed is eczema; we have written many papers and have given many presentations on this chronic disease.[1-5] (Figs. 1-4) Sulzberger found occluded eccrine ducts (Fig 5), in 1947[7] and, 60 years later, we showed these occlusions were biofilms created by normal flora staphylococci. [6] (Fig. 3) Eczema results from occluded eccrine ducts by normal flora staphylococci that make biofilms because of the salt and water present.[2] These occluded ducts then react with the inate immune system molecule toll-like receptor 2 (TRL2), (Fig.4) which produces proteinase-activated receptor 2 (PAR2) to create the "itch that rashes" which is eczema.[8-10] The biofilm/TLR2 also produces TNFα that causes spongiosis. Spongiosis is the hallmark pathological feature of eczema.[1]

We have shown how this is a double-hit phenomenon disease: one "hit" is the genetic component filaggrin (or another gene) and the second "hit" is the environmental component comprised of staphylococcal biofilms that occlude sweat ducts. The filaggrin and other genes make a faulty SC.[1]

We have shown how this process carries over to the other form of eczema where the genetic component is altered. Examples of this would include seborrheic dermatitis, where the stratum corneum (SC) is altered by yeasts (primarily Malassezia oleosa) and granular parakeratosis, where the alteration in the SC comes from the disruption caused by the myriad of granules.[12] The following table includes all the "eczema" diseases we have studied.

TABLE 1 Eczema Variations

Atopic dermatitis	atopic eczema
Seborrheic dermatitis-	seborrheic eczema
Granular parakeratosis	granular parakeratotic eczema
Tinea pedis	mycotic eczema (fig. 6)
Meyerson's nevus	nevoid eczema
Doucas Kapetanakis	pigmented purpuric eczema

Each of these disorders has shown occlusion of the sweat ducts with normal flora staphylococcal biofilms, and each disease has the characteristic pathology, microbiology, and physiology of eczema.[1] Sulzberger also found ductal occlusion in seborrheic dermatitis.[7]

The initial "itch" of eczema is caused by TLR2 that activates proteinase-activated receptor 2 (PAR2) which is a pruritogen stronger that histamine. Incidentally, the pathological finding of spongiosis is created by the interaction of the TLR2 with Myeloid Differentiation Primary Response 88 (MYD88) Pathway. Thus, the physiologic and pathological responses in eczema are generated by the innate immune system TLR2.[1]

Medium strength corticosteroid creams and ointments can be used to treat mild to moderate eczema. For more severe signs and symptoms, high patency corticoid creams and ointments can be employed. With all groups of patients, minimal use of soap and aggressive moisturization must be employed; for example, if soap (in any form) continues to be used excessively, the eczema cannot get better. And, moisturizing by itself cannot affect the overuse of soap.

Admittedly, these general measures are aimed at correcting the genetic component of the double-hit phenomenon. This may be the first time in history that clinical measures can aid in correcting the genetic component of a disease.

Further, all the foregoing has been aimed at the initiation (and presentation) of the disease; it does not include that which occurs when the epidermis is breached by scratching and the adaptive immune system comes into play in the dermis. Oral antibiotics are a helpful addition when that occurs.[1]

If all the above is carried out, antimetabolite and biological treatments are seldom necessary. Treatments such as azithromycin, methotrexate, cyclosporine, dupilumab, crisaborole, and others often have toxicities, along with being seldom necessary.

References Atopic dermatitis/eczema

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Fig.1 Flexural atopic dermatitis (AD)

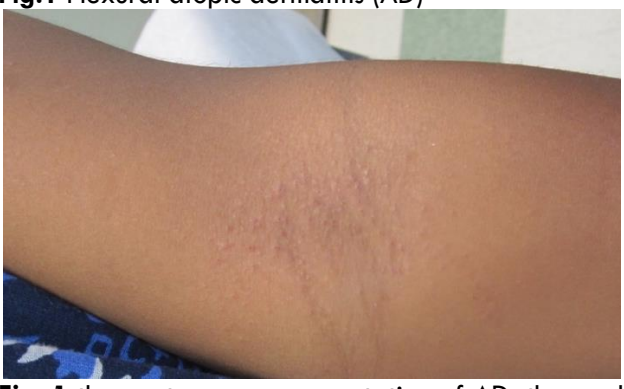


Fig. 1 the most common presentation of AD; the small papules in the midst of the rash represent occluded sweat ducts

Fig.2 Culture taken from skin of affected area of AD

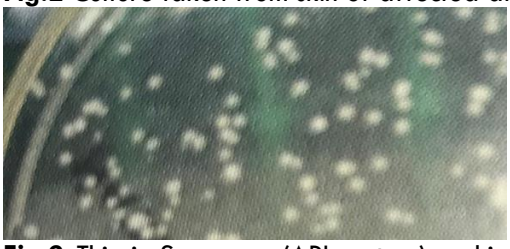


Fig.2 This is S. aureus (API system) making white colonies. 95% of all cultures had a positive XTT test implying they can produce biofilm; 90% had the biofilm forming *ica*D gene. 40% of cultures were S.aureus, 20% S.epidermidis with smaller amounts of 6 other staph species.

Fig.3 Microscopic image of skin stained with Congo red stain

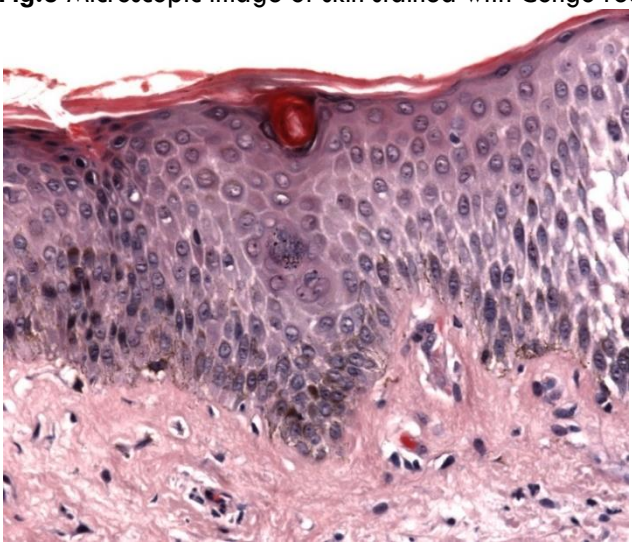


Fig.3. Biofilm occluded duct in upper epidermis; Congo red stains amyloid fibers that form the infrastructure of biofilms. Spongiosis at later edge forms the hallmark lesion of eczema. (10X)

Fig.4 Skin with CD 282 (TLR2) stain of epidermis

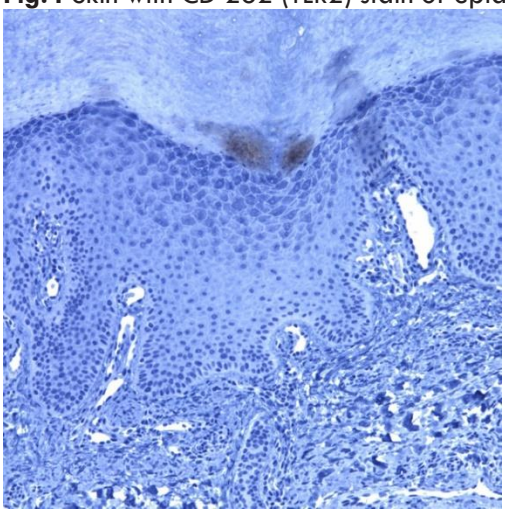


Fig. 4 TLR2 adjacent to occluded ducts represents innate immune system. It interacts with the MYD88 pathway to produce TNFα that kills micobes. TNFα is also strongest diver of spongiosis. (10X)

Fig.5 Sulzberger's original photomicrograph of occluded duct in eczema. 1947

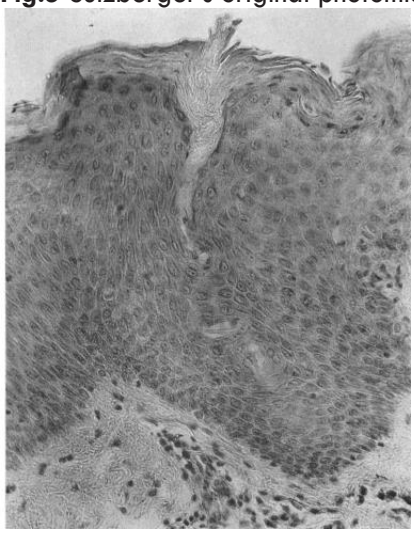
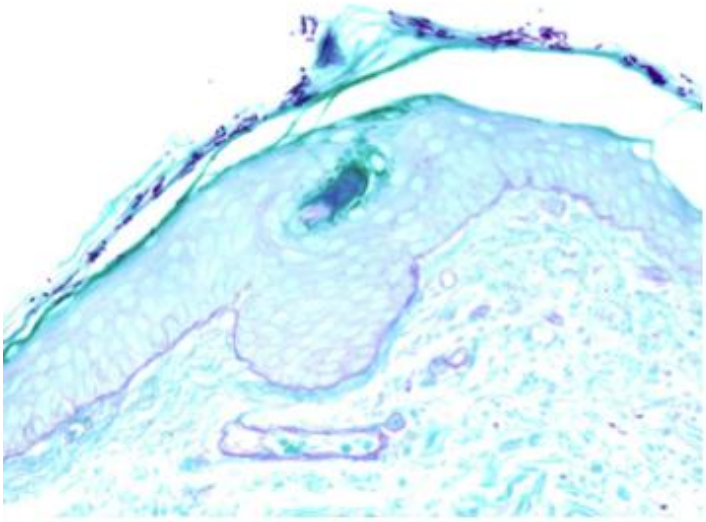


Fig.6 Fungus in SC with biofilm occluded duct.



Fig, 6 The presence of the fungi makes a faulty SC as does the filaggrin gene(10X)

PSORIASIS

Streptococcal biofilms are present in the tonsils in psoriasis. (Fig. 1,2) They are both "intra"cellular (in the ectodermal lining cells) and "extra"cellular (between the lymphoid cells). (Fig.2)[1] They cause upregulation of both the innate and adaptive arms of the immune system. Streptococcal-specific IgG has been found in the serum (Fig.3) of psoriasis patients;[2] this is likely from the ectodermal lining cells that shed into the pharynx and have their intracellular contents exposed to oral enzymes. TLR-2 is present in the dermal capillaries (Fig.4) on immunopathology of psoriatic lesions.[3] This is likely from the biofilms amongst the lymphoid cells.

Penicillin treats streptococci, but the organisms in biofilms are resistant. It appears that the serum IgG is more important than TLR2 because treatment with PCN and a biofilm disperser is minimally effective compared to penicillin given after a long course (9-12 months). After such long treatment, more than 90% of treated patients are clear (PASI 100). Saxena gave benzathine PCN every 2 weeks to achieve those results.[4,5] Given for 2 years, 80% of those patients were cured! Additional proof of streptococcus was found by McFadden who discovered locations with no streptococcus in their environments had no psoriasis.[6]

Psoriasis is also a double-hit phenomenon like eczema with the environmental component bring the streptococcal biofilms and the genetic component being one of the PSORS genes.[7] Ordinary treatment with topical corticoids plus/ minus vitamin D is moderately effective. UVB which impairs or kills Langerhans cells (antigen presenting cells) is very helpful in psoriasis.

The biologics (TNFa, IL12, 23, IL17, IL23, and T Cell inhibitors) are very effective in psoriasis and some require as little as an injection four times yearly after a loading dose.[7] We have treated multiple patients with biologics together with daily penicillin simultaneously and after 2 years have been able to discontinue the biologic successfully. Continuing one daily dose of penicillin leads to prolonged remission.[8]

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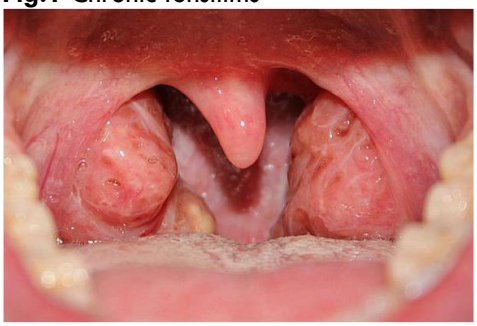
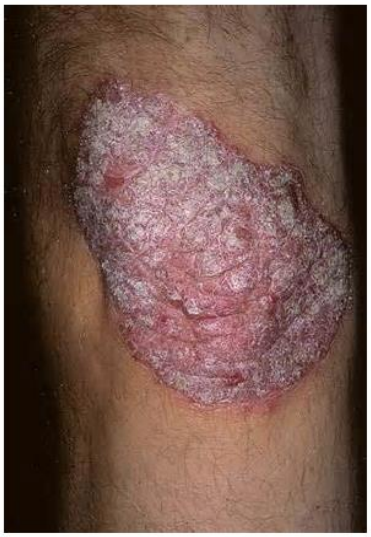
9. Personal observation

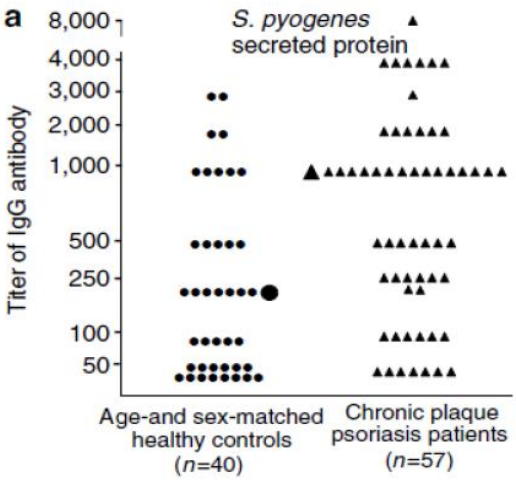
Fig.1 Chronic tonsillitis



Fig,1 Tonsillectomy resulted in 11of13 patients with both clearing and curing of psoriasis. (horleifsdottir RH, Sigurdardottir SL. Sigurgeirsson B. et al. Improvement of psoriasis after tonsillectomy is associated with a decrease in the frequency of circulating T cells that recognize streptococcal determinants and homologous skin determinants. J Immunol 2012;188(10):5160-5.

Fig.2 Plaque psoriasis; graph shows elevated anti-S.pyogenes IgG in patients vs controls





El-Rachkidy et al. *J Invest Dermatol.* 2007; 127:

Fig.3 Tonsil

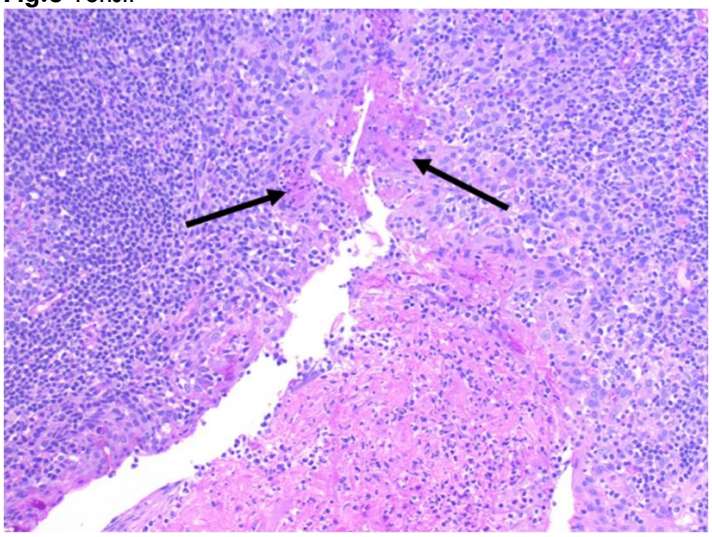


Fig.3 PAS stain reveals both intracellular (arrows show nuclei) and extracellular biofilms (large pink masses in crypt) 10X

Fig. 4 CD 282 (TLR2) from epidermis and upper dermis

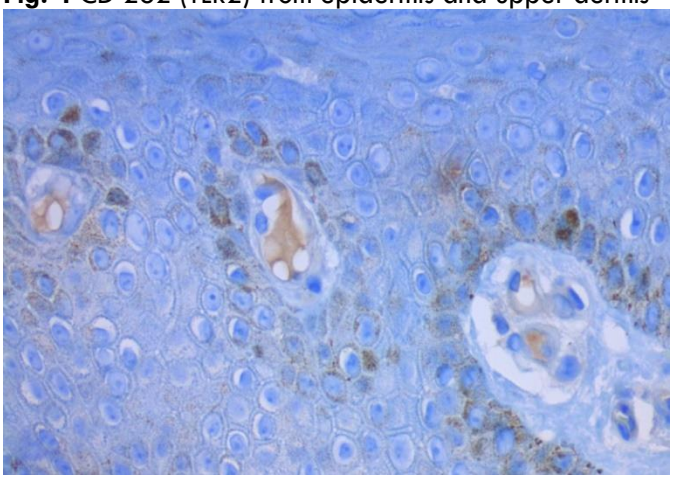


Fig.4 Brown staining TLR2 noted in upper dermal capillaries. 40X

Fig.5 Candida stained with CD282

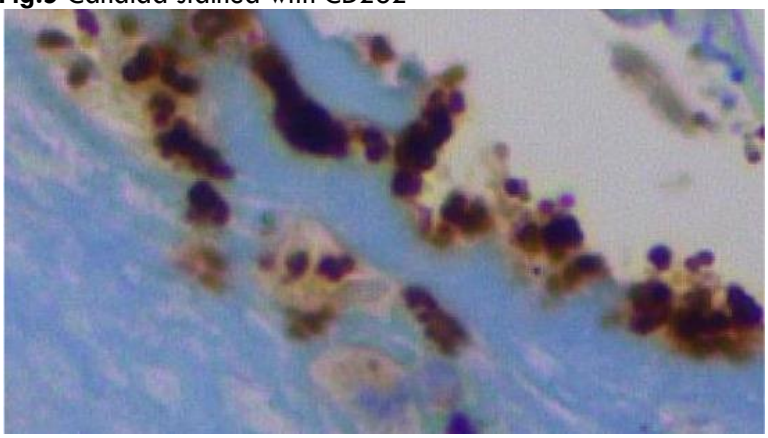


Fig. 5 Brown staining TLR2 coating yeasts in skin candidiasis; TLR2 uses MYD88 pathway to generate NFkB and TNFa to kill microbes. (40x)

Tinea Versicolor

Tinea versicolor (TV) is a yeast disease caused by Malassezia furfur/ovale. (Fig.1) It ordinarily is asymptomatic presenting with skin color change (white, pink, brown, red) and peeling.[1,2] We have shown that biofilms made by the yeast are present amongst corneocytes in the stratum corneum where they do not interact with living cells and thus do not generate TLR2.[1] (Fig.2) The biofilms are too large to fit in and occlude the eccrine sweat ducts, so they cannot generate the pathology of eczema or miliaria. (See stipulations)

The organisms generate azelaic acid which causes most of the color change. Selenium sulfide not only kills the M. furfur/ovale but it disrupts the biofilms (heavy metals topically are biofilm dispersers).[1,2,3] Ketoconazole topically works similarly, and systemically (orally).[4] It localizes in the SC, kills the organisms, and disrupts the biofilm. The azoles are biofilm dispersers, in addition to being antifungal.[4]

TV is an excellent control for the impact of TLR2 in eczema. It is activated by live cells immediately proximal to biofilms; however, in TV, the biofilms are present only in the acellular SC, so they do not generate an innate immune system response.

References Tinea versicolor

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Fig.1 Culture of M.Furfur/ovale on olive oil makes slime (biofilm) 10x

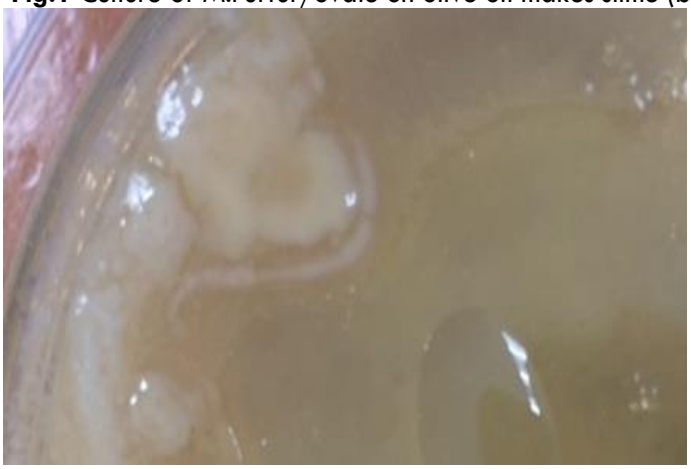
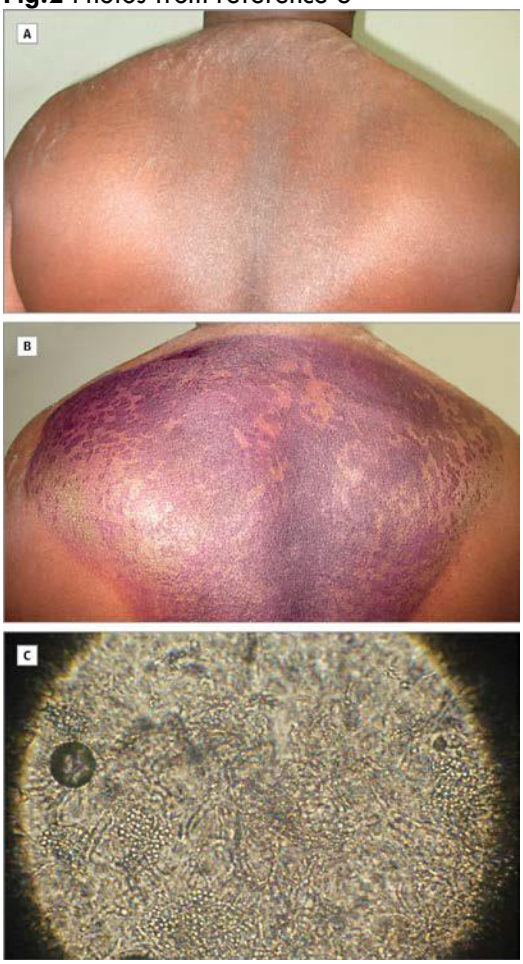


Fig.2 Photos from reference 3



Leprosy

The mycobacterium M. Leprae, makes biofilms and causes leprosy. The biofilms are initially in the liver, spleen, and kidneys. (Figs,1,2) Only in lepromatous (late stage) leprosy are biofilms found in the skin; they are in globi (histocytes filled with organisms and biofilm).[1] (Fig.3)

The addition of rifampin to dapsone has changed the incidence of 15 million worldwide 40 years ago to less than one million today. Rifampin pokes holes in the biofilm and allows the dapsone to kill the M. leprae inside.[1]

The immune reaction to M. leprae follows the bacterial load and disease progression; this has been summarized by Ridley and Jopling.[2] Britton has also reviewed the immunologic actions in depth.[3,4] Suffice it to say, the dapsone by itself would be (and was) relatively ineffective because of the biofilms made by M. leprae, but with added rifampin, the disease has been and could be controlled.[1]

There are 5 types ranging from tuberculoid leprosy (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL). The TT and LL types of leprosy have been compared based on the immune responses the elicit in the body.[2] The TT type instigates a vigorous immune response which confines bacterial load to granulomas in the dermis and peripheral nerves. This type of reaction is consistent with the Type 1 reaction in leprosy, which is mostly cellular immunity. The lepromatous types have been associated with the later stage complications, including secondary amyloidosis in the kidney, liver, and spleen (Sanz-Martín, Sharma).[5,6] The LL type is consistent with the type II immune reaction, which activates proinflammatory cytokines such as TNF, IL-1, IL-6, and IL-8 (Nery).[7]

We believe that the LL type can avoid specific cellular immunity due to the presence of biofilms, rather that with another type of evasion.. Mycobacterium leprae have been shown to form biofilms in nature (Chakraborty).[8] Biofilm formation in LL type would explain the late manifestations of disease and unusually low specific cellular immunity to M. leprae (Britton and Lockwood). This is also consistent with LL type histopathology, since

there are mostly microphages and foam cells present with large groups of leprosy bacilli.

The link between the biofilm formation in leprosy to the secondary amyloidosis is pertinent to understanding risk factors of other biofilm disease. Biofilms interact with the innate immune system through toll-like receptors on the surface of macrophages (mainly TLR2) to activate them.[9]The activation of the TLR2 receptors will elicit a proinflammatory response from the macrophage through release of cytokines, most notably TNF alpha though the MyD88 pathway.[10] As more macrophages come into contact with and are activated by the biofilm, more proinflammatory cytokines are being produces. If the biofilm is constantly stimulating the release of proinflammatory cytokines are being produced. If the biofilm is constantly stimulating the release of proinflammatory cytokines, then there is an increase of TNF alpha in the body. TNF alpha stimulates the release of serum amyloid A (SAA) from the liver. SAA in large amounts can become cleaved and deposited in the body, and certain organs are more commonly affected by secondary amyloidosis like the liver, spleen, and kidney (Simmons).[11]

The biofilm itself can also produce amyloid, and amyloid fibers protect the integrity of the extracellular matrix and the biofilm itself (Romero).[12] Therefore, biofilm and amyloid coexist with one another and diseases that are known to produce amyloid should be researched extensively to determine if there is an infectious cause.

References Leprosy

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Fig.1 PAS stain kidney shows biofilms (40X}

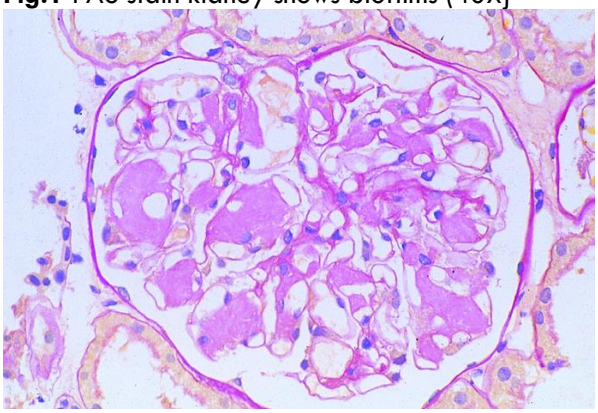

Fig.2 Congo red stain kidney shows amyloid-infrastructure of biofilm

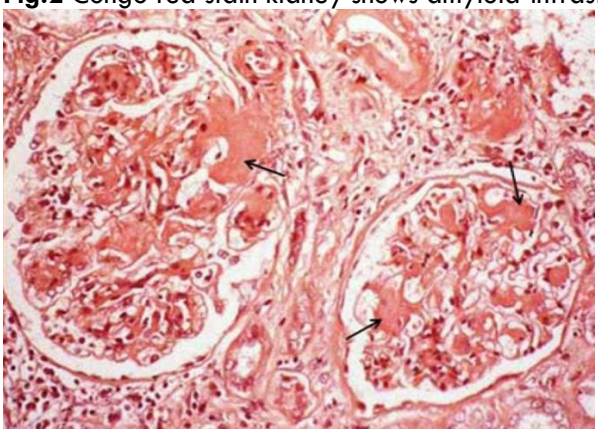

Fig.3 Congo red stain in globi-skin histiocytes

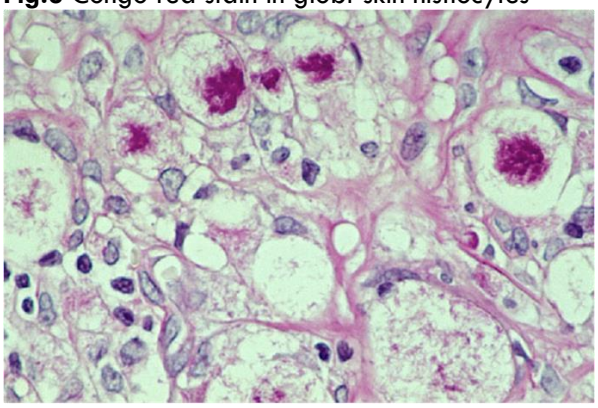

Fig. 3 M. leprae makes biofilms in the skin histiocytes in lepromatous leprosy (40X)

Tertiary Lyme Disease and Alzheimer's Disease

We have previously written about two different forms of tertiary Lyme disease. (Figs.1,2) Lyme arthritis manifests as Montauk knee (arthritic) and tertiary spirochetosis of the brain (Alzheimer's disease [ALZ]).[1] Our first patient was a premedical student doing a rotation in our department. After hearing a talk about Lyme being a biofilm disease when is becomes chronic, she said, "I have Lyme Arthritis" and revealed her knee, which was "swollen like a balloon". She had been taking doxycycline 200 MG daily for 1 month without any improvement, and in fact was gradually getting worse. After discontinuing the doxycycline, she began a regimen of Amoxicillin 500 MG 3 times daily and Rifampin 300 MG daily. Within one month, she was clear and wanted to know when she could begin running again. (Fig.3) Neither Amoxicillin nor Rifampin by themselves would have cured her, but the combination "busted apart" the biofilm and the amoxicillin was able the to kill the Lyme spirochetes inside the biofilm.

Spirochetes (Lyme and dental) (Fig.4) are found in the postmortem brain; an older woman

who died with severe dementia was analyzed.[1,2] The pathological changes in ALZ were similar in every respect to syphilitic dementia. She had biofilms in her brain intracellularly in her neurons, and extracellularly in the senile plaques.[3] (Fig.5) Research has shown the presence of Lyme spirochetes in 25% of these patients with ALZ.[4,5] More recently, it has been shown that the intracellular spirochetes made beta amyloid (Abeta) simultaneously with making a biofilm.[6](Fig.5) It has also been shown that the Abeta induces ordinary tau to make hyperphosphorylated tau (p-tau).[7] This is likely the most important development in ALZ because p-tau causes neuronal dendrite destruction.(Fig.6)This leads not only to loss of the synaptic connection from one neuron to another, but also to death of the neuron itself. This is ultimately viewed as the atrophy of the brain which is uniformly found.[8] (Fig. 7)

A pilot trial with amoxicillin 500 MG TID and rifampin 300 MG QD was done for 6 ALZ disease patients. In all, the disease stopped progressing: and, in 4 patients, there were moments of lucidity. We have proposed taking amoxicillin 500 MG daily for 2 weeks once yearly to prevent the disease.[9] Theoretically, this would include patients 65 or older and those younger who have a family history of the disease.

References Tertiary Lyme Disease and Alzheimer's Disease

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Fig.1 Montauk knee (Lyme arthritis)

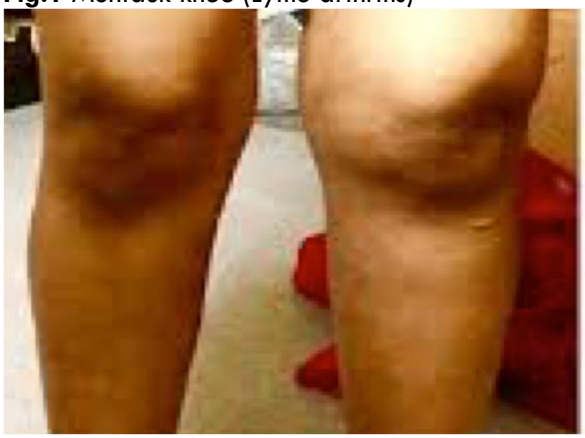


Fig.1 Patient had been on Doxycycline 200 mg/day for 1 month when this photo was taken

Fig. 2 One month later

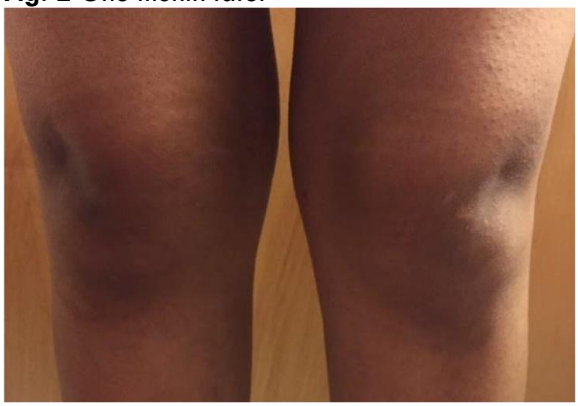
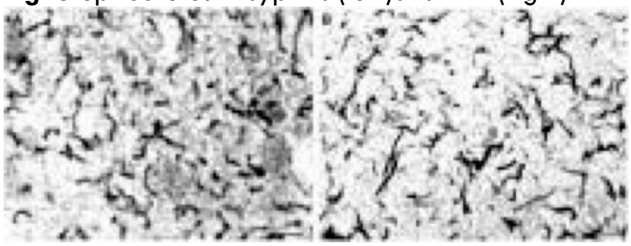


Fig.2 Doxycycline D/C'd; Amoxicillin 500 mg TID and Rifampin 300 mg/day X 1 monthled to resolution

Fig. 3 Spirochetes in syphilis (left)and ALZ (right)



From Miklossy J. Historic evidence to support a causal relationship between spirochetal infections and Alzheimer's disease. *Front Aging Neurosci*. 2015; 7:46.

Fig.4 Hippocampus of ALZ patient

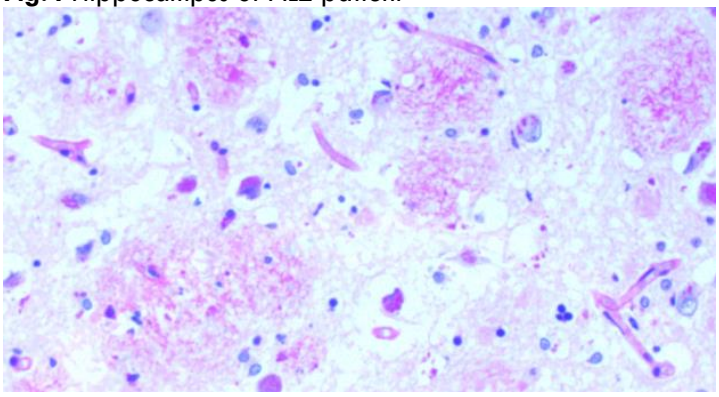


Fig.4. PAS stain shows intra and extracellular biofilms and one tau tangle on the left side of the image. (40X)

Fig.5 Hippocampus of ALZ patient

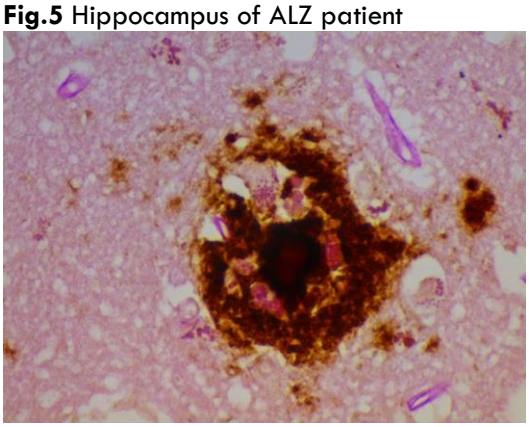


Fig.5 PAS and Abeta stains show bioflm and Abeta colocalize; also show intracellular Abeta (40X)

Fig.6 Hippocampus of ALZ patient

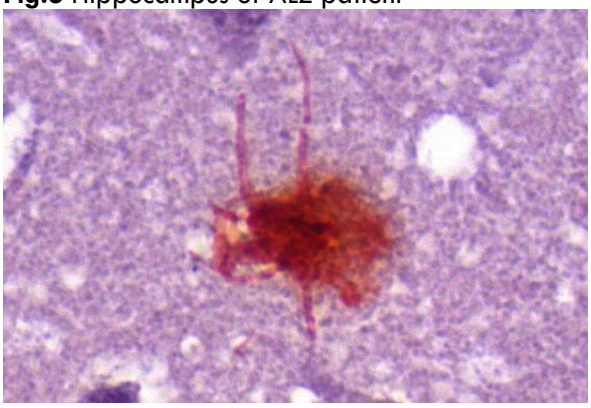


Fig.6 Neuron stained with Congo red (indicates biofilm); dendrites still elongated and not destroyed (40X)

Fig.7 Hippocampus of ALZ patient

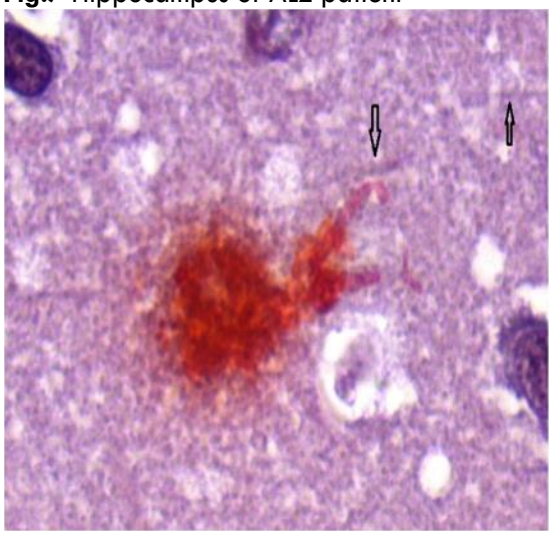


Fig.7 Dendrites destroyed

Schematic of **Fig.7**

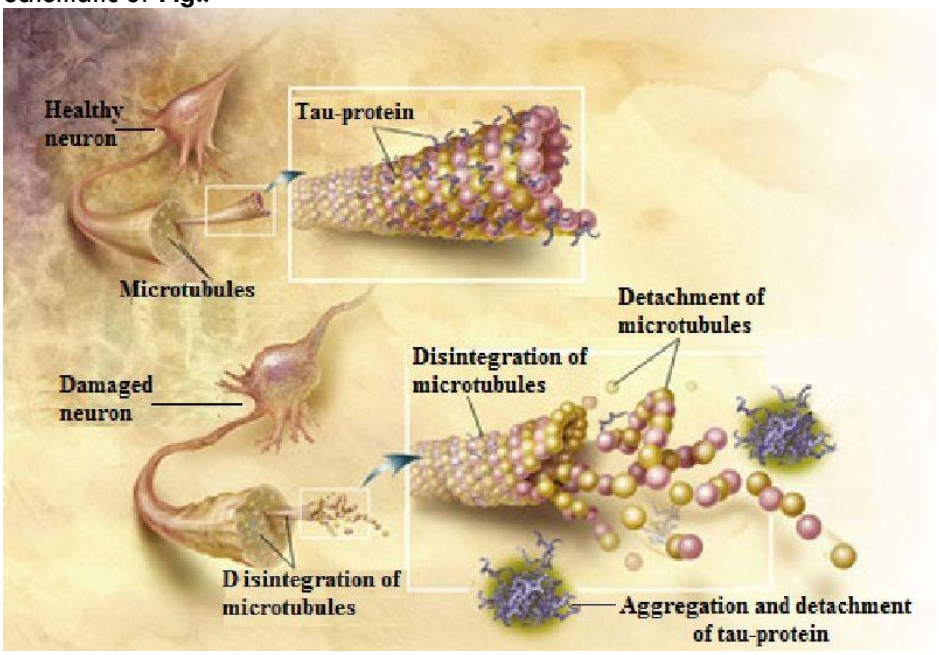


Fig.8 End result of ALZ

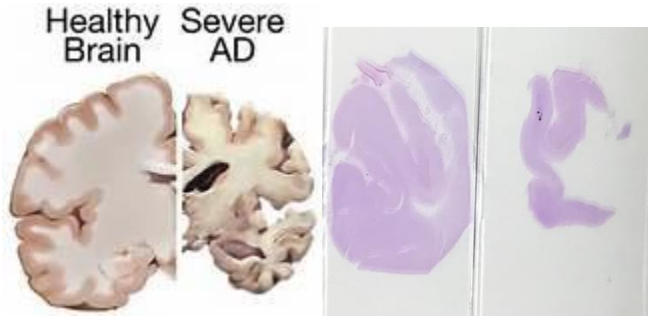


Fig. 8 Total atrophy of brain (left) and hippocampus (right).

The Deposition Diseases

Three deposition diseases have occurred in settings where biofilms have been found. First, in arteriosclerotic diseases, they have been found colocalized with cholesterol deposits in intraluminal and intramural plaques. Biofilms also create calcium deposits and attract TLR2.[1-6] (Figs.1,2 It is uncertain which is first, the biofilm or the cholesterol, but the fact is they are localized together. This was shown in multiple patients with arteriosclerotic disease (endarterectomy) specimens. The presence of calcium in the plaques makes dental organisms likely inhabitants of the plaques because they consistently make calcium in dental plaque.

Gout is another deposition disease in which uric acid deposits in gouty tophi (Fig.3) Biofilms again co-localize with the uric acid; these biofilms are made by different microbes because they stain pathologically with colloidal iron stain instead of PAS staining as seen with the other microbes.[7] Congo red staining is present in all. The biofilms, in gout then, are likely made by gram negative organisms because the staining pattern favor them. They also do not attract TLR2, so they are asymptomatic. Gouty tophi tend to develop clinically at points of trauma, but again it is unknown which comes first, the biofilm or the uric acid.

A third deposition disease is pretibial myxedema.[8] The staining pattern of the mucin in this condition is similar to gouty tophi and shows either large pools of hyaluronic acid in the upper dermis or interlacing bacterial between the collagen bundles. (Figs.4,5)The Congo red staining is less pronounced than in arteriosclerotic plaques and gouty tophi.

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Fig.1 Carotid Endarterectomy plaque

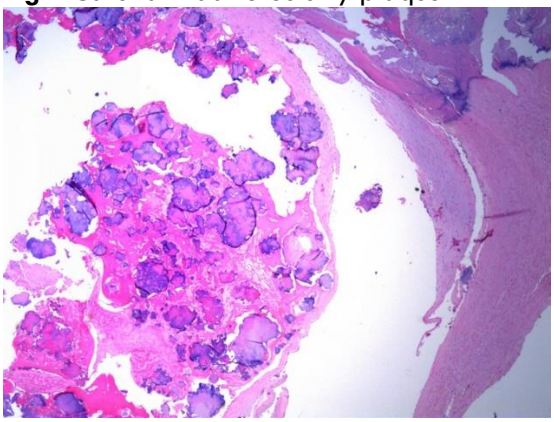

Fig.1 PAS stain shows biofilm in the plaque; calcium deposits throughout plaque 10X

Fig.2 Carotid Endarterectomy plaque

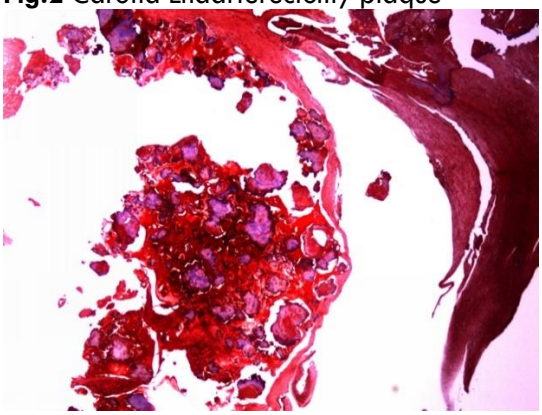

Fig. 2 Congo red stain shows biofilm in the plaque. Calcium deposits as above. 10X

Fig. 3 Carotid Endarterectomy plaque
TLR2

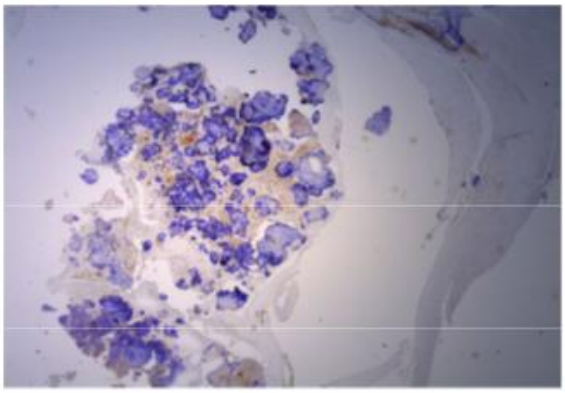

Fig. 3 TLR2 noted in the plaque. Arteriosclerosis has been associated with many organisms, such as Chlamydia pneumoniae, H simplex, P acnes, a dozen others; the same organisms are found in Alz dz brains. Biofilms have receptor/attachment sites for many organisms; is a "hotel" rather than a single-family home.

Fig.4 Tophus on ear

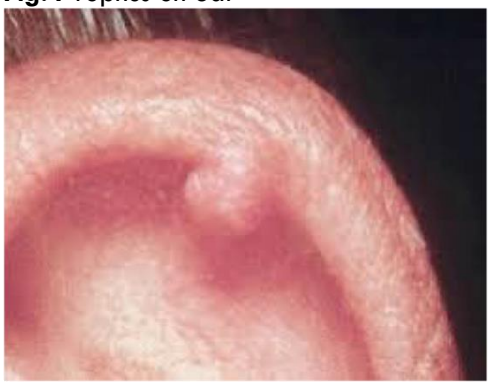

Fig.4 Tophus on ear

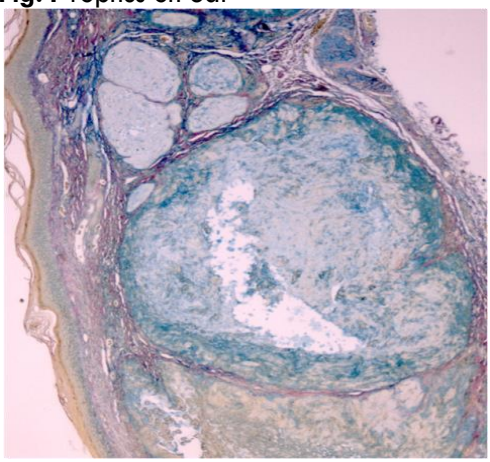

Fig.4 Colloidal iron positive where the uric acid deposited. This corresponds to PAS positivity in other biofilms. The CFe stains hyaluronic acid mucin

Fig.5 Skin biopsy of lower leg in Grave's disease (hyperthyroid) patient

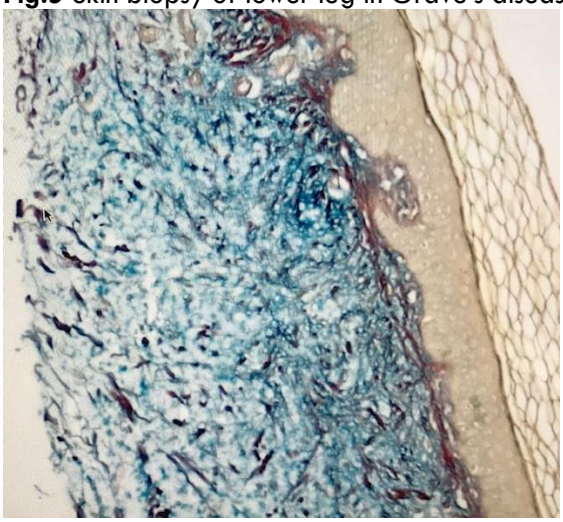

Fig. 5 Colloidal iron Stains a pool of mucin in the upper dermis; this represents the mucin in the biofilm. (10X)

Fig. 6 Skin biopsy of lower leg in Grave's disease (hyperthyroid) patient

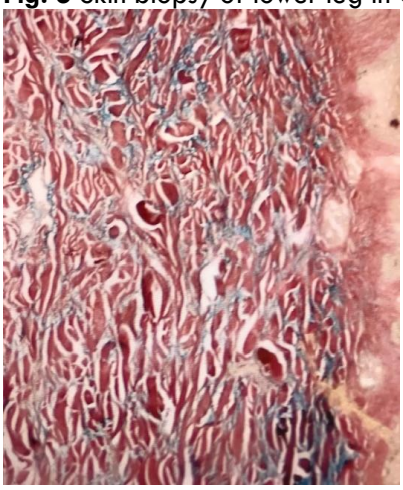

Fig. 6 Colloidal iron interspersed among collagen fibers (like Tumid LE)

Fig. 6 Skin biopsy of lower leg in Grave's disease (hyperthyroid) patient

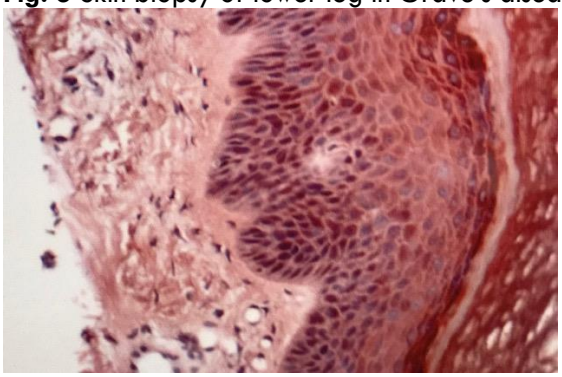

Fig. 6 Congo red positive between collagen bundles, similar to mucin.

Necrobiotic Granulomas

The necrobiotic granulomas all show biofilms in the areas of the necrobiosis. These include granuloma annulare, rheumatoid nodules, and necrobiosis lipoidica.[1-3] The pathology specimens of these three disorders stained for PAS weakly, but strongly for colloidal iron. The Congo red stained positively in all the specimens in the areas of necrobiosis. A different microbe, likely a gram-negative bacterium, is creating the biofilm in these three disorders.[2] Granuloma annulare (GA) and necrobiosis lipoidica (NL) do not have similar serologic finding as RA nodules but are biofilms none the less. (Figs1-3)

NL is linked to diabetes in 30% of patients, but correcting the diabetes has no impact on the disease. RA nodules are associated with rheumatoid factor and treatment with biologics has represented marked improvement in joint symptoms and disappearance of nodules.[2] The patients need to continue taking the biologic because it treats the symptoms and not the cause of the disease. This is like psoriasis where the biologic treats the symptoms with considerate or total clearing, but once stopped, the disease will reappear.

Biofilms have been found in other cutaneous diseases such as eczema, and systemic diseases such as leprosy,[4,5]

References Necrobiotic Granulomas

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Fig.1 Skin biopsy of lower leg; plate-like fibrosis with necrobiosis in between

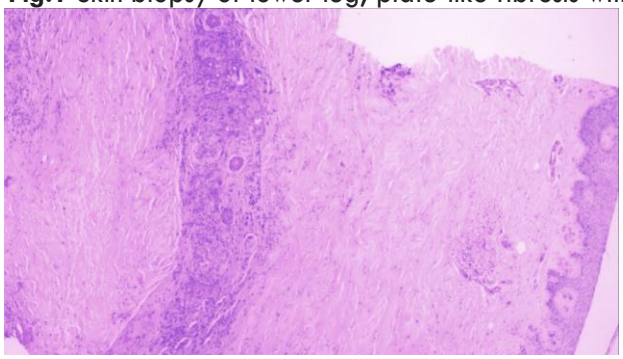

Fig.1a Skin biopsy of lower leg; plate-like fibrosis with necrobiosis in between

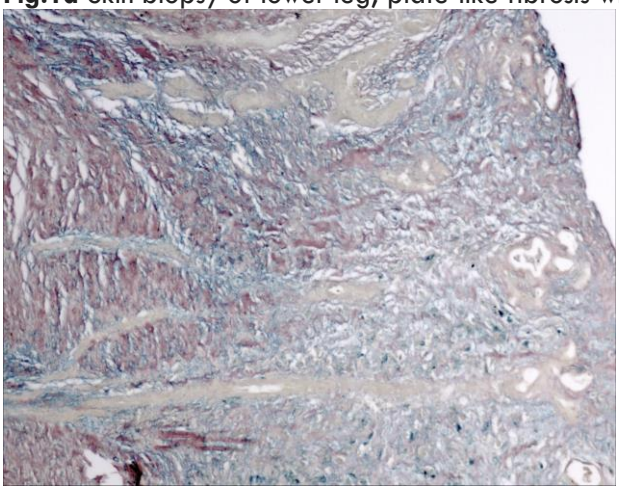

Fig.1a Colloidal iron positive in mid dermis in area of necrobiosis; represents mucin in biofilm

Fig.1b Skin biopsy of lower leg; plate-like fibrosis with necrobiosis in between

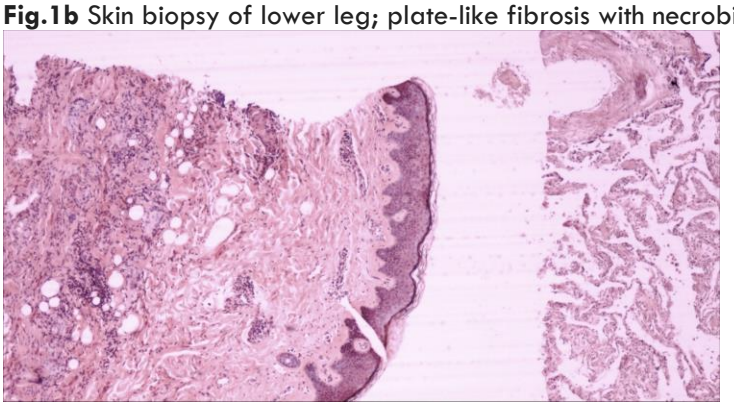

Fig.1b Congo red positive in area of necrobiosis; represents amyloid in biofilm. Control on right. 10X

Fig.2 Biopsy of rheumatoid arthritis nodule

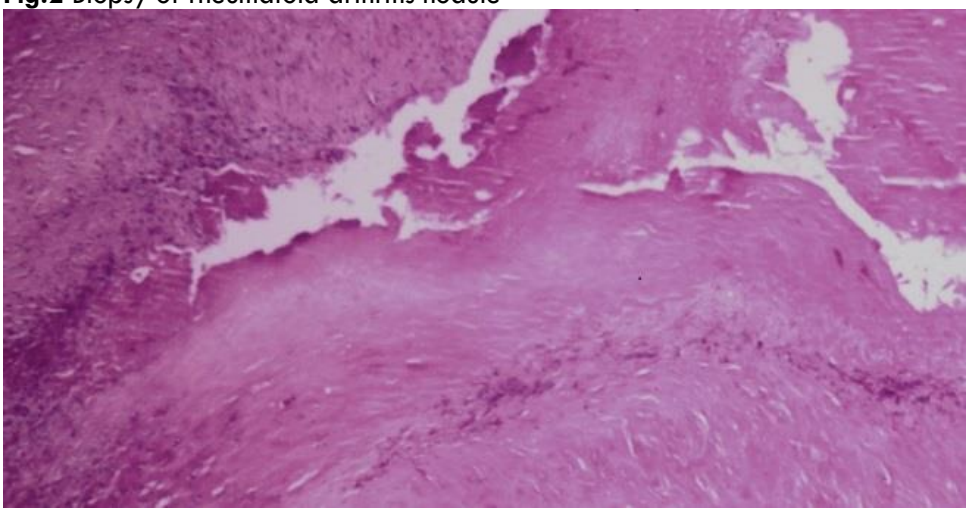

Fig. 2 Necrobiosis noted in the middle of the specimen

Fig.2a Biopsy of rheumatoid arthritis nodule

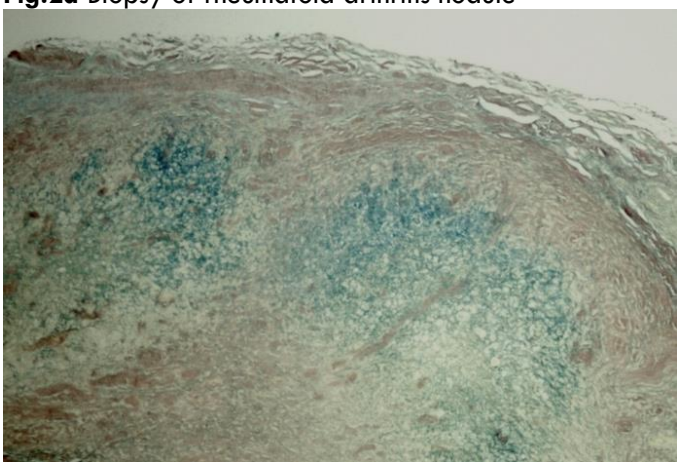

Fig. 2a Colloidal iron is positive in area of necrobiosis; this is the area of the biofilm. 10x

Fig, 2b Biopsy of rheumatoid arthritis nodule

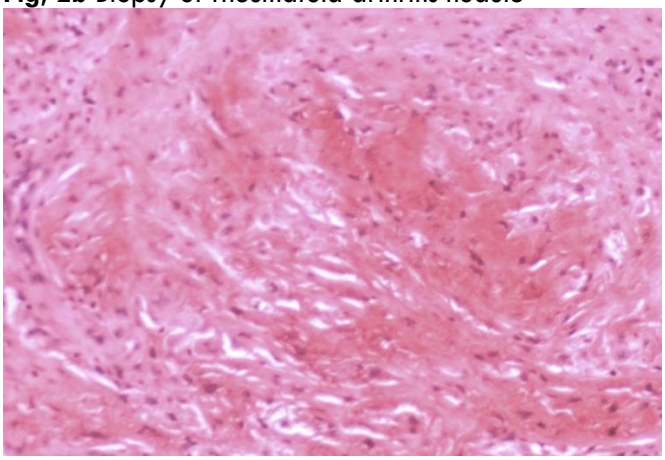

Fig. 2b Congo red positive; represents amyloid, the infrastructure of biofilms (10X)

Fig. 3 GA Stained with Congo red

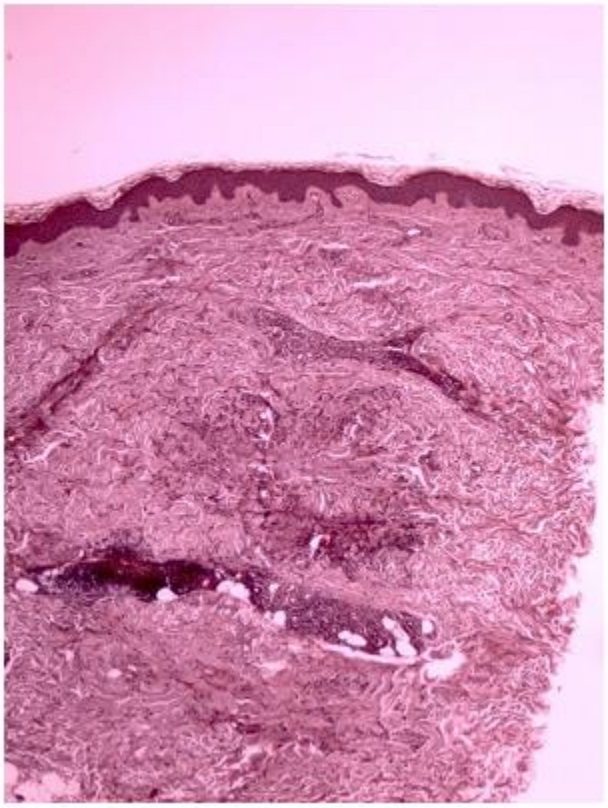

Fig. 3 Congo red staining in the same necrobiotic granulomas as colloidal iron. 5X

Viral Diseases with Biofilms

We have discovered viral biofilms in 2 diseases: one benign (molluscum contagiosum) and one malignant (squamous cell in situ in transplant patients of color).(Figs1,2) These were caused by MC virus and oncogenic human papilloma virus 16, and 18 respectively.[1,5] The pathology specimen of MC showed viral effect and intracellular staining with PAS and Congo red in all layers except the basal layer. The SCC specimens were not from sun exposed skin where they are prevalent in non-pigmented patients. These were from the wart-like lesions in non-sun exposed skin in transplant patients of color.[2] More than half of these patients had specimens that stained immunopathologically with anti HPV 16, 18 and these same specimens stained positively for PAS.(Fig.3) The Congo red staining was inhibited by transthyretin which does not allow production of amyloid, so the amyloid in biofilm was inhibited.[6] This further documents this as a malignant lesion. (Fig.4)

Both showed these biofilms to be intracellular. Being viral, they need to overtake the DNA of the cell to make more viruses. There are likely more viruses that make biofilms; the first that comes to mind are herpes zoster, which has the earmarks of a biofilm disease, and herpes simplex, which may already be present in the biofilms of ALZ made by the Lyme and dental spirochetes. This has already been shown in spirochetal biofilms with C. pneumonia in the middle of the biofilm. Perhaps something akin to that occurs with HSV.

References Viral Diseases

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Fig.! Biopsy of molluscum lesion

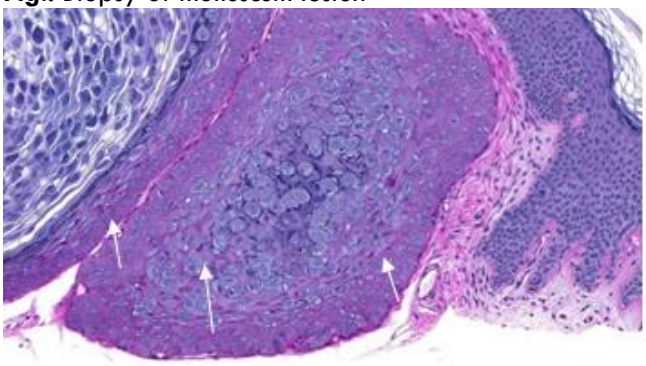

Fig.1 PAS positive at arrows. This represents the mucin in biofilms. (10X)

Fig. 2 Congo red stain

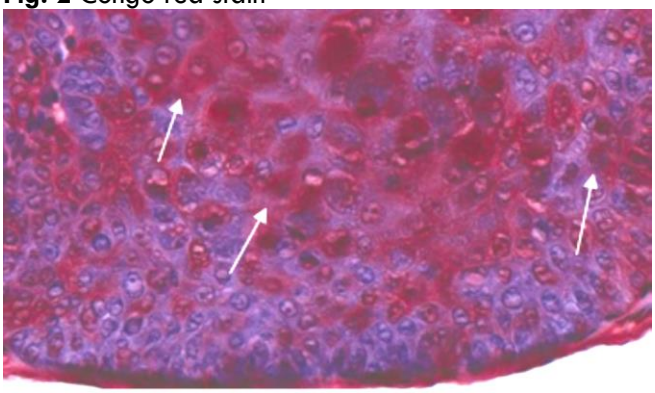

Fig. 2 Congo red positive intracellulary in Malpighian cells. This indicates the biofilm is intracellular.

Fig. 3 Skin biopsy of wart-like lesion in groin of pigmented transplant patient

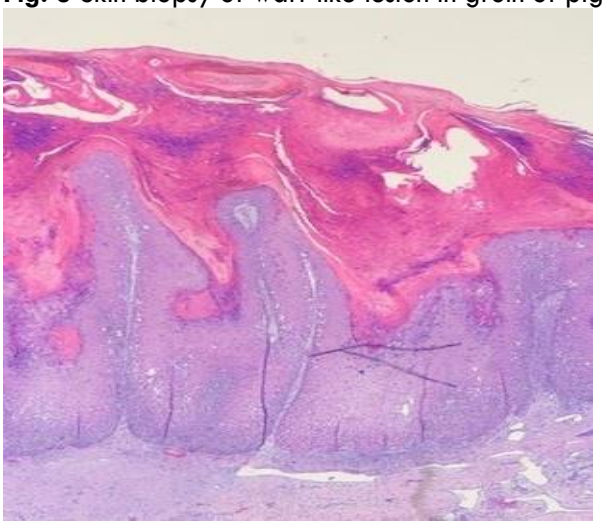

Fig. 3 H+E stain reveals Squamous cell carcinoma in situ (10X)

Fig. 4 Skin biopsy of wart-like lesion in groin of pigmented transplant patient

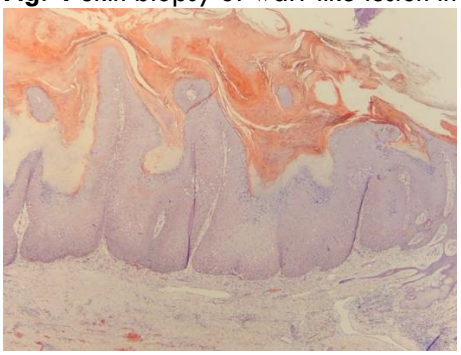

Fig. 4 PAS was positive, but Congo red was negative (above). Cancer activates transthyretin and transthyretin suppresses the formation of amyloid. This is like molluscum (intracellular PAS), except no amyloid.

Bioethical Challenges of Biofilm Diseases

We have written previously about the bioethical challenges of psoriasis, Lyme disease, and Alzheimer's disease.[1-3] Each disease has a different microbe, and each has significant challenges. Psoriasis requires a long treatment time with penicillin for treatment and cure. Further, the treatment with biologics and penicillin simultaneously (once the causative microbe is known) is very effective at treating the symptoms and signs of a particular disease.

Lyme disease, when biofilms are present, requires treatment with penicillin (or azithromycin for those allergic to penicillin) plus rifampin which pokes holes in the biofilm. It must be noted that the necessary dose of doxycycline for initial and secondary or tertiary Lyme disease is double the ordinary dose and 400 MG daily is incompatible with gastrointestinal health in nearly all patients.

Alzheimer's disease has no current effective treatment; we have recently proposed preventative treatment of penicillin for a 2-week course once yearly which logically would prevent the ALZ from developing and a clinical trial in MCI to halt the disease. This preventive approach corresponds to the treatment for primary and secondary syphilis. ALZ appears identical to (tertiary) syphilitic dementia pathologically. 200 completed clinical trials have not produced any effective treatment (mostly because they have been focused on the faulty beta amyloid theory). From the pathology, this is clearly a spirochetal/biofilm disease.

Eczema is a disease in which minimizing bathing, limiting the use of soap, and aggressively moisturizing are all helpful in the treatment of the disease. Biologics seem rarely necessary in that situation; plus, they do not have the same efficiency in eczema as in psoriasis.

As more is learned about the viral diseases, the deposition diseases, and the necrobiotic granuloma diseases, their treatments may be radically changed because of the microbe/ biofilm nature of the disorders.

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