CI - eLetter: Biofilms as a differential diagnosis for Amorphous Globs

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Sender's Name: Alan B. MacDonald M.D.

Affiliated Researcher Institution: University of New Haven, West Haven

Title: Biofilms as a differential diagnosis for "Amorphous Globs of Dr Linda Bockenstedt"

Letter:

Dear Dr Bockenstedt,

I remark with great interest your inclusion of the brief Discussion of Biofilms of Borrelia burgdorferi in your Discussion section, in connection with possible etiologies for the GFP emitting "amorphous blobs" of Borrelia burgdorferi in deep dermal sites closely apposed to the articular cartilage of the murine arthritic Lyme arthritis mice.

Our paper [6] describing for the very first time the entity of In Vitro Biofilms of Borrelia burgdorferi, and accepted for publication release on the PLOS ONE website during the week Of October 24,2012, is the first peer reviewed manuscript on Biofilm formation by borrelia of any species. It is indeed fortuitous that we, and you were working with borrelia burgdorferi.

We and you were curious to understand the implications of possible In Vivo Borrelia biofilm formation. At the level of verbal definition to fulfill the requisite (Costerton)[Ref 5] definitions for a biofilm community are the following:

1. Specialization of member microbes within the community which set these specialized forms apart from Planktonic forms of the establishing microbe.

2. Investment of the microbial members of the biofilm community by a self generated Extracellular matrix-usually including structural constituents from once living but now dead members of the biofilm community. The presence of free Extracellular DNA derived from once living now dead members of the biofilm community.

3. The existence of microbe density which exceeds the density of Planktonic microbes on an ordinary bacteriologic solid phase medium.

4. The existence of "plumbing networks" [i.e. water channels and channels for waste product elimination] in the biofilm community. [Ref 4]

5. The Resistance to antibiotic killing among specialized members of the biofilm community. [Ref4]

Now which of items 1-5 above are or might be "in Play" in your so called "Amorphous Blobs" of GFP labeled Borrelia burgdorferi in the Lyme arthritic mice?

1. Specialization:

Morphology of the microbes within the So-called "amorphous globs" differs significantly from the corkscrew shaped forms (Planktonic forms- motile forms) [Ref 4] of Borrelia burgdorferi, which are nicely demonstrated in your supplementary Videos and in Figures 1-5 (Especially in your figures 5A and 5B). You clearly demonstrate "Round bodies" among the members of the Amorphous blobs. Round form metamorphosis from pre-existing spiral forms of Borrelia burgdorferi has been elegantly demonstrated in the long list of peer reviewed publications by Drs Oystein Brorson and his cousin Dr. Sverre Henning Brorson [Ref 7] - using electron Microscopy and Phase contrast microscopy.

Round bodies were embraced by the late Dr. Lynn Margulis.[Ref 8] You specifically took the time and effort to demonstrate the formation of borrelia Round bodies in your attached supplementary videos in still photographs in Figure 5 [5A and 5B] in your manuscript. Non-spiral borrelia - i.e. roundbody borrelia are indeed legitimate shape shifted forms of the Borrelia genus, and represent a specialization by the well known spiral MOTILE form of Borreliae [Planktonic from of Borrelia]. Round bodies, granular forms, Cell wall deficient forms, and finally spiral or straightened forms of Borrelia are specialized forms of the Planktonic (spiral form).Thanks to the works of Dr Alban and colleagues [Ref 1] at the University of Rhode Island, these vital round body borreliae demonstrate a diverse protein repertoire which differ from spiral forms in their protein "fingerprint" in two dimensional SDS PAGE gel electrophoresis.[Ref 1]

2. The Overall Density of borrelia microbes in your "Amorphous blobs" in the murine deep dermis, exceeds by several orders of magnitude, the density of Planktonic (spiral/motile) forms of Borrelia burgdorferi when these are grown on solid media in the Microbiology laboratory (Preac-Mursic, V. et al)[Ref 9]

3. The assertion that All of the borrelia microbes in the "Amorphous Blobs in murine deep dermis in laboratory induced Chronic Lyme Arthritis" are ALL DEAD and merely represent "debris"....is just that--- an Assertion, buttressed by what you say are corroborative mRNA supportive data...(not presented in your paper). I have suggested to you in a personal private communication that the way to establish Live versus Dead borrelia in your "Amorphous Blobs" is to pour some Dye (Invitrogen :: Live Dead Assay) ([Ref 10]Red=dead, and Green = alive).

You have steadfastly refused to accommodate this quality control procedure, which is fast, cheap, reliable and easily accomplished. You have refused me tissue from your "amorphous Globs" dermal tissue from murine subjects, so that I might perform this simple quality control step in my own laboratory. Refusal to provide tissue to an outside scientist, is in violation of the guidelines of the NIH and other Federal funding agencies. Your Research activities were accomplished with the use of public funds. You have an incumbent obligation to provide tissue to outside scientists upon request, to verify your Experimental findings.

4. A review of all previously published manuscripts since the 1982 NEJM articles announcing the Spirochetal (Borrelial) etiology of Lyme Disease, performed by me and by pathology colleagues [Ref 3] interested in the published peer reviewed Borrelia pathology manuscripts , which specifically demonstrate with figure illustrations borrelia burgdorferi in diseased tissues-- Such a comprehensive 30 year literature review by Board Certified Anatomic and Clinical Pathologists--- reveals that there has NEVER been published a manuscript which contains images of Borrelia burgdorferi- either Living Borrelia or Dead Borrelia- which are present in Mammalian tissue in the densities which were illustrated in your figures 5A and 5B. [Ref 3] By Density criteria ALONE, these "Amorphous globs" are

Biofilm communities in murine tissue, UNTIL PROVEN OTHERWISE.[Ref 4] I therefore challenge you to voluntarily participate in Quality Control microscopic exercise--- to establish with the LIVE DEAD Invitrogen Kit that ALL of the Borrelia inside of your "Amorphous Blobs" stain Red(=Dead) and that none of the borrelia within your “Amorphous Globs” stains Green in the Invitrogen Live Dead assay.[Ref 10] If you are correct, there is nothing to lose in this quality control exercise.

Respectfully,

Alan B.MacDonald MD, FCAP, FASCP

References:

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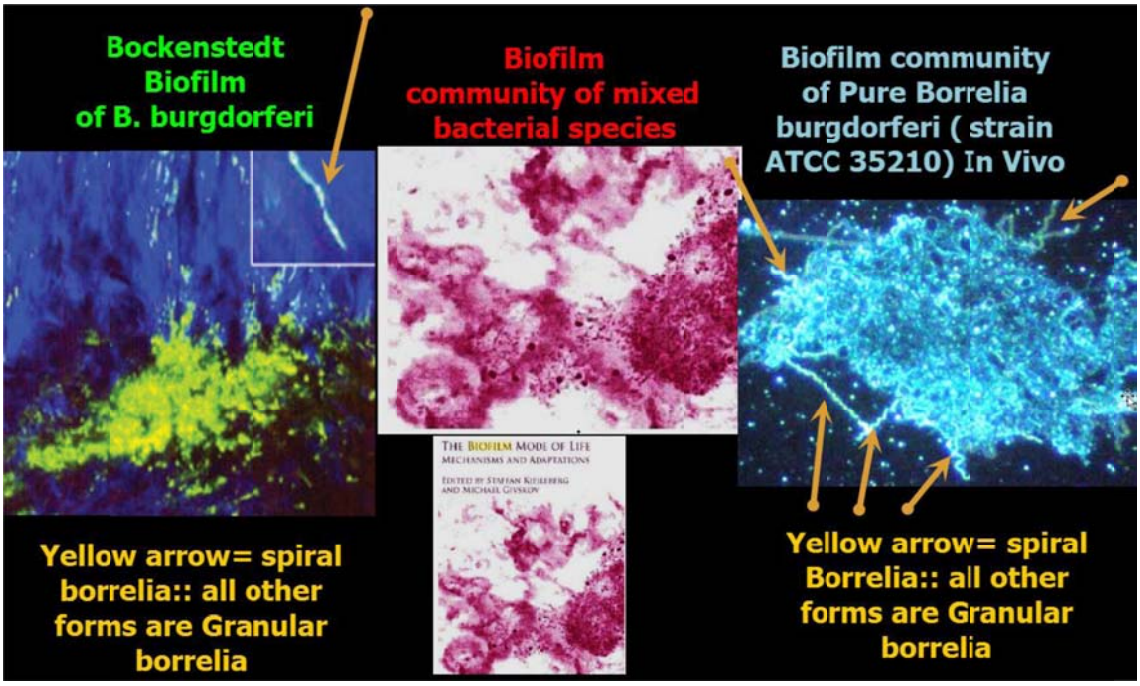
Bockenstedt
Biofilm
of B. burgdorferi
Biofilm
community of mixed
bacterial species
Biofilm community
of Pure Borrelia
burgdorferi (strain
ATCC 35210) In Vivo
THE BIOFILM MODE OF LIFE
MECHANISMS AND ADAPTATIONS
EDITED BY STEFFAN KJELLEBERG
AND MICHAEL GIVSKOV
Yellow arrow= spiral
borrelia:: all other
forms are Granular
borrelia
Yellow arrow= spiral
Borrelia:: all other
forms are Granular
borrelia