Borrelia Biofilms Dwell Inside of Amyloid Alzheimer’s Plaques:
100 consecutive Plaques Examined
By
Alan B. MacDonald, MD,
Fellow, College of American Pathologists
September 11, 2015
Free to all
Alzheimer’s Plaques: Medical Views – 50 years
PLAQUES = DEAD regions
Silver Stains – **Black to Brown color** = Dead
All remaining Brain tissue
i.e. Light colors = not yet Dead
Is Living Brain-Wounded but not Dead Yet
Take Home Message from this Research Report- September 10, 2015

- **LIVING Alzheimer’s Plaques!!!**
  - Amyloid in Plaque does not indicate Zone of DEAD brain
  - DNA Stains: *Borrelia miyamotoi* DNA probes hybridize to Plaques
- **Biofilm Communities of *Borrelia* (ALIVE) Fill the entire Plaque**
  - 100 Amyloid Alzheimer Plaques examined
  - 100 Alz. Dis. Plaques: All contain Biofilm communities of Borrelia
  - Living Biofilm Communities: Unlock Secrets

**Why do Plaques begin SMALL and Terminate as LARGE structures?**

Why do **Plaques have Empty Spaces**- in a network like arrangement?

Why is it: The number of Plaques per unit area in Alzheimer’s Disease Increases over the course to the Disease

Why do **Plaques in Early Alzheimer’s Disease Show up in the Base of Brain only** and in **End Stage Alzheimer’s Disease**: The plaques have spread to ALL Grey Matter Regions: replacing Cortical regions of the patient’s End Stage shrunken Brain at death?
Alzheimer’s Plaques: Dogma = “Dead zone”
Areas of Injury of Brain tissue
By Alzheimer’s Disease

Beta Amyloid (Beta 1-42, and others)
Dogma = “Toxic” Amyloid - disputed
First Image

Infection

Inside

Alzheimer Plaque

MacDonald, A.B. Year 2006

Alzheimer’s Disease case: Christensen, P.
Figure 6F
Hippocampus. Congo Red stain only (American Master Tech Product STCKE 90) Amyloid color red

Figure 6G
Hippocampus, Fluorescence In Situ Hybridization only (FISH) for Barretta specific DNA/mRNA (white and red). A biofilm-like community of Barretta microbes (granule Barretta) in high density, showing a rounded contour. Rounded Amyloid plaques are very similar with rounded image profiles and in plaque diameters.
Probe: TCA GCC ATA AAT GCT TCC AGA AAT AAT TCA CCA GCA CAC CGG TCT CTG CAG CTC CCG CAA ATT GAT AAC TTC CAT TCT CTC CTC
What is a Biofilm of *Borrelia*?
Essential: Teachable Moment: *Borrelia* Biofilms - Structure

1. **Specialized Borrelia**: *Inside* the Biofilm COMMUNITY

   **Granular Borrelia**-

   A. **Small, dot like Profiles**: [NO Spirals]
   B. LIVING legitimate *Borrelia* forms – “GRANULAR”
   C. “Membrane Bound” DNA containing life forms
   D. DNA and RNA – Bright Signal DNA by Probes
   E. Granular *Borrelia Growth* - Increase in number over time
   F. **Protected by Matrix of biofilm** - Gel like investment
Essential: Biofilm Borrelia Communities

• **Matrix of biofilm** - protective role –

  2. Matrix “Surround”: for Living Biofilm Community

  **Extracellular** - i.e. separate from ” Membrane Bound”

  Viscous, Thick

  Composition: **Extracellular DNA**, Extracellular Proteins etc.

  Origin: Remnants of Once Living, but now Dead microbes

  **Water Channels**: Empty spaces, in a Network:

  Nutrition Conduits, Waste (sewer) Conduits

  Supports, Protects, Insulates the Community
Biofilm Borrelia Grown In a Test Tube
Biofilm Borrelia Grown In a Test Tube
Granular **Borrelia**: What?

- Granular Infectomes:
  - Malaria model: MEROZOITE
  - Syphilis model: Granular **T. Pallidum**
  - Borrelia model: Granular **Borrelia**
Granular Borrelia: Membrane Bound—What?

- Electron Micrograph: Granular Borrelia – Magnification 126,000x
  - *Borrelia* culture – pure *B. burgdorferi* - Erythema Migrans Skin biopsy
  - Dr. Willy Burgdorfer’s EM Lab, Rocky Mtn. Lab, NIH, NIAID

Specimen provided

By

Alan B. MacDonald, MD
Membrane – surrounds Granular *Borrelia*
Granular *Borrelia* – Containing *Borrelia* DNA – WHAT?

**INDIVIDUAL MOLECULES**
- DNA
- INSIDE OF
- GRANULES
- FROM
  - *BORRELIA BURGDORFERI*

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**IMAGE**
Dr. Claude Garon
Rocky Mtn. Lab.
NIH, NIAID

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Other Strands in the Photo Are:
Linear DNA

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Granular *borrelia* – ALIVE? – What??

Granules {A} [B] with emerging spiral *borrelia* in BSK – Pure Culture – *Borrelia burgdorferi*
Granular *Borrelia* – Alive??- WHAT???

**Pure Culture**

*Borrelia burgdorferi*

**Erythema Migrans**

**Skin biopsy**

Drs. B. Berger, & A. MacDonald
Granular *Borrelia* – Alive?? What??
Biofilm Communities of *Borrelia burgdorferi* – grown in vitro from pure cultures of *Borrelia burgdorferi* ATCC 35210 American Type Culture Collection

Image credit: Alan B. MacDonald, MD
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Phase Contrast Microscopy
1000x original magnification
Note: Granular *borrelia* and Cystic *borrelia* are demonstrated in abundance indicating SPECIALIZATION by microbes from the Motile Spiral form of *Borrelia*
Return to TOPIC:

Granular *Borrelia* Biofilms Alive, inside of Amyloid Alzheimer’s’s Plaques
Images Paired - ( [A] and [B] )

[A] = Amyloid Stain

[B] = Biofilm of Granular *Borrelia*

Membrane Bound DNA

Extracellular DNA
Conclusion: *Borrelia Miyamotoi* DNA (Miya Flagellin B) is abundant INSIDE of an Amyloid Plaque in Alzheimer’s Disease—based on highly specific Fluorescence In Situ DNA Hybridization (FISH method) with Molecular Beacon DNA probe for *Borrelia Miyamotoi* Flagellin B DNA [in image B].
Green color

Thioflavin T Stain for Amyloid - Alzheimer Amyloid plaque

DNA Probe with Cy5 label - Specific for DNA of *Borrelia miyamotoi* (Red/White/Pink colors)

Note: Amyloid Region In Green - thioflavin T stain contains a *Non-borrelia miyamotoi* Spirochete(Green)
Alzheimer Amyloid Plaque stained with Thioflavin T Stain
Amyloid is bright Green Color
Note:
Voids inside of the Green plaque do not stain for Amyloid
These are present in Image [A] and (B) and are characteristic of the “empty spaces” inside of Amyloid Alzheimer’s Plaques

Alzheimer Amyloid Plaque stained with Borrelia Miyamotoi DNA Probe -Cy5 (red color)
Note:
Voids inside of the Plaque are again demonstrated, as in figure [A]
Multiple DNA Hybridizing Granular points are present throughout the plaque. These are not Amyloid [as in A] but rather are Granular borrelia forms which are very characteristic of the closely packed specialized Borrelia miyamotoi forms in a biofilm community.
Gallery of Amyloid Plaques and indwelling *Borrelia* Biofilm communities (3)

Note: No Water Channels seen in this plane of View: [A] [B]

**A**  
**Thioflavin T stain**  
Amyloid stains green color

**B**  
DNA probe binds to [hybridizes with] only *Borrelia Miyamotoi* DNA (red/pink/white)  
Excess of Granular *Borrelia* typical of specialized *Borrelia* inside of a borrelia biofilm community

Note: No Water Channels seen in this plane of View: [A] [B]
**Thioflavin T stain for Amyloid**

**DNA Probe (Molecular Beacon for *Borrelia Miyamotoi* Flagellin B DNA**

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Note: Wide Caliber Water channel Spaces Inside of the Biofilm Community of Granular *Borrelia*

--All dwelling INSIDE of the Amyloid Plaque
Congo Red Stain
Amyloid Plaque
Alzheimer’s Disease
40x objective - White polarized Light

Alzheimer’s Amyloid Plaque
DNA (Molecular Beacon) Probe
unique for
*Miyamotoi Borrelia* DNA (FlagellinB-Cy5 Fluor)
(Red colors and Red orange regions -
Dark Red-Extracellular DNA
Bright Orange/Red : DNA in Membrane
bound Living *Borrelia* forms - a Lace-like
Arrangement of *Borrelia* inside the biofilm Community

Dual Staining of Alzheimer Plaque (1)
Amyloid Alzheimer Plaque
Thioflavin T Amyloid Stain
(Green Color)

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Note: Bright Green Dot like structures; closely spaced inside of the Rounded Plaque of Green Staining Amyloid

Biofilm of *Borrelia*
showing Closely spaced Dot-like Structures which contain *Borrelia* DNA
(Miyamotoi borrelia DNA Beacon
Probe is positive with bright signals in the Granular *Borrelia* forms and less bright BUT well defined *Borrelia miyamotoi* DNA free and not membrane bound in the Extracellular MATRIX OF THE BIOFILM COMMUNITY.

Amyloid Stain {A} DNA Probe {B}
Amyloid Plaque of Alzheimer's Disease
(Congo Red Stain - White light - with Polarization)

DNA Probe specific for *borrelia miyamotoi*
DNA
Hybridizes (binds to) Borrelia DNA in the

Amyloid Plaque-AlzDis.: DNA Probe{B} +++
Experimental Methods:
1. Autopsy confirmed diagnosis – Alzheimer’s CERAD criteria, Immunostains B1-42, pTAU
2. Recut Glass slides – Hippocampus
3. Molecular Beacon Dna Probes:
   In situ DNA Hybridization [FISH method]
4. Amyloid Stains performed (CR or Tt stain)
5. Control slides run in parallel
6. Photomicrographs: Locked Field of View
   Amyloid Plaque [A] / Borrelia by Dna probe [B]
Microscopic Study of 100 consecutive Alzheimer’s Disease Amyloid plaques Demonstrated that 100/100 Contained Biofilms of *Borrelia* Dwelling Inside of the topographies Of each of the Amyloid Plaques
Borrelia in LIVE/Living Biofilm communities
Has a natural life
History very Similar to the life history of Amyloid Alzheimer’s Plaques:
Conclusion:
All Alzheimer’s Plaques Studied contained

1. Amyloid- [as expected]
2. Biofilms of Borrelia DNA { not expected}

And

The DNA of the Borrelia was
Biofilm DNA – Living- membrane Bound type
(living) Borrelia[ granular form/ cylinder form]
And extracellular DNA ( from once living but
Now DEAD Borrelia- forming Extracellular Matrix)
Biofilm community of pure culture of *Borrelia burgdorferi* (in vitro) showing Cystic forms [arrows], mixed with granular forms (dot like *red dots or blue dots*), all embedded in an Extracellular matrix.
Biofilms:
General Truths:
1. Biofilms represent a survival Adaptation of 99.9% of Microbes
2. In tissues: Biofilm formation: Always Indicates CHRONIC Infection
3. Early Biofilms are small in diameter, Late biofilms are large in diameter.
Growing over time: Plaques and Biofilms

As Years elapse:

Living Biofilm communities Grow in Size, and Increase in Complexity of Architecture
Alzheimer plaques - google

Borrelia Biofilm Units
Old biofilm Communities Are Traversed by A system of Water Canals (providing nutrient supply and Providing Waste product Removal)
A biofilm community: Empty Spaces : have a Name!!
“Water Channels”
Plumbing System for Biofilms

“Empty Spaces” : Voids
Also traverse the Alz.Dis.
Plaques { small, medium, large}
3 Dimension Reconstruction of Plaque

Plaque of Alzheimer’s Disease:
A Mix of Solid and Empty spaces
An Alzheimer’s Amyloid Plaque: Silver stain provides a Brown color in the Plaque: Dot-like Yellow empty spaces Inside of the Alz.Dis. Plaques Show the underlying YELLOW Healthy brain tissue
Silver Stain Of Alz DisAmyloid Plaque

[Brown color]

Healthy Brain tissue stains Yellow:
Diseased Brain with Alzheimer’s Plaque stains BROWN

Yellow areas INSIDE of the ALZ DIS Plaque are Empty Spaces –revealing the underlying Healthy Yellow Brain tissue Through these empty spaces [Blue arrows]

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Empty Spaces resembling Water Channels in biofilm Structural Units
Water channel formation in a biofilm

Biofilm community of pure culture of borrelia burgdorferi [in vitro] demonstrating water channel formations with corresponding water channel graphic of biofilm community of pure culture of borrelia burgdorferi [in vitro].
Alzheimer Amyloid Plaque
Stained with Thioflavin T Stain
Amyloid is bright Green Color

Note:
Voids inside of the Green plaque
do not Stain for Amyloid
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are present throughout the plaque.
These are not Amyloid [as in A] but rather are Granular borrelia forms
which are very characteristic of the closely packed specialized Borrelia
miyamotoi/forms in a biofilm community.
Alzheimer’s Plaque: 3 Dimensional Schematic View Showing Empty Spaces
Alzheimer’s Disease: A chronic biofilm type Of…… LIVING Borrelia Infection In Human Brain:
An Anecdotal Case Report – Just one!!
Dementia – What makes it Alzheimer’s

Neurofibrillary tangle  Normal pyramidal neuron
Dr Kidd

Discovers that
Tangles are Not “corpses” of
Previous Microtubules
Über eine eigenartige Erkrankung der Hirnrinde A. Alzheimer (1907)

Allg. Zeitschrift Psychiatr. 64: 146-148
Biofilm Community of *Borrelia*

: Alz.Dis : Fingerprint Preparation : antibody H9724

Specific for *Borrelia* Species including *Miyamotoi*, *Burgdorferi*, and Relapsing types

DNA in Plaques

DNA Hybridization

A “water balloon” containing Granules

Monoclonal Antibody for The Flagellin protein of Borrelia

Developed by Dr Alan Barbour
Borrelia to Tangles

Infectious Agent Inside of the Nerve cell

Inside of the cell Infection

Borrelia spirochetes inside
Hippocampal neurons in Alzheimer’s disease
Fig 1. Association of *Borrelia burgdorferi* with human cells in vitro. Confocal microscopy images of human cells incubated with *B. burgdorferi*. Human cell plasma membranes were immunostained red, and *B. burgdorferi* were labeled green according to Section 2, with the 2 images being merged whereby the yellow indicates co-localization of *B. burgdorferi* with the cellular membrane. Images represent the entire Z-stack of the sections. Panels: (A) human umbilical vein endothelial cells; (B) H4 human neuroglial cells; (C) HS-683 human neuroglial cells; and (D) human cortical neurons.
Invasion of human neuronal and glial cells by an infectious strain of *Borrelia burgdorferi*

Jill A. Livengood, Robert D. Gilmore Jr.*

*Centers for Disease Control and Prevention, Division of Vector-borne Infectious Diseases, 3150 Rampart Road, CSU Foothills Campus, Fort Collins, CO 80522, USA*

Received 13 June 2006; accepted 30 August 2006
Loss of Tau = Loss of Tubules
Herpes Zoster
“Shingles”

Dormant Virus from Childhood Chicken Pox
Stays hidden inside of Nerves for 50-80 years
and Reactivates to “March” down Nerve pathways
Trans-Synaptic Neuroborreliosis

2 Alzheimer’s neuroborreliosis with trans-synaptic spread of infection and neurofibrillary tangles derived from intraneuronal spirochetes

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Braak Stages of Alzheimer’s

Fig. 3. Stages V–VI of cortical neurofibrillary changes of the Alzheimer ty

stage VI

Heschl’s gyrus

stage IV

transentorhinal region

entorhinal region

1 cm

stage II

Alzheimer’s and Parkinson’s diseases

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Escalator Schema for Infection movements through neural Circuits
Braak Stages of Alzheimer’s Redefined as Infection in Neural Networks
Progression of Stages in Alzheimer’s Disease
Opportunities for Improvement in Alzheimer’s Patient Care

Contributions from the Molecular version of the Autopsy
Braak Stages of Alzheimer’s

Normal
Brain
Size
On Left

Alzheimer’s
Disease
Brain size
On Right

Ellison & Love: Neuropathology 2e © 2004 Elsevier Ltd.
Celebrity?? : possibly a Michael Strahan –Look-alike?

GOT

MILK

???????
Everyman: Skeptical then and now

Got It ????
Got Dementia??

What

Are we going to do about it??

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Please Bring the next Case For Diagnostic Evaluation
Lecture Ends Here

Now.. Connect the Dots...
Please..!

Dr K. Eisendle
Acrodermatitis Chronica Atrophicans
Immunohistochemistry

“Granular forms of B burgdorferi in a “colony”
With a “Reddish veil”
Patients in various states of misery, at the Ohio Insane Asylum. The photo was taken in 1946. Psychiatric medicine improved in leaps and bounds during the intervening decades.