Potential Connection of Borrelia Infection and Breast Cancer

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Borrelia burgdorferi the spirochete that causes Lyme disease

- In 1982, the etiologic agent of Lyme disease was discovered by Willy Burgdorfer who isolated spirochetes belonging to the genus Borrelia from the mid-guts of Ixodes ticks.
- He showed that these spirochetes reacted with immune serum from patients that had been diagnosed with Lyme disease. Subsequently, the etiologic agent was given the name Borrelia burgdorferi.
Let’s look at *Borrelia* exposed to penicillin
The most recognized forms of *Borrelia burgdorferi*

- Spirochetes
- Round bodies (cysts, granules)
- Attached biofilm

Photos by:
- Pabbati N MS
- Luecke DF MS
- Socarras K MS
Evaluation of in-vitro antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*

**Table 1** MIC and MBC determination by different methods

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Microdilution method/literature data (MIC) µg/mL</th>
<th>Our data (MIC) µg/mL</th>
<th>Microdilution method</th>
<th>Direct cell counting</th>
<th>BacLight™ staining</th>
<th>Microdilution method/literature data (MBC) µg/mL</th>
<th>Our data (MBC) µg/mL</th>
<th>Direct cell counting</th>
<th>BacLight™ staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>0.06–2.00</td>
<td>0.4</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>0.25–6.40</td>
<td>0.25</td>
<td>25</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.006</td>
<td>0.015</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>0.05</td>
<td>0.125</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.03–2.00</td>
<td>0.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&lt;0.03–32.00</td>
<td>5</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.06–32.00</td>
<td>0.3</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>&gt;4</td>
<td>10</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Tinidazole</td>
<td>–</td>
<td>0.09</td>
<td>&gt;62.5</td>
<td>&gt;62.5</td>
<td>&gt;128</td>
<td>10</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>
Effect of antibiotics on the aggregates of Borrelia measured LIVE/DEAD staining

Sapi E et al 2011

Red stain: Dead
Green stain: Viable
What is biofilm?

- collections of microorganisms (bacteria, yeasts or protozoa) that form on a hard surface (exception floating biofilms)
- examples: plaque that forms on teeth and the slime that forms on surfaces in watery areas (shower)
- surrounded by slimy secretions: mucoid polysaccharide structure which attaches the community to a surface
- estimated that over 90% of bacteria live in biofilm (late Costerton WJ)
Problems caused by biofilms

• Can form almost anywhere that water is present, including catheters, kitchen counters, showers, water pipes etc.
• Damage to industrial equipment
• Contamination of food, pharmaceutical and medical products
• Energy loss through inefficient energy transfer
• Medical infections and super-resistant to antibiotics
Borrelia biofilm

Sapi E et al PLoS ONE 2012
Alginate on the surfaces of *Borrelia* aggregates

Sapi E et al 2012 PLoS ONE
Can Borrelia biofilms exist in vivo?

• Double immunohistochemical staining with Borrelia and biofilm specific markers
• In situ hybridization confirmation experiments
• PCR/Whole genome sequencing confirmation

Samples:
  – Skin biopsies from Borrelia infected skin lesions
    – (Dr. B. Zelger)
  – Autopsy tissues from a Lyme disease patient
    – (Dr. K. Leigner and Dr J. Goldman)
EVIDENCE OF IN VIVO EXISTENCE OF BORRELIA BIOFILM IN BORRELLIAL LYMPHOCYTOMAS

E. Sapi¹,*, K. Balasubramanian¹, A. Poruri¹, J. S. Maghsoudlou¹, K. M. Socarras¹, A. V. Timmaraju¹, K. R. Filush¹, K. Gupta¹, S. Shaikh¹, P. A. S. Theophilus¹, D. F. Luecke¹, A. MacDonald¹, B. Zelger²

¹Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA
²Department of Dermatology and Venereology, Medical University Innsbruck, Innsbruck, Austria
Double immunostaining: Borrelia and alginate antibody from Skin biopsies from Borrelia Lymphocytoma

Sapi E et al 2016
A

DIETERLE SILVER STAINING

i

ii

iii

iv

Sapi E et al 2016

B

SILVER

BORRELIA

NO ANTIBODY

DIC
Additional IHC and *in situ* hybridization data

Sapi E et al 2016
Borrelia biofilm in infected skin tissues

Sapi E et al 2016
So could other pathogens exist in these tissues?

Velica, DevianArt
Metagenomic analyses for Borrelia Lymphocytoma Tissues

Genomic DNA analyzed by whole genome sequencing method (Perkin Elmer). Reads were aligned to human reference sequences using Burrows-Wheeler aligner software (http://bio-bwa.sourceforge.net). After Sequence Alignment/Map (http://samtools.sourceforge.net) tool was used to filter out reads not mapped to human. The remaining reads were aligned to bacterial reference genomes using Blast program (NCBI).

Reads *Borrelia burgdorferi* sensu lato, Reads for *Chlamydia spp*

Sapi E et al 2019
Borrelia  Chlamydia  Alginate  DIC

Immunohistochemistry

confocal microscopy

Borrelia  Chlamydia

Z STACKS

TOP  BOTTOM

Borrelia  Alginate

Z STACKS

Sapi E et al 2019
Mixed Biofilm in Other Infected Skin Tissues

Article

Mixed Borrelia burgdorferi and Helicobacter pylori Biofilms in Morgellons Disease Dermatological Specimens

Marianne J. Middelveen 1, Katherine R. Filush 2, Cheryl Bandoski 2, Rumanah S. Kasliwala 2, Anthony Melillo 2, Raphael B. Stricker 3,* and Eva Sapi 2
Mixed Biofilm in Infected Skin Tissues

**Figure 4.** Representative IHC images showing biofilm aggregates in MD skin sections stained for *Borrelia*, *Helicobacter* and alginate. IHC detection was performed as described in the Methods section. Panels A, F and K show skin sections treated with anti-*Borrelia* monoclonal antibody (green). Panels B, G and L show skin sections treated with anti-*Helicobacter* antibody (red). Panels C, H and M show skin sections treated with anti-alginate antibody (blue). Panels D, I and N show negative control sections treated with non-specific IgG. Panels E, J and O show sections imaged with DIC. Images were taken at 200x magnification. Scale bar = 100 μm.

Middelveen MJ et al 2019
Amyloid Changes in Mixed Borrelia Biofilms

(Middelveen MJ et al 2019)
Mixed Biofilm and Phospho-Tau in Infected Skin Tissues

Middelveen MJ et al 2019
Borrelia in Mixed Biofilms


Where else you can find Borrelia biofilm in the human body?

Hoiby N et al 2014
Borrelia Biofilm in Human Brain from in a Lyme disease /Alzheimer patient Correlates with Amyloid Changes

Senejani et al 2022
Autopsy tissues from Columbia University
Dr. James Goldman and Dr. Kenneth Liegner

• A 53-year-old woman with a 16-year history of Lyme disease who initially presented with headaches, fevers, fatigue, memory loss, cranial nerve palsies and gait disturbance and later developed progressive spastic quadriparesis and died despite the multiple antibiotic treatment

• Autopsy tissues from liver, heart, brain, kidney, lung, lymph node arrived from Columbia and the study was funded by:
CULTURE-CONFIRMED TREATMENT FAILURE OF CEFOTAXIME AND MINOCYCLINE IN A CASE OF LYME MENINGOENCEPHALOMYELITIS IN THE UNITED STATES.

Kenneth B. Liegner, Carl E. Rosenkilde, Grant L. Campbell*, Thomas J. Quan, and David T. Dennis, Armonk, NY, USA, Mount Kisco, NY, USA, and Centers for Disease Control, Fort Collins, CO, USA.

In 1987, a 37-year-old woman living in Westchester County, NY, developed spastic paraparesis, bilateral Babinski reflexes, and cranial nerve and bulbar dysfunction characterized by dysphagia, dysphonia, diplopia, absent gag reflex, and dysfunction of bowel and bladder control. CSF contained 19 WBC/mm³ (86% lymphs). A test for antibodies to *Borrelia burgdorferi* (*Bb*) in serum was negative. No etiology was established despite an extensive workup. Symptoms and signs reportedly worsened gradually from 1988 to present. There was a past history of splenectomy for idiopathic thrombotic purpura diagnosed in 1975. In 1989, the right frontal region and right basal ganglia were abnormal on brain MRI. In January 1990, CSF contained 6 WBC/mm³ (93% lymphs), but no oligoclonal bands or myelin basic protein. Paired CSF and serum tests for antibodies to *Bb*, and PCR for *Bb*-specific oligonucleotides in CSF, were negative. An empiric 21-day course of cefotaxime (3 g/12 hr i.v.) was given in January, 1990 with no clear clinical benefit. Following treatment, CSF contained 9 WBC/mm³ (93% lymphs). Four months of minocycline (200 mg/day p.o.) begun in November, 1990 also yielded no clear clinical benefit. In December, 1990 a T-cell stimulation test with *Bb* antigens was strongly positive. In December, 1991 CSF contained 6 WBC/mm³ (89% lymphs) and elevated IgG. Paired serum and CSF samples were strongly positive for antibodies to *Bb*, with a CSF-to-serum index of 1.04. Culture of this CSF specimen in BSK-II yielded a strain of *Bb*. Culture-confirmed treatment failures have been previously reported for three Lyme neuroborreliosis cases in Europe. The present case apparently is the first of this type to be reported from the United States.
Liver autopsy tissues examined for Borrelia

BORRELIA POLYCLONAL

BORRELIA MONOCLONAL

ALGINATE

NON-SPECIFIC IgG

DIC

H&E

Sapi E, Goldman J, Liegner K - unpublished data 2019
Immunohistochemical analyses of four different organs for Borrelia and for alginate (biofilm marker): Heart, Kidney, Liver, CNS.

Sapi E et al 2019
# Quantitative Analysis of Borrelia biofilms in liver, brain, heart and kidney tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th># Slides</th>
<th># Biofilms/Slide</th>
<th>Size (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>150</td>
<td>0-6</td>
<td>20-300</td>
</tr>
<tr>
<td>Brain</td>
<td>210</td>
<td>0-3</td>
<td>20-100</td>
</tr>
<tr>
<td>Heart</td>
<td>130</td>
<td>0-4</td>
<td>20-100</td>
</tr>
<tr>
<td>Kidney</td>
<td>145</td>
<td>0-4</td>
<td>20-100</td>
</tr>
</tbody>
</table>

*Sapi E, Goldman J, Liegner K 2019*
Borrelia biofilm presence corresponded with infiltrating T cells in kidney and liver tissues – inflammation response?

Sapi E, Goldman J, Liegner K 2019
Borrelia Spirochetes and Biofilms in the Heart

Sapi E, Goldman J, Liegner K 2019
Borrelia biofilm presence corresponded with inflammatory markers

Table 2 Expression of inflammatory mediators present in brain, heart, kidney and liver tissues (+++: high expression, ++: medium expression, +: minimal expression, -: no expression).

<table>
<thead>
<tr>
<th></th>
<th>CD3</th>
<th>CD8</th>
<th>CD20</th>
<th>CRP</th>
<th>CXCL-9</th>
<th>CXCL13</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Heart</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Kidney</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Liver</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Sapi E, Goldman J, Liegner K 2019 unpublished data
In summary, these results show that Borrelia burgdorferi OMVs serve to directly counter superoxide production in BE2C neurons, thereby ‘priming’ the host environment to support B. burgdorferi colonization

Effect of *Borrelia burgdorferi* Outer Membrane Vesicles on Host Oxidative Stress Response

by Keith Wawrzeniak, Gauri Gaur, Eva Sapi and Alireza G. Senejani *

Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA

* Author to whom correspondence should be addressed.

*Antibiotics* 2020, 9(5), 275; [https://doi.org/10.3390/antibiotics9050275](https://doi.org/10.3390/antibiotics9050275)
Borrelia-host interaction

- Neuron cells (BE2C) infected with Borrelia at
- 30 min (A),
- 2-hour (B), and
- 24-hour (C) time points.

Wawrzeniak K et al 2020
Where else can we find Borrelia – *Neoplastic Tissues*?
Breast cancer pilot study

• **OBJECTIVE:** 200 invasive breast cancer samples and 20 from healthy breast tissues evaluated for Borrelia burgdorferi presence by immunohistochemical methods

• **RESULTS:** Significant numbers of invasive breast ductal carcinoma and lobular invasive breasts carcinomas were positive for the presence of *Borrelia burgdorferi* spirochetes and biofilms while all of the fibroadenomas or the healthy control tissues were negative.

Gauri G and Sapi E 2021 unpublished data
Borrelia inside of breast cancer cells?

Gauri G and Sapi E 2020 unpublished data
Borrelia inside of breast cancer cells

Gauri G and Sapi E 2022
According to American Cancer Society - 15% of all cancers are caused by infectious agents

- *Helicobacter pylori* - Gastric cancer
- *Streptococcus bovis/gallolyticus* - Colorectal carcinoma
- *Salmonella typhi* – Gallbladder carcinoma
- *Chlamydia pneumonia, Mycoplasma sp.* - Lung cancer
- *C. pneumonia, C. trachomatis, C. psittaci* - Pulmonary Mucosa-Associated Lymphoid Tissue (MALT) lymphoma
- *Mycoplasma sp., C. trachomatis* - Ovarian cancer
- *Staphylococcus epidermidis, Escherichia sp. Bartonella sp.* – prevalent in breast cancer
Commensal Microbiota Promote Lung Cancer Development via $\gamma\delta$ T Cells

Graphical Abstract

Authors
Chengcheng Jin, Georgia K. Lagoudas, Chen Zhao, ..., Paul C. Blainey, James G. Fox, Tyler Jacks

Correspondence
tjacks@mit.edu

In Brief
Lung cancer development is associated with increased bacterial burden and altered bacterial composition in the lung. Depletion of microbiota or blockade of the downstream cellular or molecular immune mediators significantly suppress lung tumor growth.
 Spirochete bacteria like *Borrelia* and *Treponema* associated with different types of breast cancer

Distinct microbial communities that differ by race, stage, or breast-tumor subtype in breast tissues of non-Hispanic Black and non-Hispanic White women

Alana Smith, Joseph F. Pierre, Liza Makowski, Elizabeth Tolley, Beverly Lyn-Cook, Lu Lu, Gregory Vidal & Athena Starlard-Davenport

Scientific Reports 9, Article number: 11940 (2019) | Cite this article
Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer

Fu A et al - Cell 185, 1356–1372, 2022

Bacteria living inside tumor cells promote cancer metastasis by helping cancerous host cells against mechanical stress in the bloodstream, in turn promoting cell survival during tumor progression.

Antibiotics treatment reduced tumor metastasis more than 3-fold!
Gaylord, 1907 – *in vivo* study suggests a potential connection between spirochetes and primary breast cancer in mice
HYPOTHESIS

*Borrelia burgdorferi* plays a role in tumorigenic changes in breast epithelial cells
Effect of Borrelia on normal and breast cancer cells invasion

• Gauri G et al 2021
Effect of Borrelia infection on Breast Cancer Markers

Gauri G et al 2021
Current Projects:
*Identifying pathways and searching for markers in Borrelia infected cells*

- Molecular analyses of Infected Breast Cancer and Normal Epithelial Cells
  - RNAseq and tumor panel analyses
  - MicroRNA analyses
  - Tissue remodeling factors
  - Chemotherapeutic resistance markers
Current Projects:
New models for antibiotic studies

Ex vivo culture systems:
skin biopsies (see left)
heart valve cultures

Torres J, Sapi E 2019

Zebrafish model
Antimicrobial Testing in Zebrafish
Borrelia burgdorferi does have an antibiotic resistance form – called biofilm - which can reside in human infected tissues.

Biofilm form provides a very effective refuge strategy from antimicrobial treatments.

Borrelia can infect various of tissues and can make changes in the host cells physiology.

Borrelia can enhance cancer cell invasion.
UNH Lyme and Breast Cancer Disease Research Group
Special Thanks To:

• University of New Haven and College of A&S for funding our studies
• Philanthropic Trust, Lyme Warriers, LivLyme Foundation, Global Lyme Alliance, ILADS, Lymedisease.org, Lyme Disease Association, CT LymeRiders, for supporting our research projects
• Pink Clover Foundation and Philanthropic Trust to support our breast cancer research initiatives
• Lymedisease.org, Lyme Disease Association, Schwartz Foundation and Global Lyme Alliance for providing a “state of the art” microscopes for our morphological studies
Question?

• Please call me or send me an email:

  - esapi@newhaven.edu
  - 1-203-479-4552