

# International DERMATOLOGY Molecular Dermatopathology of LYME BORRELIOSIS

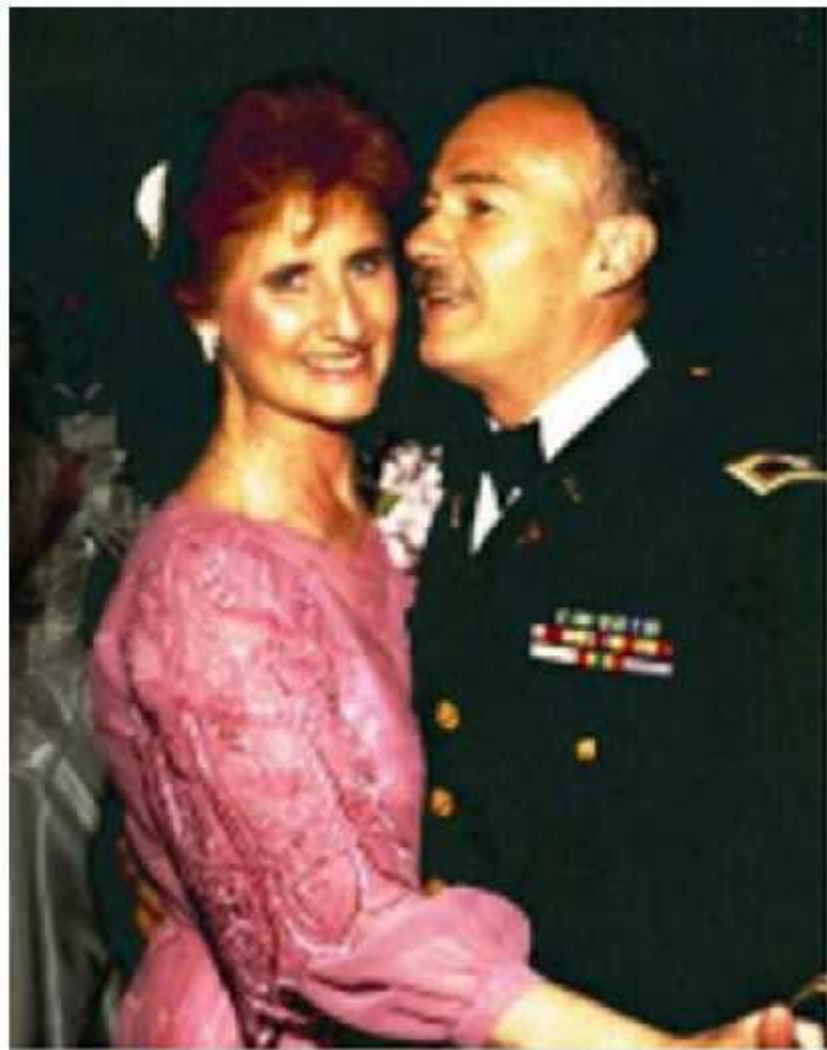
**Where in the World are Borrelia Dermatoses “at”?**



Alan B. MacDonald MD

Research Associate

**Dr Eva Sapi Borrelia Research Laboratory, University  
of New Haven, West Haven, Ct.**



*Colonel Paul Harrison  
Duray Sr., MD*

# Lyme Connecticut Problem



**WORLDWIDE  
PROBLEM**

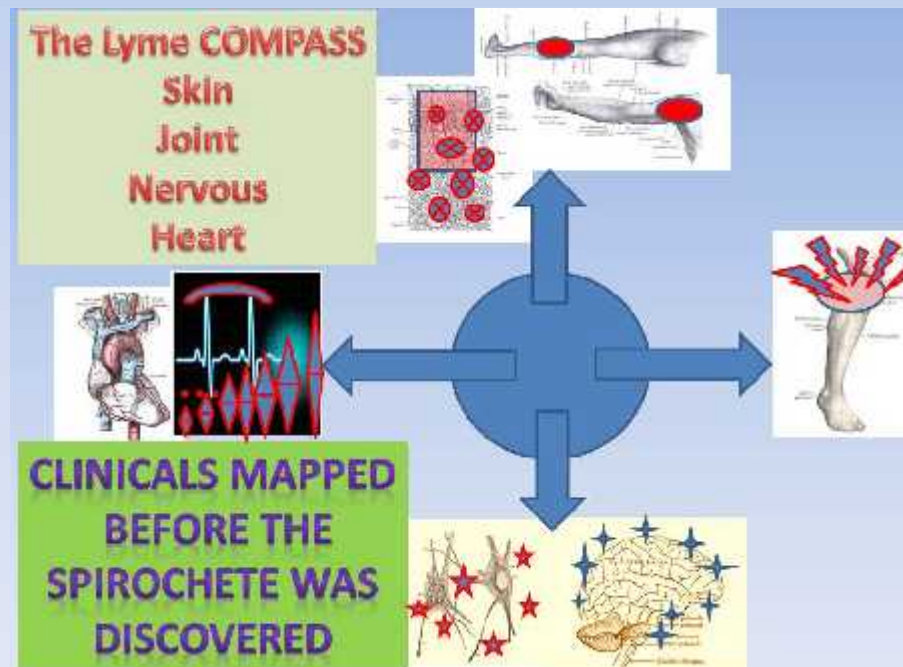
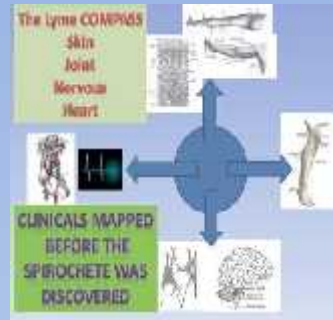
# Navigation

## MAPS

### A clinical compass

Textbooks , clinical experience

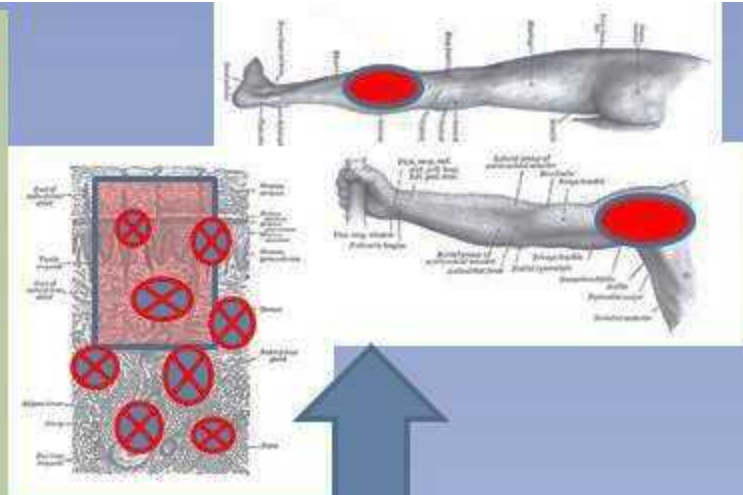
## Last century Guidance System



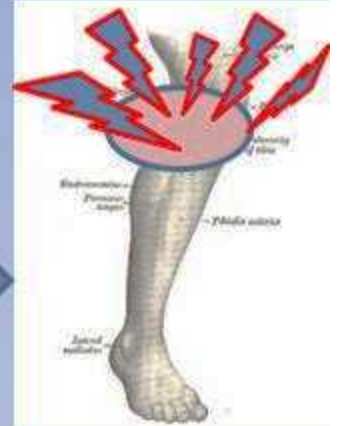
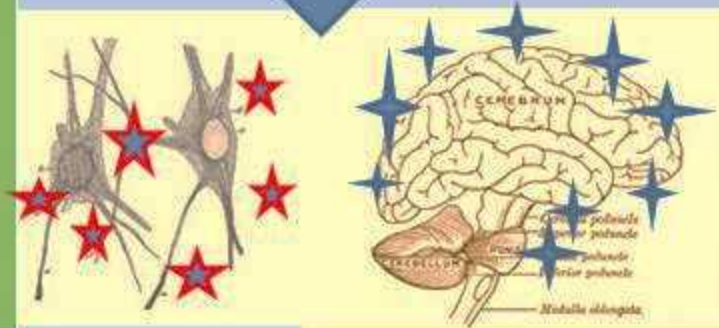


# The Lyme COMPASS

Skin  
Joint  
Nervous  
Heart



CLINICALS MAPPED  
BEFORE THE  
SPIROCHETE WAS  
DISCOVERED



# YEAR 2012 – NAVIGATION SYSTEM IMPROVEMENTS

Worldwide maps



An Expanded Cutaneous Atlas  
A Molecular Diagnostic toolbox

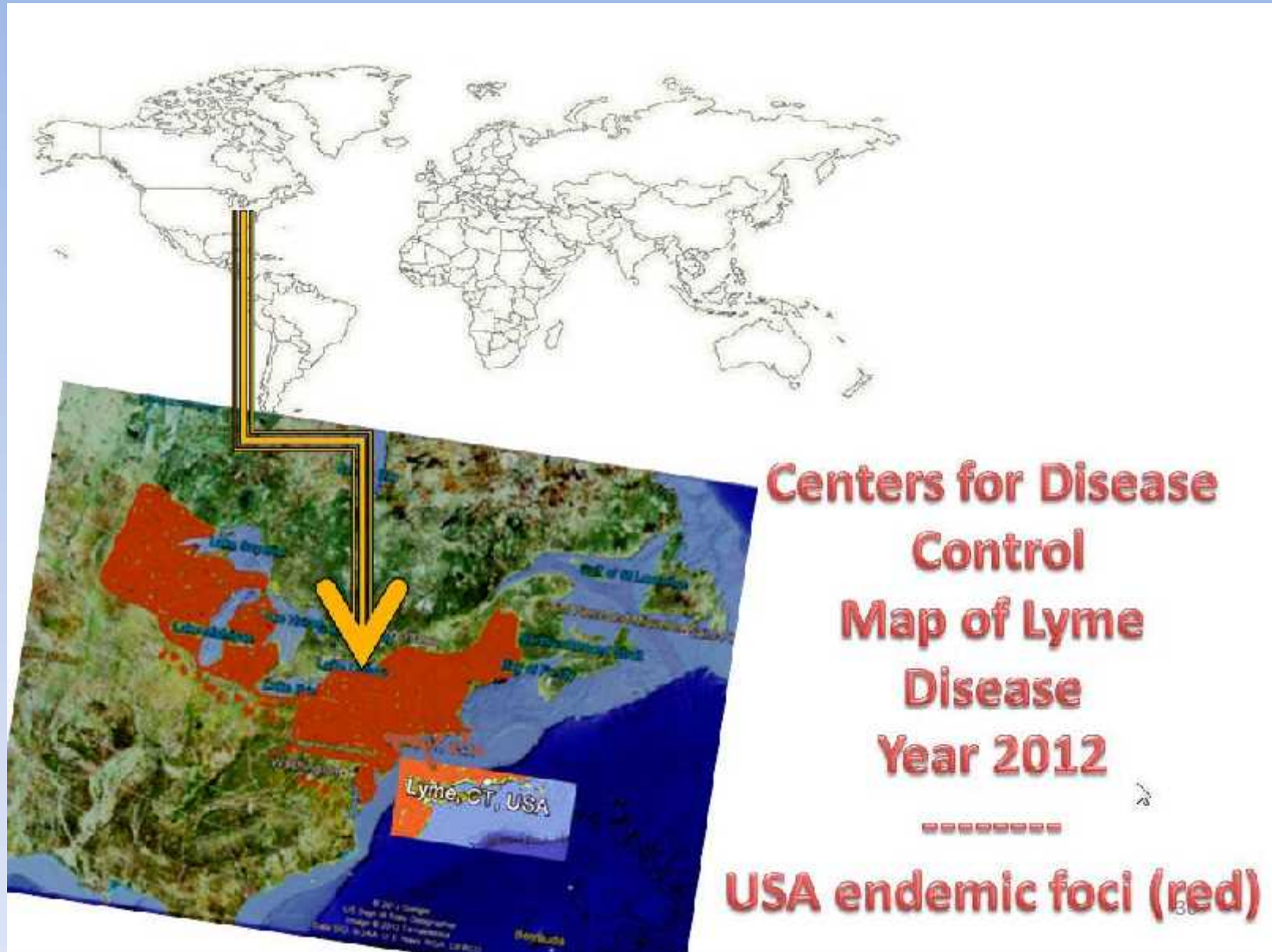


**LYME BORRELIOSIS**  
**WORLD WIDE**  
**EXTENSION\**  
**BETTER DIAGNOSIS**







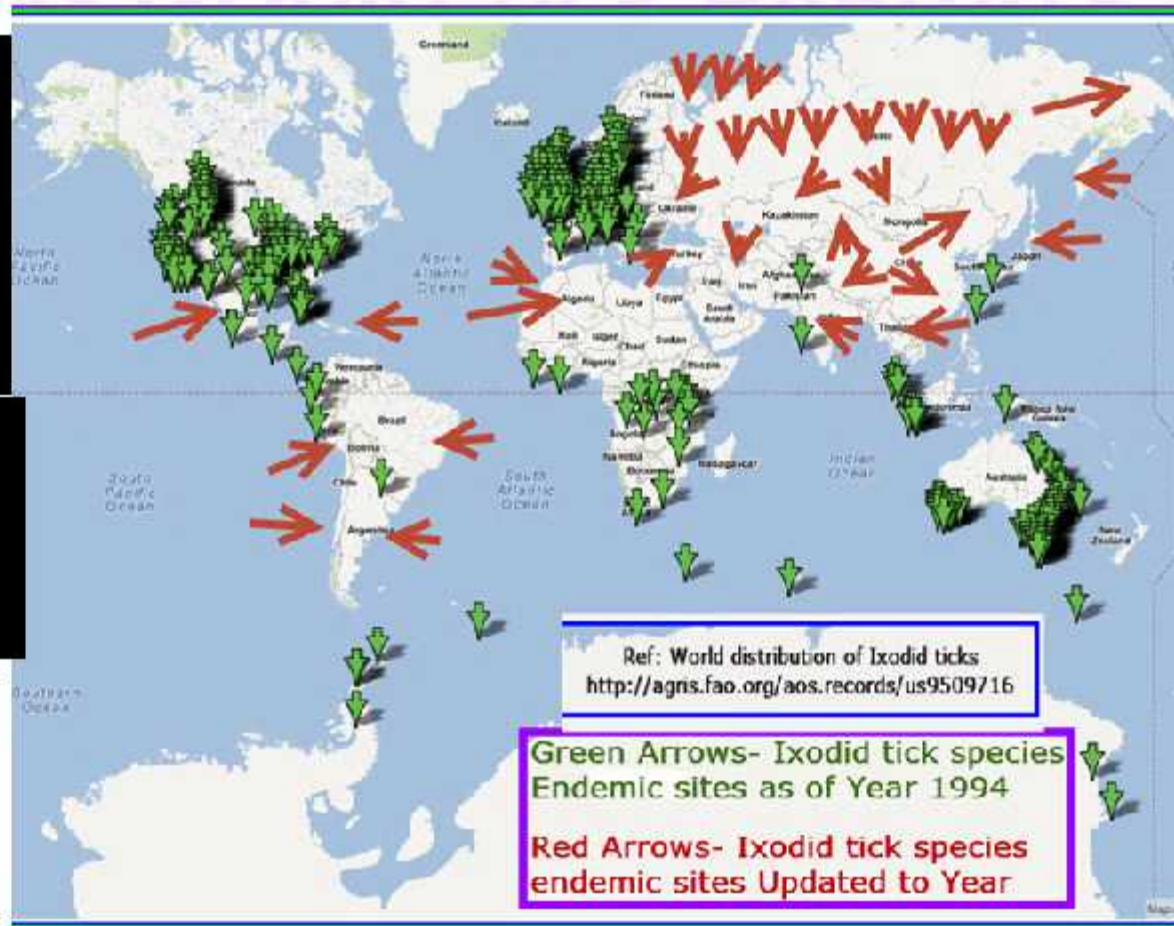


# VARIOUS IXODID TICK HABITATS

**30,000 CONFIRMED  
CDC Lyme cases  
In USA year 2009  
( with some  
alternate Estimates  
exceeding 100,000  
cases)**

**216,000 Lyme cases  
Germany per annum  
2007 (estimated  
up to 750,000 for  
year 2009)**

**20,000 cases  
China  
Per annum  
(estimate)**





**Updated to Year 2012**



**Figure 13.** Geographic distribution of pathogenic bacteria of *Borrelia burgdorferi* complex (pathogenic role of *Borrelia valaisiana* is highly suspected)

# ANATOMY OF IXODID TICK BITE SITE

Image of the Tick “feeding cavity”

Erythema Migrans : **Leukocyte infiltrations (Diverse patterns)**

**Erythema Migrans Model:** Some biopsies demonstrate **Lymphocyte-Rich Inflammatory infiltrates** ( factoring in the time interval from tick bite date to date of biopsy. Other skin biopsies( with equal time interval) Demonstrate **virtually NO LYMPHOID tissue infiltration.....**

**Neutrophilic ( pustular) infiltrates)** of EM lesions are not typical – **suspect concurrent Co-Infection when seen.**

**Eosinophilic infiltrates** are sporadically seen in EM skin biopsies.

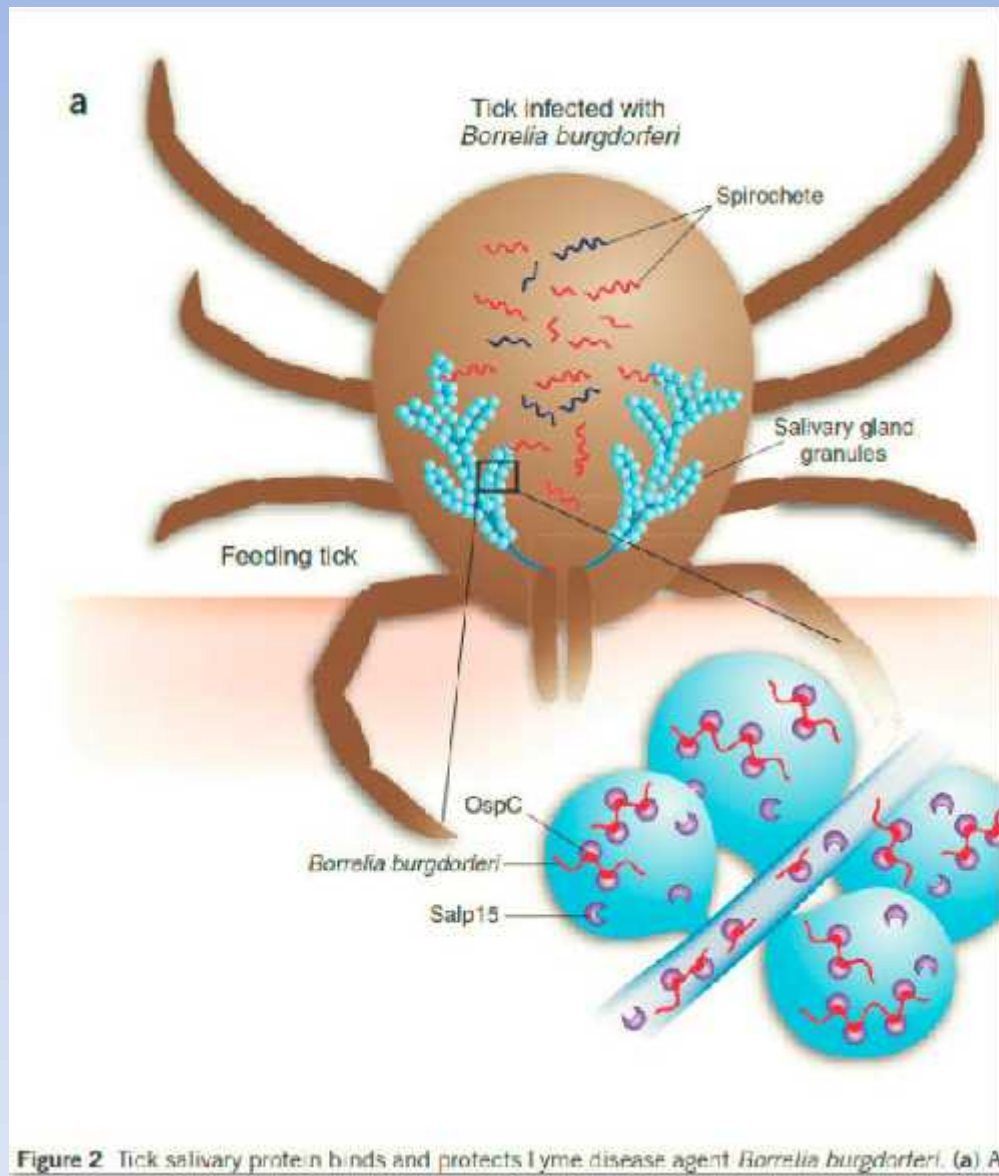
**Mature Plasma cells** are often admixed with lymphocytes in EM Skin biopsies ( ? Indicative of ongoing humoral immune response to infectious microbes and their concomitant debris”

**Follicles of Lymphoid cells (Germinal centers)** may be seen, but usually occur in the diagnosis of Borrelia Lymphocytoma [BL] (Lymphadenosis benigna cutis [LBC])

Image Gallery: E Migrans, B. Lymphocytoma, ACA

Links: Supplementary materials and manuscripts





**Image Credit:  
Patricia Rosa PhD  
et al  
Rocky Mountain  
Laboratory  
NIAID, NIH  
Hamilton, MT**

**Early Ixodid Tick bite site  
Skin of Neck**



**Photo Credit: Alan B. MacDonald MD**

**Photo credit:**  
**Alan B. MacDonald MD**  
**Copyright**  
**All rights reserved**

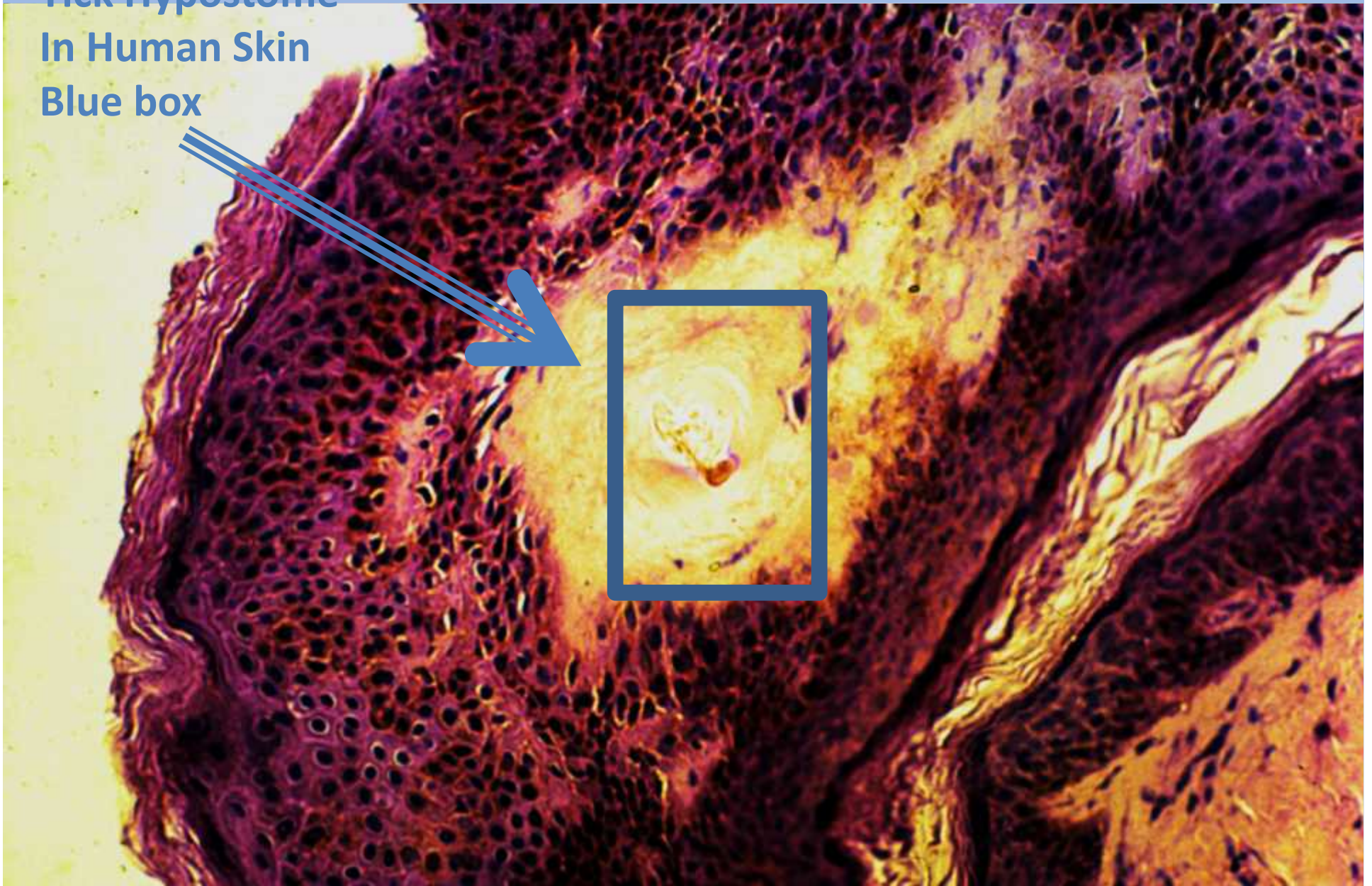
**Three Tick bite sites**  
**Each without**  
**Erythema or pain**  
**or Itching at the sites**

**Patient**  
**Removed**  
**Ixodes**  
**Scalularis**  
**Nymph**  
**form**  
**From**  
**This tick**  
**bite site**





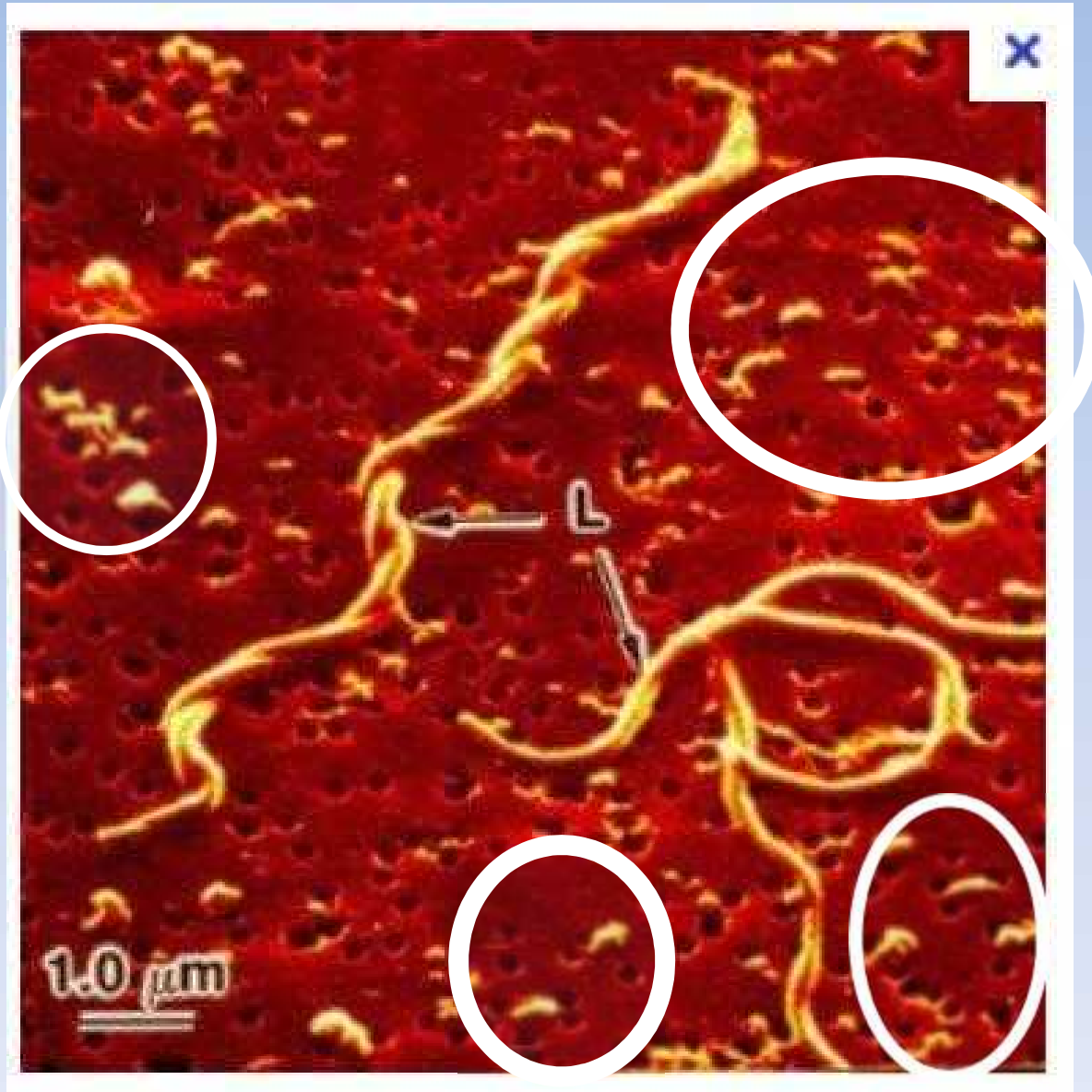
**Tick Hypostome  
In Human Skin  
Blue box**

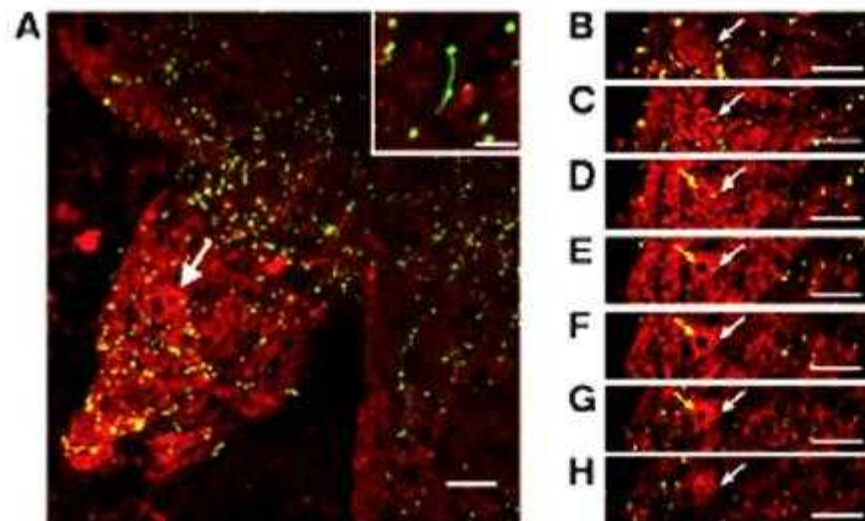




# Borrelia burgdorferi with “blebs”

Classical  
Spiral  
Forms  
Of  
Borrelia  
Burgdorferi [ L ]  
And  
Multiple  
Smaller  
“blebs”  
Or Liposomes {Circles}  
Of  
Borrelia  
Burgdorferi  
On an  
Electron  
Microscope  
Grid



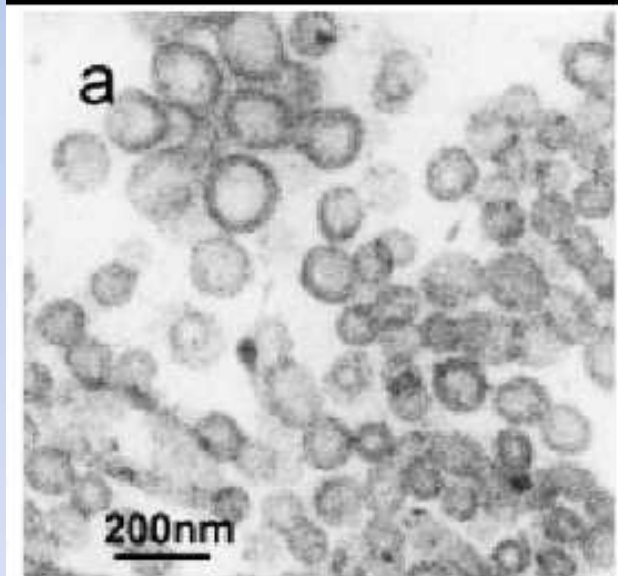


**Figure 3**

Spirochetes shed myriad blebs during early feeding (24 hours after placement). (A) Composite confocal image showing the distribution of spirochetes and numerous blebs through the full thickness of a mid-gut (45  $\mu$ m); shown in the inset is a spirochete shedding blebs. Arrow indicates the cell in the consecutive confocal sections in B–H. (B–H) Localization of an intracellular bleb (arrow) present within a spirochete.

Myriad  
Blebs  
(in green)

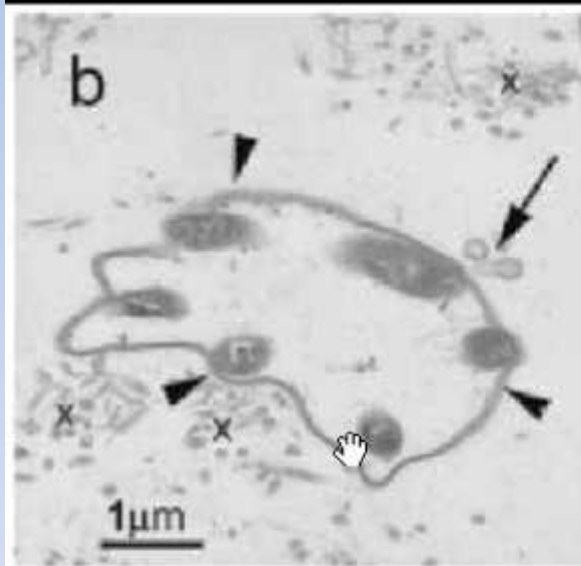
# **Borrelia burgdorferi "blebs" are LIPOSOMES**



**Dermal  
Deposits of  
"Blebs" of Bb  
by electron  
Microscopy**

**Beermann et al**

# Cystic [round body form] *Borrelia burgdorferi* shedding "blebs" [LIPOSOMES]



Lipoproteins from *Borrelia burgdorferi*  
Applied in Liposomes and  
Presented by Dendritic Cells  
Induce CD8<sup>+</sup> T-Lymphocytes *in Vitro*

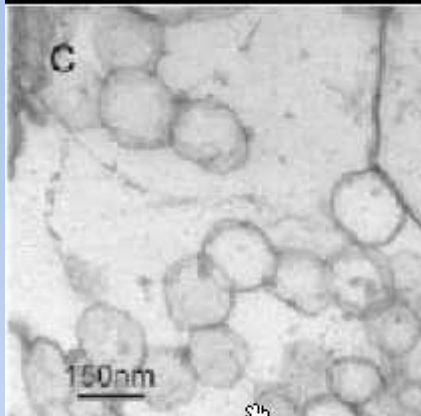
Christopher Beermann,\*

Cellular Immunology **201**, 124–131 (2000)

## Beermann et al



# **Borrelia burgdorferi LIPOSOMES also known as "Blebs"**



## **Electron microscopy Study**

**Beermann et al**

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**Borrelia burgdorferi "Blebs"**  
**[liposomes]**  
**An Electron**  
**Microscopic**  
**Study**  
**At**  
**Rocky Mtn Lab**  
**NIAID, NIH**

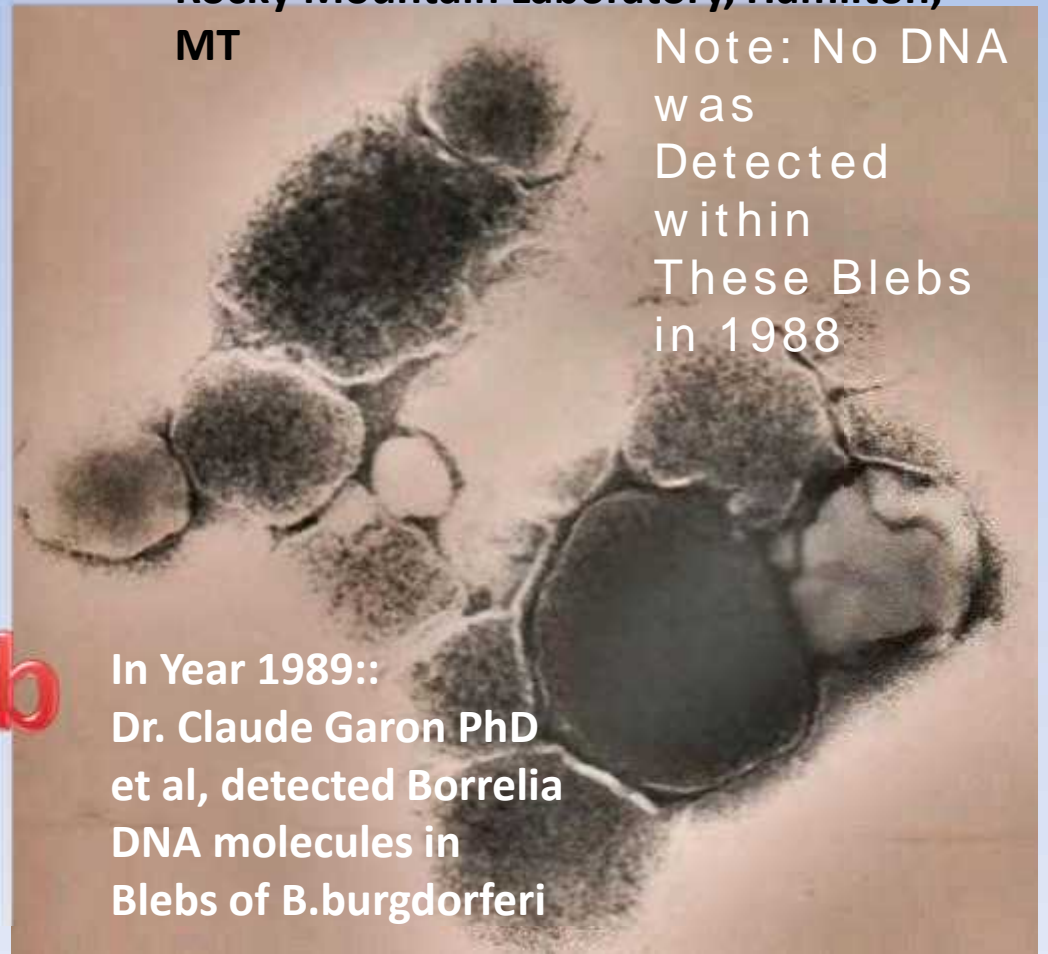
**Specimen submitted by::**

**Alan B. MacDonald, MD Year 1988**

**Electron Microscopy by:**

**S. F. Hayes, Electron Microscopist,  
Rocky Mountain Laboratory, Hamilton,  
MT**

**Note: No DNA  
was  
Detected  
within  
These Blebs  
in 1988**

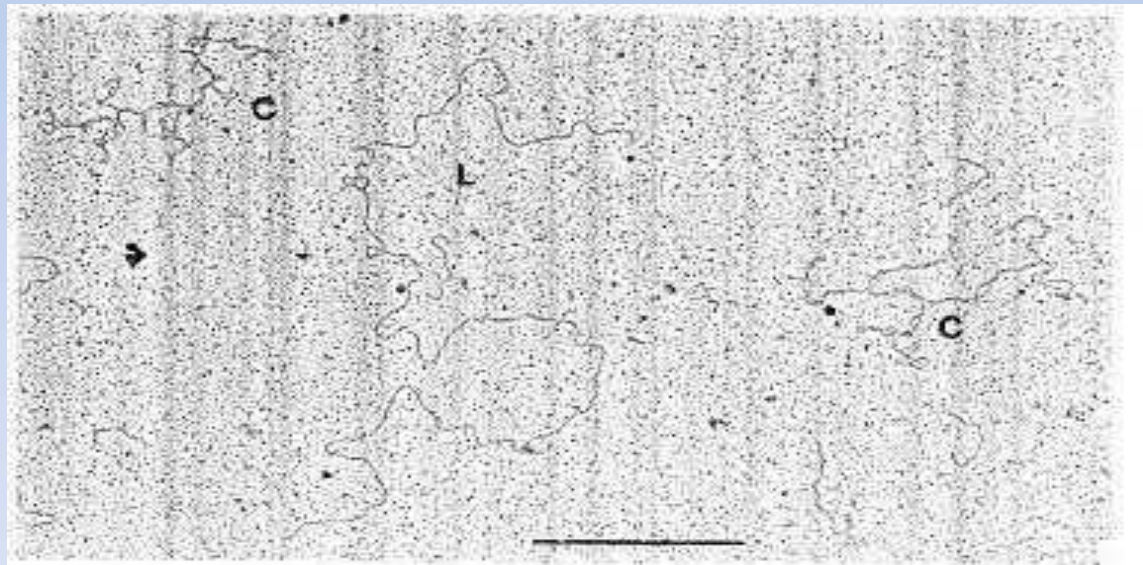


**In Year 1989::**

**Dr. Claude Garon PhD  
et al, detected Borrelia  
DNA molecules in  
Blebs of B.burgdorferi**

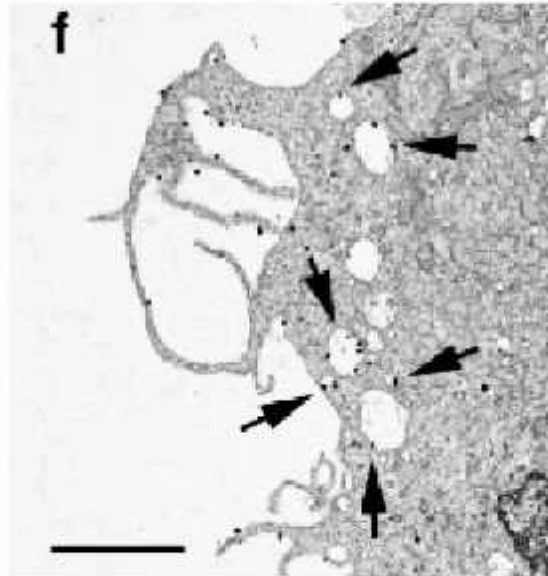
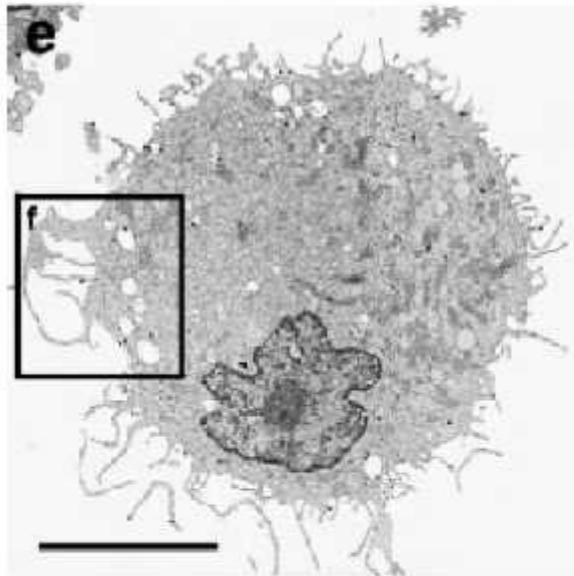
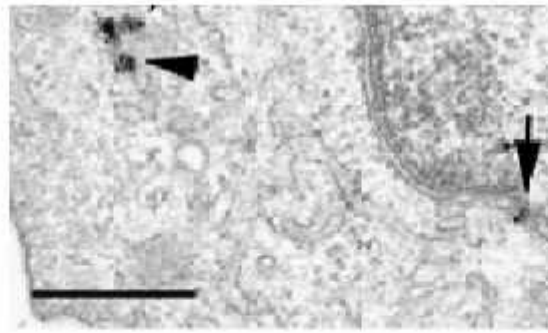
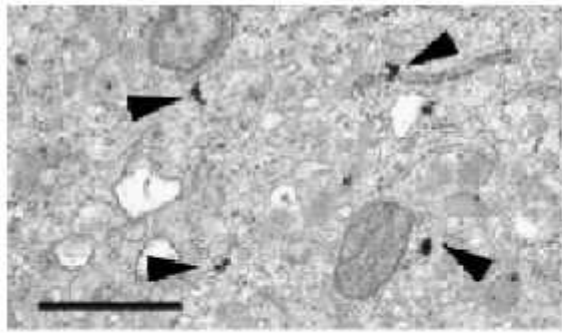


**Borrelia burgd. “blebs” contain  
DNA Molecules – Both Circular  
DNA molecules and Linear  
DNA Molecules [Probably Plasmid  
Dna and Not Chromosomal DNA]**



Purified blebs were incubated with 1.0  $\mu\text{g}/\text{ml}$  of pancreatic DNAase I (2.5 units/ $\mu\text{g}$ ) (Worthington Biochemicals, Freehold, N.J.) for 10 min at 25°C. DNA was then purified from alkaline samples by lysis with sodium dodecylsulfate, digestion with proteinase K, and extraction by phenol-chloroform as previously described [15].

Figure 8. Purified blebs were treated with an excess of pancreatic DNAase I prior to conventional DNA extraction procedures involving proteinase K, phenol extraction and alcohol precipitation. Both linear (L) and covalently-closed circular (C) molecules were observed. Molecules were mounted by the Kleinschmidt aqueous technique and rotary shadowed with platinum/palladium. Bar = 1.0  $\mu\text{m}$ .



**FIG. 4.** Uptake of Nanogold-labeled Bb-liposomes by DC was visualized with TEM: DC were incubated for 10 min with Nanogold-labeled Bb-liposomes. (a) Overview of a DC (bar: 8  $\mu$ m). (b) Higher

**Beerman et al.**

-----  
**"blebs"**  
 also known as  
**Liposomes of**  
**Borrelia Burgdorferi**  
 -----

**Transmission**  
**Electron Microscopy**  
**demonstrates the**  
**internalization**  
**of these "blebs" Into**  
**Dendritic cells of the**  
**dermis**

Lipoproteins from *Borrelia burgdorferi*

Applied in Liposomes and  
 Presented by Dendritic Cells

Induce CD8<sup>+</sup> T-Lymphocytes *in Vitro*

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# Antigen Presentation of **Borrelia** Epitopes ON the Cell Membranes of NonPhagocytic cells which have Incorporated LIPOSOMES OF BORRELIA BURGDORFERI

After incorporation, the antigenic membrane lipoproteins of Bb are expected to be located in the membrane of the incorporated vesicles. Consequently, the protein part of the lipoproteins is directed toward the cytosol, where degradation by proteasomes can corrode them to be processed toward the MHC class I pathway (47). This would also mean that peptides presented on MHC class I should be recognized by CD8<sup>+</sup>

**MHC Class I  
pathway**

**Antigens  
are  
presented  
to  
CD8  
Lymphocytes**

**Beermann et. al.**

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# Nucleus Penetration by “blebs” [liposomes] of borrelia burgdorferi

**Blebs of Borrelia burgdorferi [ liposomes of Bb]  
Actually Enter the Nucleus of the Lymphocytes and  
the Nucleus of other NON PHAGOCYTIC cells**

First, we studied the uptake of Bb-liposomes by human DC, fibroblasts, and B- and T-lymphocytes. All tested cells incorporated Bb-liposomes, as visualized by immunofluorescence microscopy. Incorporation of Bb-liposomes took place in a very rapid manner and even at 4°C. Consequently, we postulated that Bb-liposomes were taken up by nonphagocytic mechanisms. To prove this hypothesis, we constructed Nanogold-labeled Bb-liposomes. With this tool and transmission electron microscopy we could document that Bb-liposomes were incorporated within seconds. First, Bb-liposomes fused with the surface membrane of the cells, to arise subsequently in the cytosol, where most of them were found throughout. **Nanogold-labeled vesicles crossed the nuclear membrane via the nuclear pores and were then found in the nucleus. Bb-liposomes were never found in endosomes or lysosomes.**

**Beermann et al.**

**Liposomes  
are  
routinely used  
to TRANSFECT  
tissue culture  
cells**

**Liposomes of  
borrelia  
burgdorferi  
are NEVER  
found in  
Endosomes or  
Lysosomes**

Possible  
“Transfection”  
by Liposomes  
Of Borrelia  
Burgdorferi  
Scenario

-----  
As a  
Consequence  
Of Penetration  
Of the Nucleus  
Of the human  
Cell by  
Liposomes  
“Blebs”  
Of  
Borrelia  
Burgdorferi



## **Borrelia Liposomes [ "Blebs" ] , cellular penetration, and the provocation of an AUTOIMMUNE phenomenon in the human host**

It is tempting to speculate that Bb-blebs might be relevant *in vivo* for the autoimmune-like inflammation in tissues infected with Bb. One can imagine that in the environment of Bb one would expect incorporation of shedded Bb-blebs via nonphagocytic mechanisms by all cells, e.g., fibroblasts, synovial cells, and of course also DC. DC loaded with Bb-antigens through Bb-blebs might then induce cytotoxic Bb-specific CTL. Subsequently, in the tissues infected with Bb, those CTL ought to kill cells which incorporated Bb-blebs and present Bb-antigens on MHC class I surface molecules. Further investigation is required to prove this thesis in an animal model.

**Beermann et al.**

"Blebs"

**"Borrelia liposomes"  
as weapons  
in the human host**

-----

**A bleb ALONE, can provoke  
tissue injury in the  
penetrated cell  
from the "inside " "out"  
without the presence of an  
intact spiral form of  
Borrelia burgdorferi Inside  
of the Human cell  
[Non-phagocytic cell lines]**

## **Borrelia burgdorferi liposomes ["blebs"] , Human Dendritic cell Invasion, and the Induction of Borrelia burgdorferi specific CD8 T cells ( Cytotoxic T Lymphocytes -CTL)**

T-lymphocytes. With this in mind, we tested whether Bb-liposome-treated human DC, which are known to be important for the generation of CTL (35, 48), lead to Bb-specific autologous CD8<sup>+</sup> T-cells *in vitro*. Confirming our expectations, we achieved an almost pure CD8<sup>+</sup> T-cell population after 4 weeks of culture and weekly restimulation of PBMC with Bb-liposome-treated DC. To ensure that these CD8<sup>+</sup> T-cells were Bb-specific and functional CTL, we tested them in cytotoxicity assays. Thus, we were able to show that these CD8<sup>+</sup> T-cells killed Bb-liposome-treated autologous T-cell blasts, indicating that they are specific for Bb-lipoproteins.

**Cytotoxic Human T Lymphocytes [CTL] = CD8+ are stimulated in "almost PURE CULTURE" using Borrelia burgdorferi LIPOSOME treated human Dendritic Cells**

**Beermann et al**

Lipoproteins from *Borrelia burgdorferi*  
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Induce CD8<sup>+</sup> T-Lymphocytes *in Vitro*

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**MHC Class I  
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presented  
to  
CD8  
Lymphocytes**

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# Both In Vitro and In vivo *Borrelia burgdorferi* Shedding of liposomes

It is a well-known phenomenon that *B. burgdorferi* shed blebs *in vitro* (26, 27). Whether Bb-blebs are produced *in vivo* and their relevance are still debated. We show with electron microscopy that shedding of blebs by Bb also takes place in the tissue which confirms earlier observations (34). To date, the influence of Bb-blebs on the immune response has only been described for murine B-cells and the humoral immune response (46). But no data have been yet published concerning the influence of Bb-blebs on the cellular immune response.

***Borrelia  
burgdorferi*  
liposome  
immune  
response  
components**

---

**T cell arm  
B cell arm**

**Beerman et al**

# Molecular Dermatopathology Of Cutaneous Borrelioses

Keep the blood pipeline open

Anesthetize the bite Site

BLOCK ACTIVATION OF COMPLEMENT

CONCEAL BORRELIA FROM THE IMMUNE SYSTEM

IMMUNOSUPPRESSORS

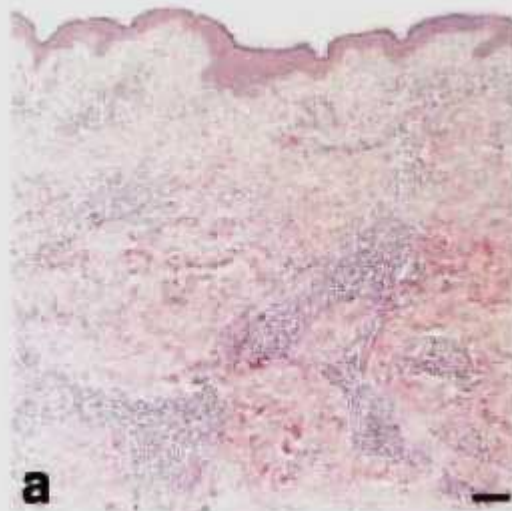
CYTOKINE RESPONSE BY HUMAN HOST

Image Gallery: Tick Salivary Gland Processing of Borrelia species

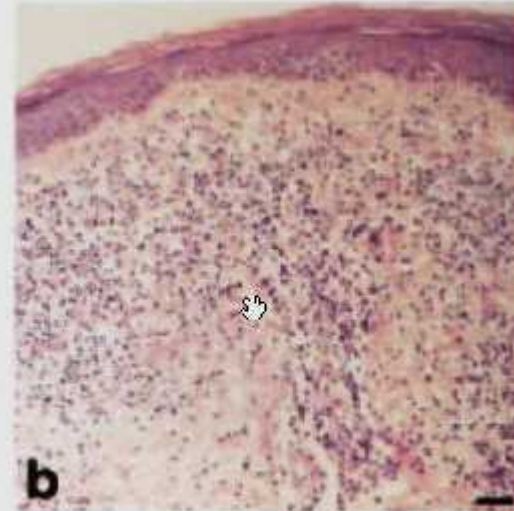
Image Gallery: Events in the Dermis after Transmission of Borrelia

Links: Supplementary materials related to tick salivary proteins





**Erythema Migrans**  
**Inflammatory**  
**Infiltrates in dermis**



**Acrodermatitis Chronica**  
**Atrophicans (aca)**  
**Inflammatory**  
**infiltrates in dermis**

**Differential Expression of Cytokine mRNA**  
**in Skin Specimens**  
**from Patients with Erythema Migrans**  
**or Acrodermatitis Chronica Atrophicans**

Robert R. Müllegger,\*† Gail McHugh,\* Robin Ruthazer,

*Key words: acrodermatitis chronica atrophicans/cytokines/  
 erythema migrans/in situ hybridization. J Invest Dermatol*  
 115:1115-1123, 2000

# Cytokines : Now visible under The Microscope

## Differential Expression of Cytokine mRNA in Skin Specimens from Patients with Erythema Migrans or Acrodermatitis Chronica Atrophicans

Robert R. Müllegger,\*† Gail McHugh,\* Robin Ruthazer,:

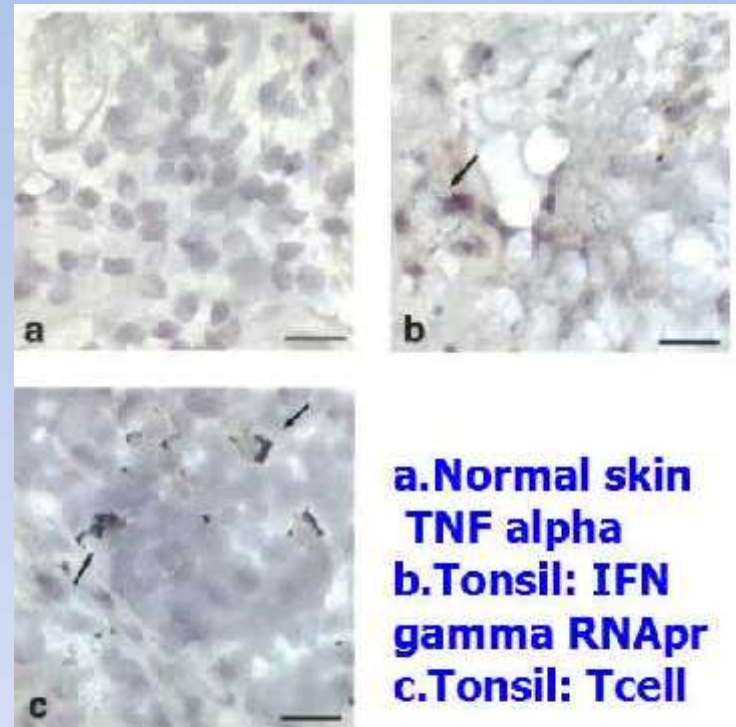
*Key words: acrodermatitis chronica atrophicans/cytokines/  
erythema migrans/in situ hybridization. J Invest Dermatol  
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**Lymphoid Cells  
Produce Cytokines  
and their Movements and  
functions are guided by Cytokines**



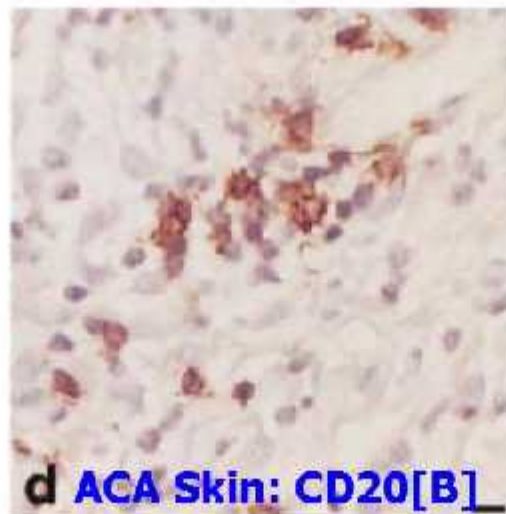
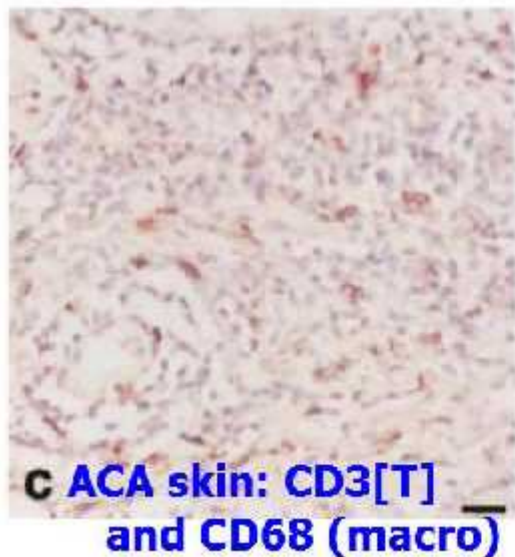
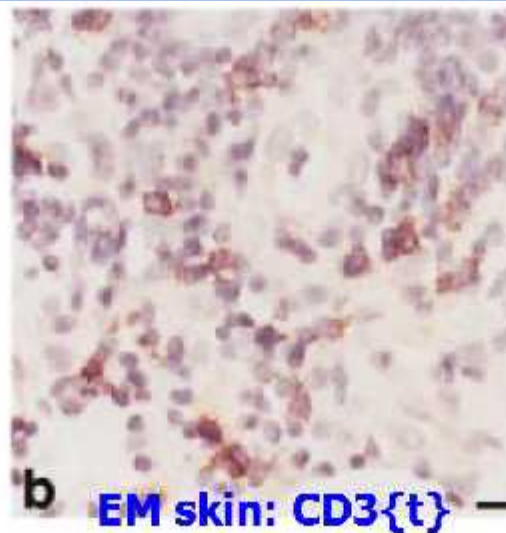
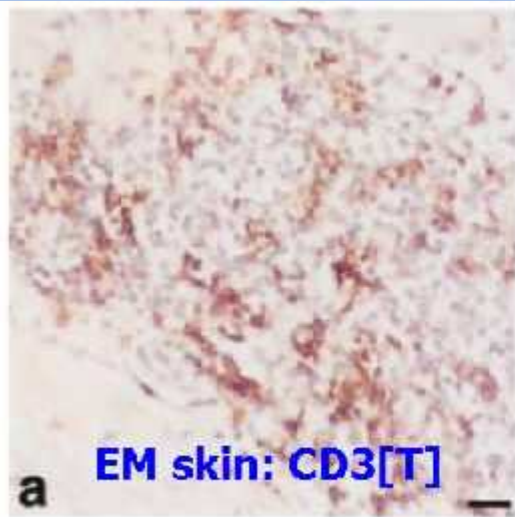


# Cytokines – Now visible under The Microscope



**Cytokine Study in Lyme Skin  
lesions:  
Normal Controls**

# Cytokines – now Visible



## Differential Expression of Cytokine mRNA in Skin Specimens from Patients with Erythema Migrans or Acrodermatitis Chronica Atrophicans

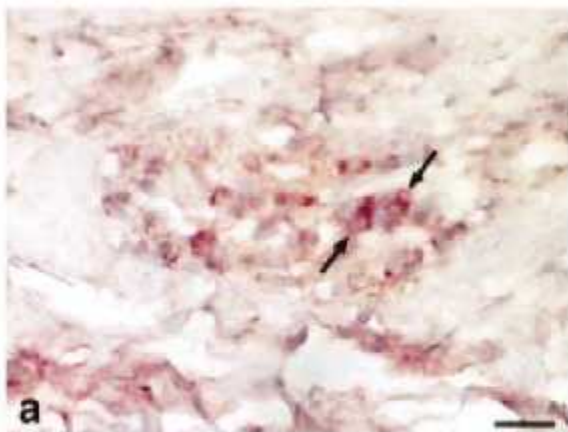
Robert R. Müllegger,<sup>†</sup> Gail McHugh,<sup>\*</sup> Robin Rützel,<sup>‡</sup>

*Key words:* acrodermatitis chronica atrophicans/cytokines/  
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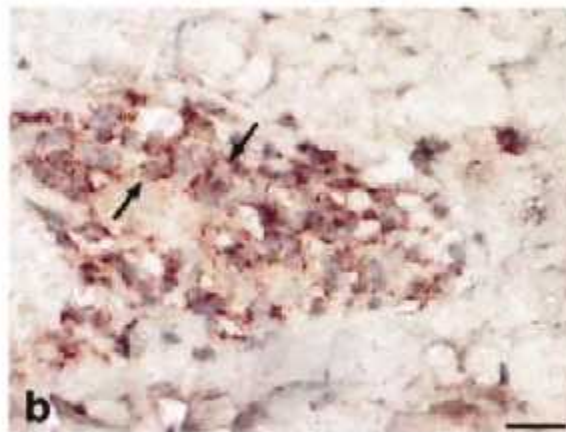
## Analysis of Leukocyte populations in Erythema Migrans and in ACA skin

# Cytokines – Now Visible

## Cytokine deposition in lesional skin Cutaneous borreliosis



**Erythema Migrans  
TNF alpha**



**Erythema Migrans  
IFN gamma**

### Differential Expression of Cytokine mRNA in Skin Specimens from Patients with Erythema Migrans or Acrodermatitis Chronica Atrophicans

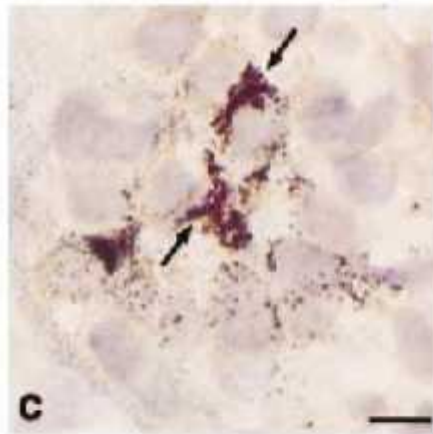
Robert R. Müllegger,<sup>1\*</sup> Gail McHugh,<sup>2</sup> Robin Ruthazer<sup>1</sup>

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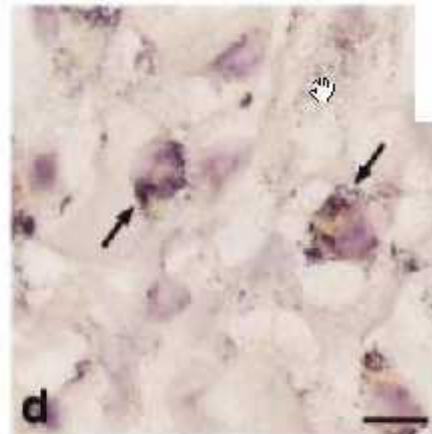


# Cytokines – now Visible

## Cytokine Deposition in Lesional Skin- Cutaneous Borrelia Infection



**Erythema  
Migrans  
TNF alpha**



**Erythema  
migrans  
IFN gamma**

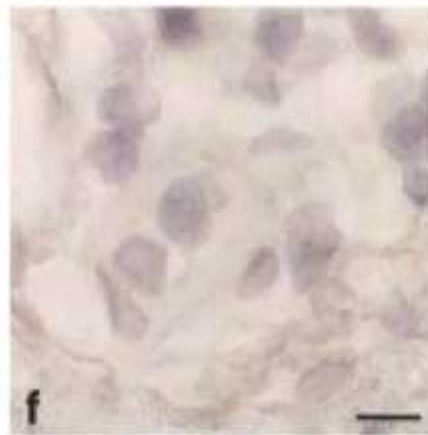
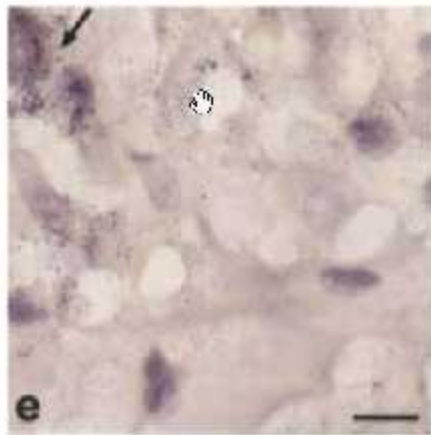
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115:1115-1123, 2000*

# Cytokines – Now Visible

## Cytokine deposition in lesional skin in Cutaneous Borrelia Infection



**Erythema  
migrans:  
IL 1Beta**

**Normal Skin:  
No cytokine  
deposits seen**

### Differential Expression of Cytokine mRNA in Skin Specimens from Patients with Erythema Migrans or Acrodermatitis Chronica Atrophicans

Robert R. Müllegger,\*† Gail McHugh,\* Robin Ruthazer,;

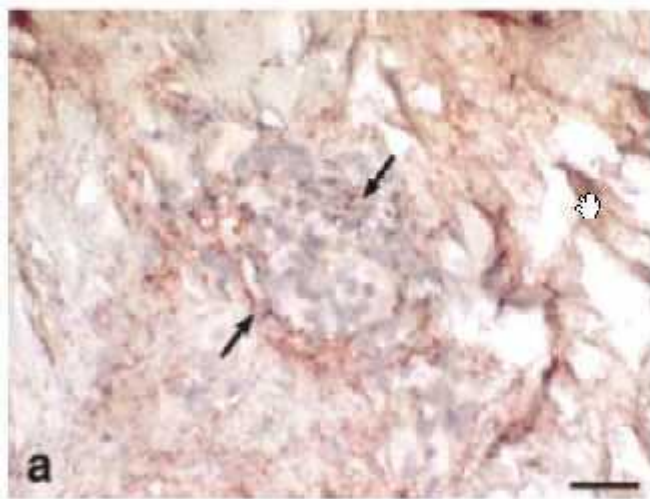
*Key words: acrodermatitis chronica atrophicans/cytokines/  
erythema migrans/in situ hybridization. J Invest Dermatol  
115:1115–1123, 2000*

# Cytokine deposition in lesional skin in Cutaneous borrelia infection

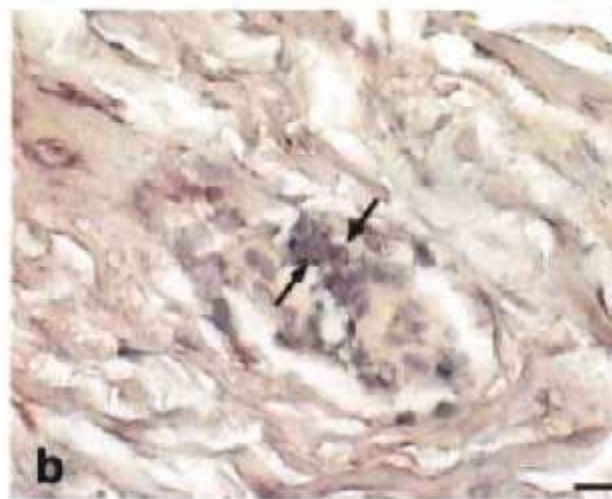
Differential Expression of Cytokine mRNA  
in Skin Specimens  
from Patients with Erythema Migrans  
or Acrodermatitis Chronica Atrophicans

Robert R. Müllegger,\*† Gail McHugh,\* Robin Ruzhazer,

*Key words:* acrodermatitis chronica atrophicans/cytokines/  
erythema migrans/in situ hybridization. *J Invest Dermatol*  
115:1115–1123, 2000



**ACA Skin:  
TNF alpha**



**ACA Skin:  
IL-4**

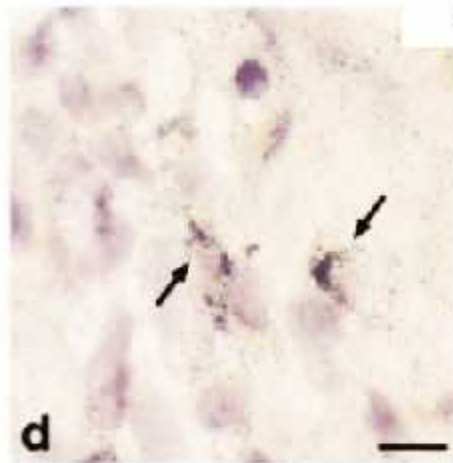
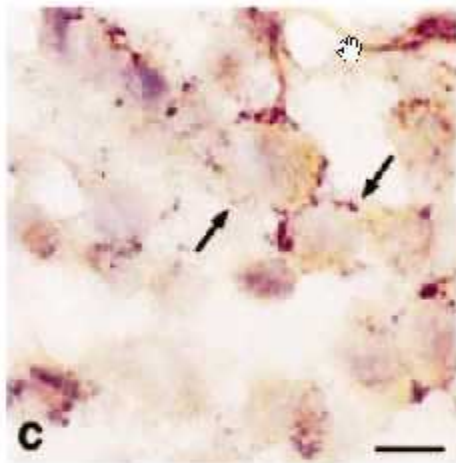


# Cytokine Deposition in Lesional skin - Cutaneous borrelia infections

## Differential Expression of Cytokine mRNA in Skin Specimens from Patients with Erythema Migrans or Acrodermatitis Chronica Atrophicans

Robert R. Müllegger,\*† Gail McHugh,\* Robin Ruthazer,‡

*Key words:* acrodermatitis chronica atrophicans/cytokines/  
erythema migrans/in situ hybridization. *J Invest Dermatol*  
115:1115-1123, 2000



**ACA skin:  
TNF alpha  
deposits**

**ACA skin:  
IL-4 deposits**

# In the BEGINNING....

**Early Ixodid Tick bite site  
Skin of Neck**



**Photo Credit: Alan B. MacDonald MD**

# Un-SEEN Tick bite sites



**Ixodid scapularis Tick attached to  
External Ear**

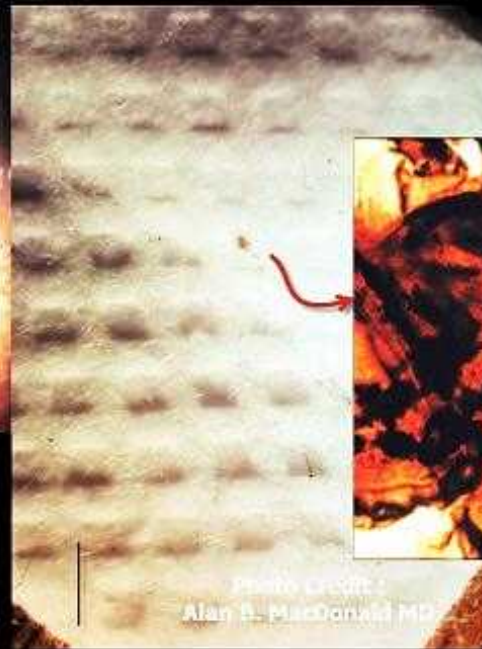
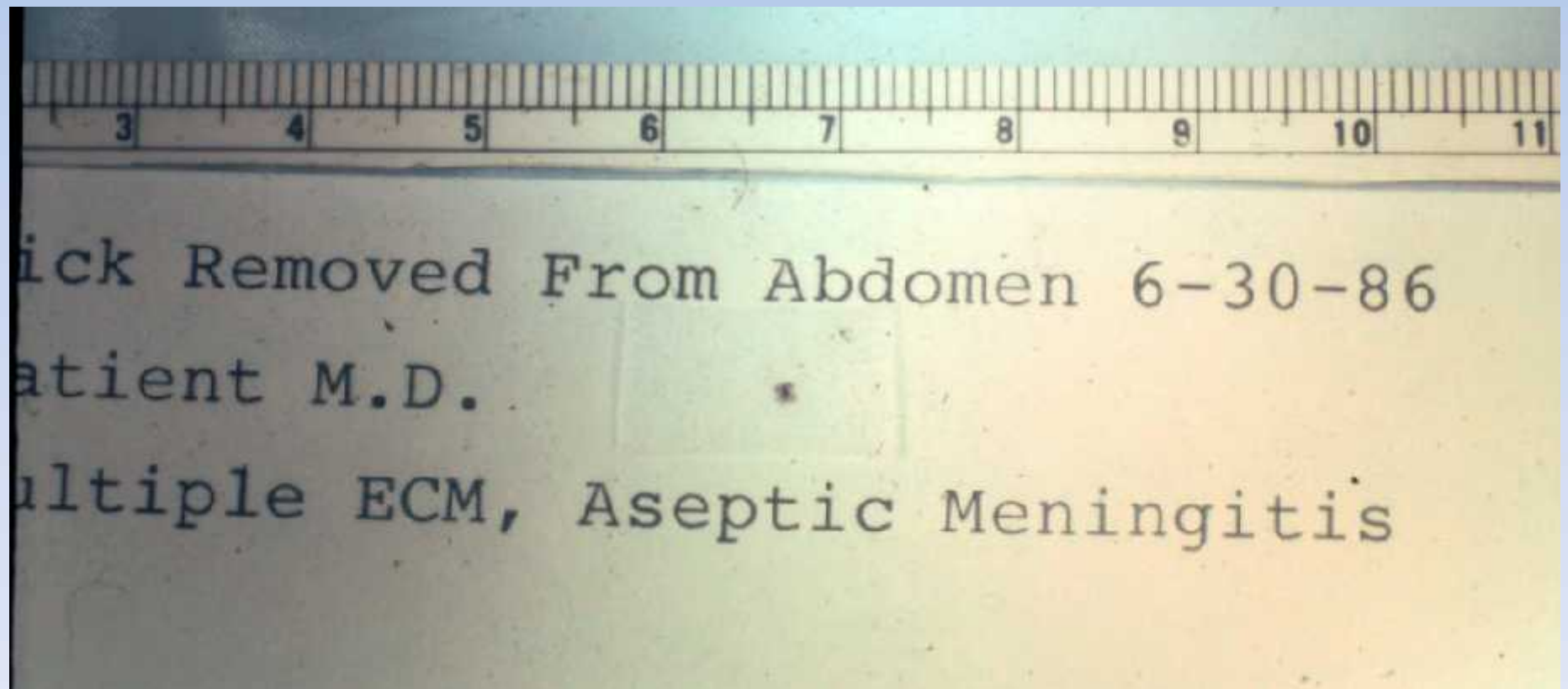


Photo Credit:  
Alan B. MacDonald MD



# Little Tick – Big time Disease



# Molecular Dermatopathology Of Cutaneous Borrelioses

Keep the blood pipeline open

Anesthetize the bite Site

BLOCK ACTIVATION OF COMPLEMENT

CONCEAL BORRELIA FROM THE IMMUNE SYSTEM

IMMUNOSUPPRESSORS

CYTOKINE RESPONSE BY HUMAN HOST

Image Gallery: Tick Salivary Gland Processing of Borrelia species

Image Gallery: Events in the Dermis after Transmission of Borrelia

Links: Supplementary materials related to tick salivary proteins

# Molecular Dermatology Of Cutaneous Borrelioses

Vertebrate Cellular Immune System - Actions following Borrelia transmission to the Dermis. The Upregulation and return of OspA in the tick bite site. **OspC is replaced by Osp A which binds Toll-like receptors.**

**OspA induces the activation of host immune cells (Lymphocytes) (Langerhans cells) (Macrophages)**

Subtypes of Osp A and biological significance of OspA variation

Image Gallery: Organization of the Proteins in the Outer Surface membrane

Links: Supplementary manuscripts and materials on Osp A



# Molecular Dermatopathology Of Cutaneous Borrelioses

*Borrelia burgdorferi* **Erp gene** activation -( Outer Surface Protein E and F lipoproteins) reside on the **Circular Plasmid 32 FAMILY** [ plasmids coding proteins helpful for survival but not mandatory for establishment of Human infectivity].

**Erp proteins** serve as **receptors for human complement protein H** [note Complement protein H is not identical to human blood group protein H which is a precursor for blood group antigens in the ABO system]

**Erp proteins** contribute to *B. burgdorferi* persistence within **CHRONICALLY INFECTED** host tissues, but it is **plausible that ERP proteins are continually produced during the entire infective cycle, especially in chronic infection.**

For Example, The **B31 strain of Bb produces Erp proteins from 17 different genes** which are grouped into 10 different ERP ORF families. **Protein ErpX is unique** in that it resides on **LINEAR PLASMID LP 56**

***Some Borrelia burgdorferi strains demonstrate TRUNCATED Erp Proteins***

Image Gallery: Erp genes and Erp proteins

Links: Supplementary materials

# Molecular Dermatopathology

***OspC is covered by Salp15 salivary protein***

Salp15 - A protein produced in the *Ixodid tick salivary gland*.

Salp 15 - *Coats the surface* of the Borrelia contained within the tick salivary gland, and facilitates "immune evasion" of the transmitted Borrelia spirochetes when they enter the vertebrate host.

**Immunosuppressive activities of SALP 15 tick salivary protein**

**Osp C induces Borrelia ACIDAL ANTIBODY PRODUCTION**

Osp C is coded for by Plasmid cp26. Excess of Ten Osp C Subtypes in Bbss

*A reciprocal relationship exists between gene expression for OSP C and OSP A*

*Osp A is expressed by spirochetes in the Tick Midgut ; OspC in the Salivary gland*

**CRASP proteins of Borrelia Strains** and Susceptibility to Complement mediated killing:

**B. Garinii is very susceptible to Complement mediated KILLING**

[ *Serum Sensitive borrelia strains are CRASP Negative* ]

**B. Afzelii is RESISTANT to Complement mediated KILLING**

[ **SERUM Resistant Borreliae are CRASP positive** ]

Image Gallery: Osp C pathobiology , CRASP pathobiology

Links: Supplementary Materials OspC



# EUROPEAN CLASSICS IN LYME BORRELIOSIS

## ROUND UP THE USUAL SUSPECTS

Erythema (Chronicum) Migrans – Benchmark Description – Year initially described

Acrodermatitis Chronica Atrophicans – Benchmark Description – Year initially described

Borrelia Lymphocytoma – (Lymphadenosis benigna Cutis) – Benchmark – Year described

## Unanswered Whys and Wherefores

Why is a Red skin sign seen early but after the red has faded persistence of Bb in skin???, Why not allow histology with immunohistochemical and DNA sequencing backup to rise to the level of “Gold Standard” culture positivity? Why Brisk Lymphoid tissue Infiltrates in some (mimicking Lymphoma cutis) and slim to none in others? Why Atrophy of epidermis in some tertiary cases? Why dense fibrosis in other long term cases, Why true cutaneous lymphomas arising contain core foci of Bb organisms by FFM in the malignant Tissues? Why not Lateral DNA transfer between Bb strains and between Bb and Human cells (horizontal DNA transfer)? Why not use multiple Bb Strains in laboratory reagent manufacture? Why not cooperate in the development of Gene chip/protein arrays which evaluate >500 epitopes to render WB methods obsolete? Why not utilize blood cultures to evaluate cases of Chronic LB for adequacy of treatment? Why ignore the CSF proteome analysis As a diagnostic tool? Why not evaluate Lyme borreliosis as a potential threat to the safety of the Blood supply? Why not maintain an independent registry of Southern Erythema migrans cases? Why have Cystic Forms and Granular forms of Bb been ignored?, Why not perform more Lyme focused Autopsies? What role for VBNC borrelia in pathogenesis??



**Europe**  
**Always miles ahead**  
**Of anywhere else**  
**On Earth**  
**With Cutaneous borreliosis**

**SURVEY OF  
THE GREATEST  
IMAGES IN  
CUTANEOUS BORRELIOSES  
AS CONTRIBUTED BY  
EUROPEAN COLLEAGUES**

# Classic Erythema Migrans

Skin of Trunk

Erythema migrans



Zentrifugal fortschreitendes  
Erythem

Inkubationszeit  
7-10 Tage (bis 4 Wochen)

***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***



# Classic Erythema Migrans

Erythema migrans

Lokalisation

Untere Extremitäten

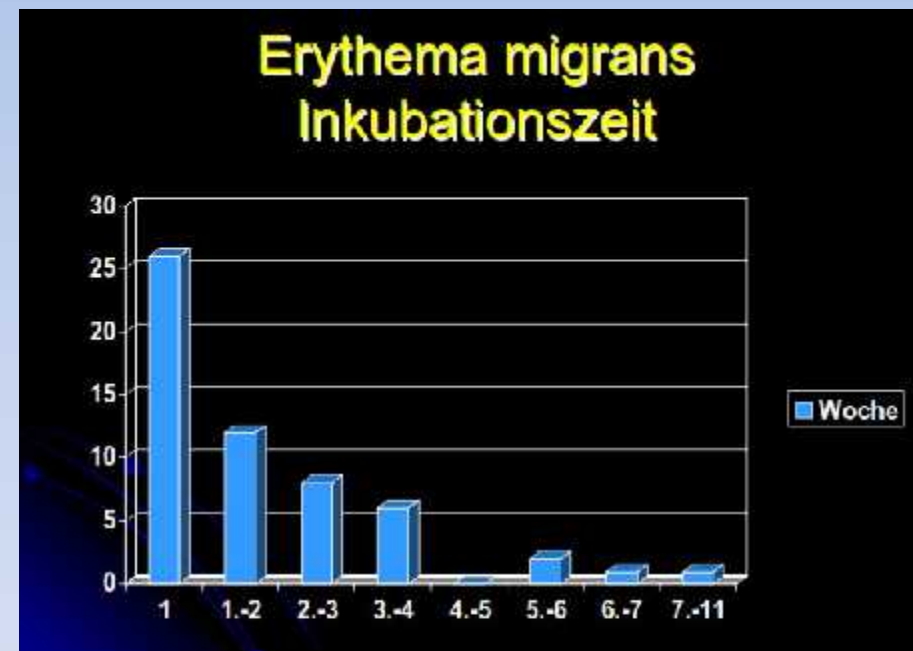
70%

Kuiper et al. Br J Dermatol 1994



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Incubation times for erythema Migrans



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Variants of Erythema Migrans

## Erythema migrans

N = 231

B. Burgdorferi Kultur +

Ringförmig	71%
Homogen	29%
Multipel	6%

Kopfschmerzen	20%
Müdigkeit	19 %
Arthralgien	11%
Myalgien	9%

Strle et al. Clin Inf Dis 1996

***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***



# Multifocal Erythema Migrans

Multifocal Erythema Migrans



Photo credit: Bernard W. Berger MD, Southampton NY  
Copyright Dr Bernard Berger MD, all rights reserved

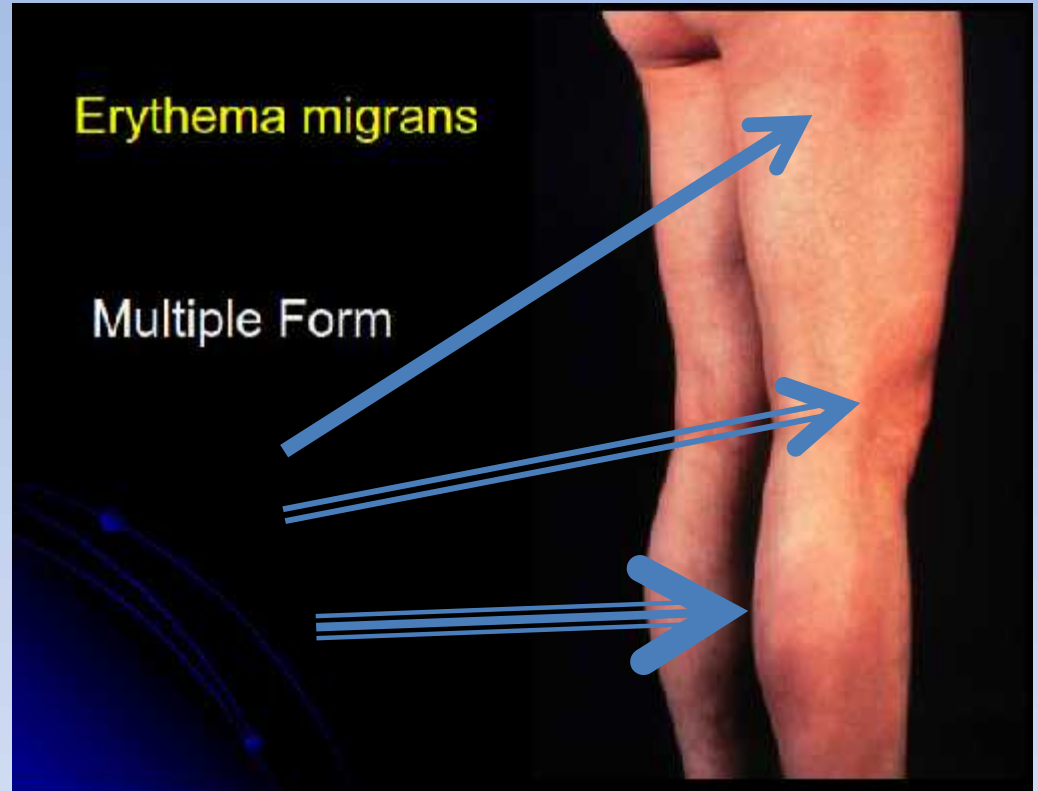
Multifocal Erythema Migrans



Photo Credit: A. B. MacDonald MD, Copyright

Erythema migrans

Multiple Form



**Image Credit:**  
**Dr Med S.A. Buchner MD**  
**Dermatologist, Basil**  
**Universitatsspital**

# Preferred sites: Borrelia Lymphocytoma

**BORRELIA  
LYMPHOCYTOMA  
ONSET  
2 DAYS TO  
6 MONTHS POST  
BITE**

## Borrelienlymphozytom

- B. burgdorferi- induzierte lymphoproliferative knotige Reaktion
- Prädilektionsstellen
  - Ohrläppchen
  - Areola mammae
  - Scrotum

Auftreten nach Zeckenstich  
2 Monate (bis 10 Monate)

***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Subtypes of borrelia Lymphocytoma

**VARIETIES OF BL**  
**SOLITARY**  
**DISSEMINATED**  
**INFILTRATING**

## Borrelienlymphozytom

- Knotig-solitär
- Disseminiert-kleinknotig
- Flächenhaft-infiltriert

***Image Credit:***  
***Dr Med S.A. Buchner MD***  
***Dermatologist, Basil***  
***Universitatsspital***



# Borrelia Lymphocytoma

**Classical  
Site and  
Classical  
Gross  
appearance**

**Soft parts of  
LOBE of EAR**

Borrelienlymphozytom

Knotig-solitäre  
Form



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# **Borrelia lymphocytoma: Atypical Gross::**

## **Pregnant pt. with BL involving**

### **Helix ONLY and SPARING Lobe of Ear**

**Comment:**  
**(ABM)**  
This case could  
Be easily  
Misdiagnosed as  
“relapsing  
Ptychondritis”  
Because the  
Soft parts of the  
Lobe of the Ear  
Are Spared.  
Incredible case!!  
Unusual for Ear  
Involvement  
In BL!!!



**Borrelia  
Lymphocytoma  
Unusual  
Because the  
Lobe of the Ear  
is spared  
and the  
Helix of the Ear  
is Red hot!!**

**Fig. 1.** Borrelial lymphocytoma. (A) Borrelial lymphocytoma before treatment

Photo Credit: Monizusko, A, et al, Borrelia Lymphocytoma: A  
Case report of a Pregnant woman  
Ticks and Tick borne disease 2012,3: 257-8

# Borrelia Lymphocytoma

Multifocal  
Borrelia  
Lymphocytoma

Borrelienlymphozytom

Knotige Form



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***



# Borrelia lymphocytoma

**Nose**

Borrelienlymphozytom



**Scalp**



**Lip**

***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Borrelia Lymphocytoma

## Classical gross Findings for Borrelia Lymphocytoma

**“Special site Skin”**

**These sites include**

**Earlobe**

**Areola**

**Eyelid**

**Genital**

**Periumbilical**



**Borrelia Lymphocytoma  
Classical form and location**

**Cutaneous manifestations  
of Lyme borreliosis**

Dieter Hassler  
General practitioner and infectious disease specialist, German Society  
for Infectious Diseases, Germany

# Atypical [flattened] borrelia Lymphocytoma of Buttock

Could this be  
confused  
With a small  
Erythema  
Migrans???

Size 2.5 cmx1.1 cm

What would your  
CDC Consultant  
Diagnose?



**Atypical borrelia  
Lymphocytoma of buttock  
Lesion size 2.5x 1.1 cm**

**Cutaneous manifestations  
of Lyme borreliosis**

Dieter Hassler  
General practitioner and infectious diseases specialist, German Society  
for Infectious Diseases, Germany



# Staph Infection of borrelia Lymphocytoma



**Microbial  
Etiologies  
were  
confirmed  
by Culture**

**Borrelia Lymphocytoma (3 lesions)  
Polytypic Variant of BL  
with Staphylococcal superinfection**

**Cutaneous manifestations  
of Lyme borreliosis**

Dieter Hasler  
General practitioner and infectious disease specialist, German Society  
for Infectious Diseases, Germany

# Borrelia Lymphocytoma

## Variants (3)

**Monotypic:: Sharp Borders,  
rubbery, Stable**

**Oligotropic :: Gradual Increase  
in Size**

**Spreads along Lymphatics**

**Miliary form:: MULTIPLE Pin  
Point lesions LYMPHATIC  
Spread**

### Variants of borrelia

#### Monotypic form

The classic monotypic form of BL consists of a variously sharply-bordered lymphocytic infiltrate with a blue-red color and a rubbery feel on palpation.

#### Oligotropic form

This form arises from the monotypic form after spreading along a lymphatic pathway. The lesions can persist for years and gradually increase in size.

#### Miliary form

Originally described by Bätverstädt (1960) as lymphadenosis cutis benigna dispersa. There are a very high number of lymphocytoma lesions, usually only about the size of the

head of a pin. Miliary lymphocytomas are only possible if there is spread of the pathogen via the bloodstream, i.e., if there is systemic infection. Differentiation from reactive lymphoma of other causes is not easy, but sometimes culture can be helpful. Immunohistology shows polyclonal stimulation.

### Cutaneous manifestations of Lyme borreliosis

Dieter Hassler

General practitioner and infectious disease specialist, German Society  
for Infectious Diseases, Germany

## **Borrelia lymphocytoma - 6 weeks duration**

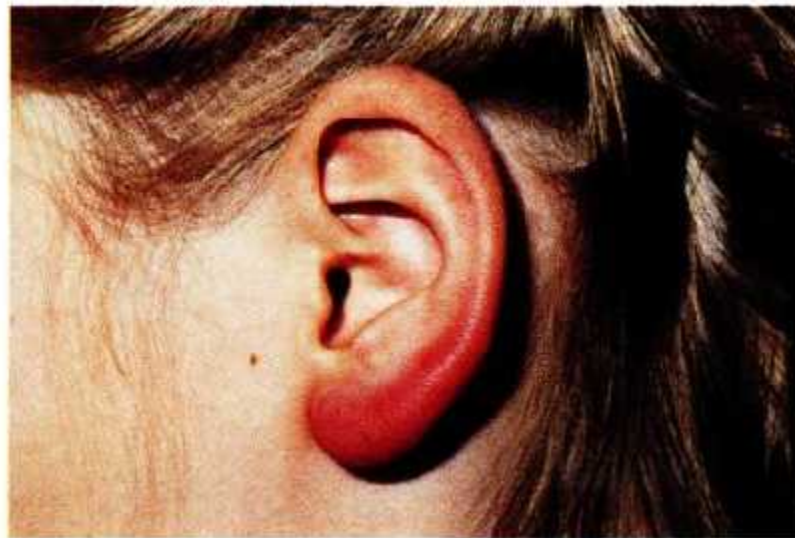


PLATE II. Spirochetal lymphocytoma of eight weeks' duration in a ten-year-old girl. A tick bite occurred six weeks prior to the beginning of the lymphocytoma.

**Image credit: Prof. Dr. Med.  
Klaus Weber, MD**



# Borrelia Lymphocytoma

6 Months  
Duration  
Ear lobe

Cartilage  
Not  
Involved  
Skin of  
Helix  
Region  
Quiescent

**FIGURE 2.** Ear lobe with *Borrelia* lymphocytoma of 6-month duration.



EVA ÅSBRINK AND ANDERS HOVMARK

*Department of Dermatology  
Karolinska Institute at Södersjukhuset  
Stockholm, Sweden*

## **Borrelia lymphocytoma - 6 weeks duration**

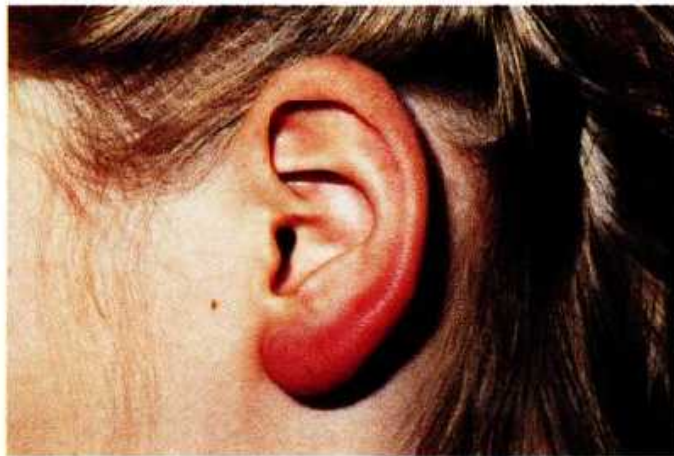


PLATE II. Spirochetal lymphocytoma of eight weeks' duration in a ten-year-old girl. A tick bite occurred six weeks prior to the beginning of the lymphocytoma.

**Image credit: Prof. Dr. Med.  
Klaus Weber, MD**

# Acrodermatitis Chronica Atropicans

## Acrodermatitis chronica atropicans

Spätstadium



Entzündlich-ödematöses Stadium  
Atrophisches Stadium

**Image Credit:**  
**Dr Med S.A. Buchner MD**  
**Dermatologist, Basil**  
**Universitatsspital**



# Acrodermatitis Chronica Atrophicans

## Acrodermatitis chronica atrophicans

Frauen 65-80%  
Einseitig 60-80%



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basel  
Universitatsspital***

# Various ACA Anatomic sites

Skin of:

Elbow

Hand

Arm

Leg

Plantar skin

**Acrodermatitis  
chronica atrophicans**

Untere Extr. 60%  
Obere Extr. 40%

- Ellbogen
- Handrücken
- Knie
- Sprunggelenk
- Plantar



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Ulnar Band formation in ACA

Acrodermatitis chronica atrophicans

„ulnar  
band“



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***



# Multiple Fibrous nodules in ACA

Acrodermatitis chronica  
atrophicans

Fibroide Knoten  
Kutan-subkutane  
Derbe Knoten

- Häufig in Gelenknähe
- Ellbogen
- Finger



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Fibrous Nodules in ACA

Acrodermatitis  
chronica  
atrophicans

Fibroider Knoten



***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Acrodermatitis Chronica Atrophicans

## Extracutaneous manifestations

### Acrodermatitis chronica atrophicans

- Periphere Neuropathie 40-60%
- Arthritis 20%
- Luxationen der kleinen Finger- und Fussgelenke 30%

Asbrink Clin Dermatol 1993

***Image Credit:  
Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***



# European Western Blot For Borreliosis

## *Borrelia burgdorferi*

P41	Flagellin	41 kDa	Frühphase
OspA	outer surface protein	31 kDa	Spätphase
OspB	outer surface protein	34 kDa	
OspC	outer surface protein	22 kDa	Frühphase
P100		100 kDa	
P39		39 kDa	
P60	common antigen	60 kDa	Kreuzreaktion

***Image Credit:***

***Dr Med S.A. Buchner MD  
Dermatologist, Basil  
Universitatsspital***

# Squamous Cell Carcinoma Arising in ACA skin



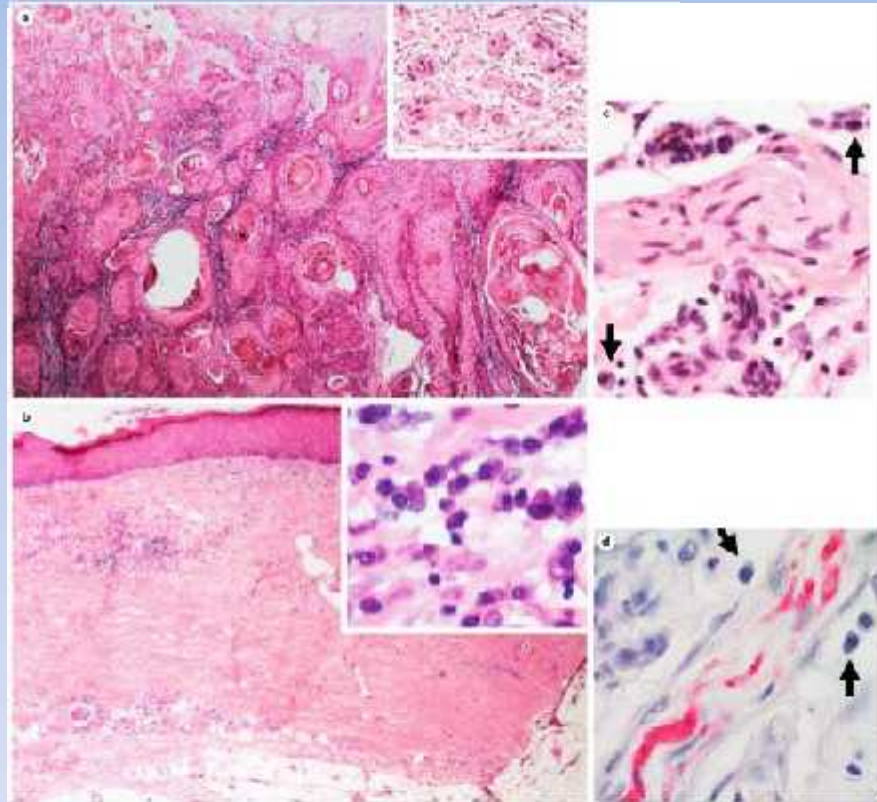
**Metastatic Squamous Cell Carcinoma  
of the Ankle in Long-Standing Untreated  
Acrodermatitis Chronica Atrophicans**

M. Leverkus · A.M. Finner · A. Pokrywka · I. Franke · H. Gollnick

Department of Dermatology and Venereology, Otto von Guericke University, Magdeburg, Germany

# Squamous Cell carcinoma

## Arising in ACA skin



### Metastatic Squamous Cell Carcinoma of the Ankle in Long-Standing Untreated Acrodermatitis Chronica Atrophicans

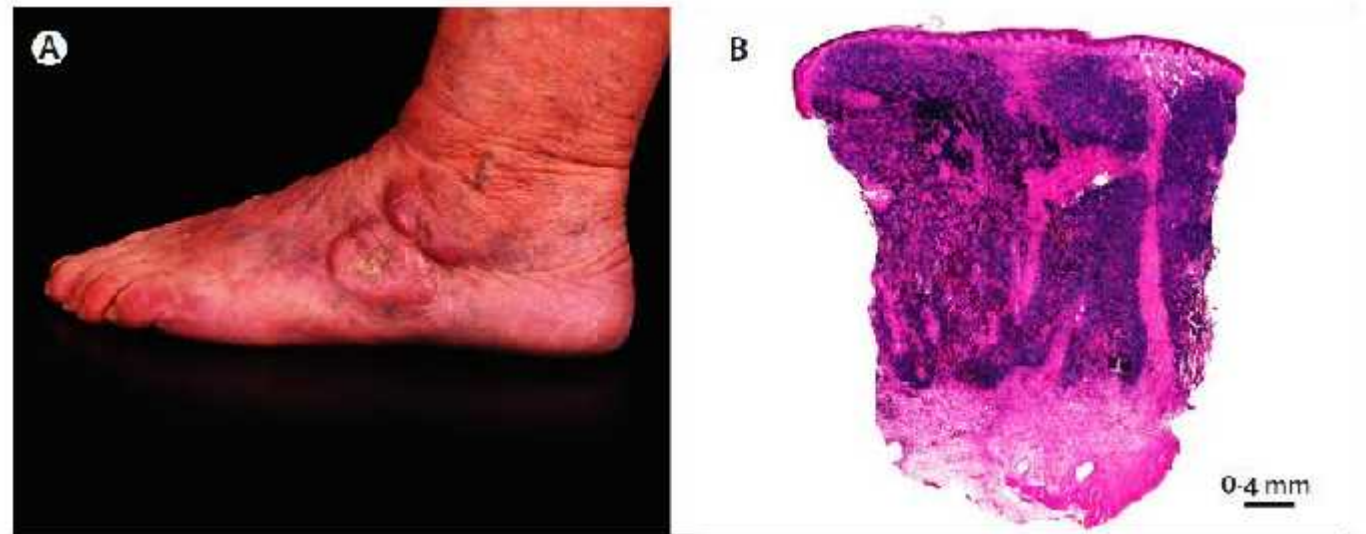
M. Leverkus · A.M. Finner · A. Pokrywka · T. Franke · H. Gollnick

Department of Dermatology and Venereology, Otto von Guericke University, Magdeburg, Germany



# Malignant Lymphoma

## Arising in ACA skin



**Figure: *B afzelii* induced cutaneous marginal zone lymphoma of the left foot**

(A) Before treatment: two large red nodules on the left foot. (B) Dense, diffuse, lymphoid infiltrates in the dermis, without involvement of epidermis.

**Lancet 2011; 377: 178**

**Department of Dermatology,  
Medical University of Graz,  
Graz, Austria (Prof E Aberer MD,  
N Wutte MD,  
Prof R Fink-Puches MD,  
Prof L Cerroni MD); and**

# Acrodermatitis Chronica Atrophicans

**FIGURE 3.** A swollen discolored heel with acrodermatitis chronica atrophicans of more than 3-year duration.



EVA ÅSBRINK AND ANDERS HOVMARK

*Department of Dermatology  
Karolinska Institute at Södersjukhuset  
Stockholm, Sweden*

# Prominent Fibroid nodules In Acrodermatitis Chronica Atrophicans

Plantar Skin  
With Prominent  
Disease

NOTE:

Very few diseases

Inflict INJURY

On the

Palmar Skin

Of Plantar Skin

---

Can you name another  
Disease which  
Involves  
Palmar/Plantar??



**Acrodermatitis Chronica  
Atrophicans with Fibroid  
NODULES  
[ of foot]**

**Cutaneous manifestations  
of Lyme borreliosis**

Dieter Hassler

General practitioner and infectious disease specialist, German Society  
for Infectious Diseases, Germany







**Metastatic Squamous Cell Carcinoma  
of the Ankle in Long-Standing Untreated  
Acrodermatitis Chronica Atrophicans**

M. Leverkus · A.M. Finnen · A. Pokryuko · I. Franke · H. Gollnick

Department of Dermatology and Venereology, Otto von Guericke University, Magdeburg, Germany

# Acrodermatitis Chronica Atopicans

**Paper thin  
Epidermis  
[Epidermal  
atrophy]  
Is a  
hallmark  
Of ACA**



Archiv für klinische u. experimentelle

Dermatologie 232, 373—383 (1968)

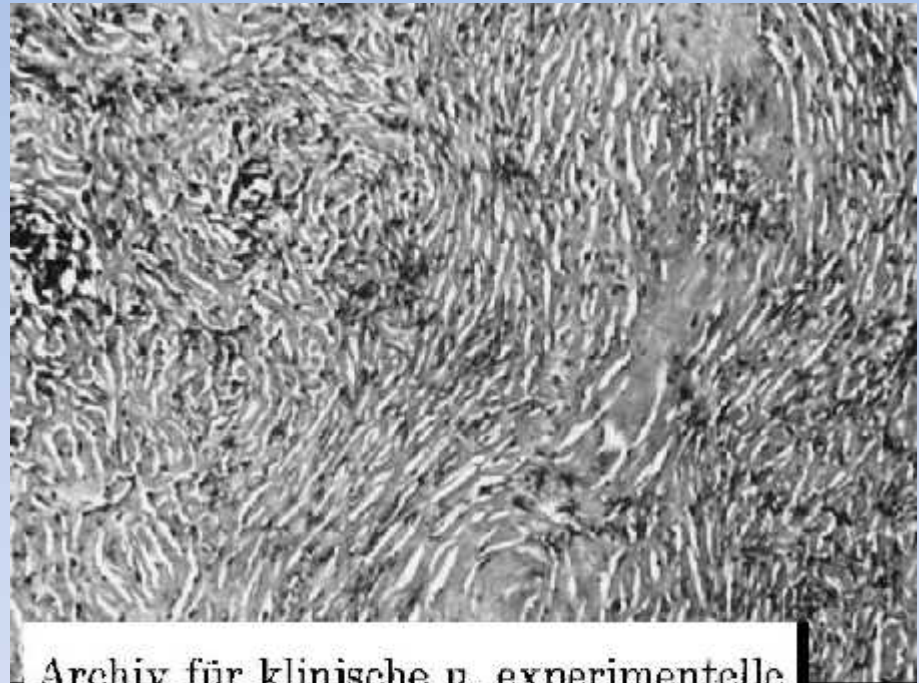
Dr. TH. HARDMEIER

Pathologisches Institut

CH-1000 Lausanne/Schweiz

# Acrodermatitis Chronica Atrophicans

Mainly Fibrotic  
Stage  
With scattered  
PERSISTENT  
Inflammatory  
Foci  
throughout  
The lesion



Archiv für klinische u. experimentelle

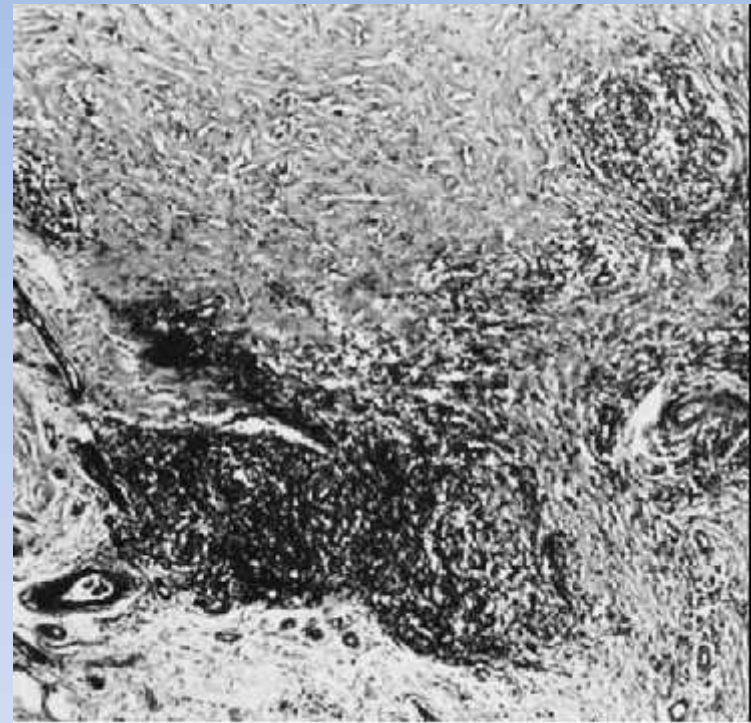
Dermatologie 232, 373—383 (1968)

Dr. TH. HARDMEIER  
Pathologisches Institut  
CH-1000 Lausanne/Schweiz



# Adrodermatitis Chronica Atrophicans

**ACA Dense  
Inflammatory  
Stage  
Little to No  
Fibrosis in this  
Stage**

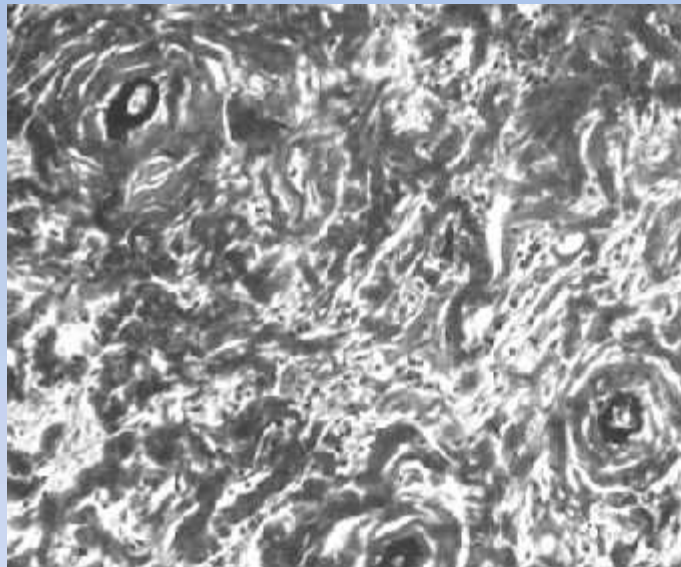


Archiv für klinische u. experimentelle

Dermatologie 232, 373—383 (1968)

Dr. TH. HARDMEIER  
Pathologisches Institut  
CH-1000 Lausanne/Schweiz

# ACA DENSE FIBROSIS Stage



Archiv für klinische u. experimentelle

Dermatologie 232, 373—383 (1968)

Dr. TH. HARDMEIER

Pathologisches Institut

CH-1000 Lausanne/Schweiz

# Acrodermatitis Chronica Atrophicans

## The Fibrosis and the Fibrous Knots

Zur Histopathologie der fibroiden Knoten  
bei Akrodermatitis chronica atrophicans

TH. HARDMEIER

26. April 1968

Archiv für klinische u. experimentelle

Dermatologie 232, 373—383 (1968)

Dr. TH. HARDMEIER  
Pathologisches Institut  
CH-1000 Lausanne/Schweiz



# ExtraCutaneous sites with ACA Histologies

***Acrodermatitis Chronica Atrophicans***  
***occurring outside of the Cutis:***

***Professor Dr. Med.Th.Hardemeier***  
***Lausanne, Switzerland Year 1964***

-----

***ACA explains 4 Idiopathic Diseases***

-----

***Thyroiditis of Reidel ( Woody Thyroiditis)***  
***Idiopathic Retroperitoneal Fibrosis***  
***Takayasu Arteritis***  
***Idiopathic MediastinalFibrosis***

# **Cutaneous borreliosis**

## **The Not so Complete List of Diagnoses**

***European Skin lesions  
which ultimately were  
linked to Borrelia  
Infection of the Skin:***

***1902 :: ACA -Herxheimer***

***1909::ECM- Afzelius***

***1922:: Paralysis -***

***Garin, Bujadoux***

***1955::Bender***

***1957: Lymphadenosis***

***Benigna Cutis (BL)***

# **FOCUS FLOATING MICROSCOPY [FFM]**

**Dr. Klaus Eisendle MD and  
Dr. Bernhard Zelger MD , PhD**

**Revolutionized the Microscopist's ability to find borrelia in the Skin**

**Expansion of the list of  
Cutaneous Borreliosis**



# Focus floating Microscopy +++



THE EXPANDING SPECTRUM OF CUTANEOUS BORRELIOSIS

GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA

**2009 April, 144 (2):157-71**

K. EISENDLE, B. ZELGER

**Lichenoid lesion of**  
**Back**  
**Lichen Sclerosus**  
**FFM Positive**

# Focus Floating Microscopy +++



THE EXPANDING SPECTRUM OF CUTANEOUS SURRELLOIDS

GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA

2009 April, 144 (2):157-71

K. EISENDLE, B. ZELGER

**Lichenoid lesion of**  
**Genital Skin**  
**Lichen Sclerosus**  
**(et atrophicus)**  
**Focus floating**  
**Microscopy**  
**POSITIVE**

# Focus Floating Microscopy +++



Figure 6.—Morphea (A) Clinical picture of early plaque morphea; B,C) histopathology of morphea at different magnifications showing dense infiltrates of lymphocytes and plasma cells and fibrosclerosis (H&E, b x4, c x100, d x200).

THE EXPANDING SPECTRUM OF CUTANEOUS BORRELIOSES

GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA

**2009 April, 144 (2):157-71**

K. EISENDLE, B. ZELGER

**Morphea**  
**skin of Leg**  
**Focus floating**  
**Microscopy**  
**POSITIVE**



# Focus floating Microscopy +++

THE EXPANDING SPECTRUM OF CUTANEOUS BORRELIOSES

GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA

2009 April, 144 (2):157-71

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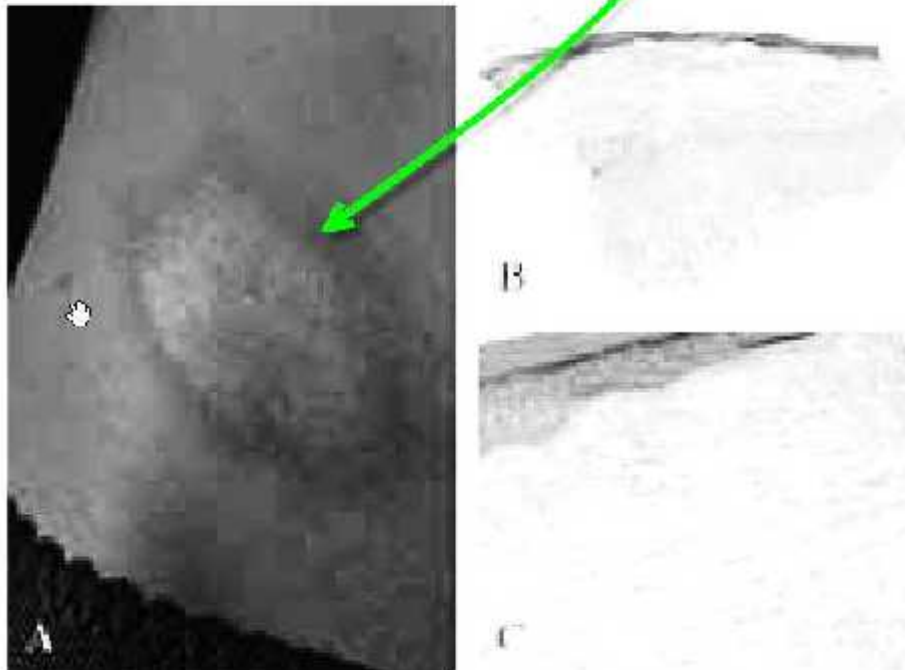


Figure 4. A) Late inflammatory poor "burned out" morphea; B, C) histopathology showing nearly absence of inflammatory infiltrates, atrophy of epidermis and marked sclerosis (H&E, b x10, c x100).

To the UnEducated Eye  
this might be confused  
with "Bull's eye type" EM

Morphea  
Variant  
"late Inflammatory  
poor type"  
"Burned OUT"

---

No Leukocytes in  
Skin biopsy  
Focus Floating  
Microscopy  
POSITIVE

**Necrobiosis Lipoidica  
Is  
Added to  
Cutaneous borrelioses  
By Drs. Eisendle and Zelger  
Year 2008**

# Focus Floating Microscopy +++

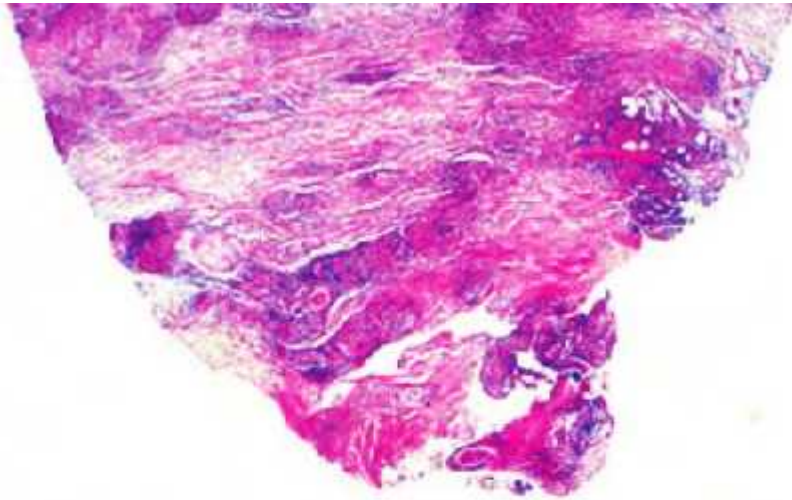


Detection of spirochaetal  
microorganisms by focus floating  
microscopy in necrobiosis lipoidica

K Eisendle, M Baltaci, H Kutzner<sup>1</sup> & B Zelger

*Histopathology* 2008; 52: 877-884.

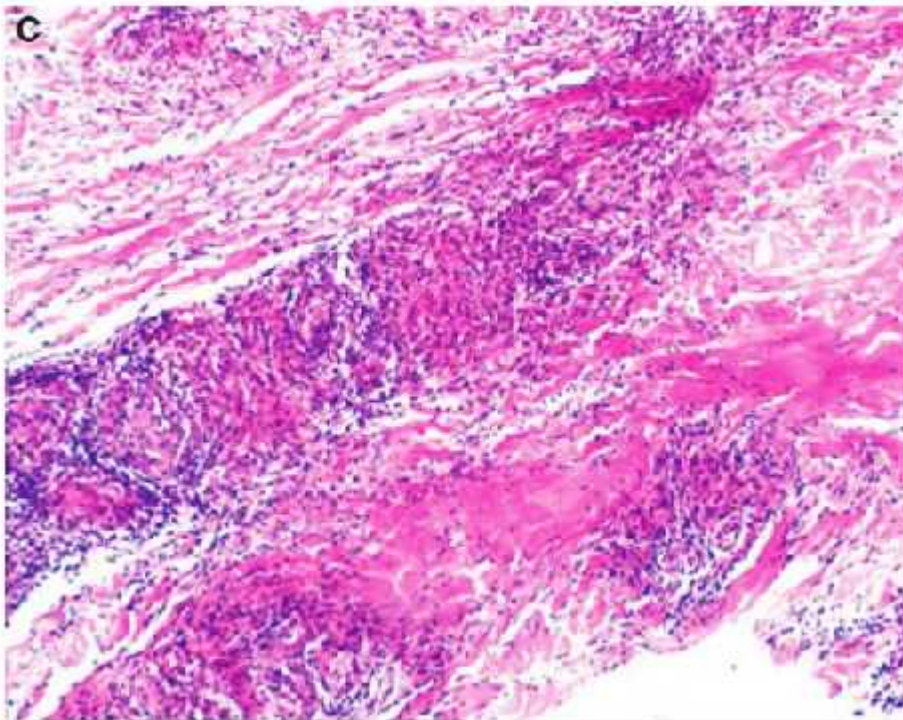




## Detection of spirochaetal microorganisms by focus floating microscopy in necrobiosis lipoidica

K Eisendle, M Baltaci, H Kutzner<sup>1</sup> & B Zelger<sup>4</sup>

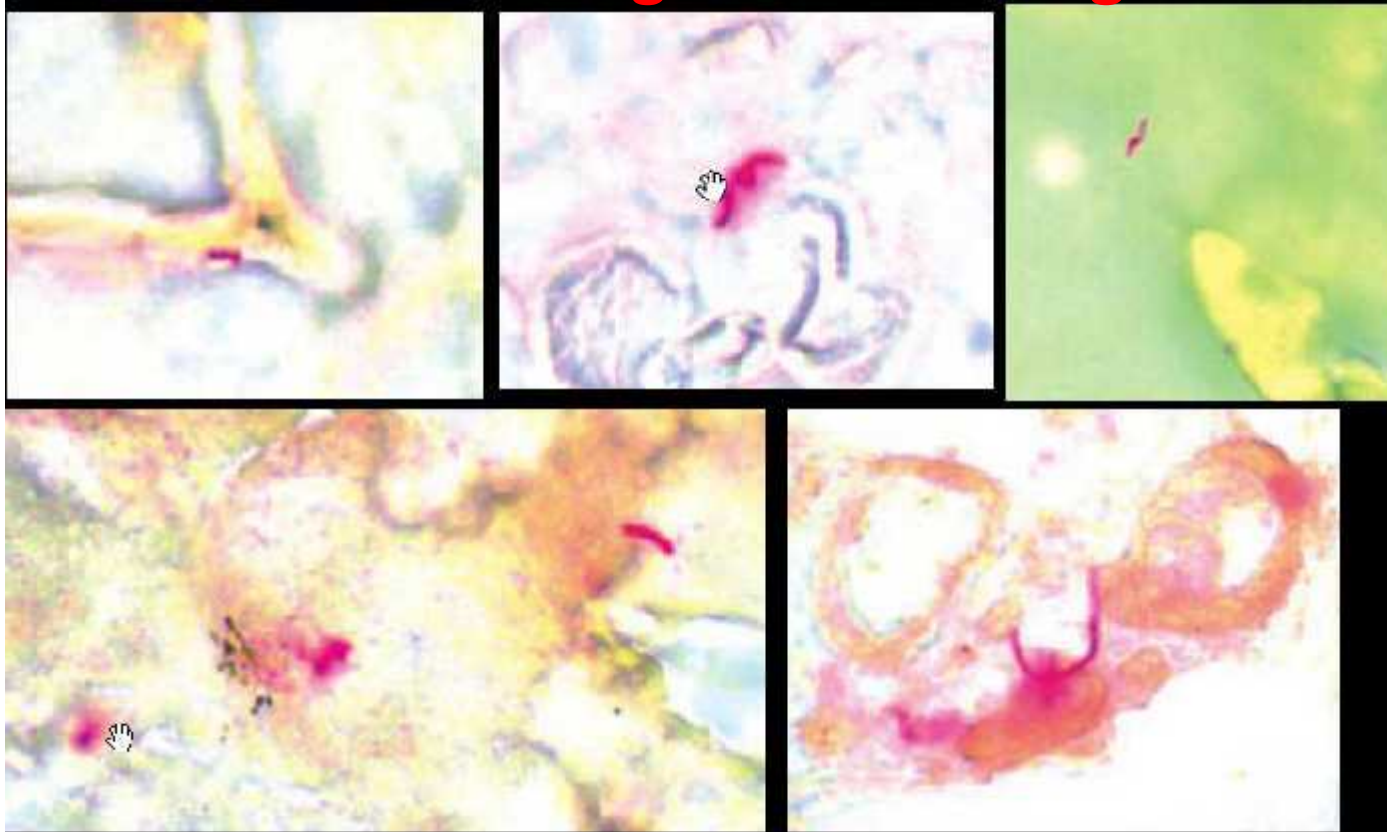
*Histopathology* 2008; 52, 877-884.



**Necrobiosis Lipoidica**  
**Microscopic view**  
**routine Hematoxylin and Eosin**

## Focus Floating Microscopy ::

Demonstrates Red staining Borrelia – single and in Groups



**Detection of spirochaetal  
microorganisms by focus floating  
microscopy in necrobiosis lipoidica**

K Eisendle, M Baltaci, H Kutzner<sup>1</sup> & B Zelger

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# Focus Floating Microscopy

Detection of spirochaetal microorganisms by focus floating microscopy in necrobiosis lipoidica

K Eisendle, M Baltaci, H Kutzner<sup>1</sup> & B Zelger

*Histopathology* 2008, 52, 877-884.



**Note:**  
**Focus Floating Microscopy**  
**demonstrates a**  
**community of**  
**borrelia with specialized**  
**[Non-Spiral] shapes**  
**which are surrounded by**  
**a "reddish veil"**



**Necrotic Xanthogranuloma**  
**Positive by**  
**Focus Floating Microscopy**  
**For Lesional Borrelia**  
**Year 2008**

**Dr. Bernhard Zelger**  
**and**

**Dr. Klaus Eisendle**

# Detection of spirochetal micro-organisms in necrobiotic xanthogranuloma

Bernhard Zelger, MD, MSc,<sup>a</sup> Klaus Eisendle, MD, PhD,<sup>a</sup> Christian Mensing, MD,<sup>b</sup>  
and Bettina Zelger, MD<sup>c</sup>  
*Innsbruck, Austria; and Hamburg, Germany*

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doi:10.1016/j.jaad.2007.05.016



# Detection of spirochetal micro-organisms in necrobiotic xanthogranuloma

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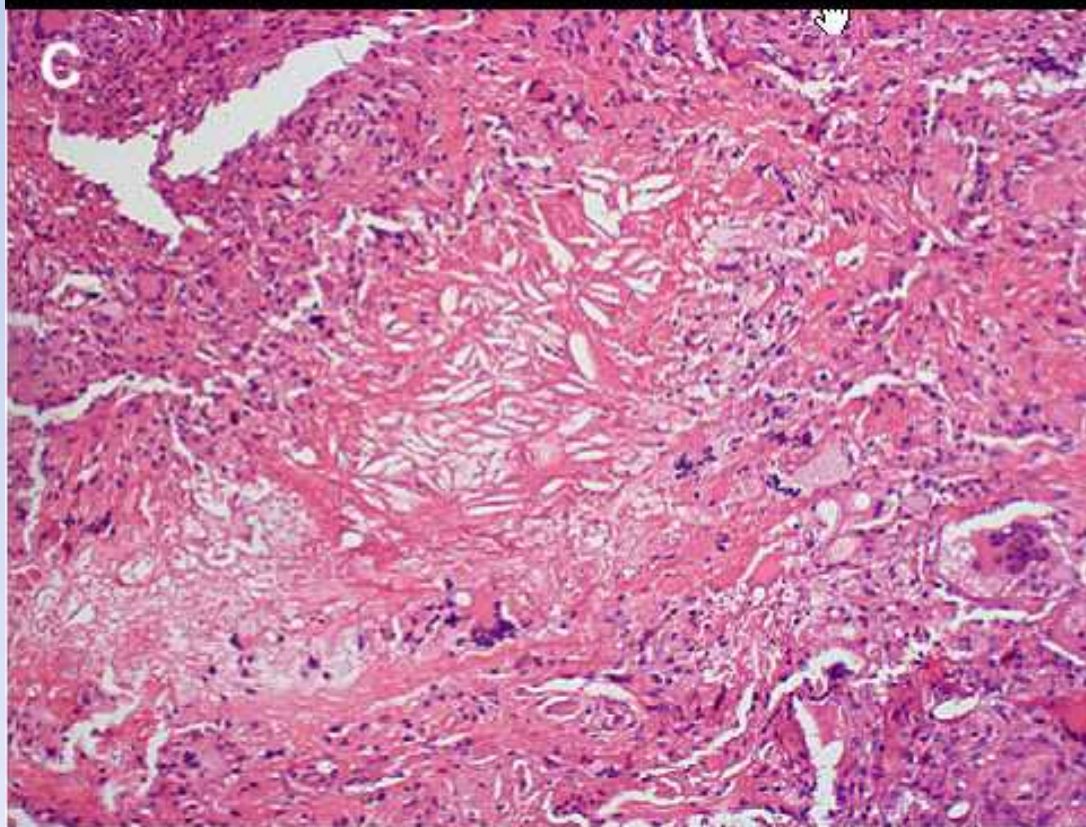


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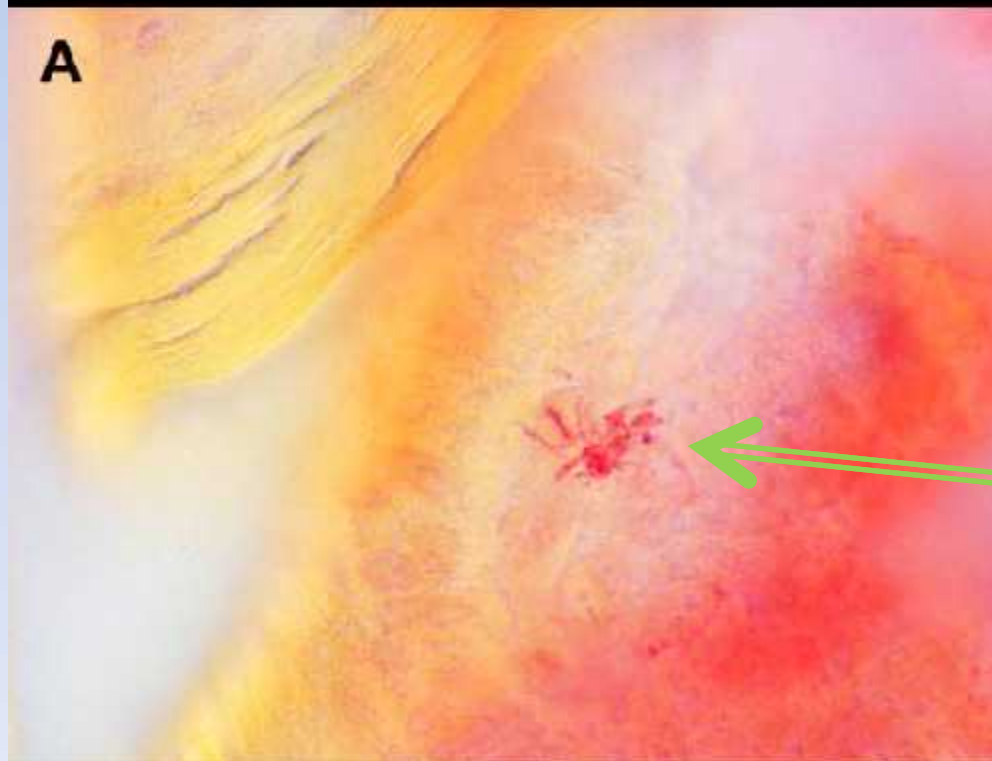


# Focus Floating Microscopy

## Detection of spirochetal micro-organisms in necrobiotic xanthogranuloma

Bernhard Zelger, MD, MSc,<sup>a</sup> Klaus Eisele, MD, PhD,<sup>a</sup> Christian Mensing, MD,<sup>b</sup>  
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doi:10.1016/j.jaad.2007.05.016



### Necrotic Xanthogranuloma

### --- Focus Floating Microscopy

---  
A Group of Borrelia  
organisms including  
Non-Spiral forms  
[ straightened and  
Granular Borrelai forms]



Paraproteinemia

N= 5 cases

Plasmacytoma

N= 2 cases

World Total Number  
Of NXG cases

N=80

FACE >> Trunk > extremities

Also in:

Myocardium, Liver, Lungs

Paraproteins :: specificity

BIND to LIPOPORTEINs

PCR [ with B31 PCR primers

:: Always negative in NXG

**NO PCR ever done with  
Primers for BB sl Group of  
Borreliae**

## Detection of spirochetal micro-organisms in necrobiotic xanthogranuloma

Bernhard Zelger, MD, MSc,<sup>a</sup> Klaus Eisendle, MD, PhD,<sup>a</sup> Christian Mensing, MD,<sup>b</sup>  
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doi:10.1016/j.jaad.2007.05.016

Patient No., sex, age at first presentation of skin lesions, y	Paraproteinemia	<i>Borrelia</i> serology	Course of disease
1, Male, 55	Since 2005 IgG lambda; no plasmacytoma	Negative	Since 1995 progressive plaques on head, face, trunk, and extremities with clinical features of morphea, necrobiosis lipoidica, and NXG; surgical reduction. Posner-Schlossmann syndrome (sarcoidosis of eye). Alive with disease 2006.
2, Female, 37, previously published <sup>2</sup>	Since 1994 IgG kappa; no plasmacytoma	NA	Since 1977-1995 progressive plaques on face, trunk, and extremities. At 1994-2000 no therapeutic benefit from clofazimine, prednisolone, dapsone, minocycline, and bath PUVA. During melphalan and prednisolone for 5 mo rapid enlargement of skin lesions. Alive with disease 2006.
3, Male, 70	None; since 2002 plasmacytoma	NA	Since 2004 first plaques in face, no therapy at this time. In due course generalization with plaques on trunk. Progression and death from plasmacytoma within 1 y despite therapy with chlorambucil.
4, Female, 42	None; no plasmacytoma	Negative	Since 2004 wax and wane of plaques on face. No therapy so far. Alive with disease 2007.
5, Female, 49	Since 1992 IgG kappa; no plasmacytoma	Negative	Since 1992 plaques of upper eyelids, later rapidly progressive to face and neck despite multimodal therapies including excision, prednisolone, clofazimine, interferon alfa, cyclophosphamide, plasmapheresis, (off-label) imiquimod therapy. Alive with disease 2006.
6, Female, 63	Since 1996 IgG kappa; plasmacytoma	NA	Since 1996 periocular plaques, lost from follow-up 2003.
7, Female, 56	Since 1984 IgM; no plasmacytoma	NA	Since 1984 first plaques of upper eyelids and face, in due course trunk and extremities. No therapy. Alive with disease 2005.

NA, Not available; NXG, necrobiotic xanthogranuloma; PUVA, psoralen plus ultraviolet A.



**Cutaneous borrelioses added since  
1980**

**Lichen Sclerosus, and genital LSA**

**Granuloma Annulare**

**Interstitial Granulomaous Dermatitis**

**Cutaneous sarcoidosis ( Chinese)**

**Necrobiosis Lipoidica**

**Necrobiotic Xanthogranloma**

**Erythema annulare centriigum**

**Lymphocytic infiltrate of Jessner**

**B cell Cutaneous Lymphoma**

**T cell cutaneous Lymphoma (M.F.)**

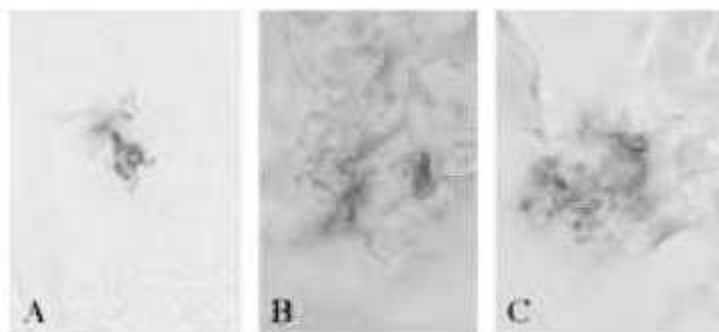


Figure 8.—A) Focus-floating microscopy and immunohistochemical staining (Acris BP 1C02, no counterstain, x1000) for various borrelial colony forms. Medusa-like cluster of “planktonic microorganisms” in a case of acrodermatitis chronica atrophicans. B) Colony of degenerating fragmented/small granula: “dying” spirochetes in a case of morphea. C) Putative biofilm formation of a borrelial colony with a mixture of medusa-like and granular spirochetal aggregations with cystic rounded forms, tubular elements or swollen granules covered by a reddish veil in a case of lichen sclerosus.

LS. For these reasons and the reliable detection of spirochetal microorganisms in morphea and LS, one must conclude that at least some cases of morphea and LS should be integrated in the spectrum of cutaneous borrelioses.

#### **Biofilms of *B. burgdorferi sensu latu* in chronic or recurrent cutaneous borreliosis?**

The hypothesis that *B. burgdorferi* might form biofilm structures in BL and ACA was recently proposed based on the finding of large colonies of *Borrelia* in classical cutaneous borrelioses shown by immuno-

histochemistry and FFM.<sup>106, 107</sup> So, *Borrelia* can grow in a “medusa colony” or in a “granular colony with a reddish veil”<sup>42</sup> (Figure 8 A-C). These forms of borrelial growth were first described in vitro by Aberer and Duray<sup>22</sup> and such colonies reveal striking similarities to previously published biofilm pictures.<sup>108</sup> It is a fascinating hypothesis to compare large borrelial aggregations in the tissues with biofilms and speculate that such biofilms of *B. burgdorferi* might be responsible for a partial resistance to antibiotic therapy in some patients with Lyme disease. Subsequently, the potential that *Borrelia* may shed from these biofilms, might thus provide a possible explanation for chronic relapsing courses of some borrelial infections. Of note, biofilm formation in the human host has already been described for other spirochetes like *Treponema denticula*,<sup>109</sup> and biofilm formation has been associated with antibiotic resistance in *Helicobacter pylori* infections.<sup>110</sup> Bacterial biofilms are responsible for several chronic diseases (*e.g.* periodontitis and chronic lung infection in cystic fibrosis patients) that are very difficult to treat because they show much greater resistance to antibiotics than their free-living counterparts.<sup>111</sup> The biofilm resistance is very unique in a sense that it requires multiple mechanisms such as incomplete penetration of the antibiotics into the matrix, inactivation of antibiotics by altered chemical microenvironment within the biofilm and an altered, protected phenotypic “spore like” state of the resistant bacteria population.<sup>112</sup> If *B. burgdorferi* is indeed capable forming biofilms, it will change the way, how we think about Lyme disease especially in patients,



# Human Babesiosis World map With Vector map

Alan MacDonald MD: Editorial Comment: Geographies with Endemic Babesiosis do not exist in a vacuum. Endemic Lyme borreliosis travels with Endemic human Babesiosis!!!!.

The NEW ENGLAND JOURNAL of MEDICINE

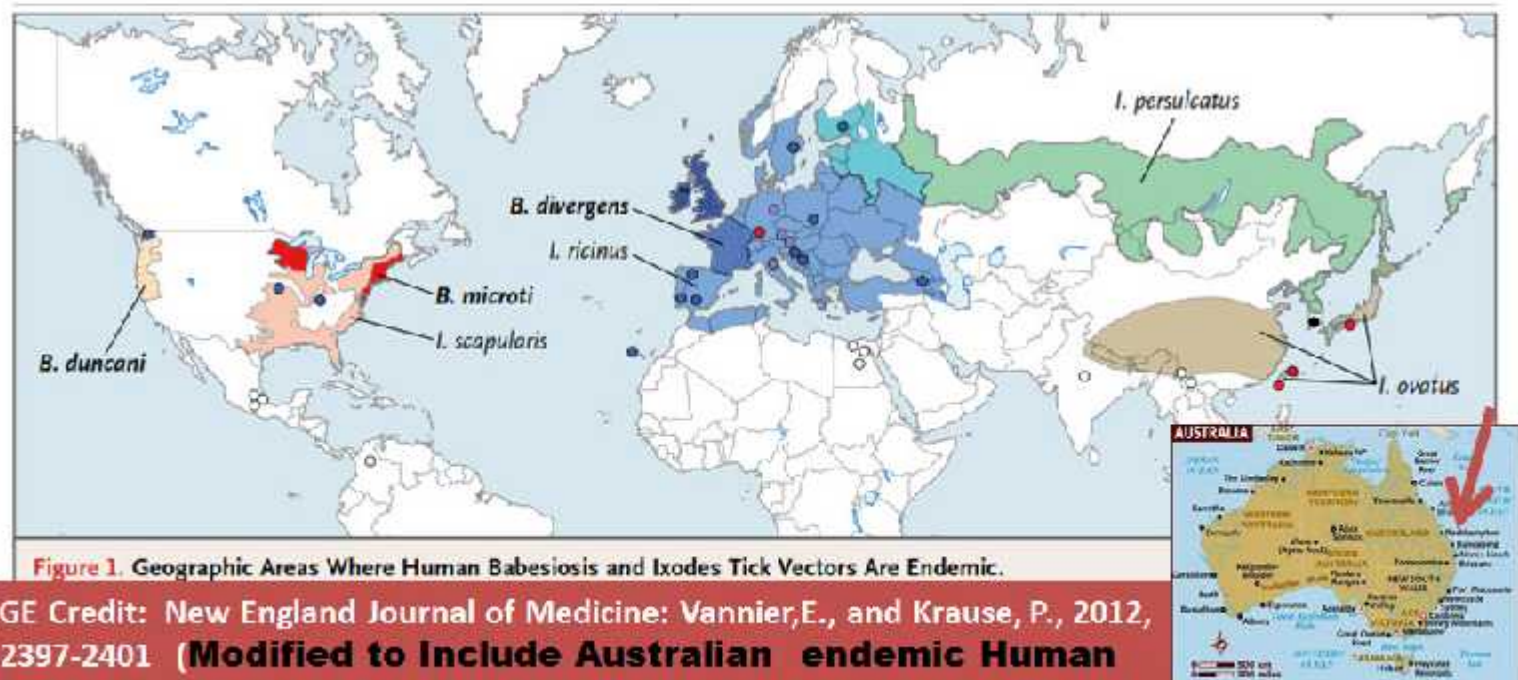


Figure 1. Geographic Areas Where Human Babesiosis and Ixodes Tick Vectors Are Endemic.

IMAGE Credit: New England Journal of Medicine: Vannier,E., and Krause, P., 2012, 366:2397-2401 (Modified to Include Australian endemic Human babesiosis )



# IXODID TICK HABITATS - EXPANDING WORLDWIDE

1975-

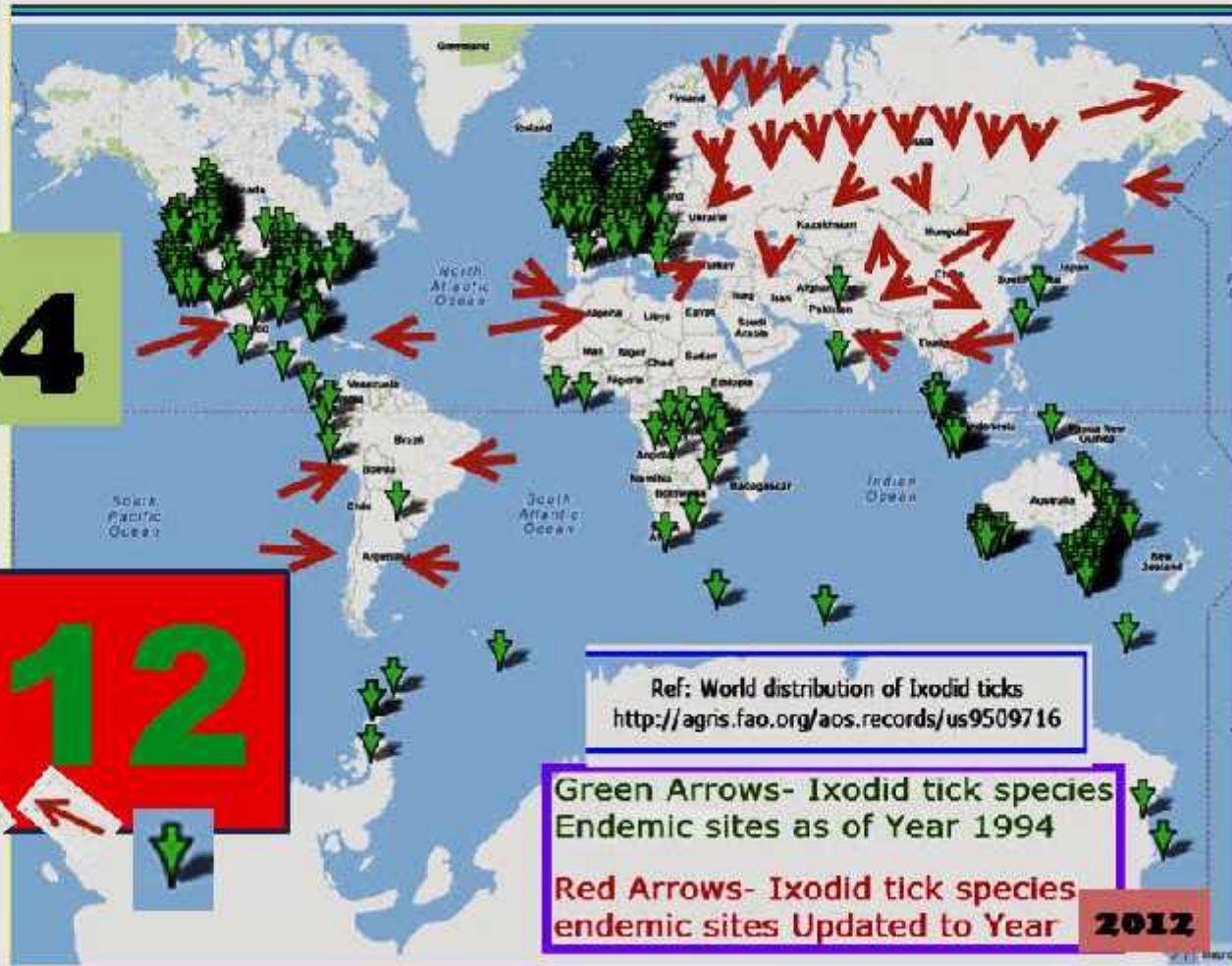
1981

No Tick maps  
posted

**1994**



**2012**





**Figure 13.** Geographic distribution of pathogenic bacteria of *Borrelia burgdorferi* complex (pathogenic role of *Borrelia valaisiana* is highly suspected)





# Who Is This clinician?



"Absence of proof  
is not  
proof of absence."

- Dr. Edwin J. Masters, M.D.

The future ain't  
what it used to be

~~Yogi Berra

## Large Missouri EM



## Culture Attempts Since 1989

1. Dorothy Feir – St. Louis University
2. Don Miles – St. Francis Hospital Cape Girardeau, MO
3. Russ Johnson – Minnesota
4. David Persing – Mayo Clinic
5. John Anderson – Conn AG Exp. Station
6. Alan Barbour – U.of Texas San Antonio
7. Paul Duray – Harvard

## Culture Attempts cont.

8. Jim Miller – UCLA
9. Julie Rawlings – Texas Dept of Health
10. Paul Fawcett – Thom Jefferson Univ.
11. Sam Telford – Harvard
12. Tom Kollars – Georgia Southern
13. Jim Oliver – Georgia Southern
14. Gary Wormser – NY Med. College

**Tables compiled by  
Dr Edwin J. Masters,  
MD  
Cape Girardeau,  
Missouri**

## Culture Variations

- A. BSK-II Variations
- B. BSK H (Sigma, St. Louis)
- C. Original BSK
- D. Kelly's Relapsing Fever
- E. Microgravity
- F. Fibroblasts
- G. Bovine Vitreous Added
- H. Pulverized *Lone stars* Added
- I. White-Footed Mice
- J. SCID Mice
- K. Rabbits
- L. Embryonated Chicked Eggs



# First Isolation and Cultivation of *Borrelia Burgdorferi*; Sensu Lato From Missouri

*J Clin Microbiol*, Jan. 1998, p. 1-5

Over 70 *B.b.*  
isolates from  
Missouri ticks



The visible EM distinctions are more for the groups, MO vs. NY. There are individual EM's from the entire spectrums of both MO and NY that are indistinguishable.

One cannot tell the geographic etiology of an individual EM by appearance alone.



**Edwin Jordan  
Masters, MD  
Internist  
Researcher  
Patient advocate  
Hero**

**Southern Erythema  
Migrans  
Collection  
Edwin Masters MD  
Cape Girardeau  
Missouri**

**Can You Tell  
Missouri type  
Erythema Migrans**

**From**

**Erythema Migrans  
New York type?????**





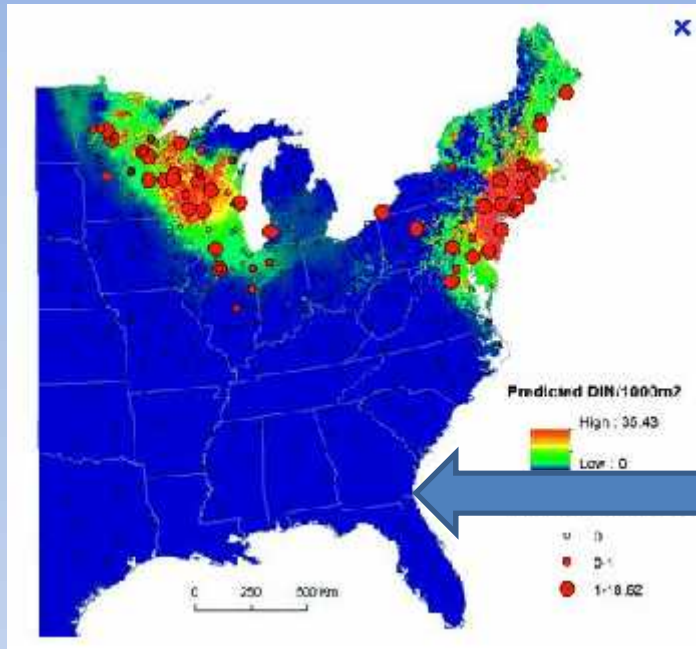
**Southern Erythema Migrans**  
**From Dr Edwin Masters' patients residing in**  
**Cape Girardeau, Missouri**

**Can you tell**  
**Erythema Migrans**  
**Missouri Type**

**From**

**Erythema migrans**  
**New York Type??**





CDC Map : National Lyme  
Disease Cases  
RED> Yellow>Green

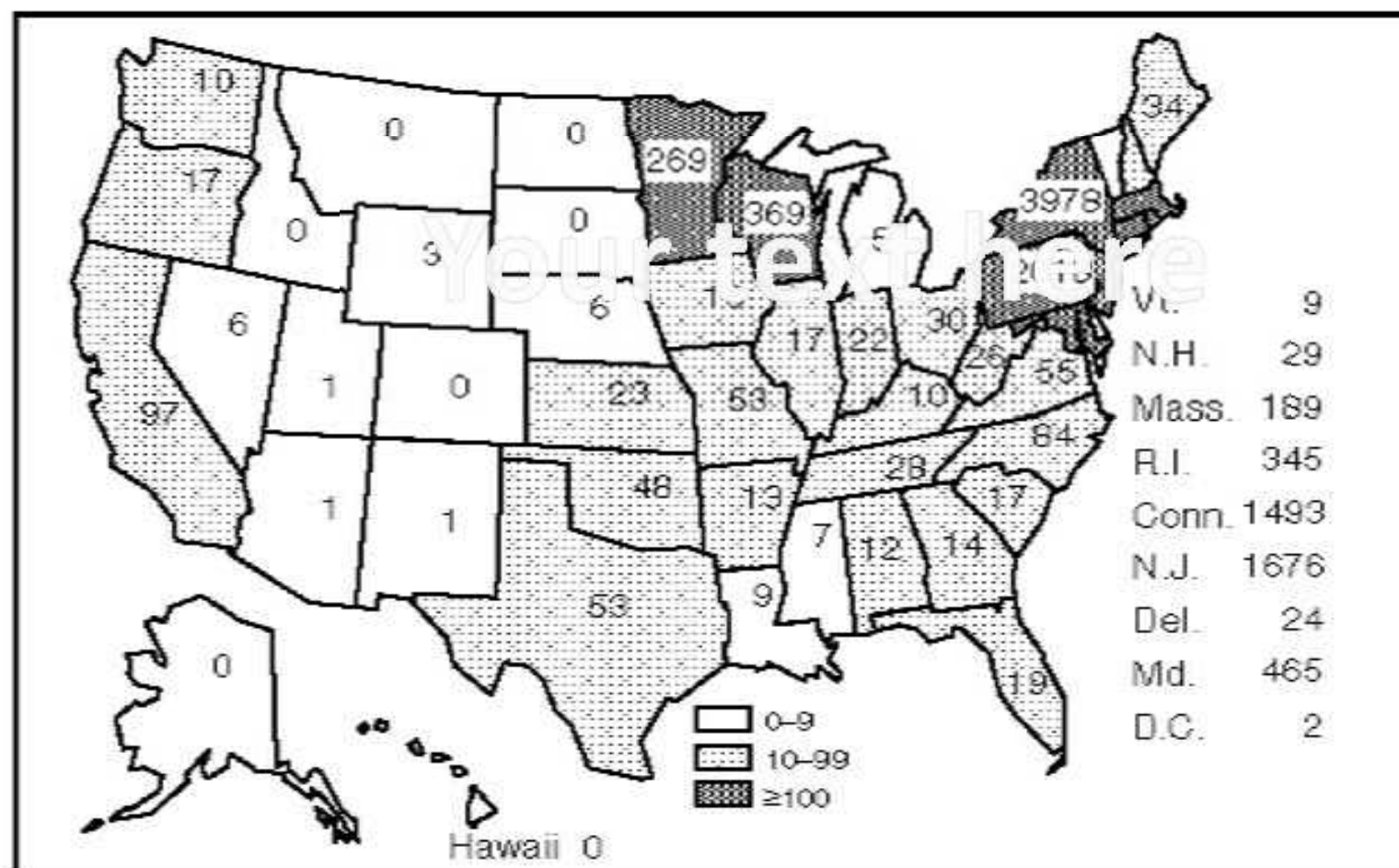
**BLUE = VIRTUALLY NO  
SIGNIFICANT LYME**

**Red =  
No Significant  
Lyme**



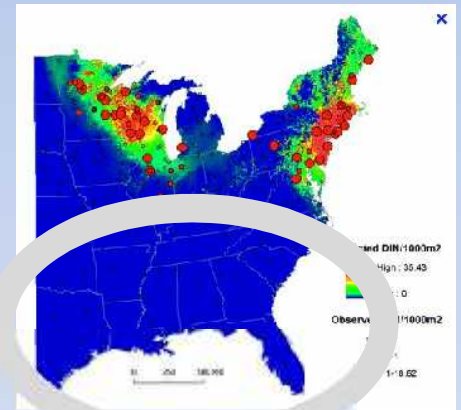
## CDC changes its rules for Accepting Lyme Reports After Year 1994

FIGURE 2. Number of reported Lyme disease cases, by state — United States, 1994





# Southern Erythema Migrans



# Southern Erythema Migrans

**A true Case – of LYME Disease in a Resident of a Southern State in the USA**

**A Reportable Case - -To your State Department of Health**

**A Statistical point on the USA Map –CDC**

**A disease which Merits –TREATMENT WITH ANTIBIOTICS**



**Not a True Case of Lyme disease**

**Southern State in the USA AND**

**Northern State in the USA**

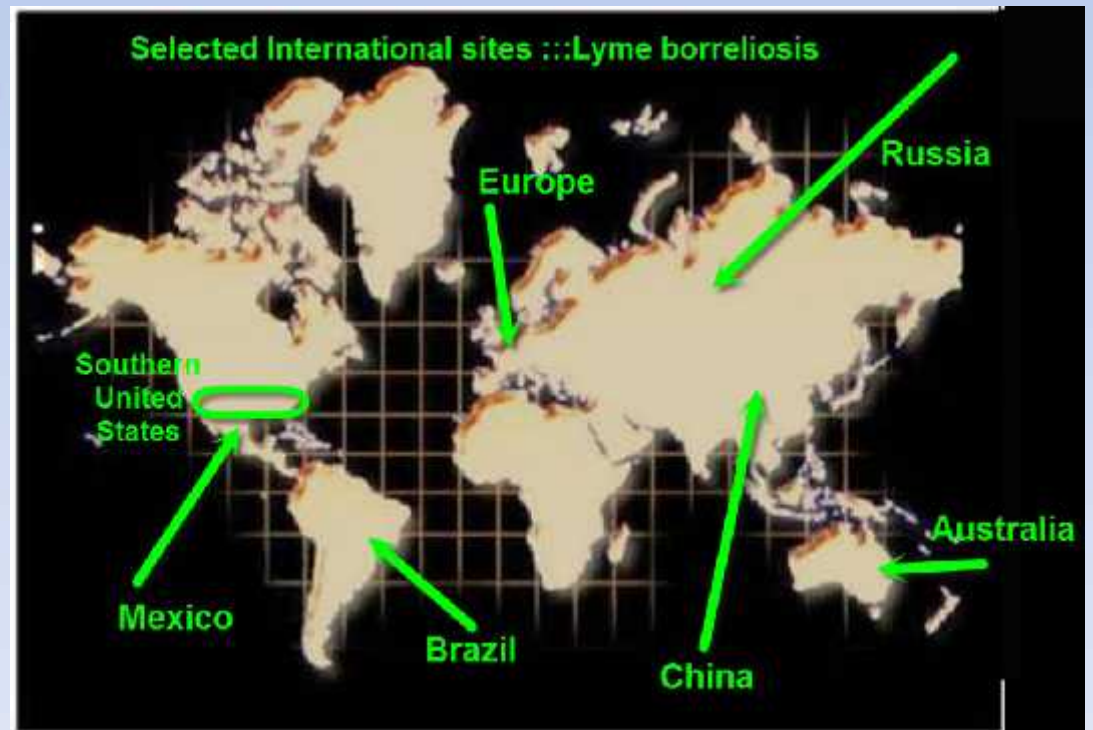
**Amblyomma Americanum [Lone Star] Tick vector Not a Reportable Case—**

**Not a Statistical point on the USA MAP –CDC**

**A disease which may encounter fiery DEBATE about the Medical Justification to Treat with Antibiotics**

# World Wide Distribution Of Cutaneous Borreliosis

ANY CONTINENT  
EXCEPT ANTARCTICA





**Who In the World is this Man??**



**HINT: BIOFILMS, FATHER OF**

# **Biofilm formation By *Borrelia burgdorferi* IN VITRO [ IN TEST TUBE ONLY] BIOFILM FORMATION BY BORRELIA BURGDORFERI**

**In Vitro biofilm formation by Bb Proven by Dr Eva Sapi et al, in  
PLOS ONE article epublished October 24, 2012**

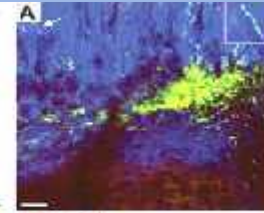
**POSSIBLE CANDIDATES FOR IN VIVO B. BURGDORFERI BIOFILMS**

**Tick Midgut :: *Ixodes Scapularis*, “a Carpet of Spirochetes”**

**Human Skin :: FFM Positive cases from Dr. Klaus Eisendle and Dr. Bernhard Zelger**

**Mouse Skin:: Dr.Linda Bockenstedt:: Yale :: Experimental Lyme arthritis model in Mice**

## Biofilms of Borrelia : In Vivo Examples : Ticks Mice and Men



Tick Mouse Man

**Biofilm** : A definition - RECITE THE Words that define the requisite parameters

Leave the *Planktonic microbial forms* behind - Biofilms are built from  
*novel NonPlanktonic microbial forms which differ in structure and in density*  
from mere *Planktonic microbial aggregates* ::::: Investment by E. Matrix

Images of biofilms of various SPIROCHETAL microbial species:

( See Image gallery : Tick gut biofilms, In vitro Biofilms, ??Human biofilm candidates  
Based on parallel structure with FFM Images from BL and ACA:: Eisendle et al FFM paper,  
Proposed Image based Biofilm examples from the Peer reviewed Medical Literature

*Helicobacter pylori* biofilms in Human Gastric tissue, structural comparisons with Bb biofilm  
Like carpets in Ixodid tick midgut

Image gallery:[Images to follow]

Links: supplementary manuscripts and materials

Note: Helpful conferences and Image data from Eva Sapl, PhD and her colleagues, Director  
of Borrelia Research, University of New Haven, West Haven Ct are gratefully received

17

**Video Lectures on Biofilm Science by the late Dr. William Costerton, are gratefully received.**

**Conversations with Mr. Richard Longman, are gratefully Received**



# Hayes and Barbour– Unique Research findings *Borrelia burgdorferi* Gemmae and Bacteriophages

392 BARBOUR AND HAYES

MICROBIOL. REV.

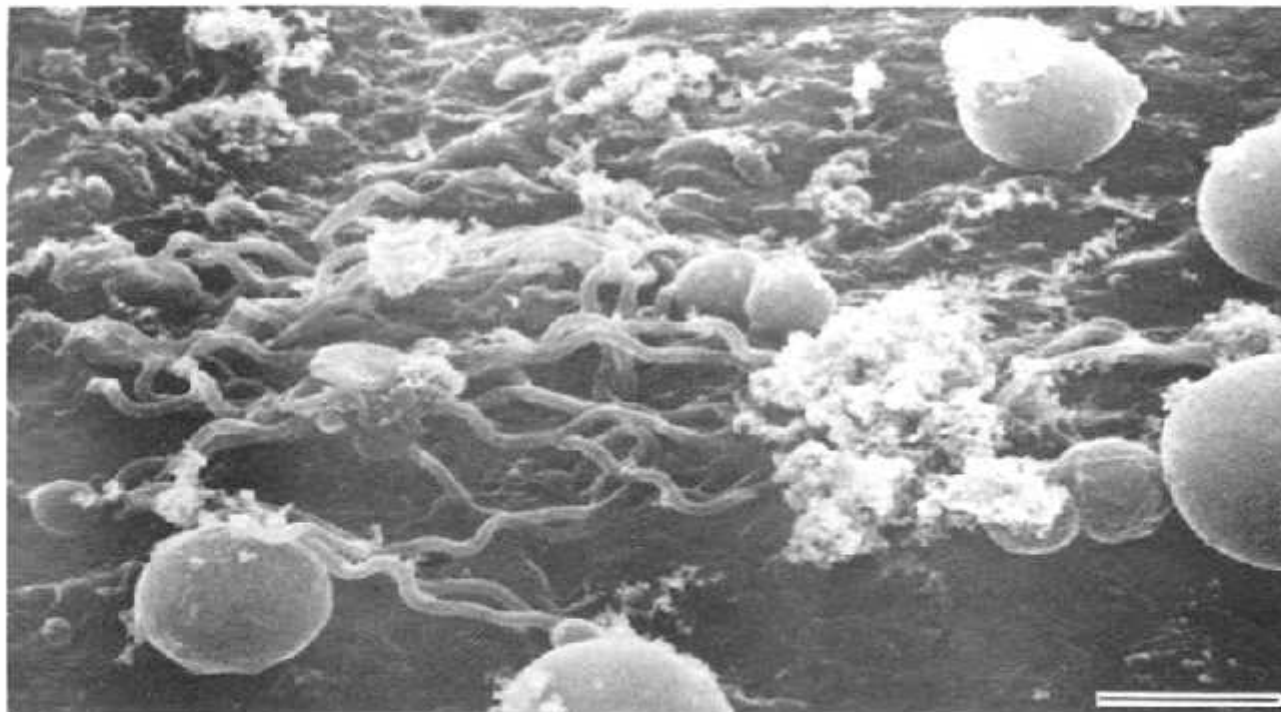
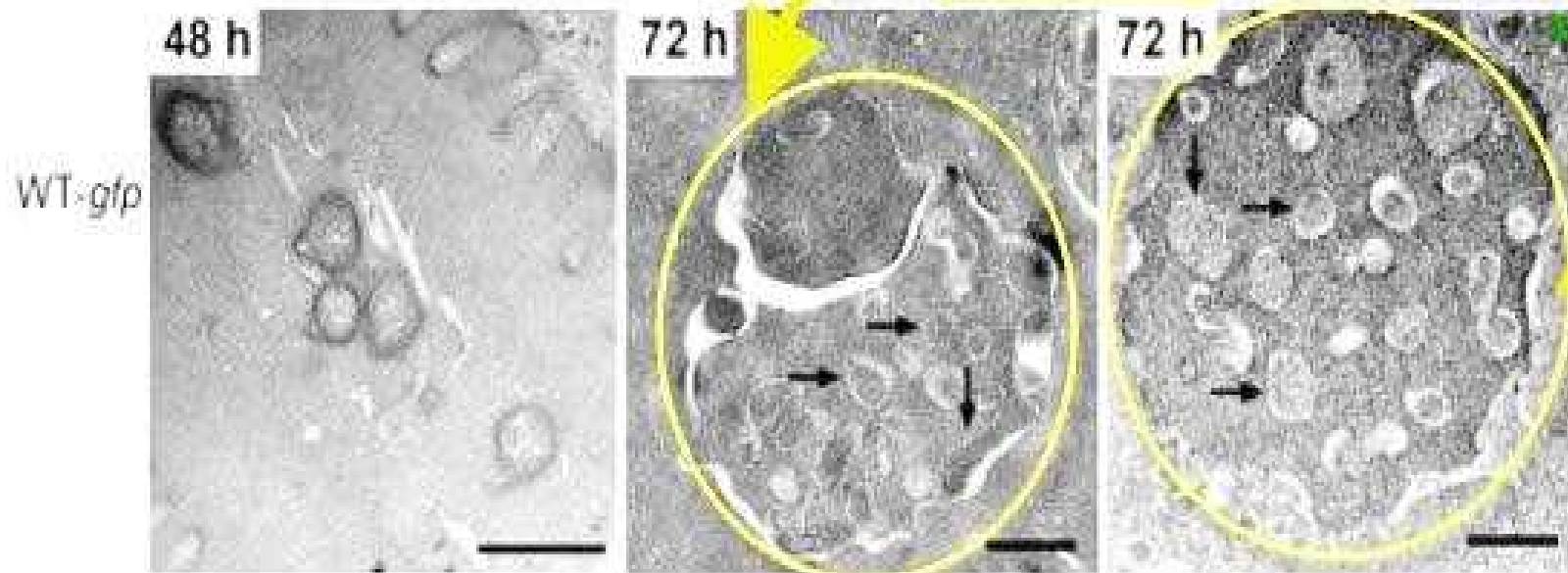


FIG. 8. Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of an *I. dammini* tick. Bar, 2.0  $\mu$ m. (Photograph courtesy of D. Corwin, Rocky Mountain Laboratories.)

# Radolf Research Group UCONN

## Round bodies of *Borrelia burgdorferi* [Arrows]

### A. Nymphal Midguts

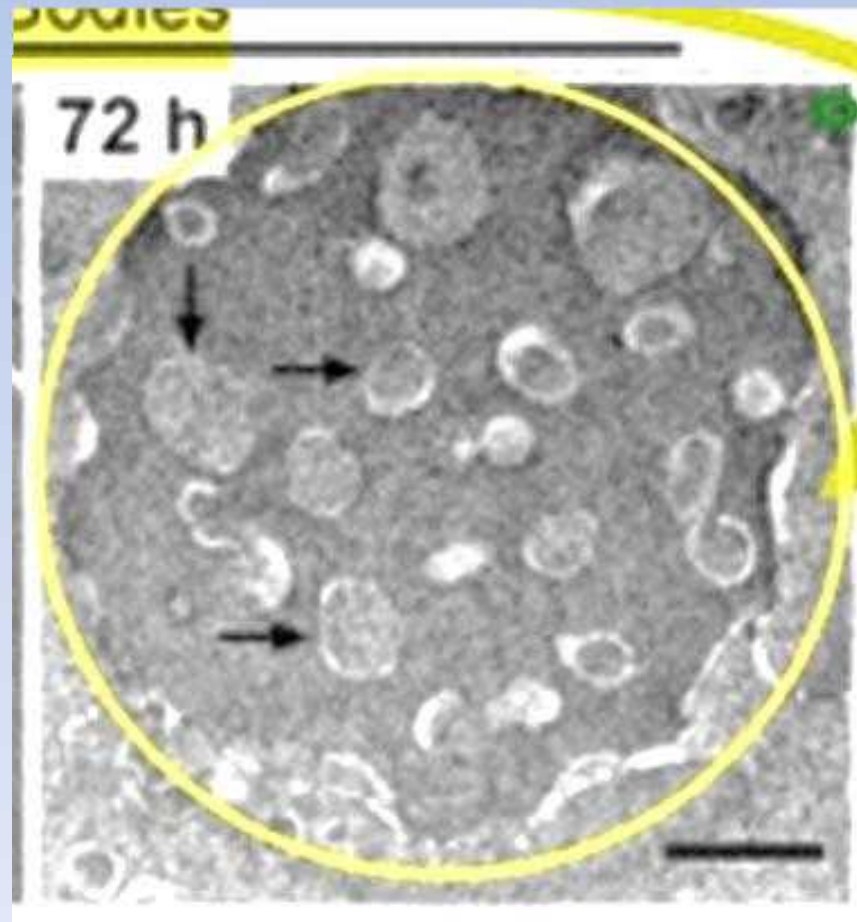


Reference: PLOS Pathogens Feb 16, 2012  
Vol 8(2):e1002532 - Dunham-Ems, S. et al

**Radolf research Group**

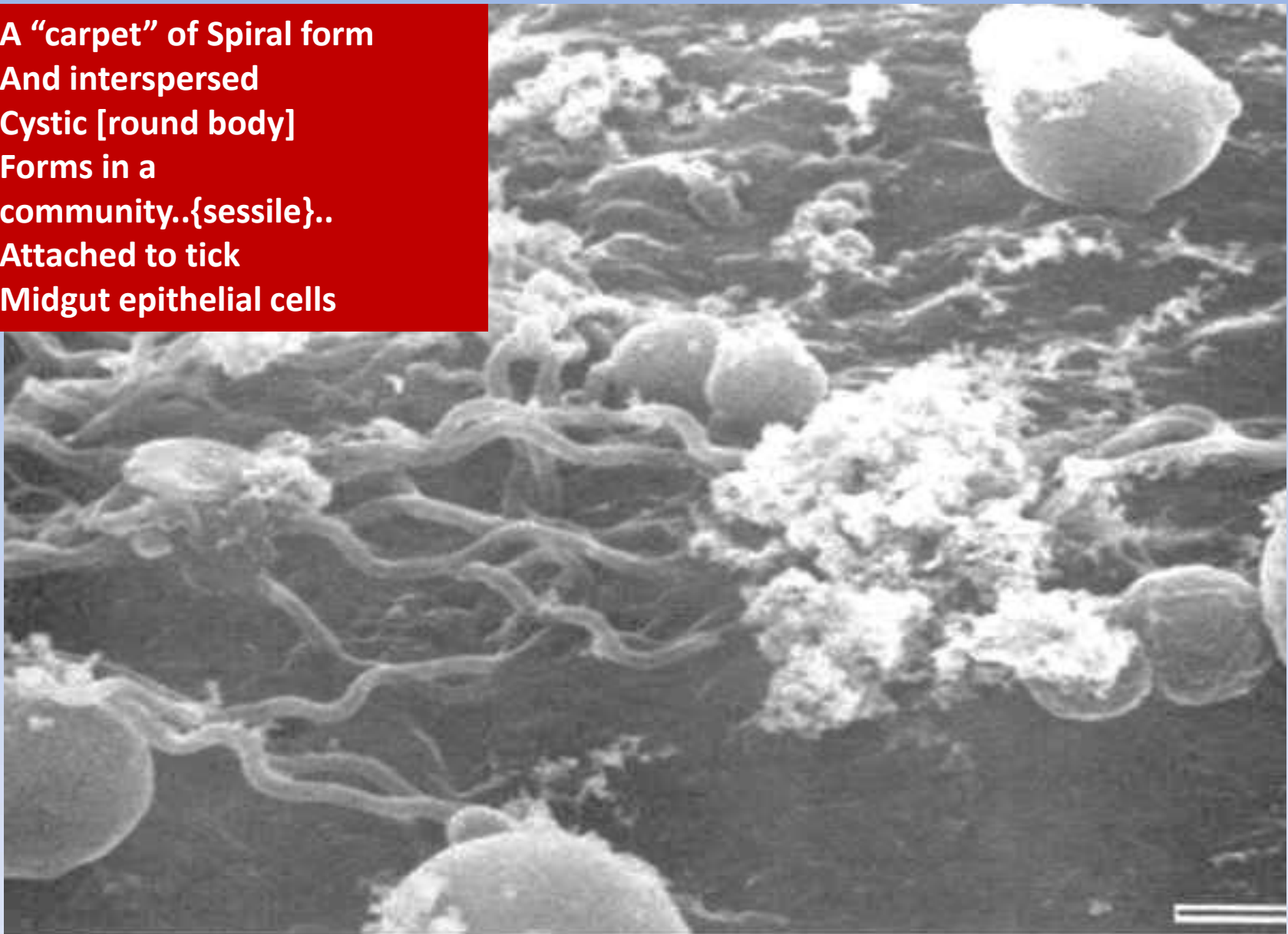
**UConn Med Ctr – Cystic borrelia**

**Tick Midgut**





A “carpet” of Spiral form  
And interspersed  
Cystic [round body]  
Forms in a  
community..{sessile}..  
Attached to tick  
Midgut epithelial cells



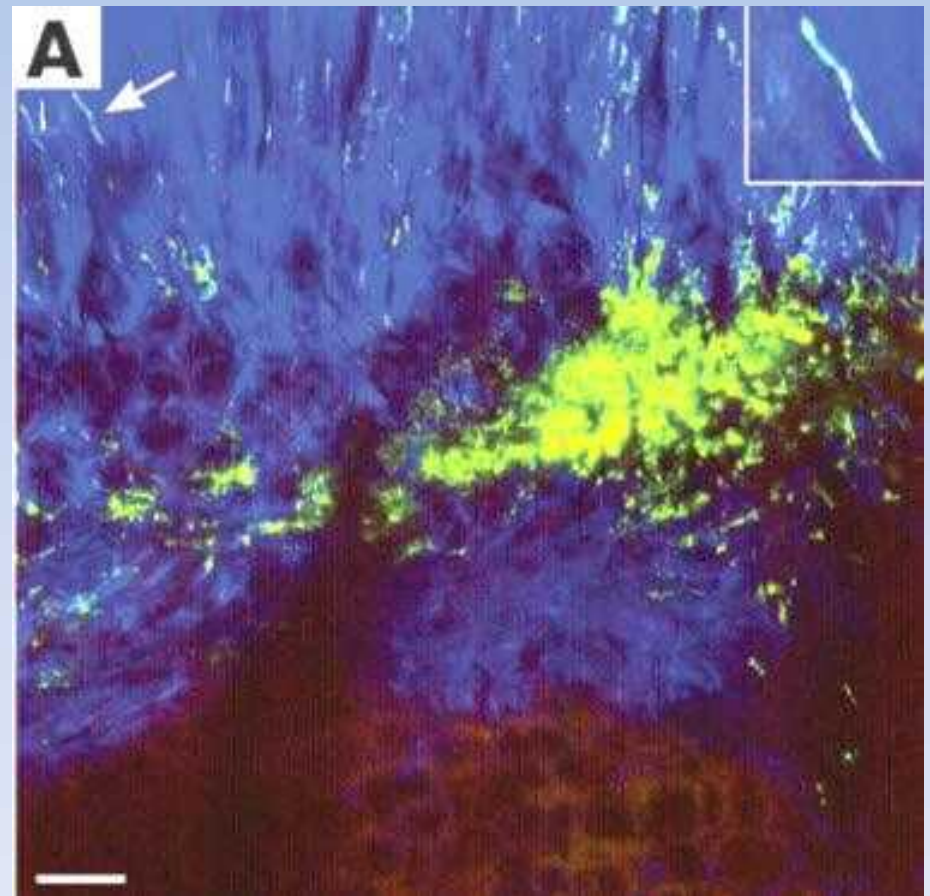
Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of a tick (micrograph courtesy of D. Corwin, Rocky Mountain Laboratories.)

# Bockenstedt Mouse model Lab induced Chronic Lyme Arthritis -Yale

**Amorphous “Globs” of Borrelia  
In Deep Mouse Dermis  
In Chronic Experimentally  
induced  
Lyme Arthritis**

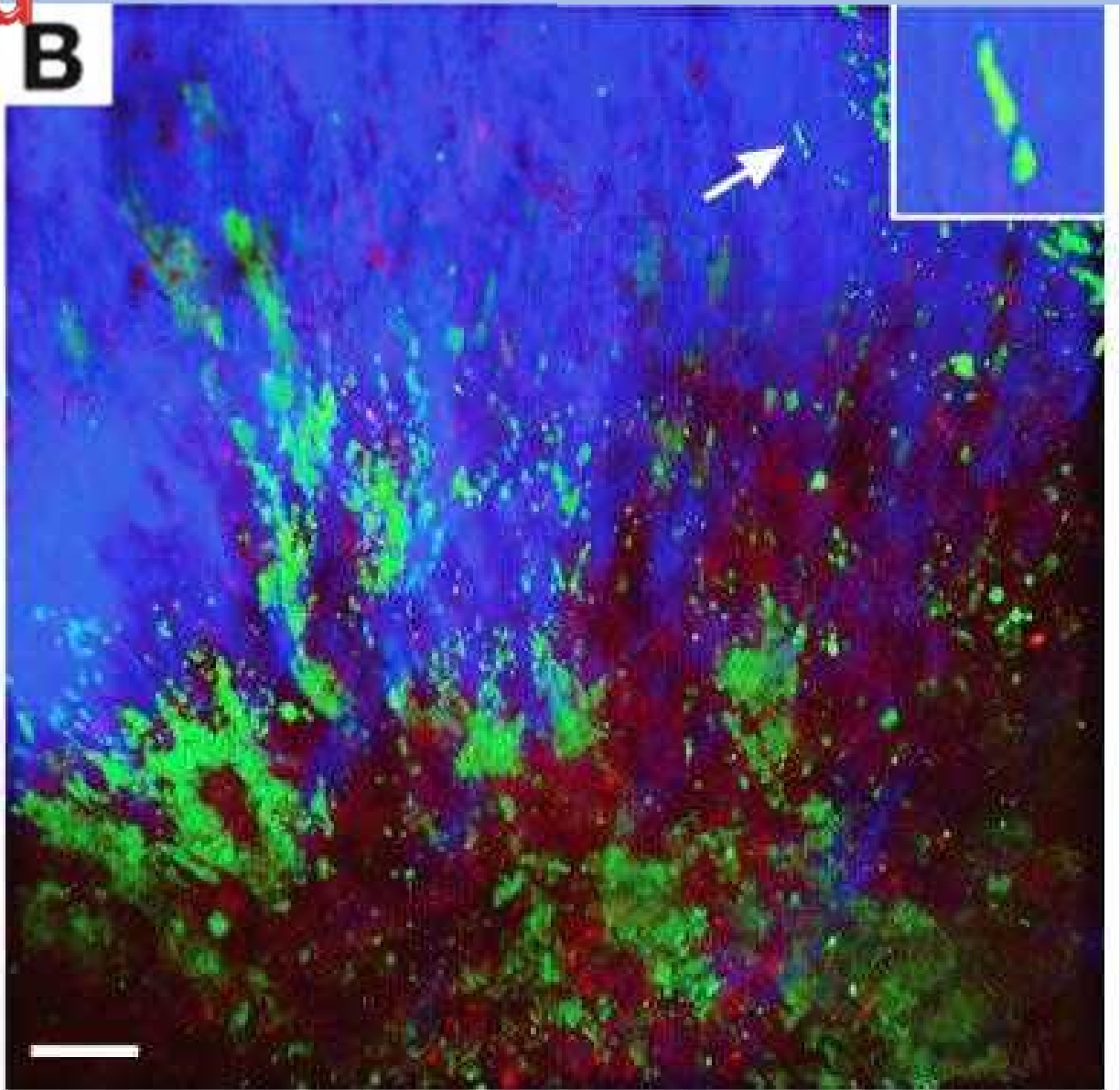
Are these so called “Globs”  
Actually biofilm Colonies in  
The Deep dermis Near the  
Arthritic Mouse Joint??

**A Community of Specialized  
[NonSpiral]  
Borrelia with interspersed  
Round Body forms in the  
mouse Dermis**



# Bockenstedt Mouse Model Of Lab induced Chronic Lyme Arthritis

**Specilaized Non-Spiral  
Borrelia , including  
Round body forms,  
Granular forms,  
In the dermis  
Forming many Colony-  
Communities in the  
Mouse Dermis—  
Highly resembling  
biofilm Communities,  
Because of their high  
Density, and lack of  
Spiral Borrelia forms**

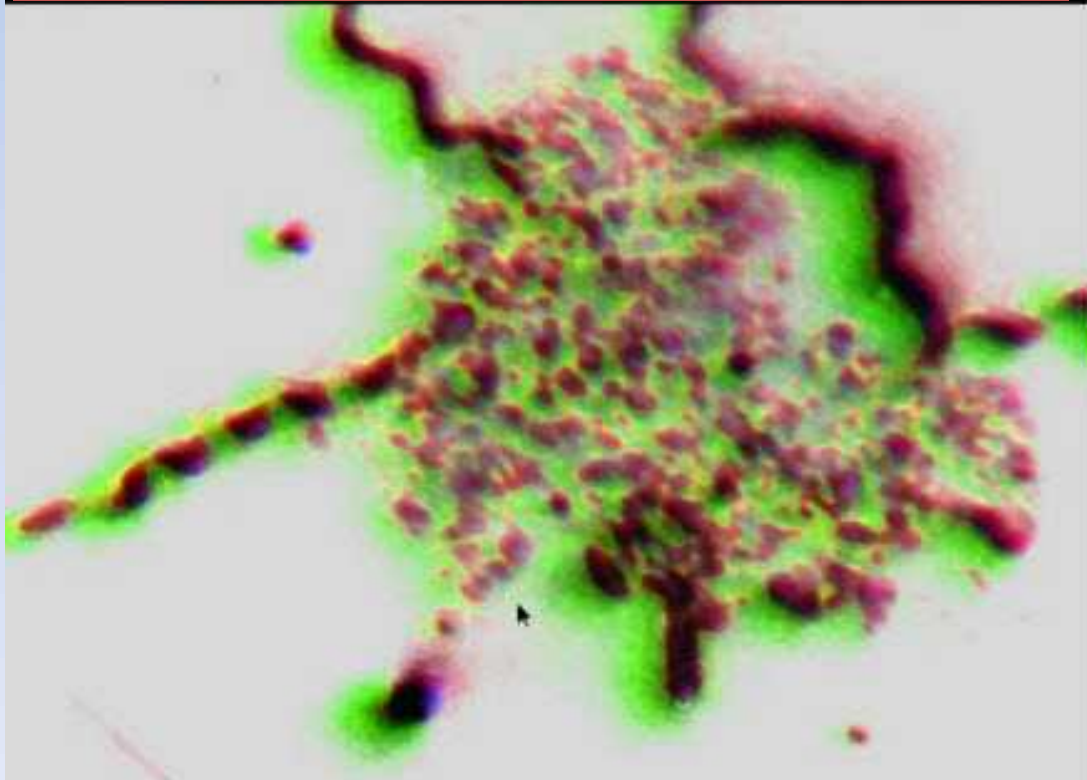




# Borrelia burgdorferi In Vitro

## Biofilm community

Spiral Borrelia forms around the Edges of a biofilm Community. The specialized borrelia forms [granular forms] dominate the central regions of the Biofilm. Planktonic forms=Spiral :: Specialized forms are INSIDE the Community



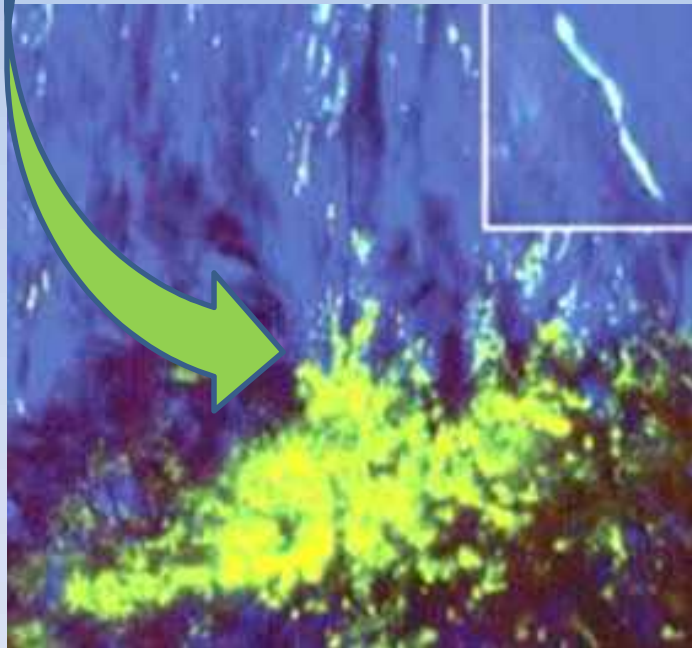
---  
**Biofilm of Borrelia burgdorferi**  
Granular borrelia forms predominate in the biofilm community

--Extracellular Matrix=green  
Viable organisms=red--  
Note: partial segmentation of spiral borrelia at 7 o'clock.  
DNA segmentation is a precursor to the emergence of Granular (round/coccoid)  
Viable forms of borrelia burgdorferi

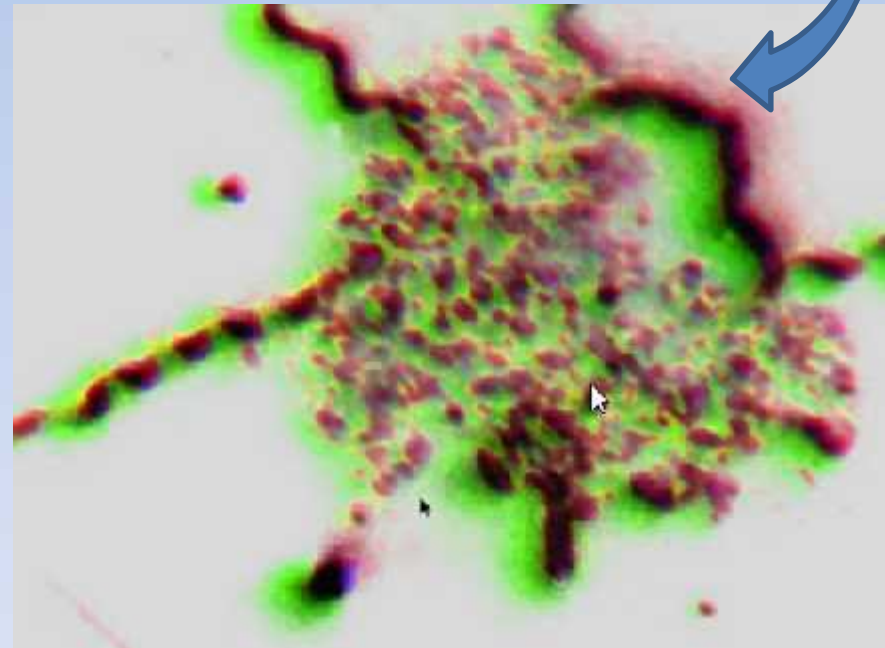
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# Compare THIS with THIS

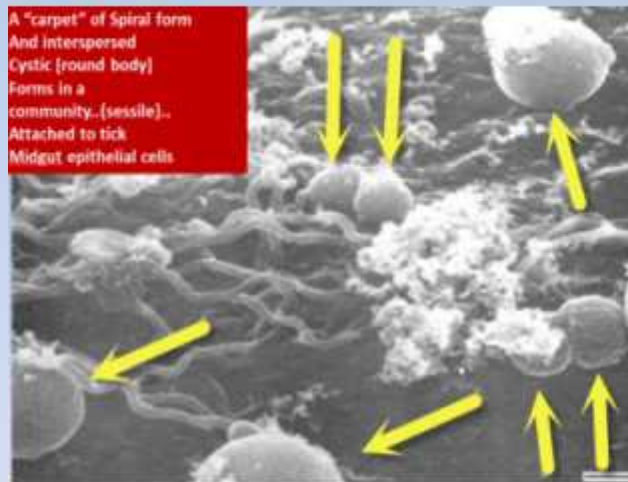


*So called “Amorphous Glob”  
of borrelia burgdorferi*



**Pure culture of ATCC 35210 [B31 strain] of  
Borrelia burgdorferi :: In vitro Biofilm**

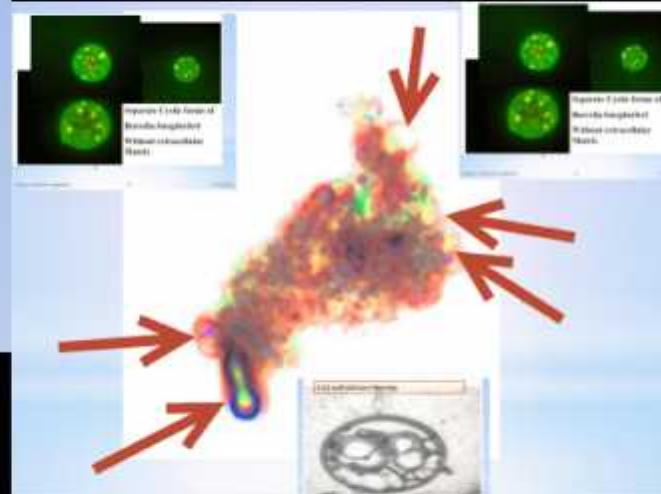
**Cystic [Round body] forms  
Of *Borrelia burgdorferi*  
In a biofilm community!!  
No Problem..**



**Yellow  
Arrows  
point to  
CYSTS  
[Round  
bodies]**

**Tick gut Model:: borrelia  
in a "carpet" ::"community"  
Consistent with a Living Biofilm**

**Prominent Cystic [round body] borrelia  
specilaized  
forms within a borrelia biofilm IN VITRO**

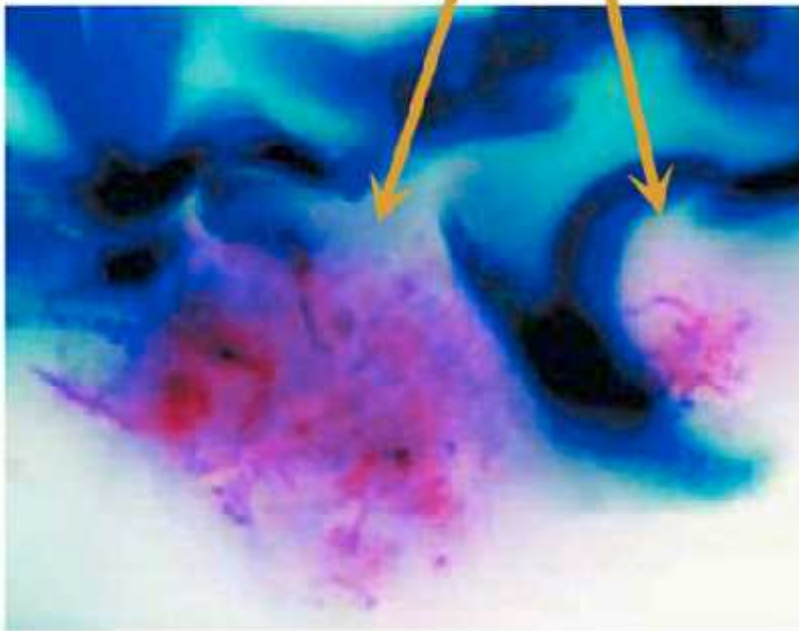


**Arrows  
point to  
Cystic  
borrelia  
within  
Biofilm of  
*Borrelia*  
In Vitro  
model**



# Human Skin : In ViVO Bb Biofilm Model

American Journal of Clinical Pathology



For Comparison Eisendle Image 5  
91x71mm (96 x 96 DPI)

**In Human  
Biofilm-like  
Communities of  
borrelia species  
in  
Human skin  
by FFM  
Eisendle and Zelger**

---  
**A Community of  
Specialized borrelia  
[non-spiral, granular,  
straightened forms]  
surrounded by a  
"REDDISH VEIL"**

---  
**The "veil" probably  
represents  
the  
Extracellular  
Matrix  
derived from dead  
members of the  
community**

**Take Home Message**  
**In Tick biofilms of borrelia**  
**In Mouse biofilms of Borrelia**  
**In Human biofilms of Borrelia**

***In VIVO borrelia biofilms***

***Not Yet Accepted in a Peer reviewed Journal –Yet***

***But the Image evidence from microscopy***

***In various articles already published***

***Describing Biofilm-like-communities in Tick, Mouse,  
Human***

***Is Very VERY Close***

***To the Biofilm Profiles – Now Proven –***

***in PLOS ONE (Sapi,E, etal , Oct24 2102 IN VITRO models***

# How Do Cutaneous Borrelia

## Lesions Instruct Us: Going Beyond the Integument::

Number of legitimate kinds of Borrelia Skin Diseases

Number of Strains of Borrelia burgdorferi SS and SL species infective for Man

Number of Tick strains – as COMPETENT to TRANSMIT

Number of Continents with Borreliosis as a Public Health problem

Number of Molecular dimensions to borreliosis in mammalian hosts

Number of "invisible" manifestations [Blebs] [Liposomes of borrelia]

Number of Extracutaneous legitimate manifestations of Borreliosis

Number of diverse forms that the borrelia spirochete can take and remain viable

Number of biofilm models {In Vitro, In TickO, In MouseO, In humanO}

Liposomes of borrelia as a model for Transfection of Borrelia DNA to Human cells

and the LIPOSOME model for activation of the Cellular immune response

with "AUTO IMMUNE" diseases

ACA dense Fibrosis/dense inflammation to explain now idiopathic fatal Fibrosing Diseases ( Retroperitoneal Fibrosis, Mediastinal fibrosis)

The "Expanding Clinical Spectrum of Duray" –Lead, Follow, or Get out of the Way!!



# Acknowledgments and Thanks

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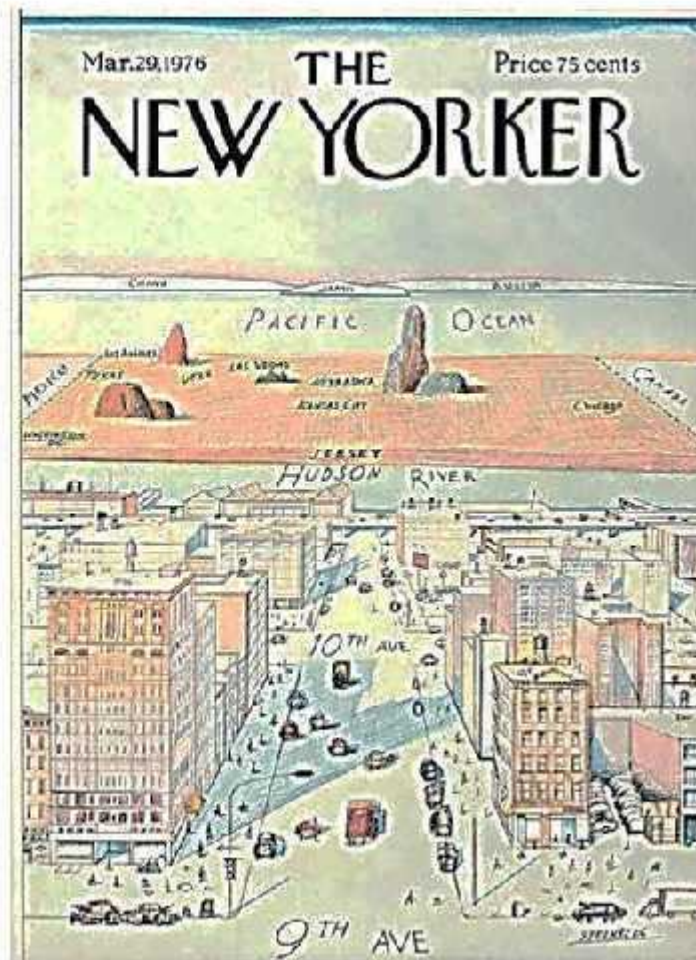
Dr. Steven Phillips, MD, Mrs. Elizabeth Schmitz, Mr. Richard Longman , Ms. Elena Cook

*Saul Steinberg and New York Magazine,*

**Sir Dr. William Costerton PhD , Dr. Paul Harrison Duray, MD**

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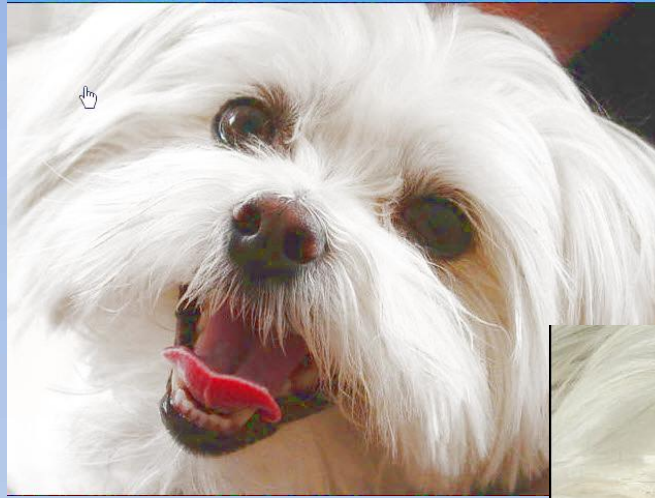


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Web link to supplementary materials: Cutaneous Lyme Borreliosis





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THANKS YOU  
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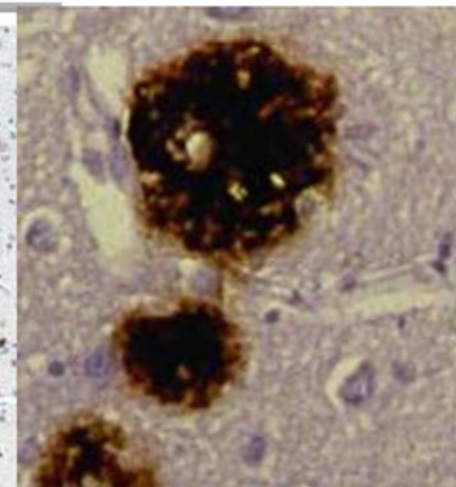
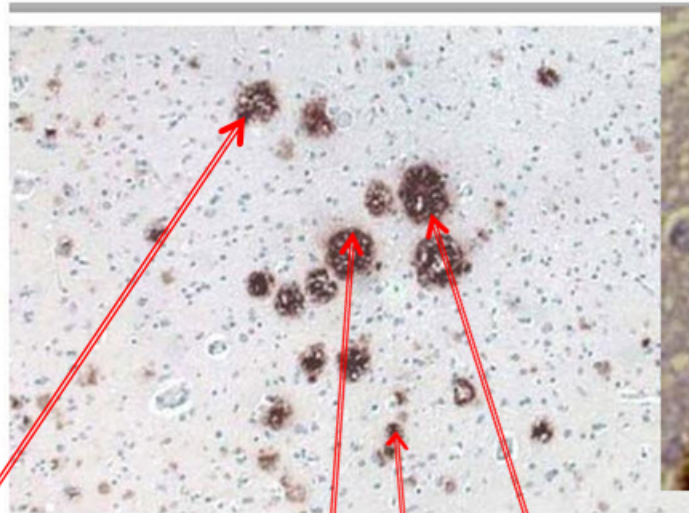
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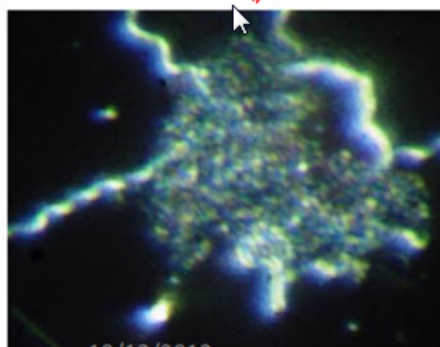


Dr Alois Alzheimer – with Morphing of Alzheimer plaques on his portrait

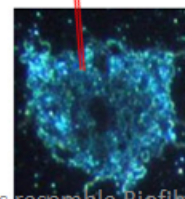
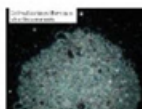


Alzheimer plaques - google

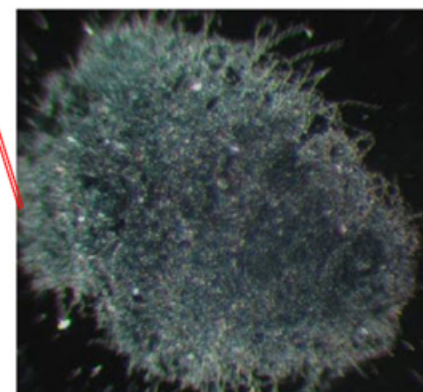
## Borrelia Biofilm Units



10/13/2012  
10/31/2012



Alzheimer Plaques resemble Biofilms of  
Molecular Doctors Research: *Borrelia burgdorferi*

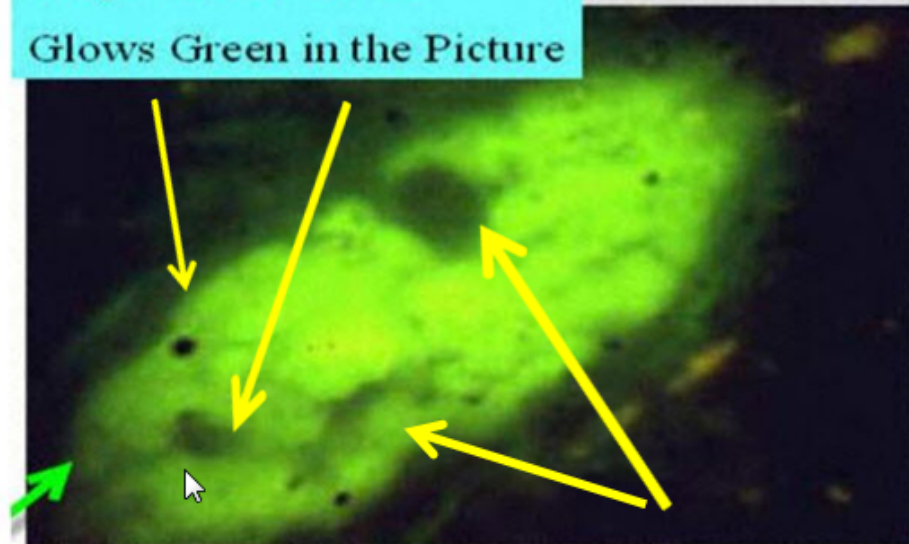




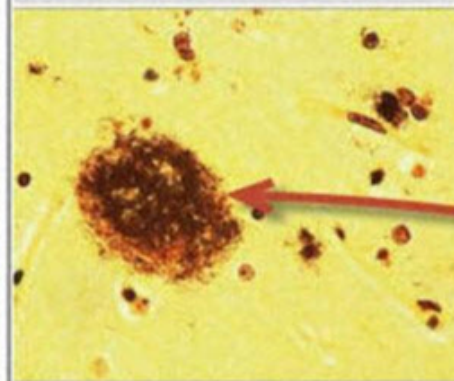
# Water Channel – like Empty Spaces – In an Alzheimer Plaque – Biofilm-like

## DNA in Plaques

Only Borrelia DNA  
Glow Green in the Picture



*Borrelia burgdorferi* Flagellin DNA , In situ hybridization, Large Plaque  
1000x original magnification



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Contrast, Darkfield,  
and Brightfield optics  
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Live borrelia  
burgdorferi are  
imaged in this picture  
on the Video monitor