# MCDB 3650
# Lyme Disease

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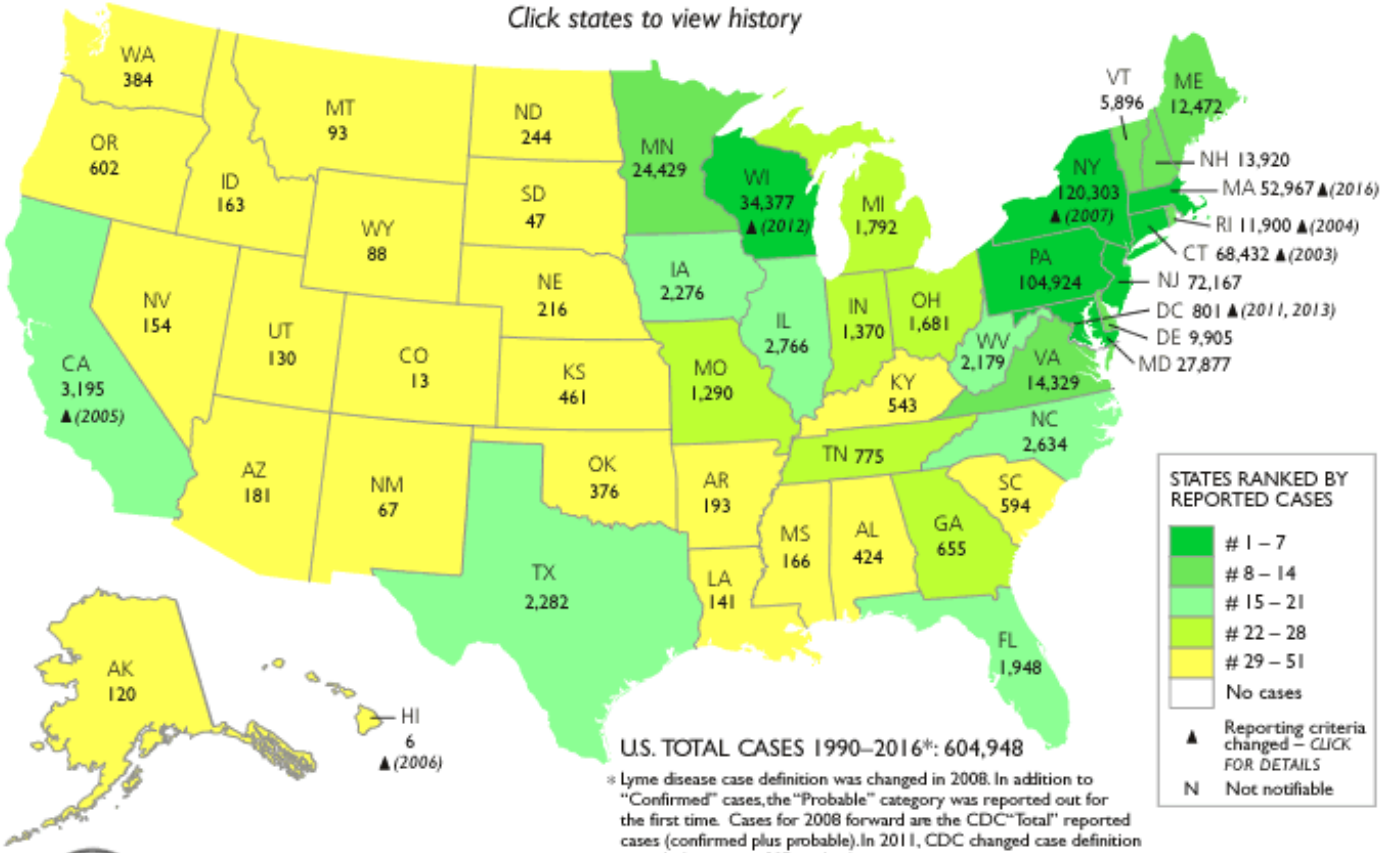
# Overview

- Review of lyme disease
- Current treatment options and their problems
- Chronic/persistent lyme disease and lyme arthritis
- Genetics of chronic lyme disease
- Molecular mechanisms of bacterial colonization
- Previous and theoretical treatment options

LYME DISEASE ASSOCIATION (LDA)

# U.S. LYME DISEASE REPORTED CASES 1990–2016*

*Click states to view history*




**U.S. TOTAL CASES 1990–2016*: 604,948**

* Lyme disease case definition was changed in 2008. In addition to "Confirmed" cases, the "Probable" category was reported out for the first time. Cases for 2008 forward are the CDC "Total" reported cases (confirmed plus probable). In 2011, CDC changed case definition to include positive CSF antibody tests.

Source: Data compiled from CDC pub data (MMWR/DVBD)



www.LymeDiseaseAssociation.org

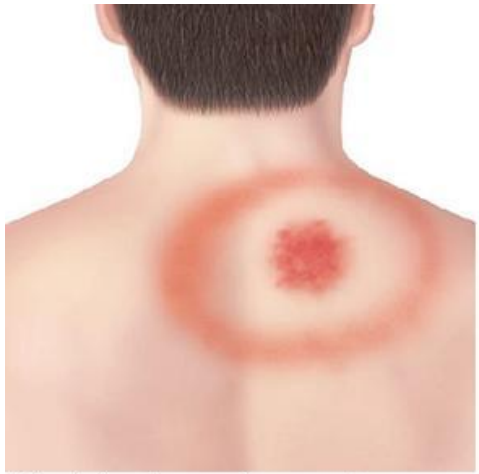
Note: CDC has stated, and confirmed in 2013, that only 10% of Lyme disease cases meeting the surveillance definition are reported – for example, if 30,000 cases are reported, 300,000 cases occurred (number does not include all the cases falling outside the stringent surveillance case definition).

# The Cause?

- Ticks!
- Ixodes scapularis- Blacklegged Tick or Deer Tick
- *Borrelia burgdorferi*

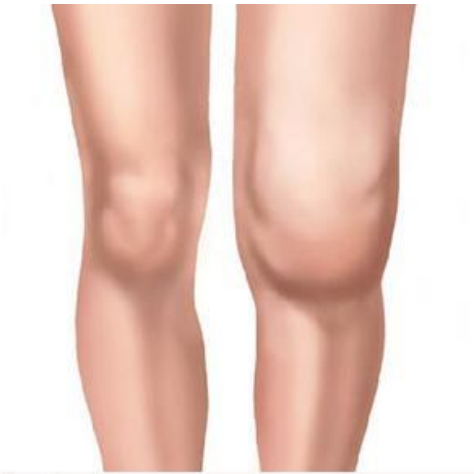
# Early stages

- Rashes- on tick bite and elsewhere
- Fatigue, achy joints, dizziness(80%), sleeping disturbances(60%)
- More developed Lymes leads to swollen joints and arthritis



"Classic" erythema migrans rash

Facial palsy



Swollen knee

# Current treatment Options

- Bacterial disease- can can be fairly easily treated with antibiotics
- Doxycycline, amoxicillin, and cefuroxime axetil
- A single dose of doxycycline administered within 72 h of a tick bite reduces the risk of *B. burgdorferi* disease by 87%
- Debate what is the best option is
- Medications are symptom specific, pain relievers for soreness and joint pain, steroids to relieve joint swelling.
- Usually not long lasting. Most will get over Lymes within months, or even years. A very small portion have persistent symptoms, with little understanding of why this happens.

# Developed/Chronic Lymes Disease

- Develops into cognitive & neurological issues(10-20%)
- Likely depends on affected area of the brain
- Cognitive- slower reaction time, difficulty concentrating, and extreme sensitivity to light
- Neurological- Loss of balance, Muscle paralysis, dementia, seizures
- Vascular- can attack heart causing light-headedness, heart palpitations, irregular beat (>1%)

# Symptoms of Chronic Lyme Disease

Chronic Lyme Symptoms - can occur weeks to months after tick bite

- Insomnia
- Joint inflammation and pain
- Memory impairments and difficulty thinking
- Irritability and panic attacks
- Bell’s Palsy - temporary facial paralysis

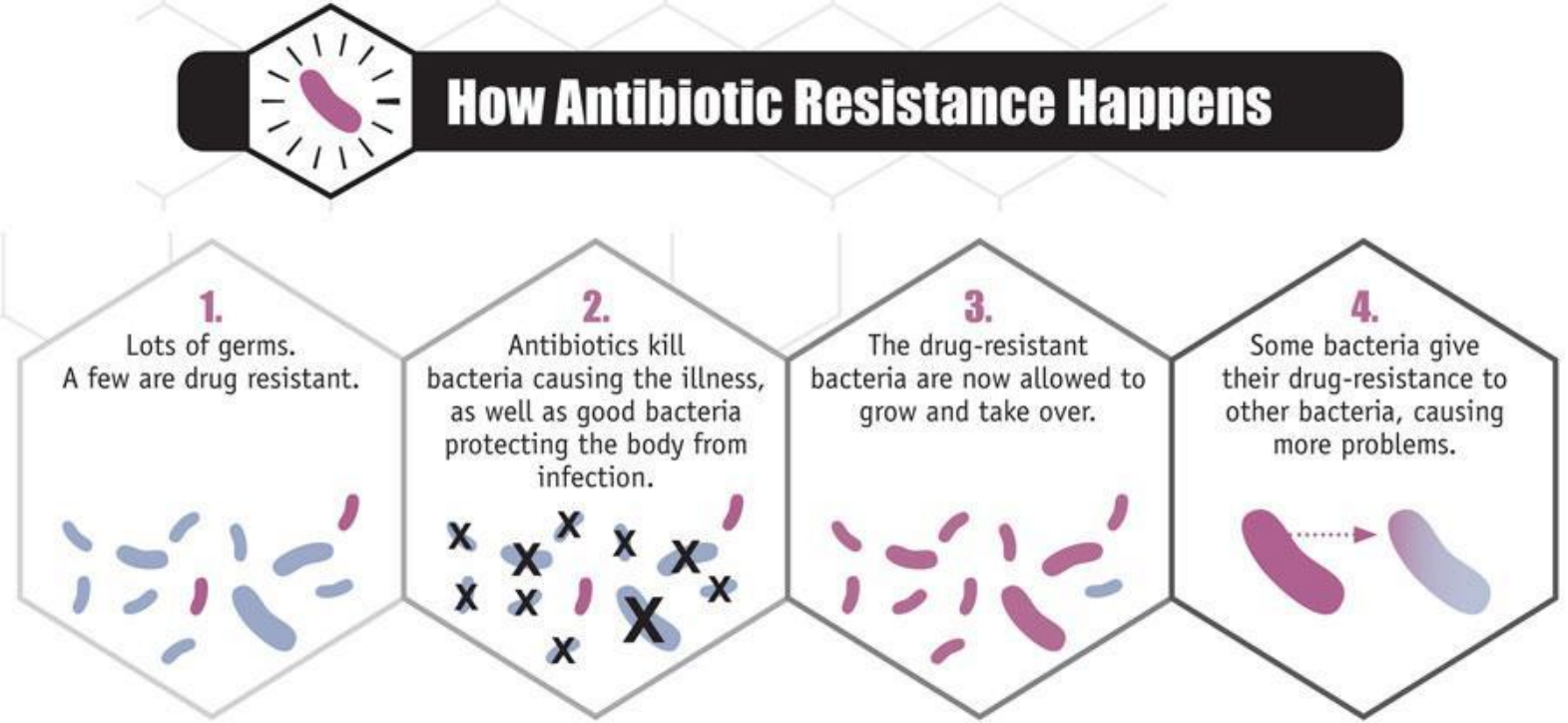
Late Stage Symptoms - can occur months to years after tick bite

- Dementia
- Seizures and/ or strokes
- Asthma and heart problems
- Parkinson’s and/ or Multiple Sclerosis type symptoms
- Vision impairment
- **Lyme arthritis - stiff, aching joints and muscles**

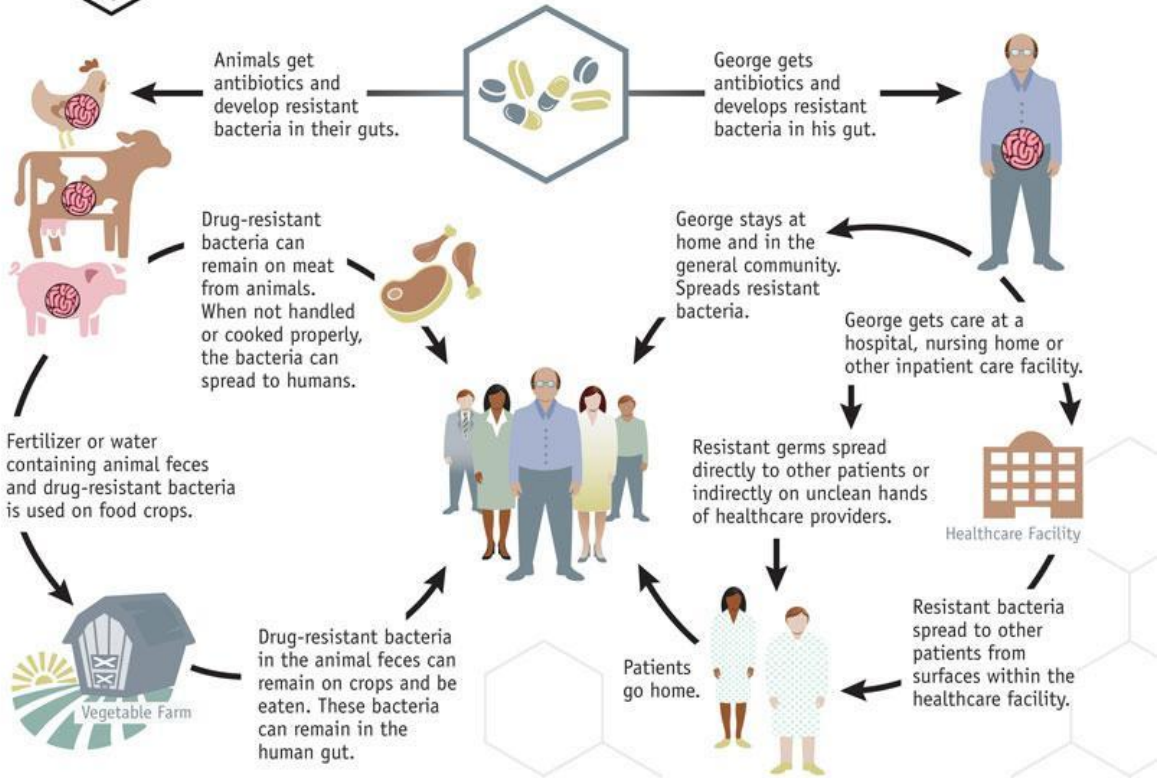
# Can you cure Lymes with antibiotics?

- Treatment for early Lyme disease is a short course of oral antibiotics, normally doxycycline or amoxicillin
- a minority of patients may still report non-specific symptoms, including persistent pain, joint and muscle aches, fatigue, impaired cognitive function, or unexplained numbness.
    - No evidence of active infection and may be diagnosed with post-treatment Lyme disease syndrome (PTLDS)
    - Closely related to neuroborreliosis and arthritis
- Approximately 10%–15% of patients with untreated Lyme disease will develop neurologic manifestations.

# Why don’t antibiotics work for everyone?



# Examples of How Antibiotic Resistance Spreads

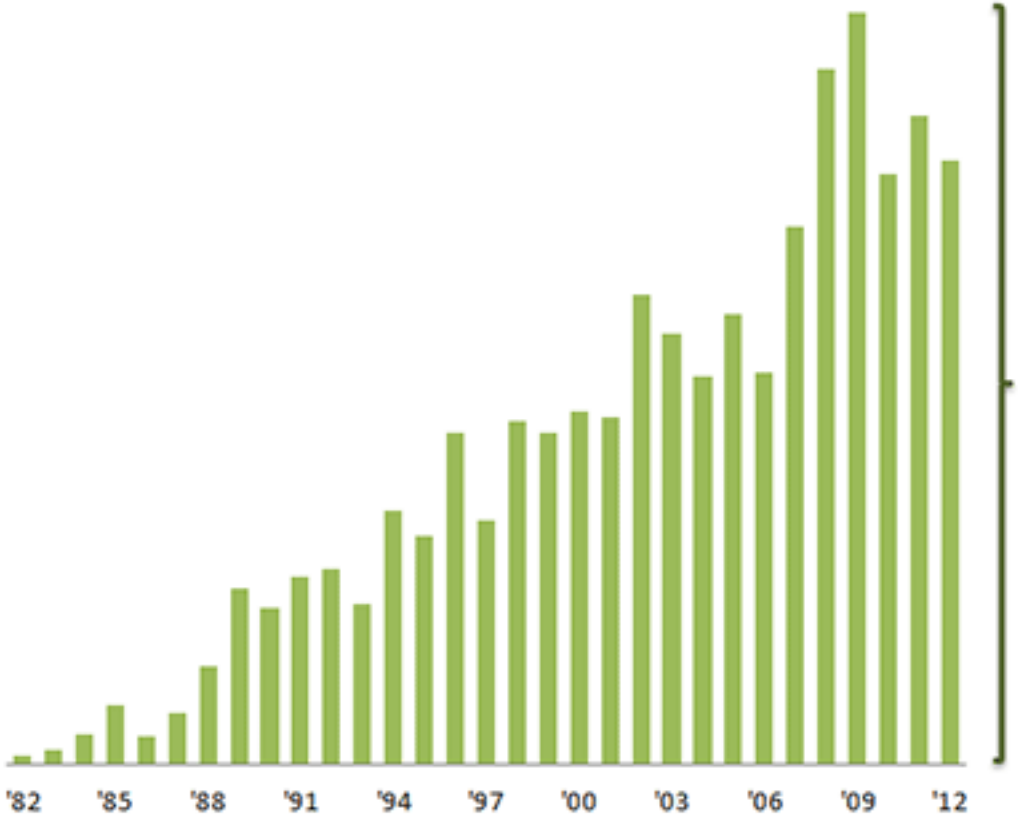



**Simply using antibiotics creates resistance. These drugs should only be used to treat infections.**

# Potential problems with current treatments

| Age Category | Drug | Dosage | Maximum | Duration, Days |
|---|---|---|---|---|
| **Adults** | Doxycycline | 100 mg, twice per day orally | N/A | 10-21* |
| | Cefuroxime axetil | 500 mg, twice per day orally | N/A | 14-21 |
| | Amoxicillin | 500 mg, three times per day orally | N/A | 14-21 |
| **Children** | Amoxicillin | 50 mg/kg per day orally, divided into 3 doses | 500 mg per dose | 14-21 |
| | Doxycycline | 4 mg/kg per day orally, divided into 2 doses | 100 mg per dose | 10-21* |
| | Cefuroxime axetil | 30 mg/kg per day orally, divided into 2 doses | 500 mg per dose | 14-21 |

# Estimated Cases of Post Treatment Lyme Disease in the US




Cumulative # Diagnosed Cases
1982 - 2012
4,396,900

*

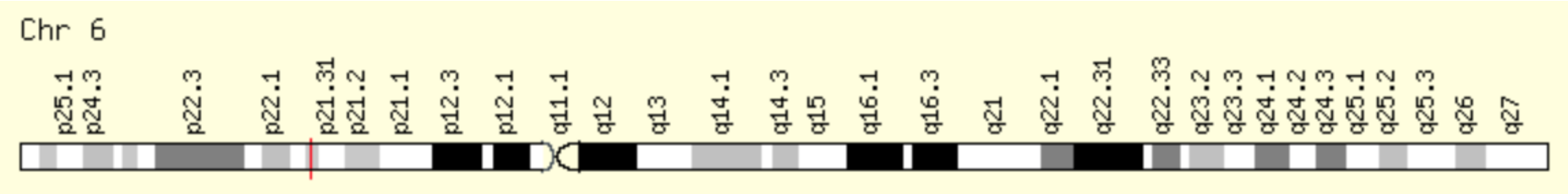
Estimated 10-20% of cases
unresolved after 24 months

=

440,000 - 880,000 Americans
continuing to experience
symptoms after treatment
(also know as
Post Treatment Lyme Disease)

# Possible Gene related to persistent Lymes

- HLA-DR is a protein coding gene for the immune system
  - B lymphocytes, dendritic cells, macrophages
  - products bind antigens
- Majority of patients with antibiotic resistant Lymes have some form of HLA-DRB1 or a closely related allele
  - HLA-DRB1 belongs to the HLA class II beta chain paralogs
  - A heterodimer consisting of an alpha and a beta chain that are both anchored in the membrane



# Current HLA-DRB Research

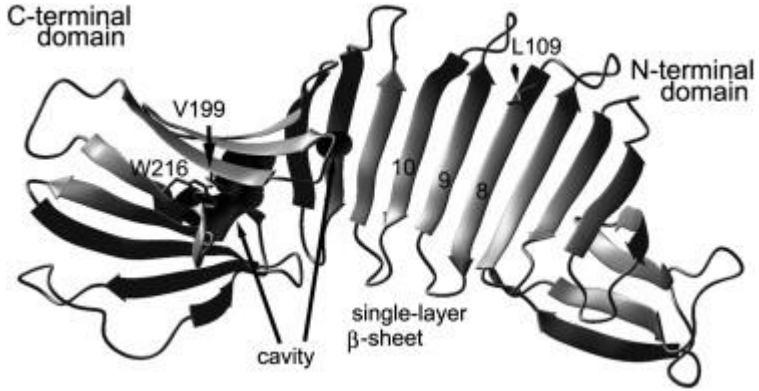
- HLA-DRB1*0401 allele was isolated as a potential cause for treatment-resistant Lymes (Steere et al.)
- Severity/duration of chronic lymes arthritic symptoms is correlated with this gene and the immune response to OspA (outer surface protein A)
- Sequencing homology with OspA and predicting binding is variable
    - Every paper identifies different base pairs to target
- One human protein identified was lymphocyte function associated antigen-1
    - hLFA-1 has homology with OspA

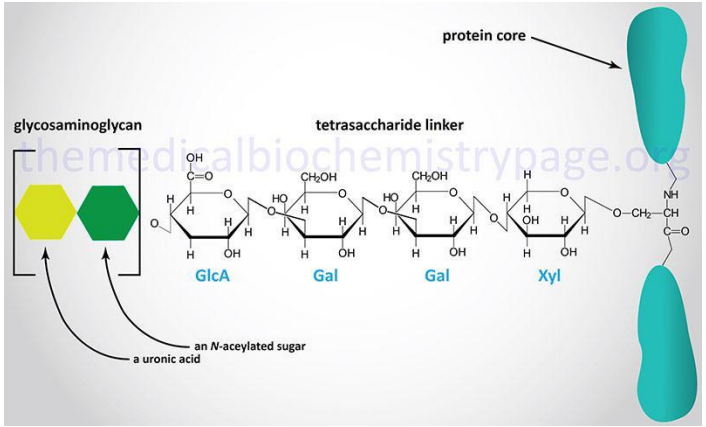
# Autoimmune mechanisms in antibiotic treatment-resistant lyme arthritis.

Steere AC[1], Gross D, Meyer AL, Huber BT.

- hLFA-1 had sequence homology with OspA at 165-173 base pairs
  - Predicted binding at DRB1*0401
- Synovial fluid T cells from most patients with treatment-resistant arthritis responded to both OspA and hLFA-1, whereas those from patients with other forms of chronic inflammatory arthritis did not.
- Molecular mimicry between a dominant T cell epitope of OspA and hLFA-1 may be an important factor in the persistence of joint inflammation in genetically susceptible patients with treatment-resistant Lyme arthritis.

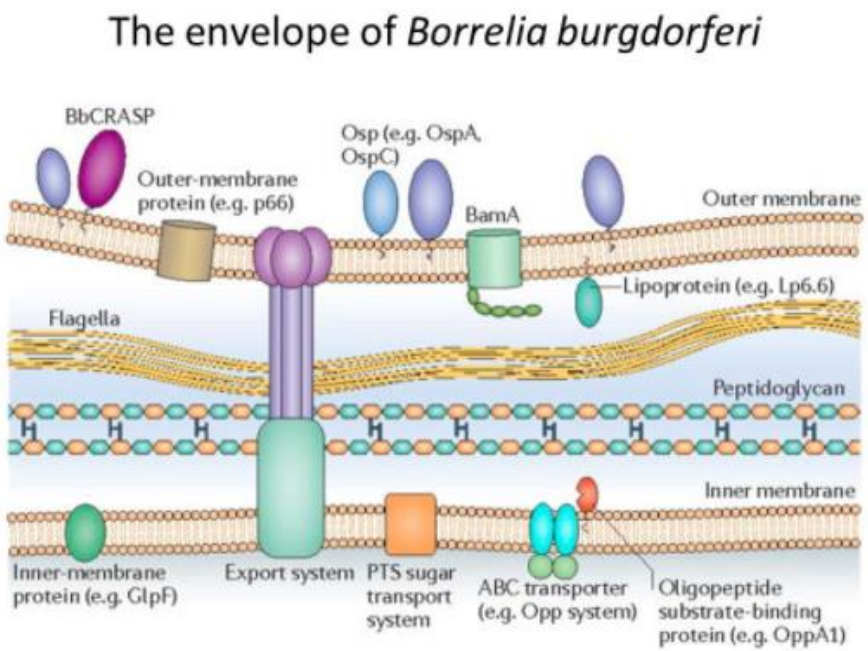
# What are PGs, GAGs, and Outer-surface Proteins?



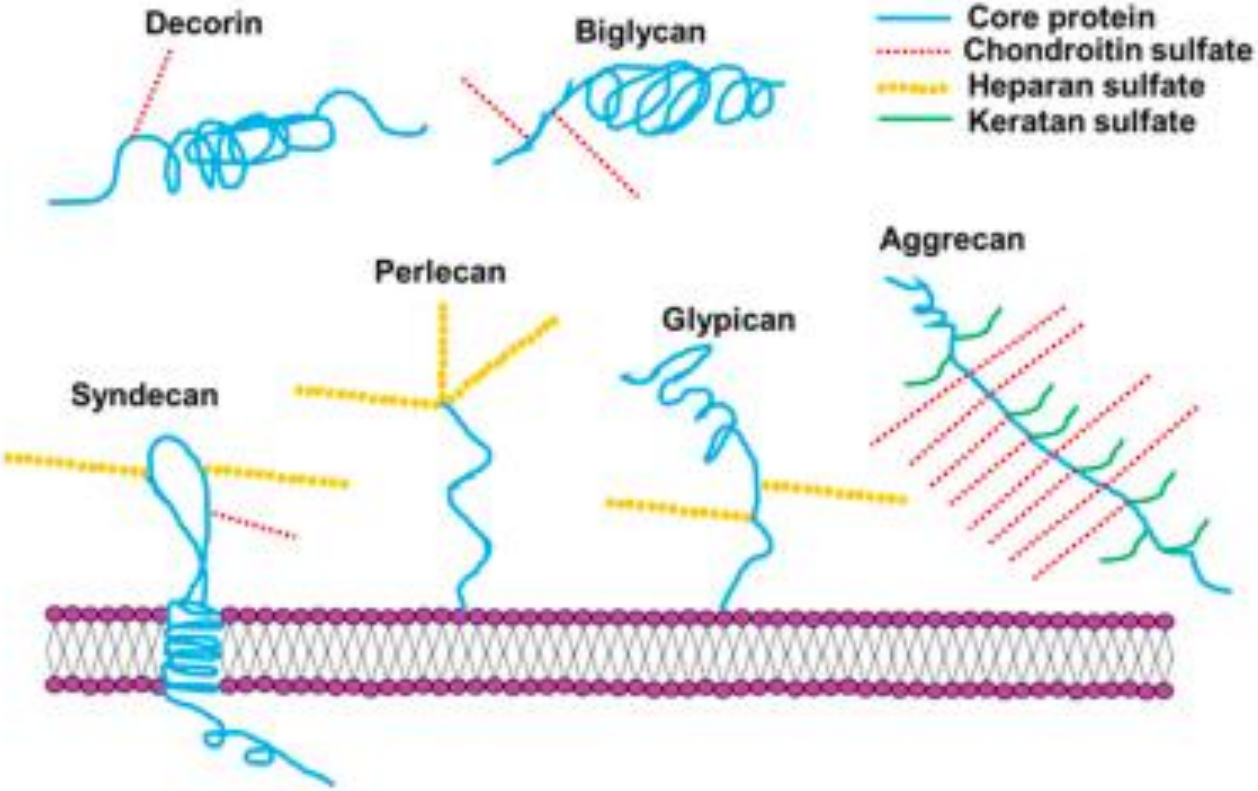

- PGs (Proteoglycans) are extracellular matrix complexes (bound to the host cell surface in our case)
    - They are composed of a core protein complex that can then bind any number of GAGs
- GAGs (Glycosminoglycans) are long, linear and negatively charged polysaccharide chains
- Outer surface proteins are just proteins that are part of the *B. burgdorferi* bacterium located on the outside surface of the spirochete
    - They have been shown to be crucial in the binding and colonization of host cells by the bacterium

# Implication of PGs and GAGs in Lyme Disease




- Borellia Burgdorferi expresses a number of proteins on  their surface (outer-surface proteins)
- These outer-surface proteins allow the bacteria to interact with the host
- This happens through interactions with GAGs and PGs
- The GAGs affect the ability of the outer-surface proteins to bind with PGs
- The binding with the PG is then what allows the bacteria to integrate itself into the host cell

# Specific PGs and GAGs involved in Lyme disease



# Some experiments that have been done

Decorin deficient mice were more resistant to spirochete colonization

- Decorin-mediated spirochete binding promotes tissue colonization

DbpA is a decorin binding protein on the surface of spirochete bacterium

- DbpA mutant bacterium were defective in binding and promoting spirochete attachment

BBK32 is an outer surface protein that binds dermatan sulfate

- BBK23 deficient spirochete bacterium showed reduces colonization

# So how can this information be used to treat Lyme disease?

If these protein complexes are the driving force behind the binding and colonization of the host cells by *B. burgdorferi* then if this interaction could be blocked, the spirochete will not be able to bind and colonize! Therefore no more Lyme disease!

# So how can this be done specifically?
# One of three potential methods

Could bind the PG so that the outer surface proteins on *B. burgdorferi* cannot bind

- A complex could be created that would bind the surface of the PGs
- This would then act similarly to a competitive antagonist preventing the *B. burgdorferi* from binding

Could bind outer surface protein so it could not bind PG

- Similarly a complex could be made that binds the outer surface proteins on the bacteria
- Preventing it from binding the PGs and thus preventing integration into host cell

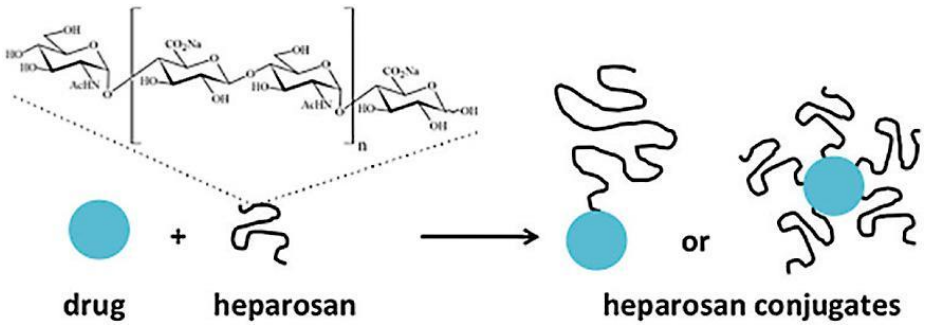
Dissociate the PG from the GAGs so they don’t have the same affinity to the B burgdorferi

- GAGs promote binding of the outer surface protein to host cell through PG interaction
- So if all GAGs were dissociated from the PGs involved in Lyme then the bacteria wouldn’t have the same binding affinity to the PG

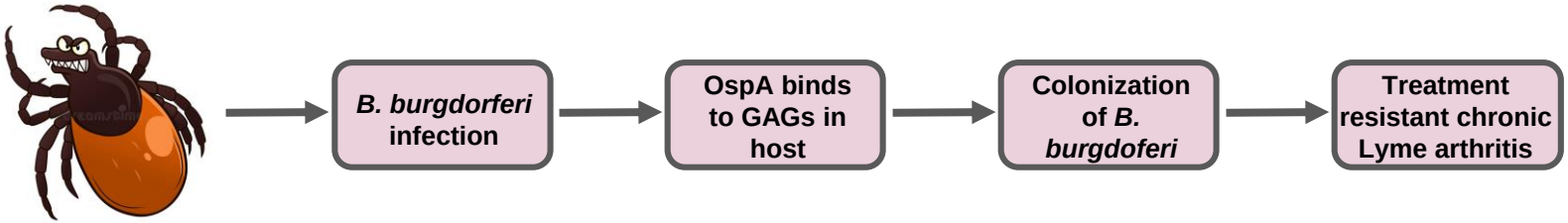
# *Borrelia burgdorferi* glycosaminoglycan-binding proteins: a potential target for new therapeutics against Lyme disease

Yi-Pin Lin,[1,2,*] Lingyun Li,[3] Fuming Zhang[4] and Robert J. Linhardt[4,5,6,*]

This was the basis behind our paper: GAG analogues or other synthetic/semi-synthetic compounds can be manufactured to bind crucial sites to prevent the binding of spirochete bacterium to host cell




# Preventing chronic Lyme disease seems simple:



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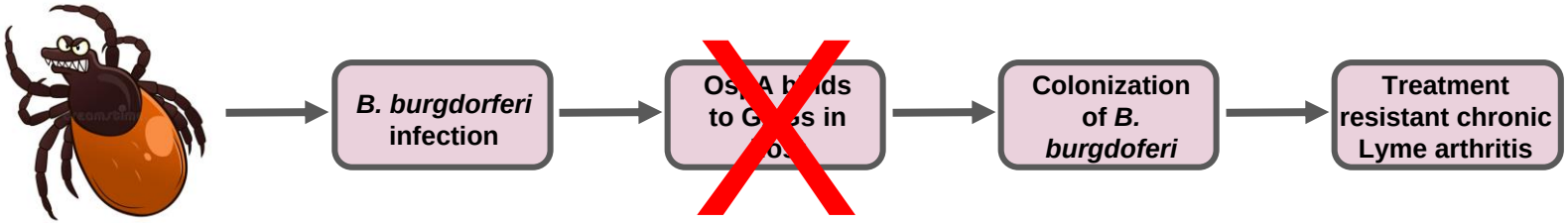



**Prevent outer surface proteins of bacteria from binding to host ligands**

**No colonization**

**No chronic Lyme disease**

# Prevent outer surface proteins of bacteria from binding to host ligands

Option 1: utilize body’s natural defenses by forming adaptive immunity to *B. burgdorferi* colonization with a vaccine

# LYMErix - the Lyme disease vaccine (1998-2002)

- OspA identified as binding protein necessary for *B. burgdorferi* colonization
- SmithKline Beecham developed OspA vaccine
  - MOA: vaccination of humans would produce circulating antibodies against OspA
  - Tick would ingest antibodies during feeding that would bind and neutralize *B. burgdorferi* spirochetes
  - No infectious spirochetes would be transferred to human through tick bite
- Phase III clinical trial led to FDA approval for LIMErix as effective Lyme disease vaccination

# LYMErix - the Lyme disease vaccine (1998-2002)

**TABLE 2.** ATTACK RATES OF LYME DISEASE AND VACCINE EFFICACY IN THE STUDY POPULATION.*

| LYME DISEASE | YEAR 1 | | | | | | YEAR 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VACCINE (N=5469) | | PLACEBO (N=5467) | | P VALUE | VACCINE EFFICACY (95% CI) | VACCINE (N=5469) | | PLACEBO (N=5467) | | P VALUE | VACCINE EFFICACY (95% CI) |
| | *No. of Cases* | *Attack Rate* | *No. of Cases* | *Attack Rate* | | | *No. of Cases* | *Attack Rate* | *No. of Cases* | *Attack Rate* | | |
| | | % | | % | | % | | % | | % | | % |
| **Definite** | | | | | | | | | | | | |
| Erythema migrans | 21 | 0.38 | 41 | 0.75 | 0.01 | 49 (14 to 70) | 15 | 0.27 | 65 | 1.19 | <0.001 | 77 (60 to 87) |
| Neurologic involvement | 0 | 0 | 1 | 0.02 | | | 0 | 0 | 1 | 0.02 | | |
| Arthritis | 1 | 0.02 | 1 | 0.02 | | | 1 | 0.02 | 0 | 0 | | |
| Carditis | 0 | 0 | 0 | 0 | | | 0 | 0 | 0 | 0 | | |
| Total definite cases | 22 | 0.40 | 43 | 0.79 | 0.009 | 49 (15 to 69) | 16 | 0.29 | 66 | 1.21 | <0.001 | 76 (58 to 86) |
| **Asymptomatic** | | | | | | | | | | | | |
| Asymptomatic infection | 2 | 0.04 | 13 | 0.24 | 0.004 | 83 (32 to 97) | 0 | 0 | 15 | 0.27 | 0.001 | 100 (26 to 100) |
| Total definite and asymptomatic cases | 24 | 0.44 | 56 | 1.02 | <0.001 | 57 (31 to 73) | 16 | 0.29 | 81 | 1.48 | <0.001 | 80 (66 to 88) |
| **Possible** | | | | | | | | | | | | |
| Influenza-like illness with seroconversion | 13 | 0.24 | 17 | 0.31 | 0.46 | 24 (−57 to 63) | 12 | 0.22 | 21 | 0.38 | 0.12 | 43 (−16 to 72) |
| Physician-diagnosed erythema migrans | 7 | 0.13 | 9 | 0.16 | 0.61 | 22 (−109 to 71) | 7 | 0.13 | 6 | 0.11 | 0.78 | −17 (−247 to 61) |
| Total definite, asymptomatic, and possible cases | 44 | 0.80 | 82 | 1.50 | 0.001 | 46 (23 to 63) | 35 | 0.64 | 108 | 1.98 | <0.001 | 68 (53 to 78) |
| **Unconfirmed** | 515 | 9.42 | 468 | 8.56 | 0.12 | | 339 | 6.20 | 326 | 5.96 | 0.61 | |

*CI denotes 95 percent confidence interval.

# LYMErix - the Lyme disease vaccine (1998-2002)

**CDC recommended use of LYMErix vaccine for people 15-70 years old who lived or worked in areas with prevalent *B. burgdorferi*-infected areas**

**Initial problems:**

- No way to assess risk for exposure to infected ticks
- Vaccine not 100% effective
- Required 3 doses over 12-month period
- No safety or efficacy data for people less than 15 years old
- Possible need for booster doses
- Diminishes perceived need to use personal protective measures against infected ticks

# LYMErix - the Lyme disease vaccine (1998-2002)

- 2001: Molecular mimicry autoimmune hypothesis
  - HLA-DR4 allele associated with development of chronic Lyme arthritis
  - Sequence homology between OspA and hLFA-1
  - Lyme disease infection may initiate autoimmune response in carriers of HLA-DR4
- Could OspA vaccination induce autoimmune arthritis in HLA-DR4 carriers?

- 1.4 million doses of LYMErix were distributed in the year following its release
  - 905 reported adverse reactions included arthralgia, myalgia, pain, and arthritis
- Anti-vaccine sentiment groups and media coverage
- Class action lawsuit against SmithKline Beecham
- FDA review of LYMErix safety
- Voluntary withdrawal of LYMErix from market in 2002

*B. burgdorferi* infection

OspA binds to GAGs in host

Colonization of *B. burgdorferi*

Activate adaptive immunity

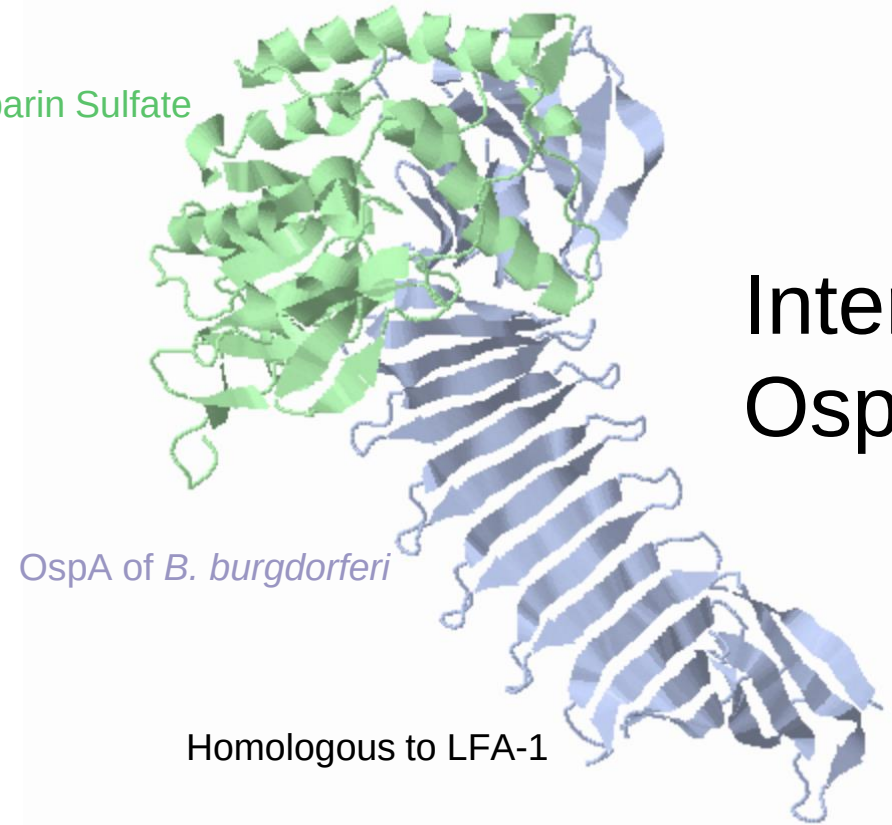
Autoimmunity to LFA-1

Autoimmune arthritis

Treatment resistant chronic Lyme arthritis

# Prevent outer surface proteins of bacteria from binding to host ligands

Option 2: remove and replace epitope of OspA that is homologous with hLFA-1

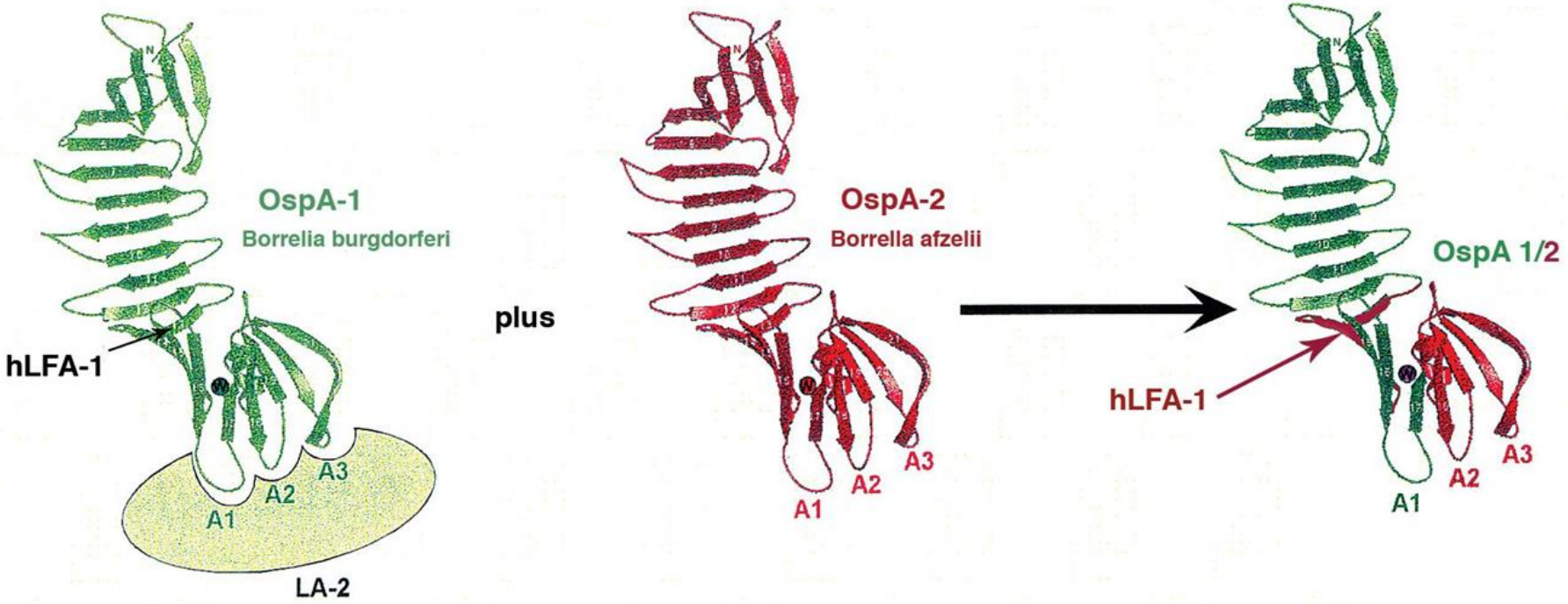



# Interaction between OspA and Heparin Sulfate

# Recombination of OspA antigen

- Multiple serotypes of OspA exist in different species of *Borrelia*
  - *B. burgdorferi* exists in the US and Europe - serotype-1
  - *B. afzelii* exists in Europe and Asia - serotype-2
- Homology between serotype-1 OspA and LFA-1
- Replace homologous regions of serotype-1 with residues from serotype-2
  - Decrease risk of autoimmune response to LFA-1
  - Expand geographical application of vaccine to other parts of the world

# Recombinant OspA-½ antigen



# New Lyme disease vaccination

- Vaccinate people with OspA-½ antigen
- Effectively transfer bactericidal antibodies to tick and neutralize OspA residues in spirochete
- Prevent binding of OspA to GAG complex in host
- Prevent colonization of *B. burgdorferi*
- No autoimmune response to LFA-1
- Decrease risk of treatment resistant Lyme arthritis

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