



# Lyme Disease

Immune system: friend or foe

**Anna Goc Ph.D.**

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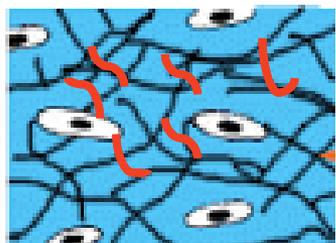
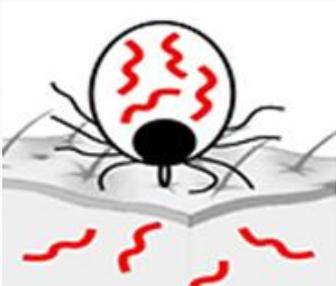
# What is Lyme Disease

- ◆ Bacterial infection with worldwide prevalence
- ◆ Systemic zoonosis – disease transmitted by animal(s)
- ◆ Disease transmitted by arthropod vector, which are ticks of the genus *Ixodes*
- ◆ Ticks harbor a bacterium of genus *Borrelia*



# Transmission of *Borrelia sp.*

A uninfected with *Borrelia sp.* tick (or its life form) feeds on an infected vertebrate



**Migration** -> into tick

**Replication** -> midgut

**Migration** -> salivary glands



**Biting and releasing** -> blood

**Colonization** -> host's skin

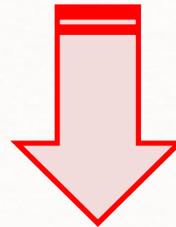
**Dissimination** -> other tissues/organs



**Colonization** -> other organs, ECM

# Infection of Host by *Borrelia sp.*

Tick's bite



- ◆ activating local inflammation
- ◆ evading host defense
- ◆ facilitating dissemination
- ◆ becoming invasive spreading quickly

*Borrelia sp.*

Immune system

**Surviving by adapting**  
(changing genes profile/surface protein)

**BODY**

**Stimulation of inflammatory cells**  
(causing acute-phase lesion - EM)

colonization

dissemination

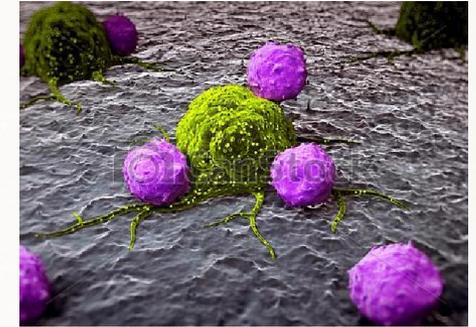
suppression

evasion

# Innate System

Response to pathogens in a generic

**Surface  
barriers**

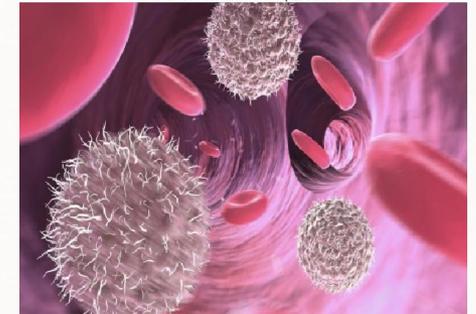


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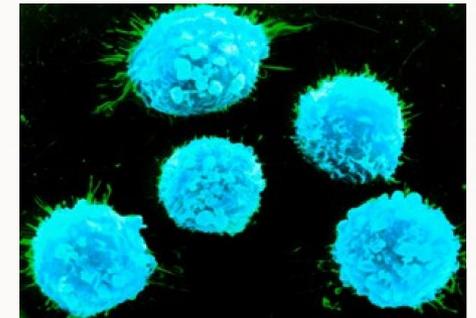
**Inflammation**



**Complement**



**Cellular  
barriers**

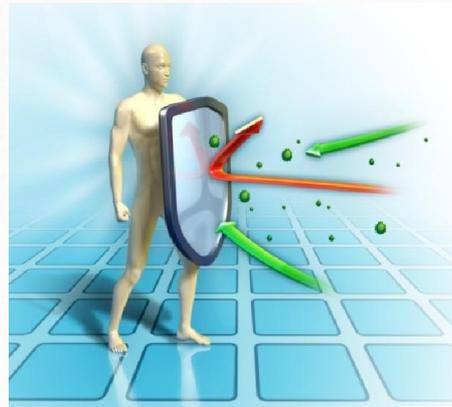
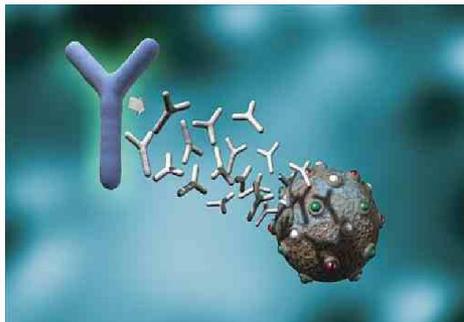


# Adaptive System

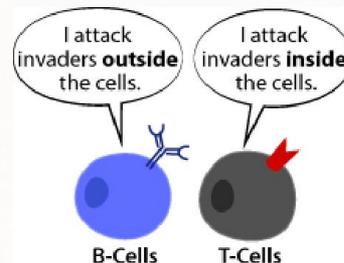
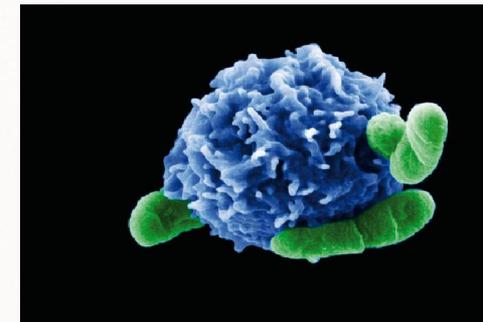
Response to pathogens not in a generic way

Types of leukocytes - lymphocytes: B and T cells derived from hematopoietic stem cells in the bone marrow

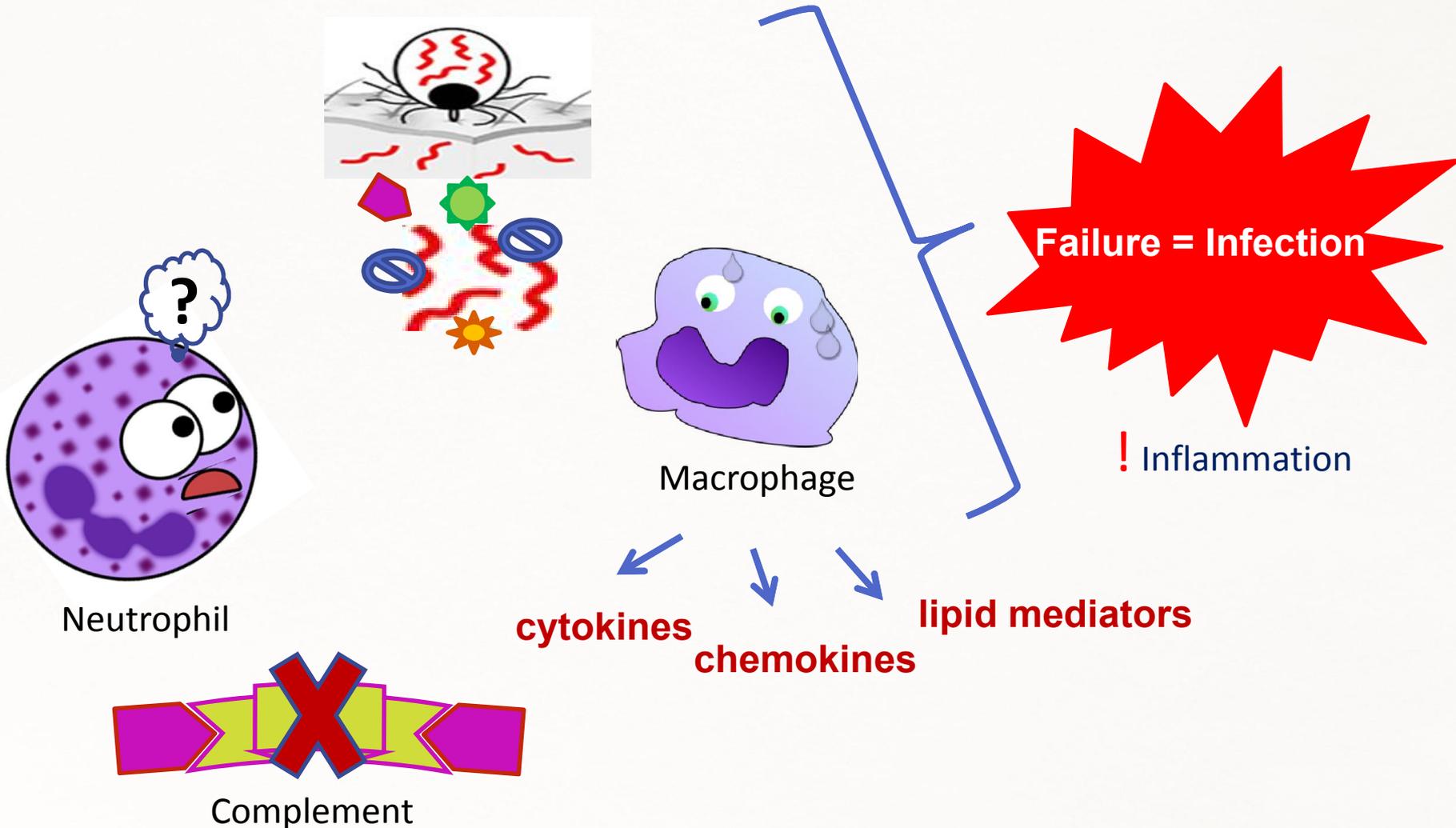
**B cells** are involved in the humoral immune response



**T cells** are involved in cell-mediated immune response



# Strategy of Infection by *Borrelia* sp.



# Adaptation of *Borrelia sp.*

Some genes of *Borrelia sp.* are expressed only in the mammals and others only when the bacteria are in the tick

## **OspA** (*bbB19*)

Helps in colonization of tick's midgut, expressed in unfed ticks, down-regulated during feeding by unknown signals

## **OspC** (*bbB19*)

Essential for initiation of infection in mammals and for colonization of certain tissues, adherence to vascular endothelium, binds to cells, host substrate(s) unknown

## **VisE** (*bbF32*)

Required for persistent infection in mammals, adherence to vascular endothelium, binds to cells, host substrate(s) unknown



Tilly *et al.*, 2008

# Survival Strategies of *Borrelia sp.*

## *Borrelia sp.* in a host

Immune system suppression

Immune system evasion/escape

### Innate:

- ◆ complement inhibition
- ◆ induction of anti-inflammatory cytokines
- ◆ tolerating of monocytes/macrophages

### Adaptive:

- ◆ induction of anti-inflammatory cytokines
- ◆ tolerating of lymphocytes
- ◆ complement inhibition; plasminogen binding
- ◆ sequestration of antibodies in immune complexes

### Phase and antigenic variations:

- ◆ gene conversion
- ◆ mutation and recombination
- ◆ viable expression of antigens/lipoproteins

### Physical isolation (seclusion):

- ◆ intracellular: fibroblasts, ECs, neuronal cells, synovial cells, phagocytes, etc.
- ◆ extracellular: latent forms, immunologically privileged sites, motility

Embers et al., 2004

# Products Required for Host Infection

**Innate immune system recognize spirochetes and control their numbers but are inadequate to completely clear an infection causing persistency**

- ◆ Antigen presenting cells (macrophages and dendritic cells) in the peripheral tissues (e.g., at the site of the tick bite), may subsequently migrate to lymph nodes and stimulate T cell and B cell responses
- ◆ Killing of *Borrelia sp.* by the phagocytes resident in the periphery and perhaps neutrophils attracted to the feeding lesion, NK cells
- ◆ Complement helps control *Borrelia sp.* numbers by opsonizing the bacteria (facilitating phagocytosis) or by direct killing via the alternative pathway

**Bacteria survive in the face of an antibody either due to “hiding” in sites protected from antibodies or evasion antibody reactivity by varying antigens or masking reactive proteins**



# Crucial Aspects of Effective Therapy

What should be taken into consideration during LD treatment

1. Eradication of pathogens
2. Boosting immunity and controlling of inflammation
3. Metabolic support for affected organs
4. Dietary support



# Tested Formula 1

Composition of formula1 selected for *in vivo* studies:

**Vitamin D3**

**Vitamin B-complex**

**Vitamin C**

**Baicalein (*Scutellaria baicalensis*)**

**10-HAD (Royal jelly)**

**Iodine/Kelp**

**Monolaurin (Coconut oil)**

**Luteolin (*Sophora japonica*)**

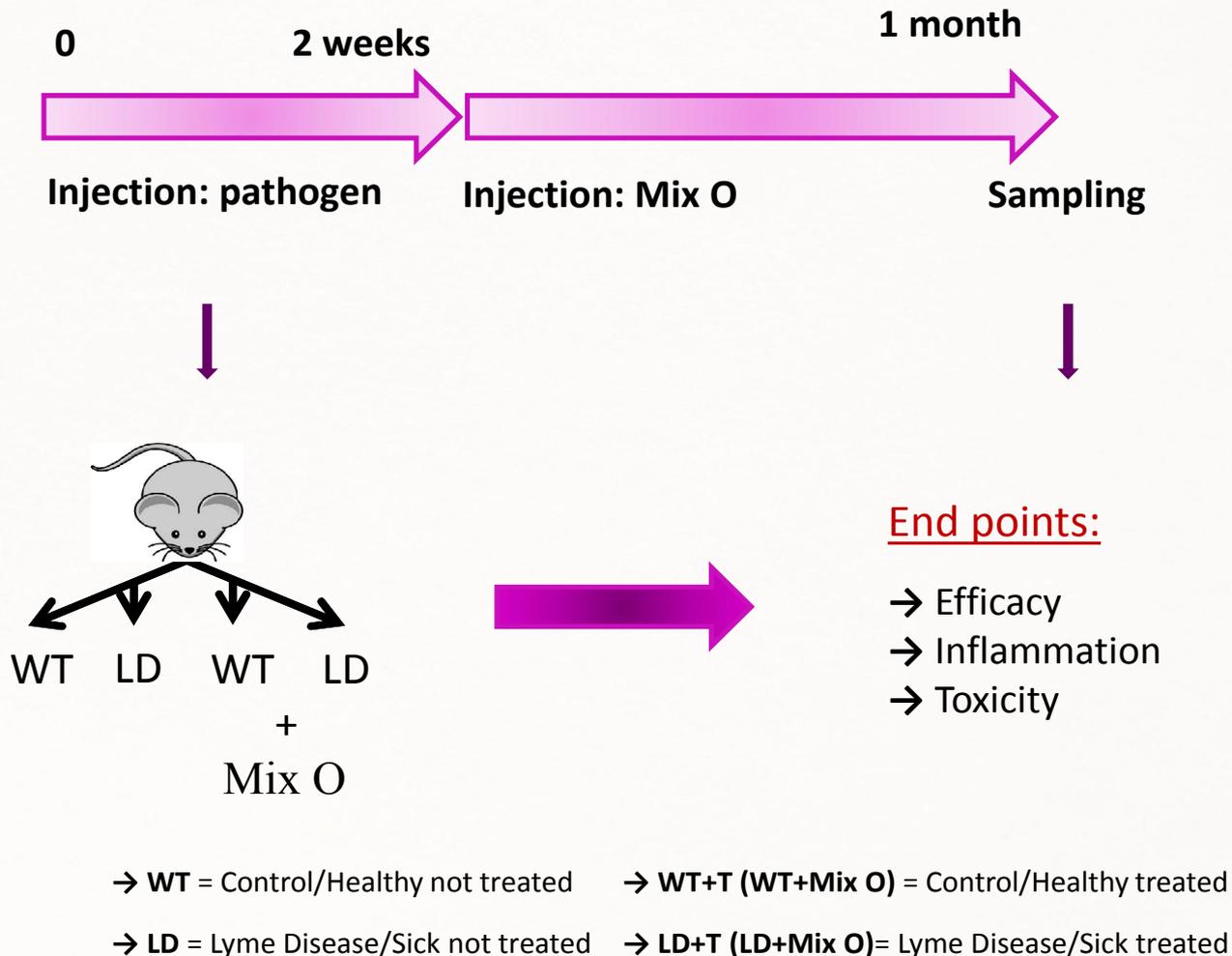
**Rosmarinic acid (*Rosmarinus officinalis*)**

*Goc et al., JAM 2015*



Aim:

# Testing of Formula 1 in Lyme Disease Animal Model



# Examinations

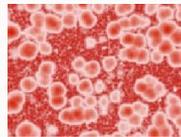
- ◆ Adverse events
- ◆ Food intake, water consumption
- ◆ Weight
- ◆ Blood and tissue sampling for determining the laboratory parameters



# Clinical Parameters



**Weight** = No change



**Morphology**



**Food/Water** = No change

WBC  
Neutrophils

RBC  
Monocytes

HGB  
Eosinophils

HCT  
Basophils

MCV  
Lymphocytes

MCH/MVHC  
Platelets

→ **WT, WT+T, LD+T** = No change

→ **LD** = Elevated level of monocytes

# Pathogen Detection

Skin

Bladder

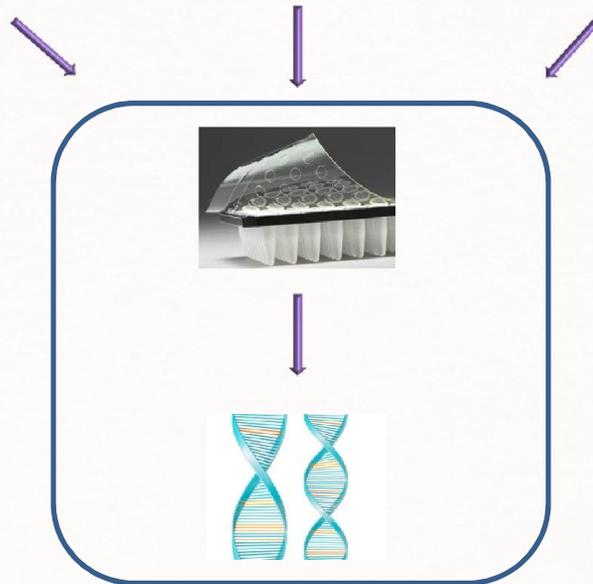
Kidney

Liver

Spleen

Joint

Heart

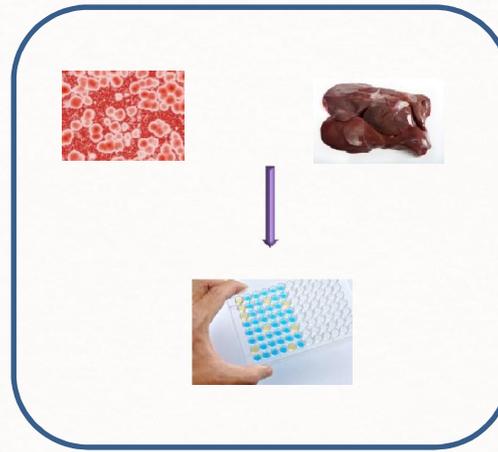


→ **WT, WT+T** = No presence

→ **LD** = Presence

→ **LD+T** = Presence reduced to 90%

# Inflammation and Toxicity



Cytokines Panel      CRP      SAA      Clusterin      KIM-1      RPA-1      Haptoglobin      Fibrinogen      GGT      Creatine Kinase      SDH      Creatinine

**Inflammation**

→ WT, WT+T, LD+T = No  
→ LD = Yes

**Toxicity**

→ No change

# Summary

- ◆ Selected formula 1 significantly reduced pathogen's presence *in vivo*
- ◆ Selected formula 1 effectively reduced levels of inflammatory markers *in vivo*
- ◆ Selected formula 1 did not displayed toxic effects *in vivo*



**“Scientific Guide in Natural  
Approach to LD for HP”**



# Thank you

Lyme Research Laboratory



[a.goc@drath.com](mailto:a.goc@drath.com)

[www.drathresearch.org](http://www.drathresearch.org)