INTEGRATIVE APPROACH FOR TESTING PATIENTS WITH LATE / PERSISTENT / CHRONIC TICK-BORNE INFECTIONS (TBI)

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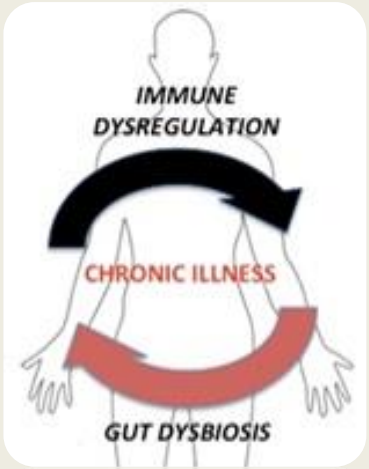
R.E.D. Labs CSO & Lab Manager

TICK-BORNE INFECTIONS

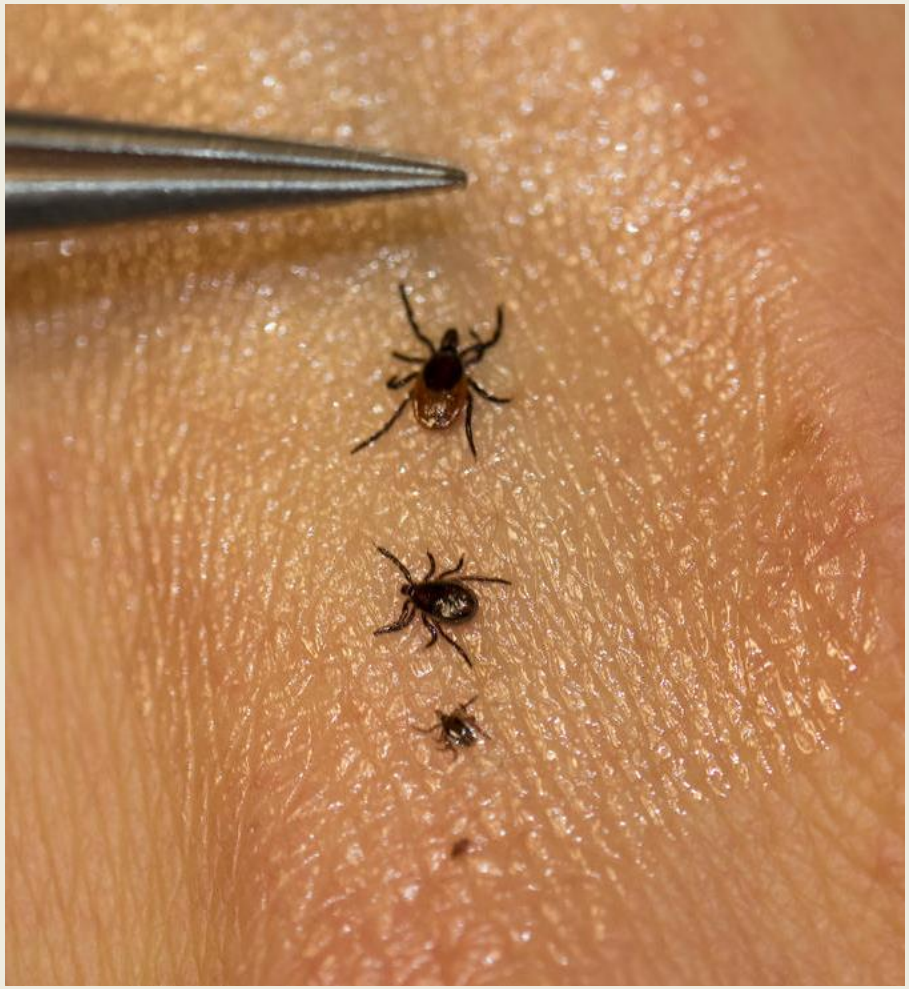
Vector-borne infections are increasing globally.

The most known, and most investigating concern borreliosis, bartonelosis, babesiosis, rickettsiosis and anaplasmosis. Many other pathogens can worsen the clinical picture and further complicate differential diagnosis.

➔ Read our updated summary, available in different languages **(www.redlabs.com)**



Tick-borne Infections



- Tick-borne diseases, which afflict humans and other animals, are caused by infectious agents transmitted by tick bites.

  ➔ **Whenever possible, get the tick tested by PCR**
- Tick-borne illnesses are caused by infection with a variety of pathogens.
- Tick-borne infections are increasing globally - Lyme disease is among the most prevalent vector borne infection in the U.S. and Europe and is reaching epidemic levels (Kugeler et al. 2015; Sykes et al. 2014).
- Download our **TICK AWARENESS BROCHURE** available in different languages (see **www.redlabs.com**)

Tick Awareness Brochure (1)

Ticks

We all like to go for a nice walk through woods, fields, laying on the grass, horse riding, golfing, etc, however exploring these natural areas may also bring the hidden danger of tick-borne infections (TBI or TBD).

Tick-borne diseases, which afflict humans and animals, are caused by infectious agents transmitted by tick bites. Lyme disease is the most widely known tick-borne disease and is caused by bacteria of the genus Borrelia. A tick can transmit multiple diseases at the same time, making appropriate diagnosis and treatment difficult. Lyme disease is among the most prevalent vector borne infection in the U.S. and Europe.
It is important to prevent the tick bite and
to know what to do in case of it.

Know where ticks are found: any tall grass, low-to-the-ground
shrubs, trees, woody plants may have ticks in them; ticks can even be
found in your own backyard, in the park. Pay special attention when hiking, horse-riding, golfing, hunting, camping, having a picnic... Cover up as much as possible: limit the amount of exposed skin! Consider using a repellent spray.

After having been outside, check your clothing for ticks: carefully inspect all outer layers of clothing and gear for ticks. Wash the clothes in hot water with soap.
Do a tick check: Inspect every part of your body for ticks, they can be as small as a poppy seed! Make sure to check between joints (behind the knees, elbows, armpits), behind your ears and any hair-covered part as tics love warm, dark places. Inspect also your animals (dogs, cats).

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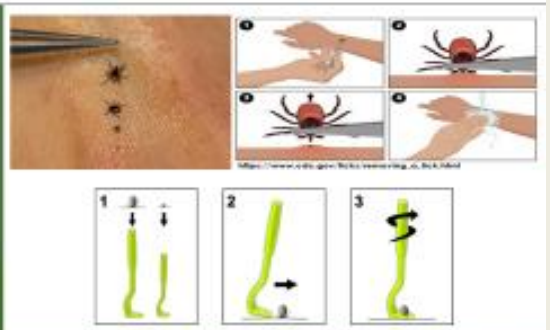
Tick Awareness Brochure (2)

Tick Bite: What To Do

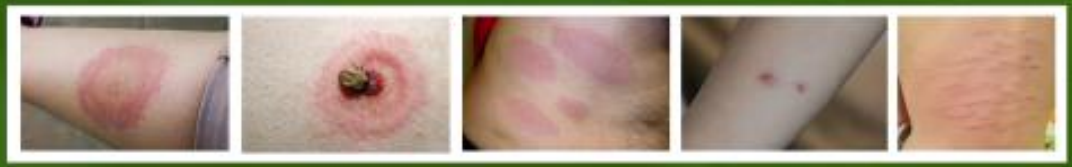
1. Do not put any chemical on the tick, use the appropriate tweezers to pull it out :
   a. special tick twist tweezers or
   b. fine-tipped tweezers to grasp the tick as close to the skin as possible and pull upward with steady, even pressure. Do not jerk the tick.
2. After removing the tick, clean the bite area and your hands with rubbing alcohol or soap and water.
1. Send us the ticks you removed from your family members or from your animals and we will test them for different tick-borne disease :
   TESTREQUESTFORM



If it is not possible to send it for the testing, the tick should be burned (in an ashtray or fireplace). Do not crush it with your fingers! This can expose you to any sort of pathogen the tick may be carrying.

Watch for symptoms for 30 days

Pay attention to the bite site - check for different skin rashes (but be aware that they occur only in 30-40% of cases):



Consider calling your healthcare provider if you get any of the following symptoms: bull's-eye rash, or general flu-like symptoms such as fever, achiness or a general lack of energy. It is important to address each tick bite even in the absence of the rash.
The long-term consequences may be very serious.

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Tick Awareness Brochure (3)

The importance of novel testing approaches for both an early and late diagnosis - Phelix Phage Test

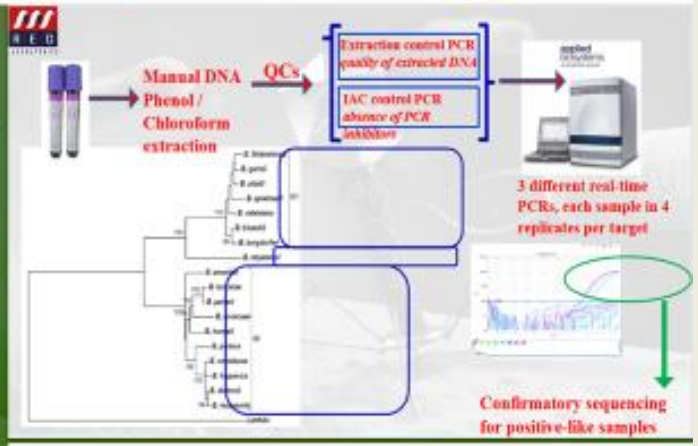
Phelix Phage Borrelia detection method consists of targeting the presence of outnumbered prophages as part of the bacteria lysogenic cycle.

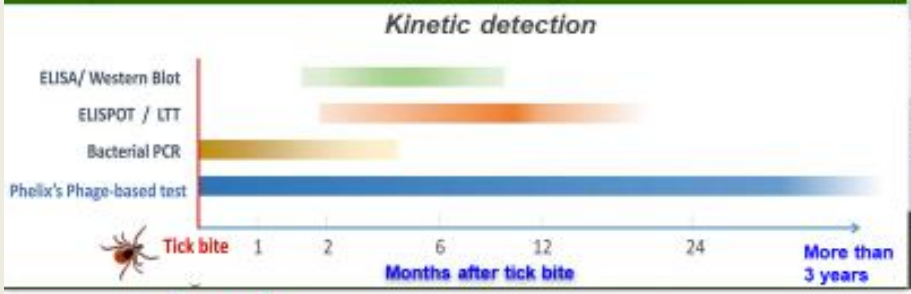
Bacteriophages could become a diagnostic tool based on the principle that if there are phages it is because there are living bacteria.

Phelix Charity together with Leicester University microbiology department have recently developed a Borrelia Phage-based PCR test (patent WO2018083491A1; publication doi: 10.3389/fmicb.2021.651217) searching for 3 major Borrelia groups:

- Borrelia burgdorferi sensu lato (including B. burgdorferi ss, B. afzelii, B. garinii, B. spielmanii, etc)
- Borrelia miyamotoi and
- Relapsing fever group (B. recurrentis, B. hermsii, etc).

The phage-based PCR test is unique as it addresses actual infection by different Borrelia subgroups. This method is efficiently used to assess both human samples and ticks. Highly sensitive and specific.
False positive results are ruled out by sequencing.





Reminder on Available Diagnostic Methods

Diagnostics	Remit
Antibody-based	• Give indirect evidence • Low sensitivity • Can't distinguish active and non-active *Borrelia* presence • Some difficulties in identifying *Borrelia* sub-types
Bacterial DNA-based	• Direct evidence of *Borrelia* presence • Low sensitivity • Can't distinguish live and dead *Borrelia* • Might be able to tell different *Borrelia* sub-types
Lymphocyte transformation test	• Provide indirect evidence • Variable sensitivity • Can only detect Lymphocytes that have been in contact with *Borrelia* within 45±15 days, thus limited in application • Distinguish active *Borrelia*? • Difficult identification of *Borrelia* sub-types?
Phage test	• Direct evidence of *Borrelia* presence • High sensitivity and specificity (Pilot study) • Can distinguish Lyme from [illegible] *Borrelia* strains • Active and non-active *Borrelia* presence

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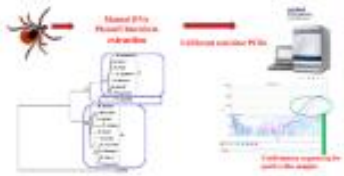
Tick Awareness Brochure (4)

R.E.D. Laboratories Procedures Testing Ticks

- Whole ticks were mechanically gridded with glass beads and DNA extracted by Phenol-Chloroform method.
- Extracted DNA was analysed by 2 methods:

HYBRISPOT TICK-BORNE BACTERIA FLOW CHIP
Simultaneous detection of 7 tick-borne bacteria genera:

Rickettsia
Ehlichia
Anaplasma
Francisella
Bartonella
Borrelia
Coxiella



PHELIX PHAGE BORRELIA TEST

- 40% of analysed ticks were negative (no detected pathogens)
- 60% of analysed ticks were positive for at least 1 pathogen

HYBRISPOT TICK-BORNE BACTERIA FLOW CHIP
Simultaneous detection of 7 tick-borne bacteria genera:

Rickettsia NONE DETECTED
Ehlichia NONE DETECTED
Anaplasma NONE DETECTED
Francisella NONE DETECTED
Bartonella NONE DETECTED
Borrelia 20% POSITIVES
Coxiella NONE DETECTED

PHELIX PHAGE BORRELIA TEST

Pathogens found in positive ticks:

Borrelia burgdorferi sl: 17% pos
Borrelia miyamotoi : 60% pos
Borrelia Relapsing Fever Group 23% pos

25% of positive ticks were for 2 pathogens

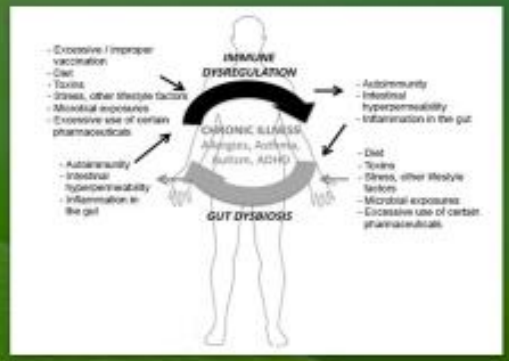
➔ Unexpected high rate of B. miyamotoi and Relapsing Fever Borrelia Phages in tested ticks

Vector-borne infections are increasing globally ⇒

Read our updated summary, available in different languages.
Lyme disease exhibits a variety of symptoms that may be confused with immune and inflammatory disorders, thus look for a Lyme disease specialist.

R.E.D. Laboratories Integrative Approach in Testing Human Samples

If an individual has any chronic health condition, ranging from arthritis to chronic fatigue syndrome to fibromyalgia, it is important to rule out tick-borne disease(s) by testing for it. It appears that many cases of fibromyalgia and chronic fatigue syndrome are actually TBD in disguise.



The all too often failure of therapies for vector-borne infections, especially in late/persistent/chronic patients, underscores the necessity to fully investigate different concurrent infections along with resulting gastrointestinal and immune dysregulations.

In order to offer better management of patients with chronic and/or persistent infections that are very difficult to uncover, it is important to focus both on direct pathogen detection as well as on indirect supportive tests.

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Tick-borne Infections

- Lyme disease is the most widely known tick-borne disease and is caused by bacteria of the genus *Borrelia.*
- Because individual ticks can harbor more than one disease-causing agent, patients can be infected with more than one pathogen at the same time, compounding the difficulty in diagnosis and treatment.
- Among neglected infections, there are few that deserve more attention and investigations, like **Tularemia**, **Yersinia**, **Mycoplasma, Chlamydia**, **Epstein-Barr virus and Herpesviruses**. Our data show that prevalence of these infections are not negligible and they should be more investigated.

Remember

- It is important to bear in mind that TBD can be acute or late stage / persistent / chronic; this is important given the diagnostic and treatment approaches might be different in these two situations.

- **Lyme disease exhibits a variety of symptoms that may be confused with immune and inflammatory disorders.**

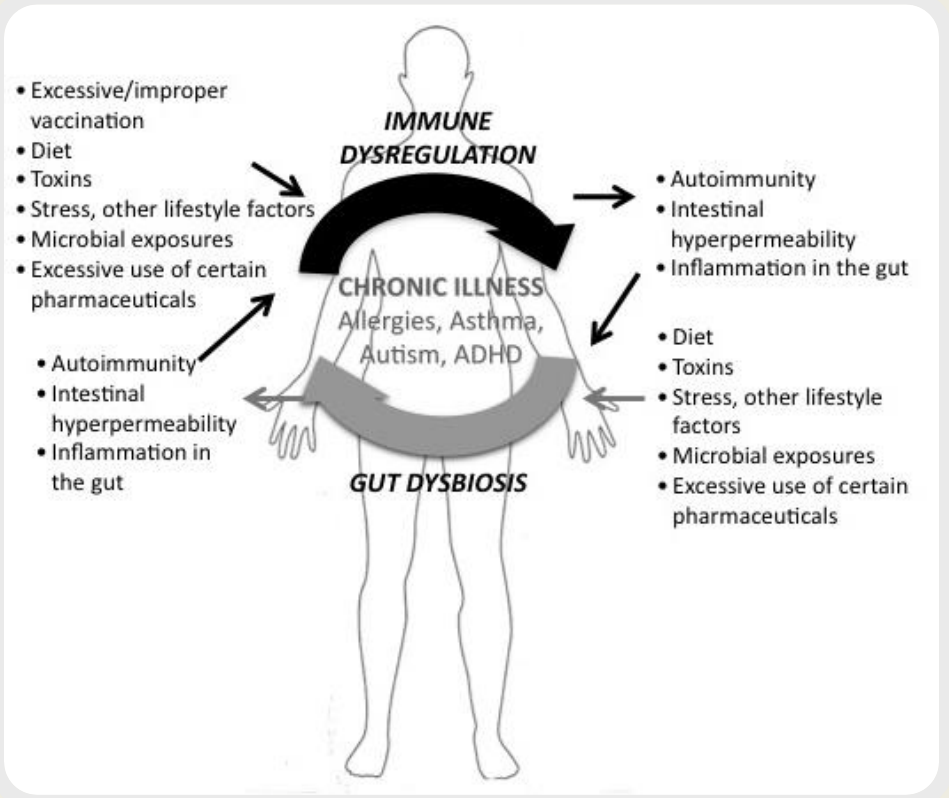
- If an individual has any chronic health condition, ranging from arthritis to chronic fatigue syndrome to fibromyalgia, it is important to rule out or diagnose tick-borne disease(s). It is apparent that many cases of fibromyalgia and chronic fatigue syndrome are actually TBD in disguise

- **Chronic patients have complex clinical picture with multiple afflictions needing thus multiple testing and careful interpretation of testing results.**

REMEBER

- The overall high failure rate of therapies for vector-borne infections, especially in late/persistent/chronic patients, underscores the necessity to fully investigate different concurrent infections along with resulting gastrointestinal and immune dysregulations.

- It is important to investigate different “co-infections” (i.e. tick-borne infections) but also other opportunistic infections (viral, bacterial, parasitic).



Chronic Lyme disease

- **Chronic Lyme disease can mimic every disease process including Chronic Fatigue Syndrome (Myalgic Encephalomyelitis), Fibromyalgia,** Autoimmune conditions including sero-negative rheumatoid arthritis and MS, Psychiatric conditions including depression and anxiety, and cause significant memory and concentration problems mimicking early dementia. **It is called the "Great Imitator“**
- If an individual has **any chronic health condition**, ranging from arthritis to chronic fatigue syndrome to fibromyalgia, it is **important to rule out or diagnose Lyme disease.** It is apparent that **many cases of fibromyalgia and chronic fatigue syndrome are actually Lyme disease in disguise**
- **Chronic Lyme sufferers also frequently house “co-infections”** such as Mycoplasma, Chlamydias, Ehrlichia, Bartonella and Babesia. These are different types of “bugs” that enjoy the company of B. burgdorferi

Testing for Tick-borne infections

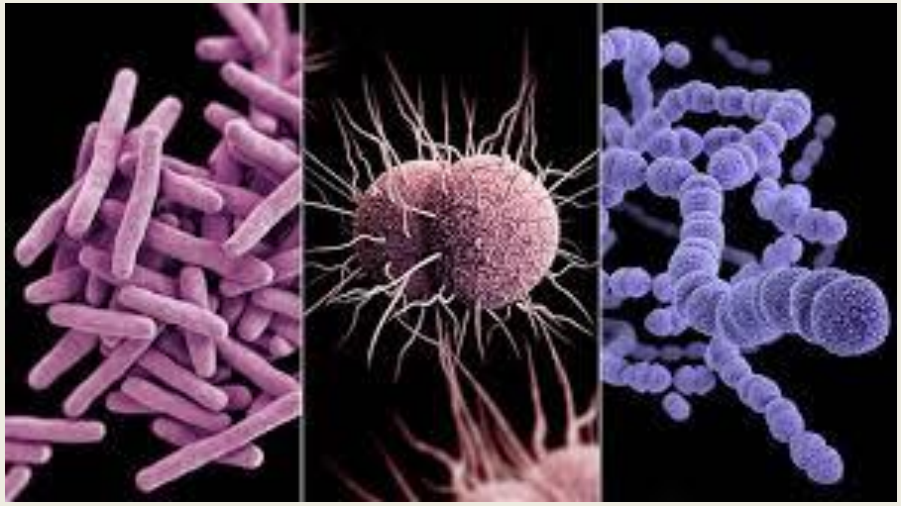
- Diagnosing Lyme and TBI-related diseases is extremely challenging.
- TBI diagnosis complications are a result of inadequate testing, mainly focusing on markers for the disease's early stages.
- Lyme cases are commonly misdiagnosed with other illnesses and even when a proper diagnosis is made, it’s often difficult to verify because accurate testing isn’t always available.
- Very few tests for Tick-borne diseases (TBD) are approved for clinical diagnosis, thus most of available testing options are “investigational” or “research” tests, aiming to help the assessment of patients with Lyme-like complaints.

Integrative Approach

- The overall high failure rate of tick-borne infection (TBI)-related testing, especially in late/persistent/chronic patients, underscores the necessity to fully investigate any potential resulting dysregulations and disabilities.
- In order to offer better management of patients with late/chronic and/or persistent infections that are very difficult to uncover, it is important to focus on an **integrative approach, inclusive of direct pathogen detection as well as indirect supportive tests focusing on immune and gastrointestinal disorders.**
- **There are many biomarkers that can help assessing the extent of gastrointestinal and immune dysregulations resulting from TBI.**

Integrative approach for TBD testing: Part 1 – pathogen detection

Infections, Co-infections, Oportunistic Infections



CURRENTLY USED TESTS FOR BORRELIA DETECTION AND THEIR LIMITATIONS

Diagnostics	Qualities and defects
Antibody-based	• Give indirect evidence • Low sensitivity (early stages) • Can't distinguish active and non-active *Borrelia* presence • *Difficult identification of Borrelia* sub-types
Bacterial DNA-based	• Direct evidence of *Borrelia* presence • Low sensitivity • Can't distinguish live and dead *Borrelia* • Might be able to tell different *Borrelia* sub-types
Lymphocyte transformation test	• Provide indirect evidence • Variable sensitivity that depends of immune system status and interfering treatments. False positive if time of incubation >24hours not reflecting real situation • Can only detect Lymphocytes that have been in contact with *Borrelia* within 45±15 days, thus limited in application • active *Borrelia*? • Difficult identification of different *Borrelia* sub-types?

Slide provided by Dr L. Teulières

NEED for NEW TESTS DEVELOPMENT

PHELIX PHAGE TEST: A Breakthrough way to detect intracellular bacteria, and an answer to non-diagnosed ill people

Phelix

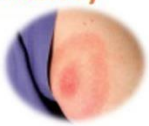
RED LABORATORIES

Context

Tick bite (30% are infected) → **Early Phase (no test available)** → **Chronic Phase (15% detected)**

30% of ticks are infected by borrelia bacteria and some others co-infections considered as infectious disease threats (CDC USA, European Commission)

10% to 40% of the patients developing an Erythema Migrans after an infected tick bite.



If not detected, Lyme Borreliosis becomes chronic and difficult to treat. Some early treated patients with ABX can still develop symptoms of a chronic infection.

Hard symptoms

- Neurological Disorders
- Joint Pain
- Tiredness
- Heart Disease
- Thyroid dysfunction

The Problem

Lack of a reliable direct test

Current diagnostics tests are not sensitive enough. Patients in the early phase of the disease or with relapsing symptoms are not treated.

Negative tests, but clinical symptoms

A medical wandering

Undiagnosed late stage cases will never be considered as patients and then properly treated despite clinical symptoms.

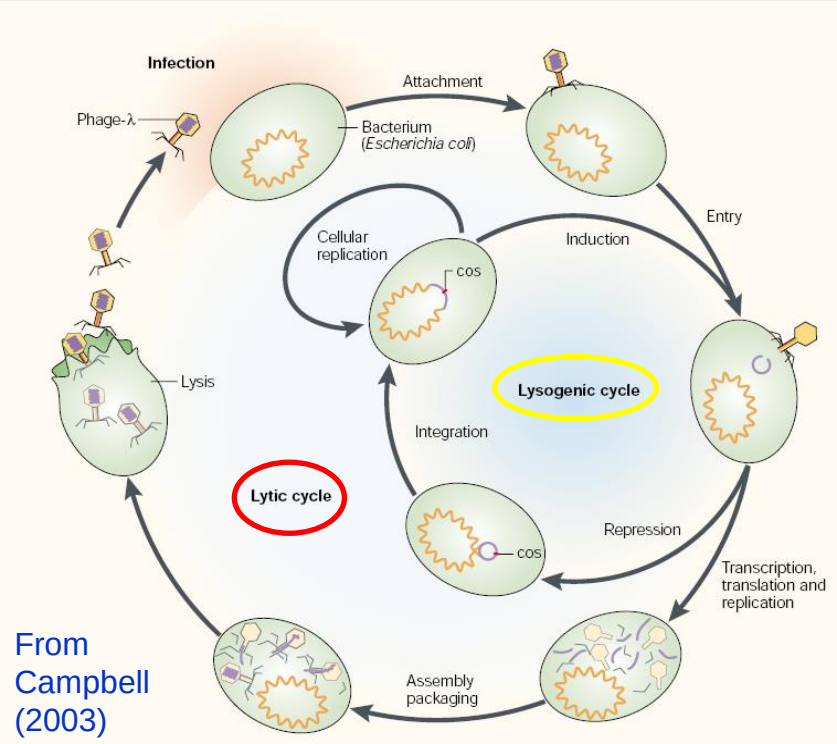
Cooperation between Academia, Testing Lab and Medical specialists

- Louis Teulières MD, PhD: *Infectious and immune diseases consultation (Paris & Lisbon); PhelixRD Charity* (*chronic infections and bacteriophages research group)*
- Jinyu Shan PhD : *Department of Infection, Immunity, and Inflammation, University of Leicester, UK*
- Martha Clokie PhD, Pr, Head: *Department of Infection, Immunity, and Inflammation, University of Leicester, UK*
- Tanja Mijatovic PhD: *CSO & Lab Manager R.E.D. Laboratories, BE*

Novel Testing Approaches : Phage-based Test

➢ The importance of novel testing approaches

The overall high failure rate of tick-borne infection (TBI)-related testing underscores the necessity for novel approaches, i.e. not relying on serology and two-tier testing. Phelix Phage Borrelia detection method (Patent WO2018083491A1) consists of targeting the presence of outnumbered prophages part of the ***bacteria lysogenic cycle****.*
Bacteriophages are present only on active bacterial infections; hence a phage-based test is a direct proof of an active infection.



From Campbell (2003)

- The **lysogenic cycle**: The phage *infects* a bacterium and inserts its DNA into the bacterial chromosome, allowing the phage DNA (now called a **prophage**) to be copied and passed on along with the cell's own DNA.
- The **lytic cycle**: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (*lyse*).

Phelix Phage Borrelia Test

- Bacteriophages could become a diagnostic tool based on the principle that if there are phages it is because there are living bacteria.
- Phelix Charity together with Leicester University microbiology department have recently developed a Borrelia Phage-based PCR test searching for 3 major Borrelia groups:

✓ Borrelia burgdorferi sl (including B. burgdorferi ss, B. afzelii, B. garinii, B. spielmanii, etc)

✓ Borrelia miyamotoi and

✓ Relapsing fever group (B. recurrentis, B. hermsii, etc).

- This method is efficiently used to assess both human samples and ticks.
- Highly sensitive and specific.
- Do not generate positive signal against other bacterial strains.
- False positive are ruled out by sequencing.

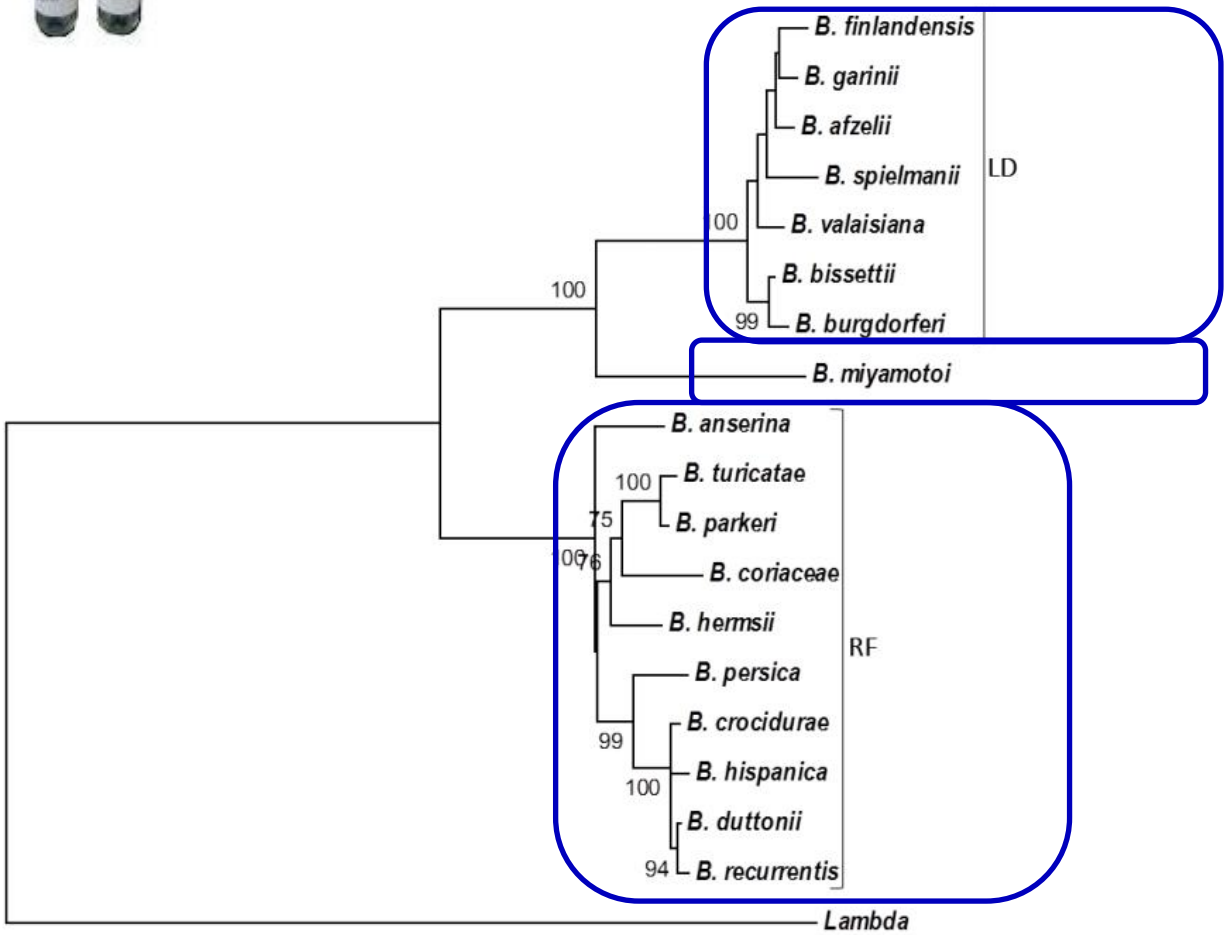
Phelix Phage Borrelia Test – How it works

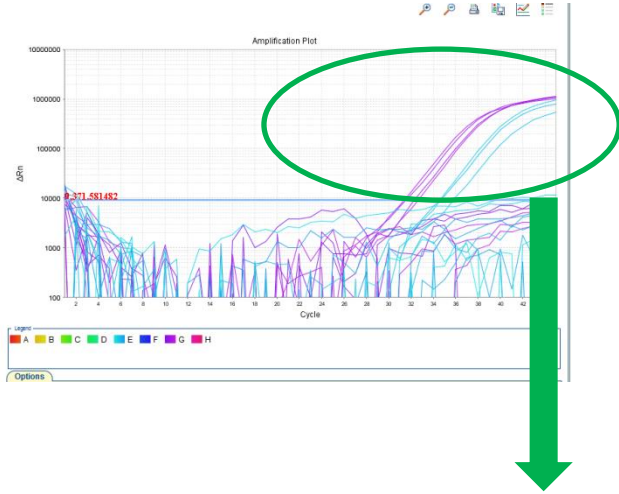
Manual DNA Phenol / Chloroform extraction

QCs

- Extraction control PCR *quality of extracted DNA*
- IAC control PCR *absence of PCR inhibitors*

3 different real-time PCRs, each sample in 4 replicates per target





Confirmatory sequencing for positive-like samples

Reminder on Available Diagnostic Methods

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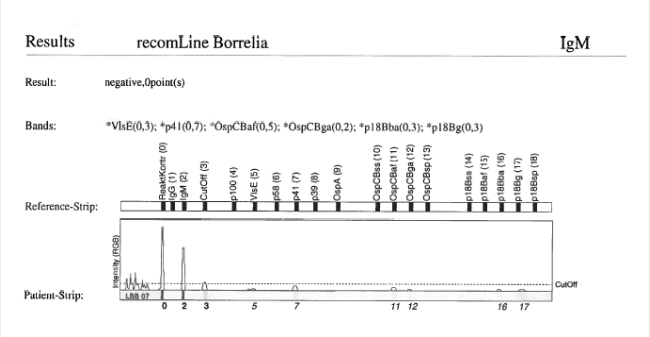
Phage test	• **Direct evidence of *Borrelia* presence** • High sensitivity and specificity (Pilot study) • Can distinguish Lyme from **Relapsing fever** *Borrelia* strains • Active and non-active *Borrelia* presence

Chronic Lyme Disease and Co-infections

- **Chronic Lyme Disease and Co-infections**
  - *In Lyme disease concurrent infections frequently occur. Co-infecting agents can be transmitted together with Borrelia burgdorferi by tick bite resulting in multiple infections but a fraction of co-infections occur independently of tick bite.*
  - *Clinically relevant co-infections are caused by Bartonella species, Yersinia enterocolitica, Chlamydophila pneumoniae, Chlamydia trachomatis and Mycoplasma pneumoniae.*
  - *Infections caused by these pathogens in patients not infected by Borrelia burgdorferi can result in clinical symptoms similar to those occurring in Lyme disease. This applies particularly to infections caused by Bartonella henselae, Yersinia enterocolitica, and Mycoplasma pneumoniae. Chlamydia trachomatis primarily causes polyarthritis. Chlamydophila pneumoniae not only causes arthritis but also affects the nervous system and the heart, which renders the differential diagnosis difficult. The diagnosis is even more complex when co-infections occur in association with Lyme disease. (from Berghoff W. Open Neurol J. 2012;6:158-78.)*
  - **Lyme disease exhibits a variety of symptoms that may be confused with immune and inflammatory disorders.**

Integrative approach for TBD testing: Part 1 – pathogen detection

- **Direct tests** for Persistent and/or chronic infections
  - **PHELIX PHAGE BORRELIA TEST**
  - **Immunoblot** for Borrelias, Chlamydias (pneumoniae, trachomatis, psittacii), Yersinia, EBV, Parvovirus, Treponema, Tropical fever (Chikungunya virus + Dengue fever virus + Zika virus), Hepatitis E Virus, etc

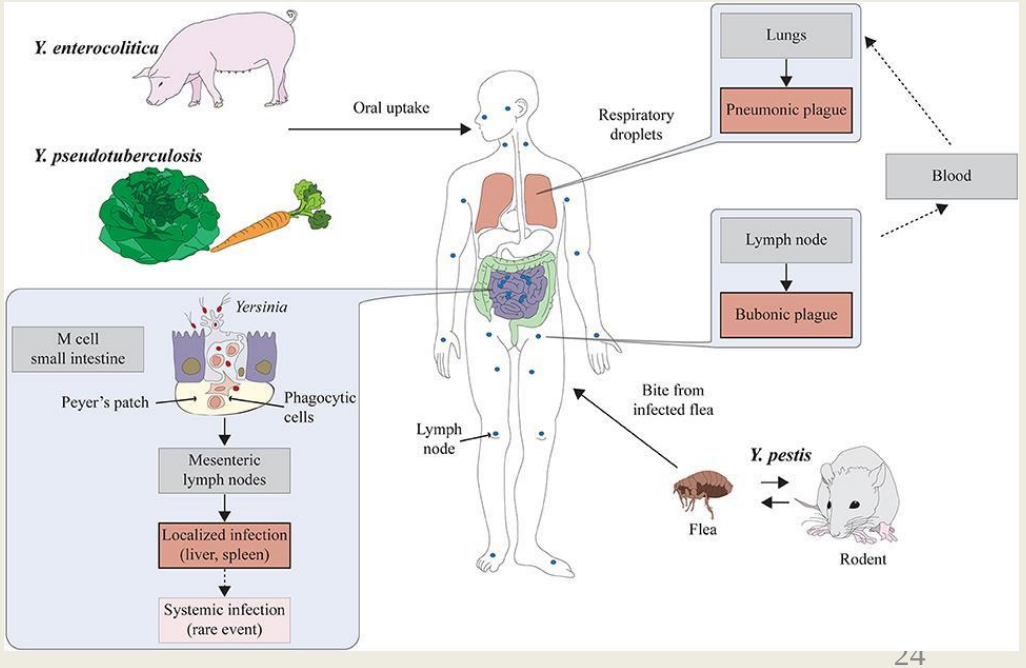


  - Serology tests for CMV, Toxoplasmosis, **Tularemia**, Leptospira, **BrucellaCapt**
  - FISH test for Babesia
  - PCRs for **Mycoplasma** spp, Mycoplasma fermentans, Mycoplasma pneumoniae, Bartonella, Brucella, Coxiella, Babesia, Anaplasma, Ehrlichia, Chlamydias, Rickettsias, Midichloria mitochondrii, etc
  - PCRs for viral infections: herpesviruses like **HHV-6**, HHV-7, HHV-8, Parvovirus, EBV, Cytomegalovirus, **Tick-borne encephalitis virus**, West Nile virus, Coxsacki virus, Enterovirus
  - **MOLDS** Serology: IgG against 6 major molds: *Candida albicans, Cladosporium herbarum, Aspergillus niger, Alternaria alternate, Penicillium chrysogenum, Aspergillus fumigatus*
  - Quantification of mycotoxins in urine
  - **Testing for parasites**
  - New tests in development

Yersinia

- The bacteria from the genus Yersinia are Gram-negative enterobacteria. From the 17 described species there are 3 know to be human pathogens:
  - ***Yersinia pestis*** causes bubonic and pneumonic plague. Bubonic plague is transmitted by the bite of infected rat fleas. Swollen, blackened lymph nodes (buboes) develop, followed by septicemia and hemorrhagic pneumonia and death. The pneumonic form spreads directly from human to human via respiratory droplets. Outbreaks are explosive in nature, and invariably lethal.
  - ***Yersinia enterocolitica*** causes severe diarrhea and local abscesses
  - ***Yersinia pseudotuberculosis*** causes severe enterocolitis.

- The most common source of Y. enterocolitica infection in humans is pork (raw or undercooked) and also contaminated water, meat, or milk.



Yersinia

- Other strains of Yersinia are also found in many other animals including rodents, rabbits, sheep, cattle, horses, dogs and cats.

- Yersinia pseudotuberculosis is a Gram-negative bacterium that causes Far East scarlet-like fever in humans.

- Y. pseudotuberculosis infections can mimic appendicitis, the disease may cause skin complaints (erythema nodosum), joint stiffness and pain (reactive arthritis), or spread of bacteria to the blood (bacteremia). Y pseudotuberculosis causes severe intestinal abscesses

- Genetically, the pathogen causing plague, Y. pestis, is very similar to Y. pseudotuberculosis. The plague appears to have evolved from Y. pseudotuberculosis

Yersinia

- Common symptoms are:
  - fever
  - abdominal pain, and
  - diarrhea (which is often bloody)
- Complications include:
  - joint pain
  - rashes (erythema nodosum)
- Some persons present mild symptoms thus difficult to uncover.
- Yersinia infections are sometimes followed by chronic inflammatory diseases such as arthritis, erythema nodosum, and reactive arthritis.
- Yersinia may be associated with Crohn's disease
- Yersinia pseudotuberculosis-derived mitogens (YpM) are superantigens, which are able to excessively activate T cells by binding to the T cell receptor. Since YpM can activate large numbers of the T cell population, this leads the release of inflammatory cytokines.

The importance of testing for Yersinia

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Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity

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- "These repeated and unregulated inflammatory challenges may profoundly remodel the immune system and thereby contribute to the increased burden of autoimmune and inflammatory disorders.
- Together, this study provides a framework to understand how previously encountered infections can induce a breakdown of tissue immune homeostasis, thereby contributing to disease later in life. Thus, in order to fully comprehend the etiology of complex diseases, it may be necessary to look beyond a patient's genetic susceptibilities and concurrent environmental stressors and examine whether immunological scarring associated with previous infections may have 'set the stage' for chronic inflammation."
- These **repeated and unregulated inflammatory challenges may profoundly remodel the immune system and thereby contribute to the increased burden of autoimmune and inflammatory disorders**."

Yersinia – testing results

- Yersinia immunoblot tests make it possible to detect past Yersinia infections, and are thus ideally suited for identification of Yersinia-induced immunopathological complications and chronic yersiniosis. Detection of IgG and IgA antibodies can be a very useful diagnostic tool if Yersinia-induced arthritis is suspected.
- An immunoblot for the detection of IgG and IgA antibodies against all pathogenic Yersinia by means of Yersinia outer proteins (YOPs). Serological differentiation of Y. enterocolitica and Y. pseudotuberculosis infections is possible for the first time with the use of new species-specific Yersinia antigens (PsaA, MyfA).
- Testing period : May 1st 2015 – May 1st 2019 (4 years)
- Immunoblot on serum
  - Total tested: 2396 samples
  - IgA positive: 412 (➔ 17.2%)
  - IgG positive: 946 (➔ 39.5%)
    - IgG, no differentiation: 624 positives (➔ 66% of IgG positives; 26.1% of all tested
    - IgG Y. pseudotuberculosis: 202 positives (➔ 21.4% of IgG positives; 28.4% of all tested
    - IgG Y. enterocolitica: 120 positives (➔ 12.6% of IgG positives; 5% of all tested

Tularemia

- Tularemia, also known as rabbit fever, is an infectious disease caused by the bacterium *Francisella tularensis*
- People can become infected in several ways, including:
  - Tick and deer fly bites
  - Skin contact with infected animals
  - Drinking contaminated water
  - Inhaling contaminated aerosols or agricultural and landscaping dust: can occur during farming or landscaping activities, especially when machinery (e.g. tractors or mowers) runs over infected animals or carcasses
  - Laboratory exposure
  - People could be exposed as a result of bioterrorism.
- Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated.
- The diagnosis of tularemia is often delayed. It may take a significant length of time to diagnose and the condition and the disease may become complicated.
- Some persons present mild symptoms thus difficult to uncover.

Tularemia

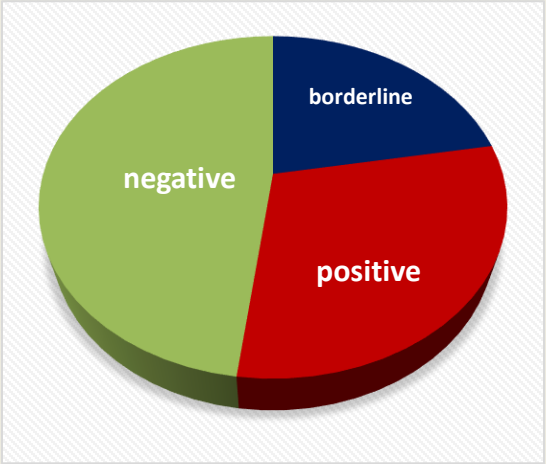
- Symptoms vary depending how the person was infected:
  - **Ulceroglandular:** occurs following a tick or deer fly bite or after handing of an infected animal. A skin ulcer appears at the site where the bacteria entered the body. The ulcer is accompanied by swelling of regional lymph glands
  - **Glandular:** Similar to ulceroglandular tularemia but without an ulcer. Also generally acquired through the bite of an infected tick or deer fly or from handling sick or dead animals.
  - **Oculoglandular:** occurs when the bacteria enter through the eye. This can occur when a person is butchering an infected animal and touches his or her eyes. Symptoms include irritation and inflammation of the eye and swelling of lymph glands in front of the ear.
  - **Oropharyngeal**: results from eating or drinking contaminated food or water. Patients with orophyangeal tularemia may have sore throat, mouth ulcers, tonsillitis, and swelling of lymph glands in the neck.
  - **Pneumonic**: the most serious form of tularemia. Symptoms include cough, chest pain, and difficulty breathing. This form results from breathing dusts or aerosols containing the organism. It can also occur when other forms of tularemia (e.g. ulceroglandular) are left untreated and the bacteria spread through the bloodstream to the lungs.
  - **Typhoidal** : characterized by any combination of the general symptoms (without the localizing symptoms of other syndromes)

Tularemia – testing results

- Testing period : July 1st 2015 – May 1st 2019

- Screening test : immunochromatography on serum
  - Total tested: 1769 samples
  - 392 (= 22.16%) were found positive and undergo confirmatory testing

- Confirmatory test: IVD Tularemia IgM ELISA
  - 392 went to confirmatory testing
  - 87 were found borderline (22,2% among those that went for confirmatory testing, 5 % of total tested samples)
  - 117 were found positive (29,8% among those that went for confirmatory testing, 6,6% of total tested samples)



Herpes viruses – HHV6 & HHV7

- **HHV-6** has a very high prevalence (close to 100% of the world's population has been exposed); primary infection is often associated with a febrile condition and sometimes with the onset of roseola (exanthem subitum).
- HHV-6 has immunomodulatory effects, including suppression of T-cell proliferation and alteration of cytokine production.
- This immunosuppression may favor the development or progression of other viral infections such as CMV, EBV or HIV.
- **HHV6 qPCR test : 3% of tested blood samples (509 samples) were found positives with high viral load, and 13% of them present HVV6 chromosomal integration.**
- **HHV-7** is closely related to HHV-6, primary infection usually occurs later in childhood than HHV-6 infection; it can also cause exanthem subitum.
- HHV-7 efficiently infects and replicates in CD4+ cells; it can be found in brain tissue but at a lower frequency than HHV-6.
- It has been suggested that HHV-7 could reactivate HHV-6 from latency. **Increased prevalence has been reported in people with autoimmune disease**.
- **HHV7 qPCR test : 34,4% of tested blood samples (128 samples) were found positives with high viral load.**

Given that a majority of the population has been exposed to HHV, qPCR testing is to be preferred over serology testing.

EBV-Epstein-Barr virus

- **EBV** (HHV-4) infects more than 90% of the world's adult population.
- It is transmitted by salivary contact; the virus first replicates in the epithelium of the oropharynx before infecting B lymphocytes where it will persist for life in a latent state.
- EBV re-activation is known to occur in times of immunocompromised state, cellular stress, and inflammation.
- Following initial infection, EBV can reactivate and has been shown to have many connections with various chronic illnesses.
- Epstein-Barr virus infection is known to cause false-positive results in Lyme disease serologic testing, particularly IgM tests.

- **EBV immunoblot test (393 samples tested):**
  - **IgM positive samples: 3%**
  - **IgG positive samples: 85%, 2% with recent infection**

qPCR testing should be added to serology testing.

Mycoplasma

- Ticks were found to carry M. Pneumonae, M. Genitalium & M. Fermentans
- These infections are exacerbating the CTBI patients, especially those with auto-immune manifestations.
- Mycoplasma spp cause B cells to be overstimulated, promoting auto-immune and rheumatoid Disease
- Mycoplasma increase production of IL-1beta & IL-6
- Poor treatment results with antibiotics
- PCR testing in body fluids and biopsies preferred to serology testing
- **PCR testing of sputum and swaps (total 111 samples) with confirmatory sequencing: 28.8% positives**

Chlamydia

- **Ticks DO NOT carry Chlamydia BUT reactivate in presence of T.B.I.**
- C. Pneumonae
- C. Trachomatis
- C. Psittaci

- HP60 is expressed ➔ arthritis, drive auto-immune reactions, create free radicals and oxidative stress

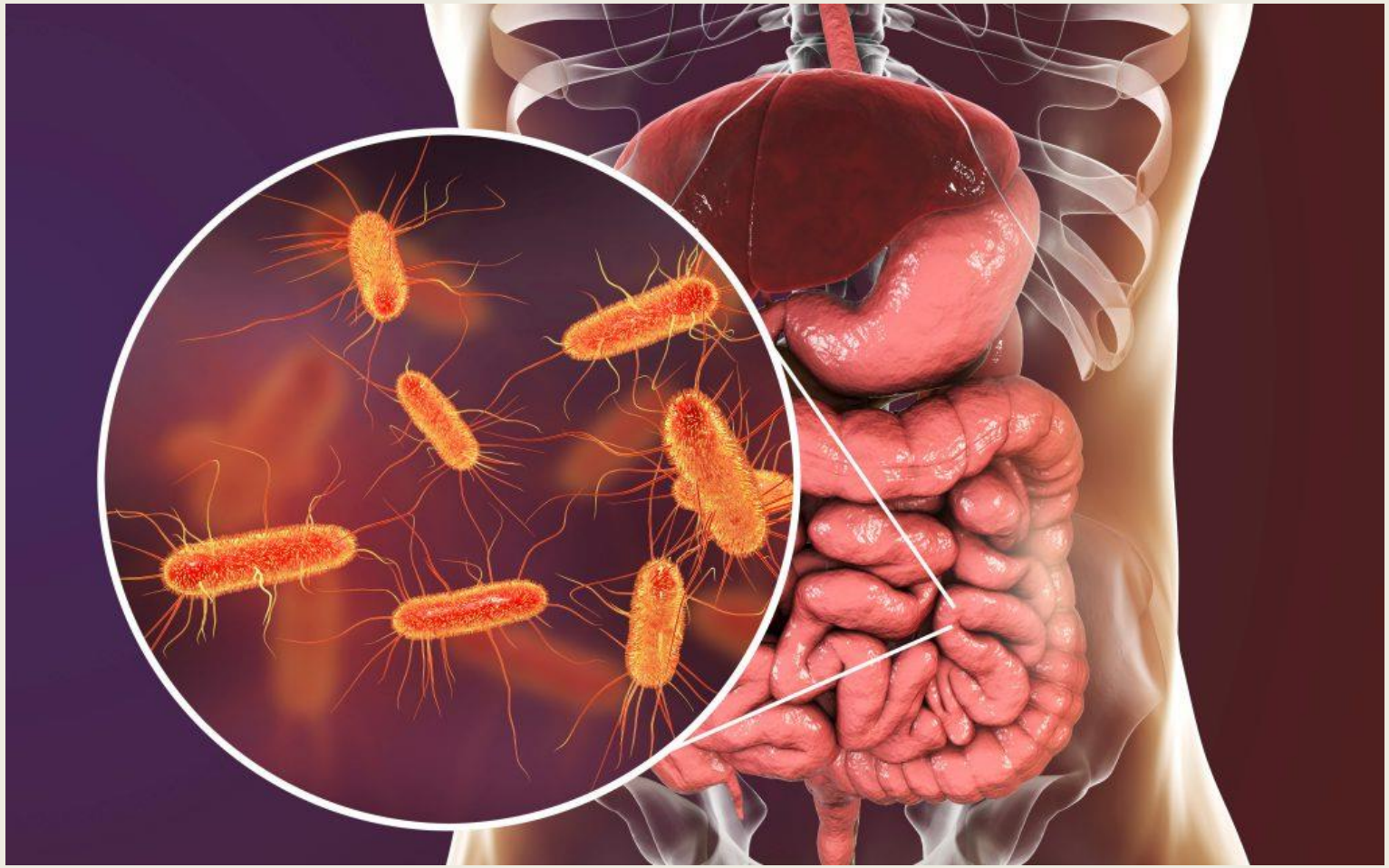
- Chlamydia ➔ turns on NF kappa B
  ➔cytokine/inflammatory molecules

- An immunoblot for the detection of IgG and IgA antibodies against Chlamydia trachomatis, Chlamydophila pneumoniae and Chlamydophila psittacci:

  1927 tested samples:

  - IgA Chlamydophila pneumoniae: 9.8% positives
  - IgA Chlamydophila psittacci: 0.4 % positives
  - IgA Chlamydila ptrachomatis: 6.7% positives

Integrative approach for TBI
Part 2 – Intestinal dysfunctions



Intestinal dysfunctions

Regulation of immune function in the gut

- **>80% of our immune cells reside in the gut**
  - Gut associated lymphoid tissue (GALT) is spread along the intestinal mucosa (Peyer's patch in the small intestine, lymphoid follicles in the colon) and hosts 80% of the body's immune cells
  - These immune cells permanently interact with mucosa-associated micro-organisms (bacteria, viruses…)
  - A delicate balance is maintained between tolerance to gut antigens (down-regulation of inflammation,…), and defense against pathogens (production of defensins,…)
- **Imbalance of gut immunity affects the whole body**
  - ***Gut barrier integrity is essential :*** Increased permeability of the mucosa causes systemic endotoxemia (chronic low grade inflammation) and abnormal immune reactions to gut antigens
  - ***Interactions host/gut flora :*** the gut microbial flora plays a major role in maintenance of host health, but can be affected by abnormal host immune function

TBI and gastrointestinal disorders

Signs and symptoms related to the gastrointestinal tract and liver may provide important clues for the diagnosis of various tickborne diseases

Manifestation	Lyme disease	Ehrlichiosis	RMSF	Tularemia	Colorado tick fever	TBRF	Q fever	Babesiosis
Anorexia	+	++	+	+	+	+	+	+
Nausea	+	++	++	++	++	+++	++	+
Vomiting	+	++	++	++	++	+++	++	+
Abdominal pain	+	++	++ to +++	++	+	++	+	+
Diarrhea	+	++	++	++ to +++	+	+ to ++	++	+
Hepatomegaly	R	+ to ++	+	+ to ++	R	+	+	+
Splenomegaly	+	+ to ++	+	+ to ++	R	R to +	+	+
Jaundice	+	+++	+	+	+	+	+	+ to ++
Elevated bilirubin level	+	+++	+ to ++	+	+	+	+ to ++	++ to +++
Elevated ALT level	++	++++	++ to +++	++	+	++	++[a]	+

NOTE. ALT, alanine aminotransferase; R, rare; RMSF, Rocky Mountain spotted fever; TBRF, tickborne relapsing fever; +, uncommon; ++ common; +++, very common; ++++, almost always present.

[a] Elevated alkaline phosphatase level is the predominant abnormality.

From: Gastrointestinal and Hepatic Manifestations of Tickborne Diseases in the United States
Syed Ali Zaidi & Carol Singer, Clin Infect Dis. 2002;34(9):1206-1212. doi:10.1086/339871

Lyme disease and gastrointestinal disorders

- **Patients with Lyme and TBDs may present primarily with GI manifestations.**
- **2015 ILADS conference, Dr. Farshid Rahbar: These patients may have complex or persistent GI symptoms involving upper, mid, or lower GI tract and have already been treated for GI issues**

  Bloating/Gas: in 76% of patients

  Abdominal Pain: in 48% of patients

  Constipation: in 42% of patients

  Food Intolerance: in 42% of patients

  Irregular Bowel Movements: in 37% of patients

- **The number of patients presenting with such symptoms is probably reaching epidemic proportions.**
- **Testing for gastrointestinal problems need to be included**
- **Useful assays to investigate intestinal dysfunctions:**
  - **BLOOD-BASED Tests: sCD14, Lactase deficiency assay, D-lactate, Ammonia in serum**
  - **BIOPSY-BASED Tests: PCR-based detection of viral and bacterial infections**
  - **STOOL-BASED Tests:**
    - **Intestinal Inflammation: sIgA, Beta-2 Defensin, EPX / EDN, Inflammation markers in stool samples**
    - **Intestinal Infections : immunochromatography antigenic testing for intestinal infections**
    - **Leaky gut: ZONULIN ELISA test in stool samples**
    - **Dysbiosis: MSA assay (metagenomic stool test)**

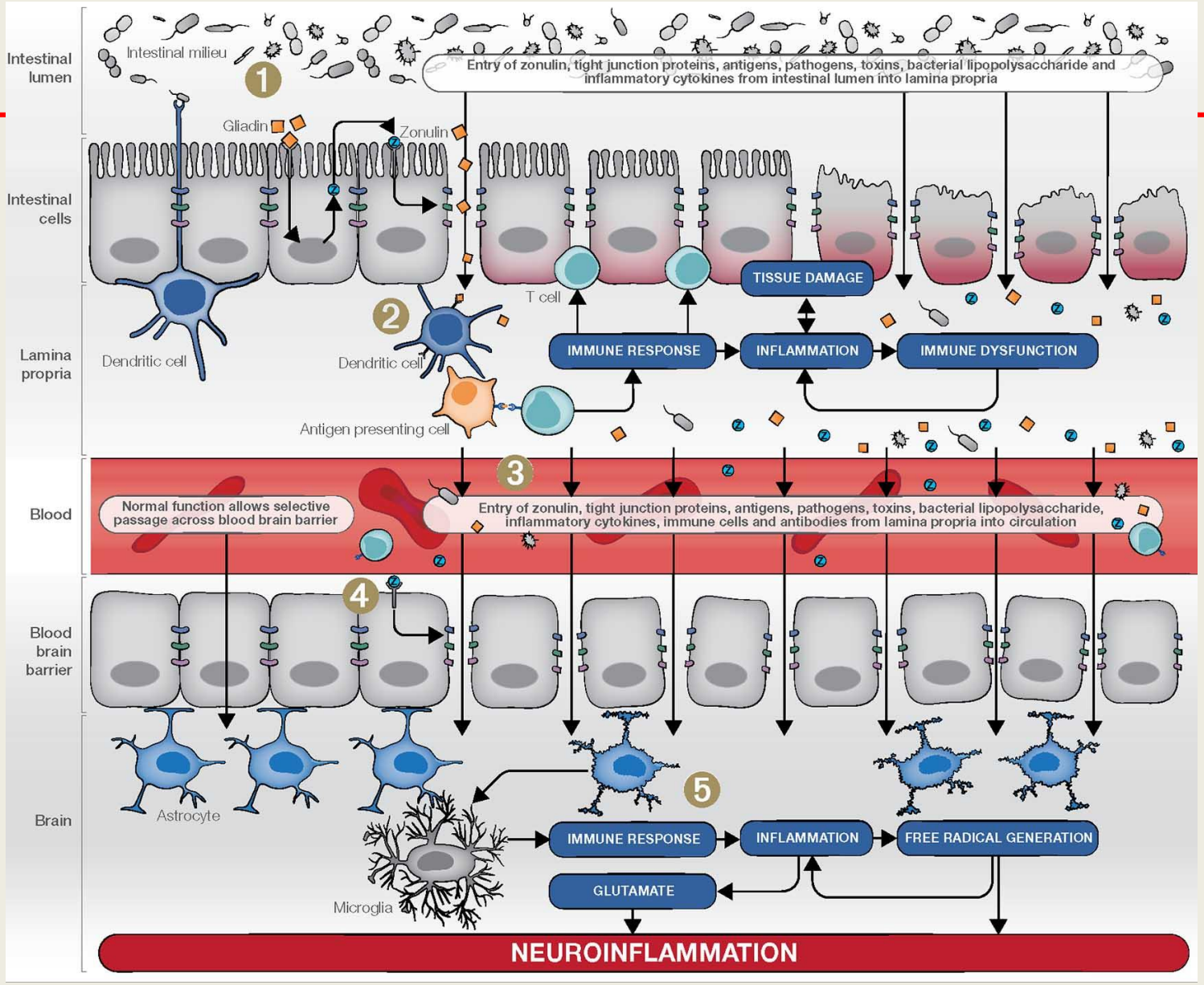
Blood-based assays for Intestinal dysfunctions

- ***sCD14 in serum***
  - sCD14 is expressed in monocytes/macrophages and plays a critical role in the recognition of bacterial cell wall components (LPS). The extracellular part of CD14 can be cleaved and released in the plasma, where it will inactivate circulating LPS. Serum soluble CD14 levels are significantly elevated in patients with leaky gut, inflammatory bowel disease, Crohn's disease, but also in patients suffering from Brucellosis or Lyme disease.
- ***Lactase deficiency assay***
  - a polymorphism in the gene coding for lactase, an enzyme responsible for the digestion of lactose (C/T-13910 polymorphism). In affected people, production of the enzyme declines during or shortly after childhood, resulting in lactose malabsorption. Undigested lactose sugars affect the development of gut microflora, leading to dysbiosis.
- ***D-lactate in serum***
  - a product of bacterial metabolism, it is neither produced nor metabolized by mammalian cells. Typically, elevated D-lactate levels are due to bacterial infection or short bowel syndrome in humans. Due to slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy.
- ***Ammonia in serum***
  - Ammonia is derived from bacterial enzymatic action on ingested amino acids. It is absorbed from the gastrointestinal tract and delivered through the portal vein to the liver, which converts most of it into urea. Abnormally high levels of ammonia can result from colic or "enteric hyperammonemia" (combination of increased bacterial production and increased gut permeability) that occurs despite normal hepatic function. Hyperammonemia is a metabolic condition characterized by elevated levels of ammonia in the blood. Increased entry of ammonia to the brain is a primary cause of neurologic disorders, metabolic disorders and some toxic encephalopathies.

Consequences of the leaky gut – Chronic activation (inflammation) of the immune system

- Leaky gut testing (Zonulin in stool) : 3 years testing period, **1301 samples 63.87% patients with increased levels!!**
- Lipopolysaccharide (LPS) - bacterial compound that can easily make its way to the blood.
- Present in the bloodstream LPS will induce a strong pro-inflammatory response in monocytes and macrophages, involving recognition by a receptor (Toll-like receptor-4) and the subsequent secretion of cytokines such as IL-1, IL-6, TNF-alpha.
- LPS also induces the NK-kB-mediated production of nitric oxide. Because NO is increased, NK function is inhibited and opportunistic infections such as mycoplasma infections are often observed.
- Herpesviruses, which tend to reactivate in a context of immune activation, will also be frequently detected.

Consequences of the leaky gut



Source: https://www.fxmedicine.com.au

Stool-based assays for Intestinal inflammation

- sIgA ELISA test in stool samples

- sIgA key function is to bind to invading micro organisms and toxins and entrap them in the mucus layer or within the epithelial cells, so inhibiting microbial motility, agglutinating the organisms and neutralizing their exotoxins and then assist in their harmless elimination from the body in the fecal flow.

- The concentration of sIgA gives us information about the intestinal immune defense:

A lack of sIgA *indicates a* ***diminished activity of the intestinal immune system***

An increased *level of sIgA shows* ***intestinal inflammation****.*

- beta-Defensin-2 ELISA test in stool samples

- Defensins exert a variable degree of antimicrobial activity against bacteria, fungi, and some enveloped viruses. The expression of ß-defensins is induced by the pro-inflammatory cytokines and also through microorganisms (e.g. E. coli, H. pylori or P. aeruginosa) and by probiotic microorganisms. A ß-defensin-2 deficiency can, for example, be observed in the intestinal mucous of patients with Crohn's disease. The defense system of the mucous membrane is therefore restricted and allows an increased invasion of bacteria, which could possibly lead to a typical infection in Crohn's disease patients. Recent results imply that ß-defensin-2 is overexpressed in active intestinal inflammation, especially in ulcerative colitis.

- EDN / EPX ELISA test in stool samples

- The accumulation of EDN in the intestine is associated with inflammation and tissue damage. Fecal EDN is considered the best of the cytotoxic granule proteins for assessment of gut inflammation. Elevated levels of fecal EDN are linked to multiple inflammatory conditions, like food allergy/sensitivity, pathogenic infections (C. difficile and H. Pylori), IBS, Eosinophilic Gastrointestinal Disorders.

- Inflammation markers in stool samples

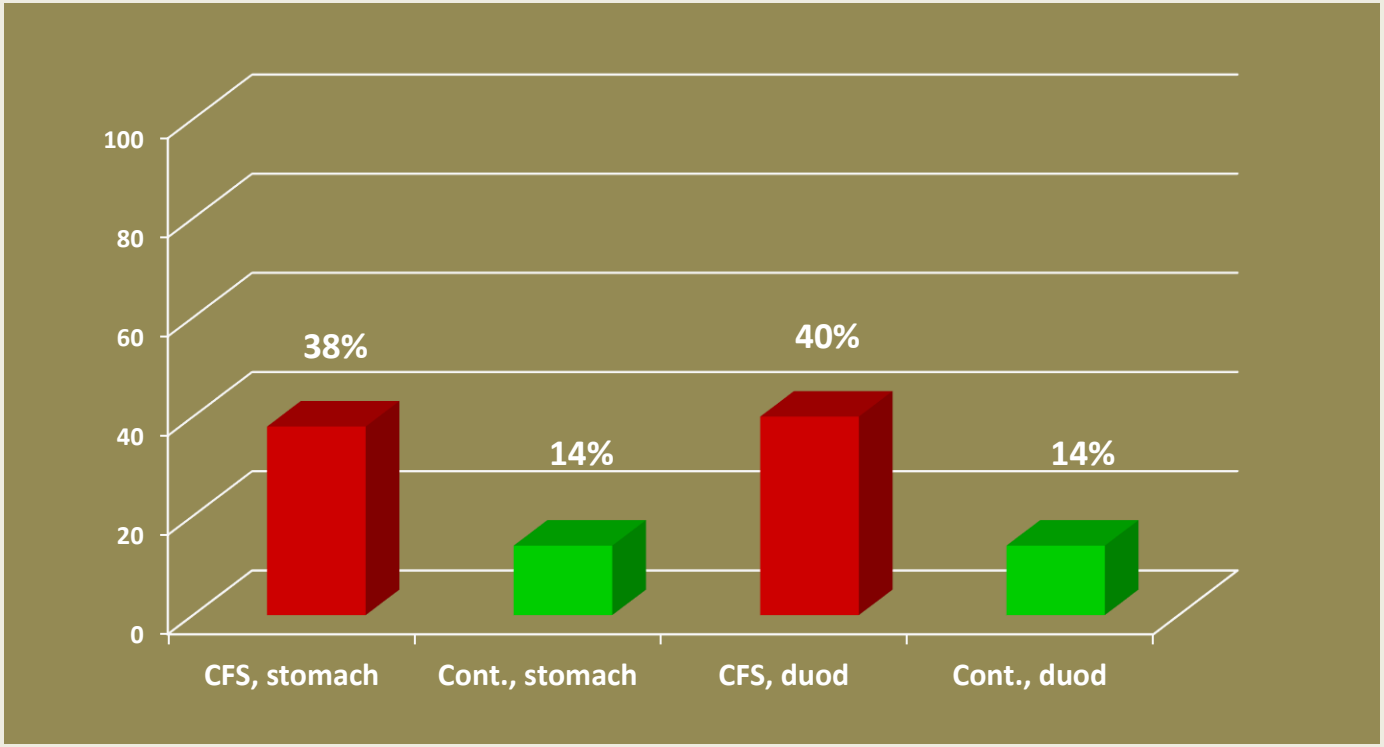
- ***Hemoglobin*** : discharged with the feces in gastrointestinal bleeding diseases
- ***Transferrin***: a blood-derived component ; a good marker for gastrointestinal bleeding
- ***Calprotectin***: a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool during bowel inflammation
- ***Lactoferrin***: a primary component of the acute inflammatory response released from fecal leukocytes; may serve as a marker of inflammation in the intestine

Assays for Intestinal Infections

- INFECTIONS - assessment in stool samples

Ag-based testing for Clostridium, Yersinia, Enterovirus, Parasites, etc

- INFECTIONS – PCR-based viral and bacterial assessment in intestinal biopsies



Frémont et al., In vivo 2009

Stool-based assays for Intestinal dysfunctions

- ZONULIN ELISA test in stool samples

Zonulin is the "doorway" to **leaky gut**. Zonulin opens up the spaces between the cells of the intestinal lining. When leaky gut is present, the spaces between the cells open up too much allowing larger protein molecules and bacteria to get into the bloodstream where an immunologic reaction can take place. As the zonulin level rises, the seal between the intestinal cells diminishes. Zonulin is the only physiological modulator of intercellular tight junctions described so far that is involved in trafficking of macromolecules and, therefore, in tolerance/immune response balance.

- MSA – Metagenomic Stool Assay

- Until recently research into microbiota composition relied almost exclusively on culture ; 40 to 80% of gut bacteria cannot be cultured
- Identification of colonies can be difficult
- Bacteria must be alive: studies of anaerobes very difficult, major loss during collection and processing of samples
- Culture approach may address only a small fraction of all bacterial species (10%?)
- E.coli once thought to be a dominant species, is a minor member…
- R.E.D. Labs scientists have developed and validated a new procedure to analyze bacterial populations in a stool sample : MSA assay
- New molecular technique involving sequencing of specific regions of bacterial DNA (metagenomics)
- Can be performed on dead organisms (exposure to oxygen, freezing are not a problem)
- Identification of each bacteria by comparing sequence with public databases: extremely precise, not subjective
- High-throughput technology allows identification of tens or even hundreds of thousends organisms in a single sample
- Bacterial DNA was extracted from stool samples, PCR amplification was performed on 16S rRNA gene regions, and PCR amplicons were sequenced Bacteria were classified by phylum, family and genus.

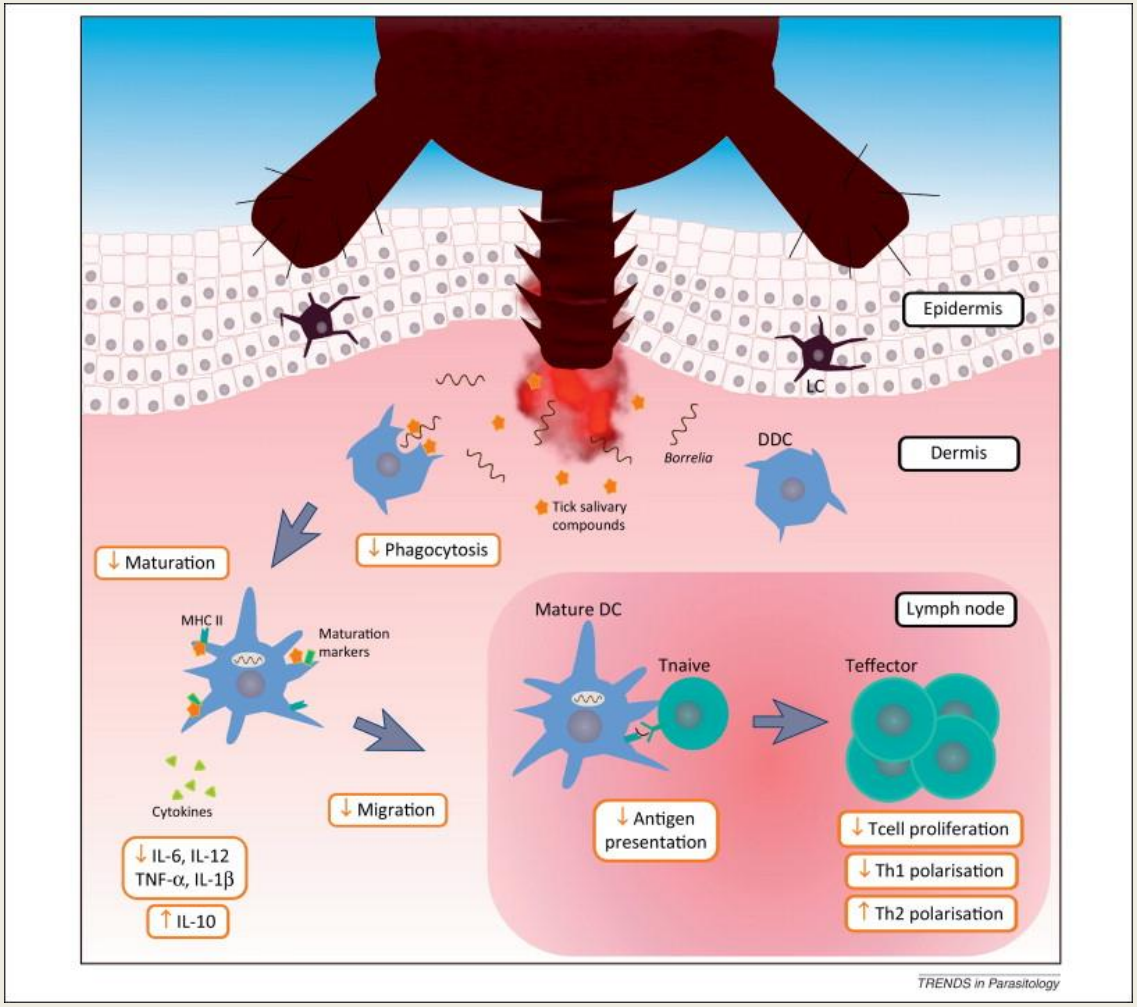
Dysbiosis testing- MSA test

Prevotella: strong hydrogen sulfide (H_2S) producers. In excess, H_2S acts as a mitochondrial poison and a potent neurotoxin. It can directly inhibit enzymes involved in the cellular production of energy. H_2S also interferes with oxygen transport by blocking hemoglobin in the red blood cells. Finally, H_2S is lowering gut pH preventing the growth of many beneficial bacteria (like Bifido).

PHYLUM	FAMILY	GENUS	Jan 2018 % of total	Oct 2018 % of total	Jan 2019 % of total	Apr 2019 % of total
		Streptococcus	18.35	4.75	1.12	0.93
	Leuconostoc	Leuconostoc	0.19	0.01	0	0.06
Bacteroidetes (gram-)	Bacteroidaceae	Bacteroides	4.42	8.53	7.3	4.41
	Rikenellaceae	Alistipes	0.11	0.65	1.19	0.69
	Porphyromonadaceae	Barnesiella	0.11	0.95	1.3	0.67
		Odoribacter	0.01	0.15	0.13	0.06
		Parabacteroides	0.23	1.77	0.66	0.36
	Prevotellaceae	Prevotella	42.02	26.07	15.61	8.96
		Xylanibacter	0.03	0	0	0.01
	Bifidobacteriaceae	Bifidobacterium	0	0.01	0	0

PHYLUM	FAMILY	GENUS	Jan 2018 % of total	Oct 2018 % of total	Jan 2019 % of total	Apr 2019 % of total
Firmicutes (gram +)	Lachnospiraceae	Anaerostipes	0.13	0.01	0.01	0.06
		Coprococcus	1.72	1.97	4.06	2.77
		Dorea	4.55	3.92	4.24	4.33
		Moryella	0.03	0	0.01	0.01
		Roseburia	3.31	3.7	3.81	15.64
		Sporobacterium	0	0	0	0
		Syntrophococcus	0	0	0	0
	Ruminococcaceae	Acetanaerobacterium	0	0	0	0
		Acetivibrio	0	0	0	0
		Ethanoligenens	0	0	0	0
		Faecalibacterium	9.08	18.55	22.95	13.27
		Papillibacter	0	0	0	0
		Ruminococcus	0.01	0.24	0.52	0.92
		Sporobacter	0	0	0	0
		Subdoligranulum	0.02	0.08	2.65	0.06
	Clostridiaceae	Butyricicoccus	2.67	2.59	5.71	7.04
		Clostridium Sensu Stric.	3.78	0.04	0.05	0
		Lactonifactor	0	0.02	0.01	0.03
	Eubacteriaceae	Anaerofustis	0	0	0	0
		Eubacterium	0	0.03	0	0.04
	Blautia	Blautia	3.68	17.15	20	27.13
	Howardella	Howardella	0	0	0	0
	Lactobacillaceae	Lactobacillus	0.42	0.01	0.08	0
	Enterococcaceae	Enterococcus	0	0	0	0
	Streptococcaceae	Lactococcus	0.03	0.02	0.01	0.29
		Streptococcus	18.35	4.75	1.12	0.93
	Leuconostoc	Leuconostoc	0.19	0.01	0	0.06
	Erysipelotrichaceae	Catenibacterium	0	0	0	0
		Coprobacillus	0.23	0.05	0.07	0.06
		Holdemania	0.01	0.01	0.02	0.01
		Turicibacter	0	0	0	0
	Veillonellaceae	Dialister	0	0	0	0.01
		Megamonas	0	0	0	0
		Megasphera	0	0	0	0
	Oscillospiraceae	Oscillibacter	0.17	1.05	2.47	2.14
	Staphylococcus	Staphylococcus	0.01	0.05	0	0.03
Bacteroidetes (gram-)	Bacteroidaceae	Bacteroides	4.42	8.53	7.3	4.41
	Rikenellaceae	Alistipes	0.11	0.65	1.19	0.69
	Porphyromonadaceae	Barnesiella	0.11	0.95	1.3	0.67
		Odoribacter	0.01	0.15	0.13	0.06
		Parabacteroides	0.23	1.77	0.66	0.36
	Prevotellaceae	Prevotella	42.02	26.07	15.61	8.96
		Xylanibacter	0.03	0	0	0.01
Actinobacteria (gram+)	Bifidobacteriaceae	Bifidobacterium	0	0.01	0	0
	Actinomycineae	Actinomyces	0.62	1.29	0.37	0.79
	Micrococcineae	Rothia	1.37	1.92	0.27	0.13
	Coriobacterineae	Asaccharobacter	0	0	0	0
		Collinsella	0.35	0.14	0.1	0.06
		Olsenella	0	0	0	0
		Slackia	0.15	0.16	0.39	1.04
Proteobacteria (gram-)	Enterobacteriaceae	Escherichia/Shigella	0.02	0	0	0
		Klebsiella	0	0	0	0
	Sutterellaceae	Sutterella	0	0	0	0
	Desulfovibrionaceae	Lawsonia	0	0	0	0

Integrative approach for TBI
Part 3 - Immune Dysfunctions in TBD



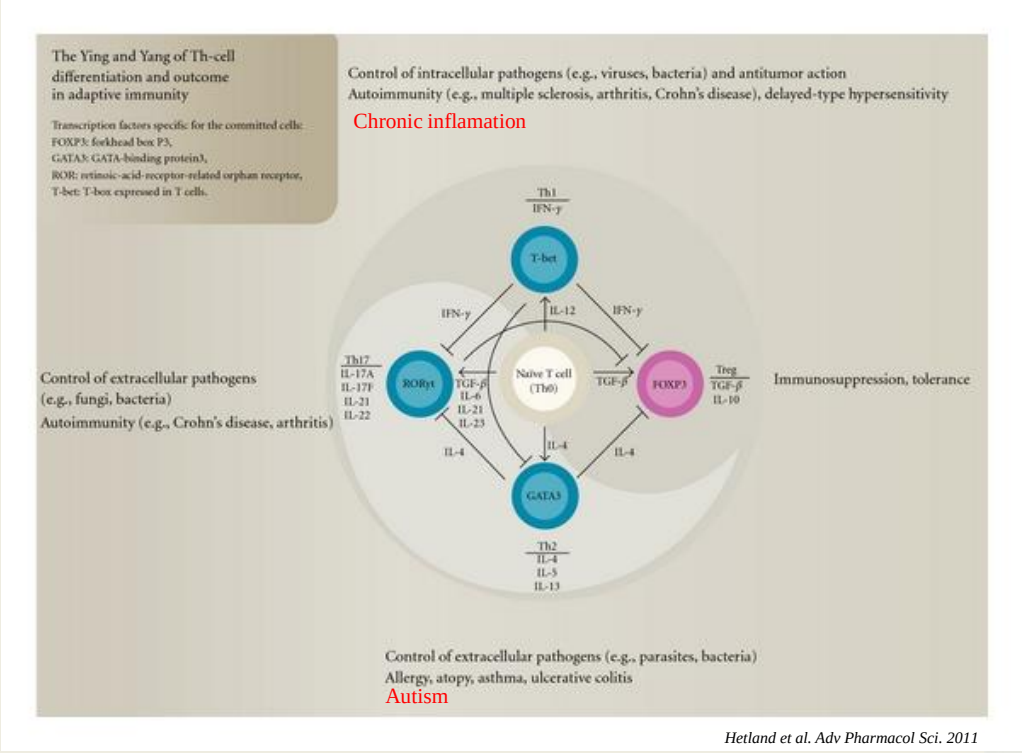
Mason et al., Trends in Parasitology 2013;
DOI:https://doi.org/10.1016/j.pt.2013.12.003

Immune Dysfunction in TBD

- A hallmark of chronic Lyme disease is an underlying immune dysfunction that not only limits the ability to accurately diagnose Lyme, leaving many such patients misdiagnosed, but also serves as a major reason for lack of treatment response to antibiotics with an inability to eradicate the chronic infection.
- The Borrelia bacteria (cause of Lyme disease) transforms from an acute to a chronic infection by transforming the body to a TH2 "extracellular" dominant response and then converting from a free swimming spirochete form in the blood into an intracellular form (L-form) to escape the elevated TH2 immunity. The suppressed and down-regulated TH1 intracellular immune response becomes an ineffective immune response by the body and an effective evasion strategy, which is the hallmark of transformation to late-stage Lyme dissemination.
- Appropriate immune modulating therapies that are able to restore normal functioning immunity may be the biggest necessary leap forward in the development of an effective treatment protocol for this multi-system illness.

Urine-based Th1/Th2 balance test

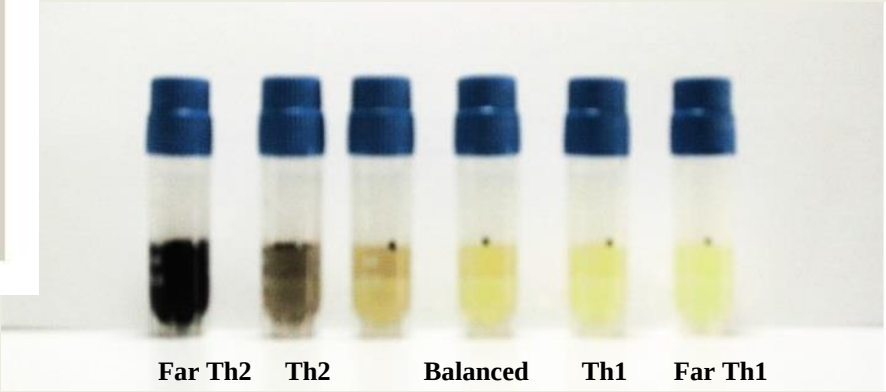
Th1/Th2 balance



Hetland et al. Adv Pharmacol Sci. 2011

urine-based Th1/Th2 balance test

- may detect disturbances of this delicate equilibrium in time in order to restore balance whenever required and before irreversible conditions are developing
- allows patients to follow-up on Th1/Th2 balance during therapy (anti-oxydants, probiotics, nutraceuticals).



Testing for immune and metabolic dysfunctions

- **The extent of the global immune and / or metabolic dysfunctions could be evaluated by testing:**
  - cytokine expression
  - elastase and perforin mRNA expression
  - oxidative stress, heavy metals, molds
  - macrophage phagocytic activity
  - sCD14 expression
  - C3a & C4a expression
  - CD57 cell subset absolute count
  - prostaglandin E2 (PGE2) synthesis
  - VEGF synthesis
  - ammonia accumulation
  - kynurenic and quinolinic acid accumulation, and many more

Testing for Immune dysfunctions

- **The extent of the global immune dysfunction is evaluated by testing:**

  (1) ***cytokine expression***

  - Testing for Th1/Th2 balance.
  - Testing for pro-inflammatory cytokines

Testing for Immune dysfunctions

- **The extent of the global immune dysfunction is evaluated by testing:**

(2) ***elastase mRNA expression : a marker of inflammation***

- Elastase is an inflammatory protease expressed in immune cells (monocytes, neutrophils) that contributes to immune defense by inactivating foreign bacteria but at the same time it causes damage to connective tissue, breaks down cytokines, immunoglobulins and immune cells receptors. An excess, chronic production of elastase is therefore detrimental.

(3) ***perforin mRNA expression : a mean to evaluate NK cell activation***

- Since NK cells play a central role in the defense against bacteria and viruses, decreased NK activity can lead to the development of opportunistic infections. NK cells exert their cytotoxic effect by releasing perforin, a protein that will destroy the cytoplasmic membrane of target cells and finally kill them.

Testing for Immune dysfunctions

- **The extent of the global immune dysfunction is evaluated by testing:**

(4) ***CD57 cell subset absolute count***

- CD57+/CD3- cells are a subset of NK cells. Their exact function, and what differentiates them from CD56+ NK cells, is not well understood. The absolute number of CD57+/CD3- cells is low in patients suffering from chronic Lyme disease (a disease that follows an infection by a bacteria called Borrelia). Patients with very low CD57 have significantly more co-infections and persistent immunologic defects than patients with higher counts. In patients that respond to antibiotic therapy, the number of cells come back to normal, hence this is a useful marker to follow the effect of a therapy.

(5) ***sCD14 expression***

- CD14 is expressed in monocytes/macrophages and plays a critical role in the recognition of bacterial cell wall components (LPS). The extracellular part of CD14 can be cleaved and released in the plasma, where it will inactivate circulating LPS. Serum soluble CD14 levels are significantly elevated in patients with inflammatory bowel disease, Crohn's disease, but also in patients suffering from Brucellosis or Lyme disease.

Testing for Immune dysfunctions

- **The extent of the global immune dysfunction is evaluated by testing:**

  (6) ***C4A expression***

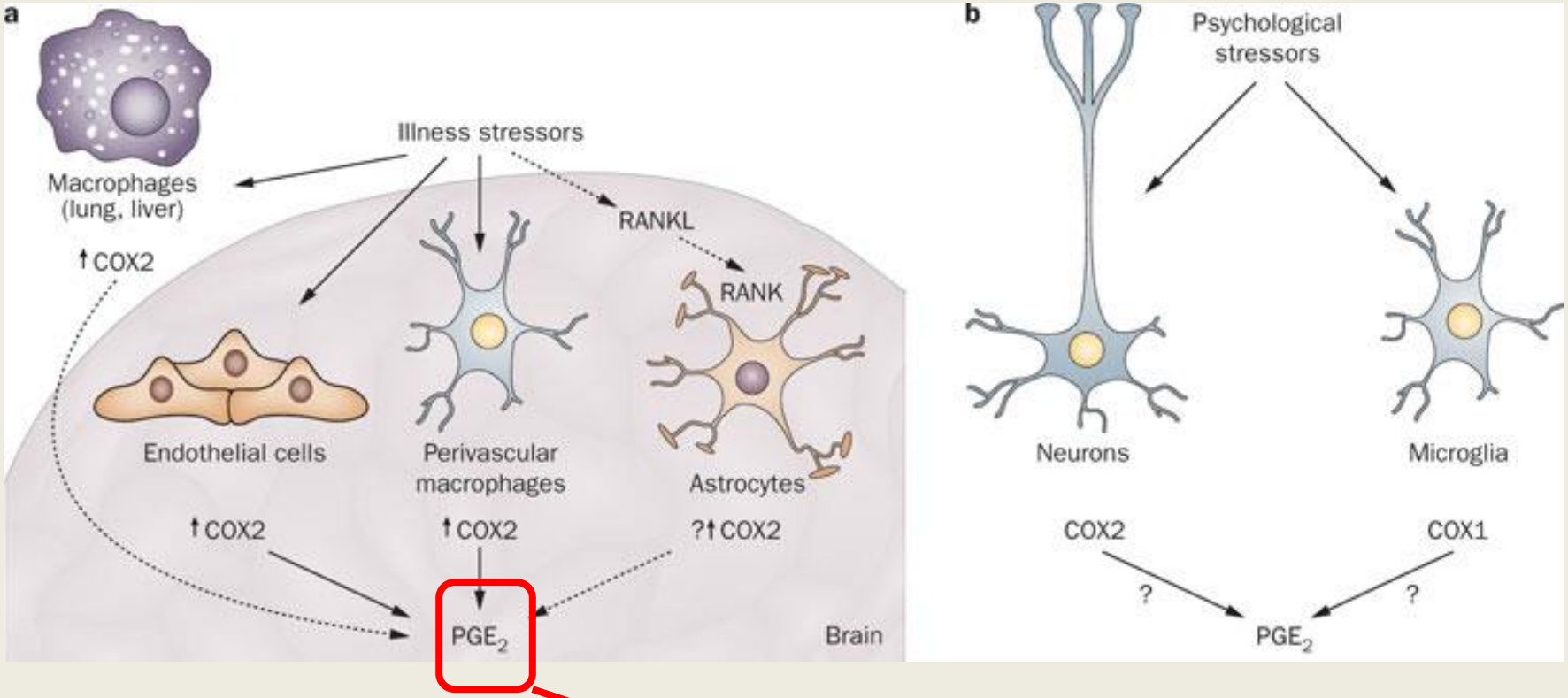
  - C4a is an anaphylatoxin generated by cleavage of complement component 4 (C4), upon activation of the complement system. C4a increase causes local inflammatory response and symptoms of hypersensitivity. US study has reported that elevated complement C4a was an early marker for Lyme disease in tick bite patients. But C4A levels are decreased in chronic/late TBD patients

  (7) ***prostaglandin E2 (PGE2) synthesis***

  (8) ***VEGF synthesis***

  (9) ***CD38 quantification***

PGE_2 production during illness and psychological stress



quantification

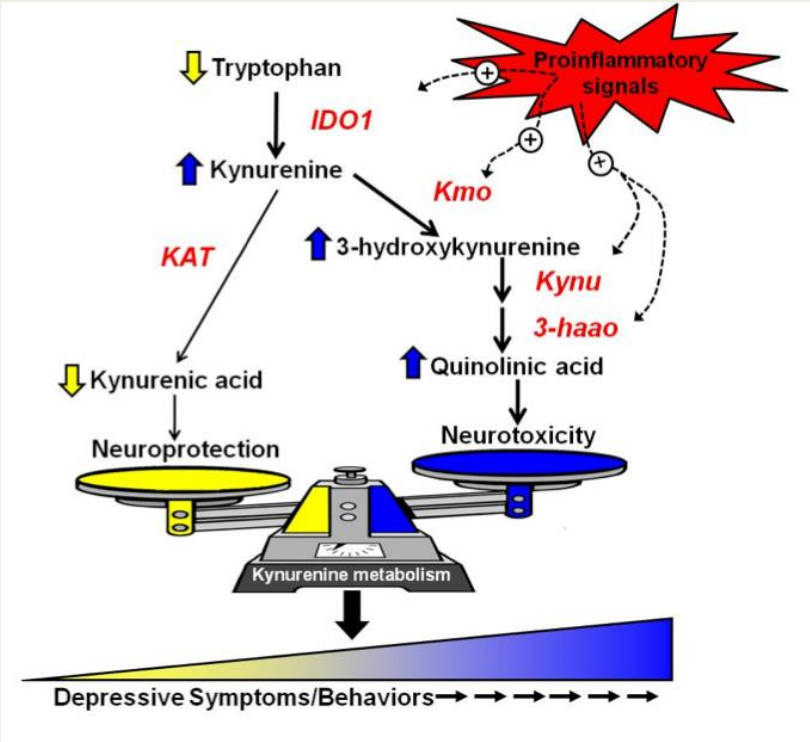
Furuyashiki, T. & Narumiya, S. (2010) Stress responses: the contribution of prostaglandin E_2 and its receptors *Nat. Rev. Endocrinol.* doi:10.1038/nrendo.2010.194

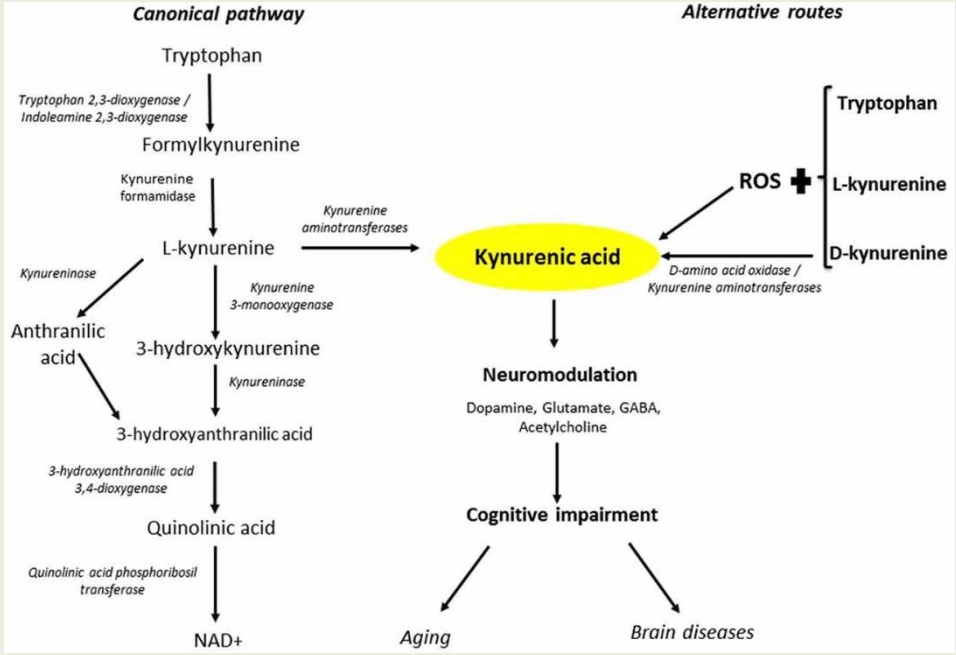
Prostaglandine E2

prostaglandin E2 (PGE2) synthesis

- PGE2 is a compound derived from membrane phospholipids
- PGE2 is also a key mediator of immunopathology in chronic infections and cancer
- PGE2 enhances its own production but suppresses acute inflammatory mediators, resulting in its predominance at late/chronic stages of immunity.
- PGE2 selectively suppresses effector functions of macrophages and neutrophils and the Th1-, CTL-, and NK cell-mediated type 1 immunity, but it promotes Th2, Th17, and regulatory T cell responses.
- PGE2 modulates chemokine production, inhibiting the attraction of proinflammatory cells while enhancing local accumulation of regulatory T cells and myeloid-derived suppressor cells.

Kynurenic and Quinolinic acids





- High levels of kynurenic acid have been identified in patients suffering from tick-borne encephalitis, schizophrenia and HIV-related illnesses. In all these situations increased levels were associated with confusion and psychotic symptoms.
- QUINO acts as a neurotoxin, gliotoxin, proinflammatory mediator, prooxidant molecule and can alter the integrity and cohesion of the blood–brain barrier. Quinolinic acid levels are increased in the brains of children infected with a range of bacterial infections of the central nervous system (CNS), in patients with poliovirus, Lyme disease with CNS involvement, traumatic CNS injury, hyperammonaemia, hypoglycaemia patients, systemic lupus erythematosus, malaria, etc.

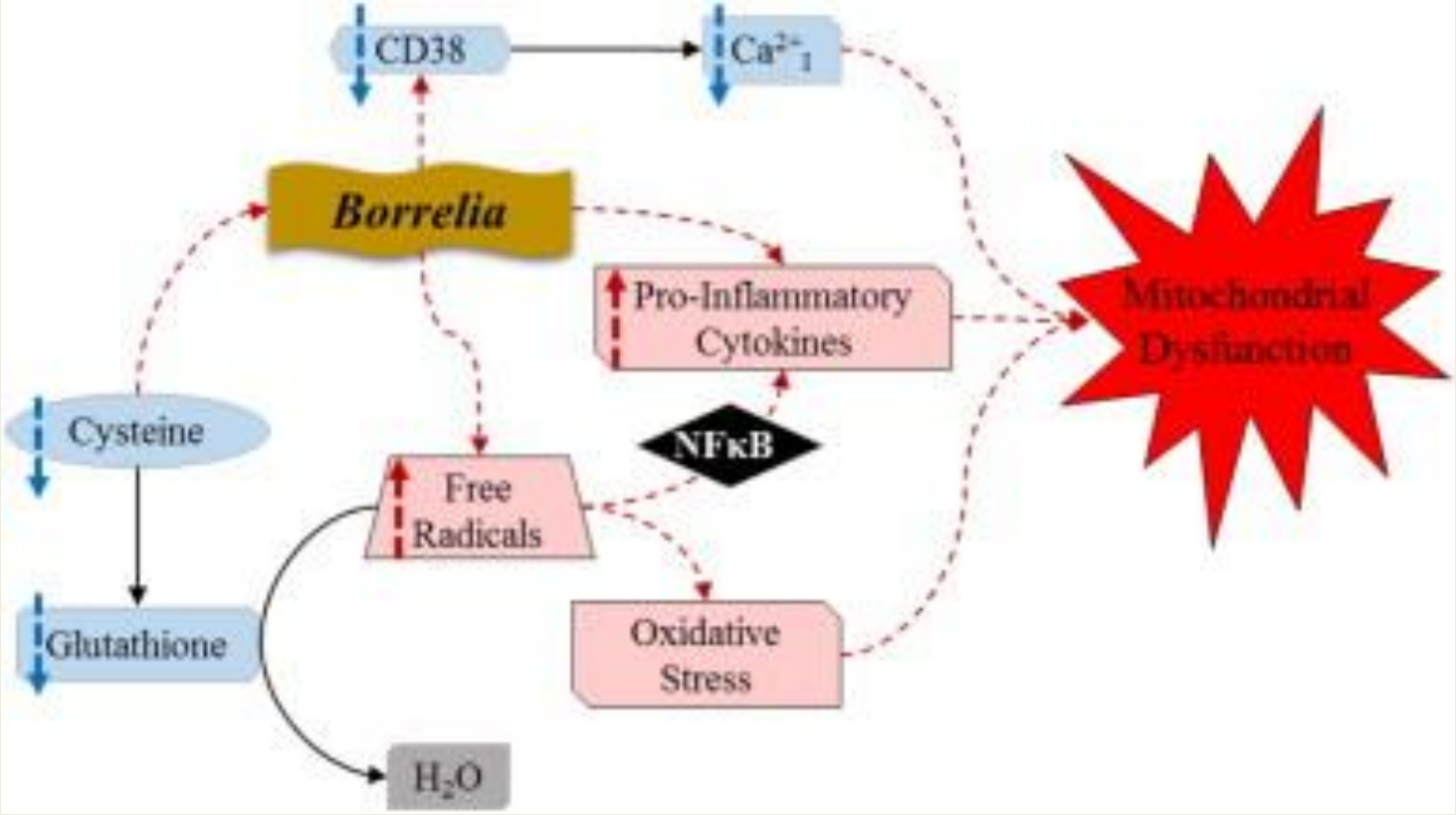
VEGF

- VEGF has a great role in pathological conditions that are associated to autoimmune diseases such as in systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.
- Serum levels of VEGF correlate with disease activity in a large number of autoimmune diseases and fall with the use of standard therapy
- Possible future therapeutic strategies in autoimmune diseases with the anti-VEGF or anti-VEGFR (receptor). So far, this therapy has been used in cancer and macular ocular degeneration in diabetes.
- Abnormally high levels of VEGF in a mold-free environment would suggest Bartonella infection.
- VEGF can go *down* in the presence of indoor molds.

CD38 in TBD

- **CD38 in TBD *(Hartiala et al. 2007, 2010)***
  - **CD38, which has an important role in dendritic cells (DC) chemotaxis and migration to lymph nodes, was strongly up-regulated by LPS of Gram - bacterias but practically not at all by Borrelia garinii (mostly inducing neuroborreliosis).**
  - **Borrelia garinii may affect crucial DC functions by blocking the up-regulation of important molecules in DC migration to lymph nodes, thus affecting further immune responses in Lyme borreliosis infection (Hartiala et al. 2007, 2010).**
  - **B. burgdorferi sensu stricto and B. afzelii are also unable to induce CD38 upregulation.**
- **Thus low levels of CD38 might indicate Borrelia infection, while high levels of CD38 might indicate other Gram- infections and/or leaky gut**

CD38 in Lyme

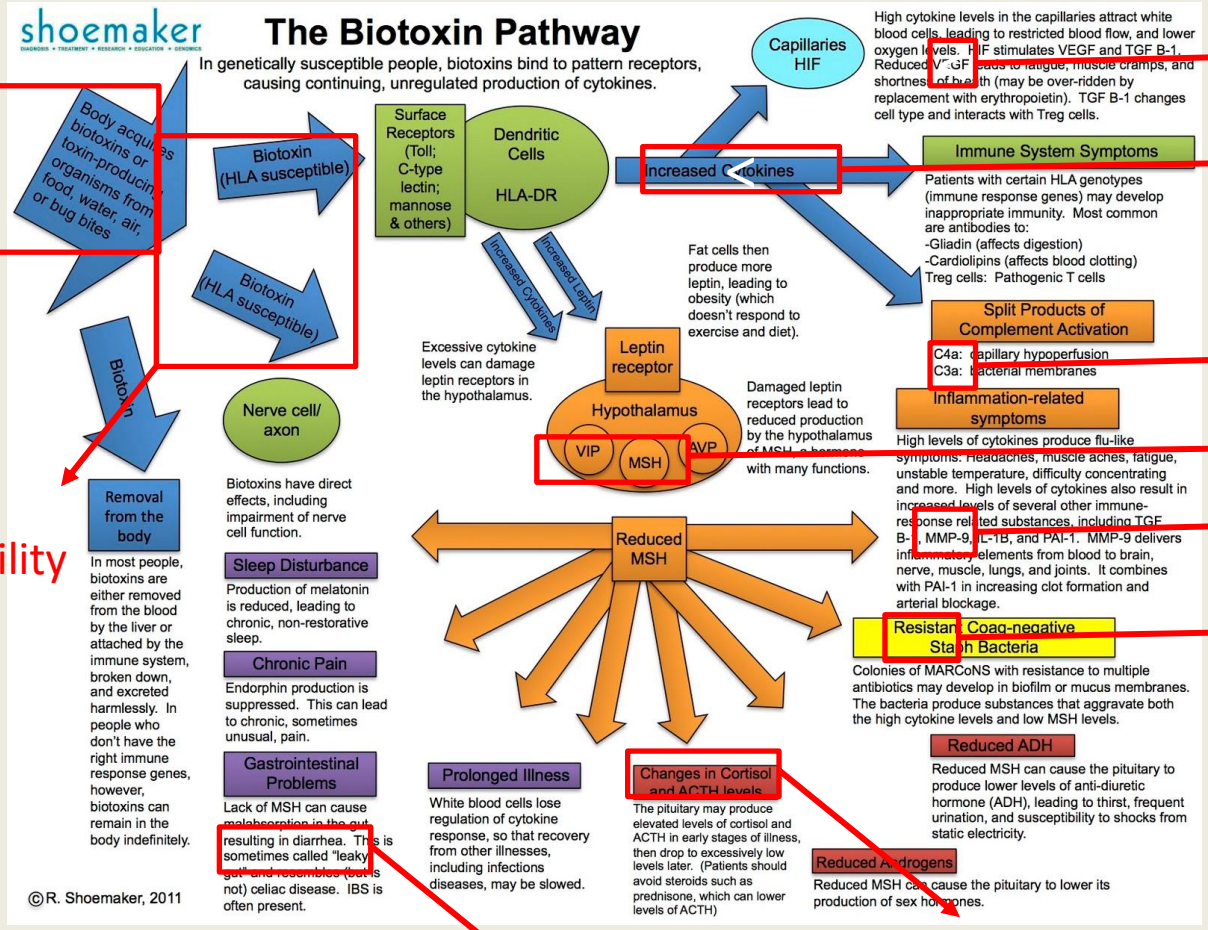


From Peacock et al. Redox Biology 2015

Focus on CIRS

- CIRS = Chronic Inflammatory Response Syndrome
- Dr. Ritchie Shoemaker: Pioneer in CIRS, Mold & Biotoxins
  - ➔ Body acquires biotoxins or toxin-producing organisms from food, water, air or bug bites
  - ➔ Lyme disease is a key trigger for CIRS
  - ➔ Biotoxins cause continuing, unregulated production of cytokines
  - ➔ Multiple damages to the body with inflammation-related symptoms, immune system symptoms, resistant bacteria, chronic pain, sleep disturbance, gastrointestinal problems,….
  - ➔ Investigation of several markers from Shoemaker biotoxin pathway

CIRS-related Testing



Biotoxins

Genetic susceptibility

VEGF

Cytokines

C3A & C4A

VIP & a-MSH

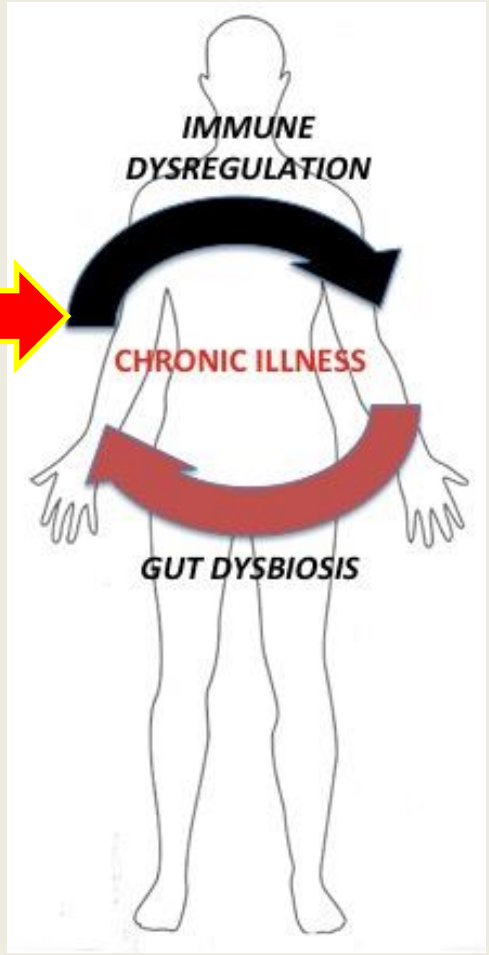
MMP-9

MARCoNS

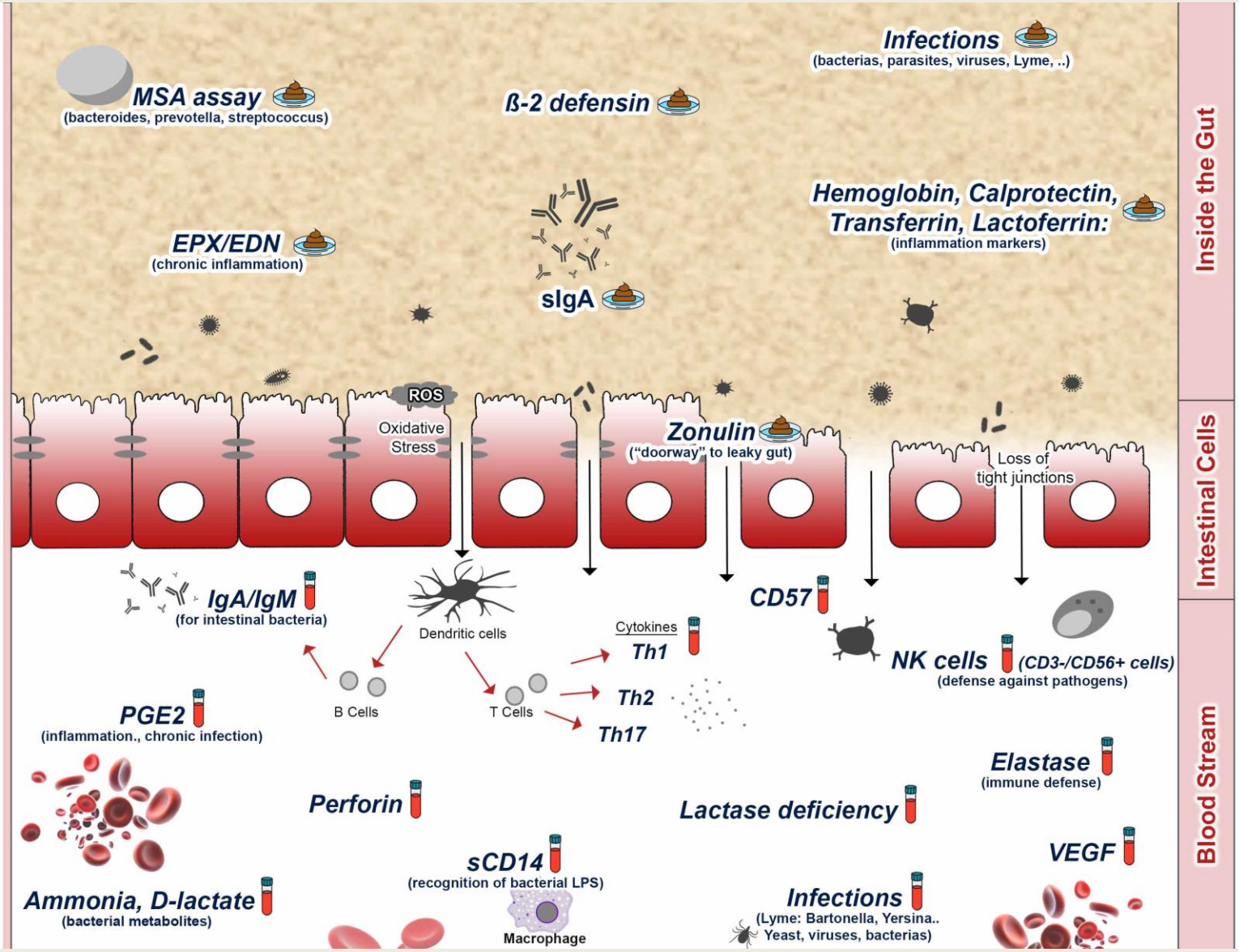
Altered homone production

Gastrointestinal problems

Vicious Circle



Consequences of TBI



Remember

- It is important to bear in mind that TBD can be acute or late stage / persistent / chronic; this is important given the diagnostic and treatment approaches might be different in these two situations.
- **Lyme disease exhibits a variety of symptoms that may be confused with immune and inflammatory disorders.**
- If an individual has any chronic health condition, ranging from arthritis to chronic fatigue syndrome to fibromyalgia, it is important to rule out or diagnose Lyme disease. It is apparent that many cases of fibromyalgia and chronic fatigue syndrome are actually Lyme disease in disguise
- **In order to offer better management of patients with late/chronic and/or persistent infections that are very difficult to uncover, it is important to focus on an integrative approach, inclusive of direct pathogen detection as well as indirect supportive tests focusing on immune and gastrointestinal disorders.**
- **Chronic patients have complex clinical picture with multiple afflictions needing thus multiple testing and careful interpretation of testing results.**

CONCLUSIONS : Integrative approach for tick-borne diseases testing

- In order to offer better management of patients with chronic and/or persistent infections that are very difficult to uncover, **focus both on direct pathogen detection as well as on indirect supportive tests (assessing immune and gastrointestinal disorders).**
- **Direct tests** for Persistent and/or chronic infections
  - PHELIX PHAGE BORRELIA TEST
  - Immunoblot for Borrelias, Chlamydias (pneumoniae, trachomatis, psittacii), Yersinia, EBV, Parvovirus, Treponema
  - Serology tests for CMV, Toxoplasmosis, Tularemia, Leptospira, BrucellaCapt
  - FISH test for Babesia
  - PCRs for Mycoplasma spp, Mycoplasma fermentans, Mycoplasma pneumoniae, Bartonella, Brucella, Coxiella, Babesia, Anaplasma, Ehrlichia, Chlamydias, Rickettsias, Midichloria mitochondrii, etc
  - PCRs for viral infections: herpesviruses like HHV-6, HHV-7, HHV-8, Parvovirus, EBV, TBE, etc
  - Testing for parasites, molds, etc
  - New tests
- **Indirect tests** for Persistent and/or chronic infections
  - CD57 cell subset absolute count
  - **CD38 quantification**
  - cytokines' expression, Th1/Th2 balance
  - sCD14 expression
  - prostaglandin E2 (PGE2) synthesis, VEGF, C4A and C3A quantification; KYNA & QUINO
  - Perforin and Elastase
  - Metal poisoning evaluation
  - **Special focus on gastrointestinal disorders** (dysbiosis, leaky gut, intestinal infections and inflammation, etc)

Questions and Contacts

- **Material available on the website (www.redlabs.com)**
- Check regularly our website (**www.redlabs.com**) for the updates
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