The Role of Pro-inflammatory Cytokines IL-6, IL-17, IL-23 and TNF-α in Maintaining Symptoms of Chronic Lyme Disease and Nutritional Interventions

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Abstract

Objectives:Lyme disease infected by the bacterium *Borrelia burgdorferi* can cause various symptoms such as pain, fatigue and cognitive problems. In Chronic Lyme Disease patients' symptoms such as pain and fatigue turned out to be chronic and persistent even after medical treatment. This mechanism review studies the role of pro-inflammatory cytokines IL-6, IL-17, IL-23 and TNF-α in chronic Lyme disease (CLD) and the nutritional interventions to down-regulate pro-inflammatory cytokine production.

Methods: Computerised databases were systematically searched for relevant studies with the intention of creating a mechanism diagram of the pro-inflammatory cytokines' role in chronic Lyme disease. Nutrients and herbs were searched based on mechanism diagrams. Searches on chronic Lyme Disease resulted in 331 studies, of which 54 review or primary research papers were accepted. Nutrient searches resulted in 608 research papers, of which 43 review or primary papers were accepted for the nutritional intervention part of the study.

Results: This mechanism review establishes that the cytokines may be an important factor in persistent symptoms in chronic Lyme disease. Increased pro-inflammatory cytokine levels were found *in vitro, in vivo* and human studies reviewed. Twelve cohort studies of chronic Lyme disease patients were found to show significantly elevated levels of IL-6 and IL-17 cytokines. For preventing pro-inflammatory cytokine production three nutrients and five herbs were studied. Quercetin, green tea, sulforaphane, resveratrol, curcumin, boswellia, andrographis paniculata and cats claw have a down-regulating effect to cytokines IL-6, IL-17, IL-23 and TNF-α according reviewed in vitro, in vivo and human studies.

Conclusion: The role of the cytokines and prolonged inflammation needs more studies but can be concluded to be a factor in constant symptoms of Chronic Lyme Disease. Reviewed nutrients and herbs can down-regulate the pro-inflammatory cytokine production and reduce the inflammation. Further research is needed to understand the mechanism of the persistent symptoms after Lyme Disease treatment and for nutritional interventions to Chronic Lyme Disease.

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Preface

Lyme disease is growing disease. It is highly endemic in Europe and North America. Lyme disease is caused by a spirochete bacterium *Borrelia burgdorferi* and the primary vector to transfer the spirochete bacteria in the human is a Ixodes species ticks. Early state treatment is antibiotic therapy, but 10-20% of the patients continue to experience several symptoms after treatment (Marques 2008). There is controversy regarding the post or chronic Lyme disease treatment and diagnosing methods. This is a genuine problem with patients that have ongoing chronic symptoms such as fatigue and pain. The mechanism of chronic Lyme disease is complex and the pathophysiology is not known. There is a demand for optional treatment methods for chronic Lyme disease.

This mechanism review studies the role of pro-inflammatory cytokines IL-6, IL-17, IL-23 ,TNF-α in Chronic Lyme Disease and the nutritional interventions to down-regulate cytokine production.

This thesis is a result of a study conducted as part of my Master of Science degree course in the Centre for Nutrition Education and Lifestyle Management (CNELM) in collaboration with Middlesex University.

1. Introduction

1.1 Overview

This dissertation is a mechanism review focusing on the role of the pro-inflammatory cytokines in Chronic Lyme Disease (CLD) and studying possible nutritional interventions to reduce inflammation in CLD. The aim of this thesis is to attempt to understand the mechanism of pro-inflammatory cytokines in CLD. The cytokine reactions seem to have an important role in managing the ongoing inflammation. The intention is to seek evidence based studies of the nutritional agents that can down-regulate pro-inflammatory cytokines in humans.

Lyme disease has been known since 1975, when it was found in Lyme city in the USA (Borchers 2014). Lyme disease is highly endemic in much of Europe, with highest incidence reported in southern Sweden, Lithuania, Germany, Austria and Slovenia (Borchers 2014). A rise in the incidences of Lyme disease similar to that observed in the USA has been reported from some European regions. The number of cases has been growing rapidly from 8000 in 1991 to 30 000 in 2009 in the USA (Nichols 2013). A French research stated in their survey from 2009-2012 the average incidence rate of Lyme Borreliosis to be 42 per 100 000 national population in France (Vandenesch 2014). Untreated Lyme disease can cause serious adverse symptoms affecting skin, nervous system, joints, energy production and heart (Zimering 2014). In general, agreement exists concerning the presentation and treatment of early localized disease. On the other hand, there is big controversy concerning the diagnosis of Chronic Lyme Disease (Nichols 2013). There is currently no clear evidence of that post or Chronic Lyme Disease syndrome is due to persistent infection with *Borrelia burgdorferi* (Marques 2008). Still 3-27% of the patients suffer residual subjective symptoms such a fatigue, arthralgias, headache, myalgias after antibiotic treatment (Borchers 2014). The complexity of Lyme disease treatment and the problems that CLD may cause is forced one to look at the mechanisms of the disease in a

more holistic way. Personalised nutrition as well as complementary and alternative medicine could offer holistic interventions and treatments to patients with CLD.

1.2 Lyme Disease.

1.2.1 Infection

Lyme disease is caused by a spirochete bacterium called *Borrelia*. The shape of the bacteria is irregularly coiled and it is highly motile. There are several Borrelia species to cause Lyme disease such as *Borrelia burgdorferi, B. sensu lato*, *B. afzelii* and *B.garinii*. The primary vector to transfer the spirochete bacteria in the human is a Ixodes species ticks (Borchers 2014). The infection is non-fatal, non-communicable from person to person, and responsive to antibiotics (Auwewaerter 2011). The most common clinical manifestation is erythema migrans that occurs at the site of the tick bite. In few weeks after the tick bite some of the untreated patients might develop also nervous system abnormalities, cardiac symptoms, joint pain and within months arthritis (Borchers 2014). There are also several other clinical manifestations of symptoms that are usual, like fatigue, arthralgia, myalgia, headache and stiff neck (Auwewaerter 2011).

1.2.2 Chronic Lyme Disease

Chronic Lyme disease has a vast range of different symptoms in different patient populations. Common persistent or relapsing symptoms are fatique, muscoskeletal pain, joint pain, and neuropathic pain (Zimering 2014). The mechanisms underlying the post/chronic Lyme disease symptoms are not known. A possible explanation is persistent infections with *B. burgdorferi.* (Marques 2008). Neuroborreliosis patients have lymphocytic meningitis, cranial neuritis and painful radiculoneuritis or other declined cognition symptoms or one or two of these symptoms (Borgermans 2014). A Lyme Arthiritis symptom is pain and swelling in the large joints (Borchers 2014).

1.3. Guidelines for Treatment

ILADS (The International Lyme and Associated Disease Society) is an international multidisciplinary medical society whose mission is to improve physicians' understanding of Lyme disease (ILADS 2014). IDSA is another society that creates guidelines for infectious disease treatments (IDSA 2015). These two associations have a different opinions of the treatment method and existence of post /Chronic Lyme disease.

1.4. Cytokines

Cytokines are a class of small proteins that act as signaling molecules at the picomolar or nanomolar concentrations to regulate inflammation. They also modulate the cell activities like growth, survival and differentiation (Ramesh 2013). Cytokines can be divided to pro- or anti-inflammatory groups. The structure and the function of their receptors specifies it. Activated immune cells produce cytokines and they act as molecular signals between immune cells (Schmitz 2008). IL-1, IL-6 and TNF-α are the inducers of acute phase response of Borrelia *burgdorferi* (Ramesh 2013). Inflammation cytokines IL-6, TNF-α, IL-1β, IL-23 and IL-17 may have an important role in persistent chronic Lyme disease.

2. Methodology

The dissertation is based on a literature search to gain an understanding of the mechanisms of the pro-inflammatory cytokine role in the chronic Lyme disease and to find nutritional interventions to down regulate inflammation. This section describes the strategy used to search, evaluate, select and study the research articles. The purpose is to make sure that all possible available relevant papers are investigated. The studies selected are used to create the mechanism diagrams presented in the result section. Primary studies used are *in vitro, in vivo* and human studies, related to Lyme disease and pro-inflammatory cytokines and nutritional interventions. The primary databases used in this study are Pubmed and ScienceDirect databases. Mendeley, reference manager program was used for referencing purpose of articles.

2.1. Quality of Researches

The quality of the researches was established by using CNELM requirements for mechanism review guidelines:

- CNELM mechanism overview was used for all primary researches included in this project.
- Sign 50 guidelines for all human studies randomised, non randomised, cohort and case control studies and systematic reviews
- Arrive quideline for all animal studies used in this project.

2.2. Limitations of search

The research included review papers, in vitro, in vivo, animal and human studies. The aim was to use more of the human papers and especially randomised controlled placebo controlled trial studies. It became evident that there is a limited amount of human studies concerning the chronic Lyme disease, cytokines and inflammation. The human studies that were found in these searches were found to be cohort studies, not randomised controlled trials. Only few studies researching nutritional aspect of the chronic Lyme disease or Borrelia *burgforfer*i were found. Because of that more searches were made to find a nutritional intervention concerning down regulation of cytokines. In the nutritional interventions section studies address also with other diseases like arthritis, diabetes and inflammation in general. Some nutrients studied in this project generated a large number of search results, and this may have caused difficulty in picking the most appropriate studies for this project concerning Lyme disease.

The in vitro and in vivo reviews and some of the primary studies are limited to understanding the outcomes of the different studies. The critical analysis and biochemical methods and analyses used in these studies are beyond the scope of this study.

3. Results

3.1 In vitro

3.1.1 *Borrelia burgdorferi* Induces Pro-inflammatory Cytokine Production in Central Nervous System

Lyme spirochete actively invade the cerebral spinal fluid (CSF), brain and meninges after infection. Generally it is accepted that the spirochetes are established in the central nervous system (CNS) within 18 days (Fallon 2010).

Table 1. Reviewed in vitro studies showed increased cytokine production in gliad, monocyte or olikodendrocyte cells after *B.burgdorferi* introduction.

Author	Results	Media	Additional data
Myers (2009)	$p < 0.05$ IL-6, IL-8, TNF-α	macaques' glia cells	Microglial cells co-cultured with B.b. induced the pro-inflammatory cytokine IL-6, IL-8, TNFα significantly
"	$p = 0.028$	macaques' glia cells	Neuronal cell apoptosis co-cultured SY cells + microglia + physical contact during co-culture with B.b
Ramesh (2008)	visualized IL-6	macaques astrocytes and glia cells	IL-6 cytokines were visualized in glial cells in situ and cytokines in tissue after B.b. induction.
Bernardino (2009	$p < 0.05$ $p < 0.005$ IL-6	human THP-1 monocytes	Antibiotics attenuated release of IL-6 in microglia/ astrocyte response to B.b. in a dose dependent, significantly.
Ramesh (2012)	$p < 0.05$ IL-6	human olikodendrocytes	B.b. induced significantly IL-6 and IL-8 in olikodendrocytes.+ B.b. induced apoptosis in human oligodendrocyte
Ramesh (2013)	visualized IL-6	dorsal root ganglia cultures of adult rhesus macaques	IL-6 cytokines were localized in sensory neurons, satellite gliad cells and Schwann cells after B.b. induction.

3.1.2 *Borrelia burgdorferi* Induces Pro-inflammatory Cytokine Production

IL-17 cytokine is a regulator of immune responses and is involved in mediating and inducing pro-inflammatory reactions in a wide range of inflammatory diseases. IL-17 is associated with severe disease progression in several autoimmune disorders, such as rheumatoid arthritis (RA) or multiple sclerosis (MS). In patients diagnosed with RA, elevated levels of IL-17 were found in synovial fluid (Shabgah 2014). The Lyme arthritis clinical symptoms are similar to RA. It has been proposed that IL-17 may have an important role in development of LA.

Table 2. Reviewed in vitro studies are indicating *B.burgdorferi* impact on increased production of IL-17. In addition cytokines IL-6 and IL-23 are needed for the process

Author	Results	Media	Additional data
Bettelli (2006)	IL-6 , TGF-β ↑	T-cells of mice	The differentiation to Th17 cells needs cytokine combination of IL-6 and TGF-β
Colodo (2008)	$p < 0,05$ IL-17	Lyme arthritis patients mononuclear cells	NapA of *borrelia burgdorferi* stimulated mononuclear cells were found to produce IL-17 cytokines
Infante-Duarte (2000)	IL-17 ↑	murine CD4+ Th cells	Microbial stimuli *B.b*. was found to induce IL-17 production
Oosting (2011)	P=0,028	peripheral blood mononuclear cells	IL-23R signaling is needed for *B.b*. induced IL-17 production
Oosting (2012)	IL-17	spleen cells	IL-17 production in spleen cells of naïve mice was increased after *B.b.* stimulation

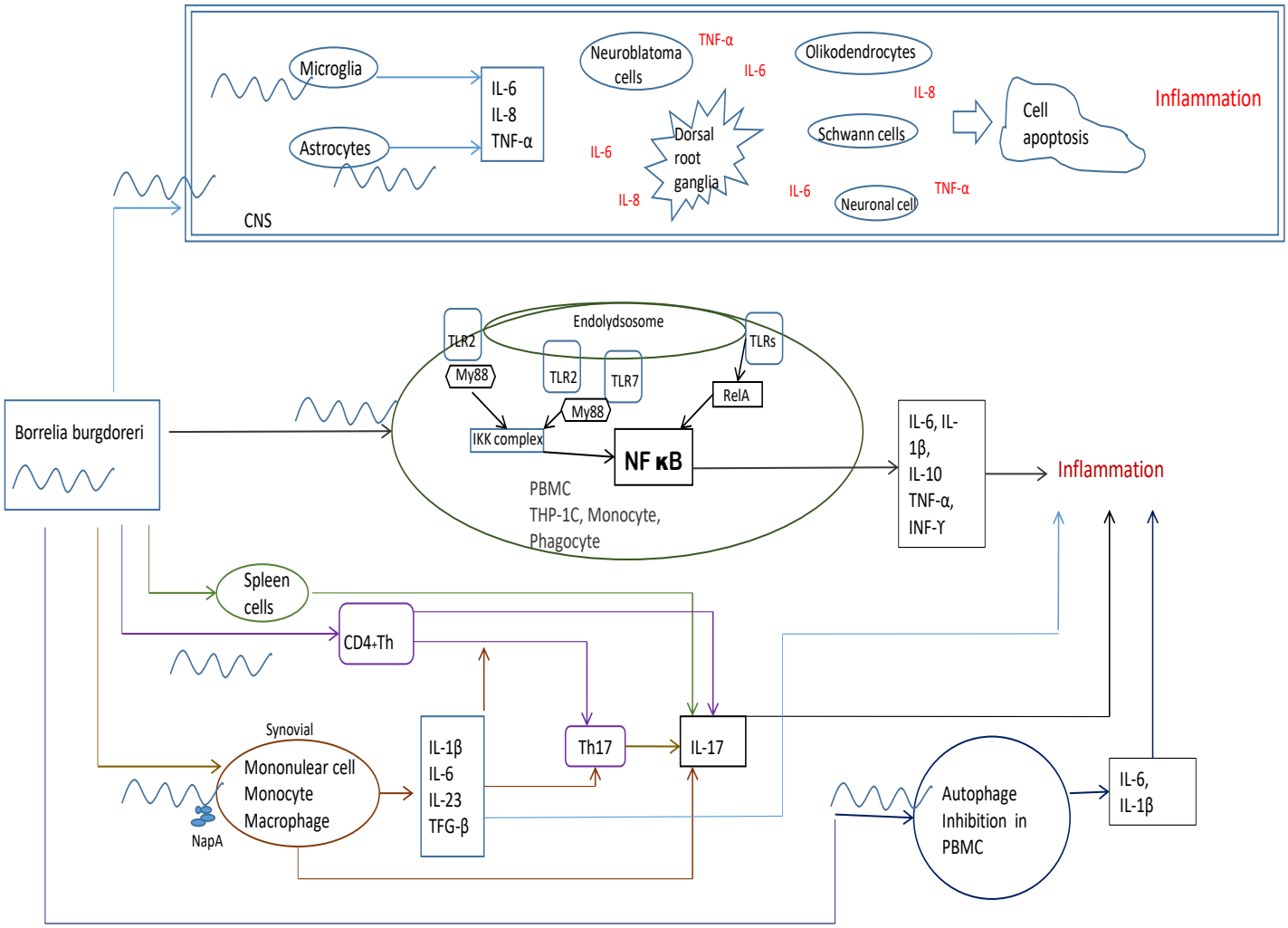
3.1.3 NF-κB pathway

NF-κB is a protein complex that controls the transcription of DNA. It is involved in cellular responses to stimulate cytokines production (Gilmore 1999). The activation of human monocytic cells by *B.burgdorferi* lipoproteins can induce the activation of the NF-κB according to Nordgard (1996) in vitro study.

Table 3. Reviewed in vitro studies *B.burgdorferi* were shown to induce the activation of the NF-κB pathway and cytokine production.

Author	**Results**	**Media**	
Nordgard (1996)	*Borrelia burgdorferi* induce NF-κB	human monocytic cells	Activation of human monocytic cells by Borrelia burgdorferi lipoproteins induce the activation of the NF-κ B
Olson (2006)	Cytokines ↑	phagocytic cells	*B.b.* is attributed to TLR-mediated signaling leads to production of cytokines NF-κB pathway
Sadik (2008)	p< 0.01 NF-κB activation	mTHP-1 cells	NF-κB activation in mTHP-1 cells when stimulated with *B.b*. is significant
Love (2014)	p< 0.001 in IL-1β,IL-6 and TNF-α	human peripheral mononuclear blood cells	Production of cytokines in PBM Cells is significant when stimulating with live *B.b*
Bernardio (2009)	p< 0.05 in IL-6, IL-8 and TNF-α	human THP-1 monocytes	The antibiotic treatment reduced significantly NF-κB binding activity after *B.b.* activation.

Diagram 1. Mechanism diagram based on above *in vitro* studies of this review. B.burgdoferi induces the cytokine production in microglia, astrocytes in central nervous system and inflammation environment causes cell apoptosis. *B.burgdorferi* induced pro-inflammatory cytokine production and inflammation via NF-κB pathway or via monocytes, macrophages, mono nuclear cells is presented in diagram. (Myers et al. 2009) (Ramesh et al. 2008) (Bernardino et al. 2012) (Ramesh et al. 2012) (Ramesh, Santana-Gould, et al. 2013)(Bettelli et al. 2006)(Codolo et al. 2008) (Infante-Duarte et al. 2000) (Oosting et al. 2012)(Oosting et al. 2011) (Norgard et al. 1996) (Olson et al. 2007) (Sadik et al. 2008) (Bernardino et al. 2012)(Buffen et al. 2013)



3.2 In vivo

3.2.1 *Borrelia burgdorferi* Induces Pro-inflammatory cytokine production in Central Nervous System

Table 4. Summary table of *in vivo* above studies.IL-6 cytokine levels were elevated in both animal studies.

Author	Results	Media	Additional data
Ramesh (2009)	visualized IL-6	euthanized rhesus macaques	IL-6 elevation in astrocytes and neurons and the dorsal root ganglia
Wang (2008)	p > 0,01 in IL-6	euthanized mice	level of IL-6 cytokines was significant in *B.b.* infected mice

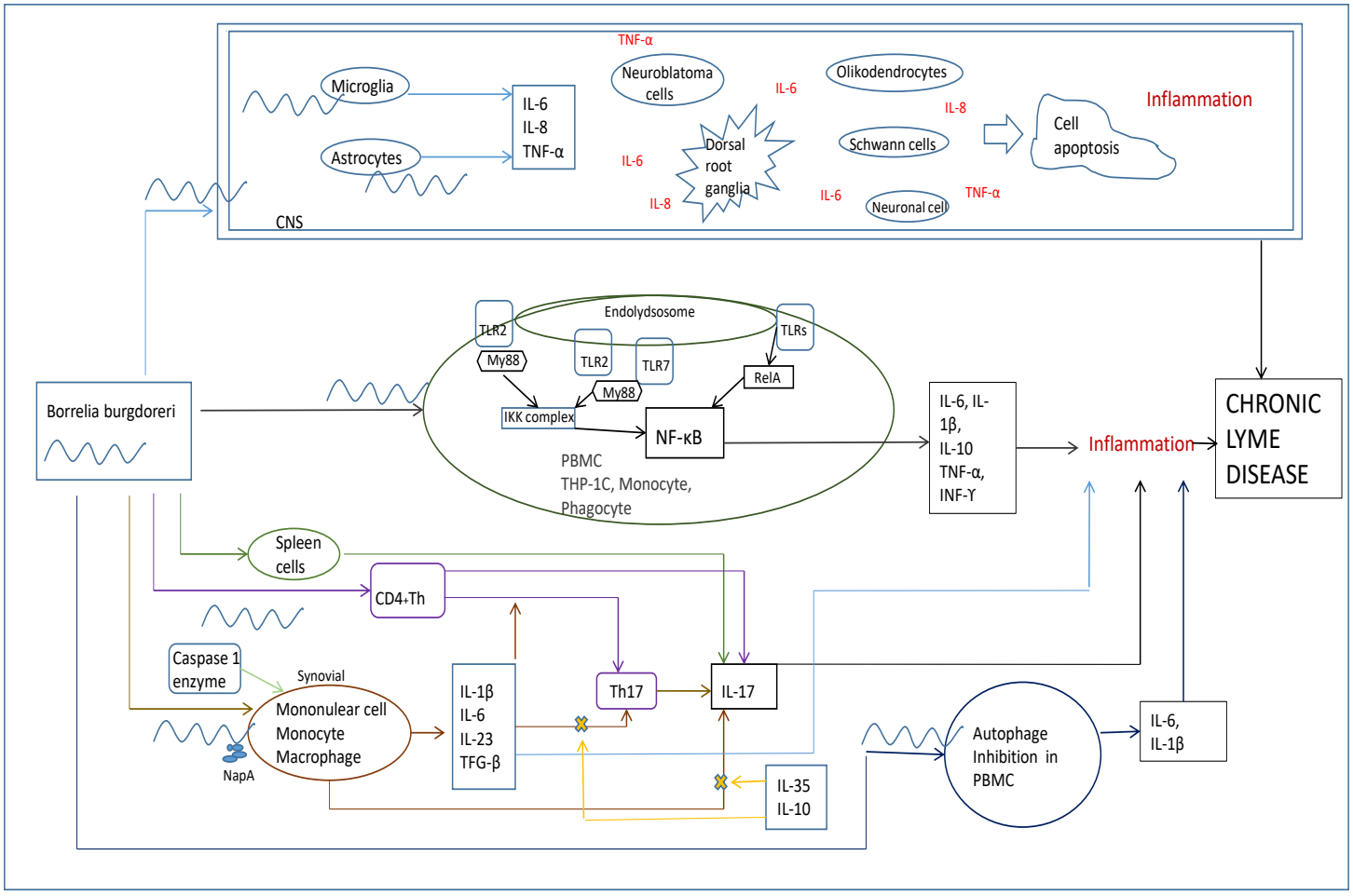
3.2.2 *Borrelia burgdorferi* Induces Pro-inflammatory Cytokine Production

Table 5. Reviewed studies show the IL-6 and IL-17 and other cytokines role as an inflammation mediator.

Author	Results	Study	Media	Additional data
Burchill (2003)	p< 0.05 inflammation ↓ with anti-IL-17 receptor	mice	joint	Delay was found in onset of swelling in hind paws in anti-IL-17 receptor
Nardelli (2008)	p < 0.05 inflammation ↓ IL-6, TGF-β and IL-17 antibody	mice	joint	Swelling decreased significantly with IL-6, TGF-β and IL-17 antibody treated mice
Nardelli (2010)	p < 0.05 in IL-17	mice	cells	IL-17 production was higher in Borrelia-vaccinated and infected mice
Kuo (2011)	p ≤ 0,05	mice	joint	IL-35 indirectly decrease inflammation by

	inflammation ↓ with IL-35			suppressing inflammatory stimulus
Hansen (2013)	$p < 0,05$ in IL-17	mice	joint	IL-17 production was found to be higher in IL-10 deficient mice
Oosting (2012)	decrease IL-6, IL-1β in caspase -1 deficient mice	mice	joint	IL-6 and IL-1β decreased with marcophages from caspase-1 deficient mice
Burchill (2003)	IL-17 ↑ with high *Borrelia burgforferi* load	mice	T-cells	IL-17 promotes no destructive arthritis unless spirochete burden is elevated

Diagram 2. Mechanism diagram based on above *in vitro* and *in vivo* studies of this review. *B.burgdoferi* induces the cytokine production in microglia, astrocytes in central nervous system and inflammation environment causes cell apoptosis. *B.burgdorferi* induced pro-inflammatory cytokine production and inflammation via NF-κB pathway or via monocytes, macrophages, mono nuclear cells is presented in diagram. Some cytokines are reducing the cytokine production (IL-10 and IL-35). Prolonged inflammation is causing symptoms in CLD. (Myers et al. 2009) (Ramesh et al. 2008) (Bernardino et al. 2012) (Ramesh et al. 2012) (Ramesh, Santana-Gould, et al. 2013)(Bettelli et al. 2006)(Codolo et al. 2008) (Infante-Duarte et al. 2000) (Oosting et al. 2012)(Oosting et al. 2011) (Norgard et al. 1996) (Olson et al. 2007) (Sadik et al. 2008) (Bernardino et al. 2012)(Buffen et al. 2013) (Wang et al. 2008) (Ramesh et al. 2009)(Burchill et al. 2003) (Kotloski et al. 2008)(Nardelli et al. 2010)(Kuo et al. 2011)(Hansen et al. 2013)



3.3 Human studies

3.3.1 Borrelia burgdorferi Induces Pro-inflammatory Cytokine Production in Central Nervous System

Human studies are an important way to assess the real function of cytokines in Lyme disease. Patients with chronic recurrent neurologic Lyme disease and prolonged symptoms such as fatigue, pain, malaise and cognitive dysfunction might have a increased cytokine levels in their CSF (Ramesh 2013).

Table 6. IL-6 and IL-17 are shown to be elevated in CSF or in serum according to reviewed human cohort studies.

Author	Results	Study	Media	
Cerar (2013)	p < 0,001 IL-6	cohort Participants 46	CSF	NB patients had significantly higher IL-6
Solonski (2014)	IL-6 ↑ after several months	cohort Participants 67	serum	Elevateted levels of IL-6 after 1-24 months after acute infection
Kondrusik (2004)	p > 0.05 before AB IL-1β, IL-6, TNF-α ↑ IL-6, TNF-α high after AB treat 4 weeks.	cohort Participants 20	serum cerebrospinal fluid	of IL-1β, IL-6, INF-Ɣ and TNF-α high
Henningsson (2011)	p < 0,0001 IL-17	cohort Participants 280	CSF	in neuroborreliosis patients
Nordberg (2010)	p< 0,0001 IL-17	cohort Participants 310	CSF	in neuroborreliosis patients

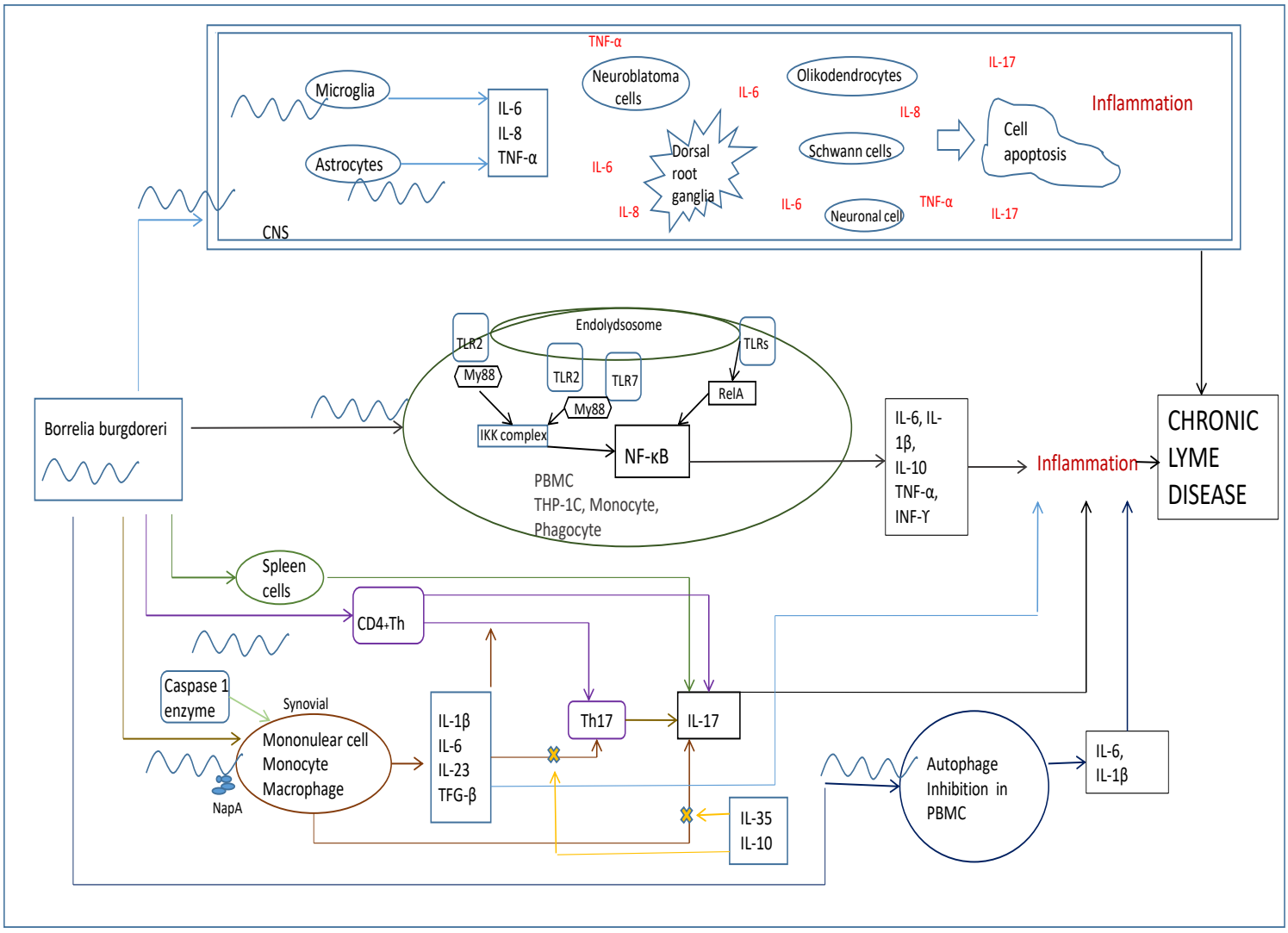
3.3.2 Borrelia burgdorferi Induces Pro-inflammatory Cytokine Production

The Lyme arthritis (LA) is chronic arthritis with persistent joint pain even after antibiotic treatment (Borchers 2014). In rheumatic arthritis the characteristic feature is cytokine and chemokine response in synovial fluid or synovial tissue (Shabgah 2014).

Table 7. Increased levels of cytokines IL-6 and IL-17 are shown in reviewed human cohort studies.

Author	Results	Study	Media	Additional data
Shin (2007)	p < 0.05 IL-1β, IL-6, TNFα	human cohort Participants 52	synovial fluid	Antibiotic-refractory LA patients had significantly higher cytokines
Skogman (2011)	IL-6 tendency to be higher	children cohort Participants 64	blood	IL-6 cytokine an increased spontaneous secretion with children with previous LD
Jablonska (2005)	p< 0.001 in IL-1β, IL-6, IL-15	cohort Participants 43	serum	CLD significantly higher cytokines concentrations
“	P < 0.001 IL-6	in vitro	LD patients PMN and PBM cells	PMN and PBM cells IL-6 higher in Lyme disease patient group
Infante-Duarte (2000)	p< 0.05 IL-17	cohort Participants 11	synovial fluid	LA patient synovial fluid T- cells production significantly high
Codolo (2008)	higher 48% NapA of B.b. value	cohort Participants 10	serum	Higher NapA of B.b. value in serum LA patients
Codolo (2013)	p < 0.001 NapA of B.b. value p < 0,001 IL-17	cohort Participants 15	synovial fluid	Significantly higher NapA synovial fluid LA patients. High IL-17 cytokine
Strle (2011)	high persistant symptoms IL-1β, IL-6, IL-10, IFNϒ and TNF-α	cohort Participants 17	serum	Persistent LD patients more symptoms and increased cytokine levels

Diagram 3. Mechanism diagram based on above *in vitro, in vivo* and human studies of this review. B.burgdoferi induces the cytokine production in microglia, astrocytes in central nervous system and inflammation environment causes cell apoptosis. B.burgdorferi induced pro-inflammatory cytokine production and inflammation via NF-κB pathway or via monocytes, macrophages, mono nuclear cells is presented in diagram. Some cytokines are reducing the cytokine production (IL-10 and IL-35). Prolonged inflammation may be cause of persistent symptoms in CLD. (Myers et al. 2009) (Ramesh et al. 2008) (Bernardino et al. 2012) (Ramesh et al. 2012) (Ramesh, Santana-Gould, et al. 2013)(Bettelli et al. 2006)(Codolo et al. 2008) (Infante-Duarte et al. 2000) (Oosting et al. 2012)(Oosting et al. 2011) (Norgard et al. 1996) (Olson et al. 2007) (Sadik et al. 2008) (Bernardino et al. 2012)(Buffen et al. 2013) (Wang et al. 2008) (Ramesh et al. 2009)(Burchill et al. 2003) (Kotloski et al. 2008)(Nardelli et al. 2010)(Kuo et al. 2011)(Hansen et al. 2013) (Cerar et al. 2013)(Soloski et al. 2014) (Kondrusik et al. n.d.) (Henningsson et al. 2011) (Nordberg et al. 2011) (Shin et al. 2007)(Skogman et al. 2012) (Jablonska & Marcinczyk 2006)(Codolo et al. 2008) (Codolo et al. 2013)(Strle et al. 2011)



3.4 **Nutritional Interventions**

Chronic Lyme disease has started to become a big public health problem in many countries during the last 10 years (Nichols 2013). The primary treatment for chronic Lyme disease is administration of various antibiotics (Burchers 2014). However, relapse often occurs when antibiotic treatment is discontinued (Vodjani 2009). The *B.burgdorferi* spirochete can become resistant to antibiotics and the spirochete bacteria may develop into inactive forms like biofilm colonies and different kind of cyst and granular or round forms and that way protect itself against antibiotic treatment (Vodjani 2009). There is a need for nutritional agents that are able to down regulate the inflammation and immune response of *B.burgdorferi* and have an effect to cyst forms of bacteria.

3.4.1 Nutrients

3.4.1.1 Sulforaphane

Sulforaphane (SFN) is a natural compound from cruciferous vegetables like broccoli, cabbage, kale, cauliflower and Brussel sprouts. It has anti-inflammatory and antioxidative properties (Schmitz et al. 2014).

3.4.1.1.1 *In Vitro* Studies of Sulforaphane

Table 8. Summary table: *in vitro* and *in vivo* studies of SFN effect to pro-inflammatory cytokines.

Sulforaphane			
Author	**Results**	**Media**	**Additional data**
in vitro			
Chan (2008)	SFN → IL-6↓	Human pancreatic duct epithelial cells	SFN inhibits the IL-6 levels in a dose dependent manner
Fragoulis (2010)	p< 0.05 in IL-1β and IL-6	Human synoviacyte cells	SFN attenuate cytokines IL-1β and IL-6 and inflammation significantly
in vivo			
Yuon (2010)	p< 0.001 in IL-6 and TNF-α	Mice	SFN reduce cytokine production and inflammation significantly
Li (2010)	p< 0.01 in IL-6 and IL-17	Mice	SFN reduce antigen specific Th17 response and production of cytokines significantly
Innamorato	p< 0.05	Mice	SFN attenuates microglia induced inflammation in

(2008)	in IL-6 and TNFα		hippocampus in LPS-treated mice and reduces cytokines

3.4.1.2 Green Tea EGCG

Green tea is a popular beverage, and it has many health effects . Green tea polyphenols include catechin, epicatechin EC, epigallocatechin EGC, epicatechin 3 gallate ECG and epigallocatechin 3 gallate EGCG. The polyphenols EGCG is found to have carcinogenic, anti-inflammatory, immonomodulatory and antimicrobial activities (Gupta 2014).

Table 11 . Summary table: *in vitro, in vivo* and human studies of EGCG effect to pro-inflammatory cytokines.

Green tea EGCG			
Author	**Results**	**Media**	**Additional data**
in vitro			
Byun (2012)	p< 0.001 in TNF-α, IL-6, and IL-1β	Dendritic cells	EGCG inhibited inflammatory cytokines TNF-α, IL-6, and IL-1β significantly
Cai (2014)	p< 0.05 in IL-1β, IL-6 and TNF-α	Rat hippocampi and microglia	ECGC decreased cytokines significantly and has neuroprotective anti-inflammatory effects
in vivo			
Yang (2014)	p< 0.01 in IL-17	Mice	ECGC reduce cytokine production significantly and can down-regulate Th17 differentiation
human			
Yoon (2013)	p< 0.05 in IL-1β, IL-1α and TNF-α	Human and Human sebocyte cells Participants 35	EGCG decreased cytokines significantly and caused improvement in acne lesion Duration: 8 weeks

Nieman (2009)	p< 0.05 in IL-6	Human Participants 39	EGCG + quersetin supplementation decrease cytokines significantly in cyclists
Basu (2011)	p= 0.3 ↓ 37% in IL-6 level	Human Participants 35	Beverage green tea showed a difference (37%) in IL-6 level. Duration: 8 weeks

3.4.1.3 Quercetin

Quercetin is one of the most plentiful dietary flavonoids and is found in a broad range of fruits and vegetables. Quercetin has been demonstrated to be anti-inflammatory (Schmitz 2014).

Table 10. Summary table: *in vivo* and human studies of quercetin effect to pro-inflammatory cytokines.

Quercetin			
Author	**Results**	**Media**	**Additional data**
in vivo			
Takashima (2014)	p< 0.05 in cytokines	Bronchoalveolar lavage fluid and alveolar macrophage cells	Quercetin internally will lead to supportative strategy for cytoprotection in lungs
human			
Boots (2011)	p< 0.05 TNF-α/IL-10 ratio	Human Participants 18	Inflammation down-regulated with quercetin antioxidant supplementation Duration: 24 hours
Bobe (2010)	concentration of IL-6 ↓	Human Participants 872	Lower concentration of IL-6 and high intake of the flavonols had a correlation. Duration: 4 years

3.4.2 Herbs

3.4.2.1 Resveratrol (RES)

The polyphenolic compound resveratrol 3,5,4-trihydro-trans-stilbene is a molecule which consists of two aromatic rings attached by a methylene bridge. It is found in several plants, grapes, peanuts, cranberries, blueberries and mulberries. Studies show that it has the ability to work as an anti-inflammatory and anti-oxidative agent (Hurley 2013).

Table 13. Summary table: *In vitro, in vivo* and human summary table of resveratrol effect to pro-inflammatory cytokines.

Resveratrol			
Author	**Results**	**Media**	**Additional data**
in vitro			
Cianciulli 2014	p< 0.005 or p< 0.01 in cytokines	microglia cells	Resveratrol down regulate the microglia activation and cytokines significantly
Wight 2012	p< 0.05 in IL-6 and TNF-α	astrocyte cells	Resveratrol suppress cytokines IL-6 and TNF-α significantly and inhibit IL-12 and IL-23 cytokine production
in vivo			
Wang (2013)	p< 0.05 in IL-1, IL-6, TNF-α	Mice	Resveratrol reduces pro inflammatory cytokine levels significantly
Imier (2009)	p< 0.05 in IL-1β, IL-1α and TNF-α	Mice	Resveratrol decreases cytokines significantly and less severe EAE and IL-17↓
human			
Ghanim (2010)	p> 0.05 in IL-6 and TNF-α	Human Participants 20	Significant decrease of pro-inflammatory cytokine levels of healthy humans. Duration 6 weeks
Tome-Carneiro (2013)	p> 0.05	Human	GE + RES supplementation cytokine level decreased significantly in Diabetes II

	in IL-6	Participants 35	patients. Duration one year
Macedo (2014)	p< 0.05 in IL-6 and TNF-α	Human Participants 60	Inflammatory cytokine levels reduced significantly after RES supplementation to firefighters Duration 3 month
Zahedi (2013)	p< 0.05 in IL-6 and TNF-α	Human Participants 20	RES reduces in cytokines significantly in basketball players in serum. Duration 6 weeks

3.4.2.2 Curcumin

Curcumin is a bioactive yellow pigment of turmeric. Curcumin is shown to be non-toxic and exhibits various biological activities such as anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-bacterial, anti-fungal, anti-viral and anti-fibrotic effects (Zhou 2011).

Table14. Summary table: *In vitro, in vivo* and human of curcumin effect to cytokines.

Curcumin			
Author	**Results**	**Media**	**Additional data**
in vitro			
Guimaraes (2013)	p< 0.005 or p< 0,01 in IL-6, TNF-α	macrophage cells	Curcumin regulate the transcription of pro-inflammatory cytokines significantly
Zhang (2014)	decreased ↓ in IL-6 and TNF-α	macrophage cells	Monocarbyl curcumin analogs are statistically best compound
in vivo			
Davis (2007)	p< 0.05 in IL-1β, IL-6 and TNFα	mice	Curcumin cytokine levels were reduced in dose dependant manner
Fu (2014)	p< 0.05 p< 0.01 in IL-1β, IL-6 and TNFα	mice	Curcumin attenuate cytokine levels significantly

Xie (2009) study	decreases in IL-6 and IL-21	rats	Curcumin ameliorate cytokine production
Kanakasabai (2012)	p< 0.01 p< 0.001 in IL-17 and IL-23	mice	Curcumin reduces cytokines significantly and have an effect to Th1/Th17 regulation
human			
Usharani (2008)	p> 0.01 in IL-6 and TNF-α	Human Participants 72	Curcumin and atorvastatin medicin decreased in Diabetes II patients significantly Duration: 8 weeks
Panahi (2015)	p< 0.001 in infl.marker CRP	Human Participants 117	Inflammation marker levels reduced after curcumin supplementation metabolic syndrome patients. Duration 8 weeks
Moreillon (2013)	p< 0.05 in IL-6	Human Participants 16	Curcumin reduces in cytokines in cronic kidney disease patients. Duration 6 weeks

3.4.2.3 Cats claw

There are alternative clinical treatments for chronic lyme disease that utilize several herbal extracts. These protocols are designed to eliminate sprirocete bacteria and its all possible forms like cyst and round bodies. Cats claw has shown to have anti-inflammatory and anti-bacterial properties (Allen-Hall 2009).

Table 15. Summary table of *In vitro* and human Summary table of Cats claw effect to cytokines.

Cats claw			
Author	**Results**	**Media**	**Additional data**
in vitro			
Datar (2010)	p< 0.05 in B.burgdorferi	Borrelia burgdorferi and cats claw extract	Cats claw extract was effective on reducing B.b. function in sprirochete and round forms

Allen-Hall (2009)	p< 0.05 TNF-α	THP-1 cells	Cats claw suppress activity of TNF-α via NF-κB
in vitro, human			
Piscoya(2001)	p< 0.01 TNF-α	Human murine macrophages Participants 45	Cats claw suppress pain in activity and TNF-α in macrophages

3.4.2.4 Andrographis paniculata

Andrographis paniculata AP is a herb that has been used to viral infections, diarrhoea, dysentery and fever (Muluye 2014).

Table 16. Summary table : *In vitro* and *in vivo* of andrographis paniculata effect to cytokines.

Andrographis paniculta			
Author	**Results**	**Media**	**Additional data**
in vitro			
Zhu (2012)	p< 0,05 in IL-1β, IL-6 and TNF-α	phosphorylated proteins	AP reduced cytokine production significantly
Chandrasekaran (2010)	p< 0,05 p< 0,01 in IL-1, IL-6	Murine macrophage	Anti-inflammatory AP mechanism prevent inflammatory and pro-inflammatory mediators to act, cytokines reduced significantly
in vivo			
Chao (2011)	p< 0,05 in IL-6 and TNF-α	Mice	AP introduction reduced cytokine production significantly.

3.4.2.5 Boswellia

Frankincence, the resin from trees of genus Boswellia, has been used as an oral anti-inflammatory agent in Eastern and Asian medicine for thousands of years (Miller 2011). Acetyl-11-keto-β-boswellic acid (AKBA) has been identified as the prominent bioactive compound (Stuner 2014).

Table 17. Summary table*: In vitro* of boswellia effect to cytokines.

Boswellia			
Author	**Results**	**Media**	**Additional data**
in vitro			
Gayathri (2007)	induction (pure or crude boswellia) ↓ IL-1, IL-6 and TNF-α	human PBMC cells	Cytokine production reduced with Boswellia extract
Stuner (2014)	p< 0.05 p< 0.0001 IL-1β, IL-17	Human cells from MS patients (naïve CD4+)	AKBA (bioactive compound od Boswellia) Decrease the differentiation of the Th17

4. Discussion

During the study process of the mechanism review it was found that there are very few reviews or primary researches on Lyme disease researching other than antibiotic treatment. This mechanism review is probably one of the first researches studying the nutritional and herbal interventions and the reduction of pro-inflammatory cytokines in Lyme Disease. It became evident that the treatment of Lyme Disease and CLD needs more non-antimicrobial optional therapy methods. This was stated in several studies that were used in this review. There are only few studies available that focus on nutritional or any other treatment method than antibiotics therapy in Lyme disease treatment.

4.1 Chronic Lyme Disease Patients

The early state treatment is easy and clear, but there is controversy regarding the post or Chronic Lyme Disease treatment methods. This is a real problem with patients that have ongoing chronic symptoms (Borgermans, 2014). Ali (2014) is studying patients´ experiences of chronic Lyme disease in the healthcare system. The study concludes that there is significant decline in the health care with chronic Lyme disease. The patients were faced with condescending and dismissive attitudes in the healthcare system.

Diagnosing criterias of the chronic Lyme disease are complex and there are no solid guidelines available (Marques 2008). The evidende-based quidelines committees disagree on the severity of the pain in chronic Lyme disease and states that post or chronic Lyme disease symptoms are “the aches and pains of daily

living" (Cameron 2009). Cameron (2009) review validates the clinical trials in persisting Lyme disease symptoms and concludes that chronic pain and other symptoms should be treated like other patients with chronic disease.

4.2 Cytokines in Chronic Lyme Disease

Cytokines have many different roles in the immune system: stimulation of synthesis, promoting the differentiation, contributing to pain by increasing the sensitivity in nerve endings (Schmitz 2008). According to studies reviewed in this project elevated pro-inflammatory cytokine levels can cause symptoms like fatigue, pain in joints and muscles, cognitive dysfunction, malaise and many other symptoms that look like normal flu-like symptoms or other inflammation reaction in the body. The sickness response is a normal body reaction to early infection period. The sickness response becomes problematic when it is prolonged (Fallon 2010). This may occur with ongoing cytokine activation due to the persistence of a triggering organism or antigen like *B. burgdorferi* (Fallon 2010).

4.2.1 Cytokines in CNS

According to the in vitro and in vivo studies of this review IL-6 has a critical role in ongoing inflammation in CNS. In reviewed in vitro and in vivo studies the gliad cells are producing pro-inflammatory cytokines IL-6 and TNF-α. Elevated levels of IL-6 in CNS were seen to cause cell apoptosis *in vitro* (Ramesh et al. 2012). The IL-6 cytokine production in CNS-immune system is shown in mechanism diagram 1. Pro-inflammatory cytokines cause the inflammation, neurologic injuries and neuritis in the DRG in the nervous system(Ramesh, Santana-Gould, et al. 2013). One function of the cytokines is to regulate neuroinflammation. Neuroinflammatory processes affect both health and disease of nervous system by regulating development of brain cells and their connections (Ramesh, Maclean, et al. 2013). In the reviewed cohort studies the CLD patients were found to have high IL-6 levels in CFS or serum. Cytokine IL-17 levels were found to be significantly high in two large cohort studies of neuroborreliosis. The *B.burgdorferi* activation of microglia and neutrophils and the ongoing cytokine production and the generated inflammation may be one reason for the difficult symptoms that CLD neuroborreliosis patients suffer.

4.2.2 Cytokines in Lyme Arthritis

Cytokine IL-17 and the T helper cell 17 are found to be mediators of chronic Lyme Arthritis (Borchers 2014). The *in vivo, in vitro* and human studies reviewed in this study have shown the important role of Th17 cells in Lyme Arthritis [diagram 2]. The differentiation of Th17 is induced by *B. burgdorferi* (Infante-Duarte et al. 2000). The production of pro-inflammatory cytokine IL-17 was shown to be increased by *B.burgdorferi* in vitro and in vivo Table 3 and 4. NapA of *B.burgdorferi* was found to have an impact to IL-17 production in vitro (Codolo et al. 2008). IL-17 production was proved to be mediated with other cytokines IL-6 and IL-23 in vitro (Bettelli et al. 2006), (Oosting et al. 2011).

IL-17, IL-6 and IL-23 cytokine were found to increase the inflammation in *B.burgdorferi* infected mice hind paws (Nardelli et al. 2008). Mediating the Th17 differentiation could be an important method of reducing the pro-inflammatory cytokine production and ongoing inflammation in chronic diseases. IL-10 and IL-35

cytokines were shown to down-regulate the IL-17 production in vivo (Kuo et al. 2011) (Hansen et al. 2013). Entzyme caspase 1 was shown to have a role in increasing the inflammation via IL-6 and IL-1β production (Oosting et al. 2012).

NapA of the *B.burgdorferi* have a role in elevated IL-17 levels in Lyme Arthritis (Codolo et al. 2008). Elevated IL-17 levels in synovial fluid can be connected to Lyme arthritis according to two cohort studies(Henningsson et al. 2011),(Nordberg et al. 2011). IL-6 concentrations were found to be elevated in serum or synovial fluid in four human cohort studies of Lyme arthritis patients Table 5.

4.2.3 NF-κB pathway

The review shows that *Borrelia burgdorferi* lipoproteins can be induced the pro-inflammatory cytokines production also via NF-κB pathway [diagram 1]. According to reviewed studies reduction of NF-κB pathway and the inflammation can be done with natural anti-inflammatory components [Table 10-17].

4.3 Nutritional Intervention

This review has looked at anti-inflammatory nutrient and herbs in vitro, in vivo and human studies trying to find enough proof to use compounds as one possible treatment method for chronic Lyme disease. The main focus on choosing the nutrition and herb components to this project has been the down-regulating feature of the pro-inflammatory cytokine production of IL-6, IL-17, IL-23 and TNF-α.

Available studies of certain herbs' or nutrients' effect on chronic Lyme disease were not found. Instead there are plenty of studies of the nutrient and herbal effect on the pro-inflammatory cytokine production in chronic diseases. These findings of the studies support the CLD treatment with nutrients and herbs protocols.

4.3.1 Nutrients

According to this review there might be several treatment possibilities to use herbs or nutrients that have an effect on down-regulating pro-inflammatory cytokines [Table 18].

Table 18. Summary table: herbs / nutrients effect to cytokine production or bacteria grow according reviewed studies. .

Pro-inflammatory cytokine / Borrelia burgdorferi	**According the studies herb / nutrient that reduces cytokine production or bacteria**	**Study type :**Studies are found in result section 3.4
IL-6	sulforaphane, EGCG, quercetin, resveratrol, curcumin, andrographis paniculata, boswellia	*in vitro* *in vivo* human
IL-17	sulforaphane, EGCG, curcumin, boswellia	*in vitro* *in vivo*
IL-23	curcumin	*in vivo*
TNF-α	sulforaphane, EGCG, quercetin, resvertatrol, curcumin, cat claw, andrographis paniculata, boswellia	*in vitro* *in vivo* human
Borrelia burgdorferi	cats claw	*in vitro*

4.3.3 Functional Medicine Model

A possible model to use in treatment of chronic Lyme disease patients is The Functional Medicine Model , which is a patient centered approach that addresses the unique interactions among genetic, environmental and lifestyle factors influencing both health and chronic disease" (The Institute of Functional Medicine). The idea of treating the person in a personalized way with balancing the whole immune system and all other functions of the body could be very beneficial. The chronic Lyme disease treatment could be done by personalised nutrition and tailored herbal protocol based on laboratory examinations and clinical findings.

4.4 Study limitations

During the research process it was noticed that there is a limited amount of literature available on chronic Lyme disease treatment methods other than antibiotic treatment. Also not every study was available. The controversial studies of the Chronic Lyme Disease could be stated as a limitation to the study. Human CLD

randomized placebo controlled trials were not found in searches and the participants in some of the cohort studies were low, < 50. This lowers the statistical power of the studies.

A very limited amount of studies were available on Lyme disease and nutrients or herbs. The nutrient or herb studies that are reviewed in this project were researching the pro-inflammatory cytokine reactions on other chronic diseases or conditions, not Lyme Disease.

It was noticed that the Chronic Lyme Disease is complex disease and the various symptoms of patients are caused by many factors. This mechanism review handles one aspect of the possible mechanisms of ongoing symptoms. The full picture of ongoing inflammation and the role of pro-inflammatory cytokines needs more researching.

4.5 Future research

The treatment of chronic Lyme disease is found to be quite difficult because of the various symptoms that patients suffer. The spirochete shaped *Borrelia burgforferi* is called a great imitator. Early diagnosis and treatment is vital for managing the pain or other symptoms. There is a gap of understanding the exact pathophysiologic mechanism that contributes to the different types of acute and chronic pain, inflammation, musculoskeletal pain, neuropathic pain in Lyme Disease (Zimering 2014). A recent study by Meriläinen (2015) is presenting the pleomorphic forms *B.burgdorferi.* The future in vitro studies should include the round bodies of cyst forms in the study agenda when searching for the different anti-spirochete agents. The different forms of the bacteria may be one explanation to difficult symptoms of the CLD.

More studies of the Chronic Lyme disease are needed. Studies are needed on the different nutrients' or herbs' effect on *B.burgdorferi* in vitro. Animal studies of the *B.burgdorferi* infected mice and the different herbs' effects are needed. Toxicity studies on the herbs are needed.

Future mechanism reviews of ongoing inflammation could help to understand to complexity of Lyme disease. Other inflammation mediators like chemokines could have been useful to study.

4.6 Conclusion

The role of the cytokines and prolonged inflammation needs more studies but can be concluded to be a factor in constant symptoms of Chronic Lyme Disease. Reviewed nutrients and herbs can down-regulate the pro-inflammatory cytokine production and reduce the inflammation. Further research is needed to understand the mechanism of the persistent symptoms after Lyme Disease treatment and for nutritional interventions to Chronic Lyme Disease.

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