Peptide Therapies for Chronic Lyme Disease

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Multisystem Disease

Goals/Objectives:

- To understand that immune dysfunction, inflammation and a TH1-TH2 shift are key components in chronic Lyme disease (CLD), CFS, FM, etc.
- To understand how immune modulatory therapies, including peptide and stem cell therapies, can be very effective in chronically ill and non-responsive patients
- Understand how the gut-brain axis and leaky gut are key dysfunctional components in CLD and how BPC-157 can be a or the key component in treatment and normalization of the GI system
- To understand the potential use of antimicrobial peptides (LL-37)
- To understand how to quickly identify key labs to test for immune dysfunction in CLD

Multisystem Disease

- Small amount of organism can stimulate huge amounts of immune modulatory cytokines and toxins
- TH1-TH2 shift
  - Increased inflammation but decreased ability to fight intracellular infections

Multisystem Disease

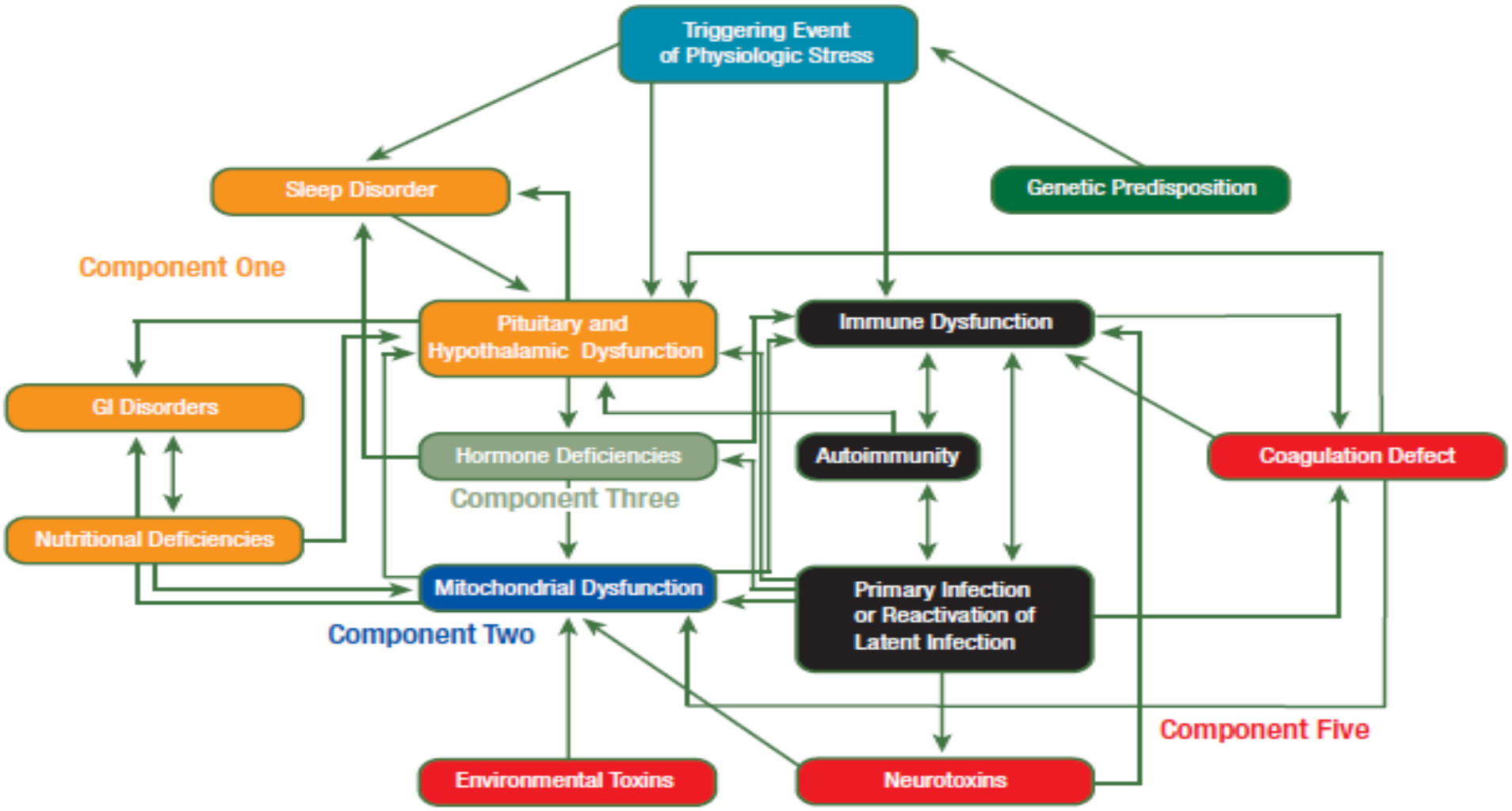
- A triggering event under the backdrop of a chronic Lyme infection sets into motion a vicious cycle pathophysiology.
- The immune dysfunction can be set off with significant stress, other infection, illness or accident.
- Each problem may trigger other problems.
- Measurable hypothalamic, pituitary, immune and coagulation dysfunction

1. Holtorf, K. A Confounding Condition: Treating chronic fatigue syndrome and fibromyalgia requires addressing the underlying problems. Healthy Aging 2008;Nov:1-7

Immune Dysregulation

- The TH1 response mainly addresses intracellular infections while the TH2 response is largely limited to extracellular infections.
- Transforming the body to a TH2 "extracellular" dominant response then converting to intracellular L-forms with a downregulated TH1 "intracellular" immune response results in an effective immune evasion strategy that may be the hallmark of transformation to late-stage dissemination.[1,19,20]

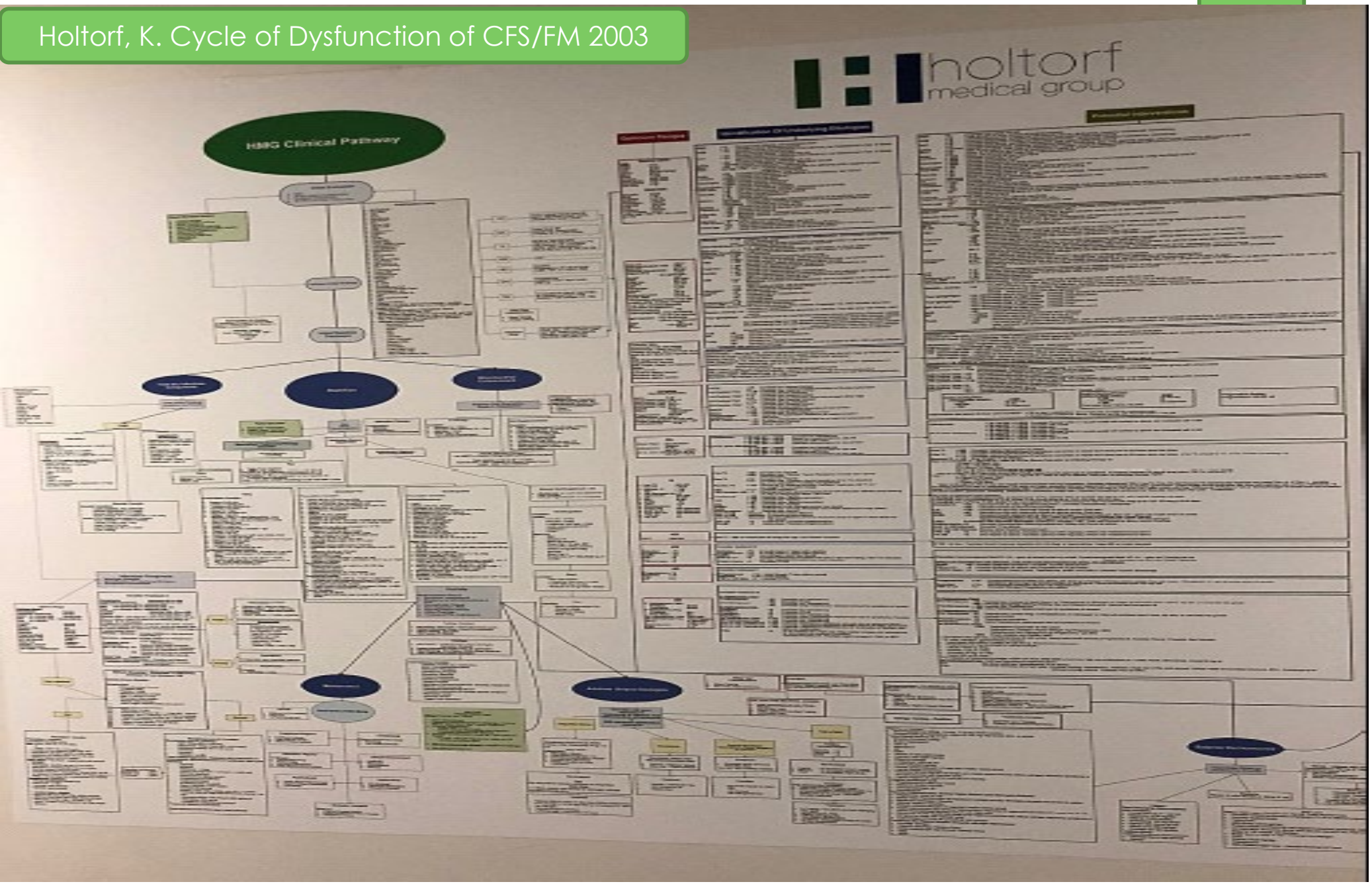
Cycle of Dysfunction with LD



Holtorf, K. Cycle of Dysfunction of CFS/FM 2003

Cycle of Dysfunction with LD

Holtorf, K. Cycle of Dysfunction of CFS/FM 2003



Multisystem Disease

- Most patients with chronic (stage III) Lyme disease are generally asymptomatic for a variable amount of time (months, years).
- Tests can be labeled as inaccurate because they are positive in "asymptomatic" patients.

Chronic Lyme Disease

- After months or years, see significant immune breakdown of TH1 and overstimulation of TH2.
- May never become symptomatic or may continually be misdiagnosed as other conditions, including CFS, FM, MS, autoimmune disease, migraines, depression, bipolar, pain syndromes, Parkinson's, ALS, Alzheimer's, CHF, obesity, OCD, ADHD, DM, lupus, Crohn's, PCOS, PMS, IBS, IC, prostatitis, sleep disorder, etc.
- The longer the duration, generally the worse the immune dysfunction, the worse the symptoms and the more treatment resistant.
- Coinfections make symptoms worse and harder to treat.

Immune Dysregulation

- With later stages, borrelia infection suppresses TH1 immunity with resultant excessive TH2 immunity, causing:
  - Ineffective intracellular immunity
  - Inability to activate natural killer (NK) cells
  - Inability to transform from IgM to IgG antibodies with a prolonged non-cytotoxic IgM response
    - ✓ Explains high incidence of IgM positive WB
    - ✓ Does not indicate that is a false positive
  - Increased autoimmunity
  - Hypercoagulation
  - Mast cell activation
  - Mitochondrial dysfunction
  - Hypothalamic/pituitary dysfunction with hormone deficiencies
  - Cascade of many effects

3. McManus M, Cincotta A. Advances in Integrative Medicine. Adv Integ Med 2015;2:81-89.

Immune Evasion

- Fast-growing infections are generally most susceptible to antibiotics; Borrelia has a very slow replication rate with stationary phase and production of persister cells.[9]
- LD is slow growing with periods of little or no growth.
  - Most antibiotics only work on bacteria in growth phase.
  - The slower the growth, the longer it takes to kill.
  - Doubling rates for Staph and Strep are about 20 minutes; Borrelia takes 12-24 hours, Bartonella 22-24 hours(35-75 times longer--can be much longer when antibiotics are introduced).
  - The standard 10-14 days of antibiotics needed to reliably clear most "regular" infections would require the antibiotic to be present 24 hours per day for **1½ to 3 years for the same curative potential.**[69]

69. Grier TM. The Complexities of Lyme Disease: A microbiology tutorial: Part 1. Lyme Disease Survival manual. LymeNet Europe

TH1-TH2 Shift in CFS

Clin Exp Immunol 2004; **135**:294–302 doi:10.1046/j.1365-2249.2004.02354.x

High levels of type 2 cytokine-producing cells in chronic fatigue syndrome

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SUMMARY

The aetiology of chronic fatigue syndrome (CFS) is not known. However, it has been suggested that CFS may be associated with underlying immune activation resulting in a Th2-type response. We measured intracellular production of interferon (IFN)-γ and interleukin (IL)-2; type 1 cytokines), IL-4 (type 2) and IL-10 (regulatory) by both polyclonally stimulated and non-stimulated CD4 and CD8 lymphocytes from patients with CFS and control subjects by flow cytometry. After polyclonal activation we found evidence of a significant bias towards Th2- and Tc2-type immune responses in CFS compared to controls. In contrast, levels of IFN-γ, IL-2 and IL-10-producing cells were similar in both study groups. Non-stimulated cultures revealed significantly higher levels of T cells producing IFN-γ or IL-4 in CFS patients. Concluding, we show evidence for an effector memory cell bias towards type 2 responsiveness in patients with CFS, as well as ongoing type 0 immune activation in unstimulated cultures of peripheral blood cells.

Keywords chronic fatigue syndrome cytokines immune activation Th1/ Th2 cytokines

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Evidence for T-helper 2 shift and association with illness parameters in chronic fatigue syndrome (CFS)

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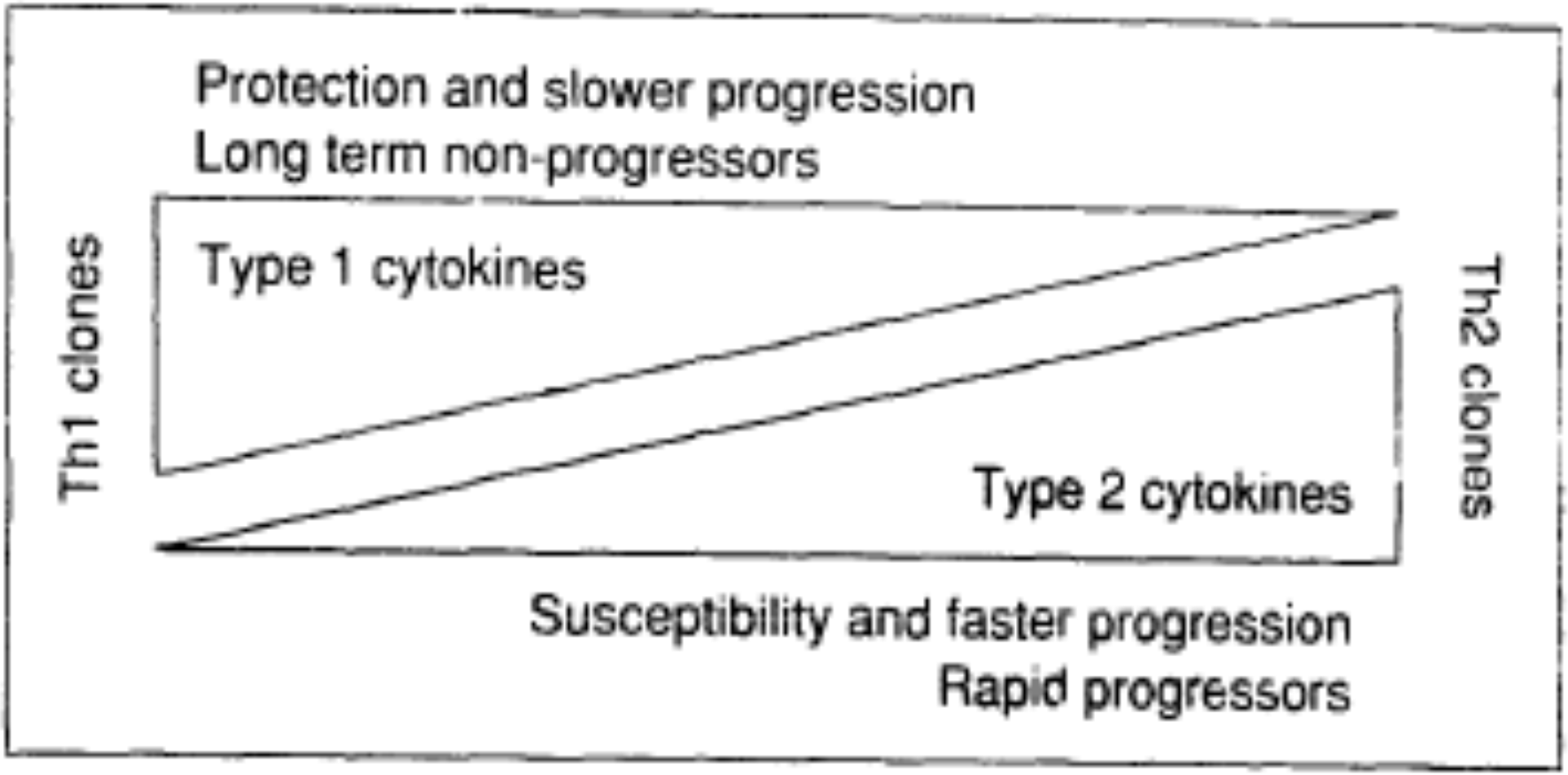
Abstract

Few immunological markers have been consistently reported in CFS. However, a shift to a T-helper 2 (Th2) type immune response has been hypothesized for individuals with CFS. The current study investigated whether individuals with CFS who exhibited a stronger shift towards a Th2 type of immune response would also exhibit more severe symptoms, poorer neurocognitive functioning, and poorer physical and psychosocial functioning. The current investigation measured the percentage of Th1-like and Th2-like memory cells using cell surface flow cytometry in 114 individuals with CFS. The associations between the ratio of Th1 and Th2 memory cells and various illness parameters measures were then examined, including symptom severity, psychiatric functioning, neurocognitive functioning, salivary cortisol levels, and chronic pain status. Results indicated that individuals who exhibited a more extreme shift towards a Th2 immune response also exhibited poorer sleep and high levels of basal salivary cortisol. The implications of these findings are discussed.

Keywords

chronic fatigue syndrome; t-helper 2 shift; immunology; salivary cortisol; cognitive functioning

TH1-TH2 Shift in HIV Determines Progression of Illness



97. Clerici M, Shearer, GM. The TH1-TH2 hypothesis of HIV infection: new insights. Immunology Today 1994;15(12):575-581

Stress and TH1-TH2 Shift

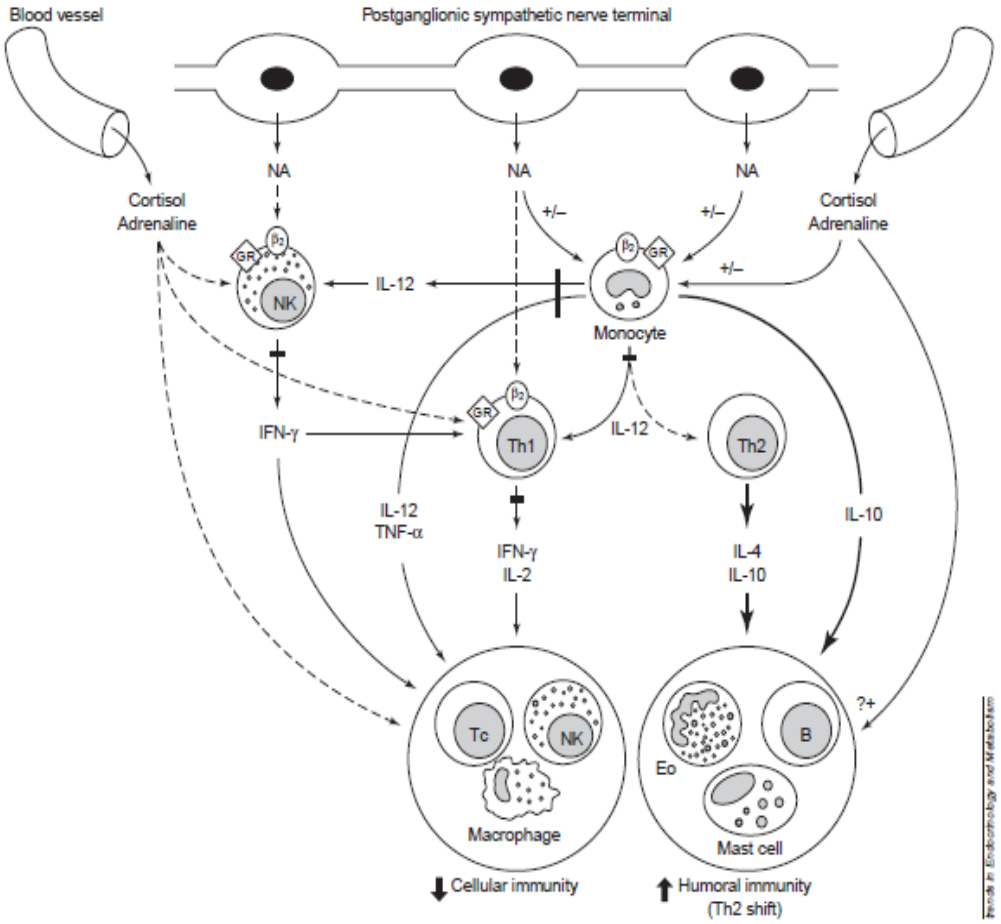


Figure 3. Stress influences immunity by stimulating cortisol and adrenaline secretion from the adrenal cortex and medulla, respectively, and the release of noradrenaline from the postganglionic sympathetic nerve terminals in blood vessels and lymphoid organs. The systemic effects of glucocorticoids and catecholamines on the production of key regulatory type 1 and type 2 cytokines, Th1 and Th2 functions, and components of cellular and humoral immunity are shown. Solid lines represent stimulation, heavy solid lines represent increased stimulation and dashed lines represent inhibition. Abbreviations: β2, β-2-adrenoceptor; +/–, stimulation/inhibition; B, B cell; Eo, eosinophil; GR, glucocorticoid receptor; IFN-γ, interferon γ; IL, interleukin; NA, noradrenaline, NK, natural killer cell; Tc, T cytotoxic cell; Th, T helper cell; TNF-α, tumor necrosis factor α

- *"In general, stress has been regarded as immuno-suppressive. Recent evidence, however, indicates that acute, subacute or chronic stress might suppress cellular immunity but boost humoral immunity."*
- *"A major factor governing the outcome of infectious diseases is the selection of TH1-versus TH2-predominant adaptive responses during and after the initial invasion on of the host."*
- **TH1-TH2 shift results in mast cell activation, which further drives the TH1-TH2 shift.**

Elenkov IJ, Chrousos. Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. *TEM 1999;10;9:359-68.*

Stress and TH1-TH2 Shift

Hypersensitivity/Mast Cell Sensitivity Syndrome

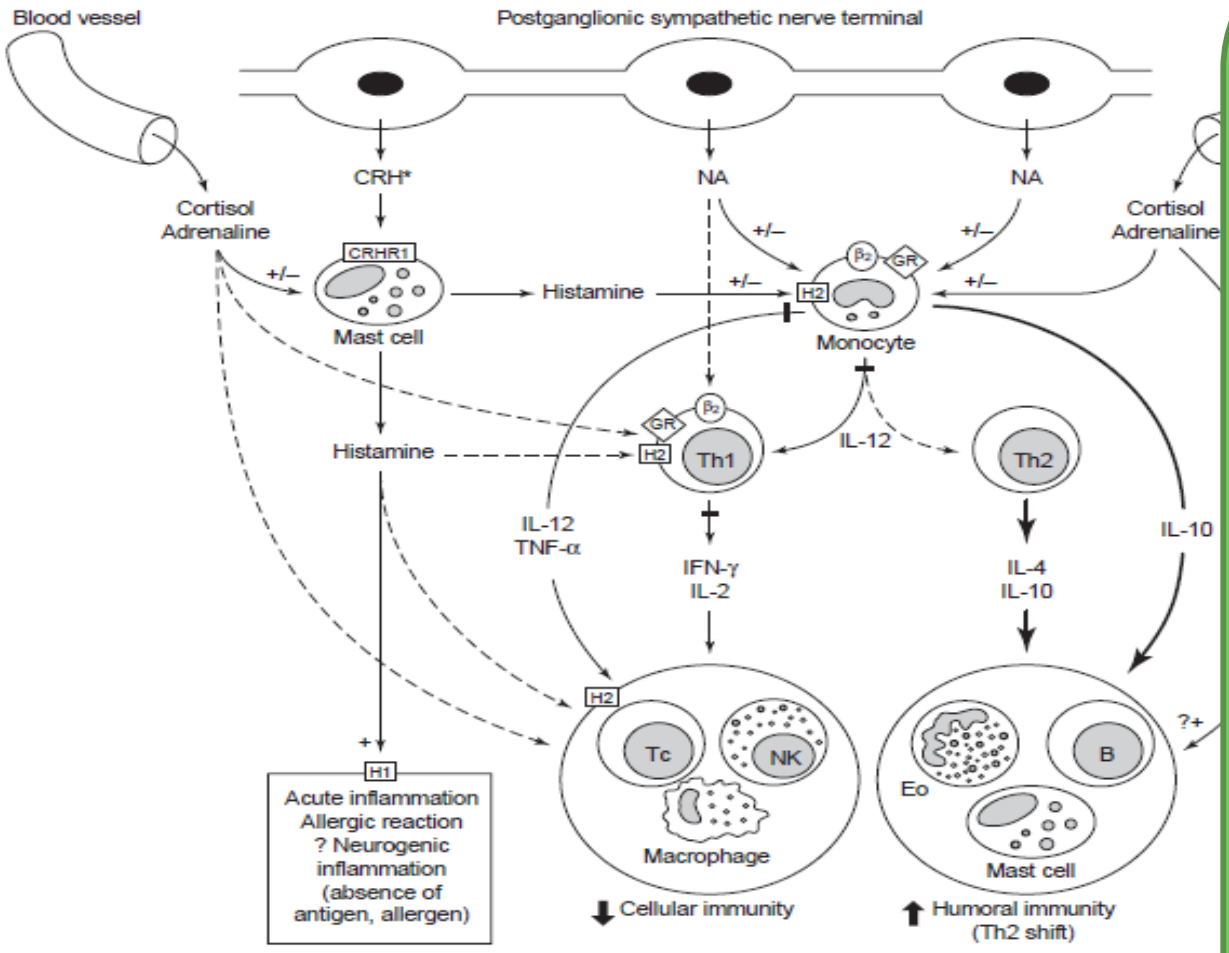


Figure 4. Stress and CRH influence immune/inflammatory and allergic responses by stimulating glucocorticoid, catecholamine (immune) CRH secretion and by altering the production of key regulatory cytokines and histamine (see text). *CRH is also releas nerves upon their activation. Solid lines represent stimulation, heavy solid lines represent increased stimulation and dashed lines r tion. Abbreviations: β2, β-2-adrenoceptor; +/–, stimulation/inhibition; B, B cell; CRH, peripheral (immune) corticotropin-relea CRHR1, CRH receptor 1; Eo, eosinophil; GR, glucocorticoid receptor; H1/H2, histamine 1/2 receptors; IFN-

- Thus, severe acute stress associated with high adrenaline concentrations and/or high local secretion of CRH could lead to mast cell degranulation.
- As a result, a substantial amount of histamine could be released, which consequently would not antagonize, but rather amplify, the Th2 shift through H2 receptors, while in parallel, by acting on H1 receptors, it could initiate a new episode or exacerbate a chronic inflammation and allergic conditions (Fig. 4).

97. Clerici M, Shearer, GM. The Th1-Th2 hypothesis of HIV infection: new insights. Immunology Today 1994;15(12):575-581

Thymic Proteins and Age

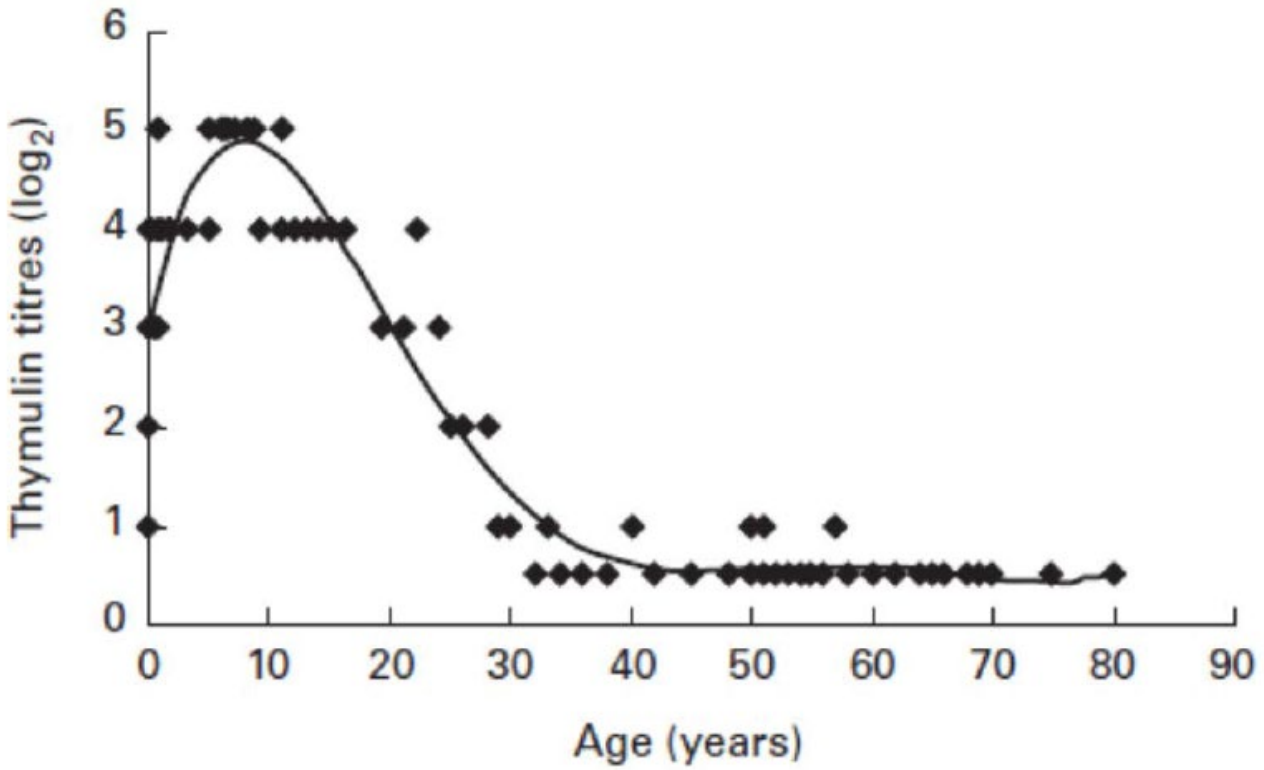


Fig. 1. Trend of thymic function through the course of the life. The thymulin titres of 93 subjects are plotted for each increment in age. Data are fitted to polynomial function using non-linear regression analysis (solid line; $r^2 = 0{\cdot}8857$).

68. Consolini R, Legitimo A, Calleri A, et al. Distribution of age-related thymulin titres in normal subjects through the course of life. Clin Exp Immunol 2000; 121:444-447

Thymic/Pineal Peptides and Age

- According to the U.S. Center for Disease Control (CDC), approximately 80 % of aged individuals are afflicted with at least one chronic disease as a result of a declination of thymic-related immune function.[73]
- Obesity and calorie intake are strongly associated with thymic involution
- The majority of people have pineal gland calcification by age 30.

72. Consolini R, Legitimo A, Calleri A, et al. Distribution of age-related thymulin titres in normal subjects through the course of life. Clin Exp Immunol 2000; 121:444-447
73. Gui J, Mustachio LM, Su DM, et al. Thymus Size and Age-related Thymic Involution: Early Programming, Sexual Dimorphism, Progenitors and Stroma. Aging Dis 2012;3(3):280-90

Thymus and Autoimmune Disease

- Looking at thymus scans and postmortem thymus biopsies, the authors showed that the overwhelmingly majority of patients with autoimmune disease (looked at myasthenia gravis, autoimmune thyroid disease and SLE)had abnormal thymus size or histology.

Irvine, WJ. The Thymus and Autoimmune Disease. Proc Roy Soc Med 1970;63:718-22

Thymic Peptides – Clinical Effects
(Thymosin alpha-1, thymosin beta-4, thymulin)

- Improved tissue repair and healing[35,36,37,41]
- Improved host defense to infection[26,27,28,29,30]
- Reverse immunosuppression of chronic infection (Lyme)[24,26,27,28,29,33,34,37,]
- Increases antioxidant and glutathione production[26,27,28,31,28,29,63,66]
- Boost NK function[26,35]
- TH2-TH1 immune modulation (infections, cancer, herxheimer, autoimmune)[26,27,28,30,31,33]
- Bind neuro/endotoxins[111]
- Cardiac regeneration and protection post-MI, CHF, etc.[39,65,98,99,100]
- Neurologic regeneration and protection post-stroke, TBI, Lyme, Alzheimer's, neuropathy, Parkinson's, etc.[63,67,42]
- Stimulate stem cell activity and proliferation[32,34,36,38,40,41,42]
- Increases longevity[89,90,91]
- Almost non-existent side effects at 100-fold dose+ excess[103,104]
- Excellent safety profile with large therapeutic window (over 1000 fold)[103,104]

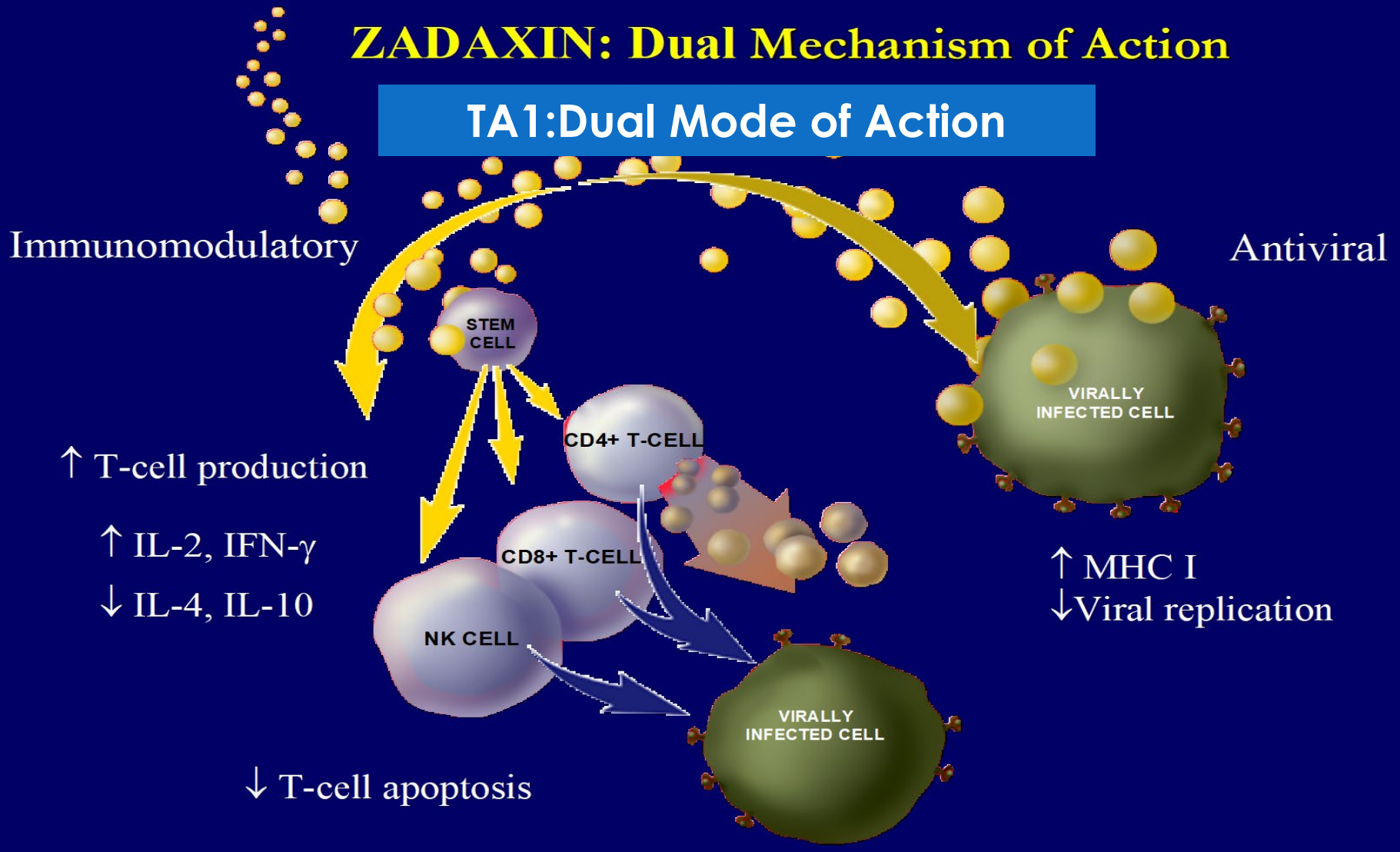
Thymosin alpha 1 (TA1)

- Clinical Effects[1,19,12,14,66]
  - Improved tissue repair and healing
  - Improved host defense to infection
  - Improved microcirculation
  - Improves stress tolerance
  - Inhibits viral replication or growth of cancer
  - Increases antioxidant and glutathione production
  - Reverse immunosuppression of CFS/FM/Lyme
  - Reduces inflammation
  - Anti-tumor effects
  - Prevents and treats autoimmunity

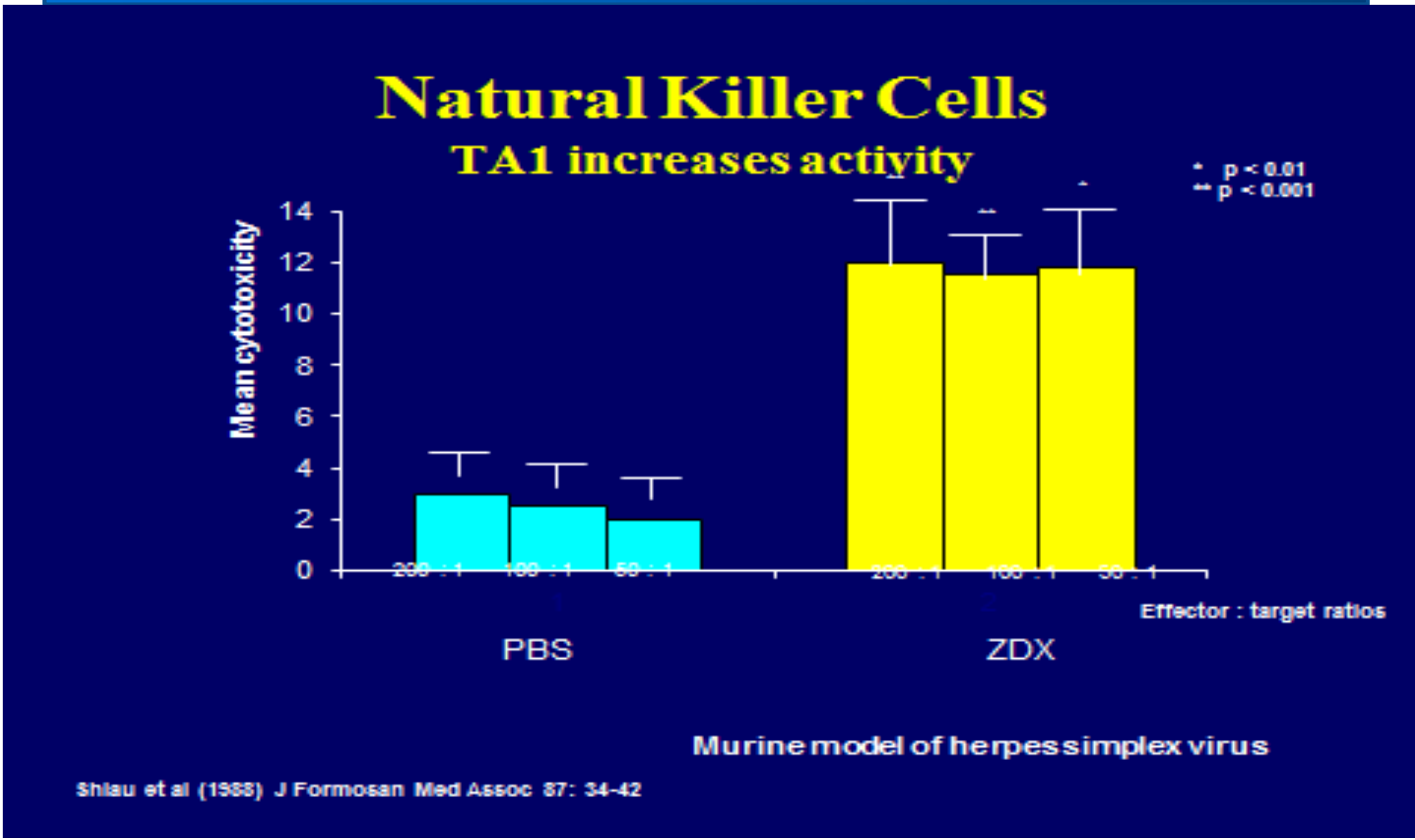
Thymosin alpha 1 (TA1)

- Potential target conditions
  - Lyme disease/HIV
  - Chronic viral or intracellular infections
  - CFS/Fibromyalgia
  - Autoimmune disease
  - Diabetes
  - Aging
  - Allergies
  - Chemical sensitivity
  - Cancer
  - Immune deficiencies
  - Prevention/Travel

TA1Effects


ZADAXIN: Dual Mechanism of Action
TA1:Dual Mode of Action
Immunomodulatory
Antiviral
STEM CELL
CD4+ T-CELL
CD8+ T-CELL
NK CELL
VIRALLY INFECTED CELL
VIRALLY INFECTED CELL
↑ T-cell production
↑ IL-2, IFN-γ
↓ IL-4, IL-10
↓ T-cell apoptosis
↑ MHC I
↓Viral replication

TA1Effects


Natural Killer Cells
TA1 increases activity
* p < 0.01
** p < 0.001
Mean cytotoxicity
0
2
4
6
8
10
12
14
200 : 1
100 : 1
50 : 1
200 : 1
100 : 1
50 : 1
Effector : target ratios
PBS
ZDX
Murine model of herpes simplex virus
Shiau et al (1988) J Formosan Med Assoc 87: 34-42

TA1 and Autoimmune Disease

- TA1 levels were checked in120 healthy controls (HC); 120 patients with psoriatic arthritis (PsA); 40 with rheumatoid arthritis (RA); and 40 with SLE.
- Found women significantly lower TA1 than men ($P < 0.0001$)
- Autoimmune patients had significantly lower TA1 levels than HC ($P < 0.0001$)
- Those on disease-modifying, anti-rheumatic drugs ((DMARD) had significantly higher TA1than autoimmune patients not on DMARDs, but lower than HC.

Pica E, Chimenti S, Gaziano, R, et al. Serum thymosin alpha 1 levels in patients wieht chronic inflammatory autoimmune disease. British Soc immunology: Clin & Experiment Immunology 2016;186;39-45

BPC-157

- Body Protection Compound-157 (BPC-157) is a pentadecapeptide (15 amino acid peptide) isolated from human gastric fluid.[BPC1-4]
- In particular, BPC-157 has displayed the unique ability to have significant rejuvenating properties throughout the gastrointestinal tract and proven to be an effective treatment for a variety of gastrointestinal conditions, including inflammatory bowel disease, leaky gut syndrome, diverticulitis, gastric reflux, irritable bowel syndrome, ulcerative colitis, Crohn's disease, persistent gastric ulcers, and stomach lesions.[BPC2,3,6-10]
- Ongoing studies have shown that BPC-157 promotes wound healing in bones, tendons, ligaments, musculature, the central nervous system, skin, and various other organs, essentially having a healing effect throughout the body.[1-4,11-20,24,26]
- It has been found to modulate pain and have novel regenerative, anti-inflammatory, gastroprotective and neuroprotective properties [BPC2,3,5]
- Huge therapeutic window. LD1 (lethal dose in 1% of population) has not been achieved in any study.
- Reverses hypocoagulability and hypercoagulability:
  - Prevents excessive bleeding and bleeding times due to a number of causes.
  - Counteracts hypercoagulability syndromes, rapidly destroying formed thrombi.
  - Reverses anticoagulant induced thrombocytopenia.

Clinical Effects of BPC-157

- Repairs the gastrointestinal tract and is shown to be more effective than H2-blockers (Zantac), proton pump inhibitors (omeprazole) and gastric coating agents (sucralfate)[BPC 2,3,7-10,32]
- Prevents and reverses inflammatory and autoimmune diseases, such as rheumatoid arthritis, Lupus and Hashimoto's [BPC 2,3,35]
- Promotes muscle, tendon, nerve and ligament healing and prevents and treats hyperalgesia [BPC 14,15,18,24,42]
- Promotes wound healing in the corneal epithelium [BPC 16,17,22,23]
- Increases serotonin and is shown more effective for depression than antidepressants. It is also shown to help to handle chronic and acute stress. [BPC 38]
- Oral and systemic administration significantly improves heart failure and hypotension (as well as hypertension).[BPC 43]
- Oral and systemic administration prevents and reverses stress urinary incontinence models in female rat. [BPC 44]

Clinical Effects of BPC-157

- Protects against numerous toxins, including alcohol, NSAIDS, Clostridium difficile toxin, mycotoxins (toxins from mold), neurotoxins, enterotoxins, and liver toxins (antibiotics, etc.) BPC 2,3,9,28,31,33,35
- Shown to be effective in traumatic brain injury, Parkinson's, Alzheimer's and multiple sclerosis BPC10,31,32,36
- Enhances the healing effects of growth hormone (increases GH receptors) BPC 37
- BPC-157 is shown to prevent and reverse a wide range of stimulated arrhythmias, including A-Fib, A-V Block, ventricular tach, premature atrial contractions and premature ventricular contractions, as well as other cardiac arrhythmias. BPC27,35
- BPC-157 outperformed acyclovir for the treatment of HSV infection in at 1/1000th the dose of acyclovir. BPC 41
- Counteracts HTN, normalizes esophageal and pyloric sphincters BPC 42
- Prevents and reverses mitochondrial damage from various toxins(8,9,20)

BPC-157

"Stable gastric pentadecapeptide BPC 157 is an anti-ulcer peptidergic agent, safe in inflammatory bowel disease clinical trials and wound healing, stable in human gastric juice and has no reported toxicity. Particularly, it has a prominent effect on alcohol-lesions (i.e., acute, chronic) and NSAIDs-lesions (interestingly, BPC 157 both prevents and reverses arthritis)… and acts as a free radical scavenger and exhibits neuroprotective properties."[42]

42. Sikiric P, Seiwerth S, Rucman R, et al. Stable gastric pentadecapeptide BPC 157: novel therapy in gastrointestinal tract. Curr Pharm Des 2011;17(16)1612-32

Oral BPC-157 for IBD and MS

- Oral BPC-157 was tested in two experimental models: one for inflammatory bowel disease and another for multiple sclerosis.
- Oral BPC-157 was found to be equally effective in the treatment of both models and multiple tissues. It was shown to have a variety of beneficial effects, including the reduction of inflammation, promoting wound healing, counteracting and healing of both gastrointestinal and central nervous system damage.
- Significant and dramatic effects were shown grossly, microscopically, functionally and behaviorally.
- This study validated oral BPC-157's usefulness as a therapeutic for both gastrointestinal and neurological damage.
- *"Its ability to be orally administered is particularly noteworthy, as there are currently no similar treatment options for inflammatory bowel disease, multiple sclerosis and other neurodegenerative diseases."*

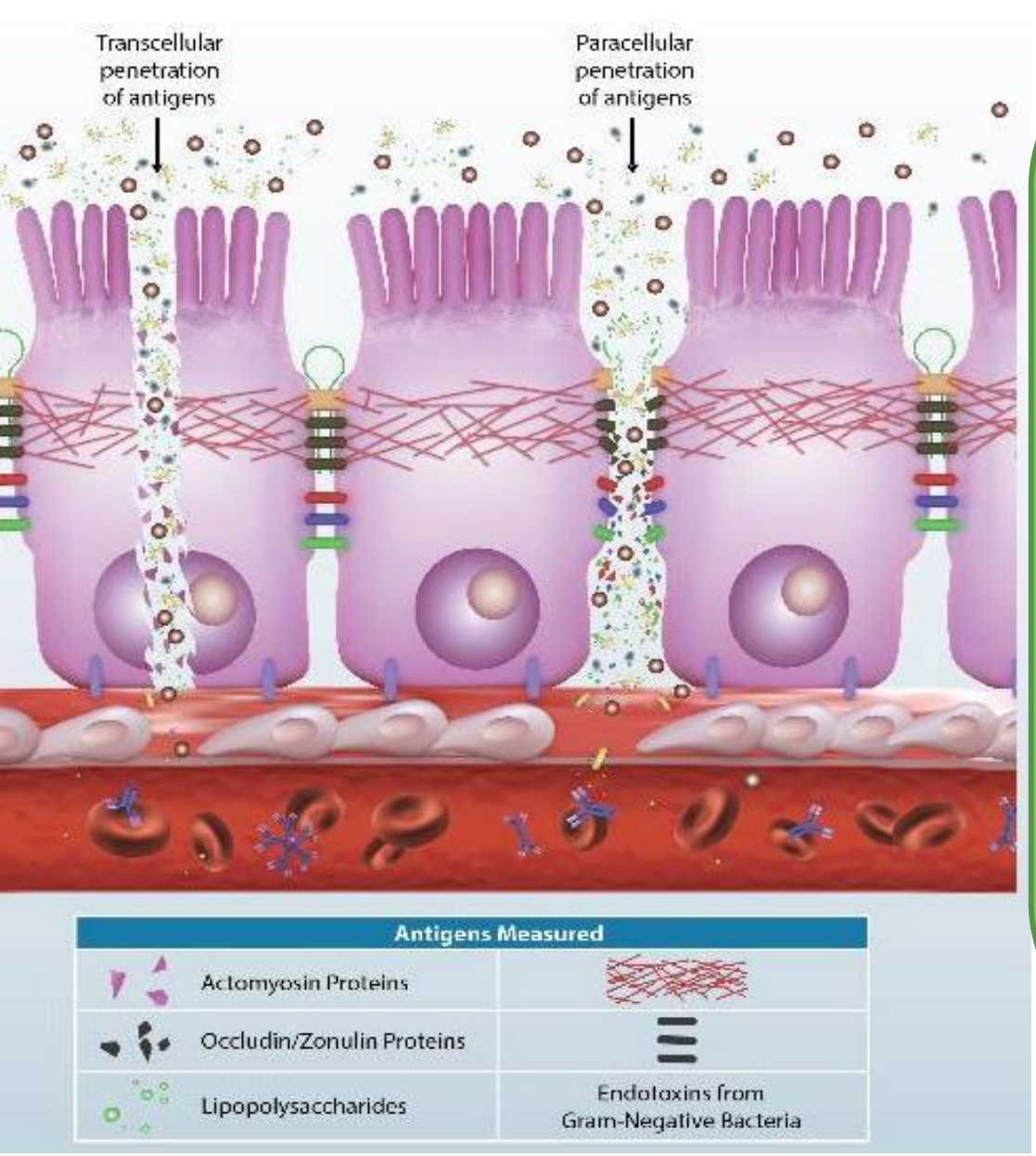
Klicek R, Kolenc D, Suran J, et al. Stable gastric pentadecapeptide BPC 157 heals cysteamine-colitis and colon-colon-anastomosis and counteracts cuprizone brain injuries and motor disability. J Physiol Pharmacol. 2013 Oct;64(5):597-61

Oral BPC-157 Improves Liver Function and Liver Regeneration

- **Aim:** "We suggest that stable gastric pentadecapeptide BPC-157 improves function and liver regeneration after 70% liver resection in rats since it can rescue otherwise severe liver lesions."
- **Materials and methods:** 70% liver resection was performed with macroscopic observation, as well as microscopic and biochemical analysis.
- **Results:** BPC-157 treated rats maintained their weight and exhibited 40% better liver rejuvenation and larger liver volume.
  - Normalized AST, ALT and bilirubin levels (confirming functional liver regeneration).
  - Microscopically, controls presented larger areas of liver steatosis necrosis and liver fibrosis, unlike BPC 157 treated rats.
  - Increased liver cell mitosis and rejuvenation
- **Conclusions:** BPC-157 rats maintained the weight, exhibited better liver regeneration and normalized serum LFTs

Crvenkovic D, Sever M, Sever AZ, et al. Pentadecapeptide BPC 157 After 70% Liver Resection in Rats. AGA Abstracts

BPC-157 for Leaky Gut and for Normalizing Gut-Brain Axis



- Numerous studies are showing the importance of healing leaky gut (increased intestinal permeability), the brain-gut axis and the microbiome in chronic illness
- BPC-157 is probably the single best treatment for leaky-gut and normalization of the brain-gut axis.
- Sikiric P, et al. Brain-gut Axis and Pentadecapeptide BPC 157: Theoretical and Practical Implications. Current Neurophamacology 2016;14:857-865

Graphic from Dr. Sam Rubar and Dr. Sandeep Gupta. SIBO, Leaky Gut and Mold Illness (Mold Illness Made Simple

AMP Overview

- AMPs serve as endogenous antibiotics that are able to rapidly kill an unusually broad range of bacteria, fungi, parasites and viruses.[76,80]
- Direct effects involve selective disruption of prokaryotic cell membrane[106]
- In addition to their direct antimicrobial activity, also have multiple modes of action, including immune modulating effects[106]
- LL-37 boosts mesenchymal stem cell migratory behavior and immunomodulatory effects.[77]
- Significant anti-biofilm activity at low levels[78,105]
- The MIC of LL-37 is much lower in vivo than in-vitro due to its immune modulating effects.
- AMPs are synergistic with antibiotics and reduce the development of drug resistance.[79]
- Effective against free borrelia spirochetes and cystic forms[76]
- Even low doses block the bioactivity of endotoxins and neurotoxins[109,110]
- Well-tolerated, very safe with little side effects and a large therapeutic window
- Caution with autoimmune disease at high doses, as with any immune modulator

78. Fuente-Nunez C, Hancock. Using anti-biofilm peptides to treat antibiotic-resistant bacterial Infections. Postdoc J 2015;3(2):1-8

AMP Mechanism of Action

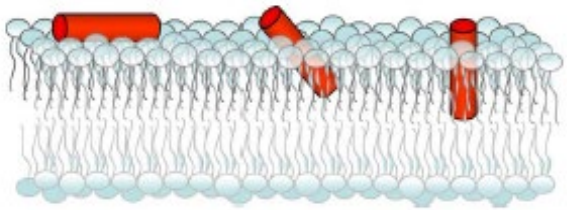


Fig. 2. Association of amphipathic α-helical peptides (cylinders) with a lipid bilayer can occur in three general orientations: parallel to the membrane surface, at an oblique angle, or perpendicular to the membrane surface (i.e., along the bilayer norma

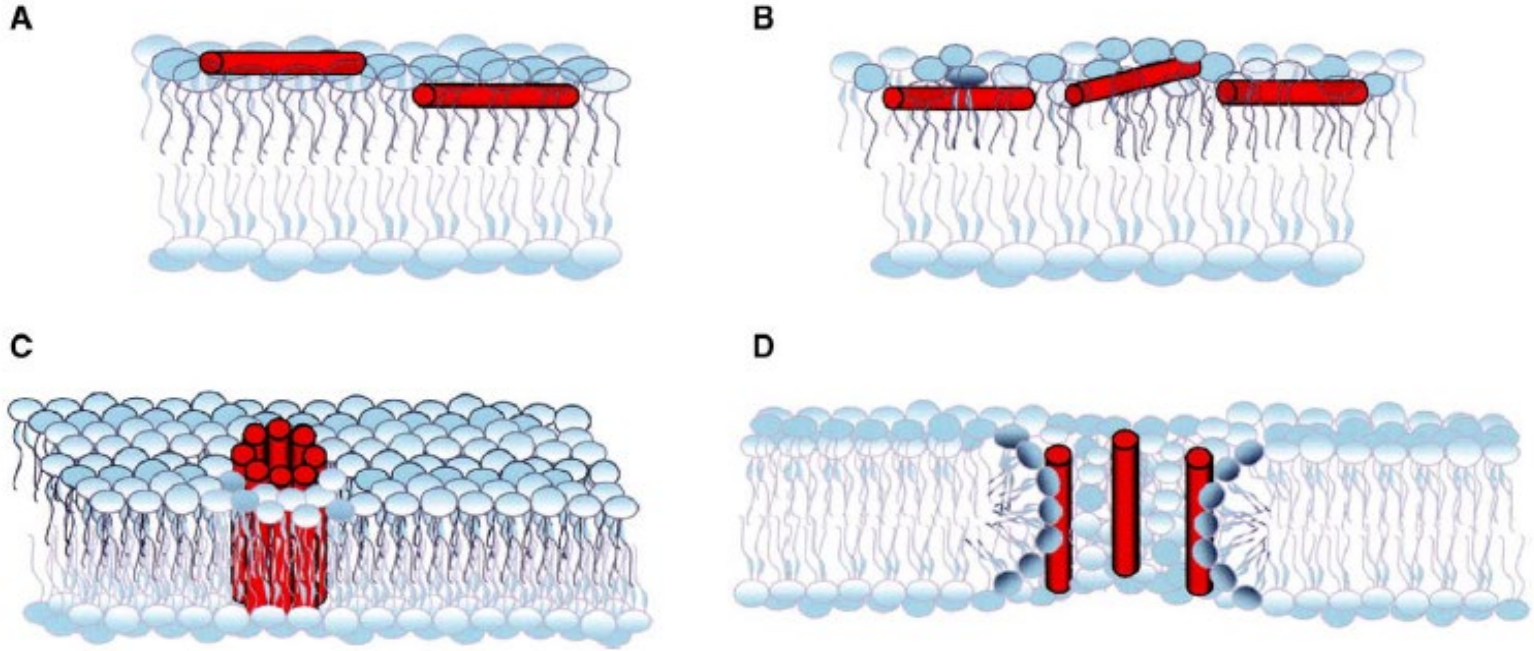


Fig. 8. Models of transmembrane channel formation. (A) Peptide α-helices (cylinders) initially associate parallel to the membrane surface, either superficially (left) or embedded just below the aqueous interface. (B) Peptides continue to accumulate at or near the bilayer surface, disrupting lipid packing and causing membrane thinning. This step may or may not involve peptide–peptide aggregation. Once a critical peptide/lipid ratio is reached, peptides either (C) insert into the membrane as a barrel-stave type pore, or (D) induce the localized formation of toroidal pores

106. Sato H, Feix JB. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic a-helical antimicrobial peptides. Biochim Biophys Acta 2006;1758(9):1245-56.

AMP-Rapid Onset of Effect (1 minute)

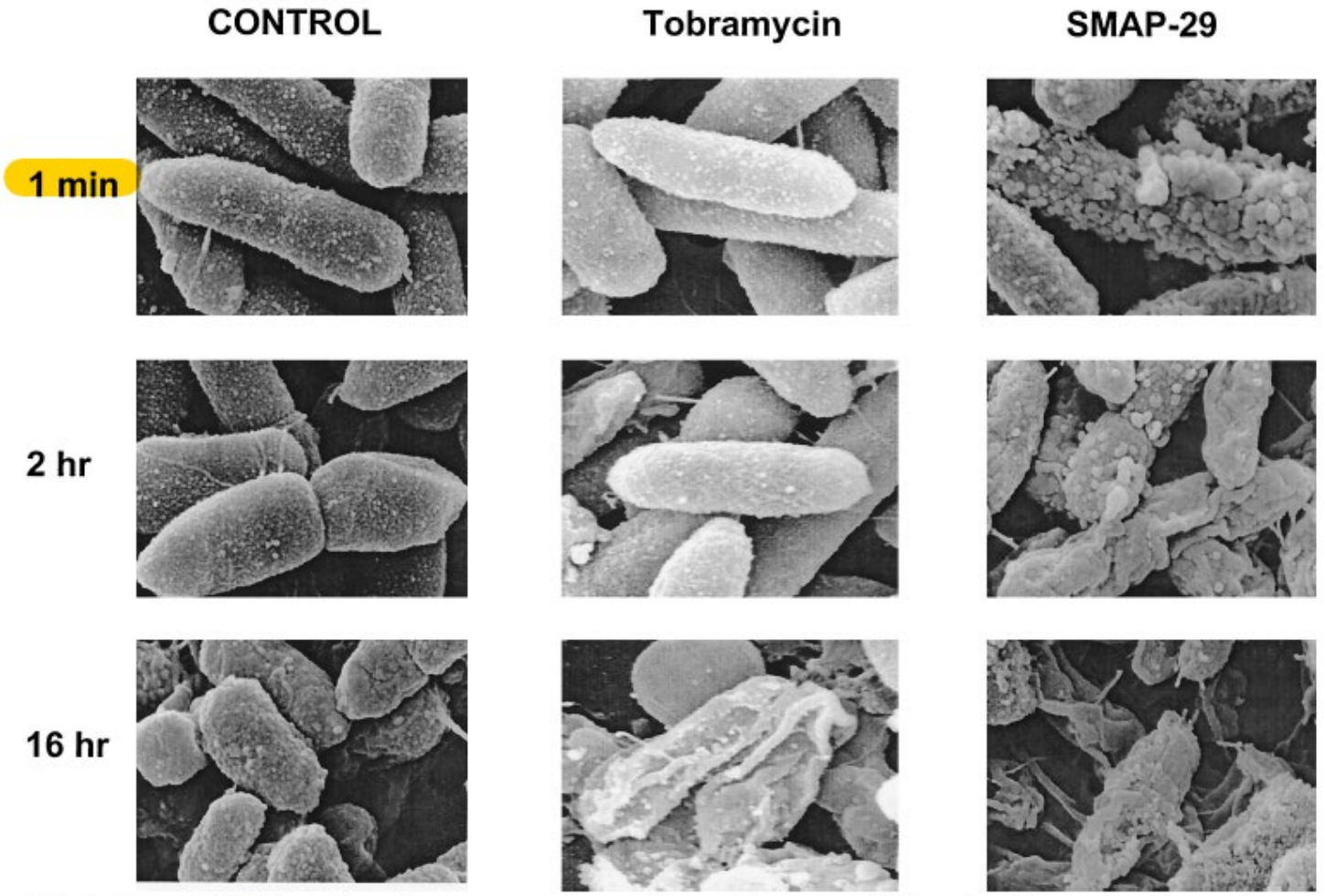
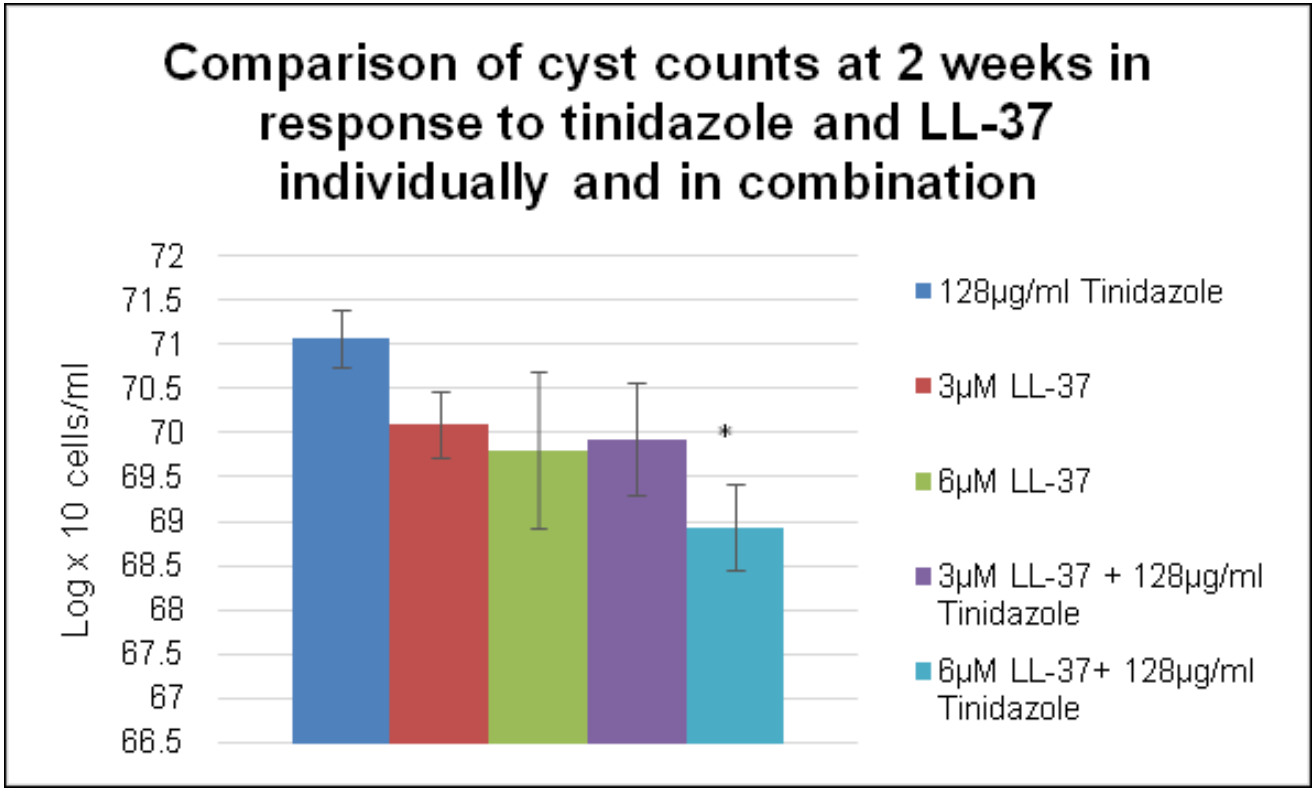


FIG. 3. Effects of SMAP29 or tobramycin treatment on the morphology of *P. aeruginosa* PAO1 evaluated by scanning electron microcopy. PAO1 was treated with media alone, tobramycin (5 μg/ml), or SMAP29 (0.5 μg/ml). At 1 min, 2 h, and 16 h the bacteria were processed for scanning electron microscopy. Within one minute, treatment with SMAP29 resulted in blebbing or blistering of the outer cell wall. The effects of tobramycin were much slower in their onset.

80. Sato H, Feix JB. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic a-helical antimicrobial peptides. Biochim Biophys Acta 2006;1758(9):1245-56.

LL37 for Lyme Disease (cystic form)



"Combining antimicrobial peptides with commonly prescribed antibiotics to treat Lyme disease may provide a new approach to the treatment of chronic infections due to the significant synergistic effect of a combination."

79. *Eckard A, Wood S. In-Vitro* Susceptibility of Different Morphological Forms of *Borrelia burgdorferi* to Common Lyme Antibiotics in Combination with Antimicrobial Peptides. J Microb Exp 2016;3(4):1-19

Peptides for Lyme

- Thymosin alpha 1 and thymosin beta 4
  - Immune modulation with reversal of TH1-TH2 shift
- BPC-157
  - Reduces inflammation and increases healing in most every tissue, including gut (probably best treatment for leaky gut), brain, skin, muscle, degenerative joints, cardiac (prevents and treats arrhythmia, & heart failure in Lyme myocarditis), neuropathic pain and protective against neuro and endotoxins.
- Semax/Selank
  - Improves brain function, memory, depression and anxiety
  - Sz treatment/prevention

Peptides for Lyme

- GHRH/GHRP
- Epithalon
  - Pineal peptide-increases longevity with significant reduction in CVD and cancer
  - Increases telomere length
  - Sleep
  - Boosts thymic function
- Delta Sleep-Inducing Peptide (DSIP)
  - Induces deep sleep and reduces pain
  - Stimulates the release of growth hormone
- LL-37
  - Effective against antibiotic resistant micobes
  - Can be use alone or with antibiotics for synergistic effects and to reduce resistance

Tβ4 and MSC Synergy

Cell Transplantation and Tissue Regeneration

Thymosin β4 Increases the Potency of Transplanted Mesenchymal Stem Cells for Myocardial Repair

Lei Ye, MD, PhD; Pengyuan Zhang, MD, PhD; Sue Duval, PhD; Liping Su, BSc; Qiang Xiong, PhD; Jianyi Zhang, MD, PhD

Background—Thymosin β4 (Tβ4) has been shown to enhance the survival of cultured cardiomyocytes. Here, we investigated whether the cytoprotective effects of Tβ4 can increase the effectiveness of transplanted swine mesenchymal stem cells (sMSCs) for cardiac repair in a rat model of myocardial infarction (MI).

Methods and Results—Under hypoxic conditions, cellular damage (lactate dehydrogenase leakage), apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labelingc cells), and caspase-8 activity were significantly lower, whereas B-cell lymphoma-extra large protein expression was significantly higher, in sMSCs cultured with Tβ4 (1 μg/mL) than in sMSCs cultured without Tβ4, and Tβ4 also increased sMSC proliferation. For in vivo experiments, animals were treated with basal medium (MI: n=6), a fibrin patch (Patch: n=6), a patch containing sMSCs (sMSC: n=9), or a patch containing sMSCs and Tβ4 (sMSC/Tβ4: n=11); Tβ4 was encapsulated in gelatin microspheres to extend Tβ4 delivery. Four weeks after treatment, echocardiographic assessments of left-ventricular ejection fraction and fractional shortening were significantly better ($P<0.05$) in sMSC/Tβ4 animals (left-ventricular ejection fraction=51.7±1.1%; fractional shortening=26.7±0.7%) than in animals from MI (39±3%; 19.5±1.7%) and Patch (43±1.4%; 21.6±0.9%) groups. Histological assessment of infarct wall thickness was significantly higher ($P<0.05$) in sMSC/Tβ4 animals (50%, [45%, 80%]) than in animals from MI (25%, [20%, 25%]) group. Measurements in sMSC (left-ventricular ejection fraction=45±2.6%; fractional shortening=22.9±1.6%; TH=43% [25%, 45%]), Patch, and MI animals were similar. Tβ4 administration also significantly increased vascular growth, the retention/survival of the transplanted sMSCs, and the recruitment of endogenous c-Kit$^+$ progenitor cells to the infarcted region.

Conclusions—Extended-release Tβ4 administration improves the retention, survival, and regenerative potency of transplanted sMSCs after myocardial injury. (***Circulation*. 2013;128[suppl 1]:S32-S41.)**

Key Words: angiogenesis ■ microsphere ■ myocardial infarction ■ stem cell ■ tissue engineering

Identification of Immune Dysfunction

- Low NK cell function < 30 LU
- Low CD 57 (LabCorp)
- Elevated C4a (Quest-Nat. Jewish on dry ice, ne)
- VEGF (increase with bartonella, suppressed by molds)
- Eosinophil cationic protein (Babesia—may only increase after treatment)
- ACE above 30
- Immune activation of coagulation (D-dimer, soluble fibrin monomer, prothrombin fragment 1+2, thrombin antithrombin complex, PAI-1) (Lyme/babesia)
- Low Igg subclasses (Secondary to TH1-TH2 shift (low IFgamma))
- Leptin above 12
- Elevated human transforming growth factor beta (HTGFB)
- Most any band on Quest WB, (even a 41 kd—selection bias on my part)
  - Provocate with antibiotics before doing indirect testing and/or treat the immune system with peptides before testing
  - Will know with a high probability who will or should test positive on further Lyme testing

Thank You

Questions?

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