LOW DOSE IMMUNOTHERAPY (LDI): A PROMISING TREATMENT FOR CHRONIC LYME DISEASE AND ME/CFS

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Note about the format: *I have used square brackets […] to differentiate material that is only my personal opinion, my elucidations, and conclusions. Material not enclosed in square brackets has been paraphrased or summarized from material in the bibliographical sources listed--without my personal input.*

ABSTRACT

Background: Conventional allergen-specific-immunotherapy (AIT) is a well-established treatment for a variety of environmental allergies that involves the administration of gradually increasing doses of allergen extracts over a period of years, given to patients by injection or sublingually. The effects of AIT leads to decreased disease severity, less drug usage, prevention of future allergen sensitizations, and a long-term curative effect. The aim of AIT is to induce long-term clinical tolerance against allergens, leading this way to a decrease of the over-reactive immune response and subsequent inflammation, both responsible for allergic symptoms. The induction of tolerance is mainly addressed by generation of allergen-specific T regulatory cells (Tregs), interleukin-10 (IL-10) and transforming growth factor beta (TGF-β); these key mediators promote the deviation of the chronic, established and pathologic inflammatory immune profile towards a more tolerogenic and anti-inflammatory response (i.e., a more proper balance among the responses Th1, Th2 and Tr1 is reached), thus ameliorating or eliminating the symptomatology.

Aside from allergy, there is extensive literature on the effectiveness of certain heterogeneous variants of the conventional AIT to treat a wide range of diseases in animals, and some positive reports in several conditions in humans observed in phase II trials, including a variety of autoimmune conditions and some chronic infectious diseases, such as multiple sclerosis, rheumatoid arthritis, Behcet's disease, inflammatory bowel diseases, or chronic HBV infection. However, this approach has yet to successfully translate to the clinic in phase III trials. Key factors for this translation will include the finding of more appropriate doses and routes of antigens (Ags) administration. There is not a standardized consensus on how to implement conventional AIT to diseases other than allergy, what might account for the mixed results observed so far. However, enzyme potentiated desensitization (EPD), low dose allergen therapy (LDA), and low dose immunotherapy (LDI) constitute variants of AIT that are not only standardized, but are also demonstrating encouraging results. Solid evidence has shown the effectiveness of EPD for a greater number of conditions than AIT has, with virtually no side effects, and promoting much longer lasting desensitization than conventional AIT. Compared with AIT, EPD uses much lower doses of Ags and employs β-glucuronidase as an adjuvant enzyme to enhance the tolerization effect. On the other

hand, as for the differences between EPD and LDA, although there are subtle differences between them, they can be considered similar approaches in practical terms and therefore, it seems that the same conditions which have shown to successfully respond to EPD, should as well improve with LDA. In the same vein, LDI constitutes a new and cutting-edge variant of LDA which, by using a wider range of Ags and more individualized doses, is exhibiting very hopeful results for many other non-allergic conditions. However, no formal data about LDA or LDI has been yet published.

Objectives: Many autoimmune diseases are initially triggered, at least in part, by microbes. Well-known examples of this, are the etiopathogenic links known to exist between *proteus mirabilis* microbes and rheumatoid arthritis or between *klebsiella pneumoniae* and both ankylosing spondylitis and Crohn's disease. In this context, LDI applied to autoimmune diseases and chronic infectious conditions, works under the rationale that molecular mimicry-mediated autoimmunity is the main underlying cause of their pathogenesis. Thus, under this paradigm, allergy may be considered as a failure of tolerance to harmless environmental allergens, while autoimmunity could be conceived as a failure of tolerance to self-Ags; therefore, as it is known to be possible to stop allergic reactions to environmental Ags through reinstating tolerance with conventional AIT, or with other forms of specific-antigen-based-immnotherapy, similarly, it should be plausible to do the same thing for certain autoimmune conditions and chronic infectious diseases, by using self-Ags or the appropriate triggering agent. Under these premises, the first goal of the current article is to compile evidence on how different approaches of AIT seem to work for these conditions, and to determine which characteristics are shared by those diseases which seem to successfully respond to these therapies. The final objective is to elucidate whether two specific conditions, i.e., chronic Lyme disease (CLD) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS), could be considered as good potential candidates to be treated with LDI.

Results: From the herein reviewed literature, it might be at least inferred that those conditions which have shown to successfully respond to either of the different AIT approaches that use much larger doses of Ags, might as well respond to LDA, including numerous autoimmune disorders, chronic inflammatory conditions and chronic infectious diseases. Likewise, the successful results obtained from clinical trials on EPD, could be extrapolated to some degree to LDI, taking into account the similarities between both techniques. On the other hand, those conditions for which distinct types of AIT have shown to be of benefit, share the following features: (1) chronic inflammatory conditions characterized by an ongoing immune activation; (2) immune deviation from the phenotype that would otherwise properly address the known/suspected trigger/s; (3) acquired molecular mimicry-mediated autoimmunity as an important pathogenic mechanism; (4) symptomatology thought to be a result of the ongoing immune activation, inflammation and related autoimmunity.

Conclusions: Although the relationship between CLD and ME/CFS has yet to be clarified, there are clear and key overlapping features between these conditions. Besides, there is extensive and compelling evidence showing that both CLD and ME/CFS are characterized by: (1) a state of ongoing immune activation; (2) an immune deviation from that that would properly address the trigger/s, thought to play a key role in the initiation and perpetuation of the condition; (3) autoimmune processes mediated by either molecular mimicry, hyper responsive T and/or B cells, or antibodies complexes (these autoimmune processes have shown in both conditions to be pathological and to correlate with the type as well as with the severity of symptoms); (4) a general agreement in the fact that symptoms are the direct result of the chronic inflammation and related autoimmunity. Taken all together, CLD and ME/CFS seem to present the common pathophysiologic characteristics to be considered as potential candidates to successfully be treated with LDI, thus corroborating the promising empirical results already reported by many doctors and patients.

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1. HYPERSENSITIVITY AND ALLERGY

1.1. IMMUNOPATHOGENESIS OF ALLERGIC REACTIONS:

The immunologic basis of allergic diseases is observed in two phases: (1) sensitization and development of memory T and B cell responses and IgE antibodies (early phase); (2) inflammation and tissue injury caused by effectors cells action (late phase).

In the process of sensitization, the allergen (any antigen (Ag) that causes allergic reactions) is recognized as dangerous, then it is taken up by a Dendritic Cell (DC) normally in the mucosae of the body, and it is then transported into the lymphatic nodes, where the DC presents the Ag to a naïve T cell (Th0) --coming from the thymus where it has matured--, and thus the Th0 becomes a Th2 effector cell, which will in turn become activated and stimulated to form millions of clones (process known as clonal expansion of allergen-specific Th2 cells), all of them, with the same specific receptor (note that for this specific antigen, also T memory and T regulatory cells will be formed at this moment). The Th2 effector and memory cells will eventually go out into the blood stream, ready to become further activated upon encountering for the second time the Ag they are specific for. These specific-Th2 cells will in turn promote both the formation of specific-B cells for the same Ag, and their further activation into Plasmatic Cells (PCs), which will synthesize soluble antigen-specific (AS)-IgEs that eventually will anchor on the surface of mastocytes (or mast cells) and basophiles. The engagement process of AS-IgEs antibodies (Abs) on mastocytes (or mast cells) and basophiles is known as sensitization. [In summary, an allergic reaction involves mainly Th2 effector cells, B cells, and sensitized mastocytes and basophils with AS-IgE Abs stuck on their membrane, all of which are "ready" to initiate a Th2 immune response when encountering the allergen they have been "trained" to fight against, so to speak. The mast cells, basophils and eosinophils keep the substances they will liberate in granules, "prepared and ready" to be released--hence the term "degranulation" of these cells upon activation].

Once the patient has been sensitized to an allergen, when he/she is exposed to that allergen for a second time, it gets "stuck" into the groove of the AS-IgE on the mast cell and/or on the basophils, which degranulate, releasing some substances (such as histamine or tryptase) which will eventually promote a positive feedback of this specific-Th2 response, including the activation and degranulation of eosinophils as well. Then, the circulating effectors Th2 cells are further activated by the mast cells and other signals, and these cells in turn will activate more B cells, perpetuating this way the Th2-inflammatory immune response. Aside from the pivotal role of the increased differentiation and clonal expansion of Th2 cells on the etiopathogenesis of allergy, it is necessary to highlight the role of Th1-cells as well, which induce the apoptosis of epithelial and/or smooth muscle cells; likewise it is also necessary to point out the important role of Th17 cells (and probably the Th22 as well) in the inflammatory process [I won't go into the details of the role these cells play because it is not required for the purpose of this article].

1.2. CLINICAL MANIFESTATIONS OF ALLERGY:

[Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. When this process becomes chronic, the inflammation becomes deleterious for the body].

The manifestations of Type I allergy (the result of the above explained activation of cells of the immune system and the severe inflammatory reactions they promote) can be very diverse, ranging from mild to severe forms and may occur locally and/or systemically, depending on the kind of allergen and on the part of the body where this process takes place, among other factors. [1, 2, 3, 4, 5]

2. ALLERGEN-SPECIFIC IMMUNOTHERAPY (AIT)

2.1. INTRODUCTION:

[Immune tolerance to allergens can be defined as establishment of a long-term clinical tolerance against allergens, which immunologically implies changes in memory type allergen-specific T and B cell responses as well as mast cells and basophils activation thresholds that do not cause allergic symptoms anymore].

In recent years, **induction of immune tolerance has become a prime target for prevention and treatment strategies for many diseases** in which dysregulation of the immune system plays an important role. **Allergen-specific immunotherapy (AIT) is a well-established treatment and is suitable for both children and adults for a variety of environmental allergies**, including pollen, pet dander, house dust mite, and venom allergies. It involves the administration of gradually increasing doses of allergen extracts over a period of years, given to patients by injection or sublingually. The **effects of AIT leads to decreased disease severity, less drug usage, prevention of future allergen sensitizations, and a long-term curative effect.**

The aim of AIT is to induce the peripheral T cell tolerance in order to modulate the thresholds for mast cell and basophil activation **and to decrease IgE-mediated histamine release**.

2.2. T REGS AND TOLERANCE:

The **induction of a tolerant state in peripheral T cells represents an essential step in AIT**. **Peripheral T cell tolerance** is characterized mainly by generation of allergen-specific T regulatory cells (AS-Tregs) and by decrease of Th2 and Th1 cells. It is **initiated by interleukin-10 (IL-10) and transforming growth factor beta (TGF-β)**, which are increasingly **produced by the AS-Tregs**, which are **known to be able to**: (1) diminish Th2 immune responses; (2) modulate the response of DCs by inhibiting their maturation and capacity of activation of T/B cells; (3) lower the response of mast cells, basophils and eosinophils--reducing this way the production of AS-IgEs and switch to the production of IgG4s and IgAs antibodies instead; and (4) directly inhibit mast cell degranulation. The **pivotal role of Tregs in inducing and maintaining immune tolerance** has been demonstrated during the last 15 years, during which their adoptive transfer was shown to prevent or cure several T-cell mediated disease models, including asthmatic lung inflammation, autoimmune diseases and allograft rejection.

There are two main Tregs subsets: "FOXP3+CD4+CD25+ Treg cells" (naturally formed in the thymus) and the "inducible-type-1 Tregs" (Tr1) (generated in the periphery under tolerogenic conditions). There are in turn 2 further subsets of inducible Tregs: "FOXP3+Tr1" and "IL10+Tr1". The two inducible Tregs subsets ("FOXP3+Tr1" and "IL10+Tr1"), play a key role in allergen tolerance.

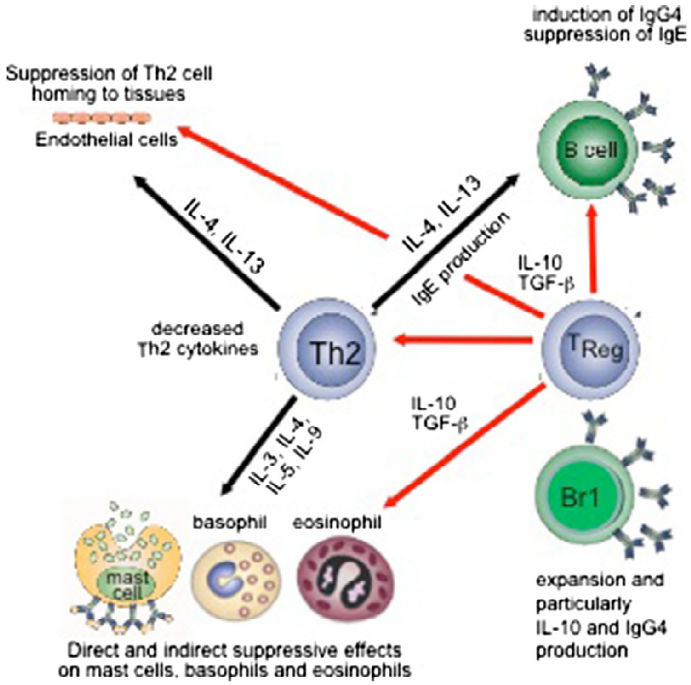
2.3. IL-10-PRODUCING REGULATORY B CELLS AND TOLERANCE:

Not only Tregs but **also B regulatory cells (Bregs) seem to play a role in inducing tolerance**. The "Human inducible IL-10-secreting B regulatory 1 cells" (10+Bregs) have shown to produce high levels of IL-10 and potently suppress AS-CD4+ T-cell proliferation. In addition, 10+Bregs showed to express IgG4 [the "allergy-protective" Ab isotype--explained later]. Moreover "IL-10-overexpressing B cells" showed a significant reduction in levels of pro-inflammatory cytokines (PICs) (TNF-α, IL-8, and macrophage inflammatory protein 1 alpha (MIP-1alpha)) and augmented the production of anti-inflammatory IL-1 receptor antagonist (IL-1RA) and vascular endothelial growth factor (VEGF). Furthermore, they secreted less IgEs and potently suppressed PICs in peripheral blood mononuclear cells (PBMCs); they also promote the regulatory [anti-inflammatory] phenotype of DCs.

2.4. MECHANISM OF IMMUNOTHERAPY:

Conventional Immunotherapy used for allergy (AIT) to different Ags (such as bee venom or cat allergens) **has shown to: (1) induce** the formation in humans of the two inducible **AS-Tregs** subsets ("FOXP3+Tr1" and "IL10+Tr1")--both by injection and by sublingual versions of the therapy--which play a key role in allergen tolerance; **(2) increase** the amount of **TGF-β**, **Bregs and AS-IgG1 and AS-IgG4 [allergy-protective Abs]** by 10-100-fold; and **(3) reduce** the production of: **AS-Th1 and AS-Th2 effector cells** (those involved in the allergy pathological vicious cycle), the levels of **AS-IgEs** (over the months or years, and after a transient increase), and the **mast cells and basophils granule content** and release of their inflammatory mediators (specially histamine and leukotrienes)--probably by reducing their threshold of activation. **Peripheral T cell tolerance is rapidly induced during AIT**. Decrease in serum IgE is relatively late and does not correlate with clinical improvement after AIT. Therefore early decrease in mast cell and basophil activity for degranulation and systemic anaphylaxis could be the first anti-inflammatory mechanism of AIT.

"ROLE OF T REG AND B REG CELLS IN THE SUPPRESSION OF ALLERGIC INFLAMMATION" (Akdis et al. 2015): [6]



IgG4 is thought to be protective against allergy--for instance by capturing the allergen before reaching the effector cell-bound IgE, thus preventing the activation of mast cells and basophils, and/or by inhibiting immune-complex formation by other isotypes. There are several other features of IgG4, which confer anti-inflammatory properties to this Ab isotype. *The evidence therefore supports the role of IgG4 in preventing the anaphylactic response not only due to its quantity but also to its blocking activity.*

IL-10 plays a key role in the induction of tolerance: IL-10-producing antigen-presenting cells (APCs), such as B cells and DCs as well as clonally expanded IL-10-producing allergen-specific Tr1 cells [AS-Tregs], all contribute to the suppressive effects of IL-10.

IL-10 down-regulates the expression of MCH-II molecules on APCs [In the context of the Type I hypersensitivity or allergic reaction, if there are less MCH-II molecules formed, there will be less Ags processed and presented by DCs and therefore less activated Th2 effector cells by DCs, what will further reduce the allergic reaction]. IL-10 also inhibits a wide range of PICs and cytokine receptors, reducing this way the degree of inflammation, and exerts inhibitory effects on activated monocytes and macrophages as well. Furthermore, IL-10 inhibits the differentiation, proliferation and activation of eosinophils [thus reducing their deleterious effects on tissues]. Besides, IL-10 regulates the allergen-specific antibody isotype formation towards a non-inflammatory direction [it promotes the formation of antigen specific IgG4 over IgEs].

TGF-β also bears a great potential in allergen-tolerance induction: It inhibits B-cell proliferation and differentiation and decreases the production of Abs by PCs--except mucosal IgAs--[what further reduces the allergic reaction, because less AS-IgEs will be formed]. TGF-β is **also essential**

for the maintenance of immunological self-tolerance. Thus, TGF-β induces the conversion of "naïve CD4+CD25− T cells" [Th0] into "CD4+CD25+ T cells" [Tregs] and it is also needed for their *in vivo* expansion and immunosuppressive capacity. [Both IL-10 and TGF-β display in addition important roles in tolerance induction at a genetic level --[what is beyond the scope of this text][.

In sum, the effects of allergen-specific immunotherapy (AIT) comprise very early effects related to APCs and adjuvants, desensitization of effector cells, antigen-specific immune tolerance induction in T and B cells, and regulation of IgE and IgG4.

2.5 AIT ADJUVANTS FOR PROMOTING T REGS FORMATION:

The role for DCs in the induction of different subsets of Tregs depends on the microenvironment: e.g., exogenous signals such as histamine, adenosine, vitamin D3 metabolites or retinoic acid can induce new populations of Tregs. In this regard, virus-like-particles as both, acellular Ag-presenting systems and as strong adjuvants are able to modulate the responses of AS-T cells [i.e.**, by injecting "viral-like-antigens" together with the specific allergy-causing-allergen, the type I hypersensitivity response can be reduced**. The rationale for this could be that a Th1 response needed to fight viruses is formed towards the Ag in question, thus preventing the characteristic infammatory-Th2 response known to occur in allergic reactions].

2.6. PEPTIDE IMMUNOTHERAPY:

In addition to conventional immunotherapy, peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. Treatment with selected epitopes from a single allergen resulted in suppression of responses to other [linked] epitopes within the same molecule. [i.e., **the same epitope can be shared by different allergens, what makes peptide immunotherapy a more efficient treatment, because the tolerance would be induced to all the Ags sharing the same epitope**].

2.7. IMMUNE RESPONSE IN HEALTHY INDIVIDUALS TO ALLERGENS:

As reviewed above, an allergic process is caused by an inflammatory-Th2 mediated response toward a harmless allergen. However, **is there any immune response in healthy individuals to these allergens?**: **Yes**; sometimes, there is a detectable immune response, **but in these cases it is mediated by Tr1 cells [Tregs] specific for common environmental allergens**. Actually **both healthy and allergic individuals exhibit all three, i.e. Th1, Th2, and Tr1 type allergen-specific subsets in different proportions. So it is the balance between Th2 and Treg cells what may lead to either allergy development or recovery** [i.e., there is an immune response that is suppressed by the Treg-mediated immunity, what prevents these subset of people from developing symptoms].

2.8. BREAKING OF TOLERANCE IN HEALTHY INDIVIDUALS:

Certain innate immune response signals and PICs break allergen-specific CD4+ T-cell tolerance in healthy subjects, which might lead to the development or exacerbation of allergic diseases after encountering microbes or under inflammatory conditions [meaning that **diseases associated with loss of tolerance might be acquired and triggered by infections and/or other inflammatory environments**]. From studies made in tonsils from healthy people, it seems that viral infections represent important candidates for breaking of allergen tolerance. [1, 2, 3, 6, 7, 28].

2.9. ALLERGEN IMMUNOTHERAPY FOR CONDITIONS OTHER THAN ALLERGY:

It is well established that mucosal administration, e.g. by the oral, sublingual or nasal routes, of many (but not all) Ags can induce peripheral tolerance, often referred to as oral tolerance, which is characterized by a decreased immune response to systemic immunization with the same Ag. **Oral (and nasal) Ag administration suppresses ANIMAL MODELS of autoimmune diseases including experimental autoimmune encephalitis, uveitis, thyroiditis, myasthenia, arthritis, and diabetes** in non-obese diabetic mice**, as well as non-autoimmune diseases such as asthma, atherosclerosis, graft rejection, allergy, colitis, stroke, and models of Alzheimer's disease**. In addition, other forms of Ag-based therapy (e.g, ligand epitope antigen presentation system (L.E.A.P.S.)) have also been successfully trialed for infectious diseases such as those caused by ***Influenza A virus***, ***Herpes Simplex Virus (HSV)*** **or** ***M. tuberculosis***. Despite the extensive literature on the effectiveness of oral tolerance to treat diseases in animals, and some positive reports in several conditions **in HUMANS such as in chronic HBV infection** or results observed in phase II trials, including **multiple sclerosis (MS), arthritis, uveitis, Behcet's disease, colitis, rheumatoid arthritis (AR), and Crohn's disease,** this approach has yet to successfully translate to the clinic in phase III trials**. The successful application of oral tolerance for the treatment of human diseases will depend on dose, developing immune markers to assess immunologic effects, route (e.g., nasal versus oral), formulation--e.g. it appears that protein mixtures may not be as effective oral tolerogens as purified proteins--, mucosal adjuvants, combination therapy, and early therapy.** It is imperative therefore to consider the full requirements for boosting the tolerogenic mechanisms induced by mucosal Ag exposure and improve protocols and regimens for oral tolerance induction and maintenance. [8, 9, 10, 11, 12, 13, 17, 18, 24]

DCs that directly interact with AS-T cells and induce autoimmune tolerance are known as tolerogenic dendritic cells (tolDCs), which bear great potential for the prevention and treatment of autoimmune disease. **Cellular therapy based on tolDCs is a novel and promising means to efficiently eradicate clinical symptoms and ameliorate immune responses in autoimmune patients** [12]. The underlying mechanism of oral tolerance induction depends on the dose of Ag administered, where, at least in preclinical models, **low repetitive doses favour the induction of Tregs** and high doses favour deletion or anergy of effector T cells. Suppressive cytokine production by Tregs (IL4, IL-10 and TGF-β) induces **bystander suppression to other (auto-)Ags presented in the close vicinity. This spreading of regulatory responses can even make it redundant to provide the primary Ag**, an advantage that could be for instance used in the context of **type I diabetes,** where several auto-Ags are enrolled in the autoimmune cascade, and the primary auto-Ag is not yet unambiguously identified [13].

In **HUMAN STUDIES**, **different forms of AIT have been shown to successfully treat a variety of autoimmune conditions, as well as some chronic infectious diseases**. For example, daily administration of the FDA-approved drug glatiramer acetate (GA) has shown beneficial effects on clinical course of **relapsing remitting multiple sclerosis**; GA is a mixture of synthetic polypeptides composed of four amino acids **resembling the myelin basic protein (MSP)** (much evidence suggests that MBP may be an auto-Ag candidate in MS [14]). Immunologic effects of GA therapy have been described, including: (1) phenotypic and functional changes in T cells and APCs which were observed as early as 4-12h after first GA injection--implying that GA induces rapid changes in immune cell effector functions; (2) immune deviation of CD4+ T cell responses from Th1 to Th2; and (3) induction of: CD4+CD25+FoxP3+ regulatory T cells [Tregs], oligoclonal cytotoxic/suppressor CD8+ T cells, immune modulatory Type II monocytes , and IL-10 producing B cells [15, 16].

Similarly, mucosal administration of peptide p331–351 of HSP60 conjugated to the non-toxic B subunit protein of cholera toxin (CTB) probed in a phase I/II clinical trial to **prevent relapse of uveitis in 5 of 8 patients with Behcet's disease** (BD) after all immunosuppressive drugs were withdrawn-- BD patients characteristically have what appear to be pathogenic T cells that specifically react with peptide p331–351 of HSP60. According to the authors, these results are consistent with the experimental findings that there is a critical threshold between naïve and memory T cells which may govern the ease

of tolerance induction: the greater the memory T cell pool the more difficult it is to induce tolerance. Control of uveitis and extra-ocular manifestations of BD was associated with: (1) lack of peptide-specific CD4+ T cell proliferation; (2) decrease in expression of Th1-cell-markers (CCR5 and CXCR3), IFN-γ and TNF-α production; and (3) decrease in CCR7+ T cells and co-stimulatory molecules (CD40 and CD28), as compared with an increase in all these parameters in patients in whom uveitis had relapsed [17].

In the same vein, the bacterial heat shock protein sequence (**dnaJp1**) has been proposed to be **cross-reactive** with corresponding self-peptides in **rheumatoid arthritis (AR)** because it is homologous with the HLA-DR Specific Epitope sequence. In a 2004 study, oral dnaJp1 had an excellent safety profile and **demonstrated immune modulatory effects**: whereas patients generally made Th1-type T-cell cytokines and proliferative responses to dnaJp1 at baseline, dnaJ-specific proliferation and IFN-γ decreased and IL-4 and IL-10 increased after the treatment. Likewise, in a placebo-controlled phase II clinical trial, the dnaJp1-treated group showed **statistically significant improvement** in *American College of Rheumatology scores* after 6 months of daily oral dnaJp1 peptide, associated with increased expression of regulatory molecules and decreased secretion of TNF in peripheral blood (PB) [18].

Additionally, induction of **oral immune regulation using colitis extracted proteins in Crohn's disease (CD)** subjects **showed successful results** in a small trial, includin (10/10 subjects had a favorable clinical response, and 7/10 subjects achieved clinical remission in 16 weeks; changes in CD4+/CD8+ lymphocyte ratio and peripheral NKT cell numbers as well as increase in serum IL-10 and IL-4 levels were associated with the improvements)[19]. Confirming these results, in 2006, a randomized, double-blind, placebo-controlled trial on administration of *Alequel*^{TM} (**an extract of autologous colonic protein-derived antigens**) to CD patients, found this type of oral immunotherapy to be **of benefit over the placebo group**. A decrease in the number of subject-specific, antigen-directed, IFN-γ spot forming colonies and an increased percentage of peripheral blood NKT cells, were only seen in the drug-treated-cohort who achieved remission [20]. In the same line, a phase II randomized, double-blind, placebo-controlled trial conducted by Israily et al. (2015), further analyzed the effect of the same autologous-Ag-mixture on CD. Again, they showed an increased ratio of CD4+/CD8+ T lymphocytes in subjects with a significant clinical response, compared with a decrease in the ratio in non-responders. As explained in this paper, the current understanding is that the primary presentation of the CD, is the result of a cascade of events and processes initiated by one or more Ags that remain unspecified. In the normal state, **low-level physiological inflammation of the gut is kept in check through an active process of immune tolerance, which might be lost in CD as evidence in humans points to an over-responsiveness and loss of tolerance of mucosal T-cells**. They further explain the concept of "**bystander suppression**" in which **regulatory cells induced by oral Ag administration can suppress immune responses stimulated by other Ags**, as long as the Ag is present in the anatomic vicinity. **The rationale for this is that regulatory cells induced by oral Ag administration secrete non-specific cytokines after being triggered by the fed Ag, and thus they can suppress inflammation in the microenvironment where the administered Ag is localized**. **Thus, it is not necessary to know the specific Ag that is the target of an autoimmune response in a human organ-specific inflammatory disease**, but rather to feed an Ag capable of inducing regulatory cells that then migrate to the target tissue and suppress inflammation. To conclude, the authors highlight--as noted by other already examined studies--that, while multiple mechanisms of tolerance are induced by oral Ag administration, **low doses favor active suppression**, whereas higher doses promote instead clonal anergy and clonal deletion [21].

On the other hand, oral immunotherapy has also shown to successfully treat chronic viral infections, such as HBV: **a vaccine composed by three surface Ags of the hepatitis B virus (HBV)** (HBsAg, PreS1, and preS2) **was given orally** every two days for 20 and 30 weeks, to 42 patients chronically infected with HBV. Induction of orally immune regulation toward viral proteins was found to be safe and **effective for amelioration of immune-mediated hepatitis in patients with chronic HBV infection**. This

effect was associated with augmentation of the antiviral immune response and with a change in the Th1/Th2 immune balance. Specifically, these improvements were confirmed by normalization of liver enzymes, significant decrease in viral load, decrease in viral surface Ags scores as well as by reduction of histological necroinflammatory score on the liver, and/or by improvement in serology markers (some patients became seronegative to HBeAg). The authors emphasized that this new concept of **oral immune regulation toward the virus, alleviated the disease, while leaving the general immunological defense of the recipient intact**. As for the explanation of the process that led to these changes, the authors state that the antiviral effect achieved in these patients was mediated by **augmentation of an anti-HBV Th1–mediated response along with a decrease in the Th2 response**, and explain that **this might have been achieved by: (1) induction of immune tolerance (oral immune regulation might have caused the removal of a deleterious T cell population (IL-10-producing), thus uncovering a more efficacious response (antiviral IFN-α producing)); (2) induction of immunity (oral immune regulation may have enhanced the effect of a beneficial subset of T cells toward the Ags that were administered); or (3) by a combination of both**. They also found a significant **increase in the number of NKT cells**--previously associated with the induction of tolerance. Interestingly the authors finally point out the importance of understanding that **pathology is not essential for the development of a protective response** [22]. These **good results of oral immunotherapy for HBV, have been also achieved by intramuscular injections of the hepatitis B vaccine**, *Sci-B-Vac™* (an aluminum hydroxide adjuvanted recombinant hepatitis B vaccine): repeated monthly I.M. injections of the *Sci-B-Vac™* co-administered with daily oral lamivudine treatment **can suppress HBV replication and lead to anti-HBs seroconversion in ~50 % of treated patients** [23].

2.10. CONVENTIONAL VACCINES: THE BEST KNOWN ANTIGEN-BASED-SPECIFIC IMMUNOTHERAPY:

[Different types of vaccines to different pathogens are broadly accepted as successful methods of "immune training" to prevent a strong reaction against newly encountered future infections. **Vaccines can be considered another form of immunotherapy, based on the inoculation of either the attenuated live pathogens or inert Ags from the microbes**]. The **general accepted mechanism induced by vaccines is as follows**: Non-live vaccines composed of protein Ags essentially activate innate responses at their site of injection, where DCs become activated and migrate toward secondary lymphoid nodes. At this location, vaccine AS-B cells are exposed to recently (<24 h) activated DCs and T cells that have up-regulated specific surface molecules. Next steps include: **(1) Antibodies protection**: This T cell help rapidly drives B cell differentiation into low affinity-Igs secreting PCs. Thereby IgG Abs appear in the blood a few days after primary immunization. Afterwards, follicular dendritic cells (FDCs) attract AS-B and AS-T cells and capture/retain the Ags for extended periods; at this point, AS-B cells undergo massive clonal proliferation. This results into the higher production of Abs of a higher Ag binding capacity. Then, acting as APCs, B cells process these vaccine Ags into small peptides that they display at their surface through MHC class II molecules which then become available for binding by follicular helper T cells (Tfh). **Interactions between antigen-specific-germinal-center (GC) B cells, antigen-bearing FDCs and antigen-specific Tfh cells result in the proliferation, survival and selection of B cells that have the highest possible antigen-specific affinity**. The peak of high-affinity-IgG vaccine Ab reaches within 4-6 weeks after primary immunization. Later on, some GC-induced PCs are attracted toward the bone marrow, where, unlike the rest of PCs that die soon, these PCs receive signals for long-term survival--vaccine Ab responses are deemed to wane and eventually decline below protective thresholds, unless repeat Ag exposure reactivates immune memory; accordingly the duration of Ab responses is proportional to the number of long-lived PCs generated by immunization. **(2) Memory B cells:** are generated in response to T-dependent Ags, during the GC reaction, in parallel to PCs (they do not produce Abs unless re-exposure to Ag drives their differentiation into Abs-producing PCs). **Memory B cells** undergo affinity maturation during several (4–6) months, and **may thus be recalled by lower amounts of Ag** and without CD4+ T cell help. In accordance, at priming, **lower Ag doses may**

preferentially drive the induction of memory B cells over PCs, whereas Ag persistence may reactivate or favor the persistence of memory B cells --which are of utmost importance for long-term vaccine efficacy (memory B cells can survive for decades even in the absence of re-exposure to Ag). **(3) T-cell response**: **All vaccines**--except polysaccharide antigens-based, **induce CD4+ T cells, e.g., Th1 and/or Th2 cells that essentially support the differentiation of B cells (Th2) or of CD8+ T cells (Th1)** (Ags are processed into small fragments and displayed at the cell surface in the grooves of MHC molecules (CD4+ T cells recognize antigenic peptides displayed by class II MHC molecules, whereas CD8+ T cells bind to class I MHC-peptide complexes); Th1-type CD4+ T cells essentially produce IFN-γ and TNF-α, participating to the elimination of intracellular pathogens both directly (cytokine responses) and indirectly via their support to macrophage activation and CD8+ T cells differentiation. On the other hand Th2-type CD4+ T cells essentially produce IL-4, IL-5 and IL-13 which are directly implicated in the defense against extracellular pathogens. Both Th1 and Th2 cells support B cell activation and differentiation during extrafollicular responses, whereas follicular (Tfh) CD4+ helper T cells provide help to GC B cells). **Lower vaccine doses have being classically associated with preferential Th1 responses,** although the main determinant of CD4+ T cell differentiation is the extent and type of DC activation by the innate system. **(4) Tregs vaccine-induced formation**: **vaccines may also elicit regulatory T cells (Tregs)**--in a Ag-specific manner--**of which CD4+CD25+ Treg cells and type 1 regulatory T (Tr1) cells are the best characterized**. CD4+CD25+ Treg cells potently suppress the proliferation and IFN-γ production by both CD4+ and CD8+ T cells, probably by direct cell-to-cell contacts and inhibition of IL-2 production. Tr1 cells **produce high levels of IL-10 and TGF-β, which mediate their suppressive function in both Th1- and Th2- responses.** Tregs are induced by DCs that capture Ags in the absence of danger signals [what might occur when lower doses of Ags are inoculated] and thus remain immature during their migration to lymph nodes. This lead Th0 cells not to differentiate into effector but into regulatory T cells. **These Tregs play essential roles in preventing autoimmune diseases as well as allergic responses. 5. Memory T cells**: Effector T cell responses are short-lived, and most (>90%) die of apoptosis within a few days. Thus, **immune memory is essential to T cell vaccine efficacy. Memory T cells may persist life-long even in the absence of Ag exposure**. (There are two types of memory T cells identified (Effector memory cells (Tem), which traffic through non-lymphoid organs and have a high cytotoxic potential, and central memory T cells (Tcm) which preferentially traffic through lymph nodes and bone marrow and do not exhibit much cytotoxic capacity, but have a high proliferative potential)). **Effector memory cells become preponderant when Ag persists, such as in chronic infections** [24].

[**Taken all together**, there seems to be **evidence for high doses of peptide-based-Ags from infective pathogens used in most vaccines to promote in the long term the development of immune memory**, including B and T memory cells. Furthermore, **IL-10 and TGF-β secreting Tregs are also formed in these high doses**. Interestingly, it has been shown that **lower doses of the Ag promote a Th1 response over the Th2 and also induce the formation of T/ B memory cells and Tregs**. Moreover, these **more tolerogenic profile of immunity seems to be associated with repeated exposure** to the Ag in lower doses. The bottom-line would be that **by using lower doses of Ags of pathogenic microorganisms, and by exposing the body to repeated doses, it seems the immune system actually can be deviated towards an anti-inflammatory and more tolerogenic immune signature**].

3. ENZYME POTENTIATED DESENSITIZATION (EPD)

3.1. INTRODUCTION:

Enzyme Potentiated Desensitization (EPD) is a method of immunotherapy developed by the clinical and academic allergist, Leonard M. McEwen, M.D., in England in the mid 60's. The method involves desensitization with combinations of a wide variety of extremely low dose allergens (10^{-14} to approximately 10^{-7}, or 1 part in 100 million to as low as 1 part in 1 quadrillion) [25, 26].

3.2. COMPARISON OF EPD TO CONVENTIONAL IMMUNOTHERAPY:

Conventional immunotherapy (AIT) uses much higher doses in comparison to EPD, which are being increased over time (from 1/1000 up to 1/100 for example). The **reason for this high doses could be that the effect of AIT is mainly achieved by raising the levels of IgGs which exert a blocking effect**--[so, the higher the dose, the higher the IgGs]. This **high dose often exerts intolerable swelling and other side effects** before clinical efficacy can be attained, as explained by Dr. W.A. Shrader (Board-certified in environmental medicine and director of the Santa Fe Center for Allergy & Environmental Medicine in Santa Fe, New Mexico, USA) [25, 27].

According to the "International consensus on allergy immunotherapy" (World Allergy Organization, 2015) [28], adverse reactions associated with AIT can be local or systemic, affecting up to 82% of patients receiving subcutaneous immunotherapy and 75% of patients receiving the therapy sublingually. In this publication the rate of fatalities per year is estimated to be around 1 in 2 to 2.5 million injections.

Dr. Shrader also points out that most deaths are due to anaphylaxis, because of the high dose of Ag used, what would not occur with EPD because the doses used are at least 10 million times less than the standard maintenance dose for conventional immunotherapy. In addition, he explains that **EPD immunotherapy can produce much longer lasting desensitization than does conventional immunotherapy [AIT],** with treatments lasting as long as 1-5 years.

In addition, EPD **uses allergens together with β-glucuronidase, an enzyme that increases the immunizing effects** of the allergens and acts directly on T-suppressor cells (it appears that this enzyme has the capacity of activating Tregs), apparently inducing a longer lasting desensitization than does any type of previously known immunotherapy [25, 29, 30, 31].

3.3. MECHANISM OF ACTION OF EPD:

While conventional immunotherapy switches the T cell response to allergen from Th2 to Th1, EPD appears to [more] specifically **induce the production of "activated" T-suppressor cells [Tregs]**--which turn off the T-helper cells that are acting inappropriately and producing the symptoms of allergy--by inducing the maturation of DCs. EPD also **redirects the cytokine network of type 2 helper T cells**; in this respect EPD has shown to **lower the inflammatory cytokine IL-6 and to raise the anti-inflammatory cytokine IL-10**. Moreover, EPD also showed to **raise the number of CD8+T cells while lowering the AS-IgEs** [25, 27, 32]. Because lymphocytes have a half-life of 12-16 weeks, the overall activity [of LDA by means of Tregs induction] appears to be long-lasting; therefore, the greater the number of activated Tregs in circulation, which is cumulative, the longer-lasting will be the response [31].

[EPD would therefore be different to AIT, in that it does not just switch the pathologically activated Th2 response into a "non-responsive to allergens" Th1 reaction, but rather it would deviate the inflammatory Th2 response into a Th regulatory predominant response, which is integrated by components of the anti-inflammatory Th2 profile (characterized by IL-10 and TGF-β); moreover, the

increase of the T-CD8+ cytotoxic cells implies a concomitant improvement of a proper Th1-like response--this process of immune response deviation towards a less reactive and inflammatory type, might also comprise changes in the Th17 response, probably towards its tolerogenic phenotype].

3.4. EPD IMMUNOTHERAPY INDICATIONS; THE AMERICAN EPD STUDY, 1993–2000:

In 1993, the Investigational Review Board of the Great Lakes Academy of Clinical Medicine approved and formally supervised a multi-centre investigation of EPD treatment in the USA, which ran **for seven years (1993-2000)**. **The paper summarizes the results of treatment with EPD of 10,372 patients for various conditions during these period of time**, by members of the American EPD Society (AEPDS)--a group of over 60 physicians specifically trained to administer EPD immunotherapy. This is **the largest outcome-based study of any type of immunotherapy ever undertaken**. At the end of this audit, a "white paper" was presented to the US Congress [33]. This study reported the **successful effects of EPD on 65 different conditions**, **including hay fever, dust mite allergy, perennial rhinitis, asthma, urticaria, eczema (dermatitis) of most all varieties, angioedema (swelling of the face, lips, etc.), food allergy or intolerance, multiple chemical sensitivity, ADHD (attention deficit/hyperactivity disorder), autism, Tourette's syndrome, irritable bowel disorders, Crohn's disease, ulcerative colitis, migraine and other headaches, rheumatoid arthritis, ankylosing spondylitis, or systemic lupus erythematosus**. The study concludes: "(...) The 'overall' response showed that 20% of patients reported excellent, 30% reported very good and 26% good, 14% reported fair and 8% reported no change. 2% of patients felt they were worse after receiving EPD than they had been prior to starting EPD. (...) **Although every condition evaluated in this study did not necessarily appear to respond dramatically to EPD immunotherapy, most responded quite favorably**. Most importantly, a large number of conditions which do not respond at all to conventional immunotherapy--and many which do not respond well to any type of therapy--appear to have responded to EPD. (...) At the end of this 7-year study of 10,372 patients who received at least 175,000 injections of EPD, the physicians **who participated in the trial conclude that the healing and health potential of EPD for use to treat allergy and autoimmune disease is immense.**(...)". [34, 35]

3.5. DOUBLE-BLINDED PLACEBO-CONTROLLED STUDIES:

The effectiveness of EPD has been reported in numerous placebo-controlled studies, all of which found statistically significant improvement of the EPD treatment group over the placebo group [25]. For instance, EPD demonstrated to be an effective treatment for **summer hay fever** in a double-blinded placebo-controlled study among 44 patients in 1990 [36]. Two years later, another double-blind placebo-controlled trial among 40 children with food-induced hyperkinetic behavior disorder found EPD useful to treat **food-induced hyperkinetic syndrome** [37]; the authors explained that EPD permitted these children to eat foods that had previously been identified as responsible for their symptoms. Later on, in 1994, the results of a double-blind clinical trial with 54 patients with pollinosis found--through immunohistochemical techniques--humoral and cellular immune modifications in a time frame of 6-8 weeks; the **authors concluded that these results seemed to confirm Dr. McEwen's hypothesis of EPD being more powerful and displaying a different mechanism of action than that of the classical immunotherapy** [38]. These conclusions had also been corroborated in a 21 subjects double-blinded study which concluded that EPD showed both good clinical efficacy and an excellent tolerability for the treatment of **pollinosis** [39]. Later on, and again supporting previous findings on EPD for pollinosis, another double-blind placebo-controlled trial among 20 patients sensitive to **parietaria and grass pollen**, not only found improvements in symptoms scores, but more importantly, also **demonstrated an increase in the mean percentage of TCD8+** PB cells and a significant post-seasonal **decrease in the mean percentage of Parietaria specific IgE**, what they explained was **proof of EPD-induced enhancement of tolerogenic mechanisms** [32]. Additionally , in 1996 the authors of a double-blind study made on a group of 35 children allergic to grass and 27 allergic to pteronyssinus and farinae

dermatophagoides verified the efficacy and tolerability of EPD in patients affected by both seasonal and non-seasonal allergies [40]. More recently, and further corroborating the good results of EPD for allergy, another double-blind randomized trial on 67 children, published on 2006, concluded: "(..) this study shows that double-dose ß-glucuronidase immunotherapy is safe, well tolerated, and efficacious in the treatment of allergic children (...)" [41].

[Please note that this is not a comprehensive review of the literature on EPD immunotherapy, but a compilation of some of the available evidence on the therapy to support the fact that EPD, being a "version" of the conventional AIT, has shown to work even more effectively than AIT, at least for some conditions, with less side effects, and seems to be a promising therapy for a wider range of conditions not just limited to allergic diseases].

3.6. CURRENT STATUS OF EPD:

EPD was used in the USA under the Investigational Review Board (IRB) from November 1993 to November 1998. When the IRB expired in 1998, delays were encountered in the submission of an Investigational New Drug (IND) proposal with the FDA, which was finally submitted in the fall of 2001 by the manufacturer of EPD (*McEwen Laboratories*, LTD, England) in an attempt to obtain its approval. The FDA banned EPD immunotherapy in the United States in April of 2002, pending approval of the drug, and placed EPD on their Import Alert List. Until early in 2004, the FDA was allowing some personal importation of EPD by patients, but importation has now been severely restricted and **EPD is no longer available under any circumstance in the USA**. **EPD is used in Canada, Europe and several other countries** [42, 43].

4. LOW DOSE ALLERGEN THERAPY (LDA)

Once the FDA closed EPD down, **Dr. Shrader created a therapy similar to EPD, called low dose allergen therapy (LDA)**, which has been used in the USA and Canada since 2002--LDA is compounded legally in the US by *College Pharmacy* [44, 45]. As stated by Dr. Shrader, **LDA formulation uses the same active components as EPD, but it has many more pollens, foods, chemicals, and other allergens (about 300).** Thus, LDA also contains the enzyme β-glucuronidase as well as stabilizers. However, **the prime difference between EPD and LDA is the absence of bacteria. Instead, LDA uses specific [dead] bacterial Ags with the main goal of treating autoimmune diseases that appear to be caused by molecular mimicry. These Ags-mixtures include *proteus, klebsiella, bacteroids, streptococcus* and *yersinia*, which are known to be related to the pathogenesis of numerous conditions such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, regional ileitis, ulcerative colitis, myositis, inflammation of tendons and connective tissue, intestinal cystitis, idiopathic thrombocytopenic purpura, and in general, reactive arthritis, PANDAS, Hashimoto's thyroiditis or psoriasis.** [31] [Throughout the next sections I review some of the specific papers linking these bacteria and others with some of these conditions].

Dr. Shrader also notes that, although no formal data about LDA has been published as yet, 10 years of experience with it by over 90 physicians indicates that **the same conditions that responded to EPD respond similarly to LDA** [26, 31]. Dr. Stephen Smith (Northwest Integrative Medicine, Washington), explains: "(...) LDA is so effective because it contains all of the major allergens found in the United States and Canada and treats the major allergens and many subclinical allergens as well. (...) The different types of LDA treatments can be mixed and matched. For example, you may only need an inhalant treatment, or you may need an inhalant and food treatment or all three. (...) LDA has worked well for many people for whom standard immunotherapy has failed. (...) One of the great advantages of LDA is its ability to reduce chemical sensitivities (...) [46].

5. LOW DOSE ALLERGEN IMMUNOTHERAPY (LDI)

5.1. LDI AND DOCTOR TY VINCENT:

During a set of **3 recent live-radio interviews** (on "AM-Impact On Your Health" show) conducted by Dr. Dennis Courtney to **Dr. Ty Vincent** (Medical Director of "Mat-Su Integrative Medicine, LLC", Alaska), Dr. Vincent talks about his large experience with LDA [47, 48, 49]. He was also **interviewed on august 28th, 2015, by Dr. Neil Nathan** (Gordon Medical Associates, California) in his radio show "The Cutting Edge of health and wellness today" [50]. More recently, Dr. Vincent gave a **webinar on LDI** [51] as well as two lectures, presented at the **conference "LDA: A new treatment option" which took place on October the 4th, 2015, in Miami (FL)** [52, 53]. Below I summarize the most relevant outcomes and information from these events/sources.

[Note that Dr. Vincent refers to LDA when using the standardized mixtures of Ags that come from the College Pharmacy, opposed to LDI (Low Dose Immnunotherapy), term that he applies to the other Ags-mixtures he prepares and uses for a wider variety of conditions, most of them non-allergic, hence the reason to change the term "allergens" for "antigens". LDI is actually a wider term that comprises LDA as well as the use of many other Ags-mixtures, not used by LDA].

5.1.1. AUTOIMMUNE DISEASES AND MOLECULAR MIMICRY:

At the conference "LDA: Ultra Low Dose Enzyme Activated immunotherapy" (October the 23th, 2014, Albuquerque, NM, USA) [54], Dr Ty Vincent had already explained his view on autoimmune diseases. According to his clinical experience and research, he presented the following key points on the subject: "(...) Many **autoimmune diseases are initially triggered, at least in part, by microbes**. (...) However, **eradicating the microbe may not stop the disease, which is now self-targeted** (...) **Desentitization to the initial microbial trigger (if possible) does stop the disease** (...)".

Throughout the set of the 3 interviews, Dr. Vincent's further elaborated on this subject, saying that he believes most (if not all) autoimmune diseases begin with an immune reaction to "something else" that is not "you" and is not "self", and then evolves into an immune response which cross-reacts with self. He goes on noting that **any chronic inflammatory condition labeled as autoimmune or otherwise, probably has a trigger, that could be "the patient themselves", or something not human, that can live in the gut, urinary tract, sinuses or anywhere else. Thereby, if you take that trigger you can use it to desensitize the patient towards that Ag through LDI, making the inflammatory reaction to go away, and therefore the symptoms.** [He notes you can either know the substance and isolate it, or just process an [autologous] sample of the patient where that trigger might reside, and work with the Ags of that sample the same way, without knowing the specific trigger, because you are in anyway desensitizing the patient to many Ags at once, among which the trigger is probably found--e.g., he uses stools as a raw material, for conditions such as Crohn's disease (CD), because the possible trigger very possibly resides in the gut--note that there is published evidence on the successful usage of autologous colonic extract to treat (CD), as depicted above [19, 20, 21]]. Dr. Vincent goes on explaining that this [**molecular mimicry-mediated autoimmunity**] is not actually new, and puts the **example of rheumatoid arthritis (RA) and the known relation of this condition with the *proteus mirabilis* microbes** in genetically susceptible individuals *[see section 6.2 for further details];* he explicates that this **is a well known case of cross reactivity, where the immune system has been "trained" to attack the pathogen, but it ends up damaging the joints because the Ags of these microbes the immune system recognizes, are very similar to those of some of our own cells, and so, the immune cells attack our own tissues causing the symptoms and signs of RA**. He brought up as an example that FDA has minocycline approved for RA because of this phenomenom--although it doesn't work very often, as he remarks--. He finally states that **LDA has about a 90% of success in treating RA** by administrating these pathogens, like a dead sterilized extract, in the same way that it is done with allergens [either with LDA or with conventional immunotherapy (AIT)].

In the same vein, **Dr. Nathan**--in the more recent interview he made to Dr. Vincent-- also talked about the experience he had with LDI. He explained that **the same cross reactivity phenomenon found in some patients with rheumatoid arthritis and the *proteus* bacteria, is also well known for ankylosing**

spondylitis [*klebsiella pneumoniae* has been found to be the possible trigger of the disease] and for ulcerative colitis and other Inflamatory Bowell Diseases (IBD) as well, in which *bacteroids* have been identified to play a key role in their pathogenesis [find more scientific details on this below]. Dr. Vincent explained why **you can actually give millions of Ags at once with LDI, with no risk at all**--like it is done for instance when stools are used as the source of the Ags; he explained that the immune system is already tolerant to most of those Ags, and therefore it won't react to them.

Safety of antigens used in LDA/LDI: Dr. Vincent remarked at the conference in Miami that the Ags used for LDI follow the **ATCC standards**, **thus guarantying that the Ags are dead**--the technique utilized, destroys the DNA of bacteria, while leaving intact the antigenic proteins.

5.1.2. TOLERANCE VS. DENSENSITIZING:

At the lecture from 2014 [54], Dr. Vincent explained that, because **allergy is a failure of "tolerance" to harmless environmental allergens, and autoimmunity is a failure of tolerance to "self"-Ags, it is possible to stop reactions to environmental Ags through reinstating tolerance with LDA immunotherapy, and in the same way, it is possible to do the same thing using "self"-Ags, or the appropriate triggering agent**.

5.1.3. LYME AS AUTOIMMUNE: NEW LYME PARADIGM:

Dr. Vincent, in April 2014, as exposed in his presentation, frustrated with the poor success of conventional Lyme treatments, decided to try a completely different treatment approach. **He wondered if Lyme Syndrome was not really the "result of an infection", but rather it had more of an "autoimmune disease",** and working under such assumption, he investigated what would happen if you "turn off" the specific immune response to the suspect bacteria in Lyme. In this vein, he explained that, **while the acute infection with *borrelia* triggers innate and inflammatory responses**--like most bacterial infections, **the chronic infection with *borrelia* triggers autoimmune inflammatory processes through coordination of auto-reactive B cells, Toll-Like receptors, and T cells**.

In order to support this new view of chronic Lyme disease, **Dr. Vincent recommended the study carried out by Soulas et al. (2005)**, on the link of chronic infections and autoimmunity [55]. In order to better understand the scientific background of LDI applied to chronic infections such as chronic Lyme disease, I have summarized the main outcomes and conclusions of this paper as well as those of other studies treating the same subject:

Clinical observations and several experimental models have suggested for decades that autoimmune diseases may be initiated or worsened by microbial infections. In theory, the mechanisms by which a microbe may activate autoreactive cells could fall into 2 categories: antigen-specific and antigen-nonspecific: (1) the antigen-specific theory relies mainly on epitope mimicry; (2) the antigen-nonspecific mechanisms are numerous and loosely grouped under the term **"bystander activation".** It has been known for decades that **bacterial or viral infection, particularly when it is persistent, leads to polyclonal B cell proliferation and Ig production**. Two different situations can be considered in this regard: (1) natural autoreactive B cells produce only low-affinity Abs that escape tolerance mechanisms because they fall below the threshold for induction of anergy, deletion, or editing; thus polyclonal activation would result in the production of more of these innocuous Abs with no role for the auto-Ag and with no clinical consequences; (2) **some anti-self B cells normally present in healthy subjects** may have sufficient affinity to bind auto-Ags and to receive some signal through the B cell receptor (BCR). Under normal circumstances such cells would remain quiescent.

Auto-Abs specific for the Fc portion of IgG are known as rheumatoid factors (RFs). Studies in mice transgenic for a human IgM RF have shown that peripheral encounter with soluble IgG leads to tolerance induction in high-affinity RF B cells via deletion. However, when T cell help is provided either simultaneously or within a few days before deletion, binding of human IgG to the B cell receptor leads instead to activation and secretion of the higher-affinity RF [56]. In this context, normal individuals express low affinity IgM rheumatoid factors (RFs) on peripheral B cells, but fail to express the higher affinity "pathologic" RFs that are associated with diseases such as rheumatoid arthritis [57].

Soulas et al. (2005), examined whether an experimental infection with *borrelia burgdorferi* (Bb), a chronic infectious disease that is known to be associated with polyclonal hypergammaglobulinemia in both humans and mice, can break this state of immunological ignorance. They showed that **chronic infection by *borrelia burgdorferi* of trangenic animals expressing the auto-Ab human rheumatoid factor (RF) on self-reactive B cell** (of low or intermediate affinities) in the absence or in the constitutive presence of the auto-Ag (a chimeric IgG with human constant region), **breaks their state of immunological ignorance, leading to the production of RFs** [i.e., these "autoreactive RF B cells" express auto-Abs IgM and IgD on their membrane, which can bind the Fc portion of a human IgG, thus forming the RF]. Indeed, B cells expressing BCRs specific for self Cγ (IgG), (RF B cells), are present in large numbers in the normal human repertoire in spite of the ubiquitous presence of IgG in different biological forms. Thus, **under normal conditions, "[autoreactive] rheumatoid factor (RF) B cells" in humans seem auto-Ag-ignorant and do not secrete RF. However RF B cells can be activated in many autoimmune conditions, but also in non-autoimmune conditions and in particular during infectious diseases**. In this assay, they used 4 different lines of transgenic mice expressing chimeric RFs that differed mainly by the affinity of the RFs for human IgG, and by the absence or the constitutive presence of human Cγ1 [the DNA sequence coding for the constant region of the IgG1]. These "sIgM+sIgD+ RF B cells" they used, develop normally in the bone marrow, localize in the B zones of the secondary lymphoid organs, are not activated in the presence of human IgG, and do not present features of anergy (i.e., they can be activated through BCR-dependent and -independent pathways). Consistently, in humans, **infectious diseases with a wide variety of pathogens, as diverse as subacute bacterial endocarditis, tuberculosis, and type C viral hepatitis, are frequently associated with a high production of RFs**. The mechanism of this production is usually considered to be nonspecific; (the mechanisms underlying nonspecific B cell activation during infectious states, include direct activation by B cell mitogens; cytokines released from activated T cells, which may substitute nonspecifically for Th cells; or even T cell help that occurs through cognate interaction but is independent of BCR specificity).

Regarding *b. burgdorferi* infection, activation of B cells through TLRs certainly plays an important role: Bb's lipoproteins including OspA and OspB possess potent B cell mitogenic properties capable of stimulating polyclonal proliferation and Ig production in vitro (these properties are dependent on the Pam3Cys modification of lipoproteins and involve TLRs, particularly TLR2). Their results showed that CD4+ T cell blockade in vivo had no effect on the nonspecific part of the RF B cell activation [through TLR interaction], since serum RFs were similar between anti-CD4–treated and untreated infected mice. However, CD4+ T cell blockade completely suppressed the auto-Ag [represented in this study by the chimeric IgG with human constant region] dependent RF production. In addition, they found that immune complexes made of anti–*b. burgdorferi* human IgG and sonicated Bb greatly enhanced the activation and proliferation of RF B cells as well as the secretion of RF compared with those induced by Bb alone--this phenomenon is auto-Ag-specific. The most straightforward explanation, the authors state, is that RF B cells, having captured Bb's Ags in immune complex form, receive T cell help through a cognate interaction with anti–*b. burgdorferi*–specific T cells, which allows them to differentiate into PCs. (Alternatively, secondary to TLR/BCR co-stimulation, B cells may be able to differentiate following non-cognate interaction with activated CD4+ T cells or with cytokines produced by them).

According to the authors, their results suggest that there is a window of BCR affinities that allow auto-reactive B cells to evade classical tolerance mechanisms and to appear ignorant but that permit their activation by the auto-Ag. Their results would also support that a physical interaction or a cross-link between the TLR and the BCR may be required. **To conclude, the paper** points out that **it has been shown that *in vivo* bacterial infectious disease can activate not only low-affinity auto-reactive B cells, but also higher-affinity anti-self B cells that have escaped central or peripheral tolerance** (these last cells are known to exist in humans during chronic infection with *mycobacteria*, but also in healthy individuals). Furthermore, these findings suggest that **chronic infection can activate auto-reactive B cells with significant affinity and creates conditions that can drive them to differentiate into memory cells. Such cells may have some physiological yet undetermined role, but in autoimmune-prone individuals, this scenario may initiate autoimmunity** [55].

5.1.4 TREATING CHRONIC LYME DISEASE WITH LDI:

Going back to the aforementioned radio interviews , Dr. Vincent further elaborated on **Lyme disease:** he affirmed again that Lyme disease is not actually an infectious disease but rather an autoimmune condition. He spent years treating Lyme with antibiotics, with no very good results for most patients and he thinks this is due to the fact that they show chronic and systemic inflammatory symptoms, not caused by the bacteria itself but rather by their own immune system over reacting and creating inflammation. In this regard, **he has created a mixture of Ags to treat Lyme disease that comprise over 70 different species, including different strains of *borrelia, bartonella, babesia, ehrlichia and coxiella*** among other pathogens usually found in Lyme disease patients; he estimates that he is getting **around a 95% of response rate [treating chronic Lyme disease with this formula]**. After observing these results, he says he has come to believe that most people carry these bacteria in our bodies forming part of our "normal flora", and what really causes symptoms in certain individuals is a chronic immune response, being the presence of the bacteria not that important [this assertion has actually been corroborated in mice, where some evidence suggests that Lyme arthritis severity does not correlate with the bacterial load [96]]. On the other hand, Dr. Vincent also explained that the patients he has treated with **multiple sclerosis (MS) with the "Lyme mixture" completely overcame their symptoms**, so he thinks that at least many MS cases are a "version" of Lyme disease. He went on explaining he had also treated successfully 5 MS patients by giving them the myelin basic protein [a protein forming part of the myelin sheath of the nerves, known to be the target of T lymphocytes in MS], so it was his opinion [at the time of the interviews] that the best treatment for MS would be to give both the Lyme antigen mixture and the myelin basic protein.

5.1.5. INDICATIONS OF LDI:

Dr. Ty Vincent explains how LDA reestablishes immune tolerance to whatever Ag you include in the mixture, even to hundreds of Ags at once. He highlights the advances he is making with this therapy, specially using autologous mixtures prepared from the patients' own bodily fluids. **Conditions he explains is successfully treating** *(he makes clear that all clinical recommendations he gives should be considered level of evidence "C", as merely expert opinion)* include very known autoimmune disorders such as **rheumatoid arthritis, myasthenia gravis, Crohn's disease, psoriasis or multiple sclerosis**. He also describes the success of LDI for infectious diseases, such as **Lyme disease and/or disorders caused by *babesia or bartonella*** infections. He talked as well about a patient diagnosed with **sarcoidosis**, who recovered with a mixture of ***micobacteria*** Ags. Finally in one of the interviews, he emphasized the good results he is getting with some chronic conditions he thinks are autoimmune in nature, including **fibromyalgia and chronic fatigue syndrome.** In the same vein, in the second lecture he gave in the recent conference in Miami (FL), he presented some clinical cases: he has found or is finding that LDI is working with different rates of success for different conditions such as **inflammatory bowel disease** (**Crohn's disease and ulcerative Colitis**), **polymyositis, autoimmune hepatis, primary biliary cirrhosis**, systemic reactions caused by ***varicella zoster virus*** [Interestingly he explained how viral Ags seem to be less immunogenic, i.e., LDI is not as efficient with virus and/or might require higher doses than for bacteria]. He also elaborated on how some patients present with conditions difficult to categorize, characterized by **systemic inflammation**; he pointed out in this respect how **widespread chronic symptoms** may be triggered by many of the myriad of microbes that live systemically in human beings. For these cases, he went on, many different Ags might be needed to be tried in order to get a satisfactory response with LDI. Next in the same lecture, he discussed the role of **skin flora** in the pathogenesis of some diseases as well as its therapeutic potential use through LDI. In this regard, the doctor presented some cases of **facial rash/atypical acne**, **impetigo** and **unexplained dermatitis**. He remarked how he was finding **psoriasis** difficult to treat unlike other conditions like acne--which responds really well to LDI. Likewise, he discussed the **oral flora** related conditions, which might manifest with **symptoms including mouth burning or numbness**, **pharingitis, dysphagia, reflux, dyspepsia, vasovagal reactions, palpitations, fatigue, mood changes** or other **psychic effects**, among many others. Afterwards, Dr. Vincent talked about those conditions that could be related to either **progesterone or estrogen sensitivity--e.g. PMS--** (both of which can be addressed with LDI), and concluded his lecture at the past conference presenting other diseases he had only **anecdotal experience** treating them with LDI, including **pyoderma gangrenosum, myaesthenia gravis, insuline antibodies, discoid lupus, lupus, cannabis allergy, dental material allergy, autoimmune hepatitis,**

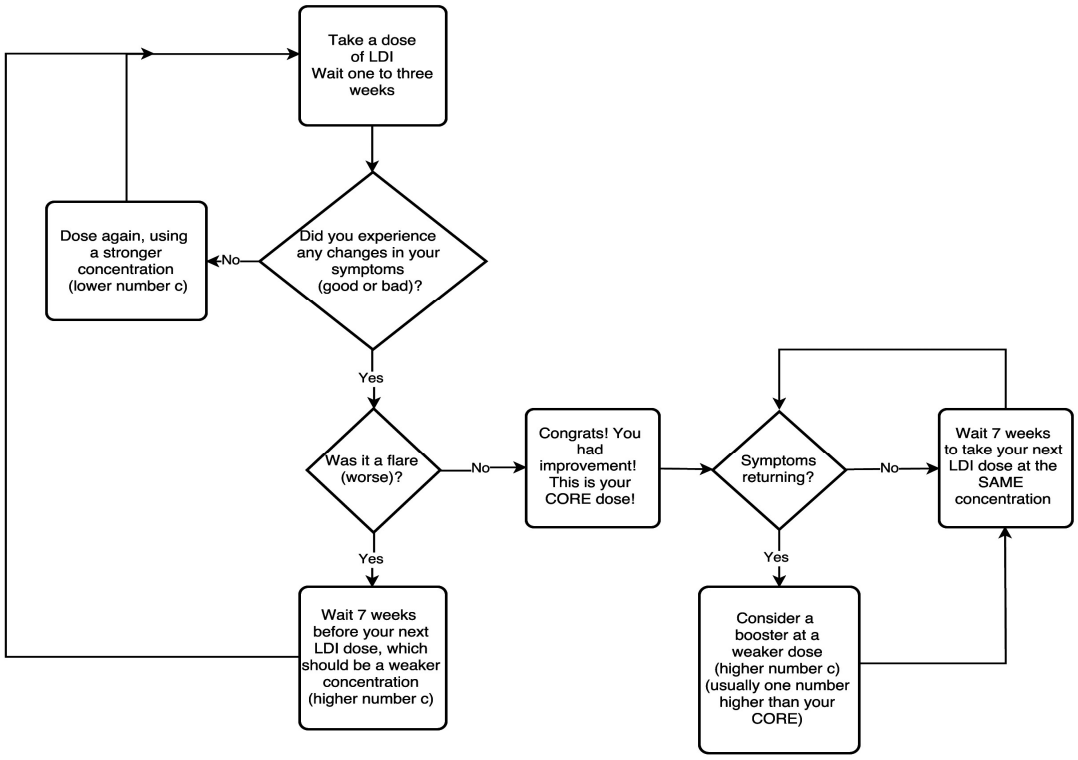
atypical Parkinson's (the non-responsive form to dopamine agonists), **alopecia areata, vitiligo or Type I DM**. For all these conditions, he explained which Ags could be the best choices in light of the available literature linking them to molecular mimicry processes, as well as according to his own experience.

Putting togethe various reviews on LDI made by Dr. Ty Vincent, the American Academy of Environmental Medicine, the NW Integrative Medicine (Washington, US) and the Breakspear Medical clinic (UK), conditions **which can be addressed with LDI therapy would include autoimmune diseases, skin and allergic conditions, chronic infections or neurologic disorders, specifically: chronic fatigue syndrome, fibromyalgia, inflammatory bowel disease (Crohn's disease and ulcerative colitis), inflammatory arthritis, reactive arthritis, ankylosing spondylitis, psoriasis, facial rash/Atypical Acne, impetigo, unexplained dermatitis, multiple sclerosis, sarcoidosis, autoimmune thyroiditis (Hashimoto's), interstitial cystitis, chronic vaginitis, idiopathic thrombocytopenic purpura (ITP), dermatitis, eczema, psoriasis, rosacea, asthma, sinusitis, rhinitis, hay fever, eczema, urticaria, anaphylaxis to peanut and food and inhalant allergy of all types, chronic pharyngitis, mouth burning or numbness, dysphagia, reflux, dyspepsia, vasovagal reactions, palpitations, fatigue, mood changes, food triggered migraines, Lyme disease, *Babesia* and *Bartonella* chronic infections, nephrotic syndrome, Alzheimer's disease, Lou Gehrig's disease (ALS), autism spectrum diseases, chronic joint and muscle pain, progesterone and/or estrogen sensitivity (e.g. PMS).** Other potential conditions that could also respond to LDI--[for which there is just anecdotal empirical experience] include: **pyoderma gangrenosum, myaesthenia gravis, insuline antibodies, discoid lupus, lupus, cannabis allergy, dental material allergy, autoimmune hepatitis, atypical Parkinson's disease** (the non-responsive form to dopamine agonists), **alopecia areata, vitiligo or Type I DM.** [46, 47, 48, 49, 50, 52, 53, 58, 59].

*[**PLEASE NOTE THAT DR TY VINCENT MAKES VERY CLEAR THAT THE LEVEL OF EVIDENCE OF LDI APPLIED TO THESE DIFFERENT DISEASES HAS TO BE CONSIDERED AS MERELY EXPERT OPINION (LEVEL "C" OF EVIDENCE")**. Also, the degree of experience Dr. Ty Vincent or other physicians may have in the application of LDI does vary for each condition. For instance the experience on some allergic diseases may be huge, while for others conditions such as dental material allergy, the experience is only anecdotal. Therefore **THIS INFORMATION MUST BE TAKEN WITH CAUTION AND IS ONLY INTENDED TO SHOW TO OTHER PHYSICIANS THE GREAT POTENTIAL LDI MIGHT BEAR**]*

5.1.6. DOSES OF ANTIGENS:

INDIVIDUAL DOSE TITRATION FOR LDI [60]



[The subject of choosing the Ags for each patient, titrating the doses, finding the proper dilution for each person, schedule a proper timing for giving the doses according to the patient's responses, etc., is beyond the scope of this article--accurate and detailed information on this regard can be actually obtained by any doctor, by contacting expert doctors in using LDA or LDI. Therefore, in this section **I am only summarizing the gist of it, in order to briefly show how this complex but essential part of the therapy works**. Nonetheless, **the above diagram does accurately reflect the basic scheme of how to implement the therapy from the beginning, and choose the proper doses according to the patient's response**].

During the interview with Dr. Nathan, Dr. Vincent explained the use the "C-scale" for dilutions [1C dilution is a 100 (10^2) to 1 dilution of a substance, 2C is 10^4 to 1 dilution, and so forth]. As for the timing, he explained that it is **necessary to wait 7 weeks between the administration of one dose and the next one, being this believed to do with the half-life of T lymphocytes** (55-60 days). If you give a dose before 7 weeks have passed from the previous one, normally the patient develops a "flare" in his/her symptoms, because it would be like giving the body a double dose. However this time frame of 7 weeks have two problems: the first one is that normally, the patient finds a quick improvement, but it wanes before the 7 weeks have passed. In general, as you give more number of doses (every 7 weeks), the improvement lasts longer and is more profound--until reaching more than 7 weeks. All the same, this **waiting time until the next dose can be given, is hard for the patients**, as they go back to their initial state of disease. The second issue is the time needed to find the proper dose for each patient (known as the "**core dose**"--that may vary significantly for each person). If you give a too high dose, you will be making the patient worse, and no immune tolerance will be achieved; on the other hand, if the dose is too weak, you are not producing any effect at all, so a titration process is needed to find the "core dose". Problem is that **this process [of finding the appropriate dose for each person] might take months**; Dr. Vincent gave the example of someone whose dose might be 10C, explaining that if you start off a dose of 20C [a 20C dose is 10^{20} times more diluted than a 10C dose] and raise it until 10C every 7 weeks, it would take you around 2 years to start the treatment. This is why the doctor is trying to make this process faster and explains how he has found that during the titration process, **if the patient does not feel anything from a certain dose, you can give the next one within 7-10 days until a response is observed**. In this titration process, if you raise the dose linearly ([e.g., 20C, 19C, 18C and so forth--note that **as the C number gets lower the concentration of the Ag given is higher**]) and the patient suffers an obvious "flare" [what it is known as a *Herxheimer's reaction*], then you know that the appropriate dose for that specific person was the previous one, although he makes clear **that it is not necessary to cause a flare in order to find the "core dose"** for most patients. Finally, **once you know the "core dose", if the patient experience an obvious improvement, but relapses before the 7th week, he is finding that it is still possible to give 1/100--sometimes 1/50-- of that dose as soon as the symptoms come back (this is known as a "Booster dose")**. You can continue taking booster doses weekly until the week number 7th, when you can give again the full core dose, to which you have to stick to for the rest of the treatment, although some adjustments might be needed--the rationale for this lies in the fact that the additional dose of Ags you are giving with the "booster doses" is only a 2-3% of the core dose [meaning it is still possible, during the time left to reach the 7th week, to stimulate the process of specific Tregs formation --this is, there are still naïve T cells (Th0) that can be converted into T regs. This is logical as the renewal of T cells is a dynamic and ongoing process].

5.1.7 ROUTE OF ANTIGENS ADMINISTRATION:

Dr. Vincent explained at the recent conference in Miami (FL), that according to his experience, both **intradermal injections or sublingually versions of the therapy works equally well**--except for rare exceptions--being the only difference that sometimes the sublingual preparation requires a higher dose for a given individual. The reason for this, he clarified, may be that there is a huge amount of immune system cells (specially Th0, capable of converting into Tregs) in the mucosa of the mouth. In this respect he notes that he started giving LDI sublingually to **autistic children**, **who, he remarks, respond great to LDI**, not just for food intolerances and/or for candida antigens--[For what EPD or LDA had traditionally been used in autism and similar kind of spectrum disorders [30]]--but **also for the "Lyme antigens mixture".** On the contrary, Dr Shrader explained he had the opposite experience with LDA, that is, the intradermal injections work much better. [probably these discrepancies have to do with both the Ags

used and the doses: while LDA normally uses higher and standardized doses for many non-infectious conditions, LDI uses much lower and tailored to each individual doses for mainly microbial Ags].

5.1.8. DR TY VINCENT'S OPINION ON LYME DISEASE AND ME/CFS:

I would like to highlight what Dr. Vincent said during the radio interviews about the **origins of the symptoms in most of these chronic inflammatory conditions, such as chronic Lyme disease or ME/CFS**. He explains that many patients notice an amazing reduction of their symptoms just a few hours after the injection. He says this is because **you are actually "switching off" the inflammatory response that is actually causing the symptoms, what leads him to the conclusion that it is not an actual damage of the tissues what causes the symptoms in the first place**--he remarks however that a real damage does take place in some cases, for example in multiple sclerosis, which he thinks is the late stage of Lyme disease; in these cases, many months might be needed in order for the muscle repairing, etc. [This might be in theory true, at least in tissues known to have unipotent stem cells, such as muscles or skin; there could also exist a much deeper degree of tissue repairing, through multipotent stem cells from the bone marrow--a branch of research I don't review in this document]. [The quick "switch off" of the chronic inflammation the doctor talks about, could be understood as a "similar" effect of NSAIDs for acute inflammation--saving the obvious differences].

5.1.9. PATHOLOGICAL INDUCTION OF TOLERANCE BY BORRELIA B. VERSUS THERAPEUTIC INDUCTION OF TOLERANCE:

While the final therapeutic goal of LDI is to induce tolerance to a pathogen, something that is mainly accomplished by inducing the formation of Tregs and the expression of key cytokines such as IL-10 or TGF-β (as previously discussed), it is necessary to **distinguish this process of "physiological induction of tolerance" from the "pathological tolerization" induced in the host by certain pathogens such as *borrelia b.* as a survival mechanism**. To fully understand these different concepts, I review in this section the host's immune response induced by Bb:

5.1.9.1. BORRELIA B. INDUCED HOST'S IMMUNE RESPONSE:

Borrelia b. or its components, may induce hyporesponsiveness in human PBMCs (tolerization). It has been demonstrated that Bb can induce the production of the anti-inflammatory cytokine IL-10 *in vitro*, suggesting that the bacteria might **inhibit early macrophage responses** [61, 62]. Likewise, the **inhibition of mononuclear and natural killer cell** function, **lymphocyte proliferation**, and cytokine production has been confirmed to be induced by Bb [63] . Moreover, Bb would also **inhibit the migration of DCs** to lymph nodes and the further migration of host's immune cells to the site of infection *(by interfering with Ca2+ mobilization* [64]*).* Furthermore, by expressing the Neutrophil Activating Protein A (NapA), Bb has shown to promote the formation of **NapA-Specific IL-10 and TGF-β releasing CD4+CD25+highFoxP3+ Tregs** with inhibitory capacity against NapA-specific T effector cells [65]. Additionally, Bb has shown to induce IL-12 production, a cytokine critical for driving cellular responses toward Th1 subset and in this way **retards Abs induction by Th2 cells.** This will protect the spirochete from destruction by the adaptive immune system [at the beginning of the infection, this way allowing the bacteria to become chronic] [66].

[About 5% of the circulating T cells in a healthy adult are γδT cells. Activation of γδT cells does not occur through presentation of antigen peptides by APCs' MHC class molecules as occurs with αβT cells (95% of T cells) . Instead, γδT cells are activated by binding of phosphorylated microbial metabolites to the TCR in a PRR-like manner. The effector functions of γδT cells are similar to those of CD4+ (secretion of Th1-like cytokines) and CD8+ cells T (cytotoxic activity)]. [Natural killer T cells (NKT cells) represent a subset of T lymphocytes that express natural killer (NK) cell surface markers. A subset of NKT cells, termed invariant NKT cells (iNKT), express a highly restricted T cell receptor (TCR) and respond to CD1d-restricted lipid ligands. iNKT cells are now appreciated to play an important role in

linking innate and adaptive immune responses and have been implicated in infectious disease, allergy, asthma, autoimmunity, and tumor surveillance] [67] .

All Bb species can activate both the classical and the alternative pathways of the complement system--complement opsonisation facilitates therefore clearance of spirochaetes by phagocytes. While neutrophils and monocytes showed a limited ability to ingest unopsonised Bb *in vitro*, mature macrophages phagocytize and kill both opsonised and unopsonised spirochaetes. Bb also stimulates proliferation of B cells and production of Abs, mainly IgG1 and IgG3. Moreover, human Vδ1γδT cells [a subset γδT cells] in synovial fluid respond to Bb lipoproteins and lipopeptides and induce maturation of DCs, resulting in secretion of IL-12p70 [normally known as IL-12] and up-regulation of co-stimulatory molecules. Furthermore, **NKT cells can become activated *in vitro* by Bb** through CD1d binding to the spirochaete. The **NKT cells can activate in turn NK cells, DCs, T cells and B cells**. This was corroborated in Erythema migrans (EM) lesions from Lyme borreliosis patients, showing an infiltrate with predominant T cells, monocytes/macrophages and DCs, a few B cells and occasional neutrophils; as for the cytokines found in these lesions, **a pro-inflammatory Th1-like pattern was found** as shown by dominant mRNA expression of the PICs: IFN-γ, TNF, IL-1β and IL-6 mRNA and Th1-associated chemokines CXCL9-11, but also of the anti-inflammatory IL-10 [67]. In the same vein, a **Th1 pattern was also found in three skin disorders of Lyme borreliosis**: acute and self-limited lesion erythema migrans (EM), subacute borrelial lymphocytoma (BL) and chronic acrodermatitis chronica atrophicans (ACA), where chemoattractants for **cells of the innate immune response, neutrophils, DCs, and macrophages were found in all three.** High levels of mRNA expression of IFN-γ were also found. However the study observed that EM and ACA showed high levels of the T-cell-active chemokines CXCL9 and CXCL10, whereas BL had high levels of the B-cell-active chemokine CXCL13. The authors concluded that **inflammatory responses may depend on tissue microenvironments and their interactions with infiltrating immune cells** [68]. On the other hand, studies on human Neuroborreliosis (NB) demonstrated **invasion of neural tissue with Th1/Th17 immune response** predominance. In addition, studies in both humans and mice that compared immune responses between Lyme borreliosis patients with good clinical outcome and patients with persistent symptoms six months post-treatment, showed that a **good clinical outcome of Bb infection is associated with strong early Th1-like immune responses followed by up-regulation of Th2-like anti-inflammatory response**, while patients with **persistent symptoms six months post-treatment had a persistent Th1 response**. This was further confirmed by increased TNF-secreting blood-derived DCs and elevated IL-12p70 [normally known as IL-12] from PBMCs, in asymptomatic Bb-infected individuals compared to NB patients. **Cytotoxic T cells, Th17 cells and Tregs** are present during the immune response or respond to Bb stimulation as well [67]. Likewise, Katchar et al. (2013) demonstrated a similar immune pattern in **patients with antibiotic-refractory arthritis**, where the frequencies of IFN-γ-producing CD56bright and CD56dim **NK cells in synovial fluid remained high**, even after spirochetal killing, suggesting that these cells contribute to excessive inflammation and immune dysregulation in joints [69]. Tregs have also been studied in patients with refractory Lyme arthritis where a **positive correlation was found between low Tregs (CD25hi+FOXP3+) and a longer post-treatment durations of arthritis.** This study exhibited as well a **higher amount of effector T cells with enhanced resistance to Tregs** [70].

On the other hand, opposite immune patterns have been also described in chronic Lyme disease (LD): Jarefors et al. (2007) found a lower expression of *borrelia*-specific-IL-12Rb2 on CD8+ cells and a lower number of *borrelia*-specific-IFN-γ secreting cells from chronic LB patients compared to asymptomatic individuals; in this line they found that the spontaneous cytokine secretion in patients with a history of **chronic LD was Th2-dominated** (the ratio of IL-4/IFN-γ secretion was higher in chronic LD patients than in controls). Furthermore, they found **higher amounts of *borrelia*-specific FoxP3 mRNA**, suggesting **a state of immunosuppression caused by the increased of Bb-specific-Treg population in chronic Lyme disease** [71]. In order to better understand the evolution of the hots's immune response during Bb infection, Henningsson et al. (2011) published a large retrospective study, assessing the Th associated cytokine/chemokine profiles in serum and in CSF in NB patients. They confirmed previous patterns and concluded: "(...) a good prognosis in NB seems to be associated with a strong T helper (Th) 1-type immune response in the cerebrospinal fluid (CSF) early in the infection, followed by a Th2-type response, capable of suppressing the Th1-type inflammation (...) **If this switching is delayed, there is a risk of tissue damage and persisting symptoms** (...)". In the same vein, in his book on Lyme disease, Dr. K.B. Singleton explains that, when Bb has successfully invaded the host, a Th1 response will

continue unsuccessfully attacking the bacteria [72]. Back in 1997, Aberer et al. had already described the **mechanism that could allow Bb to become chronic** [73]: Bb induces the secretion of the IL-10 [62] and IL-1 [64]. IL-10 (an anti-inflammatory cytokine secreted by Th2-cells) down-regulates the expression of MCH-II receptors on DCs and other APCs, and by doing so, it further **induces tolerance towards Bb**. Likewise, IL-1 promotes a tolerogenic specific immune response; then the down-regulation of MCH-II expression **would favor a switch towards a Th2 response**, given that Th1 cells might require a higher density of MCH-II receptors than Th1 cells to become activated. Thereby, the **Th1 immune reaction becomes weaker** and unable to properly get rid of the bacteria. Furthermore, **increased IL-10 might explain the low CD57+NK cells** associated with chronic LD. Moreover, high IL-10 does not impede a **polyclonal B cell proliferation** that has been shown to occur in chronic LD [62, 64, 73, 74]. Finally, as earlier discussed, increased **Bb-specific FoxP3+ Tregs have been found in chonic LD**, what would further account for the Bb-induced tolerization.

In order to shed **some light into the possible reasons for these apparently contradictory results** observed in the chronic state of the infection, Jarefors (2006) published a comprehensive medical dissertation on the Bb-induced responses in relation to disease course. She highlights how the **circumstances promoting a Th2 dominance might favor the Bb infection to become chronic**--for example the Th2-derived response displayed by postmenopausal women could explain why more women than men get re-infected with Bb. After a solid review of the available literature, the dissertation states that an initial Th1-type response with a later switch to a Th2-response is compatible with a subacute prognosis of Lyme borreliosis. On the other hand, a slowly increasing Th1-response and lack of Th2 might correspond to a chronic or prolonged disease course. Finally, **the reasons leading some individuals to develop a chronic infection** would include a mixture of factors such as **the immune status of the host, the type of infectious species, a previous infection by other pathogen or the gender** (.e.g. postmenopausal women seem to be more prone to become chronically infected). Supporting previous results, the author finally concludes that **a weak Th1-response appears to be involved in the cause of chronic Lyme borreliosis**, **where not only a Th1/Th2 balance needs to be considered but also the involvement of Tregs to the pathogenesis of Lyme borreliosis** [75].

In 2013, Amedei et al. finally published **observations connecting the still apparently contradictory previous results**. They isolated CD4+CD25+highFoxP3+ Treg cells from CSF of patients with chronic Lyme borreliosis who had had symptoms for longer than 6 months. Some of these Tregs were specific to Neutrophil Activating Protein A (NapA) expressed by Bb--which is essential for the persistence of spirochetae in ticks and has been found to sustain, at least in part, experimental arthritis. **NapA-specific-Treg derived clones from patients with chronic LB exhibited inhibitory activity on the proliferation of autologous NapA-specific T effector cells clones. Moreover, these Tregs were found to be highly expressing Foxp3 and to produce IL-10 and TGF-β but not IL-17 and IFN-γ. On the contrary, T effector clones derived from a CD4+CD25- T cell fraction, were able to produce, following NapA stimulation, a high amount of IFN-γ and/or IL-17.** In addition, this study also evaluated the microglia. The **stimulation of these CNS cells with NapA induced a strong increase of TGF-β and IL-10 secretion and only a limited increase of IL-1β, IL-6 and IL-23 [implying that Bb might induce an anti-inflammatory or immature phenotype of microglia, as it does with macrophages]. The cytokine milieu enriched in TGF-β and IL-10 may drive the differentiation of T cells towards the Treg phenotype**. The authors conclude that these results suggest that NapA is a *borrelia* virulence factor involved in the pathogenesis of chronic LB because it permits the bacterium to survive in the host by down-regulating the immune response [65] [In summary, **this study demonstrates that Bb in chronic Lyme disease inhibits the Th1 response** (that would kill it) **and promotes a Th2 anti-inflammatory profile** by inducing the formation of Bb-specific-Tregs with the capacity of inhibiting specific-Bb-T effector cells, and further drive the differentiation of Th0 cells into more Tregs by means of IL-10 and TGF-β (note that TGF-β drives the shift in the Th1/Th2 balance toward Th2 [76]). However, **at the same time, the LD patients' T effector cells shown to produce high amounts of IFN-γ and/or IL-17, what corroborates the results of numerous reports demonstrating a chronic Th1/Th17-like immune response as the possible underlying cause of reactive Lyme arthritis].**

[Taking all together, it seems to exist a paradoxical immune state in chronic Lyme disease. On the first hand, ***borrelia b*. induces immune tolerance against itself, in order to avoid an immune response that would kill it**. Thus, while a Th1 and a Th17 immune responses would get rid of the

bacteria in the acute infection, **during the chronic infection the Th1 response gets weaker and is deviated toward an anti-inflammatory Th2 response**--characterized by the secretion of IL-10--(the Th17 immunity is also driven toward to its anti-inflammatory phenotype--this won't be reviewed in the current text).

From the above examined literature, there seems to exist a **similar immune pattern in conditions caused by intracellular chronic infections**. This pattern would include **"specific-pathogen's antigens IL-10- secreting-T cells" able to--through secretion of IL-10--inhibit Th1 cells (IFN-γ secreting), promote polyclonal B cells proliferation and the subsequent autoimmune processes and related inflammation, and induce the anti-inflammatory phenotype of macrophages and microglia; these cells would in turn secrete more IL-10 as well as TGF-β, further inducing more IL-10 secreting-T cells, thus perpetuating a pathological tolerogenic state that allows the microbes to become chronic. This immune state is favored by a concomitant inflammatory and oxidative milieu that favors the instauration of autoimmune processes (mediated by auto-reactive T and B cells, molecular mimicry and/or immune complexes). At the end, the chronic inflammation and the autoimmune processes are the final causes of the symptoms.** (It is important to note that the IL-10 secreting cells are not just Tregs, but also CD4+ T cells (clearly independent from Foxp3 in their function and development). In this respect most IL-10-secreting CD4+ T cells are characteristically associated with mucosal immunity, food tolerance, and antimicrobial immunity. It has also been speculated that IL-10-secreting CD4+ T cells, which are induced in the periphery, are important for controlling immune responses to nonself-antigens, while naturally occurring CD4+CD25+ Treg cells originating from the thymus are mainly responsible for the maintenance of self-tolerance. However, there are lines of evidence suggesting that IL-10-secreting CD4+ T cells also control immune responses to self-antigens) [77]. As shown above, **in chronic Lyme disease this scenario has indeed been described**: as previously shown, **Bb-specific-Tregs able to inhibit Bb-specific-T effector cells have been found**. Likewise, a **microenvironment composed by IL-10 and TGF-β, stimulated by microglia exposed to Bb's antigens has also been described**. Similarly, **Bb has demonstrated to induce the production of the Th2 anti-inflammatory cytokine IL-10** *in vitro*, suggesting that **the bacteria might inhibit early macrophage responses**. In the same vein, **low "non-Bb-specific-Tregs" have been found to be correlated with longer duration of the disease**, while at the same time **effector T cells with enhanced resistance to these Tregs have been also demonstrated**. Furthermore, as will be discussed in the next sections, **autoimmune processes mediated by auto-reactive T and B cells, molecular mimicry and/or immune complexes, as well as an inflammatory and oxidative milieu, have largely been reported**. Finally, **a weak Th1 response together with a preponderant anti-inflammatory Th2 and Th17 immune phenotypes have also been shown (demonstrated by low cytotoxic CD8+ T cells, NKs or by low numbers of IFN-γ and IL-17 secreting cells).**

In sum, Bb induces immune tolerance toward itself while promoting at the same time a systemic and alternative over-reactive immune state characterized by chronic inflammation and autoimmunity. Thus, the "physiological induction of tolerance" achieved by LDI should be understood as the mechanism that could revert this state by: (1) inducing the formation of Tregs able to inhibit the auto-reactive T and B cells as well as the "IL-10-secreting T cells"--thus decreasing the predominant Th2 immune profile; (2) promoting (or passively allowing by eliminating their inhibition) a proper Th1 and Th17 responses; (3) lowering the systemic inflammation (driven by IL1, IL-6, TNF-α, etc.); (4) decreasing the level of oxidative and nitrosative damage in the microenviroment; and (5) reducing the autoimmune processes. Note that the mechanisms of action of LDI have not been yet studied. The conclusions I make here are just speculations formed from the extrapolation of some observations published on different types of specific-antigen-based-immunotherapy, including AIT, EPD or oral tolerization addressed by different approaches--all of which are analyzed in the present text].

6. LOW DOSE IMMUNOTHERAPY AS A NEW PROMISING APPROACH FOR CHRONIC LYME DISEASE AND FOR MYALGIC ENCEPHALOMYELITIS / CHRONIC FATIGUE SYNDROME

6.1. CHRONIC LYME DISEASE AND ME/CFS; OVERLAPPINGS:

Physicians all over the world have been for a long time linking myalgic encephalomyelitis /chronic fatigue syndrome (ME/CFS), fibromyalgia and/or multiple chemical sensitivity (MCS), with chronic Lyme disease (LD). For instance, on Wednesday, April 23th 2014, Nele Lijnen, a Belgian politician and member of the Belgian Senate, organized a round table in the Belgian Parliament, with international experts on LD, where Dr. Kenny De Meirleir (Medical Director of both: Himmunitas Clinic (Brussels), and Whittemore Peterson Institute for Neuro-Immune Disease (Reno, Nevada)), showed a presentation stating that a 95% of ME/CFS patients (fulfilling both the Fukuda and the Canadian diagnostic criteria) tested positive for *borrelia burgdorferi* by Elispot-LTT [78] --a test approved by the FDA in 2011 [79] that has proved to have both high sensitivity and specificity in several studies [80] --. In the same vein, Dr. Horowitz (Director of the Hudson Valley Healing Arts Center, New York) explains in one of the internationally most known books on LD, that the majority of his Lyme patients had been previously diagnosed with ME/CFS [81], something corroborated by Dr. Nicola Mc Fadzean (Medical Director of Restor Medicine, San Diego, CA) on her book from 2012 [82]. Moreover, Dr. Ty Vincent stated that fibromialgia and chronic fatigue issues [he was talking about ME/CFS] are technically LD if a cause has not yet been figured out [48]. In either case, it is a fact that many of the **cutting-edge "ME/CFS doctors" treat the infection with *borrelia burdogferi* as part of a whole treatment approach**; this is for instance the case of Dr. Sarah Myhill (UK) [83], Dr. Martin Lerner [84] (Beaumont Health System Treatment Center for CFS, Miami) or Dr. Neil Nathan (Gordon Medical Associates, California) [85], among many others.

There is also some **scientific literature showing a possible link between Lyme and ME/CFS**. For instance, a major internationally-recognized medical reference text (Harrison's principles of internal medicine), states that chronic LD is similar to chronic fatigue syndrome or fibromyalgia [86]. On the other hand, reviewing some medical journals, a study from 2006 emphasized this connection and pointed out an interesting observation: "(...) A relatively uniform post-infective fatigue syndrome persists in a significant minority of patients for six months or more after clinical infection with several different viral and non-viral micro-organisms. **Post-infective fatigue syndrome is a valid illness model for investigating one pathophysiological pathway to chronic fatigue syndrome.** (...) **Post-infective fatigue states have a long history and have been linked to a diverse spectrum of infections, including LD** (...)" [87]; interestingly the authors claimed to have found strong evidence implicating aspects of the host's response to infection [rather than the pathogen itself] as the likely determinant of post-infective fatigue syndrome. The same was argued in a more recent study, which stressed an intrinsic link between the host-pathogen interactions in the acute infection phase, and the genesis of the post-infective fatigue syndrome [88]. In the same line, Patrick et al. (2015) found **Lyme patients**--diagnosed according to the International Lyme and Associated Diseases Society (ILADS) guidelines [89]--**and CFS patients**--fulfilling the Canadian case definition--to **appear indistinguishable based on their medical histories, physical examination, functional scales, and a range of laboratory tests**; both groups were on the other hand clearly distinguished from healthy controls [90]. *[Please note that it is beyond the scope of this text to go into the current scientific controversy about the reliability diagnostic techniques of Bb infection]*. In the same vein, Shor (2006), emphasized in a published case study: "(...) Patients with symptoms that are consistent with chronic fatigue syndrome should be seriously evaluated for the potential of chronic Lyme infection (...)" [91]. It is also worth noting the thorough review on chronic diseases where chronic infections play an important role, published by the *Britished Journal of Medical Practioners* in 2010, where the authors conclude: "(...) **Most, if not all, CFS/ME patients have multiple chronic bacterial and viral infections** (...) **CFS/ME patients are also often infected with *B. burgdorferi*** (...)" [92]. In this same line, the results of a comprehensive review on the co-infections found in ME/CFS patients and

subsequently diagnosed with Lyme disease, are quite striking; the authors of this paper state: "(...) there is **growing awareness that CFS can have an infectious nature that is either causative, a cofactor for the illness or appears as an opportunistic infection (s)** (...) There are several reasons for this, including the nonrandom or clustered appearance of CFS, sometimes in immediate family members, the presence of certain signs and symptoms associated with infection, the often cyclic course of the illness and its response to anti-microbial therapies. (...) Recently **it has become apparent that some CFS patients have Lyme Disease** (...)". In addition, the authors found the *borrelia burgdorferi*-positive CFS group to have higher overall rates of *mycoplasmal* infections compared to controls; they also observed that the **severity of CFS signs and symptoms were related to the number of chronic infections** but not to their specific type. They further explained how in **Lyme Disease patients with multiple co-infections who had progressed to the chronic phase of the disease, symptoms can be similar in presentation to CFS**. Finally, they found very illustrative data as to how much both conditions resemble each other: "(...) In CFS, multiple infections are associated with more severe signs and symptoms, and similarly when multiple infections are present in Lyme Disease, the number of signs/symptoms and their severity and duration are usually greater in the early stages of disease (...)" [93].

In contrast to the similarities reported, **there are some other studies suggesting these two similar diseases to be different entities.** Probably the most illustrative work supporting this view was performed by Schutzer et al. (2011). They used liquid chromatography coupled to high-resolution mass spectrometry based label-free quantitative proteomics approach to **examine cerebral spinal fluid (CSF) samples from patients with CFS/ME, Lyme disease and healthy controls**, and were able to **distinguish either of each group from the others based on their unique CSF proteins** [94]. [While the authors conclude that these findings suggest a different etiology of both conditions, it is my personal opinion that they did not have important factors into account when achieving such conclusion. As it has been clearly stated by numerous papers, ME/CFS is not a statistic disease, i.e. its pathophysiology varies both over time as well as according to the degree of symptoms severity. This feature was not taken into account in this study. In addition, the used diagnosis criteria for CFS was not any of the proper international ones--such as the Canadian Consensus or the International Consensus criteria; rather, patients were selected by experts in fatigue and pain disorders, and also underwent psychiatric analysis and several blood tests to rule out common causes of fatigue; however the post-exertional malaise, an international hallmark of ME/CFS diagnosis, was not considered either for the diagnostic criteria. Furthermore, a positive result for Bb infection was included between the excluding factors for selecting the CFS patient group; this might have biased the results, given that the etiology of ME/CFS is unknown, and many infections--including Bb-- are thought to play a pivotal role in etiopathogenesis of the disease; therefore the CFS group was not representative of the whole ME/CFS community who would fall under the International criterias. Moreover, the patients included in the Lyme disease group had to fit the CDC diagnostic criteria, that according to international organizations and many physicians all over the world, might miss most patients with chronic Lyme disease. Even more, all LD patients were seropositive for Abs to Bb. However chronic LD patients normally lose the capacity of generating detectable isotypes and amounts of Abs [95], therefore patients with a preserved proper anti-humoral response towards Bb, are by no means representative of the whole chronic LD patients community, and rather, they could represent only the small percentage of patients who still preserve a strong humoral response towards the bacteria--which is usually lost over time. Finally, out of the 2768 measured proteins, the authors highlight 4 proteins related with complement activation (C1S, C4B, C1QB, C1QC) which were differentially increased in abundance consistently across the LD compared to CFS patients. While the authors expose this difference as suggestive of possible different pathogenic mechanisms, it is known that different strains of Bb and different immune hosts' responses are responsible for different degrees of complement activation by the bacteria [96] . On the contrary, the fact that the complement cascade is activated in both conditions, could indicate similar underlying mechanisms, while the higher activation observed in the LD patients could fit with a subset of patients with a preserved innate response. The

same could apply to the proteins found to be decreased in both CFS and LD patients compared to normal healthy controls, some of which were related to networks relevant to neurological functions. They found the CFS group to show a more pronounce decrease; this again would support my previous reasoning. **In conclusion, it is my opinion that the diagnosis criteria used in this study to demarcate the different groups of patients are not appropriate, as they don't consider the evolving nature of the diseases, nor the differences shown in accordance with the level of severity; thereby it is my opinion that it not possible to draw solid conclusions from these results.**

To conclude and whatever the relationship between ME/CFS and chronic LD might be, there are clear overlapping features, and until new research sheds more light on the differences/similarities of both conditions, in my opinion an eclectic view should be the best position to take].

6.2. COMMON CHARACTERISTICS SHARED BY ANTIGEN-SPECIFIC-IMMUNOTHERAPY RESPONSIVE CONDITIONS :

[While the review of specific laboratory abnormalities characteristic of each conditions is beyond the scope of the present article, the current section covers some of these main clinical features of each disease, in order to elucidate whether or not these conditions could be good candidates for specific-antigen-based-immunotherapy.

As earlier reviewed, conventional allergen immunotherapy (AIT) is an established treatment for certain allergies, caused by common Ags, to which the immune system over reacts--thus creating symptoms. Robust evidenced support the use of this therapy, and in the recent years it has become clear how the basis of this treatment is the induction of tolerance. Aside from the use of AIT for allergy, there is **solid evidence in animals and some good results in humans for the use of antigen-specific-based-immunotherapy as a successful treatment for a wide variety of conditions, including autoimmune disorders and chronic viral and intracellular bacterial infections**--however the use of AIT for such other diseases is still experimental at this point. On the other hand, **EPD or LDA** can be considered as variants of AIT--which use less doses of Ags plus the enzyme β-glucuronidase which seems to enhance the tolerization effect-- which have also **demonstrated in numerous studies to be equally or even more useful than AIT, not just for allergies, but also for certain autoimmune conditions**. In this context, LDI is also a variant of AIT, that is being used to treat many other conditions, mostly autoimmune and chronic inflammatory diseases. **While the scientific underlying mechanisms for conventional AIT as well as for other forms of antigen-specific-based-immunotherapy--including EPD--have been scientifically demonstrated in different degrees, the evidence available for the new LDI variant of immunotherapy, is at the moment only empirical**. Solid evidence for LDI in the form of peer-review trials might take many years to be performed. Nonetheless, the empirical data provided by many doctors as well as successful reports from patients on the use of LDI for a wide variety of conditions, surely deserves to be further studied. Also, the **successful results obtained from clinical trials on EPD, can be to some degree extrapolated to LDI, taking into account the similarities between both techniques.**

Several features should be taken into account when analyzing the potential usefulness of LDI: (1) antigen-specific-immunotherapy (AIT) is a conventional treatment for certain allergens that has extensively shown to display excellent results; (2) different approaches of AIT have also shown in humans to be effective for conditions other than allergy, including autoimmune conditions (such as multiple sclerosis, Behcet's disease, uveitis, Crohn's disease, ulcerous colitis or rheumatoid arthritis) and chronic infectious diseases (such as chronic HBV or *Influenza A virus*)--as earlier discussed; (3) many reviews highlight the fact that factors such as doses, administration routes or proper selection of the Ags used, can explain why this therapy clearly works on animals models, while the translation to humans seems not to be as successful as expected; (4) the use of EPD and LDA has been extensively reported as

a successful treatment for allergies with better outcomes than AIT, suggesting that using lower doses of Ags with the adjuvant enzyme β-glucuronidase, seems to enhance the effectiveness of antigen-based-specific-immunotherapy, in general; and (5) lower doses of Ags are generally associated with stronger induction of tolerance. From these assertions, **it might be at least inferred that those conditions which have shown to successfully respond to different AIT approaches that use much larger doses of Ags, might as well respond to LDI, including numerous autoimmune disorders, chronic inflammatory conditions and chronic infectious diseases.**

Which elements do conditions susceptible of been treated with AIT/EPD/LDA share in common?:

1) Chronic inflammatory conditions characterized by an ongoing immune activation.
2) Immune deviation from the phenotype that would properly address the known/suspected trigger/s.
3) Acquired molecular mimicry-mediated autoimmunity as an important pathogenic mechanism.
4) Symptomatology thought to be a result of the ongoing immune activation, inflammation and related autoimmunity].

In this regard, as both Dr. Ty Vincent and Dr. Neil Nathan explain, quite good clinical results have been achieved in **conditions for which cross reactivity** with a known Ag is known to play a key role in the pathogenesis. An example of this include the link of ***proteus mirabilis*** **microbes and rheumatoid arthritis** [97, 98]. Likewise, a large number of studies support the idea that the enteropathic pathogen ***klebsiella pneumoniae***, is the most likely triggering factor involved in the initiation and development of both **ankylosing spondylitis (AS) and Crohn's disease (CD)** [99]. In the same vein, data from both human and animal studies indicate that **inflammatory bowel diseases (IBDs)** are likely caused by dysregulated immune responses to resident intestinal microbes: certain products from ***mycobacteria*****,** ***fungi*****, and** ***clostridia*** stimulate increased effector T cell responses during intestinal inflammation, whereas other bacterial products from *clostridia* and ***bacteroides*** promote anti-inflammatory regulatory T cell responses [100].

6.3. CHRONIC LYME DISEASE AND ME/CFS: PATHOPHYSIOLOGY:

6.3.1. CHRONIC IMMUNE ACTIVATION IN CHRONIC LYME DISEASE:

The CD56dimCD16+CD57+ NK cells **[CD57+NK cells] constitute for many physicians a hallmark for the diagnosis** and monitoring of treatment evolution of chronic Lyme disease [82, 72]. Some studies have confirmed this marker to be very useful for long term Bb infection [101, 102], however other studies could not replicate these findings [103]. Stricker et al. (2002), explained that CD57+NK cells are down-regulated by the Th1 cytokines IFN-γ, IL-2 and TNF-α, and hypothesized that a **persistent decrease in CD57+NK cells may represent ongoing Th1 stimulation due to chronic infection with Bb** [102]. On the other hand, as already reviewed, patients with **persistent symptoms of Bb infection, six months post-treatment had a persistent Th1 response**, what was further confirmed by increased TNF-secreting blood-derived DCs and elevated IL-12p70 from PBMCs, in asymptomatic Bb-infected individuals compared to NB patients. In the same line, **cytotoxic T cells, Th17 cells and Tregs** are present during the immune response or respond to Bb stimulation as well [67]. Likewise, Katchar et al. (2013) demonstrated a similar immune pattern in **patients with antibiotic-refractory arthritis**, where the frequencies of IFN-γ-producing CD56bright and CD56dim **NK cells in synovial fluid remained high**, even after spirochetal killing, suggesting that these cells contribute to excessive inflammation and immune dysregulation in joints [69]. In the same vein, **higher amount of effector T cells with enhanced resistance to Tregs have been observed in** refractory Lyme arthritis [70].

Although different immune patterns have been also reported in chronic LD--as discussed above, **a chronic immune activation seems to be a constant**: Jarefors et al. (2007) found that the **spontaneous cytokine secretion** in patients with a history of **chronic LD was Th2-dominated** [71]. Likewise, in his book on Lyme disease Dr. K.B. Singleton explains that **when Bb has successfully invaded the host, a Th1 response will continue unsuccessfully attacking the bacteria** [72]. In this respect, back in 1997, Aberer et al. described the mechanism that could allow Bb to become chronic, in which IL-10 was a key part of the proposed process. High IL-10 does not impede a **polyclonal B cell proliferation that has been shown to occur in LD** [62, 64, 73, 75]. The **polyclonal activation of B cells could actually be involved in the** pathology of Lyme disease [118, **¡Error! Marcador no definido.**. Consistently, a subcategory of arthritis patients refractory to antimicrobial therapy have been shown to have **heightened and persistent humoral immune responses** to OspA [Bb's Ag-surface] [63].

6.3.2. CHRONIC IMMUNE ACTIVATION IN ME/CFS:

A hallmark of CFS is a chronic activation of the immune system [104]. Skowera et al. (2004) showed results supporting that CFS is associated with ongoing immune activation, as they found evidence **of low-grade T cell activation** in non-stimulated cultures of PB cells, which had **increased levels of both type 1 (IFN-γ) and type 2 (IL-4) cytokines** in CD4+ and CD8+ T cells (consistent with a type 0 pattern of responsiveness [an immune response that involves aspects of both Th1 and Th2]). This study found **evidence of a bias towards Th2- and Tc2-type immune responses in CFS**, what fits with the low numbers and/or activity and/or citotoxicity capacity of NK cells broadly reported in CFS, as they play a key role in the generation of Th1 type antiviral responses, and loss of their activity could result in a **Th2 bias and persistent viral activation and chronic infection**. **IL-10** production by non-stimulated CD8+ lymphocytes was **also significantly higher in CFS** patients in comparison to the control group. The authors point out that Th2 type of responses **may accompany some autoimmune disorders** [105]. In the same line, the CD26 protein is a marker of T cell activation and autoimmunity associated with Th17 cells. **Elevated CD26+CD4+ T cell numbers have been reported in people with ME/CFS compared to controls**. Likewise, the **elevated neopterin and expression of the CD26 antigen on T cells demonstrate chronic immune activation while chronically elevated IL-2 levels indicate dysfunctional T cell activation in CFS** [106, 107]. In addition, one of the effects of rituximab (used with success for ME/CFS--find more details later), besides depleting CD20+ B cells, is the inhibition of NFκB production as well as the reduction of Th17 T cells production (involved in T cell induced autoimmunity and neurotoxicity in autoimmune diseases such as MS) and to decrease IL-2 level, thus potentially **inactivating a chronically activated immune system** [108]. Finally, **CD57+NK cells have been also reported by some studies to be decreased in ME/CFS**, and low levels of these cells have been described **as an example of the "immune exhaustion"** found in ME/CFS patients cells, after 3 years of disease, as occurs in chronic infections [109, 110, 111] (on the contrary other studies have not found CD57+NK cells to be decreased in ME/CFS [112]--[what might be a reflection of the disease progression]. As explained above, **persistent decrease in CD57+NK cells may represent ongoing Th1 stimulation.**

[In short, both ME/CFS and chronic LD have shown different patterns of chronic immune activation, that might represent non-static diseases that evolve over time. A thorough review of the available longitudinal studies should be done in order to properly try to correlate the different immune patterns found on these conditions, with both the severity and the duration of the illness--note that this is beyond the scope of the current text**. However, the above reviewed evidence makes clear that for both ME/CFS and chronic LD, a chronic activation of the immune system exists, possibly as a result of chronic antigenic stimulation].**

6.3.3. CHRONIC INFLAMMATION, AUTOIMMUNITY AND ORIGIN OF SYMPTOMS IN CHRONIC LYME DISEASE:

***Borrelia b*. does not produce toxins or proteases that are directly responsible for tissue damage** upon colonization, thus, tissue spread and dissemination may be facilitated by the utilization of host's proteases. In contrast, the bacterium produces multiple molecules that activate host's responses and can lead to localized and generalized inflammatory pathogenic responses [96]. **During the late stage of Lyme borreliosis, persistent inflammation** after eradication of the pathogen (as in the antibiotic-resistant Lyme arthritis) and after the long-lasting infection evading host's immunity (as in acrodermatitis chronica atrophicans (ACA)) **has been observed** [113]. Besides, **differences in the severity and spectrum of disease among patients infected with Bb are due to both genetic differences among strains of the bacterium and differences in the host's responses**. Accordingly, results in mice showed that **symptoms might not correlate with bacterial load** [96].

As concluded by Doppenberg-Oosting in her disertation from 2013, **cytokines are highly relevant in the pathogenesis of Lyme disease**; Bb induces the production of PICs such as IL-1 and T-helper cell derived cytokines, **which are responsible for the inflammation during LD**. Among the various cytokines induced by Bb, **IL-1β and IL-17 seem to be the most important in its pathogenesis.** IL-1β is associated with the acute and chronic inflammatory processes seen in LD, whereas IL-17 seems to be confined to the more chronic forms [114]. Additionally, a **correlation between cytokine levels and severity of disease has been suggested** [115]. In this context, it seems that a **systemic inflammatory microenvironment might predominate in chronic LD**, comprised by molecules **including IL-1β, IL-6, TNF-α, NO and ROS**. IL-1 family (specially **IL-1β)** has been extensively linked with both acute and chronic symptoms in a broad variety of conditions, including **fever, loss of appetite, generalized muscle and joint aches, fatigue, gastrointestinal issues, sleep disturbances hypotension, skin rash, painful mouth ulcers and swollen lymph nodes and mental and hearing impairments** [116]. On the other hand, **IL-6 and TNF-α have been largely associated with chronic fatigue** in numerous autoimmune and malignant diseases [117]. In this respect, Horowitz explains how **TNF-α, IL-1 and IL-6 are responsible for** the **joint pain** in rheumatoid arthritis, and for the **muscle aches and pains in fibromyalgia and chronic fatigue syndrome as well as for the brain fog** associated with Alzheimer's disease and the **majority of chronic symptoms occurring in Lyme disease**. He also describes the role of **[Bb-induced] NO** and its oxidant product, peroxinitrate in LD and related inflammatory conditions. Aside from the direct damage of NO acting as a free radical and **damaging DNA** and proteins inside the cells, it can also stimulate the production of **NFκB** which can **increase in turn the production of IL-1, IL-6, IL-8, TNF-α and IFN-γ** [81]. Furthermore, it has been suggested that only ***b. burgdorferi s.s*. is able to cause a persistent and self-perpetuating inflammation** in the antibiotic-resistant Lyme arthritis [113].

Not only stimulation of cytokine production but also **polyclonal activation of B cells could be involved in the pathology of LD** [118,115]. Different mechanisms, including **permanent tissue damage and autoimmune reactions**, have been postulated to explain late manifestations of LD; these differences may result from different tissue affinity and different immunogenic and inflammatory properties of Bb species [113]. Delving deeper into autoimmunity and LD pathogenesis, a subcategory of arthritis patients refractory to antimicrobial therapy have shown to present heightened and **persistent humoral immune responses** to OspA [Bb's Ag-surface] [63]. In accordance, the "human cytokeratin 10" was identified as a cross-reactive target ligand recognized by anti-Bb's OspA Abs in a small group of patients with refractory arthritis. Likewise, several **neural proteins have been reported to induce T or B cell responses** in patients with NB or in post-Lyme syndrome (2010) [119]. Continuing with Lyme arthritis, antibiotic refractory arthritis has been extensively studied: for instance Drouin et al. further **identified 3 novel auto-Ags as targets of T- and B-cell responses**, reported in a series of 3 recently published papers. **All these auto-Abs showed specific pathologic consequences in synovial tissue in patients with antibiotic-**

refractory Lyme arthritis and the levels of these auto-Abs correlated with the severity of the symptoms. Interestingly, in order for these auto-Abs to become pathogenic and eventually lead to T and B auto-reactive cells, an inflammatory environment seems to be necessary [120, 121, 122].

In regard to the **CNS-related symptomatology**, in neuroborreliosis (NB), accumulation of B-lymphocytes, plasma cells, and activated CD8+ T cells has been identified in the CNS during early Lyme NB. In addition, the lipid moiety of the borrelial **OspA is known to induce a polyclonal B-cell activation**. On the other hand *borrelia* can adhere to murine neural and glial cell lines in animal models. Given that **OspA is known to induce apoptosis and astrogliosis**, a **direct interaction between *borrelia* and the neural cells could be responsible for the observed dysfunctions**. Furthermore, although Bb do not possess any known endotoxin, **dysfunction in Lyme NB patients might also be due to secreted substances that are induced by spirochetes, such as NO** secreted by Schwann cells **or quinolonic acid** (neurotoxic in higher concentrations) produced by macrophages (as also observed in animal models). Moreover, spirochetes **can induce cytokines like IL-6 or TNF-α in glial cells, both of which are neurotoxic and might provoke autoimmune reactions**. Finally, **autoimmune mediated mechanisms by "molecular mimicry"** could also be an important step in the observed neural dysfunction; in this regard the serum of patients with Lyme disease contains Abs against flagellin of Bb that cross-react with neural antigens [123]. Furthermore, there is evidence supporting the view of a **direct invasion of the brain by Bb** in late/chronic Lyme NB. Bb was detected and cultivated from the brains of patients suffering from tertiary Lyme NB. The detection of Bb and/or its specific Ags or DNA at sites of CNS lesions and the reproduction of Lyme meningoencephalitis *in vivo* in experimental animals are **evidences for active persistent infection**. This data reveals that Bb can evade the destruction by the innate and adaptive immune systems and **cause chronic infection, inflammation and slowly progressive tissue damage**, therefore continuous infection by Bb can cause the manifestations of late/chronic Lyme NB, including **stroke, cognitive decline and various other neuropsychiatric conditions** [66].

[In conclusion, symptoms caused by *borrelia b*. (Bb) infection are a direct result of an aberrant host's immune system reaction, induced by the bacteria's Ags. Thus, chronic Lyme disease (LD) is characterized by a systemic chronic inflammatory milieu composed mainly by elevated levels of IL-1β, IL-6, TNF-α, NO or ROS, which might account for most chronic symptoms seen in LD. Perpetuating causes of the Bb-induced chronic inflammation include the polyclonal B-cell activation and the related persistent humoral non-specific immune responses. This leads/promotes autoimmune processes mediated by auto-reactive T and B cells, and/or by autoantibodies. Both the degree of inflammation and the amounts of autoantibodies have been shown to be positively correlated with the severity/duration of different signs/symptoms of LD. The inflammatory and the autoimmune responses seem to be tissue-dependant, therefore distinct immune responses have been identified as the underlying cause of certain symptoms in different locations. A chronic Bb-induced-inflammation seems to coexist with a concomitant immunosuppression induced by the bacteria as a strategy to become chronic].

6.3.4. CHRONIC INFLAMMATION, AUTOIMMUNITY AND ORIGIN OF SYMPTOMS IN ME/CFS:

In ME/CFS a similar pattern than that observed in chronic LD appears to predominate: Maes et al. (2012) compiled the following previous **findings indicative of Inflammation in ME/CFS**: **(1) low-grade inflammation**, as indicated by an increased production of nuclear factor κB (NFκB), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), OS and NS damage to membrane fatty acids, proteins and DNA and elevated levels of cytokines (including IL-1α, IL-1β, IL-4, IL-5, IL-6 and IL-12, and lowered IL-8, IL- 13 and IL-15 levels); **(2) immune activation**, with increased expression of activation markers, e.g. CD8+ and CD38+ and HLA-DR+ markers, increased IgM responses against a number of neoepitopes formed by oxidative and nitrosative stress, dysregulation of the [antiviral] 2'-5' oligoadenylate synthetase/RNase L pathway with increased activity of PMNelastase and increased serum neopterin (which indicates activation of IFN-γ-induced pathways); **(3) immunosuppression**, as indicated by, for example, lowered natural killer cell activity, decreased *ex vivo* expression of activation markers such as CD69, impaired lymphocyte responses to phytohaemagglutinin (PHA) and delayed-type hypersensitivity skin responses; **(4) autoimmune reactions** in around 30% of the patients [other reports observed a 60%]; **and (5) others findings suggestive of inflammation** including: lowered antioxidant levels, e.g. zinc and coenzyme Q10; mitochondrial dysfunction; and bacterial translocation,

demonstrated by increased prevalences and median values for plasma IgA and IgM against the LPS of commensal bacteria, such as *Hafnia alvei; pseudomonas aeruginosa; morganella morganii; proteus mirabilis; pseudomonas putida; citrobacter koseri; and klebsiella pneumoniae*. These researchers further found in a more recent study **evidence that low-grade inflammation and cell-mediated immune (CMI) activation are hallmarks of ME/CFS** and that increases in **IL-1, TNF-α, and CMI-related pathways may be causally associated with specific ME/CFS symptoms.** The authors, additionally explain how, while lowered NK cells activity and decreased expression of activation markers, e.g. CD69, are often interpreted to indicate immunosuppression, it is known that **in inflammatory disorders signs of "immunosuppression" and inflammation/CMI activation coexist**. The study finally concludes that even though there may exist ME/CFS subgroups that are characterized by different immune responses, **inflammation is a consistent finding in ME/CFS** [106,124].

Maes et al. further **linked the leaky gut, the inflammation and the autoimmunity** found in **ME/CFS, with the symptomatology** of the disease: Increased prevalences and median values for plasma **IgA and IgM against the LPS** [major component of the outer membrane] of **commensal bacteria have been found in patient with ME/CFS**, indicating an increased translocation of gramnegative commensal bacteria subsequent to a weakening of the intestinal mucosal or tight junction barrier (leaky gut) in ME/CFS. Inflammation, through an increased production of IL-1β, IL-6, TNF-α, and IFN-γ, is known to weaken the tight junction barrier, and therefore, to induce bacterial translocation. This allows otherwise poorly invasive bacteria to cross the intestinal mucosa. This mechanism allows LPS to be translocated from the gut to the interstitium and the mesenteric lymph nodes (MLN) and eventually the blood. Consequently different pathways may be induced: (1) LPS may--through binding to the CD14-Toll-like receptor-4 (TLR4) complex--induce NFκβ and subsequently COX-2, inducible nitric oxide synthase (iNOS), and macrophage-derived PICs, including IL-1 and TNF-α, and reactive oxygen (ROS) and nitrogen species (RNS). This in turn may provoke neuroinflammation and further cause specific symptoms of ME/CFS as well as depressive symptoms; (2) the increased levels of LPS in the MLN and eventually the blood, in turn, may trigger IgA and IgM-mediated responses to LPS; (3) these gram negative bacteria may act as superantigens for T lymphocytes and also induce autoimmune responses through molecular mimicry--commensal bacteria have antigenic sites that are similar to those of the lipid structures of neuronal tissues; (4) LPS can in addition directly stimulate the production of neopterin by monocytes/macrophages and elastase by neutrophils and monocytes. **In conclusion, the increased translocation of commensal bacteria may be responsible for the disease activity in a subgroup of patients with ME/CFS through induction of a systemic inflammatory response and cell-mediated immunity activation** [106, 125].

Neuroinflammation in ME/CFS has further been confirmed with neuroimaging techniques. Nakatomi et al. (2014) used a positron-emission tomography and the radioligand 11C-(R)-PK11195 for the imaging of the translocator protein (TSPO), to map activated microglia or astrocytes in the brain of ME/CFS patients. This technique, widely used to assess neuroinflammation in neurologic diseases [neuroinflammation is evidenced by activation of microglia], allowed the researchers to conclude that **neuroinflammation is present in widespread brain areas of CFS/ME patients and was associated with the severity of neuropsychologic symptoms**. More specifically, they found inflammation of the thalamus and midbrain, what **may induce cognitive impairment and severe fatigue sensation** by perturbing the arousal and awareness states; likewise, inflammation of the amygdala was also related to the cognitive impairment score in CFS/ME patients, while impaired serotonin dynamics in the anterior cingulate cortex (as the authors showed in a previous study) were **associated with the severity of pain** in CFS/ME patients. Finally, the researchers give two plausible **explanations** for these results: first, patients could be compensating the functional loss associated with the disease, by exerting a greater effort to perform daily activities, resulting in **enhanced neural overactivation of N-methyl-D-aspartate** receptors, leading in turn to production of PICs, reactive oxygen species (ROS), and nitrogen species (NOS) that are the final cause of the inflammation. A second plausible mechanism is **the immunologic response to infectious processes**, which can also induce the production of the PIC, ROS and NOS [126].

Focusing on **the autoimmue processes found in ME/CFS** and their role in the pathophysiology, the most forceful evidence on the central role of inflammation and/or autoimmunity in the etiopathogenesis of ME/CFS has been recently shown by Fluge et al. After the results observed in a first pilot case series study of three patients showing the possible potential **of the monoclonal anti-CD20 antibody rituximab for the treatment of ME/CFS**, they performed in 2011 a second, small, randomized, double-blind placebo-controlled phase II study with 30 CFS patients with follow-up for 12 months, which results showed a major or moderate overall response in 67% of patients (significantly higher than the improvement observed in the placebo group) [127]. This study has been followed by a recent third open-label phase II trial, one-armed with no randomization, comprising 29 patients with ME/CFS, with the aim of evaluate a maintenance treatment with rituximab. At end of a follow-up of 36 months, clinically significant responses were seen in 64% of patients. (These results have been used to "fine tune" the now ongoing randomized phase III-study). The authors explain that in general, the improvements were seen from the sixth month reaching a maximum at 20-24-36 months of follow up. They also point out that **prolonged B-lymphocyte depletion [caused by rituximab] was associated with distinctly prolonged duration of clinical responses of ME/CFS symptoms**, what according to the researchers is suggestive of an influence of B-cell depletion on the mechanism for ME/CFS symptoms maintenance. On the other hand, the study concludes that the mean time lag between initial and rapid B-cell depletion in PB and start of **clinical responses may be compatible with** a mechanism involving reduction of **long-lived auto-Abs;** thus, as they stated in the previous study from 2011, the **responses occurring late after intervention could be explained by the elimination of disease-associated auto-Abs, while the early response pattern could be related to interaction of B-cells with T-cells in antigen presentation**. Exploring deeper into the subject, the authors compiled previous evidence showing a highly significant association between CFS and marginal-zone lymphomas which often arise in extra-nodal tissues, in which chronic stimulation by an Ag is thought to play an essential role in lymphomagenesis either from chronic infections or from autoimmunity [**suggesting a chronic B cell stimulation by either chronic infections or autoimmunity in ME/CFS**]. Finally the authors conclude that their observations suggest that **ME/CFS may be a variant of an autoimmune disease** [128]. In the same vein, increases in the number of mature CD19+ , CD20+ and CD21+ B cells have been reported in ME/CFS. **Activated B cells** possess a high capacity to **generate inflammation and are involved in autoimmune diseases**. Accordingly**, autoimmune reactions are highly prevalent in ME/CFS and many individuals with ME/CFS show several indicators of autoimmune responses. Consistently, numerous auto-Abs have been broadly detected in ME/CFS by different studies**, *including anti-cardiolipin, anti-nuclear envelope antigens, anti-lamine SS DNA, anti-68/48 kDa and microtubule-associated proteone, anti-muscarinic cholinergic receptor, anti-mu-opioid receptor, anti-5-hydroxytryptamine (serotinin) receptor 1A, anti dopamine receptor D2, anti-neuronal antigens antibodies, antiphospholipids and anti-gangliosides*. Furthermore, **secondary autoimmune reactions have been also reported in ME/CFS**. These reactions are directed against neoantigenic determinants (neoepitopes), which are created as a result of damage to lipids and proteins by OS and NS. This way, **self-epitopes may be damaged by exposure to prolonged OS/NS and thus lose their immunogenic tolerance and become a target for the host's immune system**. Most importantly, these **OS/ON-induced-autoimmune processes correlate positively and significantly with the severity of the ME/CFS symptoms** [112]. Finally, at this point is important to remember how **inflammation in the gut of patients with ME/CFS could also lead to molecular mimicry or superantigen-mediated autoimmunity** towards, e.g. lipid structures of neuronal tissues**, through commensal bacteria translocation**, as thoroughly described by Maes et al. (2012) in several studies [125].

As for the **origin of fatigue**, Klimas et al. (2012) concluded that the **elevated PICs IL-6 and TNF-α, the dysregulation of the HPA axis and SNS** (sympathetic nervous system) **as well as increased levels of C-Reactive-Protein (CRP)** seem to be implicated in the general mechanism of chronic fatigue, experienced in a wide variety of different conditions; **all these factors associated with fatigue have**

been extensively found in ME/CFS [117]. In addition, the **fatigue, fatigability and post-exertional malaise in ME/CFS have central and peripheral components, including increased levels of PICs, OS/NS and mitochondrial dysfunctions**. Central disorders include glucose hypometabolism and cerebral hypoperfusion; in this context, **gray matter abnormalities and astrocyte dysfunction seem to be major central contributors to fatigue**. Astrocyte dysfunction results in impaired cerebrovascular autoregulation, which is also an important cause of mental fatigue. Patients with ME/CFS display a global reduction of brain perfusion, with a characteristic pattern of hypoperfusion in the brainstem. Reduced cerebral blood flow was also observed in the brain of 80% ME/CFS patients . Moreover, **volume of white matter correlates significantly and positively with the severity of fatigue experienced by the patients**, who also display a global reduction of brain perfusion, with a characteristic pattern of brainstem hypoperfusion. **The severity of disabling fatigue is further associated with the reduction in basal ganglia activation** [108]. In addition, **bacterial translocation, inflammation and activation of CMI are associated with the severity of specific ME/CFS symptoms**. Furthermore, **inflammation and cell-mediated immunity activation are the direct causes for these symptoms**: **IL-1 and TNF-α** are known to play a role in immunologically-mediated fatigue, while TNF-α mediates the malaise that frequently occurs in CNS inflammatory disorders [106].

As for **dysregulations of the autonomic nervous system and pain**, cytokines, such as TNF-α, are also considered to be triggers of **autonomic dysfunctions**--TNF-α acts on vagovagal reflex circuits in the brainstem to disrupt the autonomic control of the gut [106]. In this respect, inflammatory pathways cause **nociceptor sensitization** with increased responsivity to catecholamines [124]. **[i.e. inflammation lower the pain threshold and favors a sympathetic response].** Morover, **neuroinflammation** (finally caused by ROS, NOS and PICs), which has been confirmed to be present in widespread brain areas in CFS/ME patients, **was associated with the severity of neuropsychologic symptoms, thus correlating with cognitive impairment, severe fatigue sensation and with the severity of pain** [126].

Other somatic symptoms might similarly be explained by **PICs** (including IL-1, IL-6 and TNF-α) as well as by Th-1 like cytokines (including IL-2 and IFN-γ), **which are known to induce depression, fatigue and a variety of somatic symptoms** ["physical in nature" as opposed to psychic] [124].

[In conclusion, symptoms of ME/CFS seem to be a direct consequence of a chronic inflammation that accompanies a ongoing week Th1-immune response, accompanied by the anti-inflammatory Th2- and probably Th17-phenotypes. While different immune signatures have been found--which might account for the non-static nature of the disease, and/or for distinct subsets of patients, the chronic inflammation is a hallmark of the disease. This homeostatic pathologic state is characterized by a microenvironment rich with pro-inflammatory cytokines and reactive species, which in turn favors the development of the well described autoimmune processes in ME/CFS. Downstream deleterious consequences of inflammation, oxidative damage and autoimmunity include: damages at a molecular level--such as mitochondrial impairment; multisystem affectations--such as hormonal imbalances, mainly observed in the HPA axis; neuroinflammation; and/or autonomic dysfunctions. These downstream abnormalities seem to constitute the final causes of the symptomatology of ME/CFS].

6.4. CHRONIC LYME DISEASE AND ME/CFS: GOOD CANDIDATES FOR LDI?

[As earlier analyzed, the huge variety of **conditions that have been shown to significantly respond to the different antigen-specific-immunotherapy approaches reviewed**, including allergic diseases, autoimmune disorders and chronic infectious conditions, **seem to share the following common features that might be required for other pathologies to be considered as potential good candidates to be successfully treated with antigen-specific immunotherapy:**

1) Chronic inflammatory conditions characterized by ongoing immune activation.
2) Immune deviation from the phenotype that would properly address the known/suspected trigger/s.
3) Acquired molecular mimicry-mediated autoimmunity as an important pathogenic mechanism.
4) Symptomatology thought to be a result of the ongoing immune activation, inflammation and related autoimmunity.

From the above performed analysis on chronic Lyme disease (LD) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS), the following conclusions might be drawn:

1. Both conditions are characterized by a state of ongoing immune activation. While in LD the logical thing to assume would be that bacteria *borrelia b*. directly induces this chronic immune response, it is actually unclear whether it participates in the late stage of the disease, or rather other factors may take place as main drivers and perpetuators of the pathophysiology, including other bacterial/fungal/viral infections or Bb-independent autoimmune processes which become persistently perpetuated. As for ME/CFS, whereas the original trigger is unknown, the ongoing immune activation is a hallmark of the disease, and actually, the immune signature observed in ME/CFS is the kind of response to be expected in chronic intracellular chronic infections that have induced over time a state of immune exhaustion--largely demonstrated in ME/CFS.

2. The predominant immune response in both LD and ME/CFS seems to be a weak Th1/Th17 responses that over time become weaker and switch to the anti-inflammatory Th2 and probably Th17 phenotypes. In LD this would be the expected profile induced by Bb in order to avoid its destruction by the immune system, and thus become chronic. On the other hand, in ME/CFS, these observations support the general believe that intracellular chronic infections might drive the perpetuation of the disease. In sum, **both diseases show an immune deviation from that that would properly address the trigger/s thought to play a key role, in the initiation and perpetuation of the conditions**.

3. Autoimmune processes have been widely demonstrated in both LD and ME/CFS, mediated by either molecular mimicry, hyper responsive T and/or B cells, or antibodies complexes, directed toward many different tissues/organs. Moreover, these autoimmune processes have shown in both conditions **to be pathological and to correlate with the type as well as with the severity of symptoms**.

4. Finally**, it is clear for both LD and ME/CFS, that symptoms are the direct result of the chronic inflammation and related autoimmunity**. Interestingly, even though LD is an infectious condition (at least in the first stages), the bacteria does not display any virulent factor. Instead, it is the host's response toward the bacteria what directly causes the characteristic inflammation and related autoimmune processes responsible for the symptoms. In this respect, as earlier discussed, there is evidence supporting chronic LD pathogenesis to be mostly autoimmune.

Taken all together, chronic Lyme disease (LD) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) present the common pathophysiologic characteristics to be considered as potentially good candidates to be successfully treated with Low Dose immunotherapy (LDI), thus corroborating the promising empirical results already reported by many doctors and patients].

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