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Including Outer Surface Proteins in the Lyme Disease Case Definition

When it comes to defining Lyme disease, it appears both sides are ignoring the disease-causing effects of the **Outer Surface Proteins (Osps)** shed by Borreliae.
Given the known effects that lipoproteins have on the immune system, both International Lyme and Associated Disease Society (ILADS) and Center for Disease Control and Prevention (CDC) should be doing extensive research to fully comprehend the effects that Osps have on the immune system.

Furthermore, Osps need to be incorporated in the definition in order to accurately describe all cases of Lyme disease. Including Osps into the Lyme disease case definition may give researchers a better understanding of why so many people relapse after treatment. It has long been documented in scientific literature that certain Osps have a direct impact on the immune system. Specifically, on the Toll-Like Receptors (TLR).

Toll-Like Receptors:

Toll-like receptors "are a class of proteins that play a key role in the innate immune system" (Toll-like receptor, 2019, p. 1). TLR "are single, membrane-spanning, non-catalytic receptors usually expressed on sentinel cells such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from microbes" (Toll-like Receptor, 2019, p. 1).

These immune receptors participate in the first line of defense against invading pathogens. They play a critical role in the innate immune response by recognizing the distinct molecular structure of invading pathogens. When it comes to Lyme Disease, both ILADS and the CDC tend to ignore the impact that certain Osps have on TLR 2.

TLR 2's mechanisms: TLR2 manages mycobacteria or fungal endotoxins

TLR receptors are responsible for managing a variety of bacterial, fungal and viral infections. In their essay, Goodridge & Underhill (2008) state that "the innate immune system utilizes multiple receptors to recognize fungal pathogens, and the net inflammatory response is controlled by interactions between these receptors" (p. 8). They found that "many fungi are recognized, at least in part, by Toll-like receptor 2 (TLR2)". (Goodridge & Underhill, 2008).

In addition, TLR 2 "agonists include bacterial lipoproteins, Gram-positive bacterial lipoteichoic acids, mycobacterial lipomannans and lipoarabinomannans, pneumococcal peptidoglycans, and Treponema-derived glycolipids"(Goodridge & Underhill, 2008). One of the key purposes of

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TLR 2 is to manage fungal endotoxins and mycobacteria when they enter the bloodstream (TLR2, 2019). For example, TLR 2/1 receptors are responsible for managing pathogens (like Tuberculosis, Brucella, Mycoplasma), and fungal antigens (i.e. Candida).

It is also important to know that, "TLR2 mediates inflammatory responses to a wide variety of lipidated microbial components, including bacterial lipoproteins, atypical lipopolysaccharides, and lipomannans" (Kelley, Ranoa & Tapping, 2013). Among these microbial agonists, bacterial lipoproteins are by far the most potent (Kelley, Ranoa & Tapping, 2013).
Moreover, "it became apparent that specific TLRs such as TLR2 and TLR4 play differential roles in the activation of the various arms of the innate immune response" (Kullberg, Meer & Netea, 2007). Given this information, we believe that it is crucial that both the CDC and ILADS include these mechanisms when establishing a case definition for Lyme Disease.

Although this may be difficult to perceive, this information is imperative when it comes to understanding how certain lipoproteins shed by spirochetes operate within the human body.

Pam3Cys/OspA

Pam3cys is a "TLR1/TLR2 agonist" (Pam3Cys, 2018). More specifically, it is a synthetic analog of the triacylated N-terminal part of bacterial lipoproteins" (Pam3Cys, 2018). Simply put, Pam3cys is a molecule that stops the immunity chain reaction in the immune systems toll-like receptors.

In a study done by Heilbrun et al (2003) from the *Department of Pathology*, University of Utah in Salt Lake City found that "OspA is one of the tripalmitoyl-*S*-glyceryl-cysteine (Pam3Cys)-modified lipoproteins abundantly expressed on the surface of *B. burgdorferi* in the gut of the unfed tick." (Heilbrun et al, 2003).

In summary, "Pam3Cys modification of bacterial lipoprotein has adjuvant properties independent of TLR2 signaling" (Heilbrun et al, 2003). When you understand that Pam3Cys and OspA are lipoproteins (can cause immunosuppression), it's clear that more research must be done by agencies like ILADS if they want to properly explain why symptoms persist in up to 50% of people after long-term antibiotic treatment.

All that said, it is especially important that both the CDC and ILADS incorporate the TLR 2 altering mechanisms in order to establish an accurate case definition of Lyme Disease.

In the *Journal of Immunology*, Akira, Hayashi, & Nobrega (2005) published a study whereby "experiments with TLR2-knockout mice confirmed that the inhibitory effects of Pam3Cys depend on the expression of TLR2" (p.6640). Furthermore, their study concluded that "**Pam3Cys keeps the precursors on a more immature stage**" (Akira, Hayashi, & Nobrega, 2005).

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The study done by Akira, Hayashi, & Nobrega (2005) "suggest that **TLR4 signaling favors B lymphocyte maturation, whereas TLR2 arrests/retards that process, ascribing new roles for TLRs in B cell physiology.**" (p. 6645).

It is alarming that both the CDC and ILADS ignore the documented mechanisms of OspA and Pam3Cys play a crucial role in understanding the effects that spirochetes have on the immune system.

Before the case definition was changed in 1994, the CDC mentioned these mechanisms in their studies. For instance, Borellia undergoes a process in which CDC officer Alan Barbour calls blebbing (Barbour, Dunn & Lade, 1990). These blebs shed surface antigens such as Outer Surface Protein A (OspA) (Barbour, Dunn & Lade, 1990).

OspA

OspA is a TLR 2 agonist (chemical that binds to a receptor and activates it to produce a biological response), making it far more toxic than bacteria managed by TLR4 (lipopolysaccharides). The toxicity of OspA or TLR 2/1 agonists are so vast, that the immune system shuts itself off to avoid septic shock (cytokine storm) (Chang et al, 2015).

Therefore, since OspA is a TLR 2 agonist, it has the ability to cause permanent immunosuppression in many who become infected (Kelley Ranoa & Tapping, 2013). In summary, OspA causes the immune system to become tolerant (unable to recognize and produce an effective antibody response) to pathogens that are managed by TLR 2/1 receptors.

TLR 2 agonists and cross tolerance

In a process known as cross-tolerance, OspA inhibits TLR 7/9 (which manage intracellular pathogens) ability to fight several other viral, bacterial, and parasitic infections. This is one of the key reasons why Coxsackie, Epstein-Barr Virus, Cytomegalovirus, HHV6, Zoster, and other herpes viruses, etc.) and secondary infections (Candida, Fungi, Mycoplasma spp., Streptococcus, etc.) are prominent in almost everyone who has been infected with spirochetes.

It was difficult to find any articles on OspA and TLR 7/9 cross-tolerance. However, other bacteria like M.Tuberculosis also shed lipoproteins that show parallel effects to Osps shed by Lyme spirochetes.

In one study, researchers found that "TLR2 signaling by Mycobacterium tuberculosis or other TLR2 agonists inhibited TLR9 induction of IFN-I and IFN-I-dependent MHC-I Ag cross processing" (Abbott, 2012, 1019).

In addition, "TLR2 also inhibited induction of IFN-I by TLR7, another MyD88-dependent IFN-I-inducing receptor, but did not inhibit IFN-I induction by TLR3 or TLR4 (both Toll/IL-1R domain-containing adapter-inducing IFN-β dependent, MyD88 independent)" (Abbott, 2012, 1019). Finally, because IRAK1 is required for TLR7/9-induced IFN-I production, we propose that TLR2 signaling induces rapid depletion of IRAK1, which impairs IFN-I induction by TLR7/9" (Abbott, 2012, 1019).
In sum, this study found that "TLR2 inhibits IFN-I induction by TLR7/9, may shape immune responses to microbes that express ligands for both TLR2 and TLR7/TLR9, or responses to bacteria/virus coinfection" (Abbott, 2012, 1019).

Therefore, if TLR 2 agonists like M. Tuberculosis can cause cross-tolerance, how come researchers continue to ignore the way similar TLR 2 agonists like OspA effect TLR 7/9. Given the parallel similarities between M.Tuberculosis and OspA, more research is needed to understand whether or not OspA has a direct affect on inducing TLR 7/9 ability to clear out viruses. It is not a coincidence that Lyme sufferers all suffer from herpes infections and other reactivated viruses and opportunistic infections that are typically managed by TLR 7/9.

TLR4 (lipopolysaccharides, known as the more typical bacteria)

One study showed that "the role of IRAK4 kinase activity in TLR2 and TLR4 homotolerance and TLR2-mediated heterotolerance of TLR" (Medvedev, Pennini, Vogel & Xiong, 2013, p. 299).

Furthermore, "IRAK4 kinase activity is dispensable for endotoxin tolerance, as evidenced by suppressed p-ERK, p-JNK, and p-p38, IκB-α degradation, induction of proinflammatory cytokines, and up-regulation of negative regulators IRAK-M and A20" (Medvedev, Pennini, Vogel & Xiong, 2013, p. 299). In contrast, IRAK4 kinase activity is critical for TLR2-elicited inhibition of Pam3Cys- and LPS-inducible p-JNK and p-p38 MAPKs. Studies are in progress to discern the molecular mechanisms by which IRAK4 kinase activity regulates TLR signaling, tolerance, and sensitivity to microbial infections, septic shock, and autoimmunity" (Medvedev, Pennini, Vogel & Xiong, 2013, p. 299). That being said, this data suggests that tolerance caused by TLR 2 agonists can also lead to cross tolerance to pathogens managed by TLR 4.

TLR5 (Flagellins)

In this study, researchers found that "lipoproteins from Borrelia burgdorferi, the agent of Lyme disease, activate inflammatory cells through TLR2 and TLR1 (Cabral et al, 2006, p. 849). Cabral et al (2006) showed that "stimulation of human monocytes with B. burgdorferi lysate, lipidated outer surface protein A, and triacylated lipopeptide Pam3CysSerLys4 results in the up-regulation of both TLR2 and TLR1 but the down-regulation of TLR5, the receptor for bacterial flagellin, and that this effect is mediated via TLR2" (p. 849).

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The results of this study indicate that diverse stimuli can cause differential TLR expression, and we hypothesize that these changes may be useful for either the pathogen and/or the host" (Cabral et al, 2006, p. 849). Therefore, TLR 5 are also affected by OspA's immune altering properties.

The mechanisms listed in the previous paragraphs can occur soon as spirochetes disseminate to the lymph nodes. (Barthold et al, 2011). Once Osps affect the B-cell germinal centers, they can collapse. As a result, B-cells are unable to properly mature (mature B-cells produce healthy antibodies) leading to a reduction in detectable antibodies (Baumgarth, Elsner, Hastey & Olsen, 2015). Simply put, the immune deficiency with Lyme Disease isn't a shortage of B-cells; rather, it seems to consist of an abundance of immature B-cells unable to produce an efficient antibody response from the affected TLRs.

In the end, there is plenty of research that shows **OspA/Pam3cys are TLR 2 agonists that can have a direct impact on the immune system**. As stated above, TLR 2 agonists can lead to immature B-cells and directly affect TLR 2/1, 4/5, and 7/9. Given the information above, it is imperative that the CDC and ILADS include the fact that OspA/Pam3Cys directly impact the production of healthy antibodies if they want to develop an accurate case definition for Lyme disease.

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