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Re: Martin Strnad Ph.D. thesis

Overview

AIM 1: Estimate Europe-wide patterns of Lyme disease *Borrelia* infection in ticks, highlight the need to undertake independent studies of genospecies within Europe, given their varying genetic content and pathogenic potential, and differences in clinical manifestation, and summarize the potential countermeasures and strategies against Lyme disease in general.

AIM 2: Delineate the underlying structure-functional mechanisms defining the delicate *Borrelia*-host interactions in order to better understand the pathogenic process at multiscale resolution using various imaging techniques.

- Mr. Strnad's thesis and associated manuscripts effectively addressed the rationale for the proposed studies, the relevance and importance of each of the review topics, and experimental progress made towards addressing the Aims outlined above.

- Mr. Strnad's writing, document formatting/presentation, and citations were appropriate in the thesis and there were no major concerns with the content. He also identified the majority of the primary abbreviations used in the text. A few minor comments are provided below for Mr. Strnad to consider if further revision is deemed to be necessary.

- Mr. Strnad demonstrated an adequate understanding of North America and European Lyme *Borrelia* background and his scientific rationale, as well as an understanding for their own work and how their findings fit into broader perspective of the *Borrelia* field. The "Conclusion and future perspectives" section was well developed and outlined areas where future studies should focus.

- The annotations that were provided by Mr. Strnad before each of the manuscripts included in the thesis successfully highlighted the importance and impact of the studies. They were effective at presenting the rationale and importance without being burdensome.

- Mr. Strnad's discussion and comparison of various imaging methods/applications was very informative. He was able to also discuss how these methods could be applied to study pathogenesis-related questions in Lyme disease (e.g., Part 2.4). The information regarding the benefits and limitations of various methods was of particular interest and helped convince the reviewer of the benefits of correlative light and electron microscopy, atomic force microscopy-based single-molecule force spectroscopy and solution nuclear magnetic resonance with respect to studying mechanisms associated with Lyme *Borrelia* infection.

- The utility of the tetracysteine/biarsenical labeling for imaging and *Borrelia* pathogenesis was very convincing and should lead to some very impactful and exciting future studies.

- One specific aspect that I did not fully consider until reading Mr. Strnad's thesis was an interesting concept connecting Lyme *Borrelia* pathogenesis and decorin-binding adhesins (Dbps). Specifically,

the hypothesis that the presence or abundance of DbpA and/or DbpB could affect relative stiffness of the bacterial cell. It seems entirely feasible that altered rigidity of the bacterial cell could affect the general swimming speed of *Borrelia*. Considering that effective translational motility is critical for the pathogenesis of *Borrelia*, it seems logical that Dbp-dependent alteration of cellular rigidity could in turn affect motility and/or chemotaxis of the spirochete, and ultimately negatively impact dissemination of Lyme *Borrelia* during infection.

- Mr. Strnad's thesis also highlighted a significant knowledge gap in the *Lyme* Borrelia field that I didn't fully appreciate. Specifically, Mr. Strnad noted that the majority of studies that described the biological functions of DbpA and DbpB (and many other virulence-associated proteins) have been carried out on North American strains of a single species (e.g., *B. burgdorferi* sensu stricto). "What applies for one *Borrelia* probably applies to other *Borrelia*" is a common misconception that exists in our field. Mr. Strnad's thesis provided excellent rationale for the need to perform similar studies on European species to address this knowledge gap.

- Mr. Strnad's discussion of future directions was very interesting and outlined several intriguing avenues for subsequent studies. Of interest are the experiments to correlate adhesins with virulence and mechanical characteristics and then determine whether distribution patterns of adhesive molecules correlated with species specific differences in LD progression.

Comments

- There are two contradictory statements on Pages 5 and 6 that might need to be clarified and/or corrected. The two statements are quoted below.
    - Page 5 states "However, despite numerous attempts, the various manifestations of LD cannot be conclusively attributed to infections with one specific genospecies".
    - Page 6 states, "It is already known that different genospecies of *B. burgdorferi* are associated with distinct clinical manifestations of LD…".

- On Page 8 and in the legend for Figure 1 there are potential typos; sentences containing "enable to…" seem to be missing a subject. Should this be "enable investigators to…"?

- At my institution, our students are required to reformat their published manuscripts to match the format of their dissertation document. Is this the case in your institution? This is just a general question. I have absolutely no objections to the approach used in this thesis (e.g., incorporating the PDF of the manuscript into the full document), and I personally find this to be more efficient (especially for more productive students).

Conclusion

- I recommend the thesis for successful defense.

Sincerely,

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