Author's response to reviews

Title: Public Health Impact of Strain Specific Immunity to Borrelia burgdorferi

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Author's response to reviews: see over
Dear editor,

Thank you for your time and effort on this manuscript. We have revised the manuscript following the recommendations of the reviewers. We have expanded our introduction to provide a better background of the evidence of type specific immunity and expanded the discussion section to better delineate the limitations of the study. We have also highlighted the need for future studies that should consider both the geographical heterogeneity in the incidence of Lyme disease and the spatial distribution of *B. burgdorferi* strains. The comments from the reviewers are written in a sans serif font and our responses in a serif font following the marker “>>>>”.

Reviewer's report

Title: Public Health Impact of Strain Specific Immunity to *Borrelia burgdorferi*

Version: 1 Date: 3 July 2015

Reviewer: Ai Takano

Reviewer's report:

Minor Essential Revisions

In the manuscript by Khatchikian and c-authors were try to clarify the significance of type specific immunity against Lyme diseases infection. The manuscript is interesting. I have some comments as follows;

1, Background is better to write more informatively. Since some readers did not know your study background, they did not understand why the authors paid attention to only human OspC type but not ticks. Moreover, are there any related studies in this field, e.g. other infectious diseases?

>>>> Thank you for this suggestion which will help to broaden the audience considerably. We have clarified the background section and expanded our introduction and discussion in multiple ways including expanding the information on *ospC*, presenting laboratory evidence of strain specific immunity to *Borrelia burgdorferi* in mice, and discussing the public health effect of type specific immunity in other disease systems.

2, In the methods, how the author selected 200 patients for this study? In the previous manuscript, more than 200 patients were listed.
We apologize for this misunderstanding. These patients consisted of the subset of patients from which strains were cultured from the erythema migrans lesions that were reported by Wormser et al. (2008) who had no prior history of B. burgdorferi infection. This set of strains, previously reported in Khatchikian et al. 2014, was used explicitly because the patients could confirm that they had no prior history of Lyme disease. These data were used to determine a conservative frequency distribution of the exposure of humans to each B. burgdorferi strain.

3, Page Line 73-75, it is better to add the reference.

>>>>> Added as suggested.

4, In the discussion Line 186-188, is there any evidence that difference was dependent on only ospC type? I could not find that description on reference 6.

>>>>> OspC has a very interesting history in the Lyme disease research fields. We have added an explicit discussion of this point. Briefly, there is indirect evidence that OspC may be the cause of the type specific immunity, but no direct evidence. Still, strain-specific immunity is unlikely to be restricted to OspC and may develop against other B. burgdorferi strain-specific surface proteins that are in genetic linkage with OspC. We have added information about the molecular causes of type specific immunity to the discussion.

5, In the discussion Line 201-203, I wonder it is better to describe the difference of OspC type between tick population and human skin population that described in reference 6.

>>>>> This is a good suggestion. We have expanded our discussion to also consider the frequency distribution of B. burgdorferi strains in ticks. A formal comparison of the frequencies of B. burgdorferi strains in ticks, human skin, and human blood is now cited (Dykhuizen et al 2008).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician. Declaration of competing interests: I declare that I have no competing interests.
Reviewer's report

Title: Public Health Impact of Strain Specific Immunity to Borrelia burgdorferi

Version: 1 Date: 27 March 2015

Reviewer: Ellen Tijsse-Klasen

Reviewer's report:

Major Compulsory Revisions

1) My main concerns regard the choice of data used to estimate the frequencies of the different OspC types: sequence data of cultured patient isolates were used. The bottleneck of culturing could affect different strains to various degrees, which might lead to skewing.

>>>> We apologize for this oversight and agree that the potential biases in the assumptions of these types of analytical models should be discussed at length. We have now discussed the potential effects of culture biases on the reported public health effect of type specific immunity.

A further source of potential skewing is that it cannot be excluded that some of the patients whose data was used here might have suffered a second or third infection with B. burgdorferi. Due to the strain specific immunity mentioned by the authors, this would lead to an underrepresentation of common OspC types in the study population. This is especially important as the data are used to estimate the number of averted cases due to strain specific immunity. It would therefore be desirable to include a discussion of such shortcomings of the data set.

>>>> Sorry for this confusion. These patients were selected in previous studies explicitly because they had no prior history of Lyme disease (Khatchikian et al 2014). This is now explicitly stated in the methods and in the discussion.

Though not all B. burgdorferi strains found in ticks are pathogenic, a comparison with OspC frequencies in tick population might help to support the assumptions of the authors. However, while I would like to see a more elaborate discussion of potential shortcomings of the study design in the final manuscript, I believe that the main message of the manuscript remains valid based on the study’s findings.
Minor Essential Revisions

2) Line 158-161: Could differences in culturability of various strains lead to such high frequencies of some strains in human isolates?

>>> Differences in culturability are possible and are now discussed. Several lines of evidence do suggest that it is unlikely that culture bias has resulted in the detection of only some strains at high frequencies. First, there are differences in the frequencies of strains in cultures of skin versus blood of humans. Second, non-culture methodologies performed with various animal species produce similar types of frequency biases, suggesting that the biases are not the result of differences in culturability. The updated manuscript discusses these topics.

3) Line 163-167: The authors state: “the frequency of a positive blood culture in patients with a recurrence of erythema migrans would be reduced by approximately 25.3%” and refer to supplementary table 1. This table does not support the statement made by the authors.

>>> We apologize for this confusion. We have rephrased this statement to better explain that these results are derived from the analytical model. The reference to the table was confusing as the information in the table represents only the assumptions used in the analyses, not the results. We have referenced the table in the proper location in the updated manuscript.

Level of interest: An article of importance in its field

Quality of written English: Acceptable Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.

Reviewer's report

Title: Public Health Impact of Strain Specific Immunity to Borrelia burgdorferi

The goal of this manuscript was to assess empirically the public health impact of strain-specific immunity to Borrelia burgdorferi in Lyme disease. The authors used analytical modeling to estimate the number of Lyme disease cases that may be prevented as a result of immunity against specific Borrelia burgdorferi OspC type if the person were to be re-exposed to the same OspC type. Assuming 3% reinfection rate and 300,000 new cases annually in the US, they concluded that between 319 and 2378 cases of Lyme disease may be averted each year due to strain-specific immunity. Although the data are derived using computational approaches, which makes it more difficult to assess their relevance in the actual disease, the findings provide a novel and unique insight into the possible impact of strain-specific immunity on B. burgdorferi infection, particularly in the northeastern US. Such modeling approaches may be applicable to estimate the effect of immunity in other regions of North America and possibly Europe where infection with B. burgdorferi is prevalent.

Discretionary Revisions: This is a well-written manuscript requiring only minor revision. The areas that would benefit from additional clarification and/or discussion are listed below.

1) This study relies on several postulates, including: that patients develop immunity following infection with any OspC type, that the immunity is targeted against the OspC of the infecting strain, and that such immunity will prevent reinfection with the same OspC type. These assumptions are in large part based on the authors’ published study of 17 patients with reinfection and on a subsequent study using computational approaches to determine the probability of being re-exposed to infection with a strain of the same OspC type. Due to the relatively small group of patients (N=17), all of whom were from northeastern US, it is not yet clear how these findings will hold up in larger numbers of patients and in other regions where the distribution of strains and the clinical picture of disease may be different. The Discussion section could be expanded to include these limitations.

>>>>>Thank you for the suggestion. We have added considerably to the discussion section on the caveats of this work.
In addition, evidence of strain-specific immunity against B. burgdorferi is primarily empirical and it will be important to directly validate this concept using laboratory approaches to determine which Borrelia components (OspC or other proteins and lipoproteins) immunity is directed against, the cellular responses that mediate the immunity, and whether immunity is generated and protective to the same degree against any OspC type. The manuscript would benefit from a brief overview of the laboratory evidence of strain-specific immunity (e.g. Probert WS et.al. 1997. J Infect Dis. 175:400-405...) and how such evidence supports the current findings derived from analytical models.

>>>>Good point. Immune responses targeting OspC have been the focus of considerable research, but evidence to support the hypothesis that these responses are the cause of type specific immunity is indirect. Further empirical studies that characterize multiple antigens are necessary to determine the mechanisms causing strain-specific immunity in humans and other species. We have made clear that *ospC* is a marker for *B. burgdorferi* lineages and discussed these points at length.

2) The potential health impact of strain-specific immunity is highly dependent on the geographical distribution of the Borrelia OspC types. Therefore, one would predict that the findings reported here are applicable primarily to the northeastern US. As stated in the manuscript, in the Lower Hudson Valley of New York approximately 80% of patients with EM are infected with one of 4 OspC types and thus the possibility of being reinfected with the same OspC type would be reduced about 25%, leading to significant impact on health. In contrast, in regions such as midwestern US where OspC types recovered from EM skin lesions are more diverse and there is not a dominant overrepresentation of one or few OspC genotypes, the impact of strain-specific immunity on human health may be minimal. Expanding the discussion to include these ideas would broaden the understanding and the implications of this work.

>>>> We find this issue extremely interesting and have expanded the discussion section to consider these points. We now discuss how differences in spatial distribution of the OspC types might potentially affect the impact of strain specific immunity.

3) The last paragraph of the Results section could use some clarification. What is the significance of reporting the recovery of spirochetes from blood versus skin? Are the frequencies of OspC types recovered from blood and EM skin lesion similar? The authors indicate that due to strain-specific immunity, the probability of positive Borrelia culture from blood in patients with recurrence of EM would be reduced by 25.3%. Would one expect a
similar decrease in probability of a positive culture from EM skin lesions? Additional discussion of the significance of Borrelia recovery from skin versus blood would be helpful.

>>>> Sorry that this was not clear. The skin cultures were used to estimate the rate of exposure of humans to each strain, which is now stated explicitly in the second sentence of the methods. We have also modified the results to clarify this point. Blood cultured *B. burgdorferi* were explicitly discussed because disseminated infections can have clinical implications.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician. Declaration of competing interests: I declare that I have no competing interests