Author's response to reviews

Title: Identification of mimotopes from Mycobacterium leprae as potential diagnostic reagents

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Author's response to reviews: see over
Reviewer's report

Title: Identification of mimotopes from Mycobacterium leprae as potential diagnostic reagents

Version: 3 Date: 8 October 2012
Reviewer: VaralakshmiVissa

Reviewer's report:
My comments are in the latter section: these are not discretionary, but important to include in the revision. They have been detailed and fairly easy to follow.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

In the revised manuscript, the authors have provided Figure 1 which has the image of the SDS-PAGE resolved M. lepraesonicate and the western blot generated from probing the same fraction with the primary pooled serum and the IgG fraction eluted from the membrane. This is helpful. The pattern of binding is familiar and expected. Lipoarabinomannan is a major contributor to the antigen binding is a big antigen and contributes to the big smear spreading from 30-40kDa.

So is the addition of Table which has the FASTA results (from querying the Leproma database with the phage peptide sequences). Seeing Ag85B as a candidate protein that binds one or more of the phage mimotopes is reassuring that the system worked (the LAM epitope/antigen as such would not be picked up by FASTA approach).

Answer: Your comments were very helpful and were included in the manuscript.

The authors still maintain that finding 'skin test antigen' alternatives was their primary goal, so they went after the DTH angle, even though the 'hook' was antibodies from Multibacillary patients, and not some other form of antigen screen. It would have been good to see more serology work and claim credit for producing a set of peptides that can assist in this.

This study was designed to identify antigens that can be used to evaluate cellular response similar to the use of lepromin. Antigens were selected from antibodies in patients. The antigens identified were then evaluated for their ability to induce cellular
response in the DTH test. The results are reported in this manuscript. Although further studies are needed involving larger samples of animals, different doses and antigen composition, we provide evidence that peptides can be skin test reagents. The value of these peptides is not at all restricted to skin tests and other T-cell assays and peptides have also been tested in serological assays. The assessment of cellular and humoral responses can help determine whether these peptides can be reagents in serological and T-cell assays. To some extent this information is included in the manuscript (lines 291–296).

The sticky point that this reviewer would still wish to pursue (and see eliminated from the manuscript) is the claim that two peptides performed as well as leprominin the DTH experiment. The data should be presented as such and not combined to yield the promising 60% similar to lepromin (i.e., 2/5 for 5A and 1/5 for 1B). The group size is too small for the numbers to be meaningful, especially for 1B, and more work needs to be done to substantiate the potential of 1B in DTH, even if 1 out of positive 5 tests is somehow encouraging. This sentence is in the Abstract (line 44) and Results and Discussion (line 266) and should be removed.

The sentence was removed from the Abstract and the Results and Discussion section.

Here are other minor points, suggestions and a list of unclear statements:

1) Title: mimotopes of M. leprae, not from

It was corrected.

2) Line 74: mouse foot pad inoculation also yields M. leprae (more viable though less numbers of cells per animal. Also the model is not as ‘specialized’ as the armadillo

M. leprae can be propagated in armadillo and common, athymic, and gene knockout mice. However, the sentence in the manuscript refers the source of M. leprae for producing lepromin.

3) Spelling: Eluted, not eluded

It was corrected.
4) Intradermally: not intradermically
It was corrected.

5) Duration and temperature for the IgG elution from PVDF membrane in presence of acidic glycine buffer?
This information was added.

6) Strategy I: A series peptides? Strategy II: B series: i.e peptide 1B?
A series peptides were identified using strategy I and peptide 1B was identified using strategy II. [Professora será que seria o caso de trocar estratégia I e II por estratégia A e B, respectivamente?]

7) Lines 219 to 221: In both strategies, an increase of reactivity was observed. However, the best result was detected between the phages and the IgG obtained in strategy II. Such results indicated that there was a selection of reactive phages with antibodies from leprosy patients.
Add this ‘with each round of panning’ at the end of ‘In both strategies, an increase of reactivity was observed’
We completed the sentence as suggested.

‘Such results……..’: this sentence is confusing or contradictory: Strategy II did not select for phage pools that was pre-depleted of clones that bind TB IgG, so the phage pool is less M. leprae specific than from Strategy I. Perhaps this sentence was meant to be placed elsewhere or should be deleted.
This sentence was removed.

8) Rewrite line 218: round was assessed – by immunoenzymatic assay ELISA – regarding its immunoreactivity (Figure 2) Just use ‘round was assessed by ELISA. (Figure 2)’.
We followed your suggestion.

9) Rewrite Line 150: amplified by infecting a culture of E. coli K91
We followed your suggestion.
10) Line 182: What is OPD: expand and name of company
Complete information on the reagent is provided on line 169 where it is first mentioned.

11) Line 212: The total IgGs from MB patients recognized proteins in M. leprae especially below 50 kDa (see Figure xxxxx). The same profile of reactivity was seen with antigen-specific IgGs, (see Figure xxxxx) however, the antibody preparation method represents an important purification and concentration step, a
Where indicated, insert figure number and lane to orient reader Figure 1A, lane 2 or 3 etc.
Also use the term gel lanes, not columns
We followed your suggestion.

12) Line 221:
This sentence was removed (see item 7).

13) Line 316: ‘the’
Review the English language was made

14) The search for similarity of peptide sequences in Leproma database showed that the peptides selected mimic mainly proteins in M. leprae, except for 5A peptide (Table 1):
Not clear: what does this mean “except for 5A peptide”?
Peptides 6A and 1B showed sequence similarity with proteins in M. leprae while peptide 5A showed similarity with a lipoprotein as well. We removed "except for 5A peptide" to make it clear.

15) Rewrite Line 302:
Recently, the development of a user-friendly assay to detect multiple cytokines[30] which can make INF-# detection assays more accessible and easier to perform was reported
The sentence was rewritten as suggested.