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

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Reviewer: Varalakshmi Vissa

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. leprae sonicate and the western blots 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 30-40 kDa.

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).

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.

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 lepomin in 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.

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

1) Title: mimotopes of M. leprae, not from
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
3) Spelling: Eluted, not eluded
4) Intradermally: not intradermically
5) Duration and temperature for the IgG elution from PVDF membrane in presence of acidic glycine buffer?
6) Strategy I: A series peptides? Strategy II: B series: i.e peptide 1B?
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’

‘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 that from Strategy I. Perhaps this sentence was meant to be placed elsewhere or should be deleted.

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)’

9) Rewrite Line 150: amplified by infecting a culture of E. coli K91

10) Line 182: What is OPD: expand and name of company

11) Line 212: The total IgGs from MB patients recognized proteins in M. leprae especially below 50 kDa (see Figure xxxx) . The same profile of reactivity was seen with antigen-specific IgGs, (see Figure xxxx ) however, the antibody preparation method represent 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

12) Line 221:

13) Line 316: ‘the’

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”?

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.