Reviewer's report

Title: Immunologically reactive M. leprae antigens with relevance to diagnosis and vaccine development.

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Reviewer: Annemieke Geluk

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General remarks:
The authors have investigated both HMI (IgG) and CMI (IFN-g) directed against M. leprae recombinant proteins that were selected based on their sequence specificity and epitope prediction pattern.

The set-up of this study is straightforward and is performed adequately. The research groups have ample experience with recruitment and diagnosis of leprosy patients and in the production of purified recombinant M.leprae proteins. However, in view of the work already performed by these groups and others the study is not really innovative. In addition, the major conclusion of the manuscript is only slightly different from the work described by these groups previously, e.g. the authors describe data on several Ag including ML0405 and ML2311 that were described previously by these authors.

A weak point of the study is that the authors have only tested individuals from one leprosy endemic region. Thus, the authors should restrict their conclusions regarding their proteins to the application of the Ag as vaccines or diagnostic tools for Brazil.

Major Compulsory Revisions

• Line 38: since 45 individuals per group were tested for Ab and 20 individuals per group for IFN-g it is not clear whether the same individuals were tested for both groups or whether separate (previous) studies on T cell assays and serology were combined. If all 20 individuals tested for IFN-g were also included in the serology tests. The ms would benefit from an addition of such data per individual for both assays in order to strengthen the hypothesis that one individual is recognized by CMI and HMI and thus can be used for diagnosis of both TT and LL leprosy.

• The authors do not provide sufficient information on the social-economic background of the EC that are used in the study, which may be crucial to the specificity for M.leprae.

• Line 355: useful for diagnosis in Brazil, as only Brazilians were investigated in the authors’ study and responses in other endemic areas need yet to be studied for these Ag.

• Line 262: the authors state that ML0840 is unique for M.leprae, this is, however,
not the case as it contains 59% homology with Mycobacterium avium subspecies paratuberculosis (MAP).

Minor Essential Revisions

• Line 35: the authors themselves and several other groups have studied the IR against M.leprae antigens even before the genome was sequenced so it is not correct to state that: “relatively little is know… during leprosy.”
• Line 37: high endemic: please provide the prevalence rate of the region of all individuals that were included in the study.
• Line 46: 16 single proteins (Table 2 idem) vs. line 49-50: n=9 + n= 3 + 3; what is the correct number meant? of these proteins. Is this exactly similar to the social-economic background of the leprosy patients and HHC?

Methods/ materials

• Line 111: please provide the number of the ethical permission obtained locally.
• Why was LID-1 (as previously described as a hybrid of these Ag) not used instead of using both ML0405 and ML2311?
• Line 125: how long after initiation of treatment were the TB recruited? In order for TB patients to be good controls their responses to PPD or MtAg should also be shown in comparison to leprosy patients’ responses to the same Ag.
• Please also show the medium values for all groups tested in a separate graph or supplemental data.
• Line 166: please provide city, state of company.
• Line 193-194: M.leprae specific…proteins: sentence is not correct.
• Line 272-283: The method can identify and predict HLA binding regions from antigen sequence. So it is not that surprising that it is not predictive for T cell epitopes as it does not predict interactions with the TCR. Instead the authors could use SYFPEITHI which also includes probability of being processed in addition to presentation in the context of a certain HLA allele. However, it remains a prediction and the authors’ conclusion is justified in that it does not imply a prediction of T cell epitopes if one uses a program that applies HLA-peptide binding motifs only.
• Table 1: please provide also PGL-I data.
• Table 2: the authors should include sequence comparisons of more sequenced (myco)bacteria like M. smegmatis, M. microti and M.paratuberculosis.
• Line 321: cross-reactivity and lack of specificity indicate similar observations, please leave one out.

Discretionary Revisions

• Line 155: cut off of 50pg/ for positive responses in a WBA that also has a detection limit of 20pg/ml is rather low.

Level of interest: An article whose findings are important to those with closely
related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.