Reviewer's report

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

Version: 1 Date: 5 November 2010

Reviewer: John Spencer

Reviewer's report:

This work examines the serological and cell mediated responses of leprosy patients at the two ends of the clinical spectrum to determine whether one can predict if any of 33 different M. leprae recombinant proteins will stimulate a response based on programs that identify potential T cell and B cell epitopes in these proteins. The study is comprehensive, well written, well executed and clearly described. Several minor points for consideration:

Minor essential revisions:

1. The study that first identified ML0405, ML0331 and ML2055 as good antigens (Ref 28) used an expression cloning system to screen reactivity of pooled LL/BL patient sera to identify potential M. leprae reactive antigens. Three members of the Ag85 complex (ML0097, ML2028, and ML2655) were reactive, some very strongly by ELISA, so it was surprising that none of these (ML0098, ML2028, and ML2655) showed any reactivity in cell mediated or serological responses in this study. Is this due to batch to batch variation or folding issues with the recombinant proteins?

2. It was mentioned in the WBA methods section that whole M. leprae cell sonicate (MLCS) was used, assumingly for a positive control, but no results were shown for this antigen (cell mediated or serological) or a T cell mitogen, such as PHA. Were such positive controls included in WBA experiments to ensure that each blood sample was capable of a good response, and if so what kinds of IFN-g levels were elicited?

3. The statement in the Abstract that “Relatively little is known about the immune responses to individual proteins of M. leprae recognized during leprosy.” is not exactly true based on a lot of earlier studies and more recent post-genomic studies cited in Refs 3, 4, 19-26, and 28.

4. Table 2, ML1685 listed as “malato” should be malate; there is an extra “y” in immunogenicity in the far right column.

Discretionary revisions:

5. Previous studies by this group examined either serological responses or T cell responses against M. leprae recombinant proteins, whereas this study links both, which is an improvement. In a previous study, 36% of the recombinant proteins
(5/14) were recognized, while in this study, 27% (9/33) were recognized as T cell antigens, so the numbers are fairly consistent. It is disappointing that some of the antigens, particularly ML2346 which is unique to M. leprae, shows good responses in TT/BT and HHC, but lacks specificity. There must be regions within these proteins that elicit this cross-reactive or nonspecific response in the other groups. Is it possible to screen the 5 potential peptides that are predicted by PROPREP for ML2346 to see if one can clean up the response in the control groups? Ditto for some of the other proteins such as ML2358 (3 predicted T epitopes), ML0276 (5 epitopes), ML2541 (5 epitopes), ML2603 (5 epitopes), and ML2380 (3 epitopes).

**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.