Reviewer's report

Title: T-cell and serological responses to Erp, an exported Mycobacterium tuberculosis protein, in tuberculosis patients and healthy individuals

Version: 1 Date: 19 May 2007

Reviewer: Katalin Andrea A Wilkinson

Reviewer's report:

General
T cell based interferon-gamma release assays based on RD1 encoded proteins represent a potentially significant advance on the tuberculin skin test for the diagnosis of tuberculosis. However, a major obstacle to the widespread introduction of these tests in endemic areas is their inability to differentiate between active and latent disease. The present article is aiming to address this issue by exploring the T cell and serological responses to Erp (exported repeated protein) of M. tuberculosis, in tuberculosis patients and controls from a non-endemic area.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The text in many parts of the manuscript is scrambled and it is very difficult to read.
2. The manuscript would also greatly benefit from editing by a native english speaker
3. The term 'latently TB BCG+ individuals' should be changed to individuals with latent TB infection (LTBI)
4. The antigen called '85B' in the manuscript is generally known as 'antigen 85B' or 'Ag85B'.
5. The introduction states that Erp has 'not yet been investigated in humans'. This is immediately followed by the sentence that 'this antigen could offer an interesting way in the management of tuberculosis disease or infection'. These two statements are contradictory and should either be expanded with further explanation and references, or the second speculative statement moved to the discussion.
6. In the Methods: Detection of specific human antibodies against recombinant M. tuberculosis proteins: the plates were coated with 3µg/ml recombinant protein in PBS, and positive values were arbitrarily decided to be >0.1 (I assume OD).

The usual control experiment to perform in order to define positive values is coating a plate with PBS alone.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. The median age in the abstract is not relevant. The median number of IFN-g spot forming cells in response to Erp in the LTBI group compared to the other groups, leading to the p value of 0.019 mentioned in the abstract would be a more relevant piece of information here.
2. 85B in the abstract should be changed to Ag85B.
3. Define Erp the first time it is used.
4. ESAT-6 was clearly included to define LTBI people, however, this is not explained. It would make the manuscript clearer if this was explained, together with the reasons why Ag85B was included in the comparison.
5. In the introduction: 'Erp is a protein encoding a cell surface component' should be deleted. Since proteins don't encode, only genes do, a statement like 'The erp gene encodes a cell surface protein with repetitive structure' would be more appropriate.
6. In Subjects and methods: the description of the 16 BCG-vaccinated individuals is very confusing and would benefit from a major rewrite and clearing up. For example 'no infected' change to 'uninfected'; 'divised' change to 'divided'; '7 displayed positive responses' -to ESAT-6 I assume, this should be clarified; 'were considered with probable latent TB' change to 'were considered to have LTBI' etc.
7. CD4 T cell purification: what method was used to determine efficiency?
8. Antigens: it is mentioned that they were tested for LPS contamination but no figures are given. This is very important when recombinant antigens are tested as high LPS leads to high TNF production and non-specific responses.
9. Why was the ELISPOP assay incubated for 40 hours? the standard assay is based on overnight incubation (about 16 hours), and detects circulating effector cells. The detection of effector cells does not increase by extending the incubation time, and 40 hours are too short for detecting memory cells. An explanation as to why a 40 hour incubation was chosen would be helpful.
10. Results: were the patients counselled for HIV testing?
11. Proliferation assays: why were only 20/22 patients tested for PPD proliferation?
12. No meaningful statistical comparisons can be performed with groups of n=3.
13. Cytokine production by CBA: a brief description of the cytometric bead assay in the methods would have been appropriate.
14. 'After Erp stimulation, high level of TNF' was detected: in order to define whether this is real, the LPS content of Erp should be stated.
15. 'In the latent TB BCG+ group, high levels of IFN-g and TNF were detected after stimulation with Erp'. This is based on n=2, and values for IFN-g 237±254, and for TNF 280±245. This obviously means that one value was very high and the other was very low. Unless numbers are significantly expanded, such comparisons are not meaningful.
16. Humoral responses: as no control experiment was performed in order to determine positive responses and non-specific binding, it is very difficult to judge the meaning of these results.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

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.