Reviewer’s report

Title: Rv1985c, a promising novel antigen for diagnosis of tuberculosis infection from BCG vaccinated controls

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Reviewer: Ajit Lalvani

Reviewer’s report:

This is clearly written manuscript that makes a useful advance in the field. Although the authors are optimistic with the results of this study, they are careful to acknowledge its limitations. Rv1985c is not absent from all strains of BCG so its diagnostic utility will be restricted to certain countries. This is acknowledged at the end of the discussion.

Major compulsory revisions

- What strain of BCG were the controls vaccinated with? This key information should be stated in the methods.
- Discussion on the global distribution of BCG Pasteur-derived strains that lack RD2 is required so readers can assess where Rv1985c would be useful and where not
- In the Introduction the authors do not adequately contextualise their study of T cell responses to this RD2-encoded antigen by omitting to mention earlier investigations of other RD2-encoded antigens for T cell-based diagnosis of TB, eg Liu et al Infection & Immunity 2004
- What is the incidence of TB in this area i.e. how likely are the controls to have been infected because of exposure in the community?
- The authors make comparisons of the diagnostic sensitivity of Rv1985c with ESAT6 and CFP10 – but they fail to discuss the very large difference in the strength of T cell response to RV1985c compared to ESAT6 and CFP10 as shown in table 2. This difference should be explicitly discussed, especially since several of the active TB and LTBI ‘responders’ has borderline responses to this antigen which would not be considered positive according the cut-off point for appositive result that is mandated by the US FDA for T-SPOT.TB
- LTBI was partly defined by a positive T-SPOT.TB. 1985c ELISpot cannot therefore be fairly compared to T-SPOT.TB as there is no objective reference standard against which the 2 tests can be compared. This limitation needs to be acknowledged and discussed.
- Ultimately, the value of any MTB antigen for diagnosis of latent TB hinges on the prognostic power of T cell responses to the antigen for predicting subsequent development of active TB disease (eg Diel at al Am J Resp Crit Care Med 2008 and Bakir et al Ann Intern Med 2008). The authors should recognise that such longitudinal data will be required to confirm the true diagnostic utility of Rv1985c
- Why would M. tuberculosis-specific antigens be a potential source of antigens for vaccine development?
- Why was the T cell assay not done on all the participants that had the antibody tests?

Discretionary revisions
- Was Rv1989c antigen used rather than peptides in the ELISpot? Why was this antigen chosen?
- The recently determined incremental diagnostic sensitivity of certain other antigens when used in combination with ESAT6 and CFP10 in ELISA and ELISpot assays is not mentioned, eg TB 7.7 in Quantiferon TB Gold In-tube and Rv3879c in ELISpot (Dosanjh et al, 2008)
- The following text in the last para of the discussion is confusing: ‘Furthermore, we also observed one T-SPOT TB-negative subject had positive ELISPOT response’. Are the authors referring to a result in the LTBI group? And what do they mean by ‘ELISPOT response’?

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

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

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

Declaration of competing interests:
I am named inventor for several patents underpinning T cell-based diagnosis. The ELISpot assay I developed and validated for diagnosis of TB infection was commercialised by an Oxford University spin-out company (T-SPOT.TB®, Oxford Immunotec Ltd) in which Oxford University and I have minority shares of equity and to which I acted as non-executive director from 2003-07.