Author's response to reviews

Title: Persistence of the immune response induced by BCG vaccination.

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Author's response to reviews: see over
Dear Editor,

Re Persistence of the immune response induced by BCG vaccination.

Following the recommendation of the editor of BMC Medicine we would like to continue the review process of this manuscript with BMC Infectious Diseases. We would like to thank the editor and the reviewers for the comments raised regarding our manuscript which we have addressed as follows.

Reviewer Dr Helen McShane

Major comments

“My major comment on this paper is there is no statistical analysis of the vaccine induced immune responses. The results comparing responses at baseline, 3 months, 1 year and 3 years after vaccination are shown, but there is no statistical analysis to show whether the post vaccination responses at the different time points are significantly different to baseline responses. This analysis needs to be performed for both PPD and antigen 85A and the results included in the figures and text. The authors present the control (unvaccinated group) data but do not perform any statistical analysis comparing vaccinated and unvaccinated groups.”

All fold increases for the vaccine induced response are measuring the size of the change in the vaccinée response compared to the size of the change in the control group. This has been made clearer in the statistical analysis section (page 6) and p values have been added to the fold increases for clarity within the text of the results section (pages 7, 8 and 10). In addition p values for the difference between the control and vaccinated groups at each time point as shown in Table 1 have been included.

“The results on antigen 85 are interesting for 2 reasons. First the higher baseline response is interesting and is well discussed in relation to environmental mycobacteria. Also the increase in antigen 85 response at 3 months in the control group (is this difference significant?) is interesting. The authors suggest it may be due to boosting by the TST, which is possible but perhaps unexpected for any TST effect to last for 3 months.”

The increase in the response to antigen 85 at 3 months in the control group was significantly different (p=0.02) compared to baseline and a statement regarding this is included on page 8, 2nd paragraph. The increase in the response to Ag85 at 3 months in the vaccinated group is greater than in the control group; however this does not reach statistical significance as shown in Table 1. The suggestion that it is unexpected for a TST effect to last for three months is interesting. We are confident that the increase in response to Ag85 at 3 months in the controls is real as the same batch to antigen was used at each time point and as this response wanes by 12 months. A study by Mawa et al 2004 (Int J Tuberc Lung Dis 8(5) 586-92) did show an
increase in IFN\(\gamma\) response to M.tb Culture Filtrate Protein in TST tested TST negative (induration <5mm) HIV infected individuals at 3 days and 3 months post TST however, this did not reach significance. We are not aware of any data in healthy (HIV negative) individuals that document such boosting effects in this type of assay at such a time point although boosting effects have been documented when tested on the day of TST reading or within one month post TST (Igari et al 2007 Int J Tuberc Lung Dis 11(7), 788-91) (as included in the discussion page 14)

“My other major comment is how can the authors be confident that the persistent responses in the subjects who responded at 3 months were not due to M.tb exposure during the 3 years since vaccination? This would seem to be a potential explanation (although I accept unlikely unless the prevalence in this community is high) but has not formally been excluded by the laboratory assays and should be mentioned in the discussion.”

The individuals in the vaccine group who responded at 3 months had been vaccinated 3 months previous to this blood test. Further to the TST at recruitment they received a second TST at 12 months post vaccination as detailed in the Methods section (pages 4 and 5).

There was no evidence that any of the vaccinees tested at 3 years post vaccination had been exposed to tuberculosis. Individuals tested at 3 years post vaccination did not undergo an additional TST at the 3 year sampling point. The annual incidence of TB in the study area was 39.8/100,000 in Redbridge PCT and 44.3/100,000 in Waltham Forest PCT in 2003 (HPA London Quarterly Surveillance Bulletin Vol 1 No 4 April 2004), which is very low. This is just at the threshold of incidence for which the introduction of universal vaccination of neonates with BCG is recommended by the Joint Committee on Vaccination and Immunisation in the UK. Though the risk of TB is considered sufficient to warrant universal neonatal vaccination it might not be considered particularly high. In addition there was no TB outbreak in these schools during this period. . In addition we have some additional data from a separate cohort of teenagers recruited from the same PCTs in which the IFN-\(\gamma\) response to an ESAT6/CFP10 fusion protein was measured using the whole blood assay. Forty nine adolescents were recruited and of these 16 had a Heaf grade >2. 3/49 had IFN-\(\gamma\) responses to this protein at >62pg/ml; of these 1/16 was in the Heaf grade >2 group and only 1/33 had a strong response of >250pg/ml to ESAT6/CFP10 fusion protein (Dr Steven Smith personal communication). We therefore think it very unlikely these subjects were exposed to TB in the 3 years since BCG vaccination and that the responders are responding as a result of BCG vaccination.

With regard to the reviewer’s comment regarding the use of abbreviations, M. tb is now used throughout the document except for the first appearance of Mycobacterium tuberculosis which appears in full in the abstract and main body of the text followed by the abbreviation in parentheses.

Reviewer Martin Ota
Major comments
“1. In their previous papers, the group deduced that lack of response to BCG in Malawi was as a result of high pre-existing immune response to PPD in vitro. They state in this paper that Ag85 is a major component of M.tb PPD. Therefore, the authors should explain why a high pre-existing response to antigen 85 in this UK teenagers, which is a component of mycobacteria as well as BCG, does not limit their immune response to BCG as it did in Malawi.”
The reviewer is correct in noting that we have previously observed only a small change in the M. tb PPD specific IFN\(\gamma\) response associated with BCG vaccination in Malawi. While we have previously observed a high prevalence of responders to other mycobacterial antigens including PPDs from non M.tb mycobacteria in the UK population the prevalence of responses and the magnitude of the responses in the UK population was lower than that seen in the Malawian population. It would therefore appear that the percentage of responders to Ag85 and other mycobacterial PPDs other than M.tb PPD is not predictive of BCG efficacy in this population. What we do observe here is that the vaccine associated change in the magnitude of the response in responders does increase in the BCG vaccinated group post vaccination compared to the control group. Therefore it is likely that a number of components including dose frequency and types of exposure to environmental mycobacteria contribute to modulating the response to BCG vaccination in adolescence. (discussion pages 13-15)

“2. The data show that the response to antigen 85 is higher at 12 than at 3 months after BCG vaccination, whereas responses peaked at one week and waned thereafter after MVA85A vaccination (reference 13). Authors should explain why BCG should induce longer lasting immune response to antigen 85 than a vaccine that consists entirely of the same antigen. Perhaps the authors should show the response to antigen 85 as part of figure 3.”

The response the reviewer refers to in reference 13 is an overnight IFN\(\gamma\) response measured in an ELISPOT system which is most likely to be measuring immediate effector cells which have been activated in vivo. Our assay measures the response in a 6 day assay which is more likely to measure IFN\(\gamma\) production from activation of a resting memory population of antigen specific cells. We have previously shown that M.tb specific memory T cell clones take a number of months to establish (Bennett et al 2006 Vaccine 24, 2617-26). When examining the response to Ag85 in this group it is important to focus on the magnitude of the response which is higher in the vaccinees than the control group at 12 months post vaccination while the response to Ag85 in both the vaccinees and controls at 3 months is higher than at 12 months. Though, when looking at the vaccine group alone (p=0.03) or when taking both the response in the control and vaccinated group into consideration (p=0.18) the median response is lower at 12 months though there is no evidence that the response wanes significantly from 3 to 12 months. However, because of the booster effect of the TST on the response at 3 months the difference between the control and vaccinated group only reaches significance at the 12 month time point (Table 1).

Regarding showing the Ag85 data as part of figure 3, we do not have 3 year post vaccination data for Ag85 in this subsequent cohort.

Minor comments

“3. The weakness is the small sample size for some of the comparisons, particularly the assessment of responses 14 years after infant vaccination. This will require interpretation of these data with some caution, which the authors should emphasize.”

This has been emphasized in the discussion page 13 paragraph 2.

“4. Figure 4 legend 13 year olds is not consistent with the text (12-14).”

This has been changed to be consistent.
“5. An opportunity to explore blocking or masking hypothesis for BCG non-response is missed here. Could authors relate individual pre- and post-BCG responses?”

The blocking and masking hypothesis is a very interesting one particularly in relation to our cohort in Malawi. It is of less relevance for our cohort in the UK which the data in this paper pertain to. While there are high numbers of responders to various different mycobacterial antigens and to PPDs from environmental mycobacteria other than M.tbc PPD in these UK subjects the percentage of responders is considerably lower and the magnitude of response is lower than in Malawi. In the UK cohort the pre vaccination response to M.tbc PPD is low and there is a large vaccine associated change in the response. We have addressed this more fully in Weir et al 2006 but wanted to focus on persistence of BCG induced immunity here.

All analysis has been carried out on individuals that have data points at each time point and analyses comparing responses at baseline and 3 and 12 months post vaccination have been included (Table 1).

Reviewer Daniel Hoft

“1) The comment in the Abstract's conclusions stating that "there is scope for boosting anti-tuberculosis immunity in BCG vaccinated children anytime from 3 months post-vaccination" is vague. What are the authors really trying to say?”

This has been reworded. We wanted to indicate that because the IFN-γ response declines after 3 months, it could theoretically boosted at any point after this time. Using ELispot assays the peak in response is even earlier (ref 13). Our data do not identify the optimal time point for boosting and further studies would be needed to define this.

“2) How was the definition of "responder" status made (i.e.-what valid statistical method was used to determine that an IFN-gamma response >62 pg/ml is a true antigen-specific response)?”

A positive IFN-γ response was defined as >62pg/ml, twice the limit of detection of the assay. The threshold aims to reduce the inclusion of false positive responses and the frequency distribution data from the pre-vaccination responses among these teenagers indicates that the response threshold is appropriate. A statement to this effect has been added to the methods section.

“3) This reviewer agrees with the authors that the increase in Ag85-specific responses seen at 3 months in the controls (Table) is probably related to the PPD tests given at screening. This phenomenon has been reported by several others in the past. This Heaf test-related boosting has to be taken into consideration when evaluating the more prolonged Ag85-specific increased responses in the vaccinated group.”

The greater increase in Ag85 response in the controls at 3 months was taken into account in the analyses of Ag85 responses due to vaccination.

“4) It would be helpful to indicate significant differences in both the Table and Figures.”

Additional p values showing the significance of the difference between the control and vaccinated group have been added to the Table and additional discussion has been inserted in the discussion section.
“5) This reviewer assumes that Figure 1 shows medians, mid-50% ranges and extreme quartiles, although it is not stated either in the legend or text.”

This has been clarified with a statement in the figure legend

“6) The end of the discussion leaves the reader flat with the statement that "information about the induction and maintenance of anti-mycobacterial immune mechanisms by BCG is also relevant for new TB vaccines currently in development as several of these vaccines are designed to act as a booster." What are the specific implications of these data for boosting?”

The text has been edited to reflect our observation that despite measured waning of the response between 3 and 12 months and 12 months and 3 years post vaccination we can still measure a persistent response in neonatally vaccinated teenagers. As the response has waned but not disappeared this would suggest there is scope or capacity within the immune system for that response to be boosted back to a greater level.

Yours sincerely

Dr Patricia Gorak-Stolinska