
Commentary

Comments on the new analysis of the Chingleput BCG trial

The trial of BCG vaccination initiated in the Chingleput district of south India was a source of major disappointment when it emerged that neither Danish nor French BCG had any protective effect. However, a new analysis provides new insights, and is a significant achievement in view of the rather simple tests that were used to categorise the individuals entered into the trial.

All subjects had clear chest X-rays at entry into the study, and were skin tested with 10 U PPD-B (prepared from *Mycobacterium intracellulare*) and 3 U of PPD-S (prepared from *M. tuberculosis*), before being allocated into groups that received placebo, or either high or low doses of Danish or French BCG. The lack of protection overall was documented previously. The new study analyses the results in relation to the skin test responses at the time of entry into the study. The authors regard individuals who had skin test responses to purified protein derivative (PPD-S) at entry of >7 mm as being infected with *M. tuberculosis* [unless in those with intermediate responses (8-11 mm) there was also a large response to PPD-B] and those with responses to PPD-B above an unstated cut-off point are as having a “protective infection with other environmental mycobacteria”. Immunologists will worry about these definitions, as discussed later, but as approximations for theorising about the meaning of the results, these are useful possibilities.

First, they considered the placebo recipients, ignoring those who received BCG. After correction for the fact that placebo recipients who had positive skin tests to PPD-B tended to be older than those who did not, it emerged that the relative risk of TB in the PPD-B positive placebo group was significantly reduced (P=0.02) compared to the risk in PPD-B negative placebo recipients. The rate of protection correlating with PPD-B positivity in the absence of BCG was 37 per cent. By contrast, in those who received BCG the relative risk of TB was not lower in subjects who were PPD-B positive at the time of vaccination.

This result implies that PPD-B positivity, presumably due to exposure to environmental mycobacteria, correlates with protection. The disappearance of the advantage in the PPD-B positive group when BCG was given could imply that the BCG was able to boost the response in the PPD-B negative group so that they could no longer be distinguished from the already partially immune PPD-B positive group.

It then emerged that this guess was probably correct. When the analysis of the efficacy of BCG included only subjects who were both PPD-B negative and PPD-S negative at intake, the 27096 BCG-vaccinated subjects in this category had significantly lower rates of tuberculosis than did the 13246 placebo recipients. Overall, the protective effect of BCG (both strains and doses) was 29 per cent. It was 37 per cent if only the higher dose of BCG was considered. Very wisely, the authors go on to doubt the clinical importance of this effect because the PPD-B negative, PPD-S negative individuals form a minority of the population, and a protection rate of 37 per cent in only 40 per cent of the community is unlikely to impact on tuberculosis control.
The simplest interpretation: One obvious interpretation, that will be considerably modified below, can be stated in the following sentences: (i) exposure to environmental mycobacteria that results in skin test responsiveness to PPD-B correlates with a degree of protection from TB; (ii) subsequent BCG vaccination does not boost this protection; and (iii) BCG can evoke similar protection in people who are “mycobacterially naïve”.

This is the first time that clear statistical evidence compatible with this view has emerged from the Chingleput study, and it is in agreement with conclusions from other trials. For instance, the apparently very successful study in the UK rigorously excluded individuals who were tuberculin skin test positive. Meanwhile it is clear that BCG vaccination of newborns and infants significantly reduces the risk of the disseminated childhood forms of tuberculosis. Presumably newborns have not yet been exposed to environmental mycobacteria, and in this situation BCG can make a difference.

However this simple interpretation is unlikely to be the whole answer. The issue is, unfortunately, much more complex.

Problems with the simple interpretation: (i) The meaning of the skin test responses to PPD-B and to PPD-S: First, the skin test results cannot be interpreted as accurate indicators of who has or has not encountered M. tuberculosis or nontuberculous mycobacterial infection. For instance, a study in The Gambia of household contacts of sputum smear-positive TB cases revealed that only 41 per cent tested positive by the PPD skin test, although we can be reasonably sure that they had all encountered M. tuberculosis. For instance, a study in The Gambia of household contacts of sputum smear-positive TB cases revealed that only 41 per cent tested positive by the PPD skin test, although we can be reasonably sure that they had all encountered M. tuberculosis. Interestingly, 65 per cent had positive PPD ELISPOT results, indicating that they had indeed encountered these mycobacterial antigens. The ELISPOT measures interferon-γ (IFN-γ) secreted within a brief (24 h) culture period, and is thought to imply the presence of already activated effector T cells that can respond very rapidly. Therefore recent boosting with mycobacterial antigen must have occurred in these people but many of them were nevertheless skin test negative. In fact, if the peripheral blood lymphocytes of donors from developing countries are incubated with PPD for 5 days or more, so that memory T cells are also activated, well over 90 per cent of donors will respond strongly (Kim, L. and the VACSIS project collaborators, unpublished data).

(ii) Regulatory T cells: This leads inevitably to a discussion of regulatory T cells (RegT) that control immune responses. There are several different types of RegT. They control both Th1 and Th2 responses, and some environmental mycobacteria are very potent inducers of RegT. This might be an important point because it has been noted repeatedly that the skin test response evoked by BCG or by recent contact with M. tuberculosis can wane very rapidly in developing countries where environmental mycobacteria are abundant. Evidence for increased RegT activity in human TB has recently appeared, and there is evidence that levels of a transcription factor, Foxp3, that is involved in RegT function, can correlate with skin test positivity (Bur J, Brookes R, and the VACSIS project collaborators, unpublished observation). Thus, skin test positivity to PPD-S or to PPD-B tell us the outcome of complex events within the immune system. Some individuals who are skin test negative, will in fact be heavily immunized by mycobacterial antigens, but with skin test responses downregulated by RegT.

(iii) Inappropriate effector mechanisms: One role of RegT is to control the balance of Th1 and Th2 responses. Immunity to M. tuberculosis is associated with Th1 activity. However, TB patients have a strong interleukin (IL-4) response, particularly in the lungs. The highest levels of IL-4 are seen in patients in those developing countries where the rates of TB are highest. In these patients IL-4 can often be measured by ELISA in serum. This represents a burden of IL-4, not seen even in asthma patients in Europe. By contrast, a splice variant of IL-4, which is a competitive inhibitor, is
expressed at elevated levels in unstimulated blood from individuals who have contacted M. tuberculosis, but not developed disease\textsuperscript{18}. These results suggest that IL-4 is detrimental in TB, and reasons why this might be are reviewed in detail elsewhere\textsuperscript{19}. Negative skin test responses seen in Chingleput in some individuals who have certainly encountered mycobacteria might be due to a Th2 bias. Similarly, in some individuals the failure of BCG vaccination might be due to a failure to turn off the detrimental Th2 component that is primed by environmental mycobacteria in many developing countries\textsuperscript{19}.

(iv) The blocking hypothesis: Another hypothesis that is not mentioned by the authors suggests that environmental organisms can evoke a response capable of inhibiting replication of BCG, but incapable of protecting from M. tuberculosis. This notion is based on work in a mouse model showing that pre-exposing mice to some strains of the M. avium complex was not significantly protective against M. tuberculosis, but could block the boosting effect of BCG vaccination, because the BCG was unable to replicate\textsuperscript{20}. This effect seen in mice might be relevant to man, but it has to be remembered that despite frequent claims that BCG protects mice from TB, true protection as defined in man has not, to our knowledge, been observed in this species. A vaccine for humans must stop disease from developing. BCG given to mice does not stop disease from developing. It merely delays inevitable death by weeks or even months. This effect would not be noticed in human vaccine trials and would certainly not be regarded as “protection”, so the relevance of these mouse experiments must remain in doubt.

(v) The masking hypothesis: We return, then, to the hypothesis favoured by the authors, but with some added twists. The masking hypothesis suggests that environmental mycobacteria can induce protective responses that BCG cannot improve, so the protective effects of BCG are seen only in supposedly mycobacterially naïve skin test negative recipients. However, in view of the above discussion, we cannot assume that the skin test negative subjects were mycobacterially naïve. Such people must be very rare indeed in the environment of south India. We can only postulate that many of these skin test negative people had responses that were suppressed by inappropriate RegT activity, or biased towards non-protective Th2 responses not manifested as delayed skin test reactions. So how did BCG protect them? It all depends on why they were skin test negative in the first place! Did BCG turn off RegT, or turn off Th2, or alter the types of effector cell generated, or simply increase the size of the existing Th1 response? The truth is that we have absolutely no idea, but these points need to be understood if we are to design vaccines that work better than BCG in the developing countries where the need for a vaccine is greatest.

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References


