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Early BCG vaccination is unrelated to pulmonary immunity against *Mycobacterium tuberculosis* in adults

To the Editor:

Vaccination with *Mycobacterium bovis* bacille Calmette–Guérin (BCG) is performed for the prevention of tuberculosis. *M. bovis* BCG vaccination is among the most commonly applied of all vaccines worldwide [1]. *M. bovis* BCG vaccination efficiently reduces the morbidity and mortality of tuberculosis in children, especially miliary tuberculosis and meningitis [2].

Although recent investigations of *Mycobacterium tuberculosis*-specific immune responses by interferon-γ release assays (IGRAs) provide evidence on the effect of *M. bovis* BCG vaccination on the prevention of primary infection with *M. tuberculosis* [3–5], it has been suggested that this effect diminishes during adolescence [2, 6]. Consequently, adults are probably not protected from pulmonary tuberculosis by BCG vaccination.

To date, no study has investigated the impact of *M. bovis* BCG vaccination performed in childhood on pulmonary immune responses in adults. The objective of this study was to assess the effect of childhood *M. bovis* BCG vaccination on systemic and pulmonary immune responses to *M. tuberculosis* in healthy adult individuals exposed to patients with acid-fast bacilli (AFB)-positive sputum smear-positive tuberculosis in Germany.

An observational, cross-sectional, multicentre study was conducted by the German Ministry of Education and Research-funded research consortium on ''Pulmonary Tuberculosis – Host and Pathogen Determinants of Resistance and Disease Progression (TB or Not TB)''. Healthcare workers (HCWs) with 1) ongoing professional contact with patients with AFB sputum smear-positive tuberculosis, 2) a cumulative professional exposure of at least 2 years, and 3) no clinical signs and/or symptoms of active tuberculosis were recruited at 18 German pulmonary medicine centres (centres are listed in the Acknowledgements section).

Furthermore, household contacts (HHCs) without evidence of active tuberculosis were enrolled at three urban municipal healthcare centres (*i.e.* Frankfurt, Hamburg and Hannover); their enrolment required 1) the absence of clinical signs and/or symptoms of active tuberculosis, and 2) cumulative exposure of >40 h to an AFB sputum smear-positive patient with culture-proven pulmonary tuberculosis. Individuals with a history of pulmonary tuberculosis who completed a standard course of tuberculosis treatment >6 months before enrolment and did not experience a relapse were recruited in a control group.

Epidemiological, clinical and demographic data, including BCG vaccination status, were captured using an *ad hoc* standardised questionnaire. An unblinded physician verified *M. bovis* BCG vaccination by clinical

examination of a scar or through the subject's vaccination passport. IGRAs were performed on cells collected from the peripheral blood of all subjects by the QuantiFERON Gold In-Tube (QFT; Cellestis Qiagen, Chadstone, Australia) or T-SPOT.TB (ELISPOT; Oxford Immunotec, Oxford, UK) at the attending physician's discretion. The use of two different test systems impaired the overall comparability of the data but reflected clinical practice in the 18 centres involved. Tuberculin skin tests were not regularly performed according to German guidelines [7]. The laboratory staff was blinded to the clinical data, vaccination status and tuberculosis disease status of the participants.

All enrolled contacts were offered a bronchoscopy with bronchoalveolar lavage (BAL), unless bronchoscopy was contraindicated for medical reasons. If the subject agreed, flexible bronchoscopy was performed according to the current German guidelines [8]. The bronchoscope was wedged into a subsegmental bronchus of the middle lobe. BAL was performed with a total volume of 200–250 mL sterile normal saline.

A T-SPOT.TB was performed on BAL mononuclear cells (BALMCs). Interferon-γ secretion was evaluated in response to the following mycobacterial antigens: 6-kDa early secretory antigenic target (ESAT-6), 10-kDa culture filtrate protein (CFP-10) and purified protein derivative (PPD; Statens Serum Institutet, Copenhagen, Denmark). The assay was conducted according to the manufacturer's instructions with the exception that 200 000, instead of 250 000, mononuclear cells were plated per well for improving the visibility of the spots.

Qualitative variables were expressed as percentages, whereas mean$\pm$SD) and median (interquartile range (IQR)) were used for parametric and nonparametric quantitative variables, respectively. The Shapiro–Wilk test, Chi-squared test, t-test and Mann–Whitney test were performed as applicable using Stata 11.0 (StataCorp LP, College Station, TX, USA).

Between June 2008 and July 2012, 512 eligible HCWs and HHCs with known BCG vaccination status were identified (263 HCWs, 166 HHCs and 83 with unclassified exposure). The exposure classification was assigned through a questionnaire, but this information was not captured in the initial phase of the study. Of the remaining 505 HIV-uninfected HCWs and HHCs, 322 (63.8%; 153 HCWs, 113 HHCs and 56 with unclassified exposure) were immunised with *M. bovis* BCG during childhood, while 183 (36.2%; 103 HCWs, 53 HHCs and 27 with unclassified exposure) were not. In 22 (4.4%) persons (12 HCWs and 10 with unclassified exposure), QFT results were unavailable. In 483 contacts with known blood IGRA status, 170 (35.2%; 92 HCWs, 55 HHCs and 23 with unclassified exposure) had a positive test result. To assess pulmonary immune responses, 88 (17.4%) out of 505 individuals (31 unvaccinated and 57 *M. bovis* BCG vaccinated) underwent bronchoscopy with subsequent BALMC IGRA. Of these, 26 (29.5%; 17 HCWs, four HHCs and five with unclassified exposure) had a positive test result, with 10 (32%) in the unvaccinated and 16 (28%) in the vaccinated group.

M. tuberculosis-specific IGRA responses were not associated with *M. bovis* BCG vaccination status (OR 1.09 ($p=0.66$) and 0.81 ($p=0.68$) for blood and BAL, respectively) (fig. 1). However, the frequency of PPD-responsive BALMCs was higher in *M. bovis* BCG vaccinated *versus* unvaccinated persons, with a median (IQR) of 22 (6–101) *versus* 8 (1–44) spot-forming cells per 200 000 BALMCs, respectively (marginal statistical significance, $p=0.0468$), indicating a possible enhanced immune response in vaccinated subjects. With respect to *M. tuberculosis*-specific immune responses, though, no vaccination-related differences were found after stimulation with the antigens CFP-10 and ESAT-6. For CFP-10, a median (IQR) of 3 (0–17) *versus* 5 (1–15) spot-forming cells per 200 000 BALMC was observed for vaccinated *versus* unvaccinated subjects, respectively; for ESAT-6, the median values (IQR) were 2 (0–19) *versus* 4 (0–11) spot-forming cells per 200 000 BALMCs for the same respective groups. No difference in systemic or pulmonary *M. tuberculosis*-specific immune responses were observed among subgroups of HCWs and HHCs (data not shown).

We investigated the impact of *M. bovis* BCG vaccination during childhood on pulmonary and systemic *M. tuberculosis*-specific immune responses in healthy adult HCWs and HHCs with recent contact with tuberculosis patients. These immune responses are considered to be correlates of infection with *M. tuberculosis* [9]. Using our methodology, the results from this study suggest that vaccination has no substantial effect on the adaptive immune system with regards to protection against *M. tuberculosis* infection in adulthood.

The interpretation of the results is based on two observational and experimental findings. First, we did not observe a difference in the frequency of latent tuberculosis infection, assessed by IGRA responses from peripheral blood cells, among recent adult close tuberculosis contacts with and without a history of *M. bovis* BCG-vaccination in childhood. Second, local pulmonary ESAT-6- and CFP-10-specific immune responses measured by BAL-IGRA did not differ between the groups, while PPD-specific pulmonary immune responses were augmented in *M. bovis* BCG-vaccinated individuals.

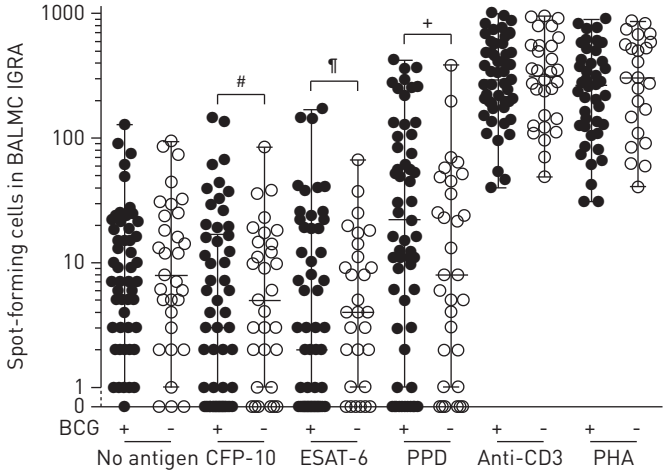

FIGURE 1 Numbers of spot-forming cells in the interferon (IFN)-γ release assay (IGRA) (T-SPOT.TB) with bronchoalveolar lavage mononuclear cells (BALMCs). *Mycobacterium bovis* bacille Calmette–Guérin (BCG)-vaccinated subjects had a higher frequency of purified protein derivate (PPD)-responsive, IFN-γ-secreting cells in bronchoalveolar lavage compared with unvaccinated subjects. ''No antigen'' results are gross values. All other results are net values, *i.e.* the ''no antigen'' value has been deducted. Long horizontal lines indicate the median, short horizontal lines the interquartile range. The logarithmic scale allows the inclusion of all values into a single figure. CFP-10: 10-kDa culture filtrate protein; ESAT-6: 6-kDa early secretory antigenic target; PHA: phytohaemagglutinin. #: p=0.59; ¶: p=0.94; +: p=0.046.

In summary, we present data from the largest cohort reported, to our knowledge, of healthy adult contacts of tuberculosis patients evaluated by bronchoscopy for pulmonary immune responses against *M. tuberculosis* and *M. bovis* BCG. In support of previous epidemiological data, we were unable to find a protective effect of *M. bovis* BCG vaccination against the development of latent infection with *M. tuberculosis* by adulthood [2]. The results underline the importance of the analysis of functional *M. tuberculosis*-specific immune responses from human lung cells for the assessment of correlates of immunity in future tuberculosis vaccine trials.

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Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India

To the Editor:

According to the World Health Organization Global Tuberculosis Report from 2013, there were 8.6 million incident tuberculosis (TB) cases globally and India alone contributed 26% to this global scenario [1]. Of the five countries with the largest number of TB incident cases in 2012, India tops the list [1]. Epidemiological data suggest that extrapulmonary TB (EPTB) constitutes about 15–20% of all TB cases, but among HIV-TB co-infection it accounts for 50% of the cases [2]. Out of 1 183 373 new TB cases notified globally, 234 029 (20%) were reported to be cases of EPTB [1].

Difficulty in sampling from the extrapulmonary sites and the paucibacillary nature of the specimens make EPTB a diagnostic challenge. Dependency on smear microscopy in these samples may lead to higher false negative rates due to the low sensitivity of this technique. *Mycobacterium tuberculosis* (MTB) culture is quite a protracted technique, requiring well-trained laboratory personnel, and delay in diagnosis can cause more harm as the treatment is often started empirically.

Rapid nucleic acid amplification tests are emerging extensively to provide better yield for rapid diagnosis of TB. The Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is an automated, hemi-nested real-time PCR for detecting MTB complex and rifampin (RIF) resistance, which was initially evaluated for pulmonary specimens in large studies [3–5].

The present communication reports the performance of Xpert MTB/RIF in EPTB samples, with a large sample size from a single centre in a country with a high TB burden.