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MODEL SYSTEMS

Childhood BCG vaccination does not influence control of *Mycobacterium tuberculosis* growth by human bronchoalveolar lavage cells

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SUMMARY

Background: Childhood vaccination with *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) reduces the risk of infection with *Mycobacterium tuberculosis* and the risk of severe forms of tuberculosis in children. The protection of adults from pulmonary tuberculosis is doubtful. This study evaluated the effect of the vaccination on the growth of *M. tuberculosis* human bronchoalveolar mononuclear cells (BALMC).
Methods: Healthy, adult healthcare workers who were regularly exposed to *M. tuberculosis*, household tuberculosis contacts, and cured tuberculosis patients were recruited in a multicentre study conducted in Germany. BALMC were co-cultured with different strains of *M. tuberculosis* in growth inhibition assays (MGIAs).
Results: MGIAs on BALMC were conducted in 90 contact persons (known vaccination status, n = 75) and 62 former tuberculosis patients (known status, n = 22). Growth rates for *M. tuberculosis* H37Rv in BALMC were independent of the vaccination status, both in healthy contacts and in cured tuberculosis patients. This finding was validated in growth inhibition assays using two different Haarlem *M. tuberculosis* outbreak strains. Subgroup analyses based on the Interferon-gamma release assay status found no impact of the vaccination on mycobacterial growth.
Conclusions: This study suggests that *M. bovis BCG* vaccination does not alter the anti-mycobacterial capacity of BALMC as assessed in *ex-vivo* growth inhibition assays.

1. Introduction

The only established vaccine against tuberculosis is *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), an attenuated live vaccine [1]. The *M. bovis* BCG vaccination is among the most commonly administered worldwide [2]. Early trials demonstrated a reduction of tuberculosis-associated morbidity and mortality in vaccinated children, mainly in miliary tuberculosis and tuberculous meningitis [3].

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Recent studies suggest an effect of *M. bovis* BCG vaccination for the prevention of primary infection with *Mycobacterium tuberculosis* as assessed using blood based interferon-γ release assays (IGRA) [4–6], but this effect seems to wean during adolescence [3,7].

Adults are not protected by the vaccination [3]. Even when individuals are re-vaccinated with *M. bovis* BCG during adolescence the incidence of or the clinical course of pulmonary tuberculosis, the most frequent clinical manifestation, is not affected [3,8].

Although tuberculosis is a pulmonary disease transmitted predominantly by inhaled aerosols, there is little understanding of the impact of *M. bovis* BCG-vaccination on pulmonary immune responses. Addressing this question may unravel some mechanisms that underlie the frustrating experiences in the worldwide quest for a more effective vaccine compound.

Immune responses to *M. tuberculosis* antigens in the human lung differ from systemic immune responses [9,10]. The induction of pulmonary, rather than systemic immune responses, may be crucial for the development of novel vaccines [11]. We investigated the adaptive systemic and local immune response following childhood vaccination with *M. bovis* BCG in a large cohort enrolled in Germany [12]. Neither in mononuclear cells from blood nor from bronchoalveolar lavage we were able to detect any significant differences between vaccinated and non-vaccinated subjects as assessed by Interferon-γ response to the antigens ESAT-6 and CFP-10 in an Elispot assay [13]. Nevertheless, interferon-γ is only one mediator involved in mechanisms contributing to human immune responses to *M. tuberculosis*. Furthermore, epidemiologic studies provide evidence of geographic variations among *M. tuberculosis* strains in co-evolution with humans of different ethnic backgrounds [14]. Therefore, the impact of *M. bovis* BCG vaccination may vary for different mycobacterial strains. Aiming to assess the sum of the anti-mycobacterial responses at the site of infection we analysed growth rates of three strains of *M. tuberculosis* co-cultured with cells from bronchoalveolar lavages. One objective of this study was to evaluate the impact of childhood *M. bovis* BCG vaccination on mycobacterial growth in cells collected from the lower airways of healthy adults in Germany, a low tuberculosis incidence country.

2. Methods and materials

This observational, cross-sectional, multicenter study was conducted by the German Ministry of Education and Research (BMBF) funded research consortium on “Pulmonary Tuberculosis – Host and Pathogen Determinants of Resistance and Disease Progression”. The trial aimed to investigate several aspects of human immune responses to *M. tuberculosis*, including the effect of childhood *M. bovis* BCG vaccination and the *ex-vivo* response to different *M. tuberculosis* strains. The first cohort of this study consisted of healthy contact persons. This included health-care workers (HCWs) recruited at 18 German pulmonary medicine centres with (a) ongoing professional contacts to patients with acid-fast bacilli (AFB) sputum smear-positive tuberculosis, (b) a cumulative professional exposure of two or more years, and (c) no clinical signs or symptoms of active tuberculosis (centres and investigators listed in the appendix).

The first cohort also included household contacts (HHCs) without evidence of active tuberculosis recruited at three urban municipal healthcare centres (Frankfurt, Hamburg, Hannover). Subjects were suitable for enrolment if they were free of clinical signs and symptoms of active tuberculosis and were exposed >40 h in total to an AFB sputum smear-positive patient, with culturally proven pulmonary tuberculosis. Patients 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 enrolled in a second cohort.

Clinical and demographic data including a history of *M. bovis* BCG-vaccination were captured on an *ad hoc* standardised questionnaire. For reasons of data protection, the initial questionnaire did not assess the type of *M. tuberculosis* exposure (household contact or healthcare worker). Amended versions of the questionnaire included this data but in some contact persons the exposure could not be classified retrospectively. *M. bovis* BCG-vaccination was verified by clinical examination of a scar or through the subject's vaccination passport. IGRAs were performed on cells from peripheral blood on all subjects by the QuantiFERON Gold In-Tube® (QFT; Cellestis Qiagen, Australia) or T-Spot.TB® (Elispot; Oxford Immunotec, Oxford, UK) as published before [12,13,15]. Tuberculin skin tests (TST) were not regularly performed according to German guidelines [16]. Subjects with known HIV infection or unknown BCG status were excluded.

All enrolled healthy contacts were offered a bronchoscopy with bronchoalveloar lavage (BAL) unless bronchoscopy was contraindicated for medical reasons. If the subject agreed, flexible bronchoscopy was performed according to current German guidelines with intravenous and local anaesthesia at the physicians' discretion [17]. The bronchoscope was wedged into a subsegmental bronchus of the middle lobe. Bronchoalveolar lavage was performed with a total volume of 200–250 mL sterile normal saline.

A T-Spot.TB® was performed on BAL mononuclear cells (BALMC) as published before [18]. If sufficient numbers of BALMC were available, mycobacterial growth inhibition assays (MGIA) with three strains of *M. tuberculosis* were conducted using a modified established assay [19,20]. In detail, triplicates of 100,000 BALMC per well at a multiplicity of infection of 5 bacteria per BALMC were set. The strains included H37Rv ATCC (reference strain), and two Haarlem strains isolated from tuberculosis outbreak clusters, 7761/01 and 9956/03 (personal communication Stefan Niemann, Research Center Borstel). Two sets of cells were incubated in a 24-well plate in 300 μl medium (RPMI with Penicillin G, Amphotericin B and 10% human serum). The first sample was harvested after 2 h, the second after 5 days. Cells were lysed with saponin 3% (final concentration 0.3%) and sonicated to disrupt mycobacterial aggregates. Serial dilutions (four log10 steps) were plated on Middlebrook 7H10 Agar. Colony forming units (CFUs) were counted two weeks later. Medium growth rates were calculated from triplicates as follows: Number of CFUs after 14 days incubation of the lysate obtained on day 5 divided by the number of CFUs after 14 days incubation of the lysate obtained at 2 h

A second cohort consisting of former tuberculosis patients was recruited for the MGIA studies if they had completed a full course of anti-tuberculosis therapy prior to entering the study.

The study protocol was initially approved by the University of Lübeck ethical board committee (reference 07-125) and was adopted by the ethical board committees of all participating centres.

3. Statistical analysis

Information collected by the above-mentioned *ad-hoc* questionnaire was entered in an Excel database. Qualitative variables were expressed as percentages, whereas mean (standard deviation) and median (interquartile range –IQR-) were used for parametric and non-parametric quantitative variables, respectively. Shapiro–Wilk test was chosen to evaluate the parametric distribution of quantitative covariates. Chi-square test, t-test, and Mann–Whitney test were applied for statistical comparisons on the basis of the selected variables. A p-value less than 0.05 was considered statistically significant. Statistical tests and descriptive computations