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Host immunity to *Mycobacterium tuberculosis* and risk of tuberculosis: A longitudinal study among Greenlanders

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ABSTRACT

Background: Human immune responses to latent *Mycobacterium tuberculosis* (*Mtb*) infection (LTBI) may enable individuals to control *Mtb* infection and halt progression to tuberculosis (TB), a hypothesis applied in several novel TB vaccines. We aimed to evaluate whether immune responses to selected LTBI antigens were associated with subsequent reduced risk of progression to TB.
Methods: We conducted a population-based cohort study in East Greenland (2012–2014) including individuals aged 5–31 years. A personal identifier allowed follow-up in national registers including the TB notification register. *Mtb* infection was defined by a positive Quantiferon test. Immune responses to LTBI antigens were assessed by whole blood antigen stimulation and interferon gamma measurement.
Results: Among 978 participants, 67 previously had TB. LTBI antigen (Rv1284, Rv2659, Rv2660c) immune response prevalence was 18%, 50%, 2% among *Mtb*-infected and 7%, 40%, 4% among non-infected (Quantiferon negative) participants. Among 911 participants without prior notified TB, 31 were notified with TB during study follow-up. Immune responses to LTBI antigens were not associated with reduced risk of subsequent TB; Rv1284 HR 0.92 (95%CI 0.28–3.04), Rv2659 HR 1.05 (95%CI 0.51–2.13), Rv2660c HR 3.06 (95%CI 0.70–13.37).
Conclusion: In this large population-based study, human immune responses to selected LTBI antigens were not found to be strongly associated with reduced risk of subsequent TB.

1. Introduction

An estimated two billion individuals have *Mycobacterium tuberculosis* (*Mtb*) infection and many will progress to clinical tuberculosis (TB) unless preventive measures are identified [1]. Thus, to meet the goal of TB elimination, the development of a TB vaccine with the ability to prevent progression from *Mtb* infection to TB is crucial as proposed in WHO's post-2015 global TB strategy [2].

Current efforts to control the development and spread of TB include more than ten vaccines in clinical testing [3,4]. Studies have reported that individuals with *Mtb* infection without progression to TB have immune responses to a specific set of *Mtb* antigens associated with latent *Mtb* infection (LTBI antigens). LTBI antigens are characteristic of the slow-replicating bacteria and it has been hypothesised that including LTBI antigens in a future vaccine could be crucial and that such a vaccine may be able to elicit host immunity capable of halting progression from *Mtb* infection to TB [5–10].

The majority of TB vaccines in clinical testing are subunit vaccines in which *Mtb* antigens are combined with a suitable adjuvant or expressed by a viral vector [11]. These vaccines rely on the preventive capability of *Mtb* antigens (such as LTBI antigens) and the immunogenic potential of the adjuvant or viral vector. However, advancement in TB vaccine development is still challenged as the clinical relevance of immune responses to *Mtb* antigens among humans is only partially understood [5,11,12]. Furthermore, no

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human studies have investigated if immune responses to one or more LTBI antigens can be found to be associated with prevention of TB.

Immune responses to *Mtb* antigens have been shown to be sensitive to cross-reactivity from non-tuberculous mycobacteria (NTM), complicating the interpretation of host immunity to *Mtb* [13]. Whereas *Mtb* infection is highly prevalent in Arctic populations such as the Greenlandic [14,15], the populations are largely free of NTM [16,17]. We took advantage of this unique situation to measure host immunity to *Mtb* in a Greenlandic population.

Our objective was to measure immune responses to three LTBI antigens (Rv1284, Rv2659, Rv2660c) which *a priori* are thought to be promising novel TB vaccine antigens, and to evaluate whether an immune response to any of these antigens could be found to be associated with a reduced risk of subsequent TB.

2. Methods

2.1. Setting

Greenland is part of the Kingdom of Denmark but governed by the Greenland Self-government. The majority of the population is Inuit (89%) [18]. The population has universal and free access to health care, including free TB treatment. The study was performed in the Tasiilaq region, East Greenland, comprising 3008 inhabitants (January 1, 2013) [18]. The average TB incidence in the period 1982–2012 was 440/100,000/year [14].

All live-born children and new residents in Greenland are assigned a personal identification number through the Civil Registration System (CRS) [19], which allows individual follow-up through all national registers and provides information on e.g. place and date of birth, sex, and TB notification status. To be categorised as Inuit in the present study, both parents of the study participant should be registered as born in Greenland.

Neonatal Bacillus Calmette-Guérin (BCG) vaccination has been a part of the Greenlandic childhood vaccination programme since 1955, but was temporarily discontinued from 1991 through 1996 due to nationwide policy changes [14,15]. A recent study concluded that 99% followed the nationwide changes in the BCG vaccination programme [14], enabling BCG vaccination status to be defined by birth cohort.

2.2. Study design and study population

The prevalence of immune responses to LTBI antigens was evaluated in a cross-sectional design. The risk of subsequent TB was evaluated using a cohort design. The study population included all Greenlanders aged 5–31 years living in the Tasillaq region in 2012. All eligible study participants were identified through the CRS and the local school registers. Overall, 1233 individuals were resident in the region and were invited to participate. Participants received a personal letter of invitation containing study information and a consent form. The invitation specified an enrolment date (from September 2012 to April 2013). Study staff enrolled the participants and obtained blood samples.

2.3. Assessment of TB and NTM

Information on TB was obtained from the TB notification system. Since 1955, TB has been mandatory notifiable in Greenland and case definitions follow WHO criteria. Prior notified TB was defined as TB notified at any time from birth to study enrolment. Subsequent TB was defined as TB notified from study enrolment to end of follow-up. The majority of all TB cases (n = 98) identified among participants were pulmonary, N = 83 (85%). Any history of a positive NTM culture among invited individuals was obtained using the CRS identification number. None of the invited individuals had a prior bacteriologically confirmed NTM infection.

2.4. Assessment of Mtb infection

Mtb infection was assessed using the interferon gamma release assay (IGRA) QuantiFERON®-TB Gold (QFT), which measures immune responses to *Mtb* antigens ESAT-6, CFP-10, and TB7.7 [20,21]. *Mtb* infection was defined as either a positive QFT at enrolment or prior notified TB, *Mtb* infection was further categorised as (a) ongoing *Mtb* infection, without prior notified TB or (b) prior *Mtb* infection, with prior notified TB. A negative QFT and no prior notified TB defined no *Mtb* infection. All QFTs were analysed at Statens Serum Institut, Denmark, following manufacturer instructions [21].

2.5. LTBI antigens and assessment of immune responses

We measured immune responses to three LTBI antigens, Rv1284, Rv2659 and Rv2660c. LTBI antigens are products of *Mtb* genes expressed during experimental LTBI conditions dominated by hypoxia and nutrient starvation [7,22]. The three LTBI antigens Rv1284, Rv2659 and Rv2660c were selected in June 2012. Previous studies reported that individuals with *Mtb* infection without progression to TB might have immune responses preferentially towards these antigens and furthermore, one antigen was already represented in a novel post-exposure TB vaccine in clinical testing (Rv2660c) [5,8,10,23–28]. In addition, we conducted a pilot study in Greenland in 2011 which indicated that the pattern of immune responses to Rv2660c and Rv2659 differed from findings reported in studies conducted in tropical settings (unpublished data). The studied antigens are specific for *Mtb* and homology was only detected between BCG and Rv1284 [8,10]. For information on Rv2659 and BCG homology; see appendix. None of the studied antigens are currently available in commercial tests.

Immune responses to LTBI antigens were measured using whole blood antigen stimulation and subsequent quantification of T-cell-induced IFN-γ by ELISA. Whole blood antigen stimulation was performed in Greenland; supernatants were stored at −20 °C before and during transport to Denmark. In Denmark, samples were stored at −80 °C until ELISA analyses. For a detailed description of antigens and laboratory analysis, see appendix.

Immune responses to LTBI antigens were defined as positive if IFN-γ was >19.5 pg/ml (the observed 95% quantile of the NIL (background) IFN-γ values) AND fulfilled at least one of the following criteria: (a) response (after NIL subtraction) >41.5 pg/ml (the observed 99% quantile of the NIL IFN-γ values) OR (b) the ratio (between response and NIL) >antigen-specific cut-points estimated by mixture models. Immune responses to purified protein derivate (PPD) originating from proteins in the *Mtb* complex [20] were assessed using the same procedure. For details, see appendix, LTBI antigen and PPD responder definitions and sensitivity analysis.

2.6. Statistical analysis

Associations between characteristics at enrolment and immune responses to LTBI antigens (responder/non-responder) were evaluated by (prevalence) and odds ratios (OR) using logistic regression. Associations between immune responses to LTBI antigens and subsequent notified TB were evaluated by Hazard ratios (HRs) using Cox regression with age as underlying timescale. Follow-up was from enrolment until the first of: subsequent TB or end of follow-up (31 December, 2014). HRs for each extra antigen immune response (trend) were estimated by including number of antigen immune responses as a continuous variable. Among

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