Immunogenicity of an inactivated mycobacterial vaccine for the prevention of HIV-associated tuberculosis: a randomized, controlled trial

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Objective: Prior to the widespread use of *Mycobacterium bovis,* Bacille Calmette-Guerin (BCG), inactivated whole cell mycobacterial vaccines had been shown effective in the prevention of tuberculosis. The present study was conducted to determine the safety and immunogenicity of an inactivated whole cell mycobacterial vaccine in persons with HIV infection.

Design: Randomized, controlled trial.

Methods: A total of 39 HIV-positive patients with prior BCG immunization and CD4 cell counts $\geqslant 200 \times 10^6$ cells/l were randomized to five doses of inactivated *Mycobacterium vaccae* (MV) vaccine or control vaccine (CV). Lymphocyte proliferation (LPA) and interferon gamma (IFN-γ) responses to mycobacterial antigens were assayed at baseline, after three and five doses of vaccine and >1 year later. Parallel studies were conducted in 10 HIV-negative subjects with prior BCG immunization.

Results: Among HIV-positive patients, 19 MV recipients had higher LPA and IFN-γ responses to MV sonicate than 20 CV recipients after three and five doses of vaccine and >1 year later. LPA responses to *Mycobacterium tuberculosis* whole cell lysate increased over time in both groups consistent with prior BCG immunization and current antiretroviral therapy; after three doses, responses were boosted to higher levels in MV subjects than CV subjects. LPA responses to WCL were also boosted in HIV-negative MV recipients. Immunization was safe and had no adverse effects on HIV viral load or CD4 cell count.

Conclusions: In BCG-primed, HIV-positive and HIV-negative subjects, MV induces durable cellular immune responses to a new mycobacterial antigen and boosts pre-existing responses to WCL. MV is a candidate for clinical trials for the prevention of HIV-associated tuberculosis.

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Introduction

Prior to the widespread adoption of *Mycobacterium bovis*, Bacille Calmette Guerin (BCG), inactivated whole cell mycobacterial vaccines had been demonstrated to be effective in preventing tuberculosis in both animals and humans [1–3]. An inactivated whole cell mycobacterial vaccine has several theoretical advantages for use in HIV-positive, BCG-primed subjects compared to other new vaccines against tuberculosis under development [4]: (1) a better safety profile than live vaccines; (2) improved immune recognition compared to subunit vaccines containing only one or two antigens; and (3) a greater likelihood of boosting BCG than live vaccines which have limited replication in subjects with prior mycobacterial immunity [5].

We have been evaluating an investigational whole cell vaccine produced by heat inactivation of a strain of *Mycobacterium vaccae* (MV), an environmental non-tuberculous mycobacterium. In animal studies MV elicits mycobacteria-specific cellular immune responses and protects against challenge with *M. tuberculosis* [6–8]. Further, protection against tuberculosis is greater in animals given MV plus BCG compared to BCG alone [9]. In human studies involving both HIV-negative and HIV-positive subjects the vaccine has been safe and immunogenic [10–13]. However, as with other inactivated vaccines, there is little detectable immune response to a single dose [11,14]. The present study represents a randomized, controlled trial to assess the safety and immunogenicity of a five-dose series of MV or control vaccine administered to BCG-primed HIV-positive subjects.

Methods

Subjects

HIV-positive subjects (n = 39) were from the Aurora Hospital HIV Program in Helsinki, Finland with a current CD4 count $\geqslant 200 \times 10^6$ cells/l. All subjects had a baseline interview, examination for BCG scar (BCG is administered routinely at birth in Finland), baseline phlebotomy, and tuberculin skin test. Subjects in the double-blinded trial were randomized 1 : 1 to receive a five-dose series of 0.1 ml intradermal MV (MV 007; SR Pharma, London, UK) or 0.1 ml intradermal control vaccine at 0, 2, 4, 6 and 12 months. Control vaccine (CV) was hepatitis B vaccine (Engerix-B; Glaxo SmithKline, Rixensart, Belgium) at 0, 2 and 12 months, and borate-buffered saline placebo (PLA 002; SR Pharma) at 4 and 6 months. Repeat phlebotomy was performed 2 months after dose 3 and dose 5 and again >1 year after dose 5 (range 15–19 months after dose 5). LPA and IFN-γ assays were performed at each of the above time-points. Repeat tuberculin skin testing was performed once 2 months after dose 5. HIV-negative subjects (n = 10) were BCG-positive Finnish healthcare workers and received MV at the same intervals. All subjects gave written informed consent for participation in the study, and the study was approved by the Ethics Review Committee, Department of Medicine, Hospital District of Helsinki.

Immunologic assays

Lymphocyte proliferation assays (LPAs) were performed on freshly isolated peripheral blood mononuclear cells (PBMC) using using a standard ^{3}H-thymidine incorporation method with media alone, 2 μg/ml *M. vaccae* sonicate (MVS), or 1 μg/ml *M. tuberculosis* whole cell lysate (WCL). Phytohemagglutinin 2.5 μg/ml (PHA; (Sigma Chemical Co., St Louis, Missouri, USA) was added as a positive control (91% of samples showed a greater than three-fold proliferation response compared to control wells). Results were expressed as net c.p.m., namely the c.p.m. for the cells stimulated with antigen (MVS or WCL) minus the c.p.m. for the control cells incubated with media alone. Results were also calculated as a proliferation index (c.p.m. of antigen stimulated cells divided by c.p.m. of unstimulated control cells).

Interferon-γ assays were performed on cell culture supernatants (stimulated as in LPA) with a commercial sandwich enzyme-linked immunosorbent assay (Diaclone Research, Besancon, France). The detection limit of the assay was between 125 and 379 pg/ml and median results of unstimulated controls were below detection limit in all study groups at all time points.

Analysis

Characteristics of the MV and CV groups were analysed as follows: vaccine site induration and erythema, Wilcoxon rank-sum test; LPA (net c.p.m.) and IFN-γ responses (pg/ml), Mann–Whitney test; adverse event rates and association between pairs of antigen response values, Fisher's exact test. For HIV-negative subjects within-group comparisons of post-dose measures versus baseline measures were made using the Wilcoxon signed-rank test. *P*-values <0.05 were considered statistically significant.

Results

Clinical features

The following baseline characteristics of HIV-positive MV (n = 19) and CV (n = 20) subjects were not significantly different: male gender, 89 versus 80%; median age, 40 versus 41 years; three or more antiretroviral drugs, 89 versus 50%; median CD4, 559 versus 631×10^6 cells/l; geometric mean plasma viral load, 219 versus 290 copies/ml; and tuberculin reactions = 5 mm, 11 and 0%, respectively. The median pre-study CD4 nadir was 206×10^6 cells/l in the MV group versus 327×10^6 cells/l in the CV group ($P = 0.03$). The 10 HIV-negative subjects had a median age of 48 years; three were male; and five had purified protein derivative (PPD) reactions ⩾ 10 mm.

Median vaccine site induration at 2 days after each of the five doses of MV ranged from 4 to 7 mm in HIV-positive subjects and 4.5 to 12 mm in HIV-negative subjects. Among 15 HIV-positive MV subjects with repeat tuberculin testing after dose 5, the only increased reaction compared to baseline was from 10 to 11 mm; two of 17 CV subjects demonstrated increased reactions: 2 to 6 mm and 0 to 4 mm. Among seven HIV-negative, BCG-positive MV subjects repeat tuberculin reactions increased in four subjects by 3 to 8 mm; no subject increased from < 10 mm to ⩾ 10 mm.

Immunologic assays

LPA responses to the new (MVS) and recall (WCL) mycobacterial antigens are shown in Figure 1. Responses to MVS were significantly higher for MV compared to CV recipients at all time points after immunization, including > 1 year after immunization. The MVS proliferation index was also higher in the MV than CV groups at all time points after immunization (data not shown); after dose 3 the MVS proliferation index was ⩾ 3 in 79 versus 35%, respectively ($P = 0.01$). Responses to WCL increased progressively in both groups consistent with prior BCG and current highly active antiretroviral therapy (HAART); responses were higher in the MV group after dose 3. In HIV-positive subjects *in vivo* vaccine site induration after MV dose 4 correlated with the *in vitro* lymphocyte proliferation response to MVS measured 2 months after dose 3 (Pearson correlation coefficient 0.622, $P = 0.008$).

HIV-negative subjects had increased responses to both *M. tuberculosis* WCL and MVS after five doses of MV compared to baseline ($P = 0.02$ and $P = 0.008$, respectively; data not shown). Prior to immunization, lymphocyte proliferation responses to WCL were higher in HIV-negative subjects than HIV-positive subjects ($P = 0.007$); this difference was preserved after immunization. Median responses to MVS after five doses were 22 547 c.p.m. in the HIV-negative group and 12 560 c.p.m. in the HIV-positive group ($P = 0.170$).

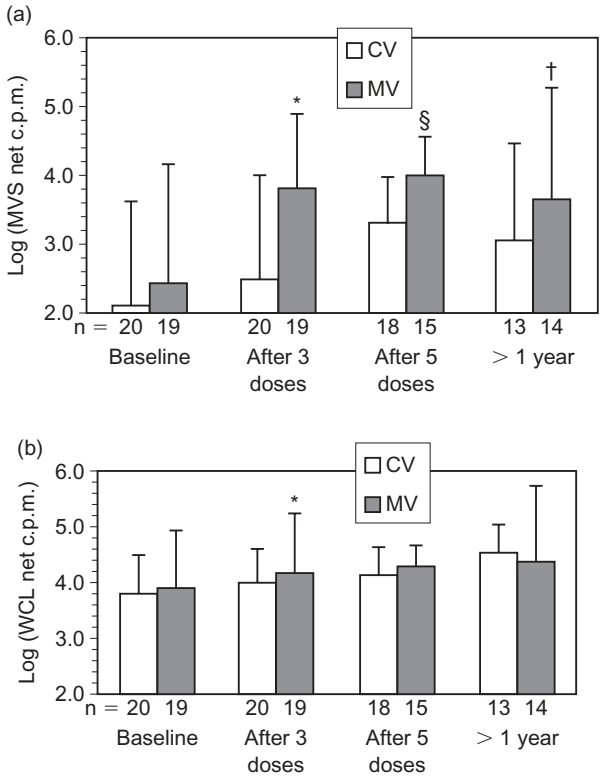

Fig. 1. Lymphocyte proliferation responses to (a) *Mycobacterium vaccae* sonicate (MVS) or (b) *Mycobacterium tuberculosis* whole cell lysate (WCL) among HIV-positive subjects who received *M. vaccae* (MV) or control vaccine (CV). Responses are shown as mean and standard deviation of $\log_{10}$ value of net counts per minute (c.p.m.). P values ⩽ 0.05 are shown: (a) *, $P = 0.0004$; §, $P = 0.0027$; †, $P = 0.0198$; (b) *, $P = 0.046$.

IFN-γ responses are shown in Figure 2. Among HIV-positive subjects with childhood BCG immunization, baseline levels of IFN-γ in response to MVS were low (geometric mean < 1000 pg/ml). After three doses of vaccine, IFN-γ responses to MVS were significantly higher in MV recipients than CV recipients (geometric mean, 4977 versus 478 pg/ml, respectively; $P = 0.001$). Similar differences were observed after five doses of vaccine and > 1 year later. IFN-γ responses to WCL were higher than responses to MVS at baseline across all HIV-positive subjects (geometric mean, 8500 pg/ml). After three doses of vaccine, responses were consistently (although not statistically) higher in MV recipients than CV recipients (geometric mean, 22 856 versus 11 561 pg/ml). Among HIV-positive MV recipients, responses to MVS and WCL were correlated ($P < 0.001$). HIV-negative MV recipients with childhood BCG immunization had high baseline IFN-γ levels to WCL (geometric mean > 100 000 pg/ml),

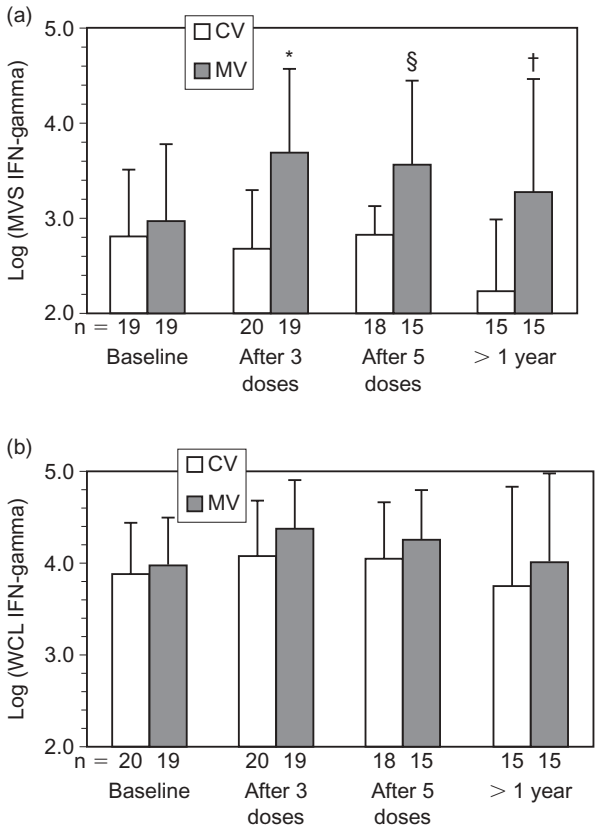

Fig. 2. Interferon-γ responses to (a) *Mycobacterium vaccae* sonicate (MVS) or (b) *Mycobacterium tuberculosis* whole cell lysate (WCL) among HIV-positive subjects who received *M. vaccae* (MV) or control vaccine (CV). Responses are shown as mean and standard deviation of geometric mean values in pg/ml. *P* values ⩽ 0.05 are shown: (a) *, $P = 0.001$; §, $P = 0.02$; †, $P = 0.008$.

with no significant increase after MV immunization (data not shown). There was a trend toward increased response to MVS after three ($P = 0.06$) and five doses ($P = 0.07$).

Safety monitoring

Two months after immunization MV and CV groups had no significant differences in CD4 cell counts (median, 682 and 641 $\times 10^6$ cells/l, respectively) or viral loads (geometric mean, 220 and 264 copies/ml). Changes in CD4 cell count and viral load before and after immunization were not significantly different between the two groups. There were no differences in the rates of any adverse event or local reaction: sore arm 16–37% of 19 MV recipients after each dose versus 10–20% of 20 CV recipients; skin breakdown (11–37% versus 10–30%, respectively); and drainage at the site (5–11% versus 0–5%). Infrequent events occurring equally among subjects in both groups included malaise (5–11%), fever (5%), and adenopathy (5%). One patient in the MV group developed a sterile abscess at the vaccine site.

Discussion

Inactivated mycobacterial vaccines were shown years ago in clinical trials to be effective for the prevention of tuberculosis [15]. Using contemporary immunologic techniques we have shown that a multiple dose series of an inactivated vaccine induces the mycobacteria-specific cellular immune responses now considered relevant for the prevention of tuberculosis [16–19]. LPA and interferon-γ responses to the non-tuberculous mycobacterial (NTM) vaccine antigen (MVS) were low at baseline, increased significantly after immunization, and were still evident at > 1 year. Since all subjects in this study had previously received BCG, these responses probably reflect both immunologic memory to common mycobacterial antigens as well as primary responses to antigens unique to MV. Extensive animal and human data indicate that both natural and vaccine-induced responses to NTM provide protection against tuberculosis [20–23]. We postulate that the responses induced by MV, an NTM, will provide similar protection.

LPA and IFN-γ responses also increased to *M. tuberculosis* WCL, a recall antigen in subjects previously immunized with BCG [24–26]. LPA responses to this antigen increased in both MV and CV recipients over time, consistent with the progressive immune reconstitution that develops in patients on HAART [27]. After three doses of MV, LPA responses to WCL were higher in MV recipients than CV recipients, consistent with MV-induced boosting of a BCG-primed response. This boosting effect was demonstrated more clearly in BCG-primed HIV-negative MV recipients whose LPA responses to WCL increased significantly after immunization. Animal studies have demonstrated the induction of a CD8 cytotoxic response following MV immunization [6]. Cytotoxic responses to BCG-infected cells were examined in a limited number of our subjects and increased significantly among HIV-negative MV subjects, with a similar trend seen in HIV-positive MV but not CV recipients (data not shown).

In this study, as previously, a multiple dose series of *M. vaccae* was safe. Immunization had no adverse effects on clinical or laboratory markers of HIV infection. Reactions at the vaccine site did occur, but were less than those reported for BCG [28]. One subject developed a sterile abscess consistent with the adjuvant properties of the mycobacterial cell wall, but, in contrast to BCG, a killed vaccine poses no risk of dissemination.

Over 60 years ago whole cell inactivated mycobacterial

vaccines were shown to be effective for primary immunization of humans against tuberculosis [1,2]. In the present study, we have demonstrated that a multiple dose series of MV, a contemporary killed mycobacterial vaccine, boosts BCG-primed immunity, does not affect PPD reactions and is safe in HIV infection. These characteristics make MV particularly suitable for immunization of HIV-infected adults residing in areas where tuberculosis is endemic and BCG is administered routinely in childhood. A large-scale efficacy trial of multiple dose MV for the prevention of HIV-associated tuberculosis is underway in Tanzania.

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