

The Effect of Delipidated Deglycolipidated (DDMV) and Heat-killed *Mycobacterium vaccae* in Asthma

PHILIPPA M. SHIRTCLIFFE, STEPHANIE E. EASTHOPE, SOO CHENG, MARK WEATHERALL, PAUL L. J. TAN, GRAHAM LE GROS, and RICHARD BEASLEY

Wellington Asthma Research Group, Department of Medicine, Wellington School of Medicine, Wellington South, New Zealand; Malaghan Institute of Medical Research, Wellington South, New Zealand; and Genesis Research and Development Corporation Limited, Auckland, New Zealand

Experimental and epidemiological evidence supports the hypothesis that exposure to mycobacteria has the potential to suppress the development of asthma and/or atopy and there are reports in the Chinese medical literature of repeated vaccination with inactivated BCG being effective in the management of asthma. Forty-three patients with stable moderately severe asthma who were skin prick test positive to house dust mite were randomized to receive two intradermal injections of either phosphate-buffered saline (placebo), heat-killed *Mycobacterium vaccae* (0.5 mg), or delipidated deglycolipidated *Mycobacterium vaccae* (DDMV) (0.05 mg). Markers of asthma severity were measured for 3 mo and blood eosinophil, IgE levels, and the T cell proliferative and cytokine responses were monitored. There were no significant differences between either treatment group and the placebo group for any of the outcome variables. There was also no difference between the treatment groups and placebo for eosinophil, IgE levels, or the T cell proliferative and cytokine response. The results indicate no effect of low dose intradermal DDMV or *M. vaccae* on asthma severity in patients with established asthma.

There is considerable epidemiological and experimental evidence to support the hypothesis that exposure to mycobacteria may suppress the development of asthma and/or atopy. This evidence includes the observation that there is a significant inverse correlation between tuberculosis notification rates and asthma prevalence in children internationally (1), that in Japanese schoolchildren the rate of current symptoms of asthma was reduced by at least one-third in positive tuberculin responders (2), and that in a murine model of allergen-induced airway eosinophilia, intranasal bacille Calmette-Guérin (BCG) infection 4 wk before allergen airway challenge results in a 90–95% reduction in eosinophilia within the lungs compared with uninfected control mice (3). There are also a number of reports in the Chinese medical literature from the past 30 yr that the repeated administration of inactivated BCG is effective in the management of asthma (4–7).

The proposed mechanism is based on the paradigm of antagonism between T-helper (Th) lymphocyte subsets and their cytokines. Mycobacteria induce a strong Th1-type immune response whereas atopic asthma may be controlled by Th2 lymphocytes. By stimulating a Th1-type immune response, a my-

cobacterial vaccine could potentially down-regulate the Th2-driven allergic process.

The potential effect of BCG vaccination in asthma also applies to *Mycobacterium vaccae* (8, 9), a nonpathogenic environmental mycobacterium that shares antigens in common with BCG and *M. tuberculosis*. Work done in the 1980s confirms immunomodulation of T cell responses *in vitro* by soluble preparations of *M. vaccae* and it has been demonstrated that a single injection of killed *M. vaccae* into ovalbumin (OVA)-preimmunized mice suppresses serum immunoglobulin E (IgE) over a wide dose range (10). Delipidated deglycolipidated *M. vaccae* (DDMV) is derived from autoclaved *M. vaccae* (HKMV) and, like HKMV, has been shown to suppress airway eosinophilia in the mouse model (communication from Genesis Research and Development Corporation Ltd).

Thus there is evidence to suggest that tuberculous infection and/or vaccination with BCG or *M. vaccae* may protect against the development of asthma and/or atopy and may have a role in the management of preexisting asthma. We conducted a trial evaluating the potential of two vaccines derived from *M. vaccae* in the management of asthma.

METHODS

Study Design

The study was a single-center, randomized, double-blind, placebo-controlled study with three treatment groups. Approval was obtained from the Wellington Ethics Committee. All patients gave written informed consent. Refer to Figure 1 for a “time line” of the study design.

Patients

Patients 16 to 60 yr old, who had had asthma for more than 1 yr, were atopic as defined by a positive skin prick test to house dust mite allergen (wheal > 3 mm than the negative control) and who were prescribed inhaled corticosteroids (less than 1200 µg/d of beclomethasone dipropionate) were enrolled. They were required to have a baseline forced expiratory volume in 1 s (FEV₁) between 60 and 100% of the predicted value (Crapo standards) and a postbronchodilator FEV₁ greater than 70% predicted. Patients also had to demonstrate two or more of the following: 12% reversibility in FEV₁ after the inhalation of 400 µg of salbutamol, current use at least two inhalations per week of short acting β₂-agonist, and a peak expiratory flow (PEF) variability of more than 15% during a 7-d screening period.

Study Vaccines

Patients were randomized to receive one of three injections: phosphate-buffered saline (0.1 ml PBS), HKMV (0.5 mg in 0.1 ml PBS per dose), or DDMV (0.05 mg in 0.1 ml PBS per dose). Injections were administered intradermally over the deltoid region by someone other than the principal investigator.

HKMV (ATCC 15483) suspensions were prepared by autoclaving *M. vaccae* for 15 min at 120° C and resuspending in PBS. For the purposes of this trial, the HKMV suspension was adjusted to 0.5 mg equivalent to 5 × 10⁸ colony-forming units (cfu) in 0.1 ml of PBS. DDMV is a delipidated and deglycolipidated derivative of HKMV suspended in PBS. HKMV yields 10% by weight of DDMV (Manufacturer’s infor-

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Correspondence and requests for reprints should be addressed to Philippa M. Shirtcliffe, Wellington Asthma Research Group, Department of Medicine, Wellington School of Medicine, P.O. Box 7343, Wellington South, New Zealand. E-mail: pip@wnmeds.ac.nz

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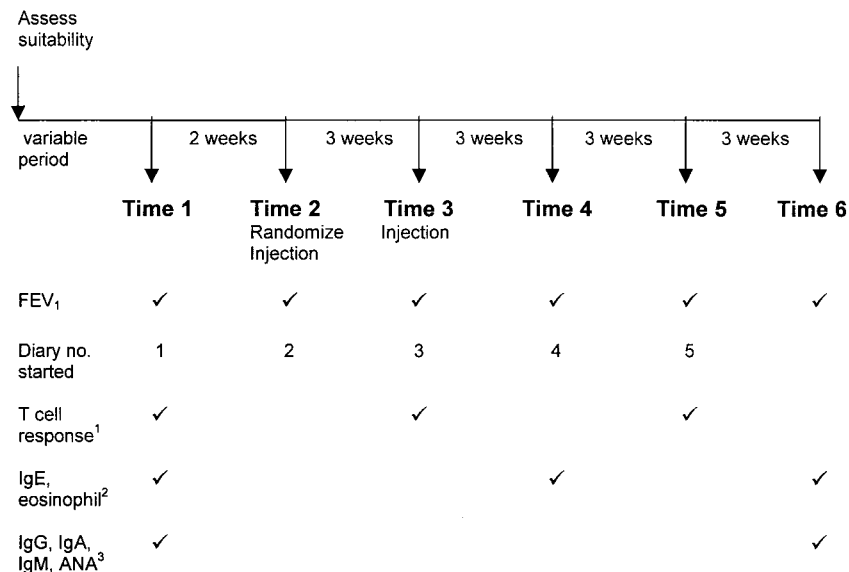


Figure 1. Time line showing study design. ¹T cell proliferative and cytokine response. ²Immunoglobulin E, full blood count, erythrocyte sedimentation rate, liver function tests. ³Immunoglobulins G, M, and A, anti-nuclear antibody, antithyroid antibodies, and rheumatoid factor.

mation). Both vaccines were supplied by Genesis Research and Development Corporation Limited, Auckland, New Zealand.

Outcome Measures

The primary outcome variable was mean morning PEF (MMPEF) as calculated from patient diary card recordings. Secondary outcome variables were defined as FEV₁ (pre- and postbronchodilator) at clinic visits, mean daytime PEF (calculated from both morning and evening PEF recordings made with a Mini Wright Peak Flow Meter), twice daily symptom scores and β₂-agonist use as recorded in patient diary cards, and mild and severe exacerbations of asthma as defined

by predetermined criteria. To investigate the systemic immune response to mycobacterial vaccination, blood eosinophil levels and immunoglobulin E (IgE) levels and the T cell proliferative and cytokine response were assessed.

Method for T Cell Proliferative and Cytokine Analysis

Peripheral blood (20 ml) was taken from subjects at Time 1, 3, and 5. The mononuclear cell fraction was isolated by Ficoll-Hypaque density gradient separation. Lymphocyte cultures were established (1 × 10⁶/ml) in complete RPMI 1640 medium supplemented with autologous serum. Lymphocytes were stimulated with purified house dust mite extract and

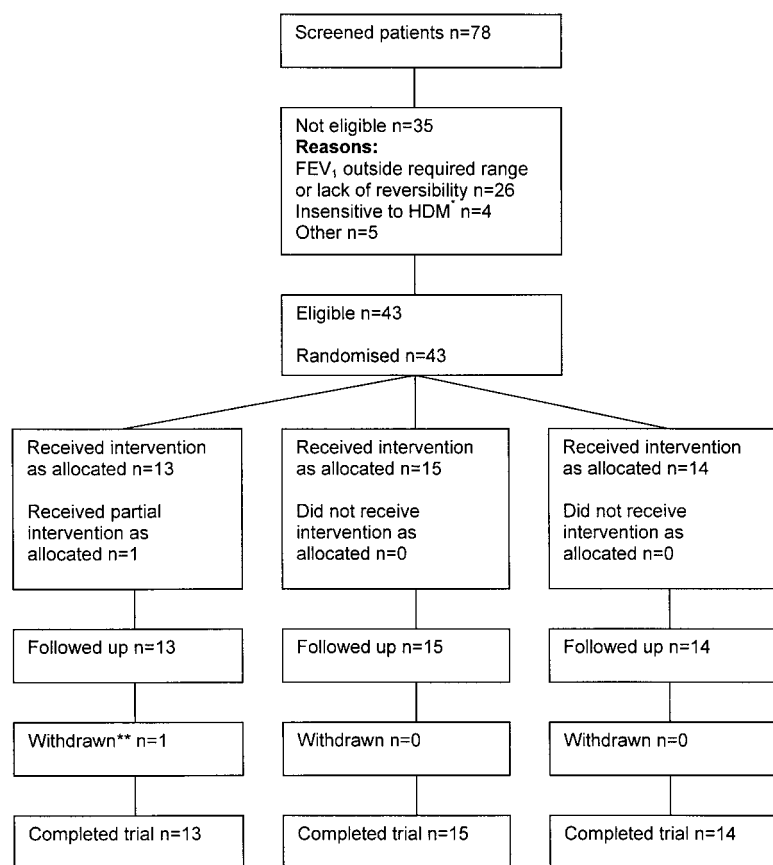


Figure 2. Trial profile. *HDM = house dust mite. **Course of prednisone for eczema.

after 6 d in culture were washed and restimulated on anti-CD3 antibody in a 48-h culture assay. The presence of interferon (IFN)- γ , interleukin (IL)-4, and IL-5 in the supernatants was assessed by specific enzyme-linked immunosorbent assay (ELISA). The allergen specific T cell response was also assessed by following the incorporation of tritiated thymidine into DNA at the end of a second set of 7-d cultures.

Statistical Analysis

The clinical data were double-entered using Microsoft Access and statistical analysis was done with PC SAS (version 6.12). The method of analysis was analysis of covariance (ANCOVA). Exploratory data analysis suggested that log transformation was necessary for daytime and nocturnal bronchodilator use, IgE levels, and eosinophil count. The response variable was the area under the curve (11) for MMPEF, FEV₁, daytime peak flow, average daytime bronchodilator use, average nighttime bronchodilator use, IgE levels, and eosinophil counts. Analysis of response variables was by the original randomization to the treatment groups (i.e., Intention to Treat Analysis). The baseline value of each response variable and log baseline IgE were used in each of the ANCOVA. For the ordinal outcome measures (daytime and nocturnal symptom scores) a generalized Cochran–Mantel–Haenszel test of conditional independence between the treatment group and the symptom score was used. For dichotomous outcome variables (mild and severe exacerbations) the percentages experiencing the outcomes were compared using a Chi-square test. Proliferation data were analyzed by calculating the area under the curve up to the peak proliferative response. Means and standard deviations were calculated for the cytokine data.

RESULTS

The trial profile is outlined in Figure 2 and baseline demographic, lung function, asthma diary data, and laboratory data are presented in Table 1.

For both primary and secondary outcome variables there were no statistically significant differences between either the

DDMV group or the HKMV group and the placebo group (refer to Table 2 and Figure 3). There was one episode that met the criteria for a severe exacerbation in both active treatment groups. There was no significant difference between the two treatment groups and the placebo group for daytime or nocturnal symptom score.

There was no significant difference between the HKMV or DDMV groups and placebo when serial eosinophil and serum IgE levels were analyzed. Consistently high lymphocyte proliferation was seen when volunteers' cells were stimulated with house dust mite extract but no significant change was seen in the proliferative response in any of the groups (data not shown). IL-4 cytokine levels were generally low with most being below the detection limit so meaningful analysis could not be performed. No significant change in IL-5 levels or IFN- γ levels was detected in any of the groups (data not shown).

Adverse Events

Response to the two vaccines ranged from small red tender lumps for a couple of days to more vigorous reactions lasting several weeks. Itch and blistering were commonly reported. Seven volunteers reported possible discharge: of these, six were receiving HKMV and the other DDMV. One of the six had a very pronounced reaction with a 1-cm abscess at each site that was continuing to discharge at the trial's conclusion. Monitoring of basic bloods did not reveal anything untoward.

DISCUSSION

This study suggests that neither of the two novel mycobacterial vaccines administered intradermally to a group with established asthma had a significant effect on asthma severity.

TABLE 1. BASELINE CHARACTERISTICS OF PATIENT GROUPS

| | HKMV (n = 15) | DDMV (n = 14) | Placebo (n = 14) |
|--|--------------------------|--------------------------|--------------------------|
| Mean age (SD, 95% CI) | 35 (11.1, 28.9–41.2) | 37 (10.0, 31.7–43.2) | 30 (8.8, 25.2–35.4) |
| Mean duration of asthma (SD, 95% CI) | 25.6 (13.7, 18.0–33.2) | 24.9 (9.5, 19.4–30.3) | 26.2 (10.5, 20.1–32.3) |
| Sex, male | 8 (53.3%) | 8 (57.1%) | 6 (42.9%) |
| Mean dose of inhaled steroid (SD, 95% CI) | 573 (393.6, 355.3–791.3) | 457 (253.3, 310.9–603.4) | 429 (230.1, 295.7–561.5) |
| Hospital admission | | | |
| Never | 12 (80%) | 8 (57.1%) | 6 (42.9%) |
| Ever | 3 (20%) | 4 (28.6%) | 8 (57.1%) |
| In the past year | 0 | 2 (14.3%) | 0 |
| Smoking history | | | |
| Current | 1 (6.7%) | 4 (28.6%) | 1 (7.1%) |
| Never | 13 (86.7%) | 7 (50.0%) | 9 (64.3%) |
| Ex-smoker | 1 (6.7%) | 3 (21.4%) | 4 (28.6%) |
| BCG (definite) | 10 (66.7%) | 11 (78.6%) | 7 (50%) |
| Mean FEV ₁ preventolin % predicted (SD) | 76% (15.6) | 76% (14.3) | 70.9% (15.6) |
| Daytime symptom score (SD, 95% CI)* | 0.9 (0.7, 0.5–1.3) | 0.9 (0.5, 0.6–1.2) | 0.8 (0.4, 0.5–1.0) |
| Daytime ventolin use (SD, 95% CI) | 1.9 (1.8, 0.9–2.9) | 1.9 (2.9, 0.1–3.7) | 0.9 (0.7, 0.5–1.3) |
| Nocturnal symptom score (SD, 95% CI)† | 0.3 (0.4, 0.1–0.5) | 0.5 (0.3, 0.3–0.7) | 0.4 (0.3, 0.2–0.5) |
| Nocturnal ventolin use (SD, 95% CI) | 0.8 (0.8, 0.4–1.3) | 0.8 (0.9, 0.3–1.3) | 0.4 (0.4, 0.2–0.6) |
| IgE (geometric mean) kU/L (95% CI) | 242.9 (123.2–478.8) | 170.3 (96.3–301.3) | 639.3 (329.2–1241.5) |
| Eosinophil (geometric mean)10 ⁹ /L (95% CI) | 0.3 (0.2–0.4) | 0.3 (0.2–0.5) | 0.3 (0.3–0.5) |

Definition of abbreviations: BCG = bacille Calmette–Guérin; CI = confidence interval; DDMV = delipidated deglycolipidated *M. vaccae*; HKMV = heat killed *M. vaccae*; IgE = immunoglobulin E.

* Six-point scale with 0 indicating no symptoms and 5 indicating incapacitating symptoms.

† Five-point scale with 0 indicating no symptoms and 4 indicating severe symptoms inhibiting sleep.

TABLE 2. AREA UNDER THE CURVE: LEAST SQUARES MEANS FOR CONTINUOUS OUTCOME VARIABLES BY TREATMENT GROUPS AND TESTS FOR SIGNIFICANCE OF TREATMENT IN ANALYSIS OF COVARIANCE

| Outcome Variable | HKMV (95% CI) | DDMV (95% CI) | Placebo (95% CI) | F (degrees of freedom, error) | p Value |
|---|---------------------------|---------------------------|---------------------------|--|---------|
| MMPEF* | 406.77 (395.14–418.39) | 391.73 (378.97–404.50) | 399.81 (386.55–413.08) | 1.60 (2,36) | 0.22 |
| FEV ₁ * % predicted | 75.65 (72.84–78.45) | 72.50 (69.39–75.62) | 74.12 (70.90–77.34) | 1.20 (2,36) | 0.31 |
| Mean daytime PEF* | 414.66 (404.75–424.56) | 401.19 (389.84–412.54) | 416.03 (404.75–427.32) | 2.16 (2,35) | 0.13 |
| Log average daytime salbutamol use ^{*,†} | 0.67 (0.53–0.80) | 0.85 (0.69–1.01) | 0.74 (0.57–0.90) | 1.57 (2,33) | 0.22 |
| Log average nocturnal salbutamol use ^{*,†} | 0.38 (0.27–0.49) | 0.42 (0.29–0.54) | 0.30 (0.17–0.43) | 0.78 (2,34) | 0.47 |
| Log IgE ^{†,‡} | 5.58 (5.50–5.67) | 5.63 (5.54–5.73) | 5.59 (5.50–5.69) | 0.33 (2,36) | 0.72 |
| Log eosinophil ^{*,†} | 0.30 (0.27–0.34) | 0.32 (0.28–0.36) | 0.28 (0.24–0.32) | 0.83 (2,36) | 0.44 |

Definition of abbreviations: DDMV = delipidated deglycolipidated *M. vaccae*; HKMV = heat killed *M. vaccae*; IgE = immunoglobulin E; MMPEF = mean morning peak expiratory flow; PEF = peak expiratory flow.

* Adjusted for baseline and log IgE (baseline).

† Variable transformed.

‡ Adjusted for baseline and age.

There are a number of features of the study that may have influenced the outcome. Randomization left an uneven distribution of covariates (e.g., BCG status, baseline IgE level) and this could have had an impact on the effect of treatment groups, although most of the covariates did not affect the outcome variables when included in the analysis of covariance. It is possible that some of our volunteers were too mild or well controlled at entry (note mean symptom score and salbutamol usage in Table 1) to demonstrate a significant improvement in some of the variables. However, use of salbutamol at least twice a week for symptom control was an inclusion criterion and the mean FEV₁ prior to treatment was 70–76% of predicted values suggesting there was room for improvement in lung function in this group. Including a further objective measure of

lung function that was more sensitive to change than morning peak flow (e.g., bronchial responsiveness) or incorporating a steroid reduction regimen into the protocol might have been helpful in detecting a treatment effect. As regards the size of this study, it had nearly 100% power to detect a 28 L/min difference (7% effect) in mean morning peak flow.

Another issue is the nature of the reagents and the dosing schedule used. In *in vitro* assays, weight for weight, DDMV is approximately 10-fold more effective than *M. vaccae* in stimulating IL-12 production from mouse peritoneal macrophages (communication from Genesis Research and Development Corporation Ltd). Initial clinical trials have reported that HKMV is effective in the treatment of psoriasis when administered intradermally in a single dose of 1 mg or two doses of

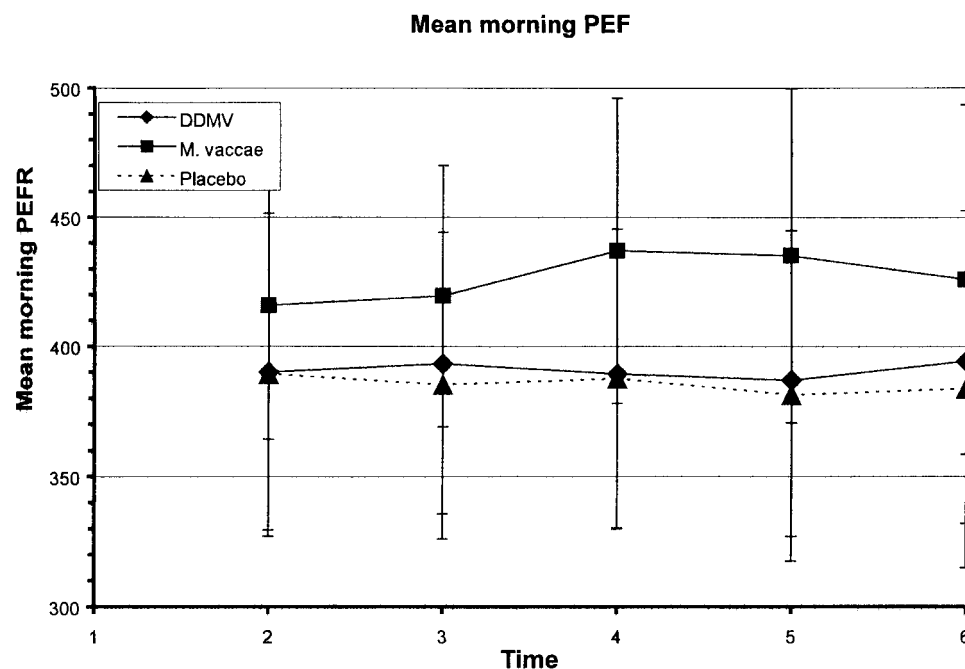


Figure 3. Mean morning peak flow. Administration of vaccine (or placebo) is at Time 2 and Time 3.

0.5 mg (12–14). A similar improvement was noted with two doses of 0.05 mg DDMV given intradermally, 3 wk apart to patients with psoriasis (unpublished data, Genesis Research and Development Corporation Ltd). The dose of the trial reagents used in this study was thus based on *in vitro* studies and the reported clinical effects of HKMV and DDMV in the treatment of psoriasis.

Our findings contrast with two recent clinical trials. In a double-blind placebo-controlled study of 40 adults with grass pollen allergy, summer rhinitis, and asthma, three intradermal injections of HKMV resulted in fewer symptoms in the treatment group, less need for salbutamol, and increased *in vitro* IFN- γ production (9). In another placebo-controlled parallel group study of 24 patients with mild to moderate asthma, a single intradermal administration of HKMV (1 mg) attenuated the allergen-induced late responses and *in vitro* IL-5 production from peripheral T cells (8).

However, inability to demonstrate efficacy with HKMV and DDMV is not inconsistent with our previous observations relating to BCG vaccination in the mouse model. First, the murine model of eosinophilia is acute: our study focused on patients with a mean duration of asthma of 25 yr. Second, route of administration may be crucial as we showed in the murine model that intranasal infection was clearly superior to either intraperitoneal or subcutaneous infection in its ability to reduce airway eosinophilia (3).

In conclusion, the results of this study indicate that neither DDMV nor HKMV has an effect on asthma severity at these doses or dosing schedules in patients with established asthma. Further studies will need to focus not only on determining the active component of mycobacteria, but also on whether different dosing schedules and routes of administration such as directly to the airways may lead to efficacy in the treatment of asthma or to prevention of its occurrence in an “at-risk” individual.

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