

Paediatric Dermatology

Effect of *Mycobacterium vaccae* on atopic dermatitis in children of different ages

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Summary

Background Exposure to environmental microorganisms is associated with variations in the prevalence and severity of atopic diseases. We have previously shown that administration of a *Mycobacterium vaccae* suspension significantly reduced the severity of atopic dermatitis (AD) in children aged 5–18 years.

Objectives This study aimed to extend these observations to younger children.

Methods Fifty-six children aged 2–6 years with moderate to severe AD were enrolled in a randomized, double-blind, placebo-controlled trial and given one intradermal injection of either killed *M. vaccae* suspension or buffer solution (placebo). Skin surface area affected and dermatitis severity score were assessed before and 1, 3 and 6 months after treatment.

Results Although a 38–54% reduction in surface area affected by dermatitis was noted at all time points after *M. vaccae* administration ($P = 0.005$), this improvement was not significantly different from that observed in the placebo group. Meta-analysis of this and our previous cohort (97 children aged 2–18 years) showed that *M. vaccae* was associated with a significant improvement in clinical severity at all ages, whereas within the placebo group, younger but not older children showed a similar improvement.

Conclusions Despite a reduction in clinical severity associated with *M. vaccae* at all ages, no benefit could be found after administering *M. vaccae* to children with AD aged 2–6 years when compared with placebo. *M. vaccae* may offer greater benefit in children over 5 years old, whose AD appears less likely to regress spontaneously.

Key words: age, atopic dermatitis, children, *Mycobacterium vaccae*, randomized controlled trial

Although the predisposition to atopic dermatitis (AD) is determined by genetic factors, exposure to certain environmental factors, including pathogenic and non-pathogenic microorganisms, influences clinical disease severity.^{1,2} Skin staphylococci and streptococci are the most common microorganisms which exacerbate AD. There is also evidence that the prevalence and severity of AD and other atopic diseases vary with exposure to mycobacteria. Previous studies have shown a lower prevalence of asthma in (i) children living in countries with higher tuberculosis notification rates,³ and (ii) Japanese children with greater tuberculin reactivity.⁴

Mycobacterium vaccae is a nonpathogenic environmental mycobacterium. In adults with asthma, a double-blind, placebo-controlled study of 40 patients showed a reduction in asthma symptoms and salbutamol inhaler use after three intradermal doses of *M. vaccae*.⁵ A similar study of 43 asthma sufferers to whom two lower intradermal doses were given found no significant benefit.⁶ In another study, 24 patients with mild to moderate asthma were randomized to receive a single dose of either *M. vaccae* or placebo before allergen airways challenge. The results indicated that pretreatment with *M. vaccae* did not significantly alter allergen-induced airways responses.⁷ We have previously demonstrated a significant improvement in disease severity 3 months after

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administration of a single intradermal dose of a killed suspension of *M. vaccae* in children aged 5–12 years with moderate to severe AD.⁸ AD is most prevalent in young children and sometimes resolves spontaneously. Based on the results of our previous trial, we embarked on a further study to extend our observations to children aged 2–6 years. The study design remained the same, except for extending the follow-up period from 3 to 6 months and, for drug licensing reasons, using a killed *M. vaccae* suspension in phosphate (SRP299) rather than borate buffer (SRL172).

Subjects and methods

Study design

The present single-centre, randomized, double-blind, placebo-controlled trial studied 56 children aged 2–6 years with moderate to severe AD between June 2001 and September 2002. All children completed the 6-month follow-up period and no child withdrew from the study.

Subjects

The children were attending the University Department of Child Health outpatient clinic at Booth Hall Children's Hospital (Manchester, U.K.), a regional referral centre for unusually severe or persistent AD.

Treatments

Sterile vials of 0.3 mL of a suspension containing 10 mg mL⁻¹ of heat-killed *M. vaccae* in phosphate buffer (SRP299) and identical vials containing buffer only (placebo) were kindly provided by SR Pharma plc (London, U.K.). In each case, 0.1 mL (1 mg) was administered by intradermal injection into nonaffected skin over the left deltoid. No child was receiving systemic corticosteroids, ciclosporin or interferon- γ , or topical tacrolimus ointment. Prescription of routine therapy, including topical corticosteroids, was not done by the study coordinator (P.D.A.), but was left to the patient's supervising paediatrician, who was not involved in the running of the trial.

Assessment

The study coordinator (P.D.A.) assessed the skin lesions in all cases and monitored the potency of the topical

corticosteroid ointment used during the trial. The severity of the children's dermatitis was assessed just prior to treatment, and then at 1, 3 and 6 months after treatment. The extent of involved skin was recorded on age-adjusted body surface area charts. Severity of the dermatitis in terms of erythema, excoriation, exudation and lichenification was graded as 1 (mild), 2 (moderate) or 3 (severe) at each body region⁹ and the 'dermatitis score' calculated by multiplying the surface area affected by this severity score to give a grade out of 300.¹⁰

Statistical analysis

Randomization was achieved by random number generation, with even numbers and 0 assigned to receive the placebo and odd numbers to receive *M. vaccae*. Allocation of children to placebo or *M. vaccae* groups was done by pharmacy staff. The investigators, patients and parents were not aware of which group the children were allocated to until after the final assessment at the end of the 6-month trial, at which stage the results were unblinded.

As data in our present study were not normally distributed (defined as mean $\pm$ SEM for skewness and kurtosis > 2), results are quoted as median and interquartile range. The Mann–Whitney *U*-test, Kruskal–Wallis test and Spearman correlation were used to examine trends in these non-normally distributed data. χ^2 analysis was used for binary data. The software package used was the SPSS (Chicago, IL, U.S.A.) statistical package.

The results of the current trial were compared with those of our previous study of children aged 5–18 years with moderate to severe AD.⁸ Both were randomized, double-blind, placebo-controlled trials conducted at the same single regional paediatric AD centre using the same outcome measures and recorded by the same observer. Data at one additional time point (6 months) were collected in the present study but the study design was otherwise identical. The dose and strain of *M. vaccae* were also identical in the two studies, except that phosphate rather than borate buffer was used in the current trial. The combined size of the two studies was 97 children.

Our previous study found a difference of 48 (95% confidence interval 32–65) percentage points between groups in the reduction in surface area from baseline to 3 months. In order to have 90% power to detect a similar difference in younger children we recruited 56 children into this study.

Ethical considerations

Permission to conduct the study was obtained from the Medicines Control Agency, Department of Health, London and the Local Research Ethics Committee. Written informed consent was obtained from parents of the children.

Results

Analysis of data on children aged 2–6 years

Baseline characteristics. Thirty-five (63%) of the 56 children were boys and 49 (88%) were white European. Forty (71%) had a parent or sibling with AD, asthma or allergic rhinitis, 20 (36%) were on inhalers for asthma and 15 (27%) had symptoms of allergic rhinitis. Groups were comparable at baseline for all the above parameters (Table 1), although the *M. vaccae* group had slightly more severe disease than the placebo group ($P = 0.05$). The median (interquartile range) duration of AD was 4 (3–5) years, the median surface area affected by AD was 34 (20–46)% and the median dermatitis score was 38 (21–67).

Table 1. Baseline characteristics of children aged 2–6 years with atopic dermatitis randomized to placebo or *Mycobacterium vaccae*

	Placebo	<i>M. vaccae</i>
No.	27	29
Age (years) ^a	3 (2–4)	4 (3–5)
Male patients, <i>n</i> (%)	15 (56)	20 (69)
White patients, <i>n</i> (%)	23 (85)	26 (90)
Family history of atopic diseases, <i>n</i> (%)	18 (67)	22 (76)
Asthma, <i>n</i> (%)	10 (37)	10 (34)
Allergic rhinitis, <i>n</i> (%)	7 (26)	8 (28)

^aMedian and interquartile range.

Table 2. Percentage body surface area affected by atopic dermatitis and dermatitis score before and after treatment

	Actual values		<i>P</i> -value	Change in scores (%)	
	Placebo	<i>M. vaccae</i>		Placebo	<i>M. vaccae</i>
Surface area					
Baseline	28 (16–43)	37 (28–48)	0.1	–	–
1 month	10 (6–33)	16 (11–26)	0.3	52 (35–70)	50 (30–64)
3 months	13 (5–30)	25 (6–44)	0.4	55 (30–75)	38 (0–78)
6 months	14 (8–28)	19 (6–35)	0.8	40 (–4–72)	54 (15–74)
Dermatitis score					
Baseline	31 (17–49)	40 (30–76)	0.05	–	–
1 month	10 (6–34)	18 (12–32)	0.2	54 (40–70)	55 (32–66)
3 months	13 (5–44)	35 (8–48)	0.4	55 (11–75)	46 (17–76)
6 months	14 (7–27)	24 (12–40)	0.2	50 (6–78)	57 (30–79)

Values are given as median (interquartile range). *P*-value (Mann–Whitney *U*-test) compares skin surface area involved or dermatitis score between the placebo and *Mycobacterium vaccae* groups.

Changes in the severity of dermatitis after administration of Mycobacterium vaccae. There was a 38–54% improvement in the median skin surface area affected by dermatitis at all time points (1, 3 and 6 months) following administration of *M. vaccae* (Table 2). The placebo group also showed a 40–55% improvement in median surface area affected at all time points. The extent of the improvement in median skin surface area affected was not statistically different between the two groups.

The trends in dermatitis score (Table 2) were similar to the changes in surface area described above. There was an improvement in the median (interquartile range) dermatitis score at 1 month [55 (32–66)%], 3 months [46 (17–76)%] and 6 months [57 (30–79)%] following administration of *M. vaccae*, and similar trends were also found in the placebo group at 1 month [54 (40–70)%], 3 months [55 (11–75)%] and 6 months [50 (6–78)%].

Alteration in the potency of topical corticosteroid ointments and frequency of oral antibiotics during the trial. There was a reduction in the potency of corticosteroids used in both study groups, but no differences between the groups ($P = 0.6$). Nine (31%) of 29 in the *M. vaccae* group [compared with nine (33%) of 27 in the placebo group] had reduced, and two (7%) of 29 in the *M. vaccae* group [compared with four (15%) of 27 in the placebo group] had increased the potency of corticosteroids used by the end of the 6-month trial (Table 3). There was also a significant reduction in the median number of courses of oral antibiotics prescribed for infective exacerbations of AD comparing the 6 months before [2 (1–3)] and after [0 (0–1)] the children started in the trial ($P = 0.001$). These changes were similar in the placebo and *M. vaccae* groups ($P = 0.8$).

Table 3. Potency of topical corticosteroid ointments prescribed to the children aged 2–6 years during the trial

	Placebo		<i>M. vaccae</i>	
	Before treatment	6 months after treatment	Before treatment	6 months after treatment
Nil	0	7 (26)	0	8 (28)
Mild	11 (41)	8 (30)	13 (45)	7 (24)
Moderate	3 (11)	4 (15)	8 (28)	9 (31)
Potent	13 (48)	8 (30)	8 (28)	5 (17)

Values are given as numbers (percentages).

Local reactions and side-effects of *Mycobacterium vaccae* administration. There were no systemic adverse effects of vaccination in either group. A local inflammatory reaction, consisting of an erythematous subcutaneous lump measuring 1–12 mm developed at the site of the injection in 13 of 29 (45%) children who received *M. vaccae* and in none of the placebo group. All reactions resolved spontaneously. The development of a local reaction did not correlate with the severity of the dermatitis or with a greater improvement in AD (data not shown). There was also no association with race or whether or not the child had received a bacille Calmette–Guérin vaccination.

Meta-analysis of data on children aged 2–5 years and 5–18 years

In order to investigate the possible reasons for the apparent effectiveness of *M. vaccae* in the previous⁸ but not the present trial, the effect of age in the total cohort of 97 children was investigated in more detail. The total population was divided into three equal groups based on age (< 5 years, 5–8 years and > 8 years). The percentage improvement in surface area affected by dermatitis over the first 3 months of the trials is shown in Figure 1. In the placebo group, the median (interquartile range) percentage improvement in surface area affected by dermatitis was 56 (16–75)% for children aged < 5 years, 32 (11–63)% for those aged 5–8 years and 10 (–12 to 29)% for those aged > 8 years ($P = 0.03$). Within the *M. vaccae* group the respective percentage improvements were 45 (15–91)%, 52 (0–78)% and 44 (12–71)% ($P = 0.7$). Thus, while there was a general trend for children of all ages to improve to a similar extent after *M. vaccae* administration, in the placebo group the younger the child the more likely they were to show a significant improvement in their AD over the same time frame ($n = 45$; $r = -0.41$; $P = 0.005$).

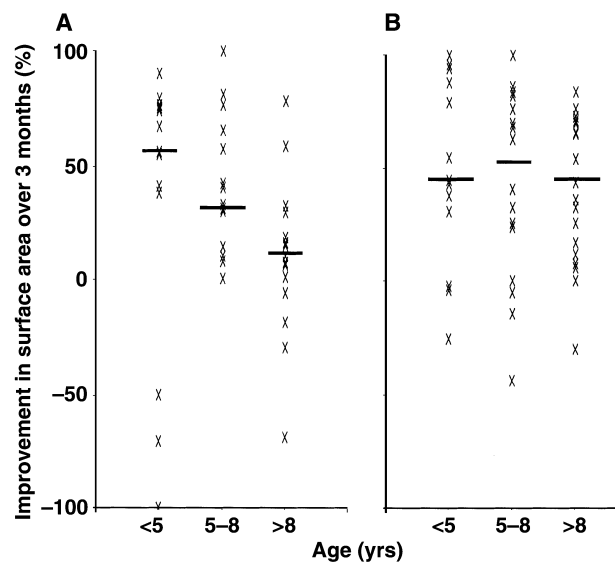


Figure 1. Improvement in percentage skin surface area affected by dermatitis in the 3 months after administration of (A) placebo or (B) *Mycobacterium vaccae* in children aged < 5 years, 5–8 years and > 8 years. Bars represent medians.

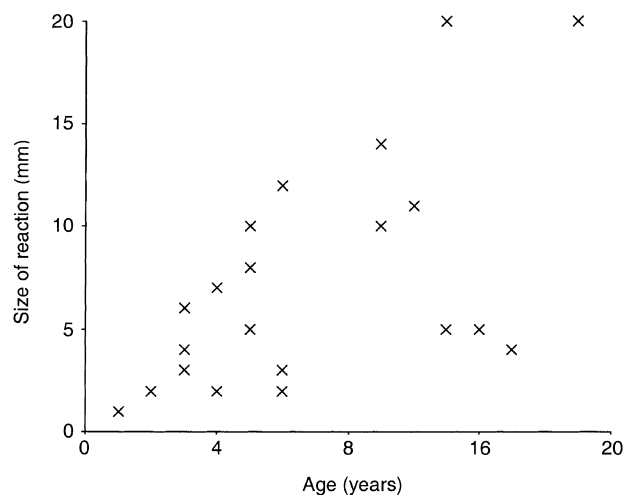


Figure 2. Size of reaction to *Mycobacterium vaccae* (mm) in children who developed a response based on age (years) of the children.

Similar numbers of children in the two trials still had a local skin reaction at 1 month after administration of *M. vaccae*: (i) present trial 13 of 29 (45%), and (ii) previous trial 13 of 21 (61%) ($P = 0.3$). However, older children developed larger reactions than younger ones ($r = 0.55$; $P = 0.004$; Fig. 2). In the group of children given *M. vaccae*, there was no difference in the percentage improvement in surface area ($P = 0.8$) or severity score ($P = 0.6$) over the first 3 months of the

trial in those who had a local reaction and those who did not.

Discussion

This randomized, double-blind, placebo-controlled trial of children aged 2–6 years with moderate to severe AD demonstrated a significant reduction in the severity of AD after a single intradermal injection of killed *M. vaccae* suspension, without any major adverse effects. However, this trial showed no added benefit of this treatment compared with a placebo. An objective improvement in the clinical severity score of AD was found in both test and control groups. Both groups also showed a reduction in the potency of topical corticosteroid used, as well as the number of courses of oral antibiotics required for the treatment of secondary bacterial infections. The design of the study ruled out confounding factors including race, gender and effects of seasonal variation. None of the patients in this study was treated with tacrolimus, ruling out this as a confounding factor.

As with our previous study,⁸ errors due to interobserver and intercentre variations were excluded by having all measurements made by one observer in one centre. A local inflammatory response in 45% of the children receiving *M. vaccae* might have biased the results towards showing a significant difference between treatments rather than the observed lack of difference. In the total cohort of children aged 2–18 years, younger children developed significantly smaller local reactions than older children, in keeping with the well-known clinical observation that younger children and infants mount a poorer cell-mediated immune response to mycobacteria and are therefore at increased risk of disseminated tuberculosis and tuberculous meningitis.^{11,12} The *M. vaccae* suspension was similar in this and our previous study except for the buffer used. The fact that there was a similar percentage improvement in dermatitis severity in the treatment group of both studies argues against one of the preparations being less effective than the other.

An explanation as to why our previous study in older children showed a significant benefit from *M. vaccae* and the present study did not, seems to lie with changes in dermatitis severity in the placebo group. As mentioned above, the observed difference in the effectiveness of *M. vaccae* and placebo between this and our previous trial is not because administration of *M. vaccae* was associated with any less of a reduction in clinical severity in the two groups, but rather that within the

placebo group, younger children showed a significant reduction in disease severity commensurate with that of the study group, while in older children the disease was generally static over the trial. AD is not a static disease especially in the age group studied in our present trial. It increases in prevalence in infancy to reach a peak in the subsequent few years, before resolving spontaneously in many individuals.¹³ It is therefore not surprising that we see an overall improvement in the dermatitis of this cohort of children aged 2–5 years. Our results highlight the importance of studying the control and test groups in parallel rather than in series, especially in younger children with AD.

Based on its natural history, AD might be viewed as a delay in the normal maturation of a child's immune system associated with hypersensitivity to usually innocuous environmental antigens turned allergens. There is growing evidence that regulatory T lymphocytes producing the immunomodulatory cytokines interleukin (IL)-10 and transforming growth factor (TGF)- β 1 are important in inducing tolerance and preventing atopy.¹⁴ We have previously shown that children with troublesome AD have polymorphisms of TGF- β 1 associated with low production of this cytokine, suggesting that inherent underactivity of this cytokine predisposes individuals to AD.¹⁵ It has also recently been shown that the immunomodulatory effects of *M. vaccae* in an animal model of allergy are mediated by these IL-10 and TGF- β 1 producing regulatory T lymphocytes.¹⁶ We propose that this regulatory pathway normally matures spontaneously in children, resulting in spontaneous resolution in their AD. In those where this process is delayed, it may be encouraged to mature by certain environmental microorganisms such as mycobacteria. If this hypothesis is correct, then it would help to explain why in young children with AD whose regulatory pathway is maturing spontaneously, administration of *M. vaccae* has no effect over and above the spontaneous improvement observed in the placebo group. Research directed at providing evidence supporting or refuting this hypothesis will resolve this issue. Furthermore, a 120-patient randomized clinical trial is under way to confirm our observation regarding the activity of SRP299 in children over 5 years old.

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