

Effect of a *Mycobacterium vaccae* derivative on paediatric atopic dermatitis: a randomized, controlled trial

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Summary

Background. The prevalence of atopic diseases in the Western world is rising while infectious diseases decline. The 'hygiene hypothesis' suggests that reduced exposure to microbes such as mycobacteria in early life is associated with increased atopic disease. Recent research showed that *Mycobacterium vaccae* reduced the severity of atopic dermatitis (AD) in children.

Objective. To evaluate the efficacy of a derivative of heat-killed *M. vaccae* in children with AD.

Methods. In total, 129 children, aged 5–16 years old with moderate to severe AD participated in this randomized, double-blind, placebo-controlled trial. Participants received an intradermal injection of either *M. vaccae* or placebo three times at 2-weekly intervals. The two groups were compared for changes in severity and extent of AD from baseline to 3 and 6 months after treatment.

Results. There was no significant difference between the two groups for change in severity of AD at 3 and 6 months ($P = 0.77$ and $P = 0.70$, respectively) or in extent of disease at 3 months ($P = 1.0$). Local injection-site reactions occurred in 47% of participants, of whom 75% received *M. vaccae*.

Conclusion. In this study, *M. vaccae* did not improve AD significantly in children with moderate to severe disease.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease. The prevalence of AD in children is between 15% and 20% in developed countries, including New Zealand.^{1,2}

Although genetic factors play an important role in the development of AD,³ the increase in prevalence observed over a short period² cannot have a genetic

basis. The 'hygiene hypothesis' suggests that reduced exposure to microbial pathogens in early life is associated with an increase in atopic diseases.⁴ Some cross-sectional studies confirmed an inverse correlation between mycobacterial exposure and atopic disease,⁵ with conflicting results for AD.⁶

Immunization with mycobacterial preparations to prevent or treat atopic disease has been explored in animals.^{7,8} More recently studies using mycobacterial preparations, particularly *Mycobacterium vaccae* for the treatment of atopic asthma and allergic rhinitis in adults, have shown variable outcomes.^{9,10}

In two randomized, controlled trials (RCTs) using a single intradermal injection of heat-killed *M. vaccae* (HKMV) for the treatment of moderate to severe AD in children, Arkwright and David found that older children showed a significant improvement in the extent and severity of dermatitis at 3 months,¹¹ but this was not seen in younger children.¹²

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Encouraged by their initial results, we conducted a similar study. The aim was to evaluate the safety, efficacy and tolerability of a delipidated, deglycolipidated and arabinogalactan depleted derivative of HKMV (AVAC™; Genesis Research and Development Corporation Limited, Auckland, New Zealand), in children with moderate to severe AD.

Methods

Approval to conduct the study was obtained from the Auckland Ethics Committee. All participants/guardians gave written informed consent prior to enrolment in the study.

This was a randomized, double-blind, placebo-controlled, parallel group, single-centre, phase II trial. Participants were recruited in Auckland, New Zealand, between October 2003 and June 2004, either by referral from paediatric and dermatology outpatient clinics, general practices, or self-referral in response to advertising. Children aged 5–16 years with moderate to severe AD were eligible to participate. AD was defined by using the UK Working Party's Diagnostic Criteria for AD¹³ and severity was defined by the Rajka and Langeland criteria.¹⁴

Participants were required to be in good general health and were ineligible for enrolment if they had a history of anaphylaxis; had used tacrolimus, ciclosporin or methotrexate in the previous 6 months; had used topical pimecrolimus within the previous 3 months; had used ultraviolet (UV)A or UVB phototherapy, systemic corticosteroids, high-dose inhaled corticosteroids or traditional Chinese medicines within the previous 6 weeks; had used very potent topical corticosteroids, changed their diet or were hospitalized for AD within the previous month; had used systemic homeopathy or systemic antibiotics or changed their AD treatments within the previous 2 weeks; had clinically infected AD at their baseline visit; or were unlikely to comply with the study requirements. Participants could use (or discontinue) their standard AD medications throughout the study providing these were not listed in the exclusion criteria.

Participants were screened and randomized into two groups 2 weeks before beginning treatment. The randomization schedule was generated (DHP Ltd, Abergavenny, Gwent, UK) using a computerized random-number generator to select permuted blocks with a size of two. In total, 129 children participated in the study; 64 in the AVAC group and 65 in the placebo group (Fig. 1). Participants in both groups had comparable baseline demographic and clinical characteristics

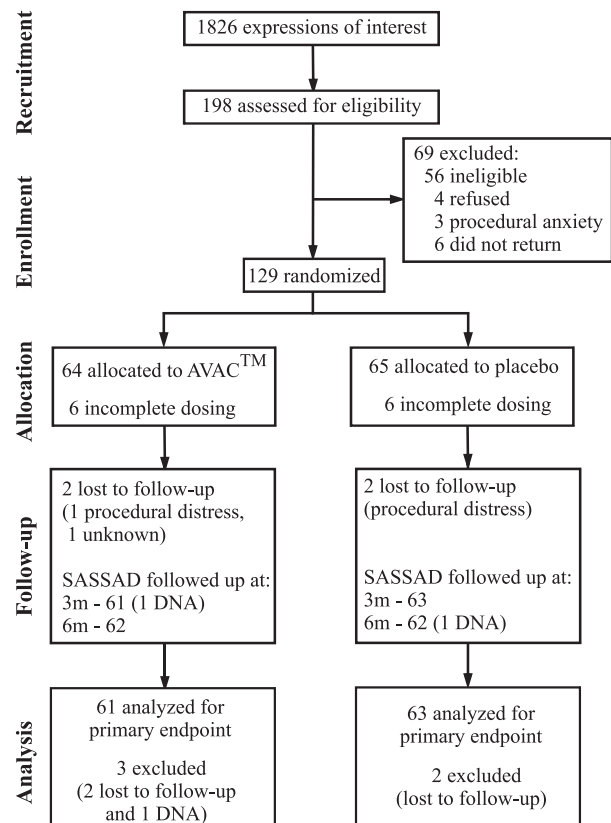


Figure 1 Participant flow diagram.

(Table 1). Study personnel, participants and parents were unaware of treatment allocation or the schedule until after statistical analyses were completed.

Each participant received an injection of either AVAC or placebo at 2-weekly intervals, resulting in a total of three injections. Both compounds were manufactured under good manufacturing practice conditions (Centre for Applied Microbiology Research, UK, under contract with SR Pharma, London, UK) and the study pharmacy was supplied with identical drug kits, containing either AVAC or placebo (Genesis). AVAC was supplied at a concentration of 250 µg/mL in phosphate-buffered saline (PBS), and the placebo was PBS. Either 12.5 µg (0.05 mL) of AVAC or 0.05 mL of placebo was administered intradermally.

Evaluations were performed at baseline (week 0), 2 and 4 weeks, and 2, 3 and 6 months (Table 2). Because of the particulate appearance of AVAC and the predicted difference in the rate of injection-site reactions in the active and placebo groups, different staff members were used as efficacy assessors and study drug administrators/injection-site assessors.

The primary efficacy endpoint was a change in disease severity from baseline to 3 months, assessed by

Table 1 Participants' baseline characteristics.

Characteristic	AVAC (n = 64)	Placebo (n = 65)
Age, years	9.5 ± 3.1	9.9 ± 3.3
Duration of AD, years	8.3 ± 3.3	8.1 ± 3.7
Female, n (%)	40 (63)	39 (60)
Ethnicity, n (%)		
European/Pakeha	18 (28)	19 (29)
Maori	15 (23)	15 (23)
Pacific Island	22 (34)	16 (25)
Other	9 (14)	15 (23)
Asthma, n (%)	16 (25)	12 (19)
Rhinitis, n (%)	16 (25)	12 (19)
SASSAD (0–108)	31.9 ± 13.4	32.1 ± 12.1
BSA (%)	31.9 ± 16.7	31.7 ± 17.0
CDLQI (0–30)	11.6 ± 5.5	10.3 ± 5.1

Data are means ± SD unless otherwise stated. AVAC, study drug. AD, atopic dermatitis; BSA, Body surface area; CDLQI, Children's Dermatology Life Quality Index; SASSAD, Six-Area Six-Sign Atopic Dermatitis.

Table 2 Study schedule.

	Week				Month		
	–2*	0†	2	4	2	3	6
Diagnosis ²⁰ and severity classification of AD ²¹	x	x					
AVAC/placebo injection		x	x	x			
SASSAD, ²² AD extent, ²³ sleep disturbance, pruritus and topical corticosteroid use assessments		x	x	x	x	x	x
CDLQI ²⁴ assessment		x				x	x
Total serum IgE	x				x	x	
Injection site evaluations			x	x	x	x	x
Adverse events assessment		x	x	x	x	x	x
Laboratory safety analyses	x					x	x

*Screening visit; †Baseline visit. AVAC, study drug. AD, atopic dermatitis; CDLQI, Children's Dermatology Life Quality Index; SASSAD, Six Area Six Sign Atopic Dermatitis.

a change in the Six-Area Six-Sign Atopic Dermatitis (SASSAD) score in the AVAC treated group relative to the placebo-treated group.¹⁵

Secondary efficacy endpoints included comparisons of relative change in: the severity of AD between baseline and 6 months; the extent of AD, topical corticosteroid use, quality of life (QOL), and sleep and pruritus between baseline and 3 and 6 months, and in total serum IgE (sIgE) between baseline and 2 and 3 months. *Posthoc* subgroup comparisons on the effect of atopy on the change in SASSAD score between baseline and 3 months were made.

The 'rule of nines' was used to assess the extent of the disease.¹⁶ QOL was assessed by using the Children's Dermatology Life Quality Index (CDLQI) questionnaire.¹⁷ Sleep disturbance and degree of pruritus were assessed using four-point and five-point symptom scales, respectively. Change in topical corticosteroid use was assessed for the week before the visit: frequency of use was assessed using a five-point scale and potency was graded as mild, moderately potent, potent and very potent.¹⁸ Blood samples for total sIgE were taken at screening, and at 2 and 3 months, and analysed by means of fluoroenzyme immunoassay (UniCAP 100 system; Phadia, Uppsala, Sweden). Atopy was defined as a total sIgE > 150 kIU/L plus a history of asthma, rhinoconjunctivitis or food allergy.¹⁹

Adverse events were reported to the study team at any time if there were concerns. Local injection-site reactions were assessed by paediatric nurses at each scheduled visit. Participant-reported reactions were also documented. Safety assessments also included full blood count, liver and renal function tests, urinalysis, physical examination and urinary pregnancy test (if there was childbearing potential).

Statistical analysis

All statistical analyses were performed using SAS software (version 9.1; SAS Inc., Chicago, IL, USA).

For the SASSAD score, which has reported standard deviations (SD) of 15–25,¹⁵ and a 10-point absolute change was considered clinically significant. Sixty patients were needed in each treatment arm to give the study 80% power 5% level of significance (two-tailed) to detect a 10-point change.

The SASSAD score, affected body surface area (BSA) percentages, QOL and total sIgE (after log transformation) were analysed using linear mixed models, with the baseline, observations at intermediate weeks and the final time point being the repeated measures. An unstructured correlation matrix was used to model the correlations between the repeated measures. The significance of the interaction of the difference in outcome between treatments at baseline and at the final time point was reported for each measure.

Sleep disturbance, pruritus measures and topical corticosteroid use were analysed using nonlinear mixed models (an ordinal logistic regression).

For safety analyses, the Mann–Whitney *U*-test was used to compare the median number of adverse events per participant between the AVAC and placebo groups by specific categories.

	AVAC		<i>P</i>	Placebo	
	<i>n</i>	Result		<i>n</i>	Result
SASSAD (0–108)					
Baseline	61	31.7 ± 13.5	0.77	63	2.4 ± 12.1
3 months		29.8 ± 12.8			30.0 ± 13.1
Baseline	62	31.8 ± 13.5	0.70	62	32.7 ± 12.1
6 months		26.0 ± 14.7			27.6 ± 14.3
BSA %					
Baseline	59*	31.6 ± 16.8	1.0	63	32.0 ± 17.3
3 months		32.2 ± 19.2			32.2 ± 19.1
Baseline	62	31.6 ± 16.7	0.17	62	32.1 ± 17.1
6 months		27.4 ± 19.7			31.0 ± 20.9
CDLQI (0–30)					
Baseline	61	11.4 ± 5.5	0.95	63	10.2 ± 5.2
3 months		8.2 ± 6.5			7.0 ± 5.1
Baseline	62	11.6 ± 5.6	0.25	62	10.3 ± 5.2
6 months		7.5 ± 6.2			7.2 ± 6.0
sIgE (kIU/L)					
Baseline	47*	714 (437–1169)	0.09	45*	398 (236–673)
2 months		569 (334–970)			480 (288–799)
Baseline	57*	642 (397–1038)	0.52	59*	484 (295–794)
3 months		633 (387–1034)			510 (305–856)

Table 3 Primary and secondary outcome measures.

AVAC, study drug. BSA, body surface area; CDLQI, Children's Dermatology Life Quality Index; SASSAD, Six Area Six-Sign Atopic Dermatitis; sIgE, total serum IgE. Data are mean SD or (for sIgE) mean of the log-transformed values then transformed back to original scale (95% confidence intervals). *Missing data for some participants.

Results

There was a mean reduction in SASSAD scores between baseline and 3 months after treatment for both groups, but no significant difference in SASSAD scores between treatment groups at 3 months ($P = 0.77$) (Table 3). There was no difference in change in SASSAD scores between baseline and 6 months between the two groups ($P = 0.70$) (Table 3). In the placebo group compared with the AVAC group, there were larger decreases in mean SASSAD score in patients with atopy than in those without ($P = 0.02$).

There was no difference between the two groups in the change in extent of AD between baseline and 3 months ($P = 0.10$) or between baseline and 6 months ($P = 1.7$) (Table 3). There was also no significant difference between the groups for change in QOL, sleep disturbance, pruritus or frequency and potency of topical corticosteroid use from baseline to 3 or 6 months (Table 3).

Baseline mean total sIgE levels were higher than normal values in both treatment groups. The observed total sIgE at 2 months had decreased to a larger extent in the AVAC group than in the placebo group, but this was not significant ($P = 0.09$). By 3 months, these values had returned to those at baseline, with no difference in change between the two groups ($P = 0.52$) (Table 3).

Table 4 Assessor-observed injection site reactions.

	AVAC	Placebo
Total doses* given, <i>n</i>	184	186
Total dose reactions, <i>n</i>	75	23
Size (mm) of reactions, (%)		
< 5	51 (68)	20 (87)
5–9.9	21 (28)	3 (13)
> 10	3 (4)	0
Reactions (mm), <i>n</i> (range)		
Ulceration	3 (3–5)	0
Induration	71 (1–12)	21 (1–6)
Erythema	16 (1–20)	2 (3–6)

AVAC, study drug. *Dose 1 + 2 + 3.

Local injection site reactions observed by assessors are shown in Table 4. They were observed in 47% of participants, of whom 75% received AVAC.

Discussion

In this trial, we found no significant differences for change in the primary outcome measure (AD severity) between the AVAC and placebo groups, or in the secondary outcome measures. AVAC was confirmed to be safe and well-tolerated in this cohort.

Retention of participants in the study was very high: only four participants withdrew from the study. Inadequate dosing occurred in 12 participants, but the numbers were equal in both groups.

Primary efficacy assessment at 3 months was performed using the SASSAD score. For 97% of the participants, the same efficacy assessor performed both the baseline and 3-month SASSAD assessment, to minimize interobserver variability.

We defined AD according to the UK Working Party's Diagnostic Criteria.¹³ Numerous trials have shown variability in how atopic AD really is, with results varying between 7% and 75%.²⁰ Our cohort with atopy had larger decreases in AD severity in the placebo group compared with the AVAC group. This analysis was performed *post hoc*, however, and we did not confirm atopy with evidence of IgE sensitization. In future trials, planned analysis could be considered to investigate whether the treatment response is different in those with and without confirmed atopy.

The use of *M. vaccae* in the treatment of AD in RCTs with blinding has shown variable results. Arkwright and David conducted two such studies, both using one dose of 1 mg of HKMV (the same strain used by us) intradermally for the treatment of AD in children and assessed them after 3 months.¹¹ We gave three doses of a *M. vaccae* derivative, assessed children 3 and 6 months after treatment, and had a larger cohort. The study populations differed, ours being recruited mainly from the community whereas theirs were recruited from a hospital centre treating unusually severe or persistent AD. A recent multicentre RCT found no difference between a single dose of intradermal HKMV (0.1 or 1.0 mg) and placebo on moderate to severe AD in children aged 5–16 years, but overall, all treatment arms improved.²¹

Unfortunately direct dose comparisons with HKMV or its other derivatives are not possible, because the products are not identical. Our cumulative dose of AVAC was 0.0375 mg. Shirtcliffe *et al.*⁹ used either 0.5 mg of HKMV or 0.05 mg of a delipidated, deglycolipidated (DDMV) derivative for their patients with asthma, resulting in no effect on their asthma severity score. HKMV yields 10% by weight of DDMV and DDMV is 10-fold more effective than HKMV in stimulating interleukin (IL)-12 release in mice.⁹ AVAC has been shown to suppress airway eosinophilia in the mouse model (Genesis R & D, personal communication). These preclinical data, along with injection site reactions observed during the trial, indicated that AVAC was biologically active.

The dose of AVAC used in our study was based on results from the paediatric phase I AD study, in which

the 'low' dose was well-tolerated, whereas clinically significant local reactions were noted with the 'high' dose. Our dose corresponded to the phase I 'low' dose. Although most injection-site reactions in our study were relatively minor, participants in the AVAC group had clinically significant local reactions, which would caution against the use of higher doses of AVAC in children.

The reason for discrepancy between the human trials^{9,12,21} and the murine trials^{7,8,22} is unclear. The timing of inoculation in the murine trials is usually before atopic sensitization or during disease establishment. In contrast, in human trials, inoculation occurs atopic disease is established. AD was well established in our trial's participants, with a mean $\pm$ SD duration of 8.2 ± 3.5 years. It is possible that established atopic disease may be less susceptible to immune regulation.

Most studies to date have used *M. vaccae* by intradermal injection. The most likely route of exposure to environmental soil microbes is the oral route, and the gut mucosa is considered an ideal site for the induction of regulatory T cells (Tregs).^{23,24} In mice, HKMV given by gavage was as effective as subcutaneous treatment in downregulating pulmonary allergic inflammation, with induction of IL-10, an immunoregulatory cytokine, also noted.²⁵ Oral administration of HKMV might allow higher doses to be given for longer periods, as it is likely to be more easily tolerated, particularly in young children. New studies have shown that loss-of-function mutations in the filaggrin gene, with resultant impaired keratinocyte differentiation, are strong predisposing factors for AD, supporting the hypothesis of a primary barrier dysfunction in AD, which allows penetration of allergens and sensitization.³ However, genetic influences alone cannot explain the increase in AD in recent decades.² Environmental influences on the epithelial barrier and immunological milieu (primed to over-react) remain important. Research should address not only immunoregulation, but also the skin barrier dysfunction.

Further research with *M. vaccae* in this field is still warranted. A number of issues need to be clarified further, including the optimum cumulative dose, standardization of potency or dose comparisons between HKMV and its derivatives, and dosing schedule and route of administration; the contribution of genotype variation or eczema phenotype to susceptibility to mycobacterial immunotherapy; whether mycobacterial immunotherapy may be more effective in primary prevention than in treatment of atopic disease; whether specific atopic diseases differ in their response; and whether the severity of AD affects the clinical response.

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