

Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease

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Background: Although a doubling in the prevalence of atopic disease, including atopic dermatitis, in the Western world over the last few generations has been paralleled by a marked reduction in infectious diseases, especially tuberculosis, it is unclear whether this increase in atopy is causally related to reduced exposure to mycobacteria.

Objectives: The aim of this study was to determine whether administration of mycobacterial antigens to atopic individuals might ameliorate their disease.

Methods: Forty-one children aged 5 to 18 years with moderate-to-severe atopic dermatitis were enrolled in a randomized, double-blind, placebo-controlled trial, where they were given either one intradermal injection of killed *Mycobacterium vaccae* (SRL 172) or buffer solution (placebo). Changes in skin surface area affected by dermatitis and dermatitis severity score were assessed before treatment and at 1 and 3 months after treatment.

Results: Children treated with SRL 172 showed a mean 48% (95% CI, 32%-65%) reduction in surface area affected by dermatitis compared with a mean 4% (95% CI, -29% to 22%) reduction for the placebo group ($P < .001$) and a median 68% (interquartile range, 46%-85%) reduction in dermatitis severity score compared with 18% (interquartile range, -2% to 34%) for the placebo group ($P < .01$) at 3 months after treatment. There were no untoward effects of the treatment, apart from a local reaction in 13 of the 21 children, which occurred 1 month after SRL 172 administration and settled spontaneously.

Conclusion: SRL 172 was associated with an improvement in the severity of the dermatitis in children with moderate-to-severe disease. (J Allergy Clin Immunol 2001;107:531-4.)

Key words: *Mycobacterium vaccae*, atopic dermatitis, atopy, T_H2

The prevalence of atopic disease (atopic dermatitis, asthma, and allergic rhinitis) in Western societies has doubled during the last half century.^{1,2} Because this has occurred in 1 to 2 generations, it cannot be due to a change in genetic susceptibility but must be due to a change within the environment.³ One of the most dram-

atic changes to the general health of Western societies over a similar time period has been a marked reduction in the prevalence of infectious diseases, particularly tuberculosis.⁴⁻⁶ It has previously been shown that reduced exposure to mycobacterial antigens is associated with an increased prevalence of atopy.⁷ On the basis of the above observations, we hypothesized that if the increased prevalence of atopic disease was causally related to reduced exposure of the community to mycobacterial antigens, then immunization of atopic individuals with these antigens would ameliorate their disease. *Mycobacterium vaccae* (SRL 172), the mycobacterial antigen used in this study, is one of over 80 species of environmental saprophytic mycobacterium. It suppresses the immune response in animal models of atopy^{8,9} and has been shown to be well tolerated when administered to children.¹⁰

METHODS

Patients and study design

This study was conducted as a single-center, randomized, double-blind, placebo-controlled trial. The children enrolled in the study, aged 5 to 18 years, attended the University Department of Child Health outpatient clinic at Booth Hall Children's Hospital, a regional referral center for unusually severe or persistent atopic dermatitis. Sixty children with moderate-to-severe atopic dermatitis were initially considered for the study. Eight children and their parents declined to take part, 7 were excluded because their dermatitis was too mild (surface area affected, $\leq 5\%$), and 3 children were excluded because they were receiving systemic steroids, cyclosporin A, or IFN- γ . Prescription of routine therapy, including topical steroids, was not done by the study coordinator (P.D.A.) but was rather left to the patient's supervising pediatricians, who were not involved in the running of the trial. Twenty-nine (71%) of 41 patients were white. Twenty-seven (66%) of 41 children had first-degree relatives affected by atopic dermatitis, asthma, or allergic rhinitis. The average length of time the children had atopic dermatitis was 8 years (range, 2-15 years). Twenty-three (56%) children had asthma requiring inhaled steroids, and 12 (29%) children had allergic rhinitis.

Permission to conduct the study was obtained from the Medicines Control Agency, Department of Health, London, and the Local Research Ethics Committee (Project No. 99060). Written informed consent was obtained from parents of the children. Sterile vials of SRL 172 (0.3 mL of a suspension containing 10^{10} heat-killed *M vaccae* per milliliter) and identical vials containing buffer only were kindly provided by SR Pharma Ltd (London, United Kingdom). Randomization and allocation (random number genera-

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tion with even numbers and 0 for control subjects and odd numbers for patients receiving SRL 172) of children to the placebo or SRL 172 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 3-month trial, at which stage the results were unblinded. Vials of SRL 172 and placebo were supplied by the pharmacy in identical boxes. Administration was always by means of intradermal injection into a dermatitis-free zone over the left deltoid. Blood samples were taken before and 3 months after immunization.

The study coordinator (P.D.A.) assessed the skin lesions in all cases and monitored the potency of the topical steroid ointment used during the trial. The severity of the children's dermatitis was assessed just before treatment and then at 1 and 3 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 mild (1), moderate (2), or severe (3) at each body region,¹¹ and the dermatitis score was calculated by multiplying the surface area affected by this severity score to give a grade of 0 to 300.¹²

Laboratory investigations

Serum total IgE determination was by means of RIA with the CAP system. Absolute blood eosinophil count was estimated with flow cytometry.

Statistical analysis

Where data were found to be normally distributed (defined as mean/SE for skewness and kurtosis <2), results are quoted as means $\pm$ SD. A 2-tailed Student *t* test was used to compare groups, and Pearson correlation was used to examine linear trends. Dermatitis scores and IgE levels were not normally distributed, even after logarithmic and square root transformations, and therefore these data are quoted as medians with interquartile ranges. The Mann-Whitney *U* test and Spearman correlation were used to examine trends in these nonnormally distributed data. χ^2 Analysis was used for binary data. The software package used was the SPSS statistical package. The power of the study was 80%, which was determined retrospectively by calculating the size effect on the basis of the paired percentage change in surface area at 3 months of the SRL 172 group ($\pm$ SD).

RESULTS

Baseline characteristics

The baseline characteristics of the children with atopic dermatitis who took part in the study are shown in Table I. There were no significant differences in the age or race of members of the placebo and SRL 172 groups. Of the nonwhite children, 3 of 7 in the placebo group were Afro-Caribbean, and the remaining 4 in this group and all 5 in the SRL 172 group were from the Indian subcontinent. There were significantly fewer boys in the SRL 172 group compared with those in the placebo group ($\chi^2 = 4.1$, $P = .04$).

The severity of the dermatitis in the 2 groups on the basis of both surface area affected and dermatitis score was similar. Male and female patients did not differ significantly in either surface area affected (male patients, 45% $\pm$ 33%; female patients, 35% $\pm$ 25%; $P = .3$) or their dermatitis score (male patients, 46 [interquartile range, 19-145]; female patients 33 [interquartile range, 19-68]; $P = .5$).

Serum IgE levels and absolute blood eosinophil counts were grossly elevated compared with normal values but did not differ between the 2 study groups. There was no significant correlation between IgE levels and blood eosinophil counts. Within the total study population, neither total IgE levels nor eosinophil counts correlated with surface area affected by dermatitis or dermatitis score.

Reduction in dermatitis severity after SRL 172 administration

Data on skin surface area affected by dermatitis are shown in Table II. Over the 3-month trial, there was no significant improvement in the surface area affected by dermatitis in the placebo group (mean, 4%; 95% CI, -29% to 22%). In contrast, there was a reduction in the surface area affected by dermatitis in the group vaccinated with SRL 172, which was significant both at 1 month (mean, 32%; 95% CI, 15%-49%; $P = .01$) and 3 months after vaccination (mean, 48%; 95% CI, 32%-65%; $P = .001$). No child showed a complete resolution of their dermatitis.

Data on dermatitis score are found in Table II. The trends in dermatitis score were similar to the changes in surface area described above. There was a small but non-significant improvement in the dermatitis score of the placebo group during the 3 months of the study (median, 18; interquartile range, -2 to 34). In contrast, there was a significant reduction in the dermatitis score of the SRL 172 group, which was noticeable at 1 month (median, 41; interquartile range, 16-74; Mann-Whitney *U* test comparing change in dermatitis score in the SRL 172 group with that in the placebo group, $P = .001$) and persisted until the end of the trial (median, 68; interquartile range, 46-85; $P = .007$).

Alteration in the potency of topical steroid ointments used during the trial

Eight of 21 patients in the SRL 172 group (compared with 3 of 20 in the placebo group) had reduced and 2 of 21 in the SRL 172 group (compared with 1 patient in the placebo group) had increased the potency of steroids used 3 months after SRL 172 administration compared with before starting the trial (not significant, Table III).

Local reaction and side effects of SRL 172 administration

One child in the placebo group was withdrawn from the study when steroid-responsive nephrotic syndrome developed soon after enrollment. No other children withdrew from the study or were lost to follow-up. There were no systemic adverse effects of vaccination in either group. A local inflammatory reaction, consisting of an erythematous subcutaneous lump at the site of the injection, which resolved spontaneously, developed in 13 (62%) of 21 children who received SRL 172 and none of the placebo group.

Of the 13 children in the SRL 172 group who had a local reaction, the reaction was 1 to 10 mm in 9 children and 11 to 20 mm in the remaining 4 children. The development of a local reaction did not correlate with the

TABLE I. Baseline characteristics of subjects with atopic dermatitis randomized to placebo or SRL 172

Characteristic	Placebo	SRL 172	P value
No.	20	21	
Age (y)	10 ± 4	9 ± 4	.3
Male patients, n (%)	12 (60)	6 (29)	.04
White patients, n (%)	13 (65)	16 (76)	.4
Surface area affected (%)	40 ± 30	38 ± 30	.8
Dermatitis score (0-300)	38 (22-86)	55 (19-69)	.8
IgE 1000 kU/mL (≤0.1)	6.6 (1.7-19.7)	9.2 (0.6-15.2)	.8
Blood eosinophil count (× 10 ⁹ /L) (0.2 [0.1-0.4])*	1.4 ± 1.0	1.0 ± 0.6	.3

Values are given as means ± SD (2-tailed Student *t* test) or medians (interquartile range) (Mann-Whitney *U* test); the χ^2 test was used for binary data.

*Normal range.

severity of the dermatitis or with a more marked improvement in the atopic dermatitis. There was also no association with the age or race of the patients or whether the child had received BCG vaccination.

Serum IgE levels and absolute blood eosinophil count after vaccination

There were no significant changes in either serum IgE or absolute eosinophil count after SRL 172 administration. After vaccination, there was still no correlation between IgE and eosinophil count or between either of these parameters and surface area affected by dermatitis and dermatitis score.

DISCUSSION

This randomized, double-blind, placebo-controlled study demonstrated that in the cohort of children studied, there was a significant reduction in the severity of their atopic dermatitis after a single intradermal injection of killed *M vaccae* suspension (SRL 172) without any major adverse effects. The lack of any placebo effect may relate to the relatively short duration of the trial and the objectivity of the assessment scores, especially the use of surface area affected by dermatitis as the main outcome measure of the study. The design of the study ruled out the clinical effect being caused by a number of confounding factors. The severity of dermatitis and the age and race of the patients were similar in the 2 study groups. Although the male/female ratio was different in the groups, there were no differences in the severity of the dermatitis between male and female patients. Any seasonal variation in dermatitis severity was taken into account by studying the SRL 172 and placebo groups in parallel at the same time of the year. Changes in potency of topical steroids were always made by the supervising pediatrician and not by the study coordinator or the parents. There were no significant changes in the potency of steroids used by either group during the study period.

Errors caused by interobserver and intercenter variations were excluded by having all measurements made by one observer in one center. Assessment of dermatitis severity is confounded by a degree of subjectivity, but calculation of the surface area affected by dermatitis

TABLE II. Skin surface area affected by atopic dermatitis and dermatitis score before and after treatment

	Placebo	SRL 172
Skin surface area affected (%)		
Before treatment	40 ± 30	38 ± 30
1 month after treatment	37 ± 29	30 ± 31
3 months after treatment	40 ± 33	21 ± 24*
Dermatitis score (0-300)		
Before treatment	38 (22-86)	55 (19-69)
1 month after treatment	40 (22-76)	22 (8-74)*
3 months after treatment	28 (14-71)	14 (6-41)*

Values are given as means ± SD (surface area affected) or median (interquartile range) (dermatitis score).

**P* < .01 comparing change in skin surface area involved or dermatitis score at 1 or 3 months after and before treatment between the placebo and SRL 172 groups.

TABLE III. Potency of topical steroid ointments prescribed to study groups during the trial

	Placebo		SRL 172	
Steroid potency	Before treatment	3 mo after treatment	Before treatment	3 mo after treatment
Nil	0 (0)	2 (10)	0 (0)	2 (10)
Mild	4 (20)	5 (25)	4 (19)	7 (33)
Moderate	9 (45)	6 (30)	6 (29)	5 (24)
Potent	7 (35)	7 (35)	11 (52)	7 (33)

Values are given as numbers (percentages).

from body surface charts is the most objective measure available and was therefore used as the major outcome measure in this study. A local inflammatory response in some of the children receiving SRL 172, but none of the children receiving placebo, may have affected the blinding of the study, but this is a very difficult issue for which to completely control. The fact that 8 (38%) of 21 children in the treatment group did not get a reaction would have reduced, but not completely prevented, any effect. Further evidence against this as a confounding effect is that although the numbers in the subgroups are small, the reduction in severity of the dermatitis in children with a local reaction was not significantly greater than that in children who had no reaction.

This study does not provide information on the long-term outcome of patients treated with SRL 172. Further studies are required to determine whether the effect of SRL 172 is sustained after 3 months. Because none of the 21 children in the SRL 172 group showed complete resolution of their dermatitis, it is obvious that a single dose is no panacea for atopic dermatitis. A reduction, rather than complete resolution, of atopic signs after a single dose of SRL 172 is not at odds with the findings of 2 Swedish studies that observed no decrease in the prevalence of atopy in children given BCG vaccination during infancy.^{13,14} These studies did not examine the relative severity of atopic disease between the vaccinated and unvaccinated groups and were only designed to detect complete protection from atopy by BCG, rather than amelioration of disease, as studied here.

Our finding of a significant improvement in the severity of childhood atopic dermatitis after SRL 172 treatment is consistent with previous observations that have shown inverse associations between tuberculin responses and atopic disorders.⁷ Although SRL 172 has been found to suppress T_H2-type allergic responses to allergens in mouse models,^{8,9} the evidence from human studies of the ability of a single dose of SRL 172 to alter T_H2 activity is less convincing.^{15,16} In this study we show no effect of SRL 172 on serum total IgE level or absolute blood eosinophil count. Studies designed to measure the activity of local cutaneous mediators of inflammation and tissue repair would be expected to correlate more strongly with disease activity than with the circulating factors measured here.¹⁷

Langerhans cells are the major antigen-presenting cells in the skin, which present protein allergens by means of classical MHC receptor pathways and thus initiate an atopic response.^{18,19} These antigen-presenting cells are relatively unusual in that they also express large amounts of the nonclassical MHC receptor CD1a on their cell surface. CD1a is known to present lipid antigens, especially those derived from mycobacteria.²⁰ Further work is required to determine whether stimulation of Langerhans cells by these lipid antigens may downregulate the atopic response to protein allergens in atopic dermatitis and thus provide a possible explanation for the clinical effects observed in this study.

Disease suppression with topical steroids is the current conventional treatment for atopic dermatitis. On the basis of our knowledge of the changes in the epidemiology of atopic disease over the past few decades and the results of this trial, therapy with mycobacterial antigens may provide a logical and simple adjuvant directly countering the consequence of the relatively aseptic, concrete, urban environments in which many of us now live.

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