

行政院國家科學委員會專題研究計畫 成果報告

比較異位性皮膚炎病人在使用局部類固醇、tacrolimus、
抗生素前後，疾病的臨床嚴重度與皮膚上金黃色葡萄球菌
的增生率及菌落密度
研究成果報告(精簡版)

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Staphylococcus colonization in atopic dermatitis treated with fluticasone or tacrolimus with or without antibiotics

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Background: The skin of patients with atopic dermatitis (AD) exhibits a striking susceptibility to colonization and infection by *Staphylococcus aureus*. Treatment with topical anti-inflammatory drugs alone can reduce *S aureus* colonization.

Objectives: To compare the clinical severity of AD and the *S aureus* colonization rate between AD patients treated with topical glucocorticoids and those treated with tacrolimus and to evaluate the effects of complementary topical antistaphylococcal antibiotic therapy and the development of fusidic acid-resistant *S aureus*.

Methods: Sixty AD patients were enrolled in a prospective, parallel, randomized study of an 8-week treatment with topical 0.05% fluticasone propionate or 0.03% tacrolimus, with or without complementary fusidic acid. Disease severity scoring of AD based on SCORing of Atopic Dermatitis (SCORAD), colonization rate and density of *S aureus* on the skin, and antibiotic susceptibility of *S aureus* isolates were evaluated.

Results: The reduction in SCORAD scores correlated with the reduction of *S aureus* numbers. Treatment with topical tacrolimus resulted in a comparable reduction in SCORAD scores to fluticasone but a slower eradication of *S aureus*. Complementary fusidic acid had no additional benefit compared with fluticasone or tacrolimus alone. Two patients developed fusidic acid-resistant *S aureus* after 8 weeks of fusidic acid treatment.

Conclusion: Tacrolimus is an appropriate alternative treatment for chronic AD. Topical anti-inflammatory therapy alone to improve the allergic skin inflammation of AD can reduce *S aureus* colonization of the skin. Topical antibiotics should be reserved for short-term use in obvious secondary bacterial infection.

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INTRODUCTION

Atopic dermatitis (AD) is a chronically relapsing pruritic inflammatory skin disorder accompanied by elevated total serum IgE levels and associated with other atopic diseases, such as asthma or allergic rhinitis.¹ The prevalence of AD is continuously increasing in developed countries. The skin of AD patients exhibits a striking susceptibility to colonization and infection by *Staphylococcus aureus*, which can secrete various exotoxins, including staphylococcal enterotoxin A and B (SEA and SEB).^{2–4} These exotoxins are superantigens. Staphylococcal superantigens (SsAgs) may penetrate the skin barrier and contribute to the persistence and exacerbation of allergic skin inflammation in AD.^{1,2,4,5} In addition, SsAgs may act as allergens and thus induce the production of functionally relevant SsAg-specific IgE antibodies in AD patients.^{3,4}

Although topical glucocorticoids are still considered the standard treatment for AD, adverse effects such as skin atrophy, striae, and telangiectasia are not negligible. Glucocorticoids are potent immunomodulatory hormones that adversely affect most cells of the immune system.¹ SsAgs were recently shown to induce glucocorticoid insensitivity of immune cells.⁶ Topical tacrolimus, a calcineurin inhibitor, is more specific in immunosuppressive action than glucocorticoids and may be highly effective in suppressing SsAg-induced inflammation.⁷ Therefore, tacrolimus provides an alternative treatment for patients with AD resistant to glucocorticoids. Previous studies demonstrated that treatment of AD exacerbations with topical anti-inflammatory drugs alone, whether glucocorticoids^{8–14} or tacrolimus,^{15,16} caused a significant reduction of *S aureus* skin colonization. However, a comparison of the effectiveness of glucocorticoids or tacrolimus in reducing *S aureus* colonization has not been reported. The aim of this study was to compare the clinical severity of AD and the *S aureus* colonization rate in AD patients treated with either topical glucocorticoids or tacrolimus.

Because *S aureus* plays an important role in the pathogenesis of AD, antistaphylococcal antibiotic therapy may be important for the disease control of AD.^{2,17} The topical antibiotic most commonly used for the reduction of *S aureus* on

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AD skin is fusidic acid, which is effective in the inhibition of methicillin-resistant *S aureus*.^{1,17} As a secondary objective of the study, we evaluated the effects of complementary topical fusidic acid therapy on the clinical severity of AD and the *S aureus* colonization rate and the development of fusidic acid-resistant *S aureus* after treatment.

PATIENTS AND METHODS

Study Populations

Sixty AD patients who visited the Department of Dermatology of the National Taiwan University Hospital as outpatients from February 2004 to February 2005 were recruited for the study. The local ethical committee approved the study, and written informed consent was obtained from each patient or a parent. Criteria for entry to the study were as follows: (1) AD diagnosed according to the criteria of Hanifin and Rajka¹⁸; (2) neither systemic nor topical antibiotics and neither systemic nor topical corticosteroids were used within 4 weeks before entry; (3) no clinical signs of overt secondary infection that obviously needed oral antibiotic therapy; and (4) the AD severity grading was moderate to severe at the time of entry into the study according to the criteria of Rajka and Langeland.¹⁹ Twenty-six of them were male and 34 were female. Their ages ranged from 9 months to 33 years, with a mean age of 15.6 years.

Treatment Protocol

The study design was parallel, randomized, and open labeled. Patients were randomly allocated to treatment with 1 of the 4 regimens: a topical application of 0.05% fluticasone propionate cream (Cutivate; Glaxo Operations Ltd, Durham, England) twice daily (in the morning and night) for 8 weeks, with or without complementary 2% fusidic acid cream (Fucidin; LEO Laboratories, Ltd, Dublin, Ireland); or a topical application of 0.03% tacrolimus ointment (0.03% Protopic; Astellas Pharma, Inc, New York, NY) twice daily (in the morning and night) for 8 weeks, with or without complementary 2% fusidic acid cream. Fluticasone propionate is a moderate-potency glucocorticoid, rated in class V in a 7-class arrangement of glucocorticoid potency. The AD patients were instructed to apply the treatment regimen to all affected areas, without occlusive dressings. In the patients with complementary fusidic acid cream use, fusidic acid was applied first to all affected areas and followed by application of fluticasone propionate or tacrolimus 20 minutes later. In addition, the use of medicated soaps or detergents was not allowed throughout the study period. The only other topical preparations allowed were the patients' usual moisturizers, which were instructed to be applied immediately after bathing throughout the study. Oral antihistamine (cetirizine; UCB Pharma, Pianezza, Italy) was given to all patients.

Clinical and Laboratory Evaluations

At the time of enrollment (day 0), both nostrils were examined for *S aureus* colonization, and the levels of total serum IgE and serum SEA- or SEB-specific IgE were measured by

the Pharmacia CAP assay (Pharmacia and Upjohn, Uppsala, Sweden) according to the manufacturer's instructions. Clinical and laboratory evaluations were performed before treatment (at day 0) and after 2 and 8 weeks of treatment. At these 3 time points, swabs (BBL, Becton, Dickinson and Company, Sparks, MD) for *S aureus* cultures were taken from the same designated skin lesion, which was the most severe local lesion at the time of enrollment (day 0). Overall clinical severity of AD was evaluated using the SCORing of Atopic Dermatitis (SCORAD) index (range, 0–103) by 2 separate clinicians (S.-H.H. and C.-Y.C.) at each visit.²⁰ Local clinical severity of AD was evaluated using the modified local SCORAD index with 6 intensity items: (1) erythema/darkening; (2) edema/papulation; (3) oozing/crusts; (4) excoriation; (5) lichenification/prurigo; and (6) local dryness. Each item was graded on a 4-point scale (0 = absent; 1 = mild; 2 = moderate; 3 = severe). Scores ranged from 0 to 18.

Bacteriological Protocol

All specimens for bacteriology were coded and processed blindly by the same bacteriologist. Cultures from both anterior nostrils were taken using one 360° clockwise rotation of a swab. The most severe local lesion was sampled using a modification of the scrub technique developed by Williamson and Kligman.²¹ Bacteria were collected using an electric rotating blade in 1 mL of tryptic soy broth (pH 7.3) wash solution. Samples of 0.1 mL of the wash solution were inoculated on trypticase soy agar with 5% sheep blood (TSA II, BBL) culture plates using a standard streak method. Plates were incubated at 35°C for 2 days, and a quantitative and qualitative analysis was then conducted. Coagulase-positive *S aureus* was identified by testing typical colonies for coagulase activity (BactiStaph Latex, Remel, Lenexa, KS). The colonization density was calculated by counting the number of colony-forming units per 1 cm² of the investigated skin surface. *S aureus* isolates with more than 10¹ CFU/cm² but less than 10⁶ CFU/cm² were considered colonized.² Antibiotic sensitivity testing of *S aureus* strains was performed on Mueller-Hinton agar (BBL), using the disk diffusion method, and was interpreted according to the Clinical Laboratory Standard Institute comparative method standard.

Statistical Analyses

The data are expressed as mean ± SEM. The data were analyzed by the principle of intention-to-treat analysis. The clinical scores were compared by nonparametric methods, including the Mann-Whitney *U* test between 2 treatment groups and the Wilcoxon signed-rank test between different time points in each treatment group. The numbers of patients who had *S aureus* skin colonization were compared by χ^2 test among different groups. Correlation between skin lesion severity and colonization density was established using the Spearman rank correlation. SPSS statistical software, version 12.0 for Windows (SPSS Inc, Chicago, IL), was used for statistical analyses. All tests were 2-tailed, and *P* < .05 was considered statistically significant.

RESULTS

Patient Characteristics Before Treatment

The initial overall clinical severity of AD by SCORAD was 55.3 ± 1.9 . The initial local clinical severity of AD by modified SCORAD was 10.8 ± 0.4 . Forty-nine (82%) of the 60 patients were colonized with *S aureus*. In 35 patients (58%), both lesional skin and anterior nostrils were colonized. In 8 patients (13%), *S aureus* colonized only the skin, and in 6 patients (10%), only the anterior nostrils. The density of *S aureus* isolated from the skin lesions varied between $10^{1.0}$ and $10^{4.7}$ CFU/cm². The mean density was $10^{3.4}$ CFU/cm². A significant but low correlation was found between the colonization density of *S aureus* on the skin lesions and the local clinical severity of AD at the time of enrollment in the study ($R = +0.320$, $P = .01$).

Before treatment, no significant difference was found in the rate or density of *S aureus* colonization and in the overall or local clinical severity of AD among the 4 treatment groups (Table 1). Serum specific IgE antibodies to SEA or SEB were detected in 33 patients (57%). Fifty-four of the 60 patients completed the study. Two patients receiving tacrolimus with complementary fusidic acid dropped out of the treatment protocol because of intolerance to a burning sensation. Two patients receiving tacrolimus only and another 2 receiving fluticasone only dropped out of the treatment protocol because of poor compliance.

Comparison Between Patients Treated With Fluticasone and Tacrolimus

Before and after treatment, no significant difference was found in body surface area involved by AD between patients treated with fluticasone and tacrolimus (Figure 1a). The patients treated with tacrolimus had higher initial subjective scores of pruritus and sleep loss than those treated with fluticasone, but no significant difference was found between the 2 treatment groups after treatment (Figure 1b).

The clinical severity scores (SCORAD) significantly decreased after 2 and 8 weeks of treatment in both the flutica-

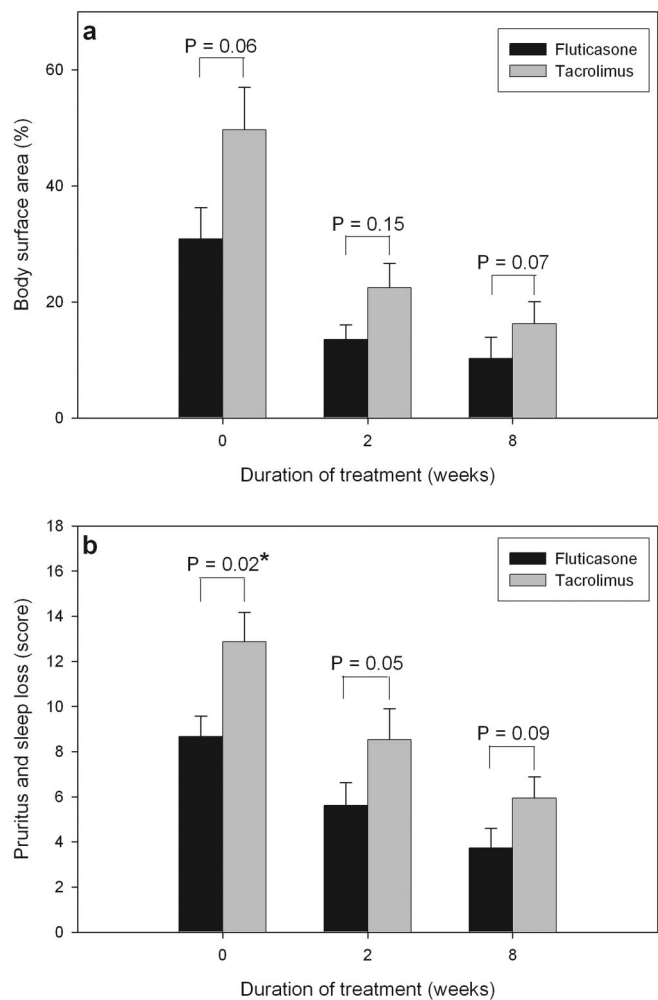


Figure 1. Comparison of patients treated with fluticasone and tacrolimus. a, Body surface area involved by atopic dermatitis before and after treatment. b, Subjective scores of pruritus and sleep loss before and after treatment. * $P < .05$.

Table 1. Characteristics of Patients Before Treatment

Characteristics	Fluticasone (n = 15)	Tacrolimus (n = 15)	Fluticasone and fusidic acid (n = 15)	Tacrolimus and fusidic acid (n = 15)
Age, mean \pm SEM, y	17.4 \pm 2.6	15.4 \pm 2.3	12.9 \pm 2.6	16.9 \pm 2.3
Sex, M:F	4:11	8:7	8:7	6:9
Overall AD severity (SCORAD), mean \pm SEM	54.7 \pm 4.3	56.7 \pm 3.7	50.0 \pm 3.2	59.9 \pm 4.2
Local lesions severity (modified SCORAD), mean \pm SEM	10.6 \pm 0.8	10.6 \pm 0.6	11.0 \pm 0.7	10.9 \pm 0.9
Colonization rate of <i>Staphylococcus aureus</i> , No. (%)	11 (73)	13 (87)	8 (53%)	13 (87)
Colonization density of <i>S aureus</i> , mean \pm SEM, log ₁₀ CFU/cm ²	2.5 \pm 2.3	3.7 \pm 3.5	3.4 \pm 3.1	3.1 \pm 2.7
Total IgE, mean \pm SEM, kU/L	4,552 \pm 1,765	3,299 \pm 1,109	4,283 \pm 1,440	3,316 \pm 936
SEA-specific IgE, No. (%)	6 (40)	8 (53)	6 (40)	7 (47)
SEB-specific IgE, No. (%)	8 (53)	9 (60)	8 (53)	7 (47)

Abbreviations: AD, atopic dermatitis; SCORAD, SCORing of Atopic Dermatitis; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B.

some group ($P = .001$) and the tacrolimus group ($P = .002$) (Figure 2a). Before and after treatment, there was no significant difference in clinical severity scores between patients treated with fluticasone and tacrolimus. Furthermore, there was no significant difference in the decrease of clinical severity scores between the 2 treatment groups.

During treatment, the reduction of *S aureus* colonization moderately paralleled the improvement of eczema ($r = .436$, $P < .001$). After 2 weeks of treatment, the patients treated with fluticasone had a lower colonization rate of *S aureus* than those treated with tacrolimus (Figure 2b). However, there was no significant difference in the colonization rate of *S aureus* between the 2 treatment groups after 8 weeks of treatment.

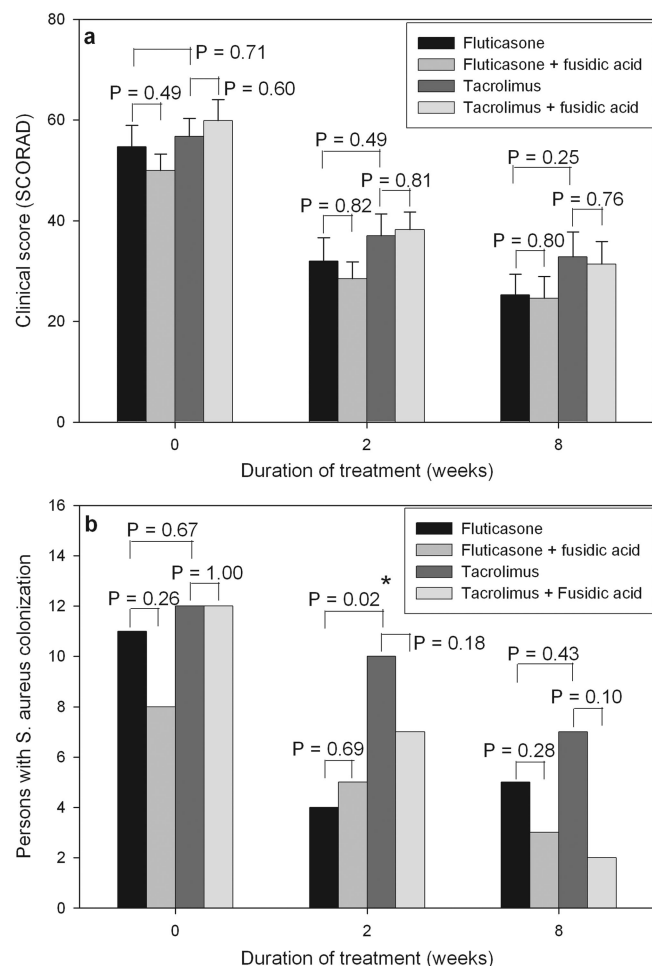


Figure 2. Comparison of patients treated with fluticasone and tacrolimus with and without complementary fusidic acid. a, Clinical severity scores (SCORing of Atopic Dermatitis) before and after treatment. b, Numbers of patients with *Staphylococcus aureus* colonization before and after treatment. * $P < .05$.

Comparison Between Patients Treated With and Without Complementary Fusidic Acid

The clinical severity scores significantly decreased after 2 and 8 weeks of treatment in the 4 treatment groups (all $P < .05$) (Figure 2a). In both the fluticasone group and the tacrolimus group, before and after treatment, no significant difference was found in clinical severity scores between patients treated with or without complementary fusidic acid (Figure 2a). Furthermore, no significant difference was found in the decrease of clinical severity scores between patients treated with or without complementary fusidic acid.

The colonization rate of *S aureus* was decreased after 2 and 8 weeks of treatment in the 4 treatment groups (Figure 2b). In the tacrolimus group, the patients treated with complementary fusidic acid had a lower colonization rate of *S aureus* than those treated without complementary fusidic acid, although the difference was not statistically significant (Figure 2b). Furthermore, no significant difference was found in the decrease in the colonization rate of *S aureus* between patients treated with or without complementary fusidic acid.

S aureus was eliminated in 82% of patients treated with complementary fusidic acid compared with 54% of patients treated without complementary fusidic acid. The colonization density of *S aureus* decreased from $10^{3.3} \pm 10^{3.6}$ to $10^{2.5} \pm 10^{3.3}$ CFU/cm² in the patients treated with complementary fusidic acid and from $10^{3.5} \pm 10^{4.0}$ to $10^{2.9} \pm 10^{3.5}$ CFU/cm² in those treated without complementary fusidic acid.

Antibiotic-Resistant Strains of *S aureus*

Ninety-eight isolates of *S aureus* were cultured from lesional skin during the study period. Among the 61 isolates cultured from patients treated without complementary fusidic acid, 43 (70%) were methicillin sensitive, 16 (26%) were methicillin resistant, and 2 (3%) were fusidic acid resistant. In the patients treated with fluticasone or tacrolimus alone, the antibiotic sensitivity pattern was not changed. However, in the patients treated with complementary fusidic acid, the percentage of both methicillin-sensitive and methicillin-resistant strains decreased significantly ($P = .04$). Of the 5 patients with persistent *S aureus* colonization, 2 (40%) developed fusidic acid-resistant strains after 8 weeks of fusidic acid treatment.

DISCUSSION

It is well established that skin colonization with *S aureus* may contribute to the persistence and exacerbation of AD.^{1,2,4,5,17} *S aureus* is not considered a member of the resident skin microflora in healthy populations whose carry rate is less than 10%.² Eighty-three percent of our AD patients were colonized with *S aureus*. The density of *S aureus* isolated from the skin lesions in our AD patients varied between $10^{1.0}$ and $10^{4.7}$ CFU/cm², which is compatible with the findings in previous studies.^{12,22} During treatment, the reduction of *S aureus* colonization paralleled the improvement in eczema, which verified earlier investigations that had shown a signif-

icant correlation between colonization density and clinical severity of AD.^{11–16,22,23}

Reitano et al²⁴ found that the efficacy of 0.03% tacrolimus ointment was higher than that of 1% hydrocortisone acetate (low-potency glucocorticoid) but lower than that of 0.1% hydrocortisone butyrate ointment (medium- to high-potency glucocorticoid). In our study, a comparable clinical improvement of AD was noted between topical 0.03% tacrolimus and 0.05% fluticasone propionate treatment, but a more rapid reduction of *S aureus* was found in patients treated with fluticasone. The slower clearance of *S aureus* in the tacrolimus group was possibly attributed to the humid and occlusive environment of the ointment, which favored *S aureus* colonization. However, ointments as vehicles enhance the absorption of the active ingredient of tacrolimus. With relatively large molecular size and high lipophilicity, the absorption of tacrolimus decreases as treatment continues and clinical improvement occurs. Therefore, tacrolimus may be more suitable than glucocorticoids for AD patients in intermittent or continuous long-term maintenance therapy.

These findings, combined with the fact that neither fluticasone nor tacrolimus has a direct antistaphylococcal activity, are consistent with the concept of the inflammatory skin condition in AD being itself a major predisposing factor for colonization with *S aureus*. Indeed, *S aureus* colonization is both a cause and a consequence of allergic skin inflammation. Mechanisms by which allergic skin inflammation of AD promotes the increase in *S aureus* colonization include skin barrier dysfunction, increased synthesis of extracellular matrix adhesins for *S aureus*, and defective innate immune responses due to decreased production of endogenous antimicrobial peptides.^{1,4,14,25}

It has been shown that systemic or topical antibiotic treatment without glucocorticoids can temporarily decrease the number of *S aureus* in skin lesions and lead to some improvement.^{9,21,26–28} However, these effects are not sustained after 4 to 8 weeks.^{26–30} In AD patients with obvious secondary skin infection or *S aureus* colonization densities above 10⁶ CFU per cm², treatment with a combination of antistaphylococcal antibiotic and topical glucocorticoid produces superior clinical effects to treatment with topical glucocorticoids alone.^{9,31} In clinically noninfected areas of AD, although one can still isolate *S aureus*, little or no evidence exists for any additional benefit from antistaphylococcal therapy when the usual dermatitis treatment is used.¹⁷

The most contentious clinical situation is where there is no overt sign of infection but the eczema is moderate to severe. Wachs and Maibach⁸ reported that the combination of topical antibiotic and glucocorticoid gave a better clinical response than glucocorticoid alone. However, others have documented no additional benefit of systemic or topical antibiotics over glucocorticoid alone in patients with moderate to severe AD.^{10,12} In our study, no significant difference was found in clinical improvement and *S aureus* clearance between AD patients treated with or without complementary topical antibiotics. A possible reason for persistent *S aureus* colonization

may be seeding from other reservoirs, such as the nasal cavity, unaffected skin, or family members.³⁰ Another possible reason is the appearance of fusidic acid-resistant strains. In our study, fusidic acid-resistant strains of *S aureus* appeared in 2 of 5 patients with persistent *S aureus* colonization after 8 weeks of fusidic acid treatment. Antibiotic resistance of *S aureus* is a serious and growing problem in dermatological practice, and fusidic acid resistance may threaten the efficacy of systemic fusidic acid for the treatment of serious *S aureus* infections.^{32,33} Therefore, fusidic acid-containing preparations should be reserved for short-term treatment of obvious secondary skin infections only.

SsAgs contribute to the persistence and exacerbation of allergic skin inflammation in AD.^{1–6,17,28,29} However, no correlation was found between levels or positive rates of serum SsAg-specific IgE antibodies and the presence of previous staphylococcal skin infection in AD patients.³ This suggests that *S aureus* induces the production of SsAg-specific IgE antibodies, not by infection but by skin penetration of exotoxins.³ Colonization of *S aureus* on the skin is a constant feature of AD, and therapeutic strategies aimed at the eradication of *S aureus* may not always be appropriate.

In conclusion, this study showed that topical 0.03% tacrolimus treatment had clinical efficacy comparable to topical 0.05% fluticasone in AD. Although initial, more rapid clearance of *S aureus* appeared in the patients treated with fluticasone, no difference was found between the 2 treatment groups after long-term use. These results suggest that tacrolimus is an appropriate alternative treatment for chronic AD. Complementary topical antibiotic treatment did not provide additional benefit compared with topical fluticasone or tacrolimus treatment alone, suggesting that topical anti-inflammatory therapy alone can improve allergic skin inflammation and reduce *S aureus* colonization in AD and that topical antibiotics should be reserved for short-term use in obvious secondary bacterial infection.

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