

Effects of Photocatalytic Agent on DS-*Nh* Mice, Developing Atopic Dermatitis-Like Eruption with an Increase of *Staphylococcus aureus*

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Key Words

Atopic dermatitis · DS-*Nh* mouse · Titanium dioxide ·
Staphylococcus aureus · Skin barrier dysfunction

Abstract

Background: *Staphylococcus aureus* (*S. aureus*) is thought to play a significant role in the exacerbation of atopic dermatitis (AD). DS-*Nh* mice, a non-hair-bearing mouse model of AD, spontaneously develop dermatitis under conventional conditions. A remarkable increase in *S. aureus* is considered to strongly relate to the induction and aggravation of this dermatitis. A topical use of anatase-type titanium dioxide (TiO₂) followed by UV irradiation, acting as photocatalyst, is believed to have antibacterial activity. We investigated the bactericidal effect of TiO₂ with UV irradiation on DS-*Nh* mice to prevent the aggravation of the dermatitis. **Methods:** Ten-week-old DS-*Nh* mice were treated with TiO₂ in petrolatum on the back, followed by UVA irradiation, for 11 weeks. The severity of dermatitis was assessed by evaluating individual lesions using a 4-grade scale and expressed as the total skin score. *S. aureus* colonizing the mouse skin was counted after isolation and incubation with agar medium. The skin barrier dysfunction was evaluated by measuring transepidermal water loss (TEWL). **Results:** The mice treated with TiO₂ and UV irradiation showed a significant increase in total skin scores, the number of *S. aureus* and TEWL values, compared

with non-treated mice. In contrast, these parameters were significantly lower in the mice treated with petrolatum and UV irradiation. **Conclusions:** A significant increase in *S. aureus* was recognized on the skin together with the aggravation of AD-like dermatitis in our mice model. Skin barrier dysfunction induced by TiO₂ and UV irradiation seems to facilitate the increase in *S. aureus*.

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Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease characterized by recurrent relapsing dermatosis with typical morphology and distribution [1]. Various factors contribute to the pathogenesis and development of AD, including immunological abnormalities, physiological and biochemical dysfunction of the skin barrier structures, environmental allergens such as mites and fungi, and psychosocial factors [2–6]. However, the pathogenesis of AD has not been elucidated as yet. It has been shown that >90% of patients with AD have *Staphylococcus aureus* (*S. aureus*) on their skin [7]. Recently, many studies have suggested that this bacterium may play a significant role in the exacerbation of AD through superantigenic exotoxins, such as staphylococcal enterotoxins A or B and toxic shock syndrome toxin-1. The spe-

cific IgE to these enterotoxins seems to be involved in the exacerbation as well [8–11].

The DS-*Nh* mice originated from a mutant male mouse with deficient hair growth in an inbred DS strain colony at the Aburahi Laboratories (Shionogi & Co., Ltd., Shiga, Japan) in 1976. These mice develop dermatitis spontaneously after 15 weeks of age under conventional conditions, but not under specific pathogen-free conditions, followed by an elevation of serum IgE [12, 13]. In these mice a remarkable increase in *S. aureus* is observed on the skin surface under conventional conditions, even before the development of the skin lesions. In a previous report, *S. aureus* applied on the skin accelerated the onset of skin eruption [13]. It has been suggested that staphylococcal enterotoxin C, acting as superantigen, produced by *S. aureus* colonizing on DS-*Nh* mice, could be responsible for the aggravation of dermatitis in these mice [14]. Therefore the colonization of *S. aureus* on the skin surface is thought to strongly relate to induction and aggravation of the dermatitis on DS-*Nh* mice. These findings indicate that the mice are useful as a model for AD with colonization of *S. aureus*, although there are many other implicated environmental factors in the exacerbation of human AD.

Anatase-type titanium dioxide (TiO₂) is known to have a photobiological activity. When exposed to UVA radiation, TiO₂ exhibits antibacterial activity by producing oxygen radicals [15–17].

In the present study we intended to investigate whether the topical use of TiO₂ followed by UV irradiation could decrease the number of *S. aureus* on the skin of mice and prevent the development of skin lesions.

Material and Methods

Animals

Five-week-old female DS-*Nh* mice raised under specific pathogen-free conditions were provided by the Aburahi Laboratories, Shionogi & Co. The DS-*Nh* mice used for this study were obtained as F1(Nh/+) from male DS-*Nh*(Nh/Nh) × female DS(+/-). They were acclimatized under conventional conditions before their use in the experiments at the Animal Experiment Center of the Yokohama City University Graduate School of Medicine.

These mice were housed in isolator cages, exposed to a 12-hour light/12-hour dark cycle, and provided standard feed and water ad libitum. This study was conducted according to the guidelines for animal experiments established by the Yokohama City University Graduate School of Medicine.

Treatment with TiO₂ and UVA

Ten-week-old mice were allocated to the following groups: the TiO₂+UVA group treated epicutaneously with 0.1% TiO₂ (ana-

tase-type; ST-01, Ishihara Sangyo Co., Osaka, Japan) in petrolatum (Pet) on the back, followed by UVA irradiation (365 nm, 10 J/cm²) with a UV lamp (Glo-Values Co., Tokyo, Japan); the Pet+UVA group treated with petrolatum on the back, followed by UVA irradiation; the Pet group treated with petrolatum on the back, and the no-treatment group. Irradiation was performed 30 min after epicutaneous treatment with TiO₂ or petrolatum. The mice were treated once a week for 11 weeks.

Evaluation of the Severity of Dermatitis

The skin lesions, including erythema, edema, excoriation/erosion, and dry skin/scale were examined macroscopically. Each lesion was individually evaluated using a grading system; grade 0: no visible skin change, grade 1: slight skin change, grade 2: moderate skin change, grade 3: severe skin change. The total skin scores were used to evaluate severity [12, 18].

Isolation of *S. aureus* from the Skin Surface

S. aureus was isolated from the dorsal skin of the mice. DD checker Seiken, MSEY agar (Denka Seiken, Tokyo, Japan), which is a medium for the isolation and identification of *S. aureus*, was used to evaluate the colonization of the skin by *S. aureus*. The plates were applied for 10 s on the skin for sampling. Next the medium plates were incubated for 24 h at 37°C. After incubation the number of colonies was counted up to 500 CFU/10 cm². The bacteria were identified according to the manufacturer's instructions.

Measurement of Transepidermal Water Loss

Transepidermal water loss (TEWL) was measured using Dermalab (Cortex Technology, Denmark) on dorsal skin without lesions. The room conditions during the experiments were kept constant (24°C and 45 ± 5% relative humidity).

Measurement of Serum Levels of IgE

Blood samples were collected from the retro-orbital plexus at 10 and 17 weeks of age, and the sera were stored at -80°C until measurement of IgE. The serum total IgE levels were measured using a sandwich ELISA kit (Yamasa Shouyu Co. Ltd., Chiba, Japan). ELISA was conducted according to the manufacturer's instructions.

Statistics

Statistical significance was determined by the Mann-Whitney *U* test for unpaired 2-group comparisons using the StatView System (version 4.5, Microsoft 2000), if not otherwise mentioned. The clinical skin scores are expressed as the mean ± standard error (SE). Other values are expressed as the mean ± standard deviation (SD). The level of significance was set at *p* < 0.05.

Results

Development of Skin Lesions

The mice treated with TiO₂ in petrolatum alone showed no significant difference in the development of skin lesions and the serum level of IgE compared with the mice treated with petrolatum only (data not shown).

The TiO₂+UVA group showed significantly severer skin eruptions, compared with the Pet+UVA, Pet or no-treatment groups at 17 weeks of age ($p < 0.05$). Figure 1 shows the most representative data of 3 independent experiments. Erythema, erosion and crusts produced by scratching were observed from 14 weeks of age not only on the dorsum but also on the face and neck in the same mice (fig. 2). Similar lesions were observed in the Pet+UVA and no-treatment groups at 17 weeks of age. In the Pet group the total skin score was significantly lower than that in the other groups at 17 weeks of age ($p < 0.05$).

The total skin score increased at 21 weeks of age in the Pet and no-treatment groups as compared to that at 17 weeks of age ($p < 0.05$), although the score in the Pet+UVA or Pet groups was still lower than that in the TiO₂+UVA group ($p < 0.05$; fig. 1).

Histopathologically the skin of 17-week-old mice in the TiO₂+UVA group showed hyperkeratosis, acanthosis and intercellular edema in the epidermis and inflammatory cell infiltration in the dermis (fig. 3).

Increase in S. aureus on the Skin Surface

A significant increase in *S. aureus* was observed on the skin of the mice in the TiO₂+UVA group, but not in the other groups at 13 weeks of age. Figure 4 shows the most representative data of 3 independent experiments. *S. aureus* increased after 15 weeks of age in the no-treatment group and after 19 weeks of age in the Pet group.

A remarkable increase in *S. aureus* on the skin surface was observed before the appearance of visible skin lesions in all groups.

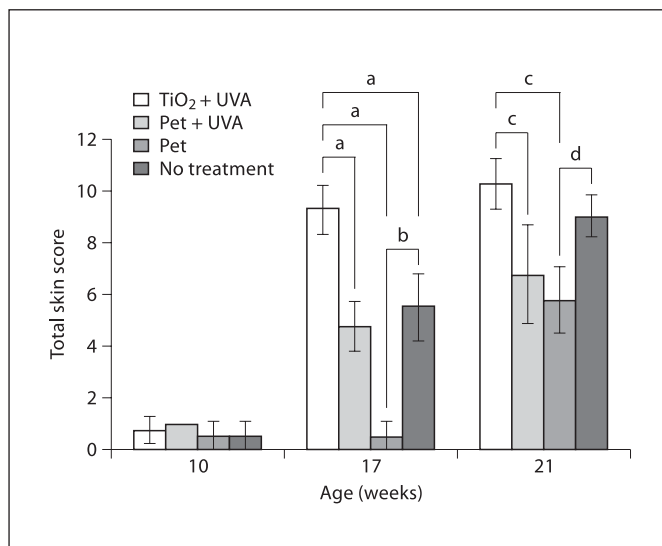


Fig. 1. Effect of the treatment with TiO₂ followed by UV irradiation and with petrolatum alone on total skin scores in DS-*Nh* mice. Ten-week-old mice were treated for 11 weeks as follows: the TiO₂+UVA group was treated epicutaneously with 0.1% TiO₂ in petrolatum on the back, followed by UVA irradiation; the Pet+UVA group was treated with petrolatum on the back, followed by UVA irradiation; the Pet group was treated with petrolatum alone on the back, and the no-treatment group was just monitored. Treatment with TiO₂ and UV induced a significant increase in total skin score, compared with the other groups (^a $p < 0.05$), but treatment with petrolatum alone showed a significantly lower score than that in the no-treatment group (^b $p < 0.05$) at 17 weeks of age. Treatment with Pet+UV or with petrolatum alone showed a still lower total skin score than that in the TiO₂+UVA group (^c $p < 0.05$). Treatment with petrolatum alone showed a significantly lower total skin score than that in the no-treatment group (^d $p < 0.05$) at 21 weeks of age. Each group consists of 4 mice.

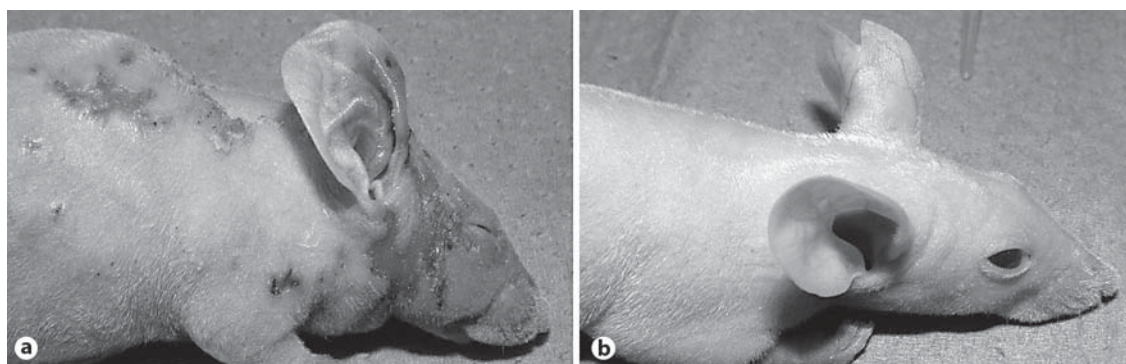


Fig. 2. Clinical features of DS-*Nh* mice after treatment with TiO₂ followed by UVA irradiation (a) and with petrolatum alone (b). Erythema, erosion, crusts and excoriations were observed on the dorsum and neck in the mice treated with TiO₂ and UV but not in those treated with petrolatum alone at 17 weeks of age.

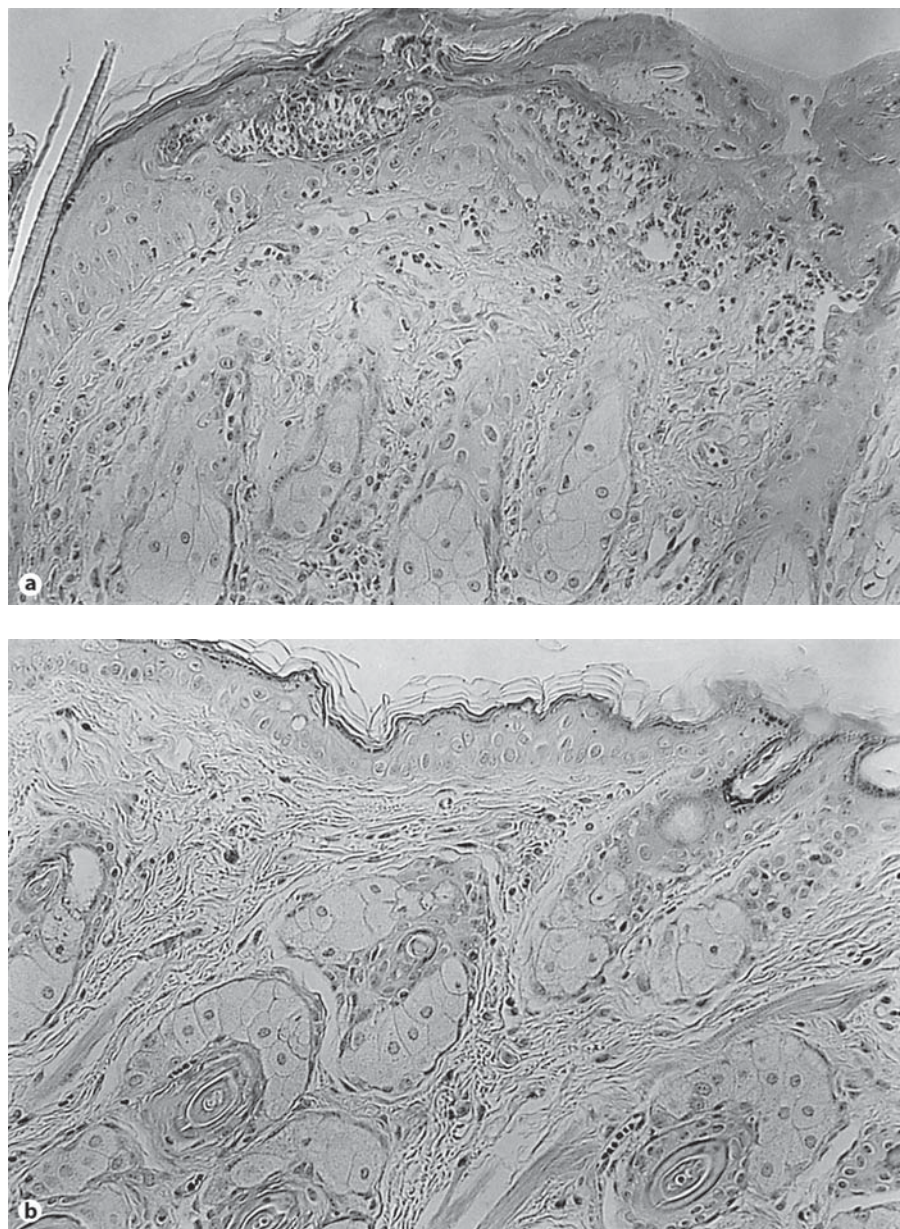


Fig. 3. Histopathological findings in skin from DS-*Nh* mice stained with hematoxylin and eosin, after treatment with TiO₂ in petrolatum followed by UV irradiation (**a**) and with petrolatum alone (**b**). Hyperkeratosis, acanthosis and intercellular edema in the epidermis and inflammatory cell infiltrations in the dermis were observed in the dorsal skin of mice treated with TiO₂ in petrolatum and UVA irradiation, but not in those treated with petrolatum alone at 17 weeks of age.

The density of *S. aureus* on the skin of the mice in the Pet+UVA group was significantly lower at 17 weeks compared with that at the beginning of treatment (table 1).

Elevation of Serum Levels of Total IgE

The levels of serum IgE in all DS-*Nh* mice were <200 ng/ml at 10 weeks of age. In the TiO₂+UVA and no-treatment groups, the levels of serum IgE ($1,096 \pm 132$ and $1,769 \pm 510$ ng/ml, respectively) were significantly increased at 20 weeks of age ($p < 0.05$). However, the in-

crease in serum IgE level was not statistically significant in the Pet group (525 ± 251 ng/ml), which developed fewer skin lesions than the other groups.

Relation between Development of Skin Lesion, TEWL Values and Increase in S. aureus on the Skin Surface Induced by Application of TiO₂ Followed by UV Irradiation

The TiO₂+UVA group showed an increase in TEWL values even on the skin without lesions and developed

Table 1. TEWL, the density of *S. aureus* on the skin surface and the total skin scores of DS-*Nh* mice in the TiO₂+UVA group

	Weeks of age	TEWL (g/h · m ²)	<i>S. aureus</i> (CFU/cm ²)	Total skin score
TiO ₂ +UVA	10	4.73 ± 0.91	259 ± 73.5	0.75 ± 0.50
	17	7.03 ± 3.96	>1,000	5.25 ± 2.06
Pet+UVA	10	3.95 ± 1.30	141 ± 44.8	1 ± 0
	17	3.8 ± 1.41	50.8 ± 47.9	3.5 ± 1.0

A significant increase of TEWL values, the density of *S. aureus* and the total skin scores was observed in TiO₂+UV-treated mice at 17 weeks of age ($p < 0.05$ vs. at 10 weeks of age), but not in the Pet+UV-treated mice at the same age. Each value represents the mean ± SE of 5 mice.

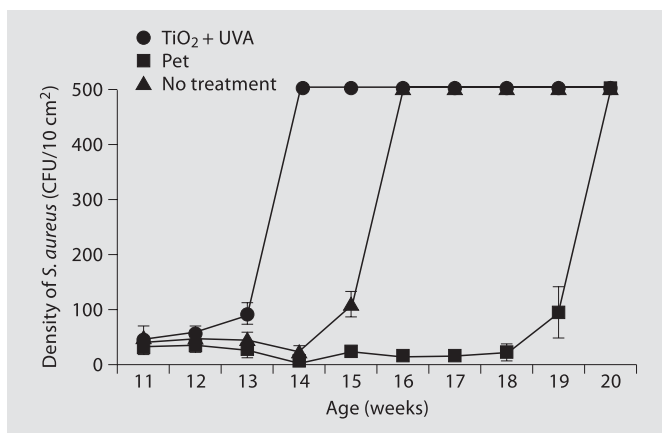


Fig. 4. Effect of TiO₂+UV on the density of *S. aureus* on the skin surface of DS-*Nh* mice. Medium plates were applied for 10 s on the skin for sampling and then incubated for 24 h at 37°C. After incubation the number of colonies was counted up to 500 CFU/10 cm². TiO₂ followed by UV irradiation induced an increase in *S. aureus* on the skin surface. A significant increase in *S. aureus* was observed on the skin of the mice in the TiO₂+UVA group, but not in the other groups at 13 weeks of age ($p < 0.05$). The Pet group showed a significantly lower number of *S. aureus* colonies than the other groups at 15 weeks of age ($p < 0.05$). Each value represents the mean ± SE for each group consisting of 5 mice.

severe skin eruptions with an increase in *S. aureus* on the skin surface at 17 weeks of age ($p < 0.05$ vs. at 10 weeks of age). In contrast the Pet+UVA group developed moderate eruption and did not show an increase in TEWL and *S. aureus* at the same age (table 1, fig. 4). This result demonstrated that the topical use of TiO₂ with UV irradiation damaged the stratum corneum and caused skin barrier dysfunction accompanied by an increase in *S. aureus* on the skin surface and the development of skin eruptions.

Discussion

Many studies have suggested that *S. aureus* contributes to the exacerbation of AD [8–11]. DS-*Nh* mice develop an eczematous skin lesion with an elevation of serum IgE levels under conventional conditions. These clinical features in DS-*Nh* mice are in accordance with those observed in human AD patients [12, 13, 18]. The colonizing *S. aureus* on the surface of these mice is thought to play a significant role in the pathogenesis of this skin lesion [12, 13]. Marked activation of T cells, which have receptor repertoires restricted to staphylococcal enterotoxin C produced by *S. aureus* colonizing on DS-*Nh* mice, is supposed to be strongly related to the exacerbation of dermatitis [14].

In the beginning we hypothesized that the induction/aggravation of AD-like eruption could be prevented by reducing *S. aureus* colonizing the skin surface with TiO₂, a photocatalytic agent. It has been clarified that when exposed to UVA radiation, TiO₂ produces hydroxyl radicals and photocatalyzes free radicals formation [15–17]. Oxidative damage in bacterial cellular RNA by means of reactive oxygen species generation in the cytoplasm has been shown to change the permeability of the cellular membrane followed by loss of potassium and calcium ions and cytoplasmic proteins, to finally result in bacterial cell death [17]. Then, in this study, DS-*Nh* mice were treated with TiO₂ epicutaneously to kill the *S. aureus* on the skin surface and prevent the development of AD-like lesions.

Contrary to our hypothesis, TiO₂ followed by UV radiation resulted in an increase in *S. aureus* on the skin surface of the mice and seemed to cause severer dermatitis compared with that of non-treated mice. TiO₂ has been reported to have photobiological activity both in

prokaryotic and eukaryotic cells [19, 20]. In this study the increase in TEWL of the TiO₂+UV group was accompanied by the development of skin eruptions. This suggested that this photocatalytic agent could induce damage of the mouse skin and cause dysfunction of the stratum corneum leading to skin eruptions.

Recent studies revealed that microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), namely fibronectin- or fibrinogen-binding protein, play a significant role in *S. aureus* attachment and binding to the skin surface in AD patients. MSCRAMMs are thought to recognize human extracellular matrix proteins such as fibronectin and fibrinogen in the skin of AD patients [21, 22]. The role of fibronectin and fibrinogen in the binding of *S. aureus* was also demonstrated in a murine model [23]. These adhesive molecules can accumulate in the stratum corneum and facilitate the binding and colonization of *S. aureus* [21]. In our study these extracellular matrix proteins might have penetrated the stratum corneum following the alteration of the epidermal skin barrier induced by TiO₂. Besides, inflammation mediated by type 2 helper T (Th2) cells was recently suggested to contribute to the increase in fibronectin in the stratum corneum and to enhance *S. aureus* binding [23]. Moreover the cytokine profile of inflammatory cells in the skin lesions of DS-*Nh* mice has been shown to shift to a Th2 cell inflammatory environment [13]. Taken together the increase in *S. aureus* on the skin

in our study might be due to an increase in extracellular matrix proteins in the stratum corneum and to the development of Th2-cell-mediated inflammation.

In our study the skin score in the Pet group was significantly lower than that in the TiO₂+UV and non-treated groups at 17 weeks. TEWL and the density of *S. aureus* on the skin were also lower in the Pet group compared with the TiO₂+UV group at the same time point. Treatment with petrolatum seemed to reduce the exacerbation of dermatitis by improving the skin barrier function. Current studies demonstrated that skin barrier dysfunction could be responsible for the pathogenesis of AD in a murine model and AD patients [24, 25]. Several studies have shown that treatment with a moisture cream could ameliorate the impairment of the skin barrier function in AD patients [26, 27]. Of interest, the density of *S. aureus* on the skin of the mice treated with Pet+UVA was lower at 17 weeks compared with that at the beginning of treatment. Then the amelioration of skin barrier dysfunction by topical use of petrolatum is expected to reduce the density of *S. aureus* on the skin of AD patients even without disinfectants and antistaphylococcal antibiotics.

In conclusion, our results suggested a significant role for *S. aureus* in the exacerbation of AD and the importance of skin care to improve the skin barrier function in the prevention of *S. aureus* colonization of the skin in AD patients.

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