

Effect of oral cyclosporin A in children with *Staphylococcus aureus*-colonized vs. *S. aureus*-infected severe atopic dermatitis

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Atopic dermatitis (AD) is frequently associated with skin colonization or infection with *Staphylococcus aureus* strains producing exotoxins. The aim of this investigation was to evaluate the effect of oral cyclosporin A (CsA) on disease severity and bacterial counts in colonized and infected patients. Eleven children with severe AD (SCORAD index >50, mean objective SCORAD score >40) were treated for 8 weeks with 2.5–5 mg/kg CsA. In five patients, the skin was only colonized with *S. aureus* whereas the remaining six patients presented clinically relevant suppurative *S. aureus* skin infections characterized by small pustules, crustings, pus and increased pruritus in the presence of *S. aureus* as determined by contact sampling and culture which regularly resulted in the indication for antibiotic treatment. Clinical and microbiological investigations were performed before and after CsA therapy. Clinical signs and symptoms of AD improved in all patients with a reduction in mean SCORAD index from 74 to 29 ($p < 0.001$). However, disease severity and bacterial counts were more reduced by CsA in the colonized patients compared with the patients with clinical overt infections. In conclusion, treatment with CsA resulted in an improvement of clinical symptoms in children suffering from severe AD. However, anti-infective treatment administered before immunomodulatory therapy is likely to be decisive for the long-term therapeutic effect.

**Rita Bunikowski¹, Martin Mielke²,
Matthias Bräutigam³, Harald Renz⁴
and Ulrich Wahn¹**

¹Department of Pediatric Pneumology and Immunology, Humboldt University of Berlin, ²Robert Koch-Institut, Berlin, ³Novartis Pharma GmbH, Nürnberg, ⁴Department of Clinical Chemistry and Molecular Diagnostics, Klinikum der Philipps-Universität, Marburg, Germany

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Rita Bunikowski, MD, Charité Campus Virchow-Klinikum, Humboldt University Berlin, Department of Pediatric Pneumology and Immunology, Augustenburger Platz 1, 13353 Berlin, Germany
Tel.: +49 30 450 566182
Fax: +49 30 450 566931
E-mail: Rita.Bunikowski@charite.de

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Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease in which T cells play a prominent modulating role (1). Eczematous skin in most patients with AD is colonized with *Staphylococcus aureus* in high densities and skin infection and invasive *S. aureus* infection is common in severe AD (2, 3). *S. aureus* strains isolated from skin of patients with AD have been shown to secrete a variety of exotoxins (SAGs), including staphylococcal exotoxins A, B, C and toxic shock syndrome toxin-1 (TSST-1) with superantigen activity. Such superantigens consist of a group of high-molecular-weight proteins defined by a unique pattern of T-cell stimulation. In contrast to conventional antigens, superantigens do not require classic presentation by

antigen-presenting cells. Instead they can directly interact with α/β T-cell receptors (TCRs). The site of engagement, resulting in the expansion and activation of certain T-cell subsets, has been mapped to the TCR V β element for some superantigens. Superantigens can induce massive T-cell stimulation and consequent release of T-cell-derived lymphokines such interleukin-2 (IL-2) or tumor necrosis factor (TNF) and these proinflammatory cytokines may have a critical role in skin inflammation (1, 4). It has recently been demonstrated that exotoxins released by *S. aureus* can modulate disease severity and dermal T-cell infiltration (5).

Antibacterial agents are widely used to treat *S. aureus*-infected atopic skin (3, 6) and can

reduce the number (2, 3, 6). The immunomodulating effect of topical steroids (7) and tacrolimus ointment is associated with an improvement in eczema symptoms and in reduction of *S. aureus* colonization at atopic skin (8).

Skin infections with *S. aureus* are not an absolute contraindication with respect to cyclosporin (CsA) therapy. However, they should be brought under control with appropriate antibacterial agents before initiating therapy. Marked improvement of AD has been reported after treatment with CsA (9). In this respect, we recently demonstrated the immunomodulatory effects of oral CsA, which significantly improved the clinical symptoms of severe pediatric AD. We measured the *S. aureus* colonization before and after CsA treatment as a safety parameter (10) and got the impression, that the therapeutic efficacy of CsA might be different in children with *S. aureus* colonization vs. overt infection. The immunomodulating effects of CsA on *S. aureus* colonization in severe AD had not been further evaluated, so we decided to examine this phenomenon in more detail.

Patients and methods

We investigated 11 Caucasian children (five female, six male; 22–197 months of age) suffering from severe AD (SCORAD index >50; mean objective SCCORAD score >40 on two baseline measurements with a minimum interval of 2 weeks) that was refractory to topical steroid therapy. All 11 patients were heavily colonized with *S. aureus*, while six of them required antimicrobial therapy for suppurative superficial *S. aureus* skin infection. All patients were treated for 8 weeks with CsA in an open-label trial. An initial daily dose of 2.5 mg/kg was given to all patients, rising to a maximum dose of 5 mg/kg per day, if CsA treatment was insufficiently effective (SCORAD reduction <35%). The treatment duration was 8 weeks, after which all responders (SCORAD reduction >35%) discontinued CsA and were followed-up for 4 weeks. The dose of CsA did not differ significantly between the *S. aureus*-colonized group (mean CsA 3.4 mg/kg; SD 1 mg/kg) and the *S. aureus*-infected group (mean CsA 3.4 mg/kg; SD 1.2 mg/kg).

Topical steroids (betamethasone 0.01% to 0.05%) were applied twice daily independently of the *S. aureus* status. No differences in the potency of steroids were observed between the *S. aureus*-colonized and the *S. aureus*-infected group. Concurrent topical steroid therapy was unchanged over this period in all patients.

After discontinuation of CsA, all patients entered a 4-week follow-up period. All patients were re-examined 4 weeks after discontinuation of CsA.

Clinical assessment

The disease severity was determined by the SCORAD index (11, 12). The SCORAD index (0–103) is based on individual items: extent (0–100%) and intensity (0–18) 7/2, and subjective items (0–20); intensity including erythema, edema/papulation, oozing/crusts, excoriations, lichenification, and dryness, each on a scale of 0–3; subjective symptoms graded by the parents/patients regarding daytime pruritus (itching) and sleep-loss, each on an analog scale of 0–10. For the objective SCORAD grading scale, objective items only were used, i.e. extent (20-point scale) and intensity (63-point scale); the total objective SCORAD score has 0 to 83-point scale.

Isolation of *S. aureus* from skin and nose

For the isolation of *S. aureus*, contact samples from both unaffected and eczematous skin were obtained before initiating CsA therapy and at the end of therapy. Neck, wrist, elbow and erosive-eczematous lesions were systematically investigated using Rodac-blood-agar-plates (diameter: 5.6 cm), which were subsequently incubated for 48 h at 32°C. Growth of *S. aureus* was identified by testing typical colonies for coagulase activity. Colonization was quantified by counting the number of colony-forming units (c.f.u.) per agar plate and subsequent calculation of colonies per cm² skin. Nasal carriers were identified by incubating standardized samples obtained from both nares by sterile cotton tips on blood and mannitol salt agar for 48 h at 32°C (5).

Detection of *S. aureus*-derived exotoxins

To identify the production of exotoxins (superantigens, SAgS): SEA, SEB; SEC, SED and TSST-1 a defined inoculum of *S. aureus* (10 colonies of a 24-h subculture on blood agar) was incubated in RPMI 1640 protein-free cell culture medium for 48 h at 37°C. The filter-sterilized supernatants (0.45 µm) were tested for the presence of exotoxins using the TSST- and SET-RPLAR[®] latex agglutination test (Unipath, Hampshire, UK[®]) according to the manufacturer's instructions. A sample of the same culture filtrate diluted 1:200 in fresh cell culture medium was used to stimulate peripheral blood mononuclear cells (PBMC) *in vitro* (5).

Identification of infected patients

Clinically relevant skin infection was defined as suppurative superficial *S. aureus* skin infection characterized by small pustules, crusting, pus and increased pruritus (3) in the presence of *S. aureus* as determined by contact sampling and culture which generally resulted in the indication for antibiotic treatment.

Microbiological investigation

Microbiological investigation was performed immediately before and after CsA treatment in all 11 patients, and 1 to 2 days after termination of antimicrobial treatment in *S. aureus*-infected children. The six infected patients generally received antimicrobial pretreatment before initiating CsA therapy. Two patients also received antimicrobials during CsA therapy because of ongoing *S. aureus* infection. Antibiotic pretreatment was applied for 4 weeks using cefuroxime axetil (80 mg/kg/day), erythromycin (50 mg/kg/day) or amoxycillin-clavulanate (60 mg/kg/day), respectively. *S. aureus* strains from all infected children were sensitive against the selected antibiotics.

Statistical analysis

Statistical analysis was performed using the chi-square test and Wilcoxon's test for independent samples.

Results

The effect of CsA treatment on SCORAD scores is presented in Fig. 1. There was no significant

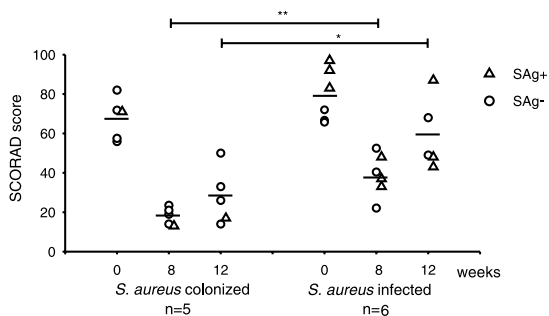


Fig. 1. Severity of atopic dermatitis (AD) as determined by the SCORAD index for the *S. aureus*-colonized (no antibiotics) and the *S. aureus*-infected group (antibiotics). Mean SCORAD index is presented for the baseline (week 0), after 8 weeks of treatment with cyclosporin A (week 8) and four weeks after cessation of treatment (week 12). Wilcoxon 2-sample test: * $p < 0.05$, ** $p < 0.01$. Δ : *S. aureus*-exotoxin producing strain; $\circ$: non-toxicogenic *S. aureus* strain.

difference in the SCORAD index between *S. aureus*-infected (see above) and colonized patients at baseline and all patients showed a clinical improvement (decrease in the SCORAD index). The mean SCORAD score (all patients) fell from 74 at baseline to 29 after CsA treatment ($p < 0.001$). One child had an insufficient response to therapy (SCORAD reduction 32%) and discontinued at week 8. However, the effect of CsA was higher in *S. aureus*-colonized patients (mean SCORAD = 18) compared with infected patients (mean SCORAD = 39; $p < 0.01$). This difference was still evident 4 weeks after cessation of CsA treatment (colonized patients SCORAD = 28 vs. infected patients SCORAD = 59; $p < 0.05$). The effect of CsA treatment on *S. aureus* colonization is presented in Fig. 2. The figure shows the degree of colonization with *S. aureus* for the most severely affected skin site.

There was a significant decrease in *S. aureus* density on lesional skin after CsA treatment in colonized patients, but not in infected patients. In addition, there was also a higher incidence of SEB-producing (toxigenic, SAg+) strains in the infected population. Three of the six infected patients harbored SEB-producing strains of *S. aureus*. One of those producing three different toxins (SEB, SEA and SED) while only one SEA- and TSST-1 producing strain was detected in the colonized group. SAg production by *S. aureus* was correlated with a higher score of AD. The mean SCORAD index in the group infected by toxigenic strains was 86 vs. 67 in the group with non-toxicogenic *S. aureus* strains; $p < 0.05$. It should be noted that complete

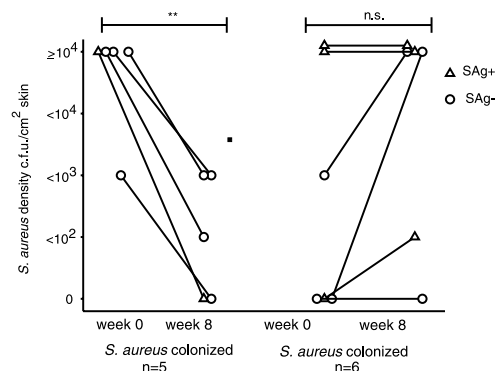


Fig. 2. Effect of cyclosporin A on the density of *S. aureus* colonization of the most severely affected skin site. Data presented for the 'colonized' group (no antibiotics) and for the *S. aureus*-infected group (antibiotics). Wilcoxon 2-sample test: ** $p < 0.01$. Δ : *S. aureus*-exotoxin producing strain; $\circ$: non-toxicogenic *S. aureus* strain.

bacterial eradication was not achieved in either group.

Discussion

The beneficial effect of CsA on the severity of pediatric AD is reduced in cases in which the disease is complicated by clinically overt infection with *S. aureus* as defined by suppurative superficial *S. aureus* skin infections characterized by small pustules, crusting, pus, increased pruritus (3) and the presence of *S. aureus* as determined by contact sampling and culture which generally resulted in the indication for antibiotic treatment.

Our results also indicate that there was a significant decrease in *S. aureus* density on lesional skin after CsA treatment in colonized, but not in *S. aureus*-infected patients.

Because CsA inhibits the production of inflammatory cytokines and decrease T cell activation but is devoid of antibacterial activity (10,13), the decrease in *S. aureus* colonization may primarily be a consequence of the improvement of the skin barrier function along with CsA-induced healing of the skin lesions. During the dermal healing process fibronectin and laminin receptors for *S. aureus* will be covered by the stratum corneum and this may decrease the adherence of *S. aureus* to the skin (8, 14, 15).

The lesser effect in the infected group may be due to (i) an infection-related lack of the anti-inflammatory effect of CsA and/or (ii) the rapid recolonization with *S. aureus* after cessation of systemic antimicrobial treatment which has generally been initiated before CsA therapy (6). With respect to point (i) it has been shown that TNF- α levels could not be reduced by CsA treatment of children with severe AD (16).

In addition a higher prevalence of *S. aureus* strains with SAg activity, especially the production of SEB, coincided with significantly higher scoring of AD, a finding supported by two other studies (5, 17). The correlation can be explained by the effect of *S. aureus*-derived SAg on increasing T-cell activation (HLA-DR⁺ and CD 69⁺) and the higher expression of T-cell skin homing factor (CLA⁺ T-cells). Furthermore analyses of TCR V β expression demonstrated increased prevalence of superantigen-reactive families in CLA⁺ T-cells in peripheral blood of infected patients with *S. aureus* (18) and in lesional T cells in patients afflicted with SAg+ producing *S. aureus* strains (5).

Anti-infective treatment administered before immunomodulatory therapy seems to be decisive for the long-term therapeutic effect.

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