

Staphylococcal α -Toxin Induces a Higher T Cell Proliferation and Interleukin-31 in Atopic Dermatitis

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Key Words

α -Toxin • Interleukin-31 • T cell proliferation •
Atopic dermatitis • Peripheral blood mononuclear cells

Abstract

Background: Patients with atopic dermatitis (AD) are frequently colonized with α -toxin-producing *Staphylococcus aureus* which is in turn positively correlated with the severity of eczema. **Methods:** In this study we addressed T cell proliferation and T cell as well as monocyte cytokine secretion upon α -toxin stimulation in peripheral blood mononuclear cells (PBMCs) from AD patients compared to healthy controls. **Results:** We found that α -toxin stimulation of PBMCs markedly enhanced T cell proliferation both in patients with AD and healthy controls and was significantly increased in AD patients compared to healthy controls. PBMCs of AD patients secreted significantly more IL-31 compared to those of healthy controls upon α -toxin and SEB stimulation. Moreover, α -toxin stimulation yielded an increase in T cell (IL-2, IL-9, IL-10 and IFN- γ) as well as monocyte (IL-1 β and TNF- α) cytokine secretion. **Conclusion:** Our results could partly explain how skin colonization and infection with *S. aureus* can contribute to chronic skin inflammation and pruritus in AD.

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Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by eczematous skin lesions with 'lichenification', pruritic excoriations and an enhanced susceptibility to bacterial skin infections, particularly with *Staphylococcus aureus* [1]. A distinct percentage of *S. aureus* strains are able to produce α -toxin, a potent 33-kDa cytotoxin which is an exotoxin that does not belong to the group of enterotoxins (superantigens). In two former studies we found (1) that 30% of *S. aureus* strains isolated from skin were α -toxin producing in untreated [2] and (2) that 63% were α -toxin-producing *S. aureus* strains from AD patients on standard anti-inflammatory and antiseptic treatment [3].

In higher concentrations, α -toxin may lead to necrosis in a wide range of human cells by forming small pores (1–2 nm diameter) into cell membranes [4]. We showed that sublytic concentrations of α -toxin were able to effectively activate T lymphocytes, which led to the induction of IFN- γ [2] and IL-22 [5]. This indicates that besides pore-forming abilities, sublytic concentrations of α -toxin have immunological properties and therefore may serve as a pathogenetic factor in AD.

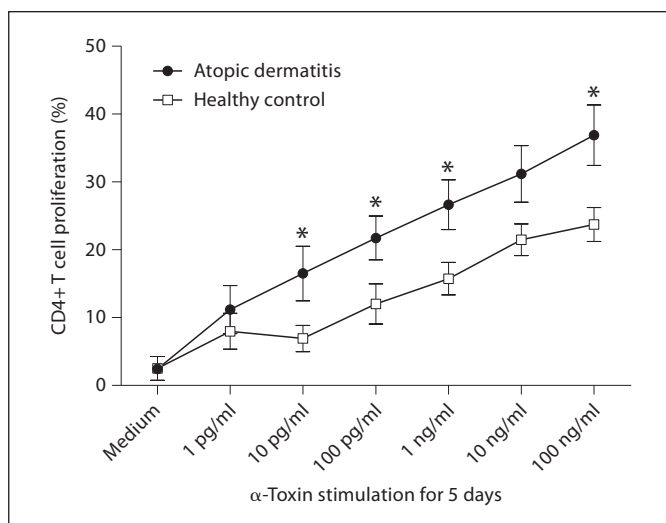


Fig. 1. α -Toxin induced a higher T cell proliferation in AD patients compared to healthy controls. PBMCs from AD patients (n = 10) or healthy controls (n = 10) were either left unstimulated (medium control) or were incubated with α -toxin as indicated for 5 days. Data are shown as a summary of percentages of proliferated CD4+ T cells that have undergone one or more cell divisions $\pm$ SEM as assessed by flow cytometry. * p < 0.05 compared to healthy controls.

IL-31 plays an important role in skin inflammation and AD and has been shown to induce skin inflammation, pruritus and severe dermatitis in IL-31-overexpressing transgenic mice [6, 7]. IL-31 is significantly upregulated in pruritic (AD) but not in nonpruritic (psoriasis) forms of chronic skin inflammation. Furthermore, exposure to staphylococcal superantigen SEB rapidly induced IL-31 mRNA expression in AD both in vivo and in vitro [6]. Recently, it has been shown that serum IL-31 levels were significantly higher in patients with AD and also correlated with disease activity [8]. A major function which has been attributed to IL-31 is the induction of pruritus in the skin via IL-31 receptors on sensory nerve cells [7]. In humans, IL-31 has been detected in the skin mainly in acute eczema [9]. Target cells for IL-31 expressing IL-31RA are activated macrophages, epithelial cells including keratinocytes as well as eosinophils in the skin [6, 7, 10, 11].

In an attempt to further understand the role of staphylococcal α -toxin in pruritic skin inflammation, particularly in AD, we investigated T cell and monocyte cytokine secretion as well as T cell proliferation upon α -toxin stimulation in peripheral blood mononuclear cells (PBMCs) from AD patients compared to healthy controls.

Materials and Methods

Peripheral blood samples were taken from informed consented healthy donors (n = 10) and patients with exacerbated AD (n = 10), presented to our outpatient and inpatient department as previously described [5]. In brief, PBMCs were isolated by density gradient centrifugation on lymphoprep (Fresenius Kabi Norge AS, Halden, Norway) and stimulated in a time- and dose-dependent manner with α -toxin (Sigma Aldrich, Deisenhofen, Germany) in sublytic concentrations as indicated. LPS was not detected in any reagent, as determined by the Limulus amoebocyte assay (Haemochrom Diagnostika, Essen, Germany). AD was determined by the diagnostic criteria of Hanifin and Rajka [12].

Proliferation of CD4+ T lymphocytes was assessed with the Vibrant CFDA SE Cell Tracer Assay (Invitrogen, Carlsbad, Calif., USA). Cells were stained according to the manufacturer's instructions. At several time points the percentages of cells with a low CFSE fluorescence which had undergone one or more cell divisions and were CD4+ (Immunotech, Marseille, France) were determined on a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany).

Cell-free culture supernatants were harvested and analyzed for IL-1 β , TNF- α , IL-31 (Duo Set; R&D Systems, Minneapolis, Minn., USA), IL-2, IL-4, IL-6, IL-10, IL-15, IFN- γ (eBioscience, San Diego, Calif., USA) and IL-9 (Antigenix America Inc., Huntington Station, N.Y., USA), using a commercially available enzyme-linked immunosorbent assay (ELISA) system following the manufacturer's instructions.

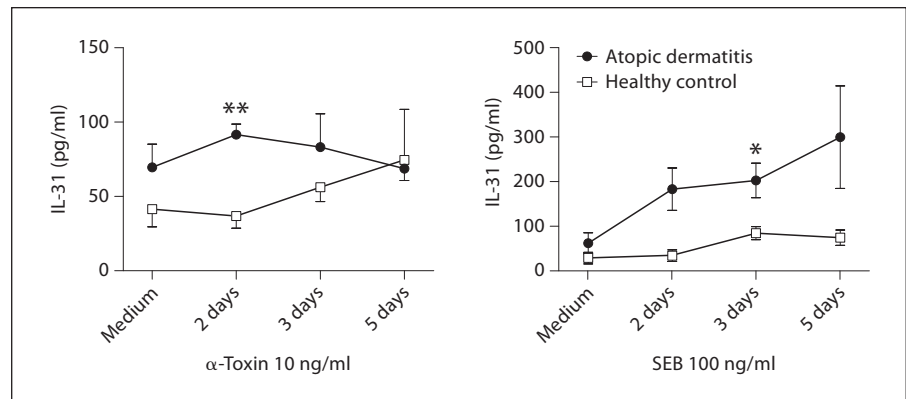
For statistical analyses, the software Sigma Stat for Windows (Systat Software, San Jose, Calif., USA) was used. Statistical analyses were performed using the Student t test.

Results

α -Toxin Induced a Higher T Cell Proliferation in AD Patients Compared to Healthy Controls

PBMCs were incubated with α -toxin in a dose-dependent manner for 5 days. Proliferation of CD4+ T cells was measured with the Vibrant CFDA SE Cell Tracer Assay. Stimulation with α -toxin in concentrations from 1 pg/ml to 100 ng/ml led to T cell proliferation in AD patients as well as healthy controls. In contrast, no T cell proliferation was detectable upon stimulation with lytic concentrations of α -toxin (≥ 1 μ g/ml; data not shown). Moreover, α -toxin induced a higher proliferation in CD4+ T cells from AD patients compared to healthy controls (fig. 1). To further explore the impact of α -toxin on T cell and monocyte cytokine secretion that might explain the higher proliferation of CD4+ T cells in AD patients compared to healthy controls upon α -toxin stimulation or might be of general interest in this disease, we investigated IL-1, IL-2, IL-6, IL-9, IL-10, IFN- γ and TNF- α secretion. We confirmed an induction of these cytokines upon α -toxin stimulation. However, we failed to detect

Fig. 2. IL-31 secretion is enhanced in PBMCs from patients with AD compared to healthy controls upon stimulation with staphylococcal exotoxins. PBMCs from AD patients (n = 4–6) or healthy controls (n = 4–6) were either left unstimulated (medium control) or were stimulated with α -toxin (10 ng/ml) or SEB (100 ng/ml), respectively, for 48, 72 or 120 h. Cell free culture supernatants were quantified for IL-31 secretion by ELISA. Data are shown as mean values $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$ compared to healthy controls.



any differences between PBMCs from AD patients compared to healthy controls (data not shown). α -Toxin stimulation did not induce IL-4 and IL-15 secretion (data not shown).

Upregulation of IL-31 Secretion by Staphylococcal α -Toxin in PBMCs from AD Patients Compared to Healthy Controls

IL-31 secretion upon α -toxin stimulation was investigated on the protein level as determined by ELISA. SEB was used as a positive control since Sonkoly et al. [6] showed a significantly enhanced induction of IL-31 mRNA in PBMCs from AD patients compared to healthy controls. PBMCs were either left unstimulated or were stimulated with α -toxin or SEB, respectively, for 48, 72 and 120 h. Stimulation with α -toxin and SEB yielded a significant upregulation of IL-31 secretion in PBMCs from AD patients compared to healthy controls (fig. 2).

Discussion

In this study, it was shown for the first time that sublytic concentrations of α -toxin induce a higher T cell proliferation in CD4+ T cells from AD patients compared to healthy controls. To find an explanation for this observation we investigated cytokines that could be responsible for the enhanced T cell proliferation in AD patients. However, we failed to detect differences in IL-2 secretion between AD patients and healthy controls upon α -toxin stimulation. Furthermore, we failed to detect IL-15 upon α -toxin stimulation which was another cytokine of interest related to T cell proliferation. An explanation for the enhanced T cell proliferation in AD patients upon α -toxin stimulation compared to healthy controls might be a

stronger induction of the inflammasome in AD patients which was recently discovered to be an important intracellular signaling complex for α -toxin [13] and should be studied in more detail in further studies.

IL-31 is a newly characterized T cell-derived cytokine which is involved in AD. A major function which has been attributed to IL-31 is the induction of pruritus in the skin [1, 6]. Exposure to staphylococcal superantigen SEB rapidly induced IL-31 mRNA expression in AD both in vivo and in vitro [6]. Our data show the induction of IL-31 by SEB in PBMCs from AD patients compared to healthy controls on the protein level. Moreover, we show the involvement of another staphylococcal exotoxin, namely α -toxin, in IL-31 induction. This effect was specific since a number of other Th2-associated cytokines (IL-4, IL-9) were not differentially regulated in AD patients compared to healthy controls upon α -toxin stimulation. In a previous study we showed an upregulation of the IL-31 receptor in human macrophages and monocytes upon stimulation with α -toxin or SEB, respectively, which is of functional relevance because IL-31 induced IL-1 β and IL-6 in these cells solely after upregulation of the IL-31 receptor with staphylococcal exotoxins [10]. Taken together, these data point to a positive feedback loop of staphylococcal exotoxins in IL-31 secretion and its functional effects, which shows an involvement of the induction of pruritus in AD.

Acknowledgements

We would like to thank Mrs. Gabriele Begemann and Mrs. Kathrin Baumert for their excellent technical assistance. This work was supported by DFG grants SFB 566 and GRK 1441 as well as the StrucMed program and a gender equality grant of Hannover Medical School.

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