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Nickel allergy and relationship with *Staphylococcus aureus* in atopic dermatitis

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ABSTRACT

Background: The increase of nickel air pollution is supposed to frequent side effects of nickel action related to virulence potential of *Staphylococcus aureus* in patients with nickel allergy in atopic dermatitis. The goal was to investigate the relationship between nickel allergy and infection by *S. aureus* in atopic dermatitis.
Methods: Nickel allergy was confirmed in atopic patients and excluded in healthy volunteers using patch testing. Infection by *S. aureus* was tested in atopic patients and healthy volunteers by use of API Staph system. The specific IgE for staphylococcal enterotoxin A and B were measured. Secretion of IFN-g, IL-2, IL-13 by PBMC under nickel sulfate and the enterotoxins A and B stimulations were studied with ELISpot.
Results: We found the increased number of infections by *S. aureus* in atopic patients with nickel allergy in comparison to atopic patients and healthy volunteers without nickel allergy. The elevated secretion of IL-2 under nickel sulfate stimulation in vitro was exclusively found in atopic patients with nickel allergy infected by *S. aureus*.
Conclusions: Our data suggest that nickel allergy and infection by *S. aureus* are linked in atopic dermatitis.

1. Introduction

The increasing concentration of nickel in the air as a consequence of urbanisation and high pollution of the air is proposed to amplify nickel—dependent processes resulting in the higher frequency of nickel sensitization in allergic diseases [1–4]. Nickel is a transition element with high biological activity and it is involved in the pathology of allergic diseases including atopic dermatitis [5,6]. Atopic dermatitis is the most frequent chronic skin disease that is regulated by genetic and environmental factors including contaminators like nickel that influence to biology of the immune cells migrating between blood circulation and skin [5–7]. The defect of skin barrier and the immune system dysfunction play a major role in the skin hyperactivity of atopic dermatitis [8]. In bacteria, nickel participates in redox enzymatic processes like hydrogen oxidation, methane biogenesis and acetate formation [9]. It is known that *Staphylococcus aureus* expresses nickel-transporters that are oligopeptide transport systems (Opp) belonging to the nickel/peptide/opine PepT subfamily of ABC transporters [10]. It has been postulated that they have a role in the human urinary tract infection by *S. aureus*, due to nickel-dependent urease increasing the pH of urine [11]. *S. aureus* also participate in development of atopic dermatitis because it can induce inflammation through extracellular vesicles containing pathogenic proteins [12]. Therefore, there might be a link between staphylococcal nickel-dependent urease and nickel allergy in pathogenesis of atopic dermatitis. The goal of our study was to examine relationship between allergy to nickel and infection by *S. aureus* in atopic dermatitis. The main aims of our study were:

1. to present modifications of cytokine millieu in nickel allergy and infection by *S. aureus* in atopic dermatitis;
2. to analyze relationship between results of patch tests to nickel and infection by *S. aureus* in atopic dermatitis.

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2. Material and methods

2.1. Participants

Twenty female patients and six male patients with diagnosed AD treated in the Department of Clinical and Environmental Allergology of the Medical College of the Jagiellonian University in Krakow, Poland and ten female and six male healthy volunteers participated in the study as a control group. In this group 62% of atopic patients (81% of of female patients and 19% of male patients) had diagnosed nickel allergy. There was no positive result for the patch test to nickel sulfate in the healthy volunteer group. A positive result for the infection by *S. aureus* had 35% of patients with AD (78 % of female patients and 22% of male patients) and 65% of patients with AD had no any Staphylococci detected. There were no positive results for infection by *S. aureus* or any Staphylococci in the healthy volunteer group. The diagnosis of atopic dermatitis was confirmed following the Hanifin and Rajka criteria. The patch test to nickel was performed with 5% $NiSO_4$ petrolatum in IQ Ultimate® chamber (Chemotechnique Diagnostics, Creutzwald, France) and the test was read after 3 and 5 days. The control group consisted of volunteers with no symptoms of allergic diseases and all with a negative history of allergic diseases. No skin lesions or eczema had been observed among the control group. The age range was from 18 to 58 years (median: 36.8 ± 14.2) in the AD group and from 25 to 61 years (median: 36.8 ± 11.2) among the control group.

2.2. Study design

Adult patients in the acute phase of atopic dermatitis and adult healthy volunteers as controls participated in the study. The secretion of cytokines in relation to diagnosed infection by *S. aureus* and nickel allergy was investigated in atopic patients. The secretion of cytokines by PBMC in vitro from atopic patients in the acute phase was compared to the secretion of cytokines by PBMC in vitro from healthy volunteers. The SCORE index determining the intensity of atopic dermatitis was graded on a scale modified by our group from 0 if any atopic eczema was observed to 4 if inflammation was very severe [13]. The result of the patch test to nickel was performed in remission, and levels of SEA- and SEB- specific IgE antibodies were measured by UNICAP 100® (Pharmacia & Upjohn, Bringwater, NJ, USA) in the acute phase of atopic dermatitis.

2.3. Cell cultures

Peripheral blood mononuclear cells (PBMC) (Fig. 1a) were isolated from the blood samples of participants through Ficoll Paque density gradient centrifugation (GE Healthcare, Wauwatosa, WI, USA). The cells were counted and tested for viability by trypane blue exclusion and cultured in Iscove's Modified Dulbecco's Medium (Biowhittaker, Lonza Walkersville, MD USA) supplemented with 50 U/ml of penicillin, 50 μg/ml of streptomycin, and 7.8 μg/ml of 1.4-dithiotreitol at 37 °C, 95% RH, 5% CO_2. The final cell density was $5 \times 10\,000$ cells/well for IFN-γ and 1000 000 cells/well for IL-2 or IL-13 for ELISpot assays (Sanquin, Amsterdam, Netherlands). The cells were cultured in triplicate with or without the presence of $NiSO_4$ (25 μM i 50 μM), and with or without the addition of L-PHA (Sigma–Aldrich, London, UK), referred to as a positive control. The concentrations of L-PHA (IFN-γ and IL-13: 5 μg/ml, IL-2: 10 μg/ml) were recommended by the Sigmaaldrich company. The concentration of staphylococcal SEA and SEB was 500 ng/ml for both stimulants.

2.4. Enzyme-linked immunospot assay

Elispot kits and A EL VIS ELISpot Reader were obtained from Sanquin (Amsterdam, Netherlands). The cells were cultured initially for 1 day in round-bottom 96-well plates (Nalge Nunc, Rochester, NY, USA) for good antigen presentation. Subsequently, the cells were transferred to 96-well polyvinylidene fluoride (PVDF) micro filter plates (Millipore, Billerica, MA, USA) each coated with respective primary antibodies. After an additional 2 days of culture for IFN-γ and 3 days for IL-2, IL-13, the ELISpot assay was performed according to the manufacturer's guidelines. Spots (Fig. 1a) were counted automatically using the A EL VIS ELISpot Reader. The stimulation index (SI) describing the change in the number of PBMC secreting a cytokine under antigen stimulation in vitro was calculated by dividing the number of PBMC secreting a cytokine under stimulant presence by the number of PBMC spontaneously secreting a cytokine (no stimulation). All experiments were performed in triplicate.

2.5. Identification of infection by Staphylococcus aureus

All tests for microbiological identification were obtained from bioMérieux (Marcy l'Etoile, France) and were performed in accordance to EUCAST (European Society of Clinical Microbiology and Infecious Diseases) recommendations for the detection of *S. aureus*. Patients and healthy volunteers did not received any antibiotics at least four weeks before the performance of microbiological tests. Microbiological material for the identification of *S. aureus* was isolated from the right and left vestibules of nose and skin lesions (right and left palms and the forearms, cheeks and the forehead) by sterile swab and were inoculated on a sterile Columbia agar plate with 5% of defibrinated sheep blood and incubated for 1 day at 35 °C for bacterial growth (Fig. 3a). Subsequently, the chosen colonies were transferred onto another selective SAID plate to find the colonies able to metabolize a chromogen. The activity of staphylococcal α-glucosidase catalyzing the reaction resulted in the appearance of green colonies that were *S. aureus* (Fig. 3a). This identification of *S. aureus* was analyzed after 1 and 2 days of incubation at 35 degrees Celsius. Additionally, the coagulase-negative Staphylococci were distinguished from *S. aureus* using an Api32 Staph test following the manufacturer's instructions (Fig. 3a). The phenotypic identification of methicillin-resistant *S. aureus* was performed using the disc diffusion method. Coagulase-positive Staphylococci were incubated for 1 day at 35 °C on agar plates with 30 mcg of cefoxitin for bacterial growth. The diameter of inhibition zone of bacterial growth was counted and interpreted according to EUCAST guidelines. All bacterial colonies that the inhibition zone was greater than or equal to 22 mm were approved as methicillin-resistant *S. aureus*.

2.6. Statistical analysis

Data were analyzed and graphed with GraphPad Prism 5.01 (Graphpad Software Inc., La Jolla, CA, USA). Data are shown as individual participants with medians and were analyzed with Mann–Whitney *U* tests and D'Agostino and Pearson omnibus normality test for a Gaussian distribution. Differences were considered statistically significant at a p value of less than 0.05.

2.7. Ethics

The study was accepted by the Ethics Committee of the Jagiellonian University in Krakow, Poland and performed in accordance with the ethical standards of the Helsinki Declaration (approval