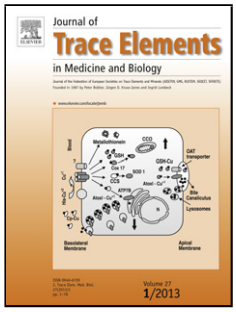
Accepted Manuscript



Title: Nickel allergy and relationship with Staphylococcus aureus in atopic dermatitis

Author: Anna Bogdali M Antoszczyk Grazyna Dyga Wojciech Obtulowicz Aleksander Bialecka Anna Kasprowicz Andrzej Magnowska Zofia Obtulowicz Krystyna

PII: S0946-672X(15)30010-9
DOI: http://dx.doi.org/doi:10.1016/j.jtemb.2015.06.009
Reference: JTEMB 25697

To appear in:

Received date: 25-3-2015
Revised date: 13-6-2015
Accepted date: 23-6-2015

Please cite this article as: Anna Bogdali M, Grazyna Antoszczyk, Wojciech Dyga, Aleksander Obtulowicz, Anna Bialecka, Andrzej Kasprowicz, Zofia Magnowska, Krystyna Obtulowicz.Nickel allergy and relationship with Staphylococcus aureus in atopic dermatitis.*Journal of Trace Elements in Medicine and Biology* http://dx.doi.org/10.1016/j.jtemb.2015.06.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Bogdali Anna M[1], Antoszczyk Grazyna[1], Dyga Wojciech[1], Obtulowicz Aleksander[2], Bialecka Anna[3], Kasprowicz Andrzej[3], Magnowska Zofia[4], Obtulowicz Krystyna[1]

[1] Department of Clinical and Environmental Allergology, JagiellonianUniversity Medical College, 31-531 Krakow, Poland, emails: am.bogdali@uj.edu.pl, grazyna.antoszczyk@gmail.com, wdyga@cm-uj.krakow.pl, [2] Department of Dermatology, Jagiellonian University Medical College, 31-066 Krakow, Poland, email:obtulowicz@poczta.fm and [3] Centre for Microbiological Research and Autovaccines Ltd., 31-016 Krakow, Poland, emails: bialecka.ania@gmail.com, a.kasprowicz@cbm.com.pl, [4]University of Copenhagen Research Centre for Control of Antibiotics Resistance-UC-Care Faculty of Health and Medical Sciences, University of Copenhagen, Stigbøjlen 4, 1870 Frb. C., 1-20 Frb. C., Copenhagen, Denmark, email: zofia@sund.ku

Nickel allergy and relationship with Staphylococcus aureus in atopic dermatitis

running head: Nickel- induced staphylococcal inflammation

Address correspondence to:
Krystyna Obtulowicz, M.D., PhD. Department of Clinical and Environmental Allergology, Jagiellonian University Medical College, Krakow, Poland
Tel.: +48 12 424 88 98 Fax: +48 12 424 88 98 e-mail:mmobtulo@cyf-kr.edu.pl

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 Staphylococcus aureus in atopic dermatitis.

Methods: Nickel allergy was confirmed in atopic patients and excluded in healthy volunteers using patch testing. Infection by Staphylococcus 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 Staphylococcus 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

ACCEPTED MANUSCRIPT

atopic patients with nickel allergy infected by Staphylococcus aureus.

Conclusions: Our data suggest that nickel allergy and infection by Staphylococus aureus are linked in atopic dermatitis.

KEYWORDS: dermatitis, atopic, nickel, Staphylococcus aureus

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 Staphylococcus aureus, due to nickel-dependent urease increasing the pH of urine [11]. Staphylococcus 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 Staphylococcus aureus in atopic dermatitis. The main aims of our study were:

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

MATERIAL AND METHODS

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

ACCEPTED MANUSCRIPT

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 Staphylococcus 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 Staphylococcus 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.

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 Staphylococcus 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.

Cell cultures

Peripheral blood mononuclear cells (PBMC) (Figure 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 degrees Celsius, 95% RH, 5% CO_2. The final cell density was 5 * 10 000 cells/well for IFN-γ and 1 000 000 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

ACCEPTED MANUSCRIPT

without the addition of L-PHA (Sigmaaldrich, 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.

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 (Figure 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.

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 Staphylococcus 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 Staphylococcus 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 degrees Celsius for bacterial growth (Figure 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 Staphylococcus aureus (Figure 3a). This identification of Staphylococcus aureus was analyzed after 1 and 2 days of incubation at 35 degrees Celsius. Additionally, the coagulase–negative Staphylococci were distinguished from Staphylococcus aureus using an Api32 Staph test following the manufacturer's instructions (Figure 3a). The phenotypic identification of methicillin-resistant Staphylococcus aureus was performed using the disc diffusion method. Coagulase-positive Staphylococci were incubated for 1 day at 35 degrees Celsius on agar plates with 30 mcg of

ACCEPTED MANUSCRIPT

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 millimeters were approved as methicillin-resistant Staphylococcus aureus.

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.

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 number: KBET/61/B/2009) and informed written constent had been obtained from patients and controls.

RESULTS

As it is listed in Table 1, the clinical characteristics varied in the studied patients concerning positive family history, eczema symptoms in childhood and a progression from atopic dermatitis to allergic rhinitis and asthma in life called the allergic march. All these clinical parameters may be related to infection by Staphylococcus aureus but there were no significant differences in these clinical parameters between atopic patients infected (SA_AD) and not infected by Staphylococcus aureus (NSA_AD) or any other Staphylococci in our studies [14]. As it has been shown in Table 2, atopic patients infected by Staphylococcus aureus (SA_AD) had colonized at least one vestibules of nose and sometimes it was accompanied by the infection of lesions on palms, forearms cheeks and the forehead but any Staphylococci were found in the nose of healthy volunteers (HV) (data not shown).

The cytokine milieu in nickel allergy and its modification by other allergic diseases in atopic dermatitis

In order to study nickel allergy in atopic dermatitis, the secretion of cytokines by PBMC under nickel sulfate stimulation (Figure 1a) in vitro from atopic patients with nickel allergy, without nickel allergy and healthy volunteers was assessed. We found no statistically significant differences in the secretion of IFN-γ, IL-2, IL-13 by PBMC from these groups (data not shown). Nevertheless,

ACCEPTED MANUSCRIPT

the reduction of IFN-γ secretion under 50 µM nickel sulfate stimulation in vitro correlated with the SCORE index in atopic patients with nickel allergy (Figure 1b) and without nickel allergy (Figure 1c) ($P< 0.05$). There was no such a correlation when the lower concentration of nickel sulfate (25 µM) was used for in vitro study (data not shown). These results suggest that the exacerbation of atopic dermatitis is related to the decrease of IFN-γ secretion and this mechanism is not specific for nickel allergy in atopic dermatitis. However, the increase of IL-2 secretion under 50 µM nickel sulfate stimulation in vitro correlated with the intensity of the result of patch test to nickel sulfate in patients with nickel allergy in AD (Figure 2a) ($P< 0.05$). If other allergic diseases corresponded with atopic dermatitis, this correlation disappeared (Figure 2b) ($P> 0.05$). No such a correlation was found when 25 µM $NiSO_4$ was used for in vitro study (data not shown). Therefore this data suggest that IL2 participates in the reaction of PBMC to nickel and can be specific for nickel allergy in atopic dermatitis.

Th1 peripheral leukocyte population is decreased in atopic patients infected by Staphylococcus aureus

We investigated whether infection by Staphylococcus aureus (Figure 3a) may be related to the changes in PBMC population in atopic dermatitis. It was found that the secretion of IL-2 under SEB stimulation in vitro was decreased in the carriers of Staphylococcus aureus in comparison to healthy volunteers ($P< 0.05$). In contrast, there were no statistically significant differences between atopic and healthy non-carriers of Staphylococcus aureus (Figure 3b) ($P> 0.05$). We also found that the reduction of IFN-γ secretion under SEB stimulation in vitro in the carriers of Staphylococcus aureus was visible even if atopic skin eczema (Figure 1a) was mild (SCORE index: at least 2) (Figure 3c). In turn, the decreased secretion of IFN-γ was found only if atopic skin eczema was acute (SCORE index: at least 3) in atopic non-carriers of Staphylococcus aureus (Figure 3d). Thus, infection by Staphylococcus aureus may be related to the reduction of IFN-γ secretion and the exacerbation of atopic dermatitis.

Infection by Staphylococcus aureus and nickel allergy in atopic dermatitis

We found that 71% of atopic patients infected by Staphylococcus aureus had diagnosed nickel allergy and only 29% of atopic carriers of Staphylococcus aureus had not diagnosed nickel allergy (Tab 3). The predominance of infection by Staphylococcus aureus in nickel- sensitive atopic patients suggests the existence of the link between nickel allergy and infection by Staphylococcus aureus in atopic dermatitis. To understand these biological relationship between nickel allergy and infection by Staphylococcus aureus we compared secretion of cytokines by PBMC in vitro from atopic

ACCEPTED MANUSCRIPT

patients inf and non-infected by Staphylococcus aureus and controls.

We discovered a reduction of IFN-γ secretion by PBMC under 25 (Figure 2c) and 50 µM (Figure 2d) of nickel sulfate stimulation in vitro from carriers of Staphylococcus aureus (SA_AD) in comparison to atopic non-carriers of any Staphylococci (NSA_AD) ($P< 0.01$). There were no statistically significant differences in IFN-γ secretion between these groups of atopic patients and healthy volunteers (Figure 2c-d) ($P>0,05$). Additionally, IL-2 secretion under 50 µM of nickel sulfate stimulation in vitro in atopic patients with nickel allergy infected by Staphylococcus aureus (SA_AD) was increased in comparison to atopic non-carriers of any Staphylococci with nickel allergy (NSA_AD) ($P< 0.05$). There were no statistically significant differences in IL-2 secretion between these groups of atopic patients and healthy volunteers (HV) (Figure 4a**).** No differences were found when 25 µM nickel sulfate was used for in vitro study (data not shown). These suggest that the cytokine milieu may be modified by nickel in case of infection by Staphylococcus aureus.

Furthermore, the increase of IL-2 secretion under SEA stimulation in vitro was found in atopic patients with nickel allergy (AD_NA) in comparison to atopic patients without it (AD) ($P< 0.05$). There were no statistically significant differences between the groups of atopic patients and healthy volunteers (HV) (Figure 4b) ($P> 0,05$). No such a difference was found when SEB was used for in vitro study (data not shown). Therefore we assume that IL-2 may be significant in the nickel allergy-related response of PBMC to staphylococcal enterotoxins in atopic dermatitis.

DISCUSSION

According to results within the last decade it is believed that Staphylococcus aureus that can adhere to the extracellular matrix in atopic skin is nickel – dependent due to the urease activity that acts as a virulence factor [10-16]. Moreover, it has been published that defects of the immune system disable proper staphylococcal clearance [17]. Furthermore, α-toxin secreted by Staphylococcus aureus preferentially destroys fillagrin-deficient keratinocytes [18] and the fillagrin defects of atopic skin is strongly related to the development of nickel allergy [19]. To understand whether nickel allergy can favor Staphylococcus aureus infection in atopic dermatitis, we need to fully explain the molecular mechanism of this phenomenon. We discovered that a reduced secretion of IFN-γ under nickel sulfate stimulation was observed in the case of patients with acute skin eczema in atopic patients with and without nickel allergy, thus we suppose that other cytokines participate in allergic inflammation initiated by nickel in atopic dermatitis. It has been published that PBMC isolated from nickel- allergic women and exposed to nickel sulfate had increased level of mRNA encoding IL-2 and secreted increased amount of IL-2 in vitro [20]. Moreover, study

ACCEPTED MANUSCRIPT

performed on murine cell line of T cells (HT-2) showed that nickel increased synthesis of IL-2 and the expression of the receptor for IL-2 [21]. Nickel also significantly increased IL-2 production by the HT-2 when the alteration of T cell activation system by cations was studied in vitro [22]. Therefore, since we have found the increased secretion of IL-2 under nickel sulfate stimulation in vitro correlated with the increased intensity of the result of patch test to nickel sulfate only in patients with nickel allergy in atopic dermatitis without any corresponding allergic diseases, we suppose that IL-2 can participate in the development of nickel allergy in atopic dermatitis. In our study we also found that some atopic patients infected by Staphylococcus aureus were nickel-sensitive because they had diagnosed nickel allergy. Molecular mechanisms of this phenomenon is unknown and needs detailed investigation. Nevertheless, it has been noted that reduction of the activity of neutrophils corresponded to reduced burden of Staphylococcus aureus from tissue in IFN- gamma defective mice [23]. Interestingly, it has been shown that nickel induced apoptosis of human neutrophils from healthy volunteers that may disrupt their antimicrobial role [24]. In our study we found a reduced secretion of IFN-γ under nickel sulfate stimulation in vitro only in atopic carriers of Staphylococcus aureus. Therefore, there may be a relationship between PBMC response to nickel and infection by Staphylococcus aureus in atopic dermatitis related to decreased secretion of IFN-γ. Furthermore, we observed increased secretion of IL-2 under nickel sulfate stimulation in vitro only in atopic patients with nickel allergy infected by Staphylococcus aureus. Therefore, IL-2 may play an important role in the infection by Staphylococcus aureus and its secretion could be dependent on the reactivity of T-cells to nickel. Importantly, our data showed the increase of IL-2 secretion under SEA in vitro found in atopic patients with nickel allergy. The molecular mechanism of this similarity in IL-2 secretion under nickel sulfate and SEA stimulation in vitro is unknown and needs further investigation. However, it is known that Staphylococcus aureus can inflame atopic skin that consequent in impaired barrier function of atopic skin and such a skin may be more predisposed for penetration by nickel [8]. It has been already published that nickel can bind to T cell receptor through a specific amino acids region in the CDR3B of a VB17+TCR that overexpression of this receptor has been found in nickel-induced CD4+ T cell lines of nickel allergic patients [25]. Furthermore, superantigens interact with TCR receptor and MHC type II through the β and α domains of TCR [26]. Therefore staphylococcal enterotoxins and nickel could activate the same intracellular pathways resulting in secretion of the same cytokine such as IL-2 that secretion is regulated by feedback loop shaping IL-2 signaling between helper and regulatory T lymphocytes that consequently may further modify secretion of other cytokines secreted by these immune cells [27]. Thus, the elevated secretion of IL-2 in our study may be sufficient to influence on functioning of helper and regulatory T cells and secretion of other cytokines creating specific mechanisms

ACCEPTED MANUSCRIPT

against bacterial invaders such as Staphylococcus aureus. However, the existence of common intercellular pathways of nickel and superantigens actions needs detailed study.

Taken together, our data suggest that PBMC response to nickel from atopic patients with nickel allergy may modify the cytokine milieu that may result in the disturbance of immune mechanisms that consequently may facilitate infection by Staphylococcus aureus in atopic dermatitis.

The existence of association between nickel allergy and infection by Staphylococcus aureus suggests that avoiding of nickel may improve the effectiveness of treatment of atopic dermatitis. Furthermore, staphylococcal nickel transporters may be promising targets for alternative non-antibiotic helper drugs therapy [28] that potentiates the activity of exisiting antibiotics used in antibiotic therapy of staphylococcal infections in atopic dermatitis.

CONCLUSIONS:

The presented study shows that:

1. The frequency of infections by Staphylococcus aureus is elevated in patients with nickel allergy in atopic dermatitis

2. Nickel can activate PBMC in a superantigen-like manner in patients with atopic dermatitis infected by Staphylococcus aureus

3. PBMC response to nickel may modify cytokine milieu that may disturb antibacterial mechanisms in atopic dermatitis.

ACKNOWLEDGEMENTS

This study was founded by the Ministry of Science and Higher Education in Poland (No. 4266/B/P01/2010/39).

Author contribution

A.M.B performed experiments, data acquisition and interpretation and drafted the manuscript. GA included patients into the study. WD measured the level of antibodies in serum and participated in data interpretation. BA performed microbiological tests and drafted the manuscript. AO participated in clinical data preparation. AK gave technical assistance, took part in study conception. ZM participated in data interpretation and drafted the manuscript. KO designed the study, performed data interpretation and drafted the manuscript. All authors contributed to revising the manuscript

ACCEPTED MANUSCRIPT

and approved the final version.

Conflict of interest

Conflict of interest: none

References

[1]. Santos R, Goossens A. An update on airborne contact dermatitis: 2001-2006. Contact Dermatitis 2007; 57(6): 353-60.

[2]. Antoszczyk G, Obtułowicz K, Wojas-Pelc A, et al. Nickel allergy in contact and atopic dermatitis. Przegl Lek 2003; 60: 334-37.

[3]. Sivertsen T, Daae HL, Godal A, et al. Ruminant uptake of nickel and other elements from industrial air pollution in the Norwegian-Russian border area. Environ Pollut. 1995; 90(1): 75-81.

[4]. Mann E, Ranft U, Eberwein G, et al. Does airborne nickel exposure induce nickel sensitization? Contact Dermatitis 2010; 62: 355-62.

[5]. Novak N. New insights into the mechanism and management of allergic diseases: atopic dermatitis. Allergy 2009; 64: 265-75.

[6]. Cameron KS, Buchner V, Tchounwou PB. Exploring the molecular mechanisms of nickel-induced genotoxicity and carcinogenicity: a literature review. Rev. Environ Health. 2011; 26(2): 81-92.

[7]. Ferran M, Santamaria-Babi LF. Pathological mechanisms of skin homing T cells in atopic dermatitis. World Allergy Organ J. 2010; 3**:** 44-47.

[8]. Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev. 2011; 242(1): 233-46.

[9]. Mulrooney SB, Hausinger RP. Nickel uptake and utilization by microorganisms. FEMS Microbiol Rev. 2003; 27(2-3): 239-61.

ACCEPTED MANUSCRIPT

[10]. Remy L, Carriere M, Derre-Bobillot A, et al. The Staphylococcus aureus Opp1 ABC transporter imports nickel and cobalt in zinc-depleted conditions and contributes to virulence. Mol Microbiol 2013; 87: 730-43.

[11]. Hiron A, Posteraro B, Carriere M, et al. A nickel ABC-transporter of Staphylococcus aureus is involved in urinary tract infection. Mol Microbiol 2010; 77**:** 1246-60.

[12]. Hong SW, Kim MR, Lee EY, et al. Extracellular vesicles derived from Staphylococcus aureus induce atopic dermatitis-like skin inflammation. Allergy 2011; 66: 351-59.

[13]. Oranje AP. Practical issues on interpretation of scoring atopic dermatitis: SCORAD Index, objective SCORAD, patient-oriented SCORAD and Three-Item Severity score. Curr Probl Dermatol 2011; 41: 149-55.

[14] Gould HJ, Takhar P, Harries HE, et al. The allergic march from Staphylococcus aureus superantigens to immunoglobulin E. Chem Immunol Allergy 2007;93:106-36.

[15]. Gonzalez H, Jensen TE. Nickel sequestering by polyphosphate bodies in Staphylococcus aureus. Microbios 1998; 93: 179-85.

[16]. Collins CM, D'Orazio SE. Bacterial ureases: structure, regulation of expression and role in pathogenesis. Mol Microbiol. 1993; 9(5)**:** 907-13.

[17]. Warner JA, McGirt LY, Beck LA. Biomarkers of Th2 polarity are predictive of staphylococcal colonization in subjects with atopic dermatitis. Br J Dermatol 2009; 160: 183-85.

[18]. Brauweiler AM, Bin L, Kim BE, et al. Filaggrin-dependent secretion of sphingomyelinase protects against staphylococcal α-toxin-induced keratinocyte death. J Allergy Clin Immunol. 2013; 131(2):421-27.

[19]. Novak N, Baurecht H, Schäfer T, et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. J Invest Dermatol. 2008; 128(6):1430-35.

ACCEPTED MANUSCRIPT

[20]. Falsafi-Amin H, Holst M, Lundeberg L. Early expression of interleukin-2 mRNA by peripheral blood mononuclear cells isolated from nickel-allergic subjects and subsequently exposed to nickel in vitro. Immunopharmacol Immunotoxicol. 2008; 30(2): 227-34.

[21]. Warner GL, Lawrence DA. The effect of metals on IL-2-related lymphocyte proliferation. Int J Immunopharmacol. 1988; 10(5): 629-37.

[22]. Smith KL, Lawrence DA. Immunomodulation of in vitro antigen presentation by cations. Toxicol Appl Pharmacol. 1988; 96(3): 476-84.

[23]. McLoughlin RM, Lee JC, Kasper DL, et al. IFN-gamma regulated chemokine production determines the outcome of Staphylococcus aureus infection . J Immunol. 2008; 181(2): 1323-32.

[24]. Testa A, Serrone M, Foti C, et al. Neutrophil activation in nickel sensitized subjects. Cytobios. 1996; 86(346): 193-200.

[25]. Thierse HJ, Moulon C, Allespach Y, et al. Metal-protein complex-mediated transport and delivery of Ni2+ to TCR/MHC contact sites in nickel-specific human T cell activation. J Immunol. 2004; 172(3): 1926-34.

[26]. Saline M, Rödström KE, Fischer G, et al. The structure of superantigen complexed with TCR and MHC reveals novel insights into superantig enic T cell activation. Nat Commun 2010; 1: 119-26.

[27]. Busse D, De la Rosa M, Hobiger K, et al. Competing feedback loops shape IL-2 signaling between helper and regulatory T lymphocytes in cellular microenvironments. Proc Natl Acad Sci U S A. 2010; 107(7): 3058–63.

ACCEPTED MANUSCRIPT

[28]. Kristiansen JE, Hendricks O, Delvin T et al. Reversal of resistance in microorganisms by help of non-antibiotics. J Antimicrob Chemother 2007; 59(6):1271-79.

Fig 1. Secretion of IFN-γ, IL-2, IL-13 in nickel allergy and SCORE index (a). Skin eczema by nickel on the forearm in a patient with atopic dermatitis (upper left) and her PBMC (2500X magnification) (upper right). IFN-γ, IL-2, IL-13 secreted by PBMC stimulated with 50 μM of nickel sulfate in ELISpot (below). PBMC from **(b)** NA_AD (n=10) or **(c)** AD (n=10) were treated with **(b-c)** 50 μM of nickel sulfate for 5 days. Secretion of IFN-γ was determined by ELISpot. Patients were grouped according to their SCORE index in both graphs. *P < 0.05

Fig 2. Effects of nickel sulfate to secretion of IFN- γ, IL-2 and nickel allergy or infection by Staphylococcus aureus. PBMC from NA_AD **(a)** but without other diseases (n= 7) **(b)** with other diagnosed allergic diseases (CA, SAR, AA, FA, AC) (n= 7) **(c)** NSA_AD (n= 7) or SA_AD (n= 7) and HV (n=7) were treated with **(a-b)** 50 μM of nickel sulfate or **(c)** 25 μM or **(d)** 50 μM of nickel sulfate. Secretion of **(a-b)** IL2 and **(c-d)** IFN- γ were determined by ELISpot. Patients were grouped according to the result of a patch test to nickel sulfate in both graphs **(Fig. 2, a-b**). *P < 0.05 ,**P < 0.01, NS- no statistical significance.

Fig 3. Effects of SEB to secretion of IL-2 and infection by Staphylococcus aureus. (a). Identification of Staphylococcus aureus: Bacteria grown on agar (upper left). Green colonies of Staphylococcus aureus that metabolized chromogen (upper right). Biochemical characterization of Staphylococcus aureus species (below). PBMC from **(b-d)** AD_SA (n= 7), AD_NSA (n=7) and **(b)** HV (n=7) were treated with SEB for 5 days. Secretion of IL-2 **(b)** and **(c -d)** IFN-γ were analyzed by ELISpot. Patients were grouped according to the SCORE index in both graphs (**Fig 3. c-d**). *P < 0.05**,** **P < 0.01, NS- no statistical significance.

Fig 4. Effects of nickel allergy, infection by Staphylococcus aureus to secretionmofmIL-2. PBMC were treated with **(a)** 50 μM of nickel sulfate **(b)** SEA for 5 days. Secretion of IL-2 by ELISpot were determined in SA_AD (n=7) or NSA_AD (n=7) and HV (n=7) with nickel allergy and AD_NA (n=10) and AD (n=10). *P < 0.05, NS - no statistical significance.

ACCEPTED MANUSCRIPT

Table 1: Comparison of clinical characteristics of atopic patients infected and not infected by Staphylococcus aureus and healthy volunteers

Clinical parameters	AD_SA (n=9) [%]	AD_NSA (n=17) [%]	HV (n=16) [%]
(+) family history	60	67	0
average SCORE index	2.5	2.4	0
Eczema symptoms in childhood	86	62	0
CD [%]	25	67	0
AA [%]	37	44	0
FA [%]	25	33	0
SAR [%]	37	33	0
AC [%]	12	0	0

AD_SA: atopic patients infected by Staphylococcus aureus

AD_NSA: atopic patients not infected by any Staphylococci

HV: healthy volunteers
CD: contact dermatitis,

AA: allergic asthma,

FA: food allergy,

SAR: seasonal allergic rhinitis,

AC: allergic conjunctivitis

ACCEPTED MANUSCRIPT

Table 2: The localisation of colonisation areas by Staphylococcus aureus in atopic patients

Localisation of staphylococcal colonisation in atopic patients (n=9)	[%] of atopic patients
the left vestibule of nose	100
the right vestibule of nose	90
palms	63
forearms	13
the forehead	13

Table 3: The clinical characteristics of patients with atopic dermatitis and healthy volunteers

Clinical parameters	AD_SA (n=9) [%]	AD_NSA (n=17) [%]	HV (n=16) [%]
asSEA IgE> 0,35 kUA/l	43	0	0
asSEB IgE> 0,35 kUA/l	43	0	0
tIgE> 150 kUA/l	75	20	0
(+) contact nickel allergy	71	55	0

asSEA IgE: allergen specific antibodies for staphylococcal enterotoxin A

asSEB IgE: allergen specific antibodies for staphylococcal enterotoxin B

tIgE: total serum IgE