Serum Biomarkers of Allergic Contact Dermatitis: A Pilot Study

Auksė Zinkevičienė^a Denis Kainov^{b, c} Eglė Lastauskienė^d
Violeta Kvedarienė^{e, f} Dmitrii Bychkov^b Myles Byrne^b Irutė Girkontaitė^a

^aDepartment of Immunology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania;

^bInstitute for Molecular Medicine Finland, FIMM, University of Helsinki, Helsinki, Finland; ^cDepartment of Environmental Research, Siauliai University, Siauliai, and ^dDepartment of Microbiology and Biotechnology, Faculty of Natural Sciences, ^eClinic of Infectious, Chest Disease, Dermatovenerology and Allergology, and

^fFaculty of Medicine, Vilnius University, Vilnius, Lithuania

Key Words

Allergic contact dermatitis · Cytokine · Serum biomarkers

Abstract

Background: Allergic contact dermatitis (ACD) is an inflammatory skin disease caused by repeated skin exposure to contact allergens. The goal of this pilot study was to identify inflammatory proteins which can serve as biomarkers for ACD. **Methods:** We measured levels of 102 cytokines, chemokines, and growth factors in the sera of 16 ACD patients during acute and remission phases, and 16 healthy volunteers. **Results:** Serum levels of adiponectin, chemokine (C-C motif) ligand 5 (CCL5), C-reactive protein (CRP), chitinase 3-like 1 (CHI3L1), complement factor D (CFD), endoglin, lipocalin-2, osteopontin, retinol-binding protein 4 (RBP4), and platelet factor 4 (PF4) were significantly higher, whereas levels of trefoil factor 3 (TFF3) were significantly lower, in ACD patients than in healthy controls. In ACD patients, serum levels of CCL5 were elevated, whereas levels of TFF3, soluble intercellular adhesion molecule-1 (sICAM-1), and platelet-derived growth factor (PDGF)-AB/BB were found to be lower during the remission phase of the disease. **Conclusions:** Serum levels of adiponectin, CCL5, CRP, CHI3L1, CFD, endoglin, lipocalin-2, osteopontin, RBP4, PF4, and TFF3 might be ex-

ploited as biomarkers for ACD, whereas levels of CCL5, TFF3, sICAM-1, and PDGF-AB/BB might be exploited for evaluation of disease progression and efficacy of ACD treatment.

© 2016 S. Karger AG, Basel

Introduction

Contact allergies are a growing problem in the world [1]. 20–25% of the European population is sensitive to at least one contact allergen [2]. These allergens are low-molecular-weight compounds that can penetrate skin, bind to epidermal proteins, and induce sensitization. Allergic contact dermatitis (ACD) is a T cell-mediated inflammatory skin disease classified as a delayed-type hypersensitivity reaction [3].

The diagnosis of ACD is based on clinical history and patch testing. The patch test determines whether a specific substance causes allergic inflammation of a patient's skin. The positive patch test indicates contact sensitization of present or past relevance. One of the disadvantages of this method is the invasiveness of the test. Moreover, sometimes the test gives false-positive and false-negative results, which can be correctly interpreted only by experienced allergists [4].

There have been several attempts to develop noninvasive tests to replace the patch test [5–7]. For example, a lymphocyte transformation test was developed for patients in whom the patch caused exacerbation of symptoms [8]. However, the lymphocyte transformation test can only be used in the diagnosis of nickel allergies on account of the low specificity and sensitivity of the test for other metals [9]. Enzymatic assays have also shown promise as diagnostic tools for ACD [10–12]. In particular, an ELISpot assay, which can detect serum levels of IL-4, IL-5, IL-10, IL-13, and IFN- γ , was developed for ACD diagnostics [13].

Here, we used rapid and sensitive immunoassays to simultaneously monitor the levels of 102 human cytokines, chemokines, and growth factors in the serum of 16 ACD patients and 16 control individuals. We showed that serum levels of 11 proteins [adiponectin, chemokine (C-C motif) ligand 5 (CCL5), C-reactive protein (CRP), chitinase 3-like 1 (CHI3L1), complement factor D (CFD), endoglin, lipocalin-2, osteopontin, retinol-binding protein 4 (RBP4), platelet factor 4 (PF4), and trefoil factor 3 (TFF3)] differed between ACD patients and healthy controls. Moreover, serum levels of 4 proteins [CCL5, TFF3, soluble intercellular adhesion molecule-1 (sICAM-1), and platelet-derived growth factor (PDGF)-AB/BB] differed in ACD patients during acute and remission phases of the disease. Our results suggest that immunoassay-based monitoring of several serum proteins might be further exploited in diagnosis and treatment of ACD.

Materials and Methods

This study was approved by the Lithuanian Bioethics Committee (ethical approval No. 158200-14-714-231), and written consent was obtained from each participant. We recruited 16 individuals (15 females and 1 male; 21–54 years of age, mean 33.1 ± 10.6) with preliminary diagnosis of acute-phase ACD by the Centre for Diagnosis and Treatment of Allergic Diseases, Vilnius, Lithuania, in January 2014. Serum samples were collected from these individuals, and then collected again during the remission phase of ACD.

The control group consisted of 16 volunteers without a previous history of ACD (15 females and 1 male; age 21–35 years, mean 27.6 ± 7.6). Volunteers did not have any chronic inflammatory or metabolic diseases. We collected serum samples from the control group twice, with a 1-month interval between sampling.

The patch tests were performed in the case and control groups using European standards (S-1000; Chemotechnique Diagnostics, Sweden). Tests for other allergens were performed according to clinical needs. Patch tests were performed on the upper back of participants. Palpable erythema, indicating a positive reaction to the patch test, was evaluated after 1–3 days. The severity of patch test reactions were graded from no reaction (–) to 3+ [14]. Importantly, patients did not use local and/or systemic corticosteroids

for at least 1 month, and treatment with antihistamines was stopped for 4–6 days before patch testing.

The relative levels of 102 cytokines, chemokines, and growth factors were determined using the Proteome Profiler Human XL Cytokine Array Kit (R&D Systems Inc., USA) according to the manufacturer's instructions. Importantly, this immunoassay was chosen because it includes several biomarkers (e.g. IL-4, IL-5, IL-10, IL-13, adiponectin, CRP, CHI3L1, CCL5, lipocalin-2, and osteopontin), which were previously associated with ACD [10–12, 15–20].

GraphPad Prism software was used for statistical analysis. The differences in protein levels between paired groups were calculated using a nonparametric Wilcoxon signed rank test. The differences between nonpaired groups were analyzed using a Mann-Whitney U test.

Results

We recruited 16 individuals with a preliminary diagnosis of ACD during the acute phase of the disease. After approximately 1 month from onset of symptoms (remission phase), we performed patch tests which confirmed preliminary ACD diagnosis (table 1). We also recruited 16 volunteers without a previous medical history of ACD. We performed a patch test for this group to confirm the absence of ACD. We collected paired serum samples from ACD patients (during acute and remission phases) and from patch test-negative volunteers (1-month interval).

We profiled 102 cytokines, chemokines, and growth factors in 4 groups of serum samples using immunoassay. We observed significant differences between the serum levels of 11 proteins of ACD cases versus healthy controls, as well as variations between the serum levels of 4 proteins of ACD patients during acute and remission phases (table 2). In particular, the serum levels of adiponectin, CCL5, CRP, CHI3L1, CFD, endoglin, lipocalin-2, osteopontin, RBP4, and PF4 were elevated, whereas the level of TFF3 was decreased, in ACD patients in comparison with healthy controls. Furthermore, the serum levels of CCL5 were increased ($p \leq 0.05$), whereas the levels of TFF3, sICAM-1, and PDGF-AB/BB were decreased in remission, versus the acute phase of ACD ($p \leq 0.005$, $p \leq 0.05$, and $p \leq 0.05$, respectively).

Discussion

Our pilot immunological analysis of 102 serum cytokines, chemokines, and growth factors identified significant differences in levels of adiponectin, CCL5, CRP,

Table 1. The results of the patch test in 16 ACD patients

Patient No.	Affected body parts	Potential source of contact dermatitis
3	Hands	Thiomersal (1+)
4	Hands	Potassium dichromate (2+); cobalt chloride (1+); fragrance mix (3+)
6	Hands	Quaternium-15 (3+)
7	Hands	Nickel sulfate (3+); Cl+Me- isothiazolinone (3+)
10	Legs	Cobalt chloride (2+)
11	Hands	Cobalt chloride (1+); nickel sulfate (1+); potassium dichromate (1+)
12	Legs	Cobalt chloride (1+); nickel sulfate (3+)
13	Hands	4-phenylenediamine (3+); toluene-2,5-diamine sulfate (3+); 2-nitro-phenylenediamine (3+); nickel sulfate (3+); 3-aminophenol (3+); 4-aminophenol (3+)
17	Face	Nickel sulfate (2+); Cl+Me- isothiazolinone (1+)
19	Legs	Cl+Me- isothiazolinone (3+); tixocortol-21-privalate (2+); cobalt chloride (1+); paraben mix (1+); euxyl K400 (1+); methylidibromo glutaronitrile (2+); 2-bromo-2-nitropropane-1,3-diol (1+)
20	Face, hands	Imidazolidinyl urea (1+); hexamethyleneteramine (3+); hexahydrol 2,5-tristriazine (3+); Cl+Me- isothiazolinone (2+); quaternium-15 (3+); diazolidinyl urea (2+); DMDM hydantoin (3+); methylisothiazolinone (2+)
21	Face, hands	Nickel sulfate (3+); lauryl glycoside (1+); Cl+Me- isothiazolinone (1+); 2-nitro-phenylenediamine (1+); ammonium persulfate (1+); 4-aminophenol (1+)
23	Hands	2-nitro-phenylenediamine (1+); ammonium thioglycolate (1+); ammonium persulfate (1+); cocamidopropyl betaine (1+)
24	Hands	Chrome (1+); cobalt chloride (2+); gold (1+); nickel sulfate (1+); cannifolia (2+); formaldehyde (3+); palladium (1+); reflin (2+)
25	Face	Octyl gallate (1+); 4-chloro 3-cresol (2+); thiomersal (3+); benzyl salicylate (3+); methylidibromo glutaronitrile (1+)
27	Face	4-phenylenediamine (2+); nickel sulfate (1+); 2-nitro-p-phenylenediamine (1+)

Table 2. Levels of 13 inflammatory markers in sera of 16 ACD patients collected during the acute and remission phases of disease and in sera of 16 healthy volunteers collected twice within a 1-month interval

Cytokine	Cases		Controls		p values (cases vs. controls)
	acute	remission	control 1	control 2	
Adiponectin	3.013±0.959	3.256±1.081	2.262±0.339	2.144±0.386	<0.005
CHI3L1	2.823±0.911	2.974±0.981	2.111±0.405	1.935±0.394	<0.05
CFD	2.213±0.635	2.343±0.635	1.851±0.274	1.673±0.243	<0.05
CRP	3.079±0.911	3.373±1.11	2.34±0.396	2.178±0.3	<0.005
Endoglin	1.683±0.606	1.815±0.565	1.425±0.226	1.215±0.393	<0.05
CCL5	2.040±0.506	2.327±0.606	1.655±0.527	1.597±0.333	<0.05
Lipocalin-2	1.638±0.39	1.729±0.42	1.394±0.232	1.268±0.197	<0.005
Osteopontin	1.395±0.397	1.479±0.533	1.106±0.147	1.008±0.152	<0.05
PF4	2.153±0.568	2.121±0.495	1.79±0.334	1.747±0.271	<0.05
RBP4	1.874±0.288	2.086±0.503	1.683±0.341	1.612±0.33	<0.05
sICAM-1	0.498±0.467	0.373±0.425	0.876±0.311	0.693±0.395	<0.05
PDGF-AB/BB	0.353±0.511	0.176±0.357	0.627±0.69	0.472±0.468	<0.05
TFF3	0.313±0.273	0.3±0.363	0.544±0.304	0.525±0.243	<0.05

CHI3L1, CFD, endoglin, lipocalin-2, osteopontin, RBP4, PF4, and TFF3 between ACD cases and healthy controls. Importantly, adiponectin, CRP, CHI3L1, CCL5, lipocalin-2, and osteopontin were shown to act as proinflammatory proteins, whereas endoglin and PF4 were shown to act as anti-inflammatory proteins in ACD [15–20]. Our results suggest that the differences in serum levels of these proteins between ACD patients and healthy individuals might be exploited in diagnosis of ACD. Moreover, immunological diagnosis of ACD might be done without restriction to contact allergens, and might be appropriate for those cases where application of the patch test is problematic.

Our analysis also revealed variations in the levels of CCL5, TFF3, sICAM-1, and PDGF-AB/BB between the

acute and remission phases in ACD patients. The roles of sICAM-1, PDGF-AB/BB, and TFF3 remain unclear in ACD; however, these proteins were shown to play immunomodulatory functions in other diseases [21–23]. Thus, the levels of CCL5, TFF3, sICAM-1, and PDGF-AB/BB might be exploited in monitoring the progression of ACD, and perhaps evaluating the effectiveness of treatment. However, further replication studies with a larger number of subjects are necessary to validate our results.

Disclosure Statement

All authors state that they have no conflicts of interest to declare. This study was supported by the J. and A. Erkkö Foundation.

References

- Thyssen JP, Linneberg A, Menne T, Johansen JD: The epidemiology of contact allergy in the general population – prevalence and main findings. *Contact Dermatitis* 2007;57:287–299.
- Simonsen AB, Deleuran M, Johansen JD, Sommerlund M: Contact allergy and allergic contact dermatitis in children – a review of current data. *Contact Dermatitis* 2011;65:254–265.
- Martin SF: Immunological mechanisms in allergic contact dermatitis. *Curr Opin Allergy Clin Immunol* 2015;15:124–130.
- Herbst RA, Lauerma AI, Maibach HI: Intradermal testing in the diagnosis of allergic contact dermatitis. A reappraisal. *Contact Dermatitis* 1993;29:1–5.
- Hansen MB, Skov L, Menne T, Olsen J: Gene transcripts as potential diagnostic markers for allergic contact dermatitis. *Contact Dermatitis* 2005;53:100–106.
- Sakaguchi H, Ashikaga T, Miyazawa M, Yoshida Y, Ito Y, Yoneyama K, Hirota M, Itagaki H, Toyoda H, Suzuki H: Development of an in vitro skin sensitization test using human cell lines; human cell line activation test (h-CLAT). II. An inter-laboratory study of the h-CLAT. *Toxicol In Vitro* 2006;20:774–784.
- Dickel H, Kreft B, Kuss O, Worm M, Soost S, Brasch J, Pfutzner W, Grabbe J, Angelova-Fischer I, Elsner P, Fluhr J, Altmeyer P, Geier J: Increased sensitivity of patch testing by standardized tape stripping beforehand: a multicentre diagnostic accuracy study. *Contact Dermatitis* 2010;62:294–302.
- Stejskal VD, Forsbeck M, Nilsson R: Lymphocyte transformation test for diagnosis of isothiazolinone allergy in man. *J Invest Dermatol* 1990;94:798–802.
- Lisby S, Hansen LH, Skov L, Menne T, Baadsgaard O: Nickel-induced activation of T cells in individuals with negative patch test to nickel sulphate. *Arch Dermatol Res* 1999;291:247–252.
- Jakobson E, Masjedi K, Ahlberg N, Lundeberg L, Karlberg AT, Scheynius A: Cytokine production in nickel-sensitized individuals analysed with enzyme-linked immunospot assay: possible implication for diagnosis. *Br J Dermatol* 2002;147:442–449.
- Rustemeyer T, von Blomberg BM, van Hoogstraten IM, Bruynzeel DP, Scheper RJ: Analysis of effector and regulatory immune reactivity to nickel. *Clin Exp Allergy* 2004;34:1458–1466.
- Bordignon V, Palamara F, Cordiali-Fei P, Vento A, Aiello A, Picardo M, Ensoli F, Cristaudo A: Nickel, palladium and rhodium induced IFN-gamma and IL-10 production as assessed by in vitro ELISpot-analysis in contact dermatitis patients. *BMC Immunol* 2008;9:19.
- Bordignon V, Palamara F, Altomonte G, Sperduti I, Pietravalle M, Cavallotti C, Cordiali-Fei P, Fuggetta MP, Cristaudo A, Ensoli F: A laboratory test based on determination of cytokine profiles: a promising assay to identify exposure to contact allergens and predict the clinical outcome in occupational allergic contact dermatitis. *BMC Immunol* 2015;16:4.
- Cavani A, Nasorri F, Prezzi C, Sebastiani S, Albanesi C, Girolomoni G: Human CD4+ T lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. *J Invest Dermatol* 2000;114:295–302.
- Cheng X, Folco EJ, Shimizu K, Libby P: Adiponectin induces pro-inflammatory programs in human macrophages and CD4+ T cells. *J Biol Chem* 2012;287:36896–36904.
- Van Vre EA, Bult H, Hoymans VY, Van Tendeloo VF, Vrints CJ, Bosmans JM: Human C-reactive protein activates monocyte-derived dendritic cells and induces dendritic cell-mediated T-cell activation. *Arterioscler Thromb Vasc Biol* 2008;28:511–518.
- Ouwehand K, Scheper RJ, de Gruijl TD, Gibbs S: Epidermis-to-dermis migration of immature Langerhans cells upon topical irritant exposure is dependent on CCL2 and CCL5. *Eur J Immunol* 2010;40:2026–2034.
- Floderer M, Prchal-Murphy M, Vizzardelli C: Dendritic cell-secreted lipocalin2 induces CD8+ T-cell apoptosis, contributes to T-cell priming and leads to a Th1 phenotype. *PLoS One* 2014;9:e101881.
- Weiss JM, Renkl AC, Maier CS, Kimmig M, Liaw L, Ahrens T, Kon S, Maeda M, Hotta H, Uede T, Simon JC: Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. *J Exp Med* 2001;194:1219–1229.
- Johansen JS: Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. *Dan Med Bull* 2006;53:172–209.
- Witkowska AM, Borawska MH: Soluble intercellular adhesion molecule-1 (sICAM-1): an overview. *Eur Cytokine Netw* 2004;15:91–98.
- Paulsen FP, Woon CW, Varoga D, Jansen A, Garreis F, Jager K, Amm M, Podolsky DK, Steven P, Barker NP, Sel S: Intestinal trefoil factor/TFF3 promotes re-epithelialization of corneal wounds. *J Biol Chem* 2008;283:13418–13427.
- Chen CF, Feng X, Liao HY, Jin WJ, Zhang J, Wang Y, Gong LL, Liu JJ, Yuan XH, Zhao BB, Zhang D, Chen GF, Wan Y, Guo J, Yan HP, He YW: Regulation of T cell proliferation by JMJD6 and PDGF-BB during chronic hepatitis B infection. *Sci Rep* 2014;4:6359.