

Stratum corneum profiles of inflammatory mediators in patch test reactions to common contact allergens and sodium lauryl sulfate

S.A. Koppes,^{1,2} S. Ljubojevic Hadzavdic,³ I. Jakasa,⁴ N. Franceschi,⁵ R. Jurakić Tončić,³ B. Marinović,³ R. Brans,⁶ S. Gibbs,^{2,7} M.H.W. Frings-Dresen,¹ T. Rustemeyer² and S. Kezic¹

¹Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ, Amsterdam, the Netherlands

²Department of Dermatology-Allergology, VU University Medical Center, Amsterdam, the Netherlands

³Department of Dermatology and Venereology, University Hospital Center, Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

⁴Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

⁵Department of Dermatology and Venereology, University Clinical Hospital Centre 'Sestre Milosrdnice', Zagreb, Croatia

⁶Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, Osnabrück, Germany

⁷Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

Summary

Correspondence

Sanja Kezic.

E-mail: s.kezic@amc.uva.nl

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Background Recent studies have demonstrated allergen-specific differences in the gene expression of inflammatory mediators in patch tested skin.

Objectives To determine levels of various inflammatory mediators in the stratum corneum (SC) after patch testing with common contact allergens and the skin irritant sodium lauryl sulfate (SLS).

Methods In total, 27 individuals who had previously patch tested positive to nickel, chromium, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) or para-phenylenediamine were retested and then patch tested with SLS and petrolatum, with petrolatum serving as the patch test control. At 72 h, the test sites were clinically graded and the SC samples collected on adhesive tape.

Results The levels of 18 of the 32 quantified mediators differed significantly from that of the control patches for at least one of the tested substances. SLS and MCI/MI induced the largest number of immunomediators. Interleukin (IL)-16 levels were significantly higher in patch test reactions in all allergens than they were in the controls, while no significant difference was detected for SLS. Furthermore, a strong negative correlation was found between strength of patch test reaction and IL-1 α levels.

Conclusions Cytokine profiles in the SC of patch tested skin did not show a distinct allergen-specific pattern. However, MCI/MI induced a larger and wider immune response than the other allergens, perhaps due to its potency as an irritant. The levels of IL-16 were significantly increased in patch test reactions to allergens but not to SLS; thus, they may help clinicians to differentiate between allergic contact dermatitis and irritant contact dermatitis.

What's already known about this topic?

- Irritant and allergic contact dermatitis share a common inflammatory pathway.
- Recent studies have shown there is an allergen-specific gene expression of inflammatory mediators in patch tested skin.

What does this study add?

- The minimally invasive stratum corneum tape-stripping technique can be used to detect a large number of inflammatory mediators.

- When applying standard patch test concentrations, methylchloroisothiazolinone/methylisothiazolinone was shown to be a more potent inducer of immune mediators than nickel, chromium and *para*-phenylenediamine.

What is the translational message?

- Further investigations into interleukin-16 in ACD might elucidate its function in the pathogenesis of ACD.
- The minimally invasive stratum corneum tape-stripping technique can be used to detect a large number of inflammatory mediators.

Allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) are frequent, especially in occupational settings.^{1–3} Although they share a similar clinical aspect, their pathogenesis and immunological mechanisms are quite different. ICD is the result of tissue damage by irritants, followed by an immunological response induced either by the direct effect of the irritant on keratinocytes or by the impairment of the skin barrier, resulting in the release of pro-inflammatory cytokines and chemokines.⁴ Individuals with a skin barrier dysfunction, such as mutations in the filaggrin gene (FLG) or atopic dermatitis, are more susceptible to developing ICD.⁵ The barrier dysfunction facilitates the easy disruption of the stratum corneum (SC).⁶

ACD is a type IV hypersensitivity reaction of the skin. It results from the activation of a previously sensitized immune system by a hapten.⁷ After penetrating the SC, haptens bind to epidermal proteins or peptides, which enables them to interact with dendritic cells (DCs), resulting in the activation of mainly CD4⁺ T cells. Metals such as nickel, cobalt and palladium are capable of directly activating DCs via Toll-like receptor-4, thereby inducing inflammatory signalling via nuclear factor- κ B.^{8,9} A complex range of inflammatory mediators is involved in both ICD and ACD. Many allergens can also induce skin irritation, which is associated with the activation of the innate immune system.¹⁰ It is likely that the early stages of ACD and ICD share cytokines associated with the innate immune system, such as interleukin (IL)-1 and tumour necrosis factor (TNF)- α .^{11–13} However, in the effector stage of ACD the adaptive immune system is mobilized and associated cytokines are upregulated.¹⁴ Vestergaard *et al.* have found that ICD and ACD show distinct histological responses, such as follicular spongiosis, which is present in early ICD but absent in early ACD reactions.¹⁵ Furthermore, differences in cytokine profiles between various allergens have also been reported, indicating that ACD cannot be regarded as a single entity.⁷ An evaluation of the differences in inflammatory profiles between ICD and ACD may give further insight into their pathogenesis and may help to differentiate between them. In addition, the detection of allergen-specific profiles may contribute to the development of more targeted anti-inflammatory drugs for treating ACD. Therefore, the aim of this study was to

determine the SC profiles of inflammatory mediators induced by the common contact allergens nickel, chromium, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), *para*-phenylenediamine (PPD) and an irritant frequently used in clinical research, sodium lauryl sulfate (SLS).

Patients and methods

Selection of patients

The experimental protocol followed the Declaration of Helsinki Principles and was approved by the Medical Ethics Committee of the University Hospital Centre, Zagreb. Patients with a previously positive (1+ or 2+) patch test reaction to either chromium, nickel, MCI/MI or PPD were identified from the database of the Department of Dermatology of the University Hospital Centre, Zagreb. Patients with a history of atopic dermatitis were excluded, and those with a positive reaction to more than one of the allergens studied were preferred over those with a single positive reaction. Eligible patients were invited to participate in the study and written informed consent was obtained from each participant. In total, 27 patients were finally included (24 female, average age 49 years).

Patch test and tape stripping

Each patient was patch tested with 8 × 8-mm Van der Bend patch test chamber[®] (Van der Bend, Brielle, the Netherlands) with: (i) one or two allergens dissolved in petrolatum (chromium, nickel, PPD) or water (MCI/MI); (ii) a 2% SLS solution (in water); and (iii) petrolatum. Petrolatum is used as a control although it is known that, very rarely, petrolatum can induce an allergic reaction and give a false-positive result when it is used as a vehicle.¹⁶ An SLS concentration of 2% was chosen to ensure a strong irritant reaction, comparable with a 2+ contact allergic reaction expected in the allergens group. The following allergen preparations were used: PPD 1%, potassium dichromate 0.5% (both Almirall Hermal, Reinbek, Germany), nickel sulfate 5% and MCI/MI 0.01% [both SmartPractice Europe, Barsbüttel, Germany (MCI/MI ratio 3:1)]. After 48 h the chambers were removed. The strength

of the patch test reaction was graded after 72 h (1+, 2+ or 3+).¹⁷ Each skin site was tape stripped using adhesive tape (1.5 cm², D-Squame, CuDerm, Dallas, TX, U.S.A.).¹⁸ Eight consecutive tapes from each skin site were collected. For the analysis, the sixth and seventh tape strips were used. The samples were extracted from the tapes by 0.5 mL of phosphate buffered saline containing 0.05% Tween20 and sonicated for 15 min, as described previously.¹⁹ After vortexing, the extract aliquots were distributed in vials and stored at -80°C until analysis.

Multiplex analysis

The analysis of inflammatory mediators from the extracts was performed using a MESO QuickPlex SQ 120 assay (MSD, Rockville, MA, U.S.A.). The following cytokines and chemokines were included: eotaxin-1/CCL11, eotaxin-3/CCL26, granulocyte-macrophage colony-stimulating factor (GM-CSF)/CSF2, IL-1 α , IL-1 β , IL-1 receptor antagonist (RA), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-8 ha, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17a, IL-18, interferon (IFN)- γ -induced protein (IP)-10/CXCL10, monocyte chemoattractant protein (MCP)-1/CCL2, MCP-4/CCL13, macrophage-derived chemokine (MDC)/CCL22, macrophage inflammatory proteins (MIP)-1 α /CCL3, MIP-1 β /CCL4, thymus and activation regulated chemokine (TARC)/CCL17, TNF- α , TNF- β and vascular endothelial growth factor (VEGF). These inflammatory mediators were analysed using off-the-shelf panels (Human V-Plex Proinflammatory Panel 1 Kit, human IL-18, vascular and growth factor panel and a cytokine panel (MSD), according to the manufactures' instructions. All these kits use human antibodies. As the amount of SC on the tape varies, the concentrations of the mediators were normalized for the total amount of protein on the tape, which was determined with a Pierce Micro BCA protein assay kit (Thermo Fischer Scientific, Rockford, IL, U.S.A.).²⁰

Statistics

Data were analysed by GraphPad prism[®] v. 6.07 (GraphPad Software, La Jolla, CA, U.S.A.). Data are shown as the mean $\pm$ SEM. The concentrations of the mediators on each skin site investigated were compared with the corresponding petrolatum control using a Wilcoxon matched-pairs rank test. Subsequently, a Kruskal-Wallis test followed by a Dunn's multiple comparison test was used to test the difference between allergens, which was also used to test the difference between allergens and SLS. The association between the clinical severity of the response and the levels of inflammatory mediators was assessed using Spearman's correlation test.

Power analysis

This was an exploratory study and the levels of most of the inflammatory mediators included in the SC have never previously been measured in ACD. In our previous study on

cytokine and chemokine levels in patients with atopic dermatitis, we found 0.16 mean values (SD 0.18) pmol mL⁻¹ of the proinflammatory cytokine IL-1 β .²¹ For the power analysis, we assumed a twofold increase. On basis of these measurements, 10 participants per allergen were required for the generally recommended level of 0.80.²²

Results

Overall, 25 individuals had a total of 34 positive patch reactions: 11 to nickel, 11 to chromium, eight to MCI/MI and four to PPD. One PPD patient had a severe reaction, so that sampling was impossible. Four of the patients tested with chromium (n = 1), MCI/MI (n = 1), nickel (n = 1) and PPD (n = 1) did not show an allergic response to the relevant allergen. The average strength of the patch test reactions was 1.5 + nickel, 1.5 + chromium, 1.8 + (MCI/MI), 2.5 + (PPD) and 1.6 + (SLS). Compared with the corresponding petrolatum controls, 18 of the 32 quantified inflammatory mediators showed significant levels of change for at least one of the investigated allergens or SLS (Figs 1, 2). In general, the largest changes compared with petrolatum controls were observed for chemotactic cytokines and T-(memory) cell recruiters (Fig. 2). The levels of TARC/CCL17, MDC/CCL22, MCP-1/CCL2 and MIP-1 β /CCL4 in patch test reactions to both SLS and allergens were elevated, although a level of significance of $P < 0.05$ was not attained in all cases. In contrast to chemokines, IL-1 cytokines and other mediators of the innate immune system showed the opposite trend. The concentration of IL-1 α was significantly decreased in test reactions to SLS as well as to allergens, with the exception of PPD, to which, however, only three individuals were positive. Other mediators with decreased levels were IL-1 β (SLS and nickel), IL-1RA (SLS), IP-10 (SLS), IL-2 (SLS), IFN- γ (MCI/MI) and IL-7 (MCI/MI).

When comparing the patch test reactions with different allergens, in general MCI/MI showed the highest magnitude of change in the levels of immunomediators, with significantly increased values for seven mediators and decreased values for four. In most cases (IFN- γ , IL-8/CXCL8, IP10/CXCL10, MDC/CCL22, eotaxin-1, IL-7 and VEGF), only patch test reactions to MCI/MI differed significantly from the control samples.

In general, there were no striking differences in the profiles of inflammatory mediators between patch test reactions to SLS and allergens. The only mediator that showed a significantly increased level in reactions to all allergens but not to SLS was IL-16. Furthermore, in contrast to the allergens, only SLS induced significant changes in the levels of IL-2, TNF- α , MIP-1a and IL-12p40 compared with the corresponding petrolatum controls.

A correlation analysis (Table 1) was performed to evaluate the association between the patch test reactions and the levels of inflammatory mediators. The strongest positive correlations were found for innate system-related cytokines IL-6 ($r = 0.63$, $P < 0.001$) and IL-8/CXCL8 ($r = 0.76$, $P < 0.001$) and for the cell recruiting mediators MIP1b/CCL4 ($r = 0.66$, $P < 0.001$) and MCP1/CCL2 ($r = 0.81$, $P < 0.001$).

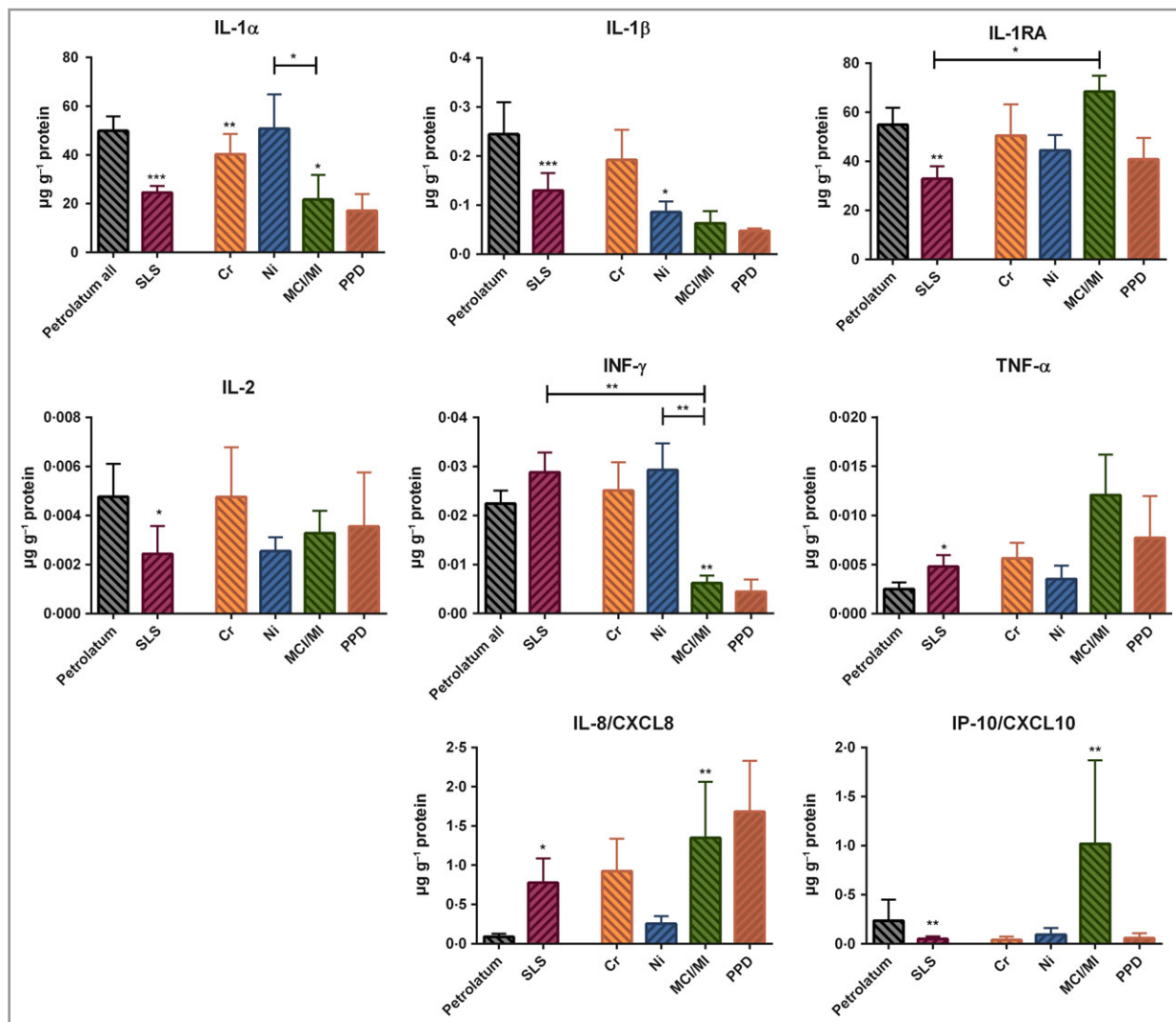


Fig. 1. Concentration of interleukin (IL)-1 and innate immunity related cytokines and chemokines of skin sites tested with chromium, nickel, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), *para*-phenylenediamine (PPD), sodium lauryl sulfate (SLS) and petrolatum. The results of petrolatum and SLS are averaged for all patients ($n = 25$). Data are given as mean with standard error of means (SEM). A Kruskal–Wallis test followed by Dunn's multiple comparison test was used to investigate differences between allergens as well as between allergens and SLS. Significant differences between allergens or SLS and their corresponding petrolatum controls, are given directly above the SEM error bar (Wilcoxon matched-pairs signed-rank test). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. INF- γ interferon- γ ; TNF- α , tumour necrosis factor- α .

Furthermore, TARC/CCL17 ($r = 0.67$, $P < 0.001$) and IL-16 ($r = 0.69$, $P < 0.001$), TNF- α ($r = 0.60$, $P < 0.001$) showed significant positive correlations. Negative correlations were found for IL-1 α ($r = -0.69$, $P < 0.001$) and IL-12p40 ($r = -0.72$, $P < 0.001$). As an illustration, data for IL-1 α , IL-16 and IL-8 have been plotted in Figure 3.

Discussion

The present study shows that contact allergens and SLS induce changes in a large number of immunomediators in the SC, which can be detected by a relatively noninvasive procedure.

Overall, the profiles of most mediators in the patch test reactions were similar among the investigated allergens and SLS, reflecting common inflammatory pathways. However, the

levels of IL-16 seemed to be indicative for ACD, as they were significantly increased in patch test reactions to all allergens, but not to SLS. Here, it may be noted that the increase of IL-16 levels did not reach significance for PPD, probably due to small number of patients in this study ($n = 3$). IL-16 plays a major role in skin hypersensitivity by the chemoattraction of CD4 + T cells.²³ Masuda *et al.* showed that IL-16 is produced by epidermal cells, especially keratinocytes, during the sensitization and elicitation phases of hapten-induced contact hypersensitivity. In agreement with the results of the present study, Masuda *et al.* found that the application of haptens and not of primary irritants or of the control vehicle induces IL-16 production in the skin. Interestingly, Reich *et al.* showed that polymorphisms in gene-encoding IL-16 influence susceptibility to contact allergy.²⁴

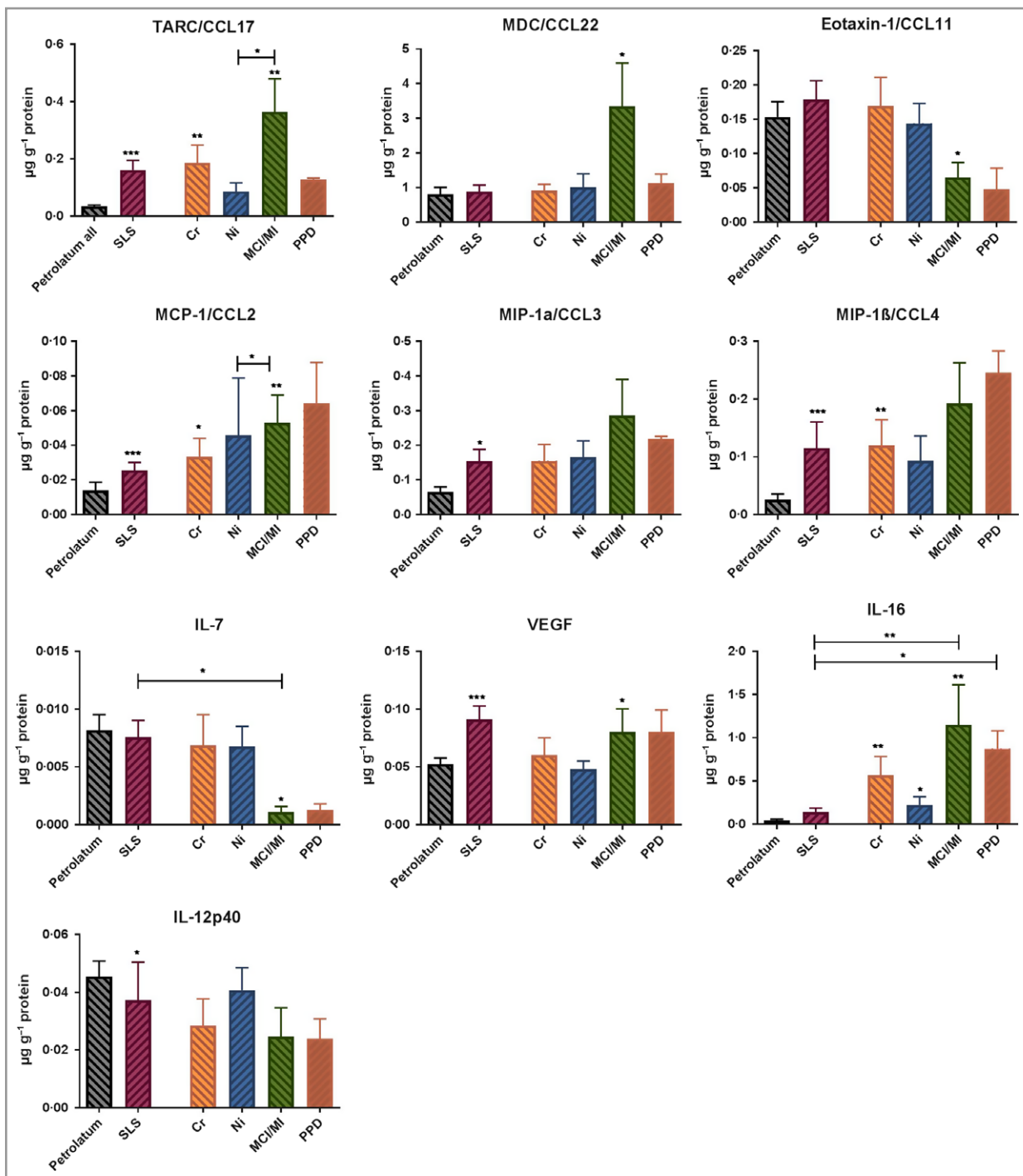


Fig. 2. Concentration of chemotactic and T-cell proliferation related cytokines and chemokines from the skin sites tested with Cr, Ni, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), para-phenylenediamine (PPD), sodium lauryl sulfate (SLS) and petrolatum. The results of petrolatum and SLS are averaged for all patients ($n = 25$). Data are given as mean with standard error of means (SEM). A Kruskal–Wallis test followed by Dunn's multiple comparison test was used to investigate differences between allergens as well as between allergens and SLS. Significant differences between allergens or SLS and their corresponding petrolatum controls, are given directly above the SEM error bar (Wilcoxon matched-pairs signed-rank test). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. TARC, thymus and activation regulated chemokine; VEGF, vascular endothelial growth factor.

When comparing allergens, patch test reactions to MCI/MI consistently showed the largest difference compared with the petrolatum controls, although similar patch test reactions were

induced by the allergens investigated. Of the 32 mediators that could be determined in the samples, 11 showed a significant difference in MCI/MI. Most of these belong to the keratinocyte-

Table 1 Correlation of patch test reaction and the levels of inflammatory mediators

	Spearman's correlation coefficient <i>r</i>	P-value
Eotaxin-1/CCL11	-0.30	0.09
Eotaxin-3/CCL26	-0.28	0.12
GM-CSF/CFS2	-0.29	0.10
IL-1 α	-0.67	0.001
IL-1RA	0.57	0.001
IL-1 β	-0.07	0.71
IL-2	0.29	0.10
IL-4	0.46	0.01
IL-5	-0.31	0.08
IL-6	0.56	0.001
IL-7	-0.06	0.73
IL-8	0.74	0.001
IL-8 (HA)	0.56	0.001
IL-10	-0.38	0.03
IL-12p40	-0.64	0.001
IL-12p70	-0.68	0.001
IL-13	-0.07	0.68
IL-15	-0.39	0.02
IL-16	0.67	0.001
IL-17A	-0.23	0.19
IL-18	0.51	0.003
IFN- γ	-0.48	0.004
IP-10/CXCL10	0.57	0.001
MCP-1/CCL2	0.80	0.001
MCP-4/CCL13	-0.36	0.04
MDC	0.36	0.04
MIP-1 α /CCL3	0.42	0.02
MIP-1 β /CCL4	0.64	0.001
TARC/CCL17	0.64	0.001
TNF- α	0.60	0.001
TNF- β	-0.39	0.02
VEGF	0.53	0.002

GM-CSF/CFS2, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IL-1RA, IL-1 receptor antagonist; IFN- γ , interferon- γ ; IP-10/CXCL10, interferon gamma-induced protein-10; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TARC, thymus and activation regulated chemokine; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

derived cytokines (IL-1 α , IL-16, TARC/CCL17, MCP-1/CCL2, IL-8/CXCL8, eotaxin-1/CCL11, IP-10/CXCL10). Among Th2-related mediators, concentrations of MDC/CCL22 and TARC/CCL17 were significantly higher than the petrolatum controls in patch test reactions to MCI/MI (MDC/CCL22) and MCI/MI and chromium (TARC/CCL17). In a recent study by Dhingra *et al.* increased mRNA expression of MDC/CCL22 and TARC/CCL17 was detected in biopsies from patch test reactions to nickel, rubber, fragrance mix, cobalt and potassium dichromate.⁷ An amplification loop has been suggested for TARC/CCL17 and MDC/CCL22, as both are regulated by IL-4 and increase their production by recruiting IL-4 releasing Th2 cells.¹⁴ In the present study, we found an increase in levels of TARC/CCL17 in

patch test reactions to MCI/MI and chromium but not to nickel, as shown by Dhingra *et al.*⁷ This discrepancy could be explained by the lower reaction severity to nickel in the present study, compared with that of Dhingra *et al.* (1.5+ vs. 2.1+, respectively).⁷

The levels of monocyte-derived chemotactic mediators (MCP-1, MIP-1a, MIP-1b) were increased in patch test reactions to allergens as well to SLS. Although not all these differences reached a level of significance, this may indicate there are common inflammatory pathways for allergens and irritants. While the concentrations of most mediators were increased in patch test reactions compared with their corresponding petrolatum controls, decreased values were found for several mediators, mainly those that are representatives of innate immunity, including IFN- γ (MCI/MI), IL-1 α (chromium, MCI/MI, SLS), IL-1 β (nickel, SLS), IL-7 (MCI/MI), IP-10 (SLS) and IL-1RA (SLS). IL-1 α levels in the SC have previously been shown to decrease after exposure to various skin irritants, which is in agreement with the effect of SLS found in the present study.^{25,26} The opposite, however, an increase of IL-1 α , has been found in the epidermis.²⁷ This discrepancy in the pattern of IL-1 α levels between these two skin strata should probably be sought in the contribution of the preformed pool of IL-1 α in the SC. Damage of the SC causes the release of these primary cytokines stored in the corneocytes and their gradual depletion in the SC intercellular matrix. This is supported by the negative correlation between IL-1 α and the strength of patch test reactions. Therefore, it may be speculated that the significant decrease of IL-1 α in patch test reactions to chromium and MCI/MI may, at least partly, be caused by the inherent skin damaging properties of these allergens.²⁸

The magnitude of changes of the inflammatory mediators in patch test reactions to MCI/MI as compared with other allergens may also be explained by its skin damaging properties, which are associated with skin barrier damage and the induction of innate immune responses. Esser *et al.* have shown a correlation between the ability of an allergen to activate innate immune responses and its allergenic potency.^{29,30} MCI is a lipophilic molecule and is small enough to cross the epidermal barrier (Kow = 2.5; MW 111 Da). Furthermore, in a previous study we demonstrated that MCI/MI induces morphological cell changes that are distinctly different from that of other allergens (Koppes *et al.*, submitted for publication) suggesting it has effects on the integrity of the skin barrier.

Recently, the important role of innate immune system in ACD has been revealed. Interestingly, metals such as nickel, cobalt and palladium are capable of directly activating innate immune signalling pathways via TLR-4 and nuclear factor- κ B.^{8,9} However, not all metals are able to do this, for instance, chromium and iron cannot.⁹ The present study did not detect major differences between nickel and other allergens; however, this may be due to the fact that sampling was limited to the SC. To gain more insight into the mechanism biopsies samples would be more suitable.

Some limitations of the present study should be considered. The levels of inflammatory mediators are probably time and

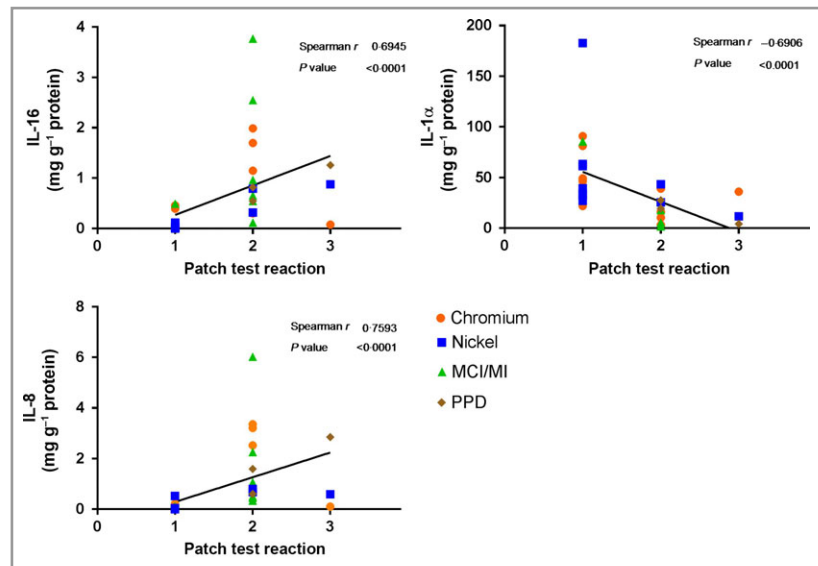


Fig. 3. Correlation between the strength of patch test reactions and the levels of IL-16, IL-1α and IL-8.

concentration dependent. For example, an initial decrease of IL-1α levels is induced by damage to the skin barrier and the gradual depletion of the preformed pool of IL-1α in the SC. However, as a consequence of inflammation, *de novo* synthesis of IL-1α will occur in epidermis, which will result in an increase in IL-1α levels. Thus, the actual concentration of IL-1α depends on the kinetics of these two processes. A longer study period and the measurement of cytokine profiles at more time points and with a series of concentration dilutions would allow for the detection of possible temporal and concentration variations existing between allergens as well as between individuals. To compensate for interindividual variation in immune response to various allergens it would be interesting to study polysensitized patients.

Moreover, we looked at the concentrations in the upper part of the skin, the dead corneocytes, and not in the viable epidermis where many inflammatory mediators are formed and where they primarily exert their activity; although, as shown here, the SC sampling method offers a non-invasive approach to measuring a wide range of inflammatory mediators *in vivo* in the skin. Lastly, objective assessment of patch test reaction, for example, by using skin bioengineering techniques (such as transepidermal water loss, erythema meter, imaging techniques, etc.) may improve the interpretation of the results. Nevertheless, current guidelines on allergy patch testing are based on clinical scoring performed by a trained dermatologist.¹⁷

In conclusion, this study suggests that differences in SC profiles of inflammatory mediators exist between ACD and ICD as well as between the allergens investigated, although most of them show similar patterns. MCI/MI induces larger and broader immune responses than the other tested allergens. The decreased levels of IL-1α in patch test reactions to MCI/MI and chromium may suggest their inherent irritant capacities play a role. Of the mediators investigated, IL-16 is the most promising marker in the SC for differentiating between ICD

from ACD. Additional investigations are needed to evaluate the possible influence of allergen or irritant concentration and interindividual variations.

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