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Differential cytokine expression in skin after single and repeated irritation by sodium lauryl sulphate

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Abstract

Background In vivo levels of cytokines and presence of neutrophils and eosinophils in skin irritation are not well known.

Objectives Our objective was to get more insight in inflammatory mediators and markers involved in single and repeated skin irritation.

Methods We sampled epidermis-derived fluid using a novel technology that includes application of a negative pressure on the skin after creation of micropores in the stratum corneum by a laser. In nine volunteers, transdermal fluid was sampled after a single 4-h 10% sodium lauryl sulphate (SLS) exposure and a repeated 3-week exposure (0.1% SLS). Twenty-seven cytokines were assessed by multiplex assay, and IL-1α, eosinophil cationic protein (ECP) and myeloperoxidase (MPO) by ELISA.

Results Levels of ECP were increased after irritation and correlated with levels of MPO. The levels of inflammatory mediators showed large interindividual differences in unexposed and exposed skin. Despite this variation, several mediators clearly showed increased levels: CC chemokine ligand (CCL)11, CXCL10 and vascular endothelial growth factor after both single and repeated exposure, interleukin (IL)-1α and basic fibroblast growth factor after single exposure and IL-1 receptor antagonist (IL-1RA) after repeated exposure. After repeated exposure, CCL5 and the ratio IL-1RA/IL-1α both increased compared to single exposure.

Conclusions We conclude that single and repeated irritation induces differential and concerted expression of various inflammatory mediators and markers.
Introduction

Repetitive contact of the skin to irritants may lead to chronic irritant contact dermatitis (ICD). Chronic ICD is often located on the hands and forearms, and the eczematous reaction is characterised by dryness, redness, scaling and hyperkeratosis.1 Workers involved in wet work or having frequent skin contact with chemicals, for example hairdressers,2,3 nurses4,5 and metal-workers,6 constitute a high-risk group for this occupational skin disease. Despite the high prevalence of ICD in high-risk occupations, little is known about the mechanism of the development of chronic ICD and the factors that predispose individuals to this skin disease.

Immunological processes play an important role in ICD: cytokines and chemokines orchestrate inflammation and repair of the skin.7-9 In vivo data on cytokine levels in skin irritation is scarce and limited. Several researchers used a single exposure with the irritant sodium lauryl sulphate (SLS) to study the cytokine response in acute ICD.10-16 In our previous study, using stratum corneum (SC) tape stripping, we found an increase in the ratio between interleukin-1 receptor antagonist (IL-1RA) and interleukin-1 \( \beta \) (IL-1\( \beta \)) in the SC after repeated SLS exposure,17 in contrast to the decreased ratio found after a single SLS exposure.18 To get a further insight in the immunological processes in the skin during acute and repeated skin irritation, sampling procedures that allow for the analysis of multiple inflammatory mediators and markers are required.

A new minimally invasive technology has recently been developed to obtain transdermal fluid (TDF) by creating micropores in the SC with an infrared laser and continuous TDF harvesting using a negative pressure.19,20 The micropores are approximately 100 \( \mu \)m in diameter and involve the SC and some tens of \( \mu \)m of the epidermis.19-21 This technology has originally been developed for real-time glucose monitoring. In the present study, we explored the feasibility of this new TDF sampling technique for studying epidermal inflammatory mediators and markers. In addition, we studied differences in levels of these substances as a response to single and repeated skin irritation.

To determine the levels of multiple cytokines and chemokines simultaneously in one single TDF sample, we used a recently developed multiplex bead array assay (Luminex, Austin, TX, USA).22 We selected a 27-plex assay in which the inflammatory mediators can be divided in five categories: (i) pro-inflammatory cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-\( \gamma \) (IFN-\( \gamma \)), IL-1\( \beta \), IL-6, IL-17 and tumour necrosis factor-\( \alpha \) (TNF-\( \alpha \)); (ii) anti-inflammatory cytokines: IL-1RA, IL-10; (iii) immunoregulatory
cytokines: IL-2, IL-4, IL-5, IL-7, IL-12, IL-13 and IL-15; (iv) chemokines: CC chemokine ligand 2 (CCL2)/monocyte chemotactic protein-1 (MCP-1), CCL3/macrophage inflammatory protein-1α (MIP-1α), CCL4/MIP-1β, CCL5/regulated upon activation normal T cell expressed and secreted (RANTES), CCL11/eotaxin, CXCL8/IL-8, CXCL10/interferon-γ inducible protein (IP-10) and (v) various growth factors: fibroblast growth factor (basic FGF), granulocyte colony-stimulating factor (G-CSF), IL-9, platelet derived growth factor (PDGF-bb) and vascular endothelial growth factor (VEGF). As IL-1α was not included in this panel, this pro-inflammatory cytokine was determined by enzyme-linked immunosorbent assay (ELISA). Besides cytokines, we also determined eosinophil cationic protein (ECP) and myeloperoxidase (MPO) which are considered inflammatory markers for presence of eosinophils and neutrophils in irritated skin.23-25

In nine healthy volunteers, we performed on the forearm both a single 4-h 10% SLS irritation test, which is commonly used as a predictive test for acute skin irritation,26 and a repeated 3-week irritation test (0.1% SLS), which is used as a model for chronic skin irritation.17,27-29 The skin reaction was monitored by measuring transepidermal water loss (TEWL) and erythema during and after these SLS exposures. One day after the single exposure and one day after the last repeated exposure, TDF was sampled from these sites and from an unexposed control site. This is the first study in which a large number of inflammatory mediators and markers have been investigated in epidermis derived fluid obtained from normal and SLS-irritated skin.

Materials and methods

Volunteers
Nine healthy volunteers, five males and four females, with a mean age of 28 years (range 20-53 years), with no visible skin abnormalities and no history of skin disease, participated in the study which was carried out in April and May 2006. Written, informed consent was obtained from all subjects prior to participation. The study was approved by the Ethics Committee of the Academic Medical Center, Amsterdam and was conducted according to the principles of the Declaration of Helsinki. The subjects were not allowed to (i) use soap or moisturizers on their forearms during the whole study, (ii) sunbathe or use a tanning bed 2 months before, and during, the study and (iii) use corticosteroids during the study.
Single 4-h exposure to SLS

The subjects were exposed for 4 h at two sites on the dominant mid-volar forearm to 10% SLS in water (200 μl, ≥ 99% purity, Fluka, Buchs, Switzerland) using patch test chambers (Finn chambers® of 18-mm diameter and Filter Paper Discs; Epitest, Tuusala, Finland) attached with adhesive tape (Scanpor® tape; Norgeplaster, Vennesla, Norway). Before application and 16-18 h after the patch removal, TEWL and erythema were measured at the exposed sites and at a control site on the volar forearm. TEWL was measured using an evaporimeter (VapoMeter SWL2g, Delfin Technologies, Kuopio, Finland). Nuutinen et al. described this measurement device in detail.30 Twenty minutes prior to the measurement, the subjects rested with their sleeves rolled up in the examination room, where the temperature was 20-22°C and the relative humidity ranged from 30% to 40%. The erythema index was measured using an erythema meter (DermaSpectrometer; Cortex Technology, Hadsund, Denmark), as described by Clarys et al.31

Repeated 3-week exposure to SLS

Two sites on the non-dominant mid-volar forearm were exposed to a 0.1% SLS solution (200 μl) for 6 h a day, 4 days a week, for 3 weeks using similar patches as for the 4-h SLS exposure. TEWL and erythema were measured at the exposed sites and at a control site before, during and after the exposure period (on days 0, 3, 7, 10, 14 and 18) (Figure 1). On the days when the patches were applied, the TEWL and erythema measurements were performed prior to patch application. The SLS concentration was adjusted after day 4 and day 11, when needed, to avoid too weak or too strong skin irritation reactions at the time of TDF sampling. When the increase in TEWL was < 10 g m⁻² h⁻¹ and in erythema was < 1.5 arbitrary units (AU) compared with the control site, the concentration was doubled. If the increase in TEWL was > 40 g m⁻² h⁻¹ and in erythema was > 3 AU, then the concentration was halved. In three subjects an adjustment was applied: in one subject, the concentration was adjusted to 0.05% in the second and third week; in two other subjects, the concentration was increased to 0.2% in the second week and readjusted to 0.1% in the third week.
Figure 1. Study design. Schedule of single and repeated sodium lauryl sulphate (SLS) exposures, measurement of transepidermal water loss (TEWL) and erythema, and sampling of transdermal fluid. SLS, sodium lauryl sulphate.

Collection of TDF

TDF samples were collected 16 h after the single SLS exposure and 18 h after the last repeated exposure (Figure 1). Also unexposed control skin on the dominant forearm was sampled. Two sampling sites were used simultaneously for TDF collection to ensure sufficient fluid for the analyses. All devices used in making micropores and harvesting TDF were manufactured and supplied by SpectRx Inc. (Norcross, GA, USA) and are described in detail elsewhere. To produce the micropores, a skin alignment ring containing a light-absorbing dye layer was placed on the collection site and subsequently the hand-held laser porator was positioned fitting inside the ring. Upon activation of the porator, four focused pulsed laser beams of infrared light are sequentially aimed at the dye spot, resulting in four micropores (<100 μm in diameter) in the SC. At each micropore position, the laser pulsed 10 times at 11 Hz with pulse duration of 30 ms. After removal of the dye paper, a harvesting head was positioned inside the alignment ring and over the pores. Next, the harvesting head was connected to a portable pump box by means of a transparent tubing, and a negative pressure of 380 mmHg was applied. After 4 h, the negative pressure was released and TDF was aspirated from the tubing using a syringe (MicroFine Insulin Syringe; BD, Franklin Lake, NJ, USA). The samples were weighed and diluted 1:1 in phosphate-buffered saline (PBS; Merck Darmstadt, Germany). After addition of 5 mM ethylenediaminetetraacetic acid (EDTA; Sigma Aldrich, Zwijndrecht, the Netherlands) and 1 mM phenylmethanesulfonyl fluoride (PMSF; Sigma Aldrich, Zwijndrecht, the Netherlands) the samples were stored at -20°C until required for analysis.
Protein assays

The IL-1α content of the TDF samples was measured using a specific ELISA kit (Human Cytosets, Biosource International, Camarillo, CA, USA). The assay was conducted according to the manufacturer’s instructions. The limit of detection was 3-4 pg/ml. Assessment of the levels of CCL2, CCL3, CCL4, CCL5, CCL11, CXCL8, CXCL10, basic FGF, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, PDGF-BB, TNF-α and VEGF in the TDF samples was performed using a Luminex-based multiplex system (Bio-Plex Human Cytokine 27-plex panel; Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer’s instructions. Samples were fourfold diluted. The levels of ECP and MPO in TDF samples were measured using an ELISA as described earlier. Total protein content was determined using the Micro BCA protein assay kit (Pierce, Rockford, IL, USA) with the bovine serum albumin supplied as standard. Samples were analysed following the instructions provided with the kit. Samples with cytokine concentrations below the limit of detection were given a value of ½ limit of detection. To take into account any possible effect of the varying sampling volume, we expressed our data as the total amount of cytokines in TDF obtained during the 4 h of sampling.

Statistics

All data are given as mean ± SD, unless otherwise indicated. To compare the data from the three treatments (control, single and repeated exposure), we used Student’s t-test for paired samples (when differences were normally distributed) and Wilcoxon signed ranks test (when differences were not normally distributed). To investigate the relation between the levels of different mediators for a specific treatment, we used Pearson’s correlation coefficient and if appropriate, normal distributions were obtained by log-transformation. To investigate the relation between differences in inflammatory mediators and markers between single and repeated exposure, e.g. ΔCXCL8_repeated - single vs. ΔECP_repeated - single, we used the Spearman’s rank correlation coefficient.
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Results

Single and repeated exposure
Sixteen hours after the single SLS exposure, the average TEWL had increased from $10.7 \pm 3.1$ (baseline) to $35.1 \pm 22.6$ g m$^{-2}$ h$^{-1}$ ($P = 0.05$) and erythema from $8.4 \pm 3.3$ to $10.3 \pm 3.5$ AU ($P = 0.006$). In the repeated exposure, TEWL increased during the first two weeks, with a partial recovery during the non-exposure days (data not shown). Remarkably, in the third week the increase in TEWL was less compared with the second week, indicating barrier recovery upon exposure. Erythema increased during the first two weeks and levelled off in the third week. Eighteen hours after the last repeated SLS exposure, before TDF sampling, TEWL had increased from $15.0 \pm 4.4$ (baseline) to $25.0 \pm 4.4$ g m$^{-2}$ h$^{-1}$ ($P < 0.001$) and erythema from $9.0 \pm 2.2$ to $11.6 \pm 2.4$ AU ($P < 0.001$). Change in TEWL ($\Delta$TEWL) after single exposure was $23.4 \pm 20.1$ g m$^{-2}$ h$^{-1}$ compared with $10.9 \pm 5.3$ g m$^{-2}$ h$^{-1}$ after repeated exposure ($P = 0.26$). Change in erythema ($\Delta$erythema) was $1.8 \pm 3.1$ AU after single exposure compared with $3.3 \pm 1.6$ AU after repeated exposure ($P = 0.12$).

TDF sampling
The median TDF sample volume was $97 \mu$l (range 63-120 $\mu$l) in unexposed skin and increased to $165 \mu$l (range 100-402 $\mu$l) after single exposure ($P = 0.02$) and to $186 \mu$l (range 140-250 $\mu$l) after repeated exposure ($P = 0.001$). No signs of oedema were visible in the subjects before and after TDF sampling. The TDF samples were always clear with no visible trace of blood. In one subject, no TDF was obtained after repeated exposure, possibly because of inadequate poration. The total protein level in TDF was $22 \pm 5$ mg/ml at both the control site and single-exposed site and increased to $26 \pm 5$ mg/ml at the repeatedly exposed site ($P < 0.01$).

ECP and MPO levels
Levels of ECP and MPO in TDF obtained from control, single and repeatedly exposed skin from each subject are shown in Figure 2. In one subject, ECP and MPO could not be determined in TDF from the control site, because of a limited TDF volume. The levels of ECP and MPO were measurable in all samples. Single and repeated exposure induced a twofold increase in the mean ECP level compared with control skin ($P = 0.04$ and $P = 0.01$, respectively). The mean MPO level also increased by a factor 2; however, this was not significant.
Levels of inflammatory mediators

In the TDF samples, we were able to detect 17 out of 28 inflammatory mediators. The results of the 13 more abundant mediators are shown in Figure 3 arranged in functional categories (pro- and anti-inflammatory cytokines, chemokines and growth factors). The levels of inflammatory mediators showed large interindividual differences, not only in absolute terms, but also in the changes as a result of single or repeated exposure. Despite this variation, several mediators showed increased levels: CCL11, CXCL10 and VEGF after both single and repeated exposure, IL-1α and b-FGF after single exposure and IL-1RA after repeated exposure (Figure 3). Interestingly, IL-9 levels dropped in both single and repeated exposed skin.

IL-1α and IL-1RA have been studied before in skin irritation. The mean levels of IL-1α almost doubled after single exposure compared with the control site (P = 0.03), whereas mean IL-1RA levels increased two- to threefold after repeated exposure compared with both single exposed and control sites (P = 0.008 and P = 0.05, respectively). This resulted in an almost threefold increased ratio between IL-1RA and IL-1α after repeated skin irritation compared
with single irritation (P = 0.05). Differential levels between single and repeated exposed skin were also found for CCL5, indicated by considerably enhanced levels after repeated exposure.

Only four mediators were present in a small number of samples (the limit of detection for the diluted TDF samples is given between brackets): CCL3 (2 pg/ml), three samples; IL-4 (10 pg/ml), four samples; IL-10 (3 pg/ml), two samples; IFN-γ (17 pg/ml), seven samples. The following mediators were not detected in any of the samples: IL-1β (4 pg/ml), IL-2 (79 pg/ml), IL-5 (78 pg/ml), IL-7 (104 pg/ml), IL-12 (88 pg/ml), IL-13 (71 pg/ml), IL-15 (4 pg/ml), IL-17 (3 pg/ml), GM-CSF (14 pg/ml), PDGF-BB (4 pg/ml) and TNF-α (16 pg/ml).
Figure 3. Amount of pro- and anti-inflammatory cytokines (IL-1α, IL-1RA, the ratio IL-1RA/IL-1α, IL-6), chemokines (CCL2, CCL4, CCL5, CCL11, CXCL8, CXCL10) and various growth factors (b-FGF, G-CSF, IL-9 and VEGF) obtained by 4-h sampling of transdermal fluid by suction. Samples were obtained from unexposed skin (control, n = 9), 16 h after a 4-h 10% SLS irritation test (single, n = 9) and 18 h after a repeated 3-week 0.1% SLS irritation test (repeated, n = 8); — indicates the geometric mean, Student’s t-test or Wilcoxon signed ranks test for paired samples. SLS, sodium lauryl sulphate.
Relations between inflammatory mediators and markers

Assuming that the presence of eosinophils and neutrophils reflects inflammation, ECP and MPO may be taken as surrogate markers of inflammation. Interestingly, ECP and MPO levels were highly correlated in control, single and repeated exposed skin (r ranging from 0.93 to 0.98, P < 0.001; data not shown), indicating similar recruitment of both inflammatory cell types during skin inflammation. We expected that ECP and MPO levels would be influenced by chemokine levels, and indeed we found strong correlations with CCL2, CCL4, CCL5 and CXCL8 as well as with non-chemokines G-CSF, IL-6 and IL-9 (r ranging from 0.72 to 0.93, P < 0.05; data not shown) in control skin. After single exposure, the correlations with most chemokine levels disappeared and only CXCL8 correlated with both ECP and MPO (r = 0.73, P = 0.03 and r = 0.86, P = 0.003, respectively) and IL-6 with only MPO (r = 0.74, P = 0.02). In repeatedly exposed skin, there was only a correlation for both IL-6 and CCL2 with ECP and MPO (r > 0.84, P < 0.01), and in addition, for CCL4 and CCL5 with only MPO (r > 0.77, P < 0.03).

We noticed that some individuals showed reduced ECP and MPO levels after repeated exposure as compared with single exposure, whereas in others the ECP and MPO levels increased. We supposed that differences in ECP and MPO levels between single and repeated exposure (ΔECP and ΔMPO, respectively) may be induced by an altered expression of chemokines and other mediators, e.g. ΔCXCL8 (data not shown). A positive correlation was found for ΔCXCL8 and ΔCCL4 (r ranging from 0.79 to 0.95, P < 0.02) with ΔECP and ΔMPO, and an inverse relation for ΔIL-1RA and ΔG-CSF (r ranging from -0.76 to -0.88, P < 0.03).

Further, no relations were found between levels of ECP and MPO obtained from the three skin sites and skin irritation response as determined by TEWL and erythema.

Discussion

In the present study, we used a novel TDF sampling method to study differences in levels of inflammatory mediators and markers after SLS-induced skin irritation in human volunteers. With this sampling technique, we obtained sufficient volumes of TDF for the multiplex cytokine assay and for the determination of IL-1α, ECP and MPO by ELISA. We were able to detect 17 different inflammatory mediators and the markers ECP and MPO in the TDF sam-
Cytokine expression in skin irritation by SLS

We found enhanced levels of ECP in SLS-irritated skin. Increased levels of ECP were also shown in lesional skin of atopic dermatitis patients. ECP and MPO are markers of eosinophilic and neutrophilic granulocytes, respectively, which are released from activated granulocytes by degranulation. In this study, the levels of ECP and MPO were determined in TDF that was frozen after sampling. Therefore, we cannot determine whether these markers were released by activated cells in the skin or originated from non-activated cells by freeze-thawing which disrupts the intracellular granules containing ECP and MPO.

In agreement with earlier studies based on measurement of cytokines in the SC, we showed that IL-1α was increased after single irritation, whereas levels of IL-1RA were increased after repeated irritation. As the pro-inflammatory effects of IL-1α are counteracted by the production of the anti-inflammatory cytokine IL-1RA, the balance between these proteins partly determines the inflammatory response. The ratio of IL-1RA/IL-1α was increased after repeated irritation, which was primarily due to an increase in the production of IL-1RA, whereas no change in the ratio of IL-1RA/IL-1α was observed in single irritation. These findings possibly suggest that, during repetitive skin contact with irritants, anti-inflammatory mechanisms come into play to suppress the early pro-inflammatory response upon a new insult. In addition to IL-1α and IL-1RA, the chemokine CCL5/RANTES also showed an increased expression after repeated exposure compared with single exposure. The abundant presence of CCL5 in a repetitive SLS exposure might indicate an increase in CCL5-producing cells, e.g. monocytes and eosinophils attracted to the site of inflammation due to the ongoing skin insults induced by irritants. CCL11/eotaxin and VEGF levels in single and repeated exposed skin were enhanced as compared with unexposed skin. Enhanced levels of CCL5 and CCL11 have been shown in skin diseases, e.g. atopic dermatitis and psoriasis. Further, a potential role of the angiogenic factor VEGF in skin irritation has also been shown earlier: in vitro levels of VEGF increased almost fivefold after a single SLS exposure of human keratinocytes. Two inflammatory mediators not previously shown were found to be increased in irritated skin: the chemokine CXCL10/IP-10 and the angiogenic growth factor b-FGF. Furthermore, we have shown the presence of IL-9, a growth factor for T-cells and mast cells, in TDF obtained from unexposed skin in six subjects. To our knowledge, this is the first time
that IL-9 was detected in normal skin, though Morhenn et al. showed the presence of IL-9 mRNA in SC tape strips.\textsuperscript{44}

Based on literature data on \textit{in vivo} SLS exposure, we had expected to detect the early pro-inflammatory cytokines GM-CSF, IFN-\(\gamma\), IL-1\(\beta\) and TNF-\(\alpha\).\textsuperscript{10,11,13,14,16} Probably, at the time of TDF-sampling in SLS exposed skin, which was 16-18 h after the irritation tests, the levels of these cytokines might have already decreased below our limit of detection. Hunziker et al. showed that the levels of TNF-\(\alpha\) measured in skin-derived lymph had approached basal levels 1 day after SLS exposure.\textsuperscript{14} IFN-\(\gamma\) is a very unstable molecule; therefore degradation of this compound during sampling and processing of TDF might have occurred.\textsuperscript{45} A remarkable finding is the absence of IL-10, a cytokine with anti-inflammatory properties, in the TDF samples of irritated skin. Various investigators showed the presence of IL-10 in skin biopsies after SLS exposure, which was already detectable at 6 h and persisted until 72 h.\textsuperscript{16} Consistent with our expectations, we did not detect the typical immunoregulatory cytokines (e.g. IL-2, IL-4, IL-5, IL-7, IL-12, IL-13 and IL-15).

The question might arise whether the cytokine levels in TDF are influenced by the sampling method, i.e. poration and suction. Upon stimulation, for example by trauma, keratinocytes and fibroblasts have been shown to release a wide array of inflammatory mediators, e.g. IL-6, CCL2, CCL4, CCL5, CXCL8, G-CSF and VEGF.\textsuperscript{38,40,46,47} These responses can occur very rapidly, for instance the production of IL-6 can increase as early as 15 min after an insult.\textsuperscript{48} On the other hand, it could be speculated that the presence of these inflammatory mediators in the non-irritated skin is caused by a process called ‘immunosurveillance’. In this process, cytokines and chemokines in the skin continuously attract inflammatory cells, e.g. natural killer cells, dendritic cells and T-cells, which guard against invading pathogens and tumors.\textsuperscript{49-52} More research is needed to elucidate to which extent the cytokine levels in unexposed skin are genuine or induced by the sampling procedure. However, regardless the fact that measured cytokine levels were possibly influenced by the sampling procedure, clear differences in cytokine levels were found between non-exposed, single and repeated exposed skin. It is remarkable that the levels of IL-9 decreased after SLS-irritation. Possibly, the cytokines produced by the keratinocytes disappear from the interstitial fluid by binding to an increased number of receptors as present on inflammatory cells, soluble receptors or proteins\textsuperscript{53} present in the irritated skin. We assume that it is unlikely that the decrease in cytokine levels was caused by a dilution effect due to oedema, as no signs of oedema were observed in our subjects.
In control skin, strong correlations were found between levels of inflammatory markers (ECP and MPO) and of inflammatory mediators (IL-6, CCL2, CCL4, CCL5, CXCL8, G-CSF and IL-9), as well as among the inflammatory mediators themselves (data not shown). This suggests a balanced interplay between these factors in the epidermis. However, after single and repeated SLS exposure, this concerted action seems to be disturbed, reflected in the loss of correlation between the inflammatory mediators and markers. When examining patterns of inflammatory mediators and markers in comparing single and repeatedly exposed skin, our data revealed that subjects showing higher levels of ECP and MPO after repeated exposure compared with single exposure, also showed the same pattern of CCL4 and CXCL8. Peculiarly, we found strong inverse relations between the markers ECP and MPO with both IL-1RA and G-CSF. The cytokine IL-1RA is known for its anti-inflammatory properties. Levels of the growth factor G-CSF, which is generally known as a stimulatory factor for granulocytes and monocytes and the production of cytokines, were in normal skin positively associated with levels of ECP and MPO. However, in irritated skin, we observed an inverse association, which might be in line with the growing evidence on the possible anti-inflammatory properties of G-CSF. Several investigators showed that the presence of G-CSF suppressed the production of pro-inflammatory cytokines of several cell types upon stimulation. The patterns described here might implicate that in irritated skin inflammation is controlled by a number of mediators only, and which may reflect individual susceptibility.

The pattern of barrier impairment and skin inflammation response induced by a 3-week repeated SLS exposure was similar as described in our previous study. This pattern was characterized by recovery of the skin barrier (‘hardening’), in the third week even though the skin was still daily exposed to the irritant. Further, we have confirmed our earlier findings on considerable interindividual differences in skin response to skin irritation. Also the levels of the inflammatory mediators and markers showed large interindividual differences. This has been demonstrated not only for normal skin, but also after single and repeated SLS exposure. This is in agreement with our previous study and that of other investigators.

For the broader application of the used sampling technique, some important issues have to be resolved. For the analysis, we needed considerable amounts of TDF, which made a sampling time of 4 h from two sampling sites necessary. This was particularly the case in unexposed skin, where the collected volume amounted to 97 μl. In SLS-exposed skin, this volume was almost two times higher. As addressed previously, the effect of the laser poration procedure on TDF cytokine levels needs to be elucidated. For example, the contribution of the po-
ration can be studied by making series of different numbers of micropores (e.g. 4, 8, 12 and 16) at the site of sampling. Another option is studying cytokine kinetics in time after poration.

In this study, we showed one of the many applications of this minimally invasive technique. This method might also be useful in studying the differences in inflammatory mediators and markers between irritant and allergic contact dermatitis. Furthermore, the levels of inflammatory mediators in other inflammatory skin diseases, e.g. atopic dermatitis and psoriasis, might be studied.

We have shown here for the first time, the presence of a wide panel of inflammatory mediators and markers in single and repeated skin irritation. We showed differences in the cytokine response after a 3-week SLS exposure compared with a single SLS exposure and we identified some mediators that may reflect individual susceptibility to irritation. However several of our findings have to be still elucidated in more extensive studies. Understanding the mechanism of chronic skin irritation is of great importance for the identification of factors associated with individual susceptibility to chronic ICD.

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