

Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF- α and IL-8/CXCL8 mRNA: new insights into the involvement of ‘alarmins’

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Background: The strip patch test is recommended if patch test results are presumed to be false-negative. It is under discussion whether the cutaneous inflammation induced by tape stripping is a pivotal element in enhancing the allergic contact dermatitis response.

Objectives: To investigate epidermal mRNA expression of mediators implicated in the regulation of inflammation induced by tape stripping according to our standardized protocol in human skin.

Patients/Methods: Thirty-one healthy volunteers (median age 26 years, range 18–62 years) participated in the study. We measured epidermal mRNAs coding for cytokines [tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin (IL)-1 α , IL-1 β , IL-33, IL-10, IL-8/CXCL8, chemokine (C–C motif) ligand 2 (CCL2)/MCP-1 (monocyte chemotactic protein-1), chemokine (C–C motif) ligand 5 (CCL5)/RANTES (regulated upon activation normal T-cell expressed, and presumably secreted), IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF)], an adhesion molecule [intercellular adhesion molecule type 1 (ICAM-1)], heat shock proteins (Hsp27, Hsp70 and Hsp90), and the inflammasome complex [NACHT-LRR-PYD-containing protein-3 (NALP3), apoptosis-associated speck-like protein containing a CARD (ASC)/CARD5 (caspase recruitment domain-containing protein-5) and caspase-1] in tape-stripped skin of the upper back relative to untreated skin of the upper back, using quantitative real-time reverse transcription polymerase chain reaction technology.

Results: Epidermal mRNA expression levels of TNF- α , Hsp90, Hsp70, IL-33, and IL-8/CXCL8 were significantly upregulated, whereas CCL5/RANTES expression was significantly downregulated 6 hr after standardized tape stripping.

Conclusions: Tape stripping the skin according to our standardized protocol shows immunostimulatory effects, with induction of keratinocyte-derived ‘alarmins’ and cytokines mounting an immune response, which may contribute to the increased sensitivity of the strip patch test versus the patch test.

Key words: epidermal barrier; heat shock proteins 70 and 90; inflammation; interleukin-8; interleukin-33; strip patch test; tape stripping; tumour necrosis factor- α . © John Wiley & Sons A/S, 2010.

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Tape stripping the skin prior to patch testing – the so-called strip patch test – is recommended if patch test results are presumed to be false-negative (1). The basic idea of this modification of patch testing is to increase the bioavailability of the test substance

in the deeper epidermal cell layers by removing the uppermost cell layers of the stratum corneum (2).

We recently proposed the first standardized protocol for uniform performance of the strip patch test (3) and subsequently verified its diagnostic

accuracy (4). In further studies, we showed a reproducible stratum corneum reduction of 31% with our strip patch test protocol (5), and its ability to considerably lower elicitation thresholds for test substances (6).

However, it is still controversial whether patch test reactions on tape-stripped skin may be false-positive because of the non-specific stimulation of the immune system (7). Thus, the exaggerated response to the test substance is believed to be irritant rather than allergic (8). In contrast, it is believed that non-persistent cutaneous inflammation, for example by mild irritant tape stripping, is a pivotal element in enhancing allergic contact dermatitis responses (9–11). In summary, the relevant mechanisms are still not fully understood (12).

To the best of our knowledge, only the study of Nickoloff and Naidu (13) has investigated acute barrier disruption of human epidermis by tape stripping, and they reported an increase in mRNA coding for tumour necrosis factor- α (TNF- α), interleukin (IL)-8, IL-10, interferon- γ (IFN- γ), intercellular adhesion molecule type 1 (ICAM-1), transforming growth factor (TGF)- α , and TGF- β . Hence, the aim of the present study was to expand our knowledge of mRNA expression changes of mediators that are involved in the regulation of inflammation and that may be induced by tape stripping according to our standardized protocol (3) in human epidermal skin. We therefore measured epidermal mRNAs coding for pro-inflammatory cytokines (TNF- α , IFN- γ , IL-1 α , IL-1 β and IL-33), an immunosuppressive/antagonist cytokine (IL-10), chemotactic cytokines [IL-8/CXCL8, chemokine (C-C motif) ligand 2 (CCL2)/MCP-1 (monocyte chemotactic protein-1), chemokine (C-C motif) ligand 5 (CCL5)/RANTES (regulated upon activation normal T-cell expressed, and presumably secreted)], growth-promoting cytokines [IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF)], an adhesion molecule (ICAM-1), heat shock proteins (Hsp27, Hsp70 and Hsp90), and the inflammasome complex [NACHT-LRR-PYD-containing protein-3 (NALP3), apoptosis-associated speck-like protein containing a CARD (ASC)/CARD5 (caspase recruitment domain-containing protein-5) and caspase-1] in tape-stripped skin of the upper back as compared with untreated skin of the upper back.

Patients, Materials and Methods

This single-centre, prospective, investigator-blinded, controlled study was approved by the ethics committee of the Medical Faculty of the Ruhr University Bochum (registration no. 2881; approved 2 February 2009). Procedures were explained in detail to all

subjects, who signed informed consent forms prior to participation.

Study population

Thirty-one healthy subjects participated in this investigation. Study enrolment took place between April and July 2009. For study inclusion, subjects had to be at least 18 years old and had to have clinically normal skin on the back. Exclusion criteria were pregnancy, lactation, intake of immunosuppressive or immune-modulating drugs such as glucocorticoids or ciclosporin, topical use of glucocorticoids on the back, intense UV exposure on the back within the 4 weeks prior to study enrolment, known local anaesthetic intolerance, and predisposition to keloid formation. The discontinuation criterion was intense discomfort in the test areas, but this criterion did not apply in any case.

Tape stripping and epidermal tissue sample

Tape stripping was performed according to our formerly proposed protocol for the strip patch test (3). Tape stripping was carried out with a 3M™ Blenderm™ surgical tape (3M™ Medica; 3M Deutschland GmbH, Neuss, Germany; metric 25 mm $\times$ 4.5 m; CE no. 0493–BSI 0086–class I; batch no. 201309, expiry date: 1 September 2013); any body hair was removed before stripping, with a 3M™ Clipper. In derogation from the original protocol, patch test substances were not applied.

On the upper back, three test areas were randomly marked with B, R and T, and received the following initial treatments: B, no tape strips (baseline value); R, tape strips down to the stratum lucidum (reference value for calculating tape strips for T according to the formula: $R_{\text{tape strips}} \times 11/26 = T_{\text{tape strips}}$ (3)); and T, calculated number of tape strips (target value). Randomization of the tape-stripped side of the upper back (comprising test areas R and T) was conducted according to a randomization list in the 31 subjects.

In brief, the adhesive tape was applied first on test area R without tension, parallel to the spine, using the fingertips to lightly smooth it onto the skin under gentle pressure for about 2 seconds (3). The adhesive tape was then removed in one smooth motion in the direction of adherence at an angle of 45°. After each stripping, a new piece of adhesive tape was applied on exactly the same test area. Stripping was performed until the stratum lucidum (break-off criterion: three small glistening spots, indicating almost complete removal of the stratum corneum) became visible. The necessary number of tape strips was then multiplied with the tape-specific correction factor, $cf = 11/26 \approx 0.42$. The calculated

Table 1. Primer sequences of all genes examined by quantitative real-time reverse transcription polymerase chain reaction

Gene symbol	Forward primer	Reverse primer
TNF- α	5'-CCCAGGCAGTCAGATCATCTTC-3'	5'-GCTTGAGGGTTTGCTACAACATG-3'
IFN- γ	5'-CAGGTCATTTCAGATGTAGCGGATA-3'	5'-TCTGTCACTCTCCTCTTTCCAATTC-3'
IL-1 α	5'-GAATGACGCCCTCAATCAAAGTAT-3'	5'-TCAAATTTCACTGCTTCATCCAGAT-3'
IL-1 β	5'-GATGGCTTATTACAGTGGCAATGA-3'	5'-CAGAGGTCCAGGTCCTGGAA-3'
IL-6	5'-CCTGAGAAAAGGAGACATGTAACAAGA-3'	5'-GGCAAGTCTCCTCATTGAATCC-3'
IL-8/CXCL8	5'-TGTGTGTAAACATGACTTCCAAGCT-3'	5'-GCAAAACTGCACCTTCACACAG-3'
IL-10	5'-GGCGCTGTCATCGATTCTT-3'	5'-CTCTTGGAGCTTATTAAGGCATTCT-3'
IL-33	5'-CACTGAAAACAGGTAGAAAAGCACAAA-3'	5'-CCTGATATACAAAAGGCAAAGCA-3'
CCL2/MCP-1	5'-CAGCAGCAAGTGTCCCAAAG-3'	5'-TCCAGGTGGTCCATGGAATC-3'
CCL5/RANTES	5'-CGTGCCACATCAAGGAGTAT-3'	5'-CGGTTCTTTCCGGTGACAAA-3'
GM-CSF	5'-CATGATGGCCAGCCACTACA-3'	5'-GATAATCTGGGTTGCACAGGAAGT-3'
ICAM-1	5'-AGCCAGGAGACACTGCAGACA-3'	5'-TGGCTTCGTCAGAATCACGTT-3'
Hsp27	5'-GCGTGTCCCTGGATGTCAAC-3'	5'-GTGTATTTCCGCGTGAAGCA-3'
Hsp70	5'-AGGAGGAACCACATGTTGAAGAG-3'	5'-TGAGAGGTCTCCATTTCTTCAGACT-3'
Hsp90	5'-GCTCCTGTCTTCTGGCTTCAGT-3'	5'-ATCTTCATCAATACCCAGACCAAGT-3'
NALP3	5'-GCTTCAGGTGTTGGAATTAGACAA-3'	5'-CTGGAGGTGAGAAGTGTGGAAAG-3'
ASC/CARD5	5'-AGCCAGGCCTGCACCTTTATAGA-3'	5'-GCAGCCACTCAACGTTTGTG-3'
Caspase-1	5'-TCACTGCTTCGGACATGACTACA-3'	5'-GGAACGTGCTGTGAGAGGTCTT-3'
RPL38	5'-TCACTGACAAAAGAGAAGGCAGAGA-3'	5'-TCAGTGTGTCTGGTTCAITTCAGTT-3'

TNF- α , tumour necrosis factor- α ; IFN- γ , interferon- γ ; IL, interleukin; CCL2/MCP-1, chemokine (C-C motif) ligand 2/monocyte chemotactic protein-1; CCL5/RANTES, chemokine (C-C motif) ligand 5/regulated upon activation normal T-cell expressed, and presumably secreted; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule type 1; Hsp, heat shock protein; NALP3, NACHT-LRR-PYD-containing protein-3; ASC/CARD5, apoptosis-associated speck-like protein containing a CARD/caspase recruitment domain-containing protein-5; RPL38, ribosomal protein L38 (housekeeping gene).

number of tape strips was then applied likewise on test area T.

Based on the epidermal mRNA expression kinetics reported by Nickoloff and Naidu (13), 6 hr after tape stripping we performed a small curettage biopsy on test areas B and T with a 4-mm round curette blade following minimal injection of local anaesthetic. A subgroup of 11 subjects additionally received a curettage biopsy on test area R.

Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)

Quantitative real-time RT-PCR was performed as previously suggested (14). Total cellular RNA was isolated from epidermal tissue samples with an RNeasy[®] Mini Kit (Qiagen, Chatsworth, CA, USA), following the manufacturer's protocol. Prior to cDNA synthesis, mRNA was digested with RNase-free DNase I (Roche Applied Science, Mannheim, Germany). cDNA was synthesized by reverse transcription from DNase I-treated RNA, using Multi-Scribe[™] reverse transcriptase enzyme and random hexamer primers (TaqMan[®] Reverse transcription reagents; Applied Biosystems, Foster City, CA, USA). RT-PCR was performed with a Power SYBR[®] Green PCR Master Mix (Applied Biosystems) and StepOne[™] Real-Time PCR System (Applied Biosystems). PCR primers for the 18 mediators and the housekeeping gene, the ribosomal protein L38 gene (RPL38), were

designed with the PRIMER EXPRESS 3.0 program (Applied Biosystems) and produced by the custom oligonucleotide synthesis service TIB MolBiol (Berlin, Germany). All primers were checked for cDNA specificity using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>). The primers are shown in Table 1.

PCR amplifications were performed in a total volume of 25 μ l, containing 5 μ l of cDNA sample, 0.5 μ M each primer and 12.5 μ l of Power SYBR[®] Green PCR Master Mix (Applied Biosystems). PCR was started with 2 min at 50°C and an initial 10-min denaturing temperature of 95°C, and this was followed by a total of 40 cycles of 15 seconds of denaturing and 1 min of annealing and elongation at 60°C.

Melting curve analysis was performed to identify all amplified PCR products. Target gene mRNA levels were normalized to the homogeneously expressed housekeeping gene RPL38. Expression levels of mRNA were calculated using the comparative Δ - ΔC_t method (15). In order to make the mRNA levels more illustrative, the ΔC_t values (logarithms) were retransformed.

Blinding

The biologist performing the RT-PCR (M.S.) was blinded with respect to the kind of treatment of the respective test area. Analysis of unblinded data was eventually performed by T.G.

Statistical analysis

Data analysis was performed using the statistical package MedCalc (Mariakerke, Belgium). Distribution of data was assessed by the D'Agostino-Pearson test. Medians including the range and 95% confidence intervals (CIs) were calculated. mRNA data were analysed using the Kruskal–Wallis ANOVA and the Wilcoxon *post hoc* test for paired data. Exploratory assessment of correlation was performed using the Spearman and Kendall Tau procedures, including CIs. As this was an exploratory study, no α -adjustment (Bonferroni) was performed. Statistical significance was set at the 5% level (two-sided).

Results

Study population

All 31 subjects finished the study according to the protocol. No adverse events from performing tape stripping were reported. Demographic and clinical characteristics of these subjects and their subgroup ($n = 11$) are summarized in Table 2. Test sides of the upper back receiving tape strips were balanced. Appearance of the stratum lucidum in test area R required a median number of 24 tape strips. After multiplication by the tape-specific correction factor [$cf = 11/26$ (3)], the 'median' subject received 10 tape strips in test area T.

Table 2. Demographic and clinical subject characteristics of the total ($N = 31$) and the subgroup ($n = 11$)

Characteristic	Value	
	Total	Subgroup
Age (years), median (min.–max., Q1, Q3)	26 (18–62, 24, 34)	30 (23–54, 26, 34)
Sex, n (%)		
Female	19 (61.3)	6 (54.5)
Male	12 (38.7)	5 (45.5)
Fitzpatrick skin type, n (%)		
I	4 (12.9)	1 (9.1)
II	20 (64.5)	8 (72.7)
III	7 (22.6)	2 (18.2)
IV–VI	0 (0.0)	0 (0.0)
Tape-stripped side of the back, n (%)		
Left	15 (48.4)	5 (45.5)
Right	16 (51.6)	6 (54.5)
Tape strips down to the SL (R), median (min.–max., Q1, Q3)	24 (15–35, 21, 28)	24 (15–35, 22, 30)
Tape strips calculated (T),* median (min.–max., Q1, Q3)	10 (6–15, 9, 12)	10 (6–15, 9, 13)

Q1, 25th percentile; Q3, 75th percentile; SL, stratum lucidum; R, test area with tape strips down to the stratum lucidum (reference value); T, test area with calculated numbers of tape strips (target value).

*Strips down to the SL $\times$ 11/26.

Epidermal mRNA expression levels

The RT-PCR data were non-normally distributed. For the total, medians with their range and differences of medians, including their 95% CIs, of epidermal mRNA expression levels are presented in Table 3. Six hours after tape stripping, epidermal mRNAs of TNF- α , IL-33, Hsp70, Hsp90, and IL-8/CXCL8 showed significantly higher expression in the test areas with the calculated numbers of tape strips (T) than in the test areas with no tape strips (B). In contrast, mRNA of CCL5/RANTES showed significantly lower expression in the epidermis 6 hr after tape stripping.

Among these, correlations of epidermal mRNA expression levels between IL-33 and Hsp70 ($r = 0.47$, 95% CI 0.14–0.71, $P = 0.011$), IL-33 and Hsp90 ($r = 0.63$, 95% CI 0.34–0.81, $P = 0.001$) and Hsp70 and Hsp90 ($r = 0.88$, 95% CI 0.76–0.94, $P < 0.0001$) were highly significant. Epidermal mRNA expression levels of Hsp90 were also significantly correlated with those of TNF- α ($r = 0.38$, 95% CI 0.03–0.65, $P = 0.039$). Epidermal mRNA expression levels of IL-8/CXCL8 were inversely correlated with those of CCL5/RANTES ($r = 0.54$, 95% CI 0.22–0.75, $P = 0.003$). Other significant correlations were not found (data not shown). Only differences in the median mRNA expression levels of Hsp90 and IL-33 were significantly correlated with the interindividually different calculated numbers of tape strips (Hsp90, $r = 0.45$, 95% CI 0.11–0.69, $P = 0.014$; IL-33, $r = 0.38$, 95% CI 0.03–0.65, $P = 0.035$). However, no correlations were seen between epidermal mRNA expression levels and age, sex or the Fitzpatrick skin type (data not shown).

For the subgroup of subjects with additional curettage biopsies from the test area with tape strips down to the stratum lucidum (R), the medians and range of epidermal mRNA expression levels are given in Table 4. Six hours after tape stripping, epidermal mRNAs of IL-1 β and Hsp90 showed significantly higher expression in the test areas with tape strips down to the stratum lucidum (R) than in the test areas with the calculated numbers of tape strips (T).

Discussion

This study is unique in presenting data on the upregulation of epidermal mRNA expression levels of TNF- α , Hsp90, Hsp70, IL-33 and IL-8/CXCL8 in human abraded skin of the upper back with calculated numbers of tape strips according to our proposed standardized strip patch test protocol (3). Tape stripping the skin of the upper back to the stratum lucidum as a simple-to-judge visual endpoint indicating almost complete removal of

Table 3. Epidermal mRNA expression levels in untreated skin (test area B) versus tape-stripped skin with calculated numbers of tape strips (test area T) of 31 subjects

Gene symbol	Untreated skin (B), median mRNA (range)	Tape-stripped skin (T), median mRNA (range)	Difference, median mRNA (95% CI)	T–B, <i>P</i> -value
TNF- α	0.37 (0–2.03)	0.48 (0–3.59)	0.08 (–0.05–0.27)	0.033
IFN- γ	0 (0–0.039)	0 (0–0.256)	0 (0–0)	0.743
IL-1 α	0.025 (0–0.073)	0.025 (0–0.107)	0 (–0.001–0.004)	0.450
IL-1 β	0.08 (0–0.54)	0.10 (0–0.89)	0.01 (–0.02–0.05)	0.519
IL-6	0 (0–0.020)	0 (0–0.015)	0 (0–0)	0.992
IL-8/CXCL8	0 (0–0.150)	0.006 (0–1.342)	0 (0–0.05)	0.013
IL-10	0.005 (0–0.254)	0.008 (0–0.523)	0 (–0.001–0.004)	0.487
IL-33	0.23 (0.04–0.54)	0.26 (0.03–1.14)	0.03 (–0.01–0.07)	0.036
CCL2/MCP-1	0.28 (0–0.75)	0.32 (0.10–1.87)	0.04 (–0.03–0.11)	0.100
CCL5/RANTES	0.007 (0–0.113)	0.006 (0–0.066)	–0.003 (–0.005–0)	0.002
GM-CSF	0.0005 (0–0.0024)	0.0003 (0–0.0031)	0 (–0.0006–0)	0.450
ICAM-1	0.011 (0–0.033)	0.014 (0–0.223)	0 (–0.003–0.007)	0.340
Hsp27	28.48 (8.12–192.93)	29.08 (9.59–115.51)	–1.87 (–6.85–3.99)	0.688
Hsp70	0.49 (0.21–4.70)	0.56 (0.19–4.60)	0.08 (–0.04–0.18)	0.045
Hsp90	1.71 (0.52–9.79)	2.19 (0.67–10.57)	0.46 (0.22–0.76)	<0.0001
NALP3	0.0002 (0–0.0024)	0.0005 (0–0.0031)	0 (0–0.0001)	0.390
ASC/CARD5	1.70 (0.51–12.31)	1.76 (0.40–22.19)	0.09 (–0.22–0.22)	0.625
Caspase-1	0.14 (0–0.96)	0.11 (0–0.28)	0 (–0.05–0.01)	0.227

CI, confidence interval; TNF- α , tumour necrosis factor- α ; IFN- γ , interferon- γ ; IL, interleukin; CCL2/MCP-1, chemokine (C–C motif) ligand 2/monocyte chemotactic protein-1; CCL5/RANTES, chemokine (C–C motif) ligand 5/regulated upon activation normal T-cell expressed, and presumably secreted; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule type 1; Hsp, heat shock protein; NALP3, NACHT-LRR-PYD-containing protein-3; ASC/CARD5, apoptosis-associated speck-like protein containing a CARD/caspase recruitment domain-containing protein-5.

the stratum corneum (3) further increased epidermal mRNA expression levels of Hsp90 and, more importantly, of IL-1 β as compared with abraded skin of the upper back with calculated numbers of tape strips.

Within the epidermis 6 hr after tape stripping, keratinocytes were most likely the major sources of TNF- α , Hsp90, Hsp70, IL-33, IL-8/CXCL8 and IL-1 β (16–19). In contrast, Langerhans cells constitute only up to 5% of the total epidermal cell

Table 4. Epidermal mRNA expression levels in tape-stripped skin with calculated numbers of tape strips (test area T) versus skin tape-stripped down to the stratum lucidum (test area R) of the subgroup of 11 subjects

Gene symbol	Tape-stripped skin (T), median mRNA (range)	Tape-stripped skin (R), median mRNA (range)	Difference, median mRNA (95% CI)	(R–T), <i>P</i> -value
TNF- α	0.53 (0.27–3.59)	1.07 (0.60–2.52)	0.52 (–0.51–0.80)	0.123
IFN- γ	0 (0–0.005)	0 (0–0)	0 (–0.002–0)	0.250
IL-1 α	0.001 (0–0.051)	0.014 (0–0.175)	0.004 (–0.015–0.017)	0.496
IL-1 β	0 (0–0.22)	0.26 (0–0.94)	0.26 (0.08–0.51)	0.002
IL-6	0 (0–0)	0 (0–0)	NC	NC
IL-8/CXCL8	0 (0–0.054)	0 (0–2.739)	0 (0–0.703)	0.125
IL-10	0 (0–0.075)	0 (0–0.080)	0 (–0.056–0.035)	0.938
IL-33	0.32 (0.03–1.14)	0.56 (0.12–2.30)	0.25 (–0.14–1.09)	0.084
CCL2/MCP-1	0.36 (0.10–1.87)	0.17 (0.02–0.85)	–0.17 (–1.28 to –0.01)	0.148
CCL5/RANTES	0 (0–0.009)	0 (0–0.012)	0 (–0.008–0.002)	0.563
GM-CSF	0 (0–0.0031)	0 (0–0.0015)	0 (–0.0009–0.0001)	0.438
ICAM-1	0.007 (0–0.223)	0.006 (0–0.310)	0.006 (–0.032–0.022)	0.496
Hsp27	19.45 (9.59–115.51)	13.95 (3.79–74.13)	–5.35 (–24.84–0.38)	0.175
Hsp70	0.47 (0.19–4.60)	1.31 (0.35–3.56)	0.16 (–1.32–1.01)	0.465
Hsp90	1.88 (0.67–10.57)	3.98 (1.46–11.49)	1.49 (–1.32–4.41)	0.032
NALP3	0 (0–0.0021)	0 (0–0.0034)	0 (–0.0018–0.0005)	0.844
ASC/CARD5	1.38 (0.73–22.19)	0.62 (0.21–2.74)	–0.49 (–6.31–0.15)	0.098
Caspase-1	0.08 (0–0.28)	0 (0–0.48)	–0.08 (–0.14–0)	0.313

CI, confidence interval; TNF- α , tumour necrosis factor- α ; IFN- γ , interferon- γ ; IL, interleukin; CCL2/MCP-1, chemokine (C–C motif) ligand 2/monocyte chemotactic protein-1; CCL5/RANTES, chemokine (C–C motif) ligand 5/regulated upon activation normal T-cell expressed, and presumably secreted; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule type 1; Hsp, heat shock protein; NALP3, NACHT-LRR-PYD-containing protein-3; ASC/CARD5, apoptosis-associated speck-like protein containing a CARD/caspase recruitment domain-containing protein-5; NC, not computed.

population (20), and it has also been reported that mechanical skin irritation caused by tape stripping exhibits no inflammatory cell infiltration up to 24 hr after the procedure is performed (13, 21, 22). Thus, keratinocytes sense the danger signal of 'tape stripping' and can activate a number of effector mechanisms to trigger cutaneous inflammation.

As is widely believed, TNF- α plays an important pro-inflammatory role by inducing numerous mediators (23) without being specific to allergic or to irritant reactions (21).

To the best of our knowledge, we report for the first time a considerable increase in expression of the heat shock proteins Hsp90 and Hsp70 after skin irritation by tape stripping. Both heat shock proteins have been only recently classified as 'alarmins' (17, 24, 25), a subgroup of the damage-associated molecular pattern family (26). In contrast to what was formerly believed, Hsp90 and Hsp70 themselves have no cytokine-inducing activities (27). Heat shock proteins may be upregulated by pro-inflammatory cytokines such as TNF- α and be crucial in protecting keratinocytes against numerous types of environmental stress and inflammation, although their role in inflammation is still unclear (28). The molecular chaperone functions of heat shock proteins are obviously necessary not only for the folding of newly synthesized proteins in the keratinocytes but also for the protection of proteins during exposure to irritant or stressful situations such as tape stripping, which may cause proteins folded previously to unfold, and extracellular active secretion or passive release of heat shock proteins at sites of skin irritation (26, 27). However, many irritants other than tape stripping [e.g. sodium lauryl sulfate (29)] can induce heat shock proteins, but not all heat shock proteins are induced by any given irritant (16). Thus, we could not find Hsp27 as a marker for skin irritation (29) induced by tape stripping. In our study, Hsp90 expression increased considerably with an increasing number of tape strips. This emphasizes that epidermal mRNA upregulation of heat shock proteins may also depend on the intensity of barrier disruption (21).

IL-33, a junior member of the IL-1 family (17, 30), also plays a pivotal role in response to tissue injury (31). Recently, IL-33, with its chemotactic and immunomodulating (i.e. anti-inflammatory) abilities, has been discussed as a further member of the 'alarmin' family, because of its predominant appearance in the nuclear compartment of keratinocytes (17, 30). It might function as an early warning system at sites of skin irritation, reflecting secretion from keratinocytes that are no longer stable. TNF- α and IL-1 β can induce IL-33 expression in keratinocytes, but the precise biological role of IL-33 is still largely unknown (31).

IL-8/CXCL8 and CCL5/RANTES are some of the most abundant chemokines involved in chemotaxis of T-cells into the skin (19), and are released by TNF- α -activated keratinocytes (32). In contrast to the findings of Onoue et al. (33) in murine skin, we observed no increase of CCL5/RANTES expression in tape-stripped human epidermis; in fact, the opposite was true.

Keratinocytes can activate IL-1 β independently of the immune system after physical insult (34). Activated through a keratinocyte-based inflammasome-mediated process, IL-1 β has been proposed by Watanabe et al. (35) to be crucial for haptenized Langerhans cell migration to the lymph nodes. This parallels the common belief that early synthesis of IL-1 β is required for the sensitization phase of allergic contact dermatitis, inducing a cytokine cascade including TNF- α , GM-CSF and IFN- γ (19, 36, 37), even though IL-1 β is not specific for allergic reactions (20, 21). TNF- α can significantly increase some of the inflammasome components, such as caspase-1, in keratinocytes (34). In this study, IL-1 β was only induced by tape stripping to the stratum lucidum, suggesting that over-intense epidermal barrier disruption might increase the risk of sensitization by the strip patch test procedure itself. However, epidermal mRNA of the inflammasome complex was not upregulated at the investigated point in time, regardless of the number of tape strips.

We acknowledge several methodological aspects that represent strengths and limitations of this study. First, we performed curettage biopsies with minimal blemish and dermal haemorrhage at biopsy sites. This is a practical method for the separation of *in vivo* epidermis from dermis. Consequently, we could explore mRNA expression changes of regulatory mediators of inflammation in epidermal fragments rather than full-thickness skin biopsies, with varying contributions of resident dermal cells or infiltrating cells. However, the potential influence of the minimal injection of local anaesthetic (38) and the surgical procedure itself on the RT-PCR analysis was not evaluated. Second, for technical reasons, the analysable epidermal tissue sample was limited. Therefore, we had to restrict the mediator panel to 18 genes, giving us only limited insights into the complex epidermal inflammation profile. For example, we excluded from real-time quantitative RT-PCR analysis further keratinocyte-derived cytokines that may be partly secreted in response to non-specific stimulation: TGF- α , TGF- β , IL-1RA, IL-7, IL-12, IL-15, IL-18, CXCL1/Gro- α and CXCL10/IP-10 (39–41). Third, we can make no comment on the kinetics of mediator expression, because only one time point after tape stripping (6 hr) was measured. Further studies evaluating the

kinetics of mediator expression in human skin after tape stripping are needed, but will be hardly feasible for ethical reasons. However, this shortcoming might be overcome, for example, by performing cutaneous microdialysis (42, 43). Finally, we did not include methods that offered the possibility of confirming the presence of expressed genes at the protein level as well. Although RT-PCR is a far more sensitive method for the detection of gene expression than immunohistochemistry, the latter method should be included in future studies.

In vivo studies analysing epidermal mRNA expression in humans after acute barrier disruption by tape stripping are scarce (21). In 8 healthy volunteers, Nickoloff and Naidu (13) found increased epidermal mRNA expression levels of TNF- α , IL-8, IL-10, IFN- γ , ICAM-1, TGF- α , and TGF- β 6 hr after tape stripping; IL-1 β , IL-2, IL-4 and IL-5 mRNAs, as additionally assayed with RT-PCR, were not detected. However, direct comparison with our data is somewhat hampered because of a different test site (buttock skin), a higher number of tape strips (80–100 times in the majority of subjects) and a mediator panel of genes that did not completely overlap, even though the time of biopsy was identical. Nevertheless, our data showed also an upregulation of TNF- α and IL-8 mRNAs in the epidermis.

In summary, not only the physical epidermal barrier but also the inflammatory epidermal barrier is influenced by tape stripping according to the proposed standardized strip patch test protocol (3). Our own data suggest that the defined reduction of stratum corneum of 31% (5) in association with the increased expression of epidermal mRNAs, including those of TNF- α , Hsp90, Hsp70, IL-33 and IL-8/CXCL8, may be in large part responsible for the considerably increased sensitivity of the strip patch test versus the patch test (4, 6). However, we cannot estimate how much the observed stratum corneum reduction or changes in epidermal cell immunology contribute to this enhanced sensitivity. The pathophysiological significance of all of these findings needs to be further clarified. Finally, it should not be forgotten that the extent to which sensitization is expressed clinically depends always on a complex interaction between the sensitizing potency of the test substance, exposure and skin susceptibility (44). Against this background, one may also argue that test substances can elicit patch test reactions if only because the change in skin susceptibility caused by tape stripping has been sufficient to permit this; that is, a true-negative test result is turned into a false-positive test result. Patients might be diagnosed as being allergic to environmental agents to which they are not. This again emphasizes the task of diagnosing allergic

contact dermatitis (45), in which the strip patch test will be a useful tool if a careful patient history and case-dependent use tests are also taken into consideration (46).

Conclusion

Standardized tape stripping of the skin prior to patch testing (3) not only facilitates the penetration of test substances (5) but also enhances skin immunoreactivity to the test substances by inducing the expression of keratinocyte-derived ‘alarmin’ and cytokine mRNAs. With this expanded but still far from complete knowledge, the strip patch test has the potential to detect the presence of allergic contact dermatitis in patients with negative patch test results (4).

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