Injury to the Stratum Corneum Induces *In Vivo* Expression of Human Thymic Stromal Lymphopoietin in the Epidermis

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TO THE EDITOR

The formation of a competent barrier at the interface to the terrestrial environment is a fundamental function of the skin. Acute or sustained insult to the stratum corneum results in perturbation of the barrier integrity and generates an array of positive and negative downstream alarm signals that initiate both homeostatic and proinflammatory responses (Nickoloff and Naidu, 1994; Elias, 2005). The compromised barrier integrity therefore might not be merely an end point but also trigger the release of cytokines of critical importance to the initiation of allergic skin inflammation and the manifestation of common disease entities such as atopic dermatitis (AD) (Elias *et al.*, 1999; Elias and Steinhoff, 2008).

Of particular interest in that context is thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine preferentially expressed by the epithelial cells at the interfaces such as the skin, airways, and gut (Liu *et al.*, 2007). The critical role of TSLP in the initiation of allergic inflammation is well established and although there has been progress in unveiling the factors triggering TSLP expression, the underlying mechanisms are not fully understood (Li *et al.*, 2006; Liu 2006, 2007; Lee *et al.*, 2008). Cultured skin explants and primary human epithelial cells were shown to release TSLP upon inflammatory, bacterial, and

Table 1. Noninvasive bioengineering assessment of the barrier-function parameters at baseline and following irritant application or tape stripping *in vivo*

A. 96-h repeated open application test

Parameter	Baseline SLS-treated field	Baseline control field	+ 96 h SLS-treated field	+ 96 h control field
TEWL, $g\,m^{-2}\,h^{-1}$	5.49 ± 0.38	5.45 ± 0.32	33.64 ± 2.56$^{a***, b***}$	5.64 ± 0.32
Chroma a*-, AU	5.82 ± 0.5	5.76 ± 0.5	12.05 ± 1.34$^{a***, b***}$	5.64 ± 0.61
Skin pH, AU	5.09 ± 0.16	4.97 ± 0.13	6.11 ± 0.19$^{a**, b**}$	5.12 ± 0.15
Capacitance, AU	37.3 ± 1.78	37.36 ± 1.76	19.22 ± 2.10$^{a***, b***}$	36.63 ± 1.76

B. Single 24-h application of 2% SLS

Parameter	Baseline SLS-treated field	Baseline control field	+ 24 h SLS-treated field	+ 24 h control field
TEWL, $g\,m^{-2}\,h^{-1}$	6.21 ± 0.18	5.51 ± 0.36	42.16 ± 7.76$^{a**, b**}$	5.41 ± 0.30
Chroma a*-, AU	6.76 ± 0.24	6.69 ± 0.31	12.23 ± 1.54$^{a*, b**}$	5.93 ± 0.25
Skin pH, AU	4.96 ± 0.35	5.26 ± 0.19	7.15 ± 0.08$^{a*, b**}$	4.92 ± 0.12
Capacitance, AU	51.84 ± 2.28	50.49 ± 2.77	32.20 ± 5.31$^{a**, b*}$	49.29 ± 2.95

C. Tape stripping

Parameter	Baseline TS field	+100 Strips TS field	Control field
TEWL, $g\,m^{-2}\,h^{-1}$	4.83 ± 0.66	61.57 ± 1.92$^{a***, b***}$	4.82 ± 0.65
Chroma a*-, AU	3.66 ± 1.21	10.87 ± 1.84$^{a**, b**}$	3.80 ± 0.99

Abbreviations: AU, arbitrary units; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; TS, tape stripping.
All data are presented as mean ± SEM and differences are considered significant when $P<0.05$.
[a]Values compared with baseline; [b]values compared with control field; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Abbreviations: AD, atopic dermatitis; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss TS, tape stripping; ROAT, repeated open application test

viral stimuli or trauma (Allakhverdi *et al.*, 2007; Bogiatzi *et al.*, 2007). In human skin, TSLP is expressed by keratinocytes in lesional skin of AD patients, but not in nickel-induced allergic contact dermatitis and systemic lupus erythematosus (Soumelis *et al.*, 2002). Compromised barrier function is characteristic for AD, therefore we aimed at investigating the role of tape stripping (TS) and irritant damage to the stratum corneum for the expression of human TSLP *in vivo*.

For the purpose, we analyzed TSLP expression in skin biopsies obtained from healthy, adult, nonatopic volunteers following: (1) repeated open application (ROAT) of 2% sodium lauryl sulfate (SLS) for 96 hours ($n=8$); (2) single 24-hour application of 2% SLS under occlusion ($n=4$); (3) sequential TS until removal of the stratum corneum ($n=4$). A biopsy from adjacent nontreated field served as control (normal skin).

Visual scoring and bioengineering assessment of the barrier function of the test and control (nontreated) fields were performed as follows: (1) ROAT baseline and every 24 hours up to 96 hours; (2) patch test baseline and 2 hours after removal of the test chamber; (3) TS baseline and upon complete removal of the stratum corneum. Erythema was measured using Chromameter CR200 (Minolta, Osaka, Japan) and expressed in the L*a*b* system. Transepidermal water loss (TEWL) was measured with the open chamber system (Tewameter TM300), skin pH was monitored using the skin pH meter PH905 and skin hydration was assessed by measuring capacitance (Corneometer CM825), all devices from Courage and Khazaka Electronics (Cologne, Germany). For each parameter, three consecutive measurements per field were performed by the same observer under controlled ambient conditions (room temperature $21 \pm 2\,^{\circ}C$; relative humidity 35–45%). At the end of the observation period (96 hours for ROAT, 24 hours for the patch test and 48 hours for TS), 5-mm punch biopsies from the test and control fields were taken for immunohistochemistry and quantification of TSLP mRNA by real-time quantitative PCR. The study was conducted according to the Declaration of Helsinki principles. The protocol was approved by the Ethics Committee of the University of Lübeck and all participants gave written consent beforehand.

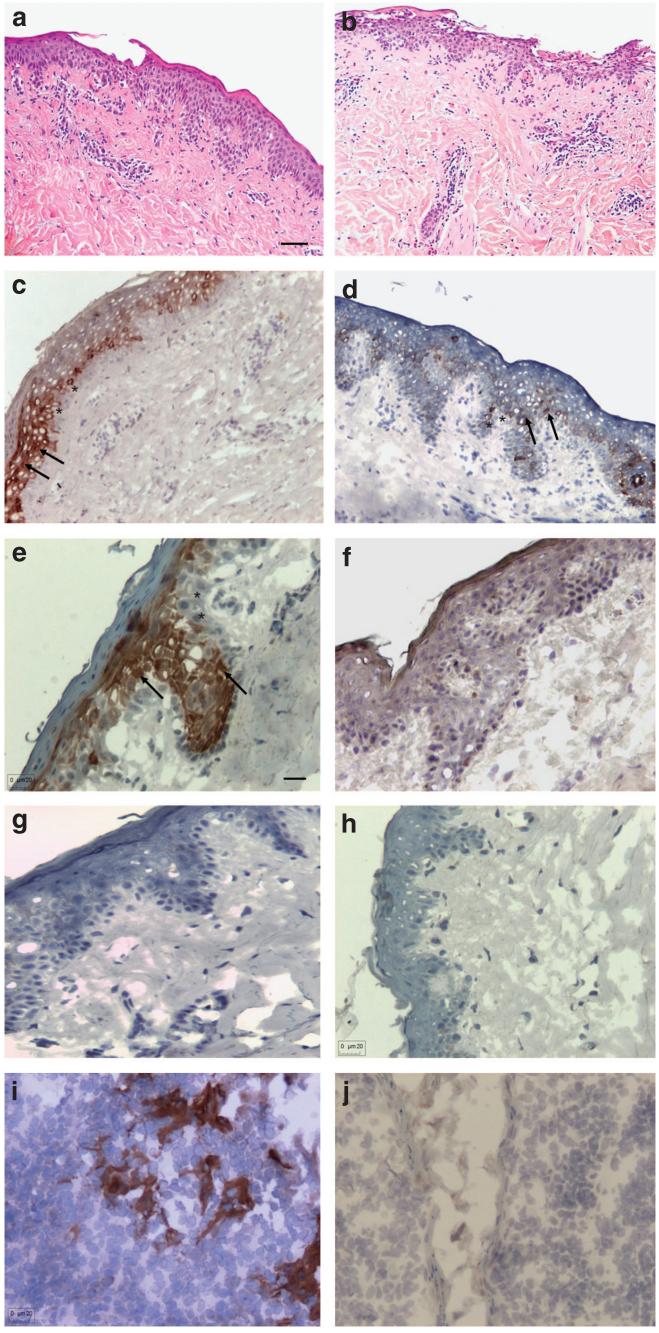

Figure 1. TSLP expression in human skin after tape stripping and SLS application *in vivo*. (**a**, **b**) Hematoxylin–eosin staining of SLS-treated field: (**a**) 96-hour repeated application; (**b**) 24-hour patch test. (**c**–**e**) Cryosections from SLS-treated and tape-stripped skin stained with sheep or rat anti-human TSLP mAb (R&D Systems and kind gift from Rene de Waal Malefyt, respectively). TSLP expression is restricted to the epidermis and detectable in the suprabasal cell layers (arrows), while the basal layer (asterisk) remains negative: (**c**) 96-hour open test; (**d**) 24-hour occlusion test; (**e**) 48 hours after tape stripping. (**f**–**h**) No detectable TSLP immunoreactivity in frozen sections from adjacent field (normal skin) of the same volunteers. (**i**) TSLP expression by crypt epithelial cells of human tonsil: positive control. (**j**) Adjacent section of human tonsil: isotype control. Scale bar = 15 μm.

Tape stripping and SLS application resulted in moderately severe inflammatory reaction reflected by significant increase in the visual score of the test fields compared with baseline and the nontreated field. The compromised barrier integrity was evidenced by the significant increase in TEWL, chromametry a*-value, skin pH, and significant decrease in capacitance of the test fields compared with baseline, while the barrier-function parameters of the control field remained unchanged (Table 1). Histopathology changes compatible with irritant dermatitis were present on hematoxylin-eosin-stained sections from SLS-treated skin (Figure 1a and b) and absent in the control samples.

TSLP-specific focal immunoreactivity was present in seven out of eight samples obtained from the test field on which barrier damage was induced by repetitive SLS application (Figure 1c), in one out of four samples obtained from the SLS patch test field (Figure 1d) and in four out of four samples obtained from the TS field (Figure 1e), whereas the samples from the control field remained consistently negative (Figure 1f–h). TSLP was expressed by keratinocytes in the suprabasal cell layers, mainly the spinous and granular layers, and absent in the basal layer and these findings were consistent across the samples (Figure 1c–e). These observations are in agreement with previously published data suggesting that TSLP expression is a feature of keratinocytes undergoing terminal differentiation (Soumelis *et al.*, 2002; Liu 2007). Similarly to earlier reports, we found dissociation in TSLP expression at the protein and mRNA levels (Bogiatzi *et al.*, 2007) as TSLP mRNA levels in TS samples were comparable with control skin (data not shown).

The pathways leading to human TSLP expression upon tape stripping or irritant damage to the epidermis *in vivo* remain to be determined. Previously, human TSLP was shown to be induced under the synergistic effects of proinflammatory and Th2 cytokines (Bogiatzi *et al.*, 2007). Recent evidence for TSLP release by Notch-deficient keratinocytes in engineered mice showing skin barrier defects and eczema-like phenotype (Demehri *et al.*, 2008, 2009) suggests a link between barrier integrity and TSLP. In agreement with these observations, the results of our study make reasonable to speculate that alterations in the permeability barrier function play a role for human TSLP expression. Injury to the stratum corneum, barrier integrity, and inflammatory signaling are intricately interrelated and mechanistic separation of the downstream events in *in vivo* human skin system is difficult as perturbation in one function ultimately impacts the other.

In summary, our findings provide evidence that injury to the stratum corneum by tape stripping and irritant application induces the expression of human TSLP in the epidermis. The pattern of immunoreactivity is consistent independently of the mechanism to induce barrier damage. To the best of our knowledge *in vivo* inducible upregulation of TSLP in human skin has not been previously reported.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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