

Irritant threshold and histological response of epidermis to irritant application

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Individuals vary in their ability to react to irritants, which can be demonstrated for sodium lauryl sulfate (SLS) using the irritant threshold (IT) test. We aimed to study whether the histological and immunohistochemical features of the skin following SLS exposure varied with subject's IT. 8 subjects were recruited. Their IT was measured. Biopsies were taken after 2 hr and 4 hr of occlusion with 20% SLS and control. The specimens were stained with haematoxylin and eosin and for Langerhans cells. At 4-hr, low-threshold subjects developed changes to a greater extent than high-threshold subjects. The relationship of histological reaction to IT could be related to a differential pro-inflammatory cytokine response in subjects. Low IT has been previously associated with a tumour necrosis factor alpha promoter region polymorphism.

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Subjects can be shown to vary in their susceptibility to the irritant surfactant sodium lauryl sulfate (SLS) using a variety of protocols (1–3). One study applied 5% SLS as a patch test for 48 hr to volar forearm skin in 10 healthy subjects and examined biopsies by light microscopy (3). Despite the uniformity of exposure, reactions varied clinically from mild (minimal surface change) to severe (erythema and oedema). The histopathology of mild reactions was dominated by parakeratosis, spongiosis and a mild inflammatory cell infiltrate. In severe reactions, necrosis of basal layers of the epidermis with an extensive inflammatory cell infiltrate occurred.

A contrasting method of assessing subject irritant susceptibility is to apply SLS at varying concentrations under occlusion to the upper inner arm for 4 hr. At 24 hr, some patients develop erythema to concentrations as low as 0.3%, while others fail to react to 20% (1). The lowest concentration of SLS reacted to is the subject's irritant threshold (IT). This SLS threshold test is a development of a proposed human predictive irritant assay, which uses 20% SLS applied for 4 hr to the upper inner arm as a control. This work provided the basis for the biopsy timings and concentration of SLS exposure in this study (2).

The response of epidermal Langerhans cells (LCs) to experimental application of SLS is predominantly that of migration. Some studies have, however, shown either no response or increased LC numbers. This seems likely to reflect differences in experimental method (4–9).

We hypothesized that the SLS threshold of a subject could be related to the histopathological changes seen after exposure to SLS. Those subjects with a low threshold might develop extensive epidermal change compared with high-threshold subjects. Furthermore, low-threshold subjects may develop this response at an earlier time point. It was also postulated that changes in LCs could be related to threshold.

Materials and Methods

Local ethical committee approval was gained. Volunteers who had active dermatitis or a past history of atopic dermatitis were excluded. Respiratory atopics, without dermatitis, were not excluded, there being some evidence that they have a similar barrier function to healthy controls (10). Systemic illness or regular medication resulted in exclusion. The volunteers' IT was tested following an established protocol (1). In brief, 20 µl of descending concentrations of SLS

from 20% to 0.1% aqueous, plus a control of distilled water, was applied for 4 hr to the upper inner arm using 8-mm Finn Chambers[®] (Epitest, Tuusula, Finland) and read after 24 hr for clinical inflammation. The lowest concentration of SLS, which induced erythema was taken as the subject's IT.

Patients with high thresholds ($\geq 20\%$) or low thresholds (0.1% or 0.5%) were then retested using only 2 chambers: 20% SLS and water. The chambers were applied for either 2 hr or 4 hr. Any erythema was noted. Biopsies were excised from the centre of each reaction with a 6-mm punch biopsy, after injection of 1% plain lignocaine. All biopsies were fixed in neutral-buffered formalin for 24 hr and processed whole through a routine 15-hr cycle to paraffin wax embedding. 4- μ m thick serial sections were cut using a sledge microtome and mounted on poly L-lysine-coated slides. Sections were dried overnight at 37°C. All slides for staining were dewaxed in xylene.

From each block 8 sections were stained for haematoxylin and eosin (H&E) and CD1a. Immunocytochemistry entailed blocking sections for endogenous peroxidase for 10 min in 3% H₂O₂ in methanol. Following this, heat-mediated antigen retrieval was employed using 0.01 M sodium citrate pH 6.0 as a buffer. Sections were microwaved for 10 min at 650 W. Sections were then transferred to this buffer source. Primary antibody dilutions for immunocytochemical staining were 1/10 CD1a (Vector Labs, Peterborough, UK). A universal linking 2nd and 3rd layer was used in all cases. Final reaction products were visualized using 3,3'-diaminobenzidine. Sections were then counterstained for 1 min in Harris haematoxylin, dehydrated, cleared and mounted. A known positive control was run in tandem with all test slides. A negative control involved the omission of primary antibody.

All slides were assessed blind by a histopathologist. Langerhans cells exhibiting a brightly stained cytoplasm with a dark nucleus were counted along the CD1a-stained biopsy length using an accepted method and then an average taken (11).

Results

8 volunteers with known thresholds were biopsied, 4 at 2 hr and 4 at 4 hr. Visual inspection of the patch test sites after removal showed that 2 volunteers with low thresholds had erythema after 4 hr of occlusion with 20% SLS. At each time point, the subjects were split equally between high- (20%) and low (0.1% or 0.5%)-threshold subjects. Table 1 summarizes the results found in

the 4 patients (a–d) biopsied after 4 hr. The 2 low-threshold subjects (a, b) showed epidermal necrosis when compared with control. The 2 high-threshold subjects (c, d) showed minimal changes compared with control (Fig. 1). Dermal changes on H&E at 4 hr in all active biopsies showed a minimal perivascular infiltrate compared with controls. In all subjects at 4 hr, the CD1a count was lower in active biopsies compared with control – this was not statistically significant.

At 2 hr, no trend was seen between active and controls after staining with H&E and no trend for changes in LC numbers. Dermal LCs were not demonstrated at 2 or 4 hr.

Discussion

In this study, after identical exposure to the irritant SLS, low-IT subjects developed epidermal necrosis while high-IT subjects did not. This finding in otherwise normal subjects distinguished only by their response to the recently proposed IT test is worthy of report.

However, the sample size of each group studied was small, effectively 2 at each biopsy time point. The high interindividual variation in response to SLS would usually make such small numbers difficult to interpret. However, this study was carefully controlled and the design divided the groups by IT into what were hypothesized to be high and low SLS responders. The numbers at each time point could have been increased by selecting only 1 biopsy time point at 4 hr. However, one of the aims of this study was to assess if low-IT subjects developed reactions at an earlier stage than high-IT subjects. No evidence for this was shown.

The authors argue that this work provides part of the explanation for the histological variability to SLS noted by Willis et al. (3). We hypothesize that the severe reactions to SLS reported by these authors in their group of 10 subjects would have occurred in low-IT subjects.

A non-significant trend to decreased LC numbers after SLS application compared with controls was seen in all subjects at 4 hr. This is in keeping with other work, but provides no basis for a relationship for LC migration between IT and irritant application (4–9).

These findings may relate to recent work, which has shown a link between a tumour necrosis factor- α (TNF- α) cytokine polymorphism and irritant susceptibility in humans. Those reacting to low concentrations of SLS (low IT) showed a significant increase in expression of an allele that is associated with an excessive inflammatory response in other clinical settings (12). Human

Table 1. Assessment of histopathological and immunohistochemical changes in active and control biopsies from 4 subjects (a–d) after 4 hr of patch test application

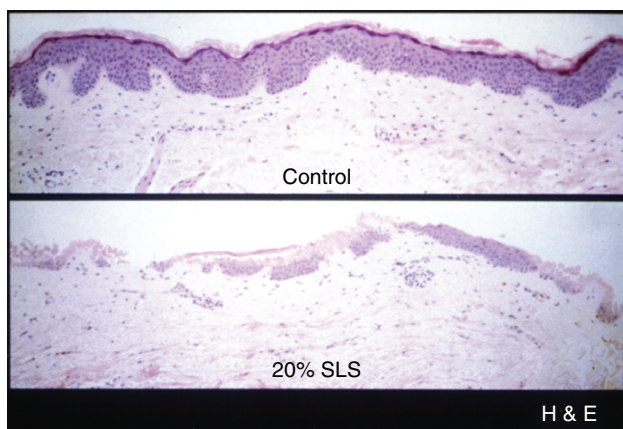
Subject/irritant threshold	Patch test applied: active (20% SLS) /control (H ₂ O)	Clinical assessment after 4 hr	H&E assessment	CD1a count average
A/0.1	Active	Erythema	Full-thickness epidermal necrosis	70
	Control	No change	Normal	140
B/0.5	Active	Erythema	Partial epidermal necrosis	93
	Control	No change	Normal	130
C/20	Active	No change	Minimal upper epidermal necrosis	70
	Control	No change	Normal	89
D/20	Active	No change	Normal	88
	Control	No change	Normal	140

H&E, haematoxylin and eosin; SLS, sodium lauryl sulfate.

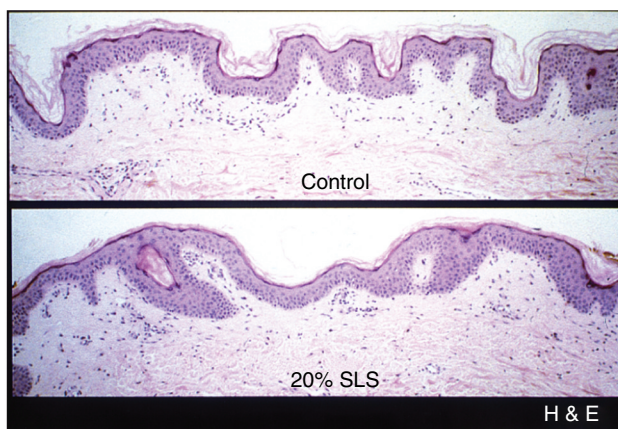
keratinocytes are a source of TNF- α following a variety of stimuli (13–15). This cytokine plays a role in experimental irritant contact dermatitis in humans (16). Tape stripping of the human epi-

dermis results within 6 hr, with an increase in keratinocyte messenger RNA TNF- α (17). Human lymph drainage studies of SLS-induced dermatitis show a rapid and significant rise in

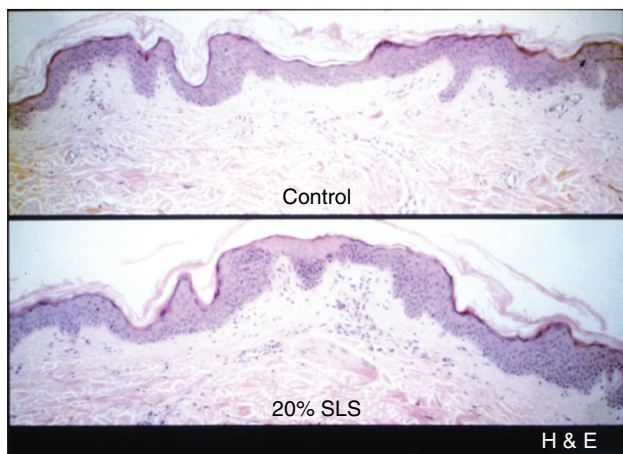
Subject A



Subject B



Subject C



Subject D

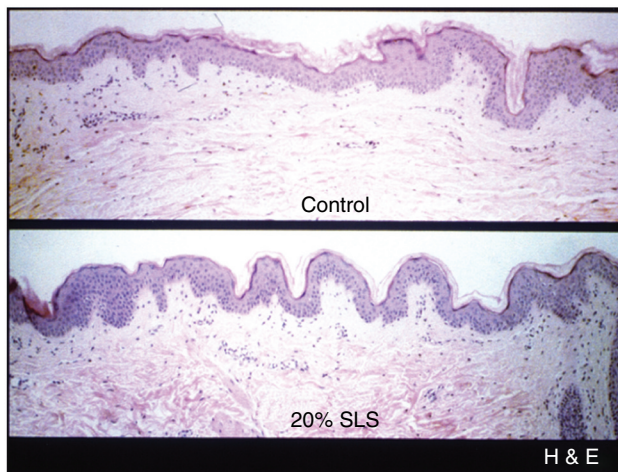


Fig. 1. Haematoxylin and eosin (H&E) staining of 2 biopsy specimens at 4 hr taken from subjects a–d (Table 1). Lower specimen 20% sodium lauryl sulfate (SLS), upper control. Full-thickness epidermal necrosis seen in low-threshold subject (a, b). Minimal epidermal change in high-threshold subjects (c, d).

TNF- α in parallel with the clinical symptoms of contact dermatitis (18). We hypothesize that relative over-production of this cytokine in the low-IT subjects played a part in the extensive epidermal necrosis that they developed.

The demonstration of a relationship between IT and histopathological response to SLS is important as it strengthens the validity of this recently proposed test.

References

- Judge M R, Griffiths H A, Basketter D A et al. Variation in response of human skin to irritant challenge. *Contact Dermatitis* 1996; 34: 115–117.
- Basketter D. Contact irritation models. In: *Toxicology of Contact Dermatitis*, Basketter D, Gerbrick F, Kimber I, Willis C (eds): Chichester, John Wiley & Sons, 1999: 39–56.
- Willis C M, Stephens C J M, Wilkinson J D. Epidermal damage induced by irritants in man: a light and electron microscopic study. *J Invest Dermatol* 1989; 93: 695–699.
- Lindberg M, Färm G, Scheynius A. Differential effects of sodium lauryl sulphate and nonanoic acid on the expression of CD1a and ICAM-1 in human epidermis. *Acta Derm Venereol (Stockh)* 1991; 71: 384–388.
- Lisby S, Baadsgaard O, Cooper K D, Vejlsgaard G L. Decreased number and function of antigen-presenting cells in the skin following application of irritant agents: relevance for skin cancer? *J Invest Dermatol* 1989; 92: 842–847.
- Ferguson J, Gibbs J H, Beck J S. Lymphocyte subsets and Langerhans cells in allergic and irritant patch test reactions: histometric studies. *Contact Dermatitis* 1985; 13: 166–174.
- Mikulowska A, Andersson A. Sodium lauryl sulphate effect on the density of epidermal Langerhans cells. Evaluation of different test models. *Contact Dermatitis* 1996; 34: 397–401.
- Brand C U, Hunziker T, Limat A, Braathen L R. Large increase of Langerhans cells in human skin lymph derived from irritant contact dermatitis. *Br J Dermatol* 1993; 128: 184–188.
- Cumberbatch M, Dearman R J, Griffiths C E M et al. Langerhans cell migration. *Clin Exp Dermatol* 2000; 25: 413–418.
- Conti A, Di Nardo A, Seidenari S. No alteration of biophysical parameters in the skin of subjects with respiratory atopy. *Dermatology* 1996; 192(4): 317–320.
- Bieber T, Ring J, Braun-Falco O. Comparison of different methods for enumeration of Langerhans cells in vertical cryosections of human skin. *Br J Dermatol* 1988; 118: 385–392.
- Allen M H, Wakelin S H, Holloway D et al. Association of *TNFA* gene polymorphism at position –308 with susceptibility to irritant contact dermatitis. *Immunogenetics* 2000; 51: 201–205.
- Barker J N W N, Mitra R S, Griffiths C E M et al. Keratinocytes as initiators of inflammation. *Lancet* 1991; 337: 211–214.
- Wilmer J L, Burleson F G, Kayama F et al. Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. *J Invest Dermatol* 1994; 102: 915–922.
- Kock A, Schwarz T, Kirnbauer R et al. Human keratinocytes are a source for tumour necrosis factor α : evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med* 1990; 172: 1609–1614.
- Hunziker T, Brand C U, Kapp A et al. Increased levels of inflammatory cytokines in human skin lymph derived from sodium lauryl sulphate-induced contact dermatitis. *Br J Dermatol* 1992; 127: 254–257.
- Nickoloff B J, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol* 1994; 30: 535–546.
- Brand C U, Braathen L R. Investigation of skin-derived lymph: an approach to get insight into the skin immune system. *Dermatology* 1996; 193: 283–288.

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