

# A Twenty-four-hour Occlusive Exposure to 1% Sodium Lauryl Sulfate Induces a Unique Histopathologic Inflammatory Response in the Xerotic Skin of Atopic Dermatitis Patients

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**Twenty-four-hour occlusive exposures of 1% aqueous sodium lauryl sulfate (SLS) produced unique functional and histological responses in patients with atopic dermatitis. Disruption of the stratum corneum barrier, measured by transepidermal water loss, was much greater and longer lasting than in normal controls. In contrast to controls the histologic pattern induced reproduced the typical features of the disease with spongiosis, exocytosis of mononuclear cells and a perivascular infiltrate containing eosinophils. The perivascular infiltrate consisted of CD1a<sup>+</sup>, CD4<sup>+</sup> and HLA-DR<sup>+</sup> cells, which was much greater and more persistent in atopics. Eosinophilic major basic protein was abundant in atopics but absent in controls. SLS provocation of atopic dermatitis is a striking experimental example of Koebnerization, in which disruption of the stratum corneum barrier as well as cytokine activation of keratinocytes reproduces the clinical diseases. Key words: EG2; patch test; sodium lauryl sulfate; transepidermal water loss.**

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Anionic soaps and surfactants are classic irritants which are capable of inducing strong inflammatory reactions on human skin. Sodium lauryl sulfate (SLS) is the archtypical example of an anionic surfactant which has been extensively studied by numerous researchers. The structural and functional changes induced in human skin by SLS have been reported in many publications, using biochemical, ultrastructural and bioengineering techniques (1, 2). Patients with atopic dermatitis (AD) show a greater vulnerability to the damaging effects of SLS (3, 4). For example, positive patch test reactions are provoked by lower concentrations applied to apparently uninvolved skin.

It is appreciated that non-dermatitic skin of AD patients is not completely normal. A fine scaling is often present (atopic xerosis); the stratum corneum barrier is impaired, as evidenced by increased diffusional water loss along with decreased capacity to take up and hold water. Hence, the entire integument is involved though the changes may be very subtle (5).

In connection with a study of atopic xerosis in children living in Philadelphia, Pennsylvania, occlusive patch tests to 1% SLS were applied for 24 h to the lateral mid-legs in winter. This produced a variable erythema followed by prolonged scaling. An unexpected finding in 2 of 9 children with active AD was the development of a persistent, erythematous, pruritic, scaling dermatitis, confined exactly to the site of exposure, lasting for 2 to 4 months. Two children, ages 5 and 7, were biopsied 3 and

5 weeks, respectively, after the patch test. The histopathology was a spongiotic dermatitis, consistent with AD. These unpublished observations prompted the studies which are the subject of this report.

## MATERIAL AND METHODS

Two studies were conducted, one on white subjects in Philadelphia and one on Orientals in Sendai, Japan. In both places, the patients had AD according to the criteria of Hanifin & Rajka (6). Informed consent was obtained.

### Philadelphia study

Six patients with active long-standing AD, ages 9, 11, 14, 21, 30 and 31 (4 males and 2 females) participated and five healthy controls ages 8, 12, 13, 16 and 17 (4 males and 2 females) were recruited.

One hundred microlitres of 1% aqueous SLS (Sigma, 98% purity) was applied on cotton discs (Webril) in large Hilltop chambers to the uninvolved mid-volar forearms for 24 h. The test sites were evaluated for redness, scaling and pigmentation 30 min after removal, the next day and 1 week post-removal. Two punch biopsies were taken from each subject, i.e. 3-mm specimens were obtained 24 h after removing the chambers, along with control biopsies from nearby untreated sites. These were formalin-fixed, cut at 6  $\mu$ , and stained with haematoxylin and eosin (H and E) and PAS-Giemsa. Microscopically, parakeratosis, spongiosis and cell infiltration were globally evaluated without statistical analysis owing to small sample size.

### Sendai study

Thirteen AD patients (4 males and 9 females), with a mean age 27 (range 16–56) participated. All had moderate to severe disease. The control group consisted of 13 normal subjects with a mean age of 29 (range 20–33). One hundred microlitres of 1% SLS was applied on filter paper discs in large Finn chambers (12 mm in diameter) to the uninvolved mid-volar forearms for 24 h as above. Distilled water served as a control. The reaction sites were observed for the presence of redness, scaling and pigmentation 30 min and 1 week post-removal. Transepidermal water loss (TEWL) was measured at baseline, 30 min and again 7 days later. Measurements of TEWL were also made at untreated sites and those exposed to water. Measurements of TEWL were made following international guidelines, at 19–22°C room temperature and 23–29% relative humidity (7).

Three mm punch biopsies were taken from SLS-treated and nearby sites as follows: (i) before SLS exposure (1 AD and 3 normals), (ii) 24 hours post-exposure (3 AD and 3 normals) and (iii) 7 days post-exposure (3 AD). Actually two sets of the above tests were carried out in cases in which biopsy was performed on Day 2 to avoid interference with the Day 7 TEWL measurements.

The specimens were fixed in 2% paraformaldehyde embedded in OCT compound (Miles Inc. Elkhart, IL, USA), snap-frozen in liquid nitrogen, cut at 6  $\mu$ , and stained with H and E and toluidine blue.

Immunohistochemical evaluations were performed with the following mouse monoclonal antibodies: CD1a, CD4, CD8, CD20, HLA-DR, LFA-1, ICAM-1 (Becton Dickinson, San Jose, CA, USA) and EG2 (Nichirei Co., Tokyo, Japan). After blocking with rabbit serum

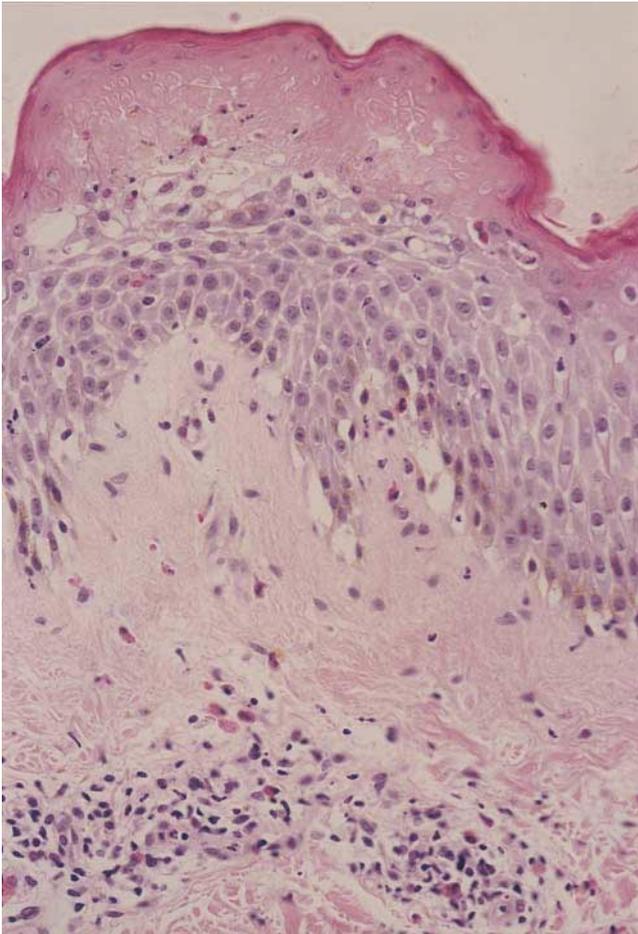


Fig. 1. Twenty-four-hour biopsy after 24-h exposure to 1% sodium lauryl sulfate in one patient with atopic dermatitis (AD). There is diffuse eosinophilic necrosis in the upper epidermis with exocytosis of mononuclear cells and eosinophils and inflammatory changes in the underlying tissues typical for active AD, namely spongiosis and exocytosis of inflammatory cells and a fairly intense perivascular mononuclear infiltrate with scattered eosinophils (H & E,  $\times 120$ ).

diluted in phosphate-buffered saline (PBS), the sections were serially treated with the above antibodies (at a dilution of 1 : 100 or 1 : 200) or isotype-matched control mouse monoclonal antibody at 4°C overnight. After 15 min of incubation the sections were treated with biotinylated anti-mouse Ig for 30 min, followed by peroxidase conjugated avidin for 30 min. Each step was followed by washing three times with PBS. The avidin-biotin peroxidase technique kit was obtained from Histofine SAB-PO(M) kit (Nichirei, Tokyo, Japan). Finally, the sections were developed with diaminobenzidine solution and 1% hydrogen peroxide and counter-stained with Mayer's haematoxylin. EG2 anti-

body was used at a dilution of 1 : 10. Alkaline phosphatase conjugated goat anti-mouse IgG was used as a secondary antibody. The staining reaction was developed with naphthol AS-MX phosphate and fast red TR with 1 mM levamisole to inhibit endogenous alkaline phosphatase. Qualification of eosinophils was carried out for sections stained for EG2. We counted the number of EG2<sup>+</sup> cells in at least six fields under 150 magnification in the upper dermis and lower dermis. Other immunohistochemical data could not be expressed quantitatively because of high site to site unreliability.

The Wilcoxon rank sum test and Wilcoxon signed rank test were used for statistical analysis of TEWL between AD group and controls and within each group AD patients and controls, respectively. *P*-values < 0.05 were considered significant.

## RESULTS

### Philadelphia study

*Clinical.* In both groups, the test sites were moderately red after 24 h exposures to SLS. The reactions were slightly more intense in the AD group. However, 7 days post-exposure, most of the AD patients still exhibited mild erythema and all showed mild to moderate scaling. The control subjects showed only scaling at that time.

*Histopathology.* In 3 of 6 patients with AD, spongiotic foci with exocytosis of mononuclear cells was noted in the epidermis 24 h after removal of SLS. In normal controls, only cytotoxic changes were observed in the upper epidermis consisting of poorly staining eosinophilic, swollen, keratinocytes with blurred cell margins. A similar degree of cytotoxicity was observed in AD but in one case there was diffuse eosinophilic necrosis in the upper epidermis with exocytosis of mononuclear cells and eosinophils (Fig. 1).

A fairly intense perivascular mononuclear infiltrate was present in 5 of the 6 patients with AD. In normal controls, only 2 of the 5 subjects showed a slightly increased mononuclear cell infiltrate, decidedly less than in AD. More importantly, scattered eosinophils were found in the infiltrate in the same 3 subjects who showed spongiotic foci (Fig. 1).

### Sendai study

*Clinical features.* After SLS for 24 h, the test sites became moderately red and scaly, with no difference between the two groups. Seven days later the sites showed variable hyperpigmentation and scaling, somewhat more pronounced in the AD patients.

*TEWL.* In AD patients and controls TEWL values of SLS-treated sites were higher than those of water-treated sites and nearby untreated sites. Table I shows the difference between patients with AD and controls. The TEWL values had greatly increased in AD patients and persisted at high levels even 7 days later. By 7 days, the increase in TEWL was much lower, approaching normalcy in the controls. These differences were statistically significant (Table I).

*Histopathology.* Histological changes of SLS-treated skin were mainly observed in the upper and mid dermis. In normals a mild perivascular mononuclear cell infiltrate was present compared to greater, moderate infiltrate in AD patients. Scattered mononuclear cells were intermingled with melanophages due to pigment incontinence of melanin in a linear pattern along the dermo-epidermal junction in AD patients. A mild epidermal acanthosis was noted in both groups, perhaps

Table I. Results of transepidermal water loss (mean  $\pm$  SD)

	Initial	1 day	7 days
AD (mean $\pm$ SD)	6.4 $\pm$ 3.3*	25.4 $\pm$ 3.6**	13.4 $\pm$ 3.2***
Control (mean $\pm$ SD)	3.1 $\pm$ 1.2*	13.0 $\pm$ 2.1**	5.7 $\pm$ 0.19***

\*, \*\* and \*\*\* *p* < 0.05; <sup>a</sup>*p*; <sup>b</sup>*p* < 0.05.

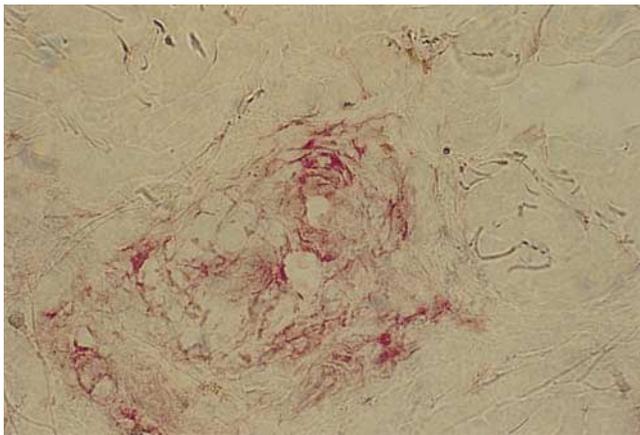


Fig. 2. EG2 staining skin biopsy taken 24 h after patch testing uninvolved atopic dermatitis skin with SLS. EG2<sup>+</sup> eosinophils scattered in the dermis and the staining showed in the surrounding tissues ( $\times 250$ ).

slightly greater in AD, along with mild spongiosis. Exocytosis of mononuclear cells into the epidermis was found only in AD. The dermal mononuclear infiltrate was still present after 7 days in AD but scarcely evident in controls. In AD, the epidermis was moderately acanthotic with parakeratotic segments in the stratum corneum. Parakeratotic corneocytes were not seen in mildly acanthotic epidermis in controls.

**Immunohistochemistry.** At baseline, only a few, scattered CD1a<sup>+</sup>, CD4<sup>+</sup> and HLA-DR<sup>+</sup> infiltrating cells were observed. These were more abundant in AD than in normals. At 24 h, an inflammatory infiltrate consisting of CD1a<sup>+</sup>, CD4<sup>+</sup> and HLA-DR<sup>+</sup> cells extended from the perivascular regions toward the epidermis. The infiltrate was greater and more extensive in AD patients. In contrast to controls it was still present in AD patients at 7 days, limited to the perivascular area. A trace of ICAM-1 was noted in a few blood vessels and clear expression of ICAM-1 was found on the keratinocytes at the sites of lymphocytic infiltration. This expression of ICAM-1 was found only in the SLS-applied sites regardless of AD or normal subjects but never before SLS application. Almost all infiltrating cells were LFA-1 positive. No CD80<sup>+</sup> or CD86<sup>+</sup> cells were observed. CD20<sup>+</sup>B cells were completely lacking in both groups. Expression of HLA-DR was not observed in the keratinocytes either in AD patients or in controls.

EG2<sup>+</sup> eosinophils, sequentially studied in 3 AD patients and 3 controls were found only in AD patients (Table II). Moreover, there was diffuse EG2<sup>+</sup> staining in the surrounding tissue,

Table II. EG2<sup>+</sup> cells in involved skin after SLS application (mean  $\pm$  SD)

	Untreated skin	1 day	7 days
AD 1	ND	12.0 $\pm$ 6.0	10.0 $\pm$ 4.6
AD 2	0.5 $\pm$ 1.1	3.1 $\pm$ 2.9	5.5 $\pm$ 4.4
AD 3	ND	6.6 $\pm$ 3.8	0.8 $\pm$ 1.6
Control 1	0	0	ND
Control 2	0	0	ND
Control 3	0	0	ND

ND = not done.

suggesting a release of major basic protein (Fig. 2). By contrast, EG2<sup>+</sup> eosinophils were lacking before and after SLS for 24 h in control skin obtained from three healthy subjects.

## DISCUSSION

Past studies have demonstrated that the apparently uninvolved integument of patients with AD shows increased susceptibility to anionic surfactants as well as exogenous injuries (8–12). The current study has confirmed the prior findings and has added observations which further illuminate our understanding of the nature of this increasingly prevalent and still mysterious disorder. Our salient findings were:

(1) A 24-h exposure to 1% SLS greatly increased TEWL in Japanese patients with AD, in comparison to modest increases in controls. TEWL was still increased 7 days later, much more so in AD patients. Agner (4) did not find any specific increase in TEWL in AD patients using a much lower concentration of SLS. An abnormal stratum corneum in AD patients has been demonstrated ultrastructurally and biochemically (12, 13). We confirmed the abnormal stratum corneum in AD functionally.

(2) Histopathologically, in Philadelphia, 1% SLS induced in AD patients, but not in controls, focal spongiotic foci with exocytosis of lymphocytes along with a perivascular mononuclear infiltrate at 48 h. Eosinophils were noted in the perivascular mononuclear cell infiltrate of the same subjects but not in controls. In the Japanese patients, only mild spongiosis was found in the epidermis after 1% SLS. This disparity in the epidermal changes might be due to racial differences as well as to the utilized methods, e.g. patch testing method and timing of biopsy for acute changes (48 h for US versus 24 h for Japanese). Our data contrast with a previous report which showed that focal spongiosis could be induced by 5% SLS patch test in normals (14). With a higher concentration of 2% SLS, Scheynius & Fischer (15) reported the persistence of a perivascular T-lymphocyte infiltrate well beyond 7 days in normal individuals.

The histopathology of early AD lesions is characterized by mild spongiosis, exocytosis of lymphocytes and parakeratosis (16). We believe that we have induced a miniature AD by exposure to 1% SLS, based on histopathologic findings which seem to be similar to the native disease. This accords with earlier findings in Philadelphia in which a persistent site of histologically verified AD was induced by acute occlusive exposure to SLS. We judge these reactions to be examples of Koebnerization, which clinicians realize is fairly common in patients with AD. The presence of a low grade dermatitis and an impaired barrier in the apparently uninvolved skin of AD patients helps explain Koebnerization (3). Psoriasis is another disease in which subtle, subclinical abnormalities can be demonstrated in apparently uninvolved skin. Focusing only on visible lesions obscures the fact that the entire integument is involved (17).

So far, Koebnerization has not been experimentally induced in AD patients by other investigators. In Sendai, stripping to the glistening layer with adhesive cellophane tape failed to provoke Koebnerization, unlike that reported in psoriasis (18). The SLS response, which excited an inflammatory response in addition to damaging the stratum corneum barrier, may be peculiar to this substance (19).

(3) The immunohistochemical studies in Japan gave further evidence of Koebnerization. The presence of EG2<sup>+</sup> activated eosinophils in the perivascular mononuclear infiltrate at 24 h was a striking finding, completely absent in controls. EG2<sup>+</sup>

eosinophils were apparently in an activated state releasing eosinophil-cationic-protein (ECP). Bruynzeel et al. (20) demonstrated that EG2<sup>+</sup> eosinophils infiltrated the dermis and epidermis after patch tests with aeroallergens in AD patients. As mentioned above, in Philadelphia abundant eosinophils were observed even in ordinary H and E sections at 48 h. Further, CD4<sup>+</sup> T cells and CD1a<sup>+</sup> dendritic cells migrated towards the epidermis from the infiltrate, which was more abundant and longer lasting than in normals. HLA-DR<sup>+</sup> infiltrating cells are believed to consist of activated T cells, macrophages and dendritic cells.

A recent study (21) showed ICAM-1 and several integrins expressed in the keratinocytes after a 1% SLS patch test for 24 h. We observed focal expression of ICAM-1 on keratinocytes after SLS, but there was no difference in intensity between AD patients and controls. A prior study (22) reported that cultured keratinocytes produced several cytokines after stimulation by SLS. We postulate that after SLS exposure eosinophil chemoattractants may be released by mast cells and activated T-cells with a Th2 cytokine profile in AD patients (22, 23) in blood as well as the skin.

The eosinophilic infiltrate in AD persisted for at least 5–7 days after SLS. This suggests that strong damage to the stratum corneum barrier will facilitate subsequent penetration of other environmental allergens, hence inducing AD lesions. Interestingly, we observed a similar eosinophil infiltrate 24 h after 1% SLS exposure conducted on a 34-year-old female with allergic rhinitis. Thus, atopic respiratory disease may have some kinship with AD. Clinicians are aware of overlapping syndromes. Recently, Elias et al. (24) speculated that acute perturbations to the barrier from insults such as organic solvents, tape stripping, and surfactants stimulate a series of homeostatic responses, resulting in a downstream cascade of chemokines, adhesion molecules and other mediators, culminating in inflammation, angiogenesis and fibroplasia.

We have demonstrated two unique findings after exposure to SLS. The first is a stronger and longer lasting damage of the stratum corneum barrier as measured by diffusional water loss. Low levels of intercellular lipid ceramides (25, 26) are thought to be partly responsible for this unusual proclivity to SLS-induced irritation (27). The second and more important finding is that the SLS inflammatory reaction is histologically similar to that which occurs in the native disease and in the patch test reaction to specific allergens.

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