

Differential Effects of Sodium Lauryl Sulphate and Non-anoic Acid on the Expression of CD1a and ICAM-1 in Human Epidermis

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Normal human skin was exposed to two different detergents, sodium lauryl sulphate in distilled water and non-anoic acid in isopropanol at different concentrations. The detergents were applied under occlusion in epicutaneous tests for 24 h and biopsies were taken at 24 or 48 h. Frozen sections were labelled with monoclonal antibodies against CD1a, CD3 and ICAM-1. The evaluation of the labelled sections showed that there were differential effects on the expression of ICAM-1 and CD1a+ cells in epidermis. After non-anoic acid application ICAM-reactivity could not be detected and there was a decrease of staining for CD1a after exposure to 80% non-anoic acid. Sodium lauryl sulphate treatment, however, induced ICAM-1 expression on keratinocytes and had minor effects on the number of CD1a+ cells. ICAM-1 expression was also detected in normal epidermis in 3 of 9 unexposed control biopsies and after occlusion with the vehicles distilled water and isopropanol. An increased amount of CD3+ cells was found in the skin exposed to both detergents. The results show that there are dose and time dependent variations in the epidermal response to irritants which might influence the immunological events taken place in the epidermis. **Key words:** Irritant contact dermatitis; Detergents; Occlusion; Immunohistochemistry.

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During the past decade it has become evident that the epidermis fulfills a function as a micro milieu for immunological events. Several dermatoses including skin associated lymphomas are characterized by the migration to and the presence of T-lymphocytes in

the epidermis. The epidermal keratinocytes can express the intercellular adhesion molecule-1 (ICAM-1) and this molecule acts as a ligand for the lymphocyte function associated antigen-1 (LFA-1). Interactions between LFA-1 and ICAM-1 are crucial events in numerous immunological and inflammatory processes (cf.1). In normal skin ICAM-1 is found only occasionally on keratinocytes (2). On the contrary, the expression of ICAM-1 on keratinocytes has been described to be increased in several inflammatory dermatoses including allergic contact dermatitis and in skin associated lymphomas (3-6). In a comparison of allergic and irritant contact dermatitis (3) ICAM-1 was increased in the allergic reaction but not in the irritant. Recently it has been described that the ICAM-1 expression on keratinocytes precedes and is closely linked to the migration of T-lymphocytes into epidermis in the evolving delayed hypersensitivity reaction (7, 8). The expression of ICAM-1 on the keratinocytes can be manipulated both in vitro and in vivo by gamma-interferon (1, 9-12), tumour necrosis factor in vitro (1, 9, 11), and phorbol ester in vitro (12). Thus the presence of ICAM-1 has been considered an important mechanism for the induction, maintenance, and resolution phases of skin diseases (12).

Contact dermatitis can be divided in allergic or irritant contact reactions. The allergic reaction is associated with a "memory" and is elicited at each contact with the allergen in question. On the contrary, the irritant contact reaction is considered non-specific. Both are characterized by an inflammatory process involving mediators and a cell infiltrate dominated by T-lymphocytes (cf. 13, 14, 15). It is thus possible that irritants has the capacity of altering the epidermal pre-requisites for immunological events. At the ultrastructural level it has recently been dem-

Table I. The ocular evaluation of the irritant reactions at the time of biopsy. 0 = negativ; + = redness; ++ = redness and oedema.

Group 1 (48 h) n = 3	Contr 0	Distilled water 0	2% SLS +	4% SLS ++
Group 2 (48 h) n = 3	Contr 0	Isopropanol 0	20% NAA 0 - +	80% NAA +
Group 3 (24h) n = 3	Contr 0	80% NAA + - ++	4% SLS + - ++	

onstrated that in the initial phase of the irritant reactions there are differences in the effects on epidermal cells between different substances (16) indicating diverse effects on cellular functions. Using energy dispersive X-ray microanalysis (EDX) we have previously demonstrated that different irritants cause diverse responses in the epidermal cells at a functional level as measured by the elemental content of the cells (i.e. the sodium, magnesium, phosphorous and potassium concentrations) (17). The possibility of differences in the interactions between different irritants and immunological parameters in epidermis was approached in the present investigation where we used two different detergents at two concentrations to induce irritant reactions in human skin. The reactions have been evaluated by immunohistochemistry using monoclonal antibodies directed against CD1a, CD3 and ICAM-1.

MATERIAL AND METHODS

Subjects

Irritant reactions were induced in 9 healthy volunteers without any clinical or anamnestic signs of skin diseases or atopy. All had given their informed consent. The investigation was approved by the Ethics committee at Karolinska hospital.

Patch testing

As irritants we used two different types of detergents: sodium lauryl sulphate (SLS) in distilled water and nonanoic acid (NAA) in isopropanol. The test substances were applied under occlusion in small Finn-Chambers®, diameter 8 mm, (18) on the gluteal area to ascertain non sun-exposed skin. Fifteen µl of the test solutions were applied on filter paper in each chamber. The strength of the test reactions was graded visually at the time of biopsy according to a scale from 0 to +++ where +++ represents redness, oedema and papules-vesicles, ++ equals redness and oedema, + stands for redness and 0 is a negative skin reaction. Skin biopsies, diameter 3 mm, were taken after dermal injection of local anesthesia (Lidocaine®, ASTRA, Sweden). The biopsies were immediately placed in transport medium (Histocon®, HistoLab, Sweden) on ice and

subsequently snap-frozen in chilled isopentane and stored at -70°C.

Experimental design

The experiments were divided in three groups:

Group 1: On each of three persons (2 males, 1 female, age 26-52 years) 2% SLS, 4% SLS in distilled water (w/v) and distilled water alone were applied under occlusion for 24 h. Biopsies were taken at 48 h.

Group 2: On each of three persons (3 males, age 23-37 years) 20% NAA, 80% NAA in isopropanol (v/v) and isopropanol alone were applied under occlusion for 24 h. Biopsies were taken at 48 h.

Group 3: On each of three persons (3 males, age 24-25 years) 4% SLS in water and 80% NAA in isopropanol were applied under occlusion for 24 h. Biopsies were taken at 24 h just after the removal of the Finn Chambers.

In all groups unexposed skin served as control.

Immunocytochemistry

Vertical frozen sections were cut in a cryotome at -30°C. Acetone-fixed sections, 6 µm thick, were stained with a 3-stage monoclonal antibody peroxidase anti-peroxidase (PAP) technique (19). The monoclonal antibodies used were: Leu-4 (working dilution: 1:256; cluster determinants: CD3; mainly pan T cells) and Leu-6 (1:64; CD1a: Langerhans' cells) from Becton Dickinson, Sunnyvale, CA, USA, and anti-ICAM-1 (1:80; CD54) from Serotec, Oxford, UK. The peroxidase reaction was developed with 3-amino-9-ethylcarbazole and the sections were counterstained with Mayer's haematoxylin. The dilutions of antibodies were determined using sections from normal lymph nodes and skin. Specificity tests included omission of the primary antibodies, and staining was not observed in these tests. The examination was performed under coded conditions by one investigator. The sections were evaluated twice at different times. Serial sections were obtained and consecutive sections were taken for the staining so that every fifth section was stained with one and the same antibody. At least two sections were included for each antibody and biopsy. The sections were viewed in a light microscope at X400 using a standard eye-piece. Only the interfollicular part of epidermis was evaluated. The CD1a positive cells were counted in at least 10 fields per biopsy. Cells with dendrites and a detectable cell nucleus were considered positive. The result is expressed as the number of positive cells per field. The amount of CD3+ cells in dermis and the amount of ICAM-1 positivity in epidermis were estimated semi-quantitatively using a scale from 0 to 3 (0 = no, 1 =

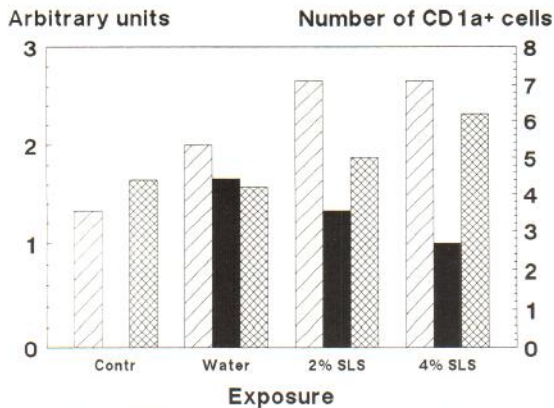


Fig. 1. The results in group 1. Exposure to SLS for 24 h, biopsies at 48 h. The means for three persons are given. ■ CD3+ cells; ■ ICAM-1+ cells; ☒ CD1a+ cells.

few, 2 = moderate, 3 = many cells). The scale was adjusted for each antibody so that the grading 3 refers to the maximal numbers of positive cells within all specimens.

RESULTS

The visual evaluation revealed that the skin reactions induced were in agreement within the three different experimental groups (Table I) with no marked interindividual differences. At 48 h (groups 1 and 2) the reactions to the 4% SLS had a marked oedema compared to the reactions to the NAA. At 24 h (group 3) the reactions to SLS and NAA were similar. Non of the persons showed macroscopical reactions to the vehicles (water and isopropanol).

The results of the immunohistochemical analysis are given in Figs. 1, 2, and 3. The exposure to both NAA and SLS caused an increase in the number of CD3+ cells in the upper part of dermis at 24 h and at 48 h. At 48 h there was also a minor increase in CD3+ cells after water exposure but not after isopropanol. Few CD3+ cells were also found in the epidermis after both SLS and NAA exposure.

The ICAM-1 expression in epidermis did, however, differ between the two detergents and so did the number of CD1a+ cells. After SLS exposure ICAM-1+ keratinocytes were increased at 24 and 48 h. At 48 h after 4% SLS treatment there was a tendency to increased number of CD1a+ cells. In the case of NAA the CD1a+ cells were clearly decreased after the exposure to the 80% solution. ICAM-1-reactivity could not be detected in the epidermis exposed to 20% or 80% NAA. Compared to the controls both water and isopropanol exposed epidermis showed increased levels in ICAM-1 ex-

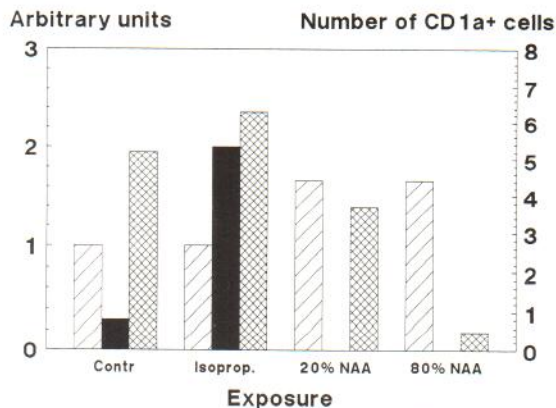


Fig. 2. The results in group 2. Exposure to NAA for 24 h, biopsies at 48 h. The means for three persons are given. ■ CD3+ cells; ■ ICAM-1+ cells; ☒ CD1a+ cells.

pression. The ICAM-1 reactivity seen after SLS, water and isopropanol was not homogenous in distribution but was found on keratinocytes in groups in the epidermis. In the control biopsies, 3 of 9 specimens displayed ICAM-1 reactivity on single cells or few keratinocytes in dispersed groups.

DISCUSSION

In the present investigation we have considered the possibility of dose and time differences in the epidermal response to different irritants. SLS and NAA belongs to two different groups of detergents and thus have different chemical properties. We applied concentrations of the detergents that has been shown to induce similar clinical reactions (21). The primary irritant reaction induced by the patch tests

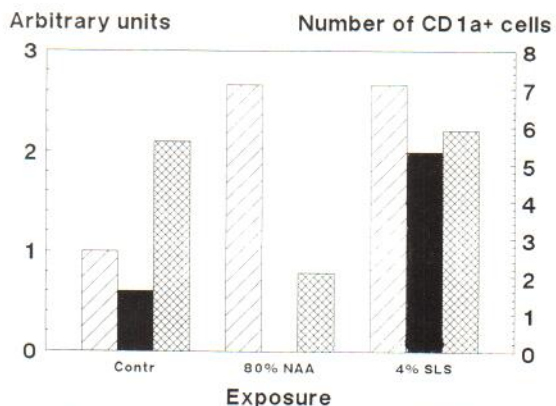


Fig. 3. The results in group 3. Exposure to 80% NAA and 4% SLS for 24 h. Biopsies at 24 h. The means for three persons are given. ■ CD3+ cells; ■ ICAM-1+ cells; ☒ CD1a+ cells.

did express similar clinical pictures with erythema and oedema for the two substances with a lymphocyte infiltrate in dermis and epidermis (measured as CD3+ cells) of similar magnitude. There were, however, differences in the response regarding dose and time (cf. Table I, Figs. 1–3). The result of the present investigation show that the expression of ICAM-1 on epidermal keratinocytes do differ when the skin is exposed to different irritants. The same reaction pattern was seen for the Langerhans' cells (CD1a+ cells). In spite of the lymphocyte infiltrate, there was no ICAM-1 expression in epidermis in the case of NAA. After SLS treatment there was a detectable ICAM-1 positivity on epidermal keratinocytes and ICAM-1 was also detectable in epidermis after occlusion with the vehicles water and isopropanol. Since the discovery of the intercellular adhesion molecules it has been reported by several groups that ICAM-1 positivity on keratinocytes is linked to the presence of a lymphocyte infiltrate (3–8). Some data in the literature, however, indicate that the interactions between the ICAM-1 expression and the presence of a lymphocyte infiltrate can be more subtle than a direct association. The fact that adhesion molecules have been reported to be absent in normal epidermis was recently disputed by Konter et al. (2) who found some ICAM-1 positive keratinocytes in an extensive study on different classes of adhesions molecules. They concluded that the expression of adhesion molecules in cutis follows a constant distribution in different cell systems and that their results indicate that the ICAM-1 molecule is not a prerequisite for lymphocyte epidermotropism. This is in agreement with our findings of 3 out of 9 unexposed control biopsies with ICAM-1 positive keratinocytes. In a report on inflammatory skin lesions Vejlsgard et al. (3) did not find ICAM-1 on the keratinocytes after exposure to SLS or croton oil in more than 4 out of 27 biopsies. The tested concentrations of the two irritants were not given but it can be assumed that the primary irritant reactions were associated with a lymphocytic infiltrate (cf. 13, 14, 15). In agreement with our results, Willis (personal communication, 20) has demonstrated that SLS and NAA did produce different expressions of ICAM-1 on the epidermal keratinocytes when applied under occlusion for 48 h with detectable ICAM-1 after SLS but not after NAA exposure. Furthermore, it has been shown that the phorbol ester has the capacity to induce ICAM-1 expression on keratinocytes *in vitro* (12). This induction is mediated via a protein

kinase C signal transduction. It is of interest that the phorbol ester do not induce HLA-DR expression as does the interferon-gamma. The phorbol ester is a derivative of croton oil which was reported not to induce ICAM-1 expression.

The discrepancies in the occurrence of CD1a+ cells after SLS and NAA applications seen in the present study is confirmed by the results of Willis et al. (22). They could also confirm the disappearance of CD1a+ cells at the ultrastructural level (22). It is thus possible that the two substances, SLS and NAA, interacts with the epidermal cells in different ways regarding both the point of action on the cellular level and the time course.

There are several possible explanations for our findings. Different chemicals might interact directly on the transduction of membrane signals leading to differences in the expression of cell surface molecules (12). This can not fully explain the reduction in CD1a+ cells as it has been demonstrated that this is indeed a reduction of cells (22). Another possible factor is the time and dose effect, meaning that the time and dose response for the expression of cell surface molecules might vary between different chemical substances. This implies variations in the complicated interplay between cytokines and cells. A factor that has to be further investigated, and that is linked to the above discussed possibilities, is the active dose at the cellular level of the applied substances. That is the effects on the cellular metabolism and membrane functions. To obtain this information there is a necessity to measure cellular function from a physiological point of view. This can be obtained by the EDX-technique where the elemental content of cells can be determined. In an early EDX study on irritant effects of di-nitrochlorobenzene it was indeed possible to demonstrate dose and time dependent alterations in the elemental content of epidermal cells after the application on the skin of the di-nitrochlorobenzene (23). Using the EDX technique it has also been possible to follow the induction and regression of chemically induced hyperplasia in guinea-pig epidermis (24).

In conclusion, the present investigation demonstrates that different irritants applied to the skin surface might induce different responses in epidermis measured with markers for immunological components although the clinical picture is that of primary irritancy. This implies a more complex interaction between environmental factors and the epidermis in the context of irritant reactions. This

opens up the very interesting question of how low grade irritant stimuli might influence the immune response of the skin. This is of a direct clinical interest in the management of contact dermatitis.

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