View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Differential Effects of Detergents on Keratinocyte Gene Expression

Fred van Ruissen, Mai Le, Joseph M. Carroll,* Pieter G. M. van der Valk, and Joost Schalkwijk

Department of Dermatology, University Hospital Nijmegen, Nijmegen, The Netherlands; *Genetics Institute, Andover, Massachusetts, U.S.A.

We have studied the effect of various detergents on keratinocyte gene expression *in vitro*, using an anionic detergent (sodium dodecyl sulfate), a cationic detergent cetyltrimethylammoniumbromide (CTAB), and two nonionic detergents, Nonidet P-40 and Tween-20. We measured the effect of these detergents on direct cellular toxicity (lactate dehydrogenase release), on the expression of markers for normal differentiation (cytokeratin 1 and involucrin expression), and on disturbed keratinocyte differentiation (SKALP) by nothern blot analysis. As reported in other studies, large differences were noted in direct cellular toxicity. In a culture model that mimics normal epidermal differentiation we found that low concentrations of sodium dodecyl sulfate could induce the expression of SKALP, a proteinase inhibitor that is not normally expressed in human epidermis but is found in hyperproliferative skin. Sodium dodecyl sulfate caused upregulation of involucrin and downregulation of cytokeratin 1 expression, which is associated with the hyperproliferative/inflammatory epidermal phenotype found in psoriasis, wound healing, and skin irritation. These changes were not induced after treatment of cultures with CTAB, Triton X-100, and Nonidet-P40. This effect appeared to be specific for the class of anionic detergents because sodium dodecyl benzene sulfonate and sodium laurate also induced SKALP expression. These *in vitro* findings showed only a partial correlation with the potential of different detergents to induce clinical, biophysical, and cell biologic changes *in vivo* in human skin. Both sodium dodecyl sulfate and CTAB were found to cause induction and upregulation of SKALP and involucrin at low doses following a 24 h patch test, whereas high concentrations of Triton X-100 did not. Sodium dodecyl sulfate induced higher rates of transepidermal water loss, whereas CTAB treated skin showed more signs of cellular toxicity. We conclude that the action of anionic detergents on epidermal keratinocytes is qualitatively different from the other detergents tested, which might have implications for *in vitro* toxicology studies that use cell biologic parameters as a read-out. We would hypothesize that detergents cause skin injury by several mechanisms that include direct cellular toxicity, disruption of barrier function, and detergent specific effects on cellular differentiation, as demonstrated here for sodium dodecyl sulfate, sodium dodecyl benzene sulfonate, and sodium laurate.* ***Key words: cultured cells/human keratinocytes/irritancy. J Invest Dermatol 110:358–363, 1998***

Exposure of the skin to irritants can induce various physiologic and cell biologic changes (Willis *et al*, 1990, 1991, 1993). Application of irritants disrupts the barrier function of the horny layer that leads to activation of the keratinocyte. High concentrations of irritants may lead to direct cytotoxicity or necrosis as assessed by morphologic and biochemical criteria for cell death. Furthermore, application of irritants may lead to an inflammatory process and a cellular infiltrate of polymorphonuclear leucocytes and mononuclear cells (Andersen *et al*, 1987; Thestrup Pedersen *et al*, 1989), depending on the strength of the detergent stimulus (Willis *et al*, 1993). These actions cannot be strictly separated from a mechanistic point of view because they are interdependent. Disruption of the barrier function is associated with loss of cohesion of corneocytes and desquamation and with increasing transepidermal water loss (TEWL), and consequently dehydration of the skin. The skin reacts through changes that are aimed at restoration of the epidermal barrier function. These changes include upregulation of differentiation associated proteins (e.g., involucrin) (Le *et al*, 1996), increased keratinocyte proliferation in the basal layer (Proksch *et al*, 1993), increased lipid synthesis (Proksch *et al*, 1993), and a strong upregulation of epidermal fatty acid binding protein (Le *et al*, 1996). This sequence of events finally leads to a new steady state of the cutaneous barrier. Several mediator systems have been implicated in the process of epidermal changes and inflammation. The release of cytokines by activated keratinocytes could be considered as a potential marker for irritation. Upon irritation and/or barrier disruption [e.g., by sodium dodecyl sulfate (SDS), acetone, or tape stripping], but also under pathologic conditions (e.g., psoriasis), enhanced production of pro-inflammatory cytokines on both protein and mRNA levels has been reported (Enk and Katz, 1992b; Nickoloff and Naidu, 1994; Wood *et al*, 1996). Other investigations have focused on the expression of integrins and intercellular adhesion molecule-1 (ICAM-1). Under inflammatory conditions with involvement of the epidermis, and in wound healing models, upregulation and suprabasal expression of $\alpha2\beta1$, $\alpha3\beta1$, $\alpha6\beta4$, $\alpha v\beta5$, and induction of $\alpha5\beta1$ has been demonstrated (Adams and Watt, 1991). In line with these findings ICAM-1, a specific ligand for $\beta2$ integrins, is expressed by keratinocytes *in vivo* in irritant contact dermatitis (Griffiths and Nickoloff, 1989; Griffiths

Manuscript received August 20, 1997; revised November 8, 1997; accepted for publication December 10, 1997.

Reprint requests to: Dr. Fred van Ruissen, Department of Dermatology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Abbreviation: CTAB, cationic detergent cetyltrimethylammoniumbromide.

0022-202X/98/$10.50 · Copyright © 1998 by The Society for Investigative Dermatology, Inc.

et al, 1990; Luger and Schwarz, 1990; Willis *et al*, 1991). This effect may be secondary, induced by the release of TNF-α (Willis *et al*, 1991). It has also been demonstrated that the plasminogen activator system is locally activated *in vivo* at the site of SDS induced skin irritation. The plasminogen activator system (active plasmin) is thought to exert pro-inflammatory capacity via activation of further enzyme systems, and/or via generation of chemotactic peptides and nonspecific tissue destruction (Reinartz *et al*, 1991). *In vitro* SDS/alkyl sulfates are capable of strongly activating keratinocyte derived plasminogen.

Irritancy tests in general are based on the concept of keratinocyte activation, i.e., that keratinocytes provide a signaling interface that transforms nonspecific harmful stimuli from the environment into endogenous signals, thereby activating local and systemic repair and defence mechanisms. These mechanisms include both hyperproliferative and inflammatory events (Kupper, 1989; Sauder, 1989; Luger and Schwarz, 1990). Keratinocyte activation is thought to initiate a humoral cascade originating from immediate release of preformed key mediators such as arachidonic acid and its metabolites and IL-1α. These mediators in turn trigger the biosynthesis and release of other cytokines and proinflammatory mediators (Kupper, 1989; Griffiths *et al*, 1990; Barker *et al*, 1991; Hunziker *et al*, 1992).

The different effects of irritants on keratinocytes are difficult to disentangle *in vivo*, and we have therefore applied an *in vitro* culture model to study the direct effects of irritants on keratinocytes without the involvement of inflammatory cells, and without the effects secondary to barrier disruption. Furthermore, *in vitro* irritants do not have to pass the skin barrier and bioavailability is not variable. An *in vitro* model allows discrimination between keratinocyte gene expression and cytotoxicity. Finally, environmental conditions can be controlled, a high degree of reproducibility can be obtained, and a wide range of concentrations of various test agents can be applied. In this study we compared the potential of different detergents to induce the hyperproliferative/inflammatory differentiation phenotype (as described by van Ruissen *et al*, 1996). Using keratinocyte cultures three different classes of detergents were evaluated: e.g., anionic detergents [SDS, sodium dodecyl benzene sulfonate (SDBS), and sodium laurate (SL)]; cationic detergents [cetyltrimethyl-ammoniumbromide (CTAB)]; and nonionic detergents [sorbitan mono-oleate (Tween-20), nonylphenyl-polyethylene glycol (Nonidet-P40), and polyethylene glycol tert-octylphenyl ether (Triton X-100)]. The *in vitro* effects of these detergents were measured on direct cellular toxicity [lactate dehydrogenase (LDH) release] and on the expression of several markers for normal differentiation [cytokeratin 1 (CK1) and involucrin]. As an inducible marker for disturbed differentiation we used the expression of the protease inhibitor SKALP/elafin, a member of the recently described Trappin gene family (Zeeuwen *et al*, 1997). Furthermore, induction of inflammation *in vivo* was evaluated by the assessment of erythema and TEWL. In addition, expression of involucrin and SKALP was evaluated immunohistochemically. Our results reveal differential effects of detergents on cell biologic markers, which stresses the complexity of irritant reactions *in vivo*.

MATERIALS AND METHODS

Keratinocyte cultures Keratinocytes were seeded in 6 well culture dishes and cultured until confluence in keratinocyte growth medium (KGM) as described before (van Ruissen *et al*, 1996). KGM was composed of keratinocyte basal medium (Biowhittaker, Walkersville, MD; 0.15 mM calcium), supplemented with ethanolamine (0.1 mM) (Sigma, St. Louis, MO), phosphoethanolamine (0.1 mM) (Sigma), bovine pituitary extract (0.4% vol/vol) (Biowhittaker, Walhusville, MD), insulin (5 μg per ml) (Sigma), hydrocortisone (Collaborative Research, Lexington, MA), mouse epidermal growth factor (10 ng per ml) (Sigma), penicillin (100 U per ml) (Gibco, Breda, the Netherlands), and streptomycin (100 μg per ml) (Gibco).

Keratinocyte detergent treatment At confluence, keratinocyte differentiation was established by switching the cells to KGM supplemented with 5% vol/vol fetal calf serum (Seralab, Nistelrode, the Netherlands) (KGM/FCS) or to KGM depleted of growth factors (bovine pituitary extract, insulin, and epidermal growth factor) and hydrocortisone (KGM/–GF) for 48 h. Addition of KGM/FCS induced a hyperproliferative/inflammatory differentation marked by strong expresssion of SKALP, involucrin, and transglutaminase and absence of CK1 expression. Cultures grown in KGM/–GF exhibited a normal differentiation characterized by expression of CK1, involucrin, and transglutaminase and the absence of SKALP expression (van Ruissen *et al*, 1996). Detergents were added at different concentrations to KGM/–GF. After 48 h of incubation, media were collected, cultures were washed twice with phosphate-buffered saline, and total RNA was isolated. The following detergents were applied: SDS, Triton X-100, Tween-20 (Biorad Laboratories, Richmond, CA), Nonidet P40 (BDH Chemicals, Poole, U.K.), CTAB, SDBS, and LS (Sigma).

Cytotoxicity measurement using LDH release The LDH release was measured in media of cultured human keratinocytes treated with detergents. Aliqouts of 100 μl media were transferred to a 96 microtiter plate and mixed with 100 μl reaction mixture (Boehringer, Mannheim, Germany). After 30 min incubation, performed in the dark at room temperature, the absorbance of the formazan salt (red) was measured at 495 nm. As a reference wavelength we used 655 nm. LDH release was measured at different time points during treatment (0, 3, 6, and 24 h after treatment). To exclude errors due to the substances, we performed a control for both LDH activity contained within the test substance and whether the substance itself interfered with the LDH activity. Cytotoxicity measurements were perfomed in triplicate, the values were calculated as suggested by the manufacturer, including a background control for LDH activity in the media, a control for untreated cells (0% lysis of the cells), and a control for maximum LDH release (100% lysis of the cells).

RNA isolation and northern blot analysis Cells were lysed in 1 ml RNase-All [2.1 M Guanidine-thiocyanate (Research Organics, Cleveland, OH), 8.5 mM N-lauroylsarcosine (Sigma), 12.5 mM NaAc pH 5.2, 0.35% vol/vol β-mercaptoethanol (Merck, Darmstadt, Germany), and 50% vol/vol Tris-saturated biophenol pH 8.0 (Biosolve, Amsterdam, the Netherlands)]. After lysis, 100 μl chloroform was added. The samples were centrifuged for 15 min (13000 rpm, 4°C) and the aqueous phase was precipitated with 500 μl isopropanol on ice for 45 min, and subsequently centrifuged at 4°C for 15 min. The pellet was washed with 70% ethanol and dried at room temperature for 5 min. This RNA pellet was resuspended in 150 μl NSE (50 mM NaAc, 0.2% SDS, and 2 mM ethylenediamine tetraacetic acid) and 562.5 μl 100% ethanol was added. For quantitation 62.5 μl of this RNA suspension was pelleted, resuspended in 1 ml sterile H_2O, and spectrophotometrically analyzed at 260/280 nm. Equal quantities of this total RNA (10 μg) were loaded on a 1% agarose gel and electrophoretically separated in 10 mM sodium phosphate buffer (Sambrook *et al*, 1989). The pH of the electrophoresis buffer was kept within acceptable limits by constant circulation of the buffer. The gels were blotted on positively charged nylon membranes (Boehringer) using 10 × sodium citrate/chloride buffer (1.5 M NaCl, 0.15 M NaCitrate). After RNA transfer, the membranes were washed in 2 × sodium citrate/chloride buffer, and the RNA was cross-linked to the membrane using ultraviolet irradiation (312 nm, 0.2 J per cm^2). Subsequently, blots were stained with ethidium bromide dissolved in sterile water to visualize the RNA. The blots were (pre)-hybridized overnight at 65°C as described by Church and Gilbert (1984) using random labeled probes for CK1, SKALP, involucrin, and human acidic ribosomal phosphoprotein PO (hARP), which functions as a control to quantitate the amount of total RNA loaded in each lane (Laborda, 1991). Autoradiography was performed using Kodak X-Omat X-ray films and Kodak Biomax MS films (Eastman Kodak, Rochester, NY).

Subjects A group of six healthy volunteers with no past or present history of skin disease participated in the study. Their ages ranged from 21 to 27 y, and all subjects gave written informed consent.

Detergent application Detergents were applied (day 0) for 24 h using a patch test. Aliquots of 200 μl detergent solutions were pipetted on to several patches as previously described (Le *et al*, 1996). Patches were applied to the skin of the upper back parallel to the vertebral column. The detergents were applied at the following concentrations: SDS, 1%; CTAB, 2%; and Triton-X100, 10%.

Clinical grading and TEWL measurement Visual examination and TEWL measurements were performed at 1 h (day 1) and 24 h (day 2) after removal of the patches. The erythema was graded using the following visual scoring; 1, mild patchy erythema; 2, diffuse mild erythema; 3, moderate erythema; 4, intense erythema; and 5, intense erythema with edema. TEWL measurements were performed using a Tewameter TM 210 (Courage & Khazaka, Germany), according to the standard guidelines (Pinnagoda *et al*, 1990). Before the actual assessments, a delay period of 15 min was introduced to reduce the effects of sweating (Baker and Kligman, 1967). During the measurements the room temperature was kept at a constant temperature of 20°C. Relative humidity varied from 35% to 49% (mean 39.3%).

Biopsy procedure Punch biopsies (3 mm in diameter) were taken from each of the patch tests. A maximum of two biopsies per volunteer were taken and a total of 12 biopsies were obtained per time point. After 4 h of fixation in formalin the samples were embedded in paraffin, sectioned at 6 μm, and prepared for immunohistochemistry.

Immunohistochemical stainings Sections were deparaffinized in xylene for 20 min, followed by rehydration through an ethanol series ranging from 100% to 50%, and a final wash in phosphate-buffered saline during 10 min. Sections stained for anti-involucrin received a pretreatment with citrate buffer and needed antigen retrieval in the microwave oven. Additionally, sections were preincubated for 15 min with 20% normal rabbit serum for anti-involucrin (MON-150) or 20% normal swine serum for anti-SKALP staining. Sections were incubated for 60 min with anti-involucrin (MON-150; 1:15) and anti-SKALP (SSK-9201; 1:500) diluted in phosphate-buffered saline/azide containing 1% bovine serum albumin. Finally, sections were incubated for 30 min with either peroxidase-conjugated rabbit anti-mouse or peroxidase-conjugated swine anti-rabbit immunoglobulins diluted in phosphate-buffered saline containing 5% human serum. All sections were developed using 3-amino-9-ethylcarbazol as chromogenic substrate for 10 min at 37°C. Stained sections were washed twice with demineralised water and mounted in glycerol-gelatine.

RESULTS AND DISCUSSION

Keratinocyte gene expression is induced *in vitro* after application of detergents Detergents were tested during a period of 48 h on confluent keratinocyte cultures in a protein free medium (KGM/–GF). Using northern blot analysis we evaluated the effect of detergents on keratinocyte gene expression as described previously (van Ruissen *et al*, 1996). The concentrations of detergents ranged from 0 μg per ml to 50 μg per ml for SDS, from 0 μg per ml to 3 μg per ml for CTAB, Nonidet-P40, and Triton X-100, and from 0 mg per ml to 3 mg per ml for Tween-20. We used gene expression induced by KGM/FCS (hyperproliferative phenotype) and KGM/-GF (normal phenotype) as a positive and negative control, respectively. Northern blot analyses of keratinocyte cultures treated with five different detergents are summarized in **Fig 1**. Addition of SDS leads to a strong induction of SKALP expression, upregulation of involucrin expression, and downregulation of CK1 as illustrated in **Fig 1(*d*)**. The induction of SKALP expression was found in a narrow concentration range between 3.13 and 6.25 μg per ml. At concentrations of 12 μg per ml and higher, cytotoxicity was induced and RNA isolated from these cultures was totally degraded as shown by the absence of any positive signal of the control household gene (hARP). The strong induction of SKALP and the downregulation of CK1 appears to be coupled. This demonstrates the switch from a normal differentiation program where SKALP is absent and CK1 is highly expressed, to a hyperproliferative/inflammatory differentiation where CK1 expression is downregulated and SKALP expression is strongly upregulated. The strong induction of SKALP expression and the changes in involucrin and CK1 expression are similar to those found under conditions of regenerative maturation, as described by Mansbridge and Knapp (1987). Examination of the northern blots demonstrates that CTAB, Triton X-100, Nonidet-P40, and Tween-20 do not induce changes in SKALP, involucrin, or CK1 expression, compared with the control medium without detergent.

To investigate if the induction of the hyperproliferative/inflammatory gene expression is due to the physicochemical properties of anionic detergents, we examined the induction of SKALP expression by other anionic detergents, e.g., SDBS and SL. **Figure 2** illustrates the induction of SKALP expression by SDBS. SDBS and LS induce SKALP expression in a similar concentration range as found for SDS. SKALP expression is induced at concentrations ranging from 3.13 μg per ml to 6.25 μg per ml. In addition, involucrin expression showed the same expression pattern as described by SDS. Using SDBS we see involucrin expression at concentrations ranging from 1.56 μg per ml to 6.25 μg per ml. Also the toxicity profile was similar as judged by degradation of RNA.

From these data we conclude that anionic detergents like SDS, SDBS, and SL are able to induce the hyperproliferative/inflammatory differentiation program, whereas the others do not show any signs of aberrant gene expression. It was previously shown in two other studies that anionic detergents like SDS could affect cellular gene expression (Lindberg *et al*, 1991; Reinartz *et al*, 1991). Reinartz *et al* reported

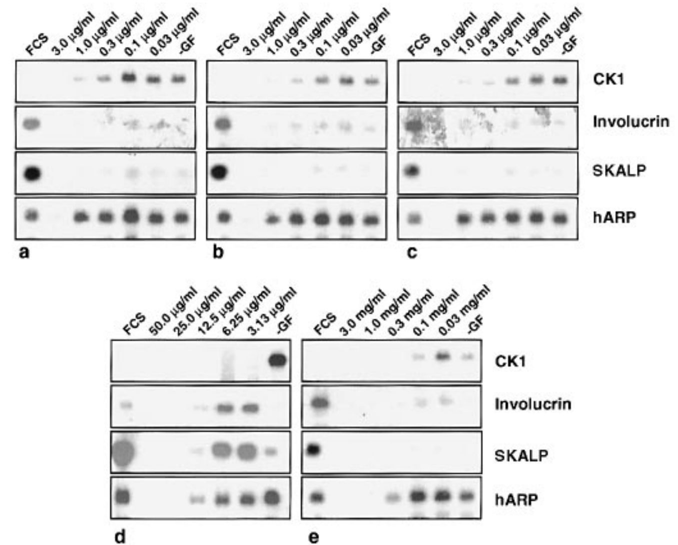

Figure 1. Keratinocyte gene expression in cultured human keratinocytes after treatment with different detergents. Human keratinocytes were grown to confluence and normal keratinocyte differentiation was established by switching the cultures to KGM/–GF. Subsequently, detergents were added at different concentrations to KGM/–GF and after 48 h of incubation total RNA was isolated. Induction of the hyperproliferative/inflammatory phenotype by the addition of 5% fetal calf serum served as a positive control (*lane 1*). Northern blots were hybridized using the probes CK1, involucrin, SKALP, and hARP. The human acidic ribosomal phosphoprotein PO (hARP) probe was used for normalization of the amount of total RNA that was used. (*a*) CTAB, (*b*) Nonidet-P40, and (*c*) Triton X-100, respectively, were applied at concentrations ranging from 3 μg per ml to 0 μg per ml; (*d*) SDS was applied at concentrations ranging from 50 μg per ml to 0 μg per ml; and (*e*) Tween-20 was applied at concentrations ranging from 3 mg per ml to 0 mg per ml. Note the difference in concentration range between Tween-20 and the other detergents.

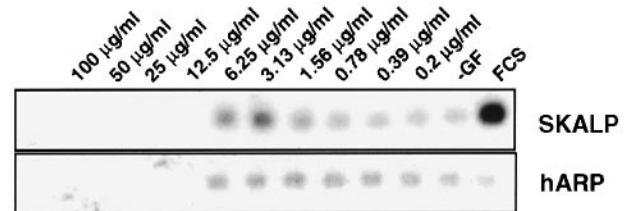

Figure 2. SKALP expression is induced in cultured human keratinocytes after treatment with SDBS. Human keratinocytes were grown to confluence and normal keratinocyte differentiation was established by switching the cultures to KGM/–GF. Subsequently, SDBS was added at different concentrations to KGM/–GF and after 48 h of incubation total RNA was isolated. Induction of the hyperproliferative/inflammatory phenotype by 5% fetal calf serum served as a positive control (last lane). Northern blot analysis was performed using the SKALP and hARP as probes. SDBS was applied at concentrations ranging from 100 μg per ml to 0 μg per ml.

differential effects of chain length of anionic detergents. Differential action of detergents on cellular metabolism could be caused by differences in interaction with the signal transduction components (Reinartz *et al*, 1991). Alternatively, differences in subcellular distribution, degradation, and kinetics of gene expression could play a role.

The possible involvement of primary cytokines in the induction of SKALP expression has recently become evident (Barker *et al*, 1991). From preliminary experiments we obtained evidence that SKALP expression could be induced by addition of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and transforming growth factor (TGF)-α. It has been reported that TNF-α expression is upregulated during irritant reaction to SLS (Hunziker *et al*, 1992). Furthermore it has been demonstrated that TNF-α, IFN-γ, and GM-CSF are upregulated after nonspecific stimuli, and that other cytokines like IL-1α, IL-1β, MIP-2, IP-10, or I-Aα were upregulated by specific contact sensitisers (Enk and Katz, 1992b). The data provided by Enk and Katz (1992b) and

Luger and Schwarz (1990) indicate that the induction of irritation is probably not caused by a single cytokine but a complex array of signals that determines that process is initiated. The cytokine profile induced by *in vitro* exposure to anionic detergents in our model requires further investigations to define in more detail the concentration of different inflammatory cytokines.

Cytotoxicity is differentially induced by various concentrations of detergents Morphologic examination of the cultures showed signs of cell damage at high concentrations of detergent, which was reflected in the inability to isolate intact RNA (see above). To check if equal amounts of RNA were loaded on the gel we hybridized the blots using hARP as control probe (Laborda, 1991). Comparison of northern blots after hybridization with hARP showed that CTAB, Nonidet-P40, and Triton X-100 induced cell damage at concentrations equal or greater than 1 μg per ml, as indicated by the empty lanes on the northern blots. Cell damage induced by Tween-20 occurred at concentrations above 0.3 mg per ml, whereas SDS and SDBS induced cell damage at concentrations higher than 12 μg per ml. These results suggest that detergents have a direct cytotoxic effect on human keratinocytes. From these data we can conclude that cytotoxicity of the detergents based on the ability to isolate intact RNA can be graded as follows: CTAB, Triton X-100, Nonidet-P40 > moderate cytotoxic (SDS, SDBS, SL) > noncytotoxic (Tween-20). Although the quality of the isolated RNA from the cultures is probably a good reflection of cellular integrity, it is not a quantitative measure for cellular damage. In order to quantitate the detergent induced effects, we evaluated cytotoxicity using the release of LDH by keratinocyte cultures as a marker for cell damage. LDH is a stable cytoplasmic enzyme present in all cells. It is rapidly released into the cell culture supernatant upon damage of the plasma membrane. Therefore, measurement of LDH is an easy and rapid method to assess cytotoxicity. Keratinocyte cultures were grown to confluence and treated with the previously described detergents at different concentrations. **Figure 3** illustrates the percentages of viable cells after treatment with SDS, CTAB, and Tween-20. SDS, CTAB, and Tween-20 all have a different range in which they are cytotoxic. For the different detergents we can express the cytotoxic effect as $^{24h}Ld_{50}$ (lowest concentration leading to a 50% reduction of viable cells after 24 h of treatment). Based on these values the cytotoxicity of the detergents can be arranged in the following order: CTAB ($^{24h}Ld_{50}$ = 3 μg per ml), SDS ($^{24h}Ld_{50}$ = 18 μg per ml), and Tween-20 ($^{24h}Ld_{50}$ = 125 μg per ml). Surprisingly, the cytotoxicity data of Triton X-100, Nonidet-P40, and LS based on the LDH release did not correlate well with the cytotoxicity data as reflected by isolation of intact RNA. At low doses of these detergents RNA was totally degraded; however, no significant LDH release could be measured. Morphologic examination of the cultures revealed blebbing and vesicle formation that might have encapsulated LDH. These data suggest that the ability to isolate intact RNA from keratinocyte cultures provides more accurate information about cell damage than measurement of LDH release, at least for some of the detergents studied.

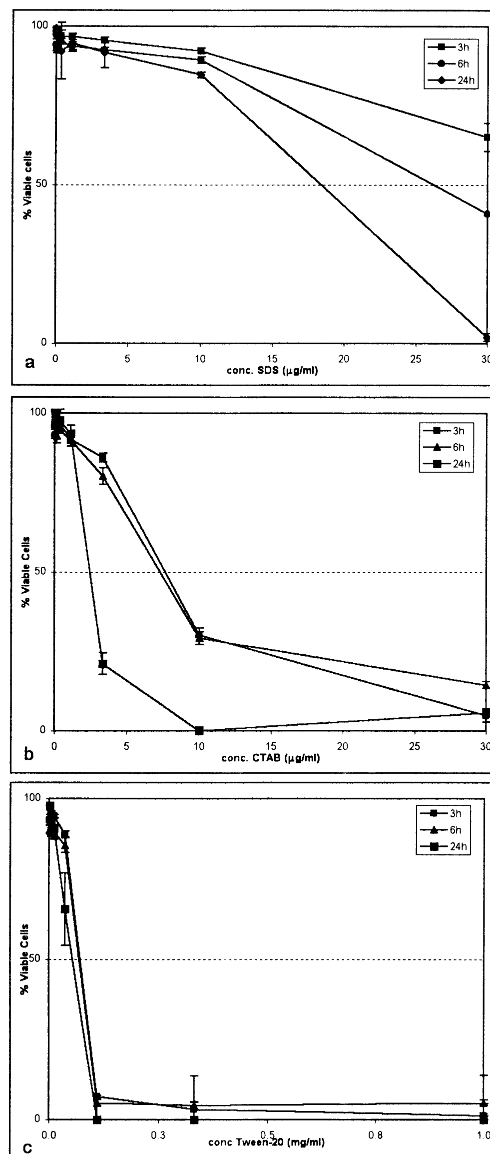

Figure 3. Differential cytotoxicity by detergents. Human keratinocytes were grown to confluence and normal keratinocyte differentiation was established by switching the cultures to KGM/–GF. Subsequently, detergents were added at different concentrations to KGM/–GF. Culture supernatants were collected at 3, 6, and 24 h after addition of the detergents and LDH release was measured. (*a*) SDS and (*b*) CTAB, respectively, were applied at concentrations ranging from 30 μg per ml to 0 μg per ml; and (*c*) Tween-20 was applied at concentrations ranging from 1 mg per ml to 0 mg per ml. Error bars indicate SEM of three experiments.

Clinical, biophysical, and cell biologic alterations are induced in human skin following application of different detergents In view of the differential effects of various types of detergents on cultured human keratinocytes with respect to gene expression and cytotoxicity, we have examined the *in vivo* effects of application of SDS, CTAB, Triton X-100, and Tween-20 on human skin. In a dose finding pilot study we found that following a 24 patch test, only SDS and CTAB induced severe redness and an increase of TEWL. Triton X-100 induced moderate TEWL and erythema at high concentrations (10%), and Tween-20 was without effect even at concentrations higher than 10% (data not shown). The cell biologic impact of detergent application on human skin and the resulting clinical effect (erythema) is likely to result from the combined effects of barrier disruption (as measured with TEWL), cytotoxic effects, and direct effects on keratinocyte cellular metabolism. On the basis of the dose finding study we have chosen concentrations of SDS and CTAB that induced similar clinical effects (erythema), and we have investigated the cell biologic alterations and disruption of barrier function. **Figure 4**(***a***) shows that application of 1% SDS or 2% CTAB, respectively, gives similar effects on erythema, both on day 1 and on day 2 after application. The effect on barrier disruption, however, was different because 1% SDS induced a higher increase of TEWL than 2% CTAB (**Fig 4*b***). Histopathologic examination revealed that overt cellular toxicity, ranging from cells with pycnotic nuclei to intraepidermal blister formation, was present only in the biopsies from CTAB treated skin. Immunohistochemical evaluation for the induction of markers associated with the regenerative/inflammatory differentiation program of the epidermis, such as induction of SKALP expression or an increase in involucrin expression, revealed no gross differences between SDS and CTAB. **Figure 5** shows that SKALP is induced in the stratum granulosum and upper stratum spinosum in SDS treated skin at 24 h after application. Skin treated with CTAB shows similar results, and skin treated with 10% Triton X-100 does not show induction of SKALP or upregulation of involucrin. In a separate study we compared very low doses of SDS and CTAB, which induced a similar rate of TEWL comparable with

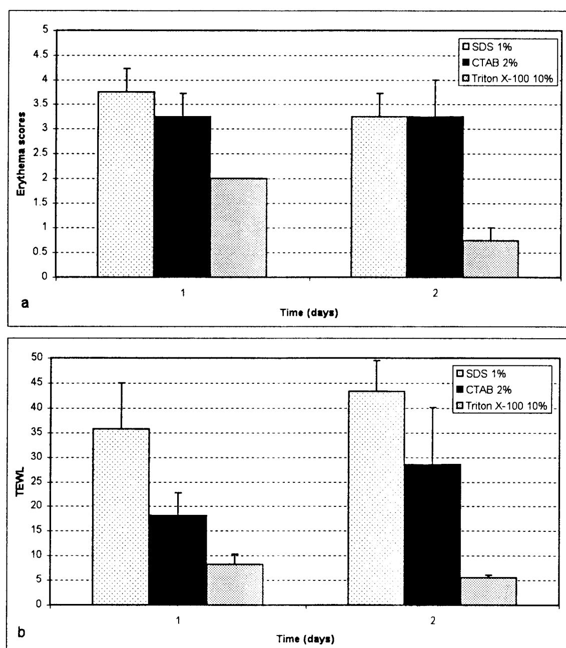

Figure 4. *In vivo* induction of erythema and TEWL after application of detergents. Detergents were applied on normal human skin during a 24 h patch test. We compared the effects of 1% SDS, 2% CTAB, and 10% Triton X-100. Erythema (*a*) and TEWL (*b*) scores were obtained 1 h and 24 h after removal of the patch. Note that 1% SDS and 2% CTAB induced similar degrees of erythema, whereas a high concentration of the non-ionic detergent Triton X-100 only induces moderate erythema at day 1. A marked difference in TEWL between SDS and CTAB was found despite the fact that similar erythema scores were induced. TEWL values of Triton X-100 are given for comparison. No significant increase in TEWL was found at any time. Error bars indicate mean ± SEM of six volunteers.

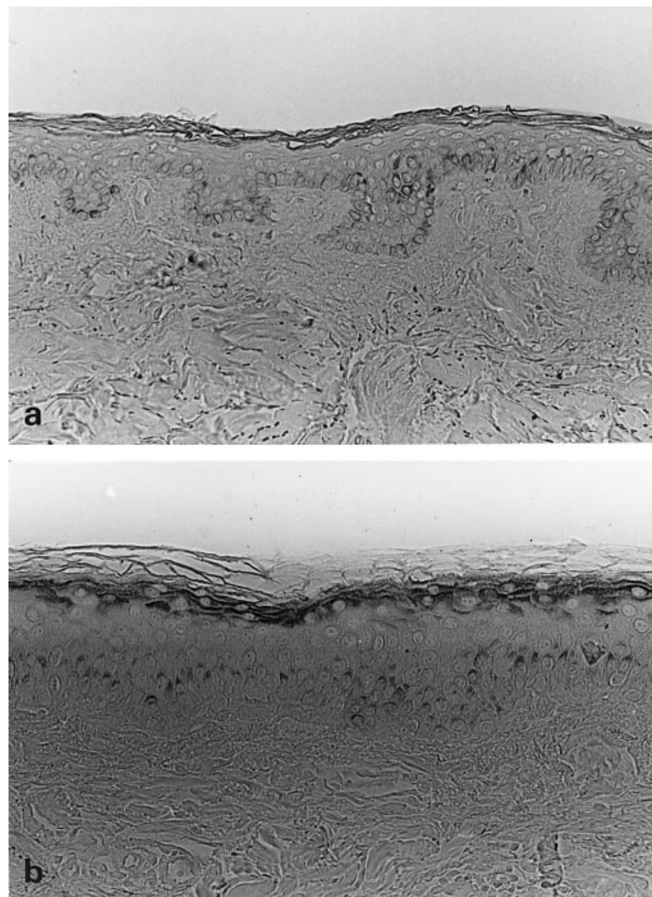

Figure 5. SKALP expression in normal and SDS treated skin. SDS was applied on normal human skin during a 24 h patch test. Biopsies were taken 24 h after removal of the patch and sections were immunostained with a polyclonal anti-SKALP anti-serum. SKALP, which is absent in normal skin (*a*), is induced in the stratum granulosum of SDS (1%) treated skin (*b*). Note that the apparent signal in the basal layer does not represent SKALP immunostaining, but is caused by melanin granules.

that of 10% Triton X-100. Here we found a very mild expression of SKALP and upregulation of involucrin both with SDS and with CTAB, and no effect of Triton X-100 (not shown). These findings basically show two things: first, a similar clinical outcome, as assessed by erythema, is not necessarily accompanied by similar findings in TEWL and cellular toxicity, and second, similar rates of TEWL are not necessarily accompanied by similar cell biologic changes, depending on the detergents studied.

Disruption of the barrier results in activation of keratinocytes within hours (Proksch *et al*, 1993; Nickoloff and Naidu, 1994; Wood *et al*, 1996). This results in upregulation of CK16 and keratinocyte proliferation accompanied by the production of a specific cytokine and adhesion molecule profile. It has been demonstrated that the dermal cytokine response is delayed in comparison with the epidermal cytokine response. In addition, the dermal response was deficient in several cytokines and was quantitatively distinctive from the epidermal response. These results indicate that keratinocytes can be directly activated within hours of addition of exogenous stimuli, and they thereby influence the underlying dermal compartment (Nickoloff and Naidu, 1994). In previous studies we have shown that disruption of barrier function by tape-stripping leads to upregulation of dermal tenascin expression that is probably the result of keratinocyte signaling to the dermal cells (Schalkwijk *et al*, 1991). Furthermore, direct action of detergents on keratinocytes with respect to the cytokine release depends on the type of detergent/stimuli used. As described by Enk and Katz (1992a, b), detergents/stimuli cause a selective change in the signal strength of the mRNA of several cytokines. The upregulation or induction of cytokines takes place over a short period of time, ranging from 15 min to 6 h. Here we use a 24 h patch test and evaluated changes 24 h after removal of the patch. In this model it is not possible to distinguish between barrier disruption, direct action on keratinocytes, or inflammatory changes. Because the extent of barrier disruption and the regenerative responses following cell damage also influence the cell biologic alterations, it is very difficult to draw conclusions on the contribution of direct actions of detergents on keratinocyte metabolism *in vivo*. *In vitro* models using human keratinocytes, as described here, enable us to study the direct interactions between irritant and cells, which are difficult to dissect *in vivo*. Further investigations are required to assess whether the information derived from these *in vitro* systems, together with the physicochemical properties of the detergent/irritant, can be used to predict the response of human skin exposed to these stimuli.

This work was supported by grant PAD 96-03 from the Alternatives to Animal Experimentation Platform, The Netherlands.

REFERENCES

Adams JC, Watt FM: Expression of β1, β3, β4, and β5 integrins by human epidermal keratinocytes and non-differentiating keratinocytes. *J Cell Biol* 115:829–841, 1991

Andersen KE, Benezra C, Burrows D, *et al*: Contact dermatitis. A review. *Contact Dermatitis* 16:55–78, 1987

Baker H, Kligman AM: Measurement of transepidermal water loss by electrical hygrometry. Instrumentation and responses to physical and chemical insults. *Arch Dermatol* 96:441–452, 1967

Barker JN, Mitra RS, Griffiths CE, Dixit VM, Nickoloff BJ: Keratinocytes as initiators of inflammation [see comments]. *Lancet* 337:211–214, 1991

Church GM, Gilbert W: Genomic sequencing. *Proc Natl Acad Sci USA* 81:1991–1995, 1984

Enk AH, Katz SI: Early events in the induction phase of contact sensitivity. *J Invest Dermatol* 99:39S–41S, 1992a

Enk AH, Katz SI: Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci USA* 89:1398–1402, 1992b

Griffiths CE, Nickoloff BJ: Keratinocyte intercellular adhesion molecule-1 (ICAM-1) expression precedes dermal T lymphocytic infiltration in allergic contact dermatitis (Rhus dermatitis). *Am J Pathol* 135:1045–1053, 1989

Griffiths CE, Esmann J, Fisher GJ, Voorhees JJ, Nickoloff BJ: Differential modulation of keratinocyte intercellular adhesion molecule-I expression by gamma interferon and phorbol ester: evidence for involvement of protein kinase C signal transduction. *Br J Dermatol* 122:333–342, 1990

Hunziker T, Brand CU, Kapp A, Waelti ER, Braathen LR: Increased levels of inflammatory cytokines in human skin lymph derived from sodium lauryl sulphate-induced contact dermatitis. *Br J Dermatol* 127:254–257, 1992

Kupper TS: Mechanisms of cutaneous inflammation. Interactions between epidermal cytokines, adhesion molecules, and leukocytes [published erratum appears in Arch Dermatol 1989 Dec; 125 (12):1643]. *Arch Dermatol* 125:1406–1412, 1989

Laborda J: 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. *Nucl Acids Res* 19:3998–3998, 1991

Le TK, Schalkwijk J, van Siegenthaler G, de Kerkhof PCM, Veerkamp JH, van der Valk PGM: Changes in keratinocyte differentiation following mild irritation by sodium dodecyl sulphate. *Arch Dermatol Res* 288:684–690, 1996

Lindberg M, Farm G, Scheynius A: Differential effects of sodium lauryl sulphate and non-anoic acid on the expression of CD1a and ICAM-1 in human epidermis. *Acta Derm Venereol* 71:384–388, 1991

Luger TA, Schwarz T: Evidence for an epidermal cytokine network. *J Invest Dermatol* 95:100S–104S, 1990

Mansbridge JN, Knapp AM: Changes in keratinocyte maturation during wound healing. *J Invest Dermatol* 89:253–263, 1987

Nickoloff BJ, Naidu Y: Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol* 30:535–546, 1994

Pinnagoda J, Tupker RA, Agner T, Serup J: Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 22:164–178, 1990

Proksch E, Holleran WM, Menon GK, Elias PM, Feingold KR: Barrier function regulates epidermal lipid and DNA synthesis. *Br J Dermatol* 128:473–482, 1993

Reinartz J, Boukamp P, Schickel E, Fusenig NE, Kramer MD: Activation of the plasminogen activator system in a keratinocyte cell line (HaCaT) by alkyl sulfates. *Biomed Biochim Acta* 50:743–748, 1991

van Ruissen F, de Jongh GJ, Zeeuwen PL, van Erp PE, Madsen P, Schalkwijk J: Induction of normal and psoriatic phenotypes in submerged keratinocyte cultures. *J Cell Physiol* 168:442–452, 1996

Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning. A Laboratory Manual*. Cold Sprin Harbor Laboratory Press, New York, 1989

Sauder DN: Interleukin 1. *Arch Dermatol* 125:679–682, 1989

Schalkwijk J, Steijlen PM, van Vlijmen-Willems IMJJ, Oosterling B, Mackie EJ, Verstraeten AA: Tenascin expression in human dermis is related to epidermal proliferation. *Am J Pathol* 139:1143–1150, 1991

Thestrup Pedersen K, Larsen CG, Ronnevig J: The immunology of contact dermatitis. A review with special reference to the pathophysiology of eczema. *Contact Dermatitis* 20:81–92, 1989

Willis CM, Stephens CJ, Wilkinson JD: Differential effects of structurally unrelated chemical irritants on the density and morphology of epidermal CD1+ cells. *J Invest Dermatol* 95:711–716, 1990

Willis CM, Stephens CJ, Wilkinson JD: Selective expression of immune-associated surface antigens by keratinocytes in irritant contact dermatitis. *J Invest Dermatol* 96:505–511, 1991

Willis CM, Stephens CJ, Wilkinson JD: Differential patterns of epidermal leukocyte infiltration in patch test reactions to structurally unrelated chemical irritants. *J Invest Dermatol* 101:364–370, 1993

Wood LC, Elias PM, Calhoun C, Tsai JC, Grunfeld C, Feingold KR: Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. *J Invest Dermatol* 106:397–403, 1996

Zeeuwen PLJM, Hendriks W, de Jong WW, Schalkwijk J: Identification and sequence analysis of two new members of the SKALP/elafin and SPAI-2 gene family. *J Biol Chem* 272:20471, 1997