
Confocal histopathology of irritant contact dermatitis in vivo and the impact of skin color (black vs white)

Shari P. Hicks, MD,^a Kirsty J. Swindells, MRCP,^a Maritza A. Middelkamp-Hup, MD,^a
Martine A. Sifakis, MEd,^a Ernesto González, MD,^b and Salvador González, MD^a
Boston, Massachusetts

Background: The pathogenesis of irritant contact dermatitis and its modulation according to skin color is not well understood. Reflectance confocal microscopy (RCM) enables high-resolution, real-time, in-vivo imaging of human skin.

Objective: The goal of our study was to use RCM to determine whether susceptibility to irritant contact dermatitis differs between black and white skin.

Methods: Participants were placed in groups on the basis of skin color and the volar aspects of their forearms exposed to 1% and 4% sodium lauryl sulfate using Finn Chambers (Allerderm Laboratories Inc, Petaluma, Calif). They were evaluated at 6, 24, and 48 hours by RCM, transepidermal water loss, laser Doppler velocimetry, and routine histology.

Results: Participants with white skin had more severe clinical reactions than those with black skin. RCM revealed microscopic changes even without clinical evidence of irritation. Confocal features included parakeratosis, spongiosis, perivascular inflammatory infiltrate, and microvesicle formation, and these features were confirmed by routine histology. Also, participants with white skin had greater mean increases in transepidermal water loss after exposure to 4% sodium lauryl sulfate than did participants with black skin.

Conclusion: In-vivo RCM can track early pathophysiologic events revealing differences between black and white skin during the development of irritant contact dermatitis, and may support the theory that those with black skin are more resistant to irritants. (J Am Acad Dermatol 2003;48:727-34.)

Irritant contact dermatitis (ICD) is the most common form of dermatitis and is defined as non-specific damage to the skin after exposure to an irritant. Clinical manifestations are influenced by several factors including the concentration of the chemical, duration of exposure, temperature, humidity, and anatomic location. In addition, these manifestations can be influenced by individual char-

acteristics such as age, sex, pre-existing skin disease, and ethnic origin.^{1,2}

Our work investigates further the influence of skin color (black vs white). Several reports demonstrate that people with black skin may be more resistant to skin irritants than those with white skin.^{3,4} Tape stripping eliminates this difference, suggesting that susceptibility to irritants relates to stratum corneum (SC) function.⁴ Structural studies support the theory that the SC of people with black skin provides a more resistant barrier because of an increased intercellular cohesiveness of the SC; possibly related to a more dense, compact SC.^{5,6} Also, Reinertson and Wheatley⁷ demonstrated a higher lipid content in the SC of people with black skin and this may provide better barrier function. A more recent study also found a greater lipid content in black versus white skin, and a more compact but equally thick SC.⁸ However, other authors have shown conflicting data on the relative susceptibility to irritants in black and white skin.⁹⁻¹² These differences probably reflect the variable response of hu-

From the Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Harvard Medical School,^a and Contact Dermatitis Unit, Department of Dermatology, Massachusetts General Hospital.^b

Supported by National Institutes of Health, National Institute for Occupational Safety and Health Grant RO1 OH04029.

Conflict of interest: None identified.

Accepted for publication June 15, 2002.

Reprint requests: Salvador González, MD, Wellman Laboratories of Photomedicine, Bartlett Hall 814, Massachusetts General Hospital, Boston, MA 02114. E-mail: sgonzalez3@partners.org.

Copyright © 2003 by the American Academy of Dermatology, Inc.

0190-9622/2003/\$30.00 + 0

doi:10.1067/mjd.2003.220

Table I. Visual scoring scale used to assess clinical development of irritant contact dermatitis after exposure

Score	Term	Meaning
0	Negative	No response
1	Minimal	Slight erythema
2	Mild	Mild erythema
3	Moderate	Moderate erythema + edema
4	Severe	Severe erythema + edema + vesicles
5	Necrosis	

man skin to irritants in general, making it difficult to draw definite conclusions about a specific subpopulation.

Reflectance confocal microscopy (RCM) has been used to noninvasively image human skin, providing a virtual window into tissues in vivo without obvious artifacts or destruction.^{13,14} The device operates by tightly focusing a low-power, near-infrared laser beam on a specific point in the skin, and then detecting only the light reflected from the focal point through a pinhole-sized spatial filter.¹⁵ The beam is then scanned horizontally over a 2-dimensional grid to yield a horizontal section. The measured lateral resolution of 0.5 to 1 mm and measured axial resolution (section thickness) of 2 to 5 mm yield images comparable with routine histology.¹⁶ Penetration depth is sufficient for imaging the epidermis, and the papillary and upper reticular dermis.

The goals of this investigation were to define the major histologic features of ICD in real time by in vivo RCM and to determine whether racial or ethnic differences exist in the susceptibility to the early development of ICD by comparing 2 participant groups: those with black skin and those with white skin. Transepidermal water loss (TEWL) measurement and laser Doppler velocimetry (LDV), standard noninvasive research techniques for ICD evaluation, were also used as additional means of study.

MATERIALS AND METHODS

Participants

A total of 14 healthy volunteers between the ages of 18 and 40 years were recruited for this study, which was approved by our institutional review board. There were 8 participants with white skin (6 with Fitzpatrick skin phototype II and 2 with Fitzpatrick skin phototype III), and 6 participants with black skin (5 with Fitzpatrick skin phototype V and 1 with Fitzpatrick skin phototype VI). Exclusion criteria included age greater than 40 years and a positive history of atopy.

Exposure to irritant

The volar aspect of forearm skin was exposed to 35 mL of 4% and 1% sodium lauryl sulfate (SLS), and 10X phosphate-buffered saline using a strip of 4 10-mm Finn Chambers (Allerderm Laboratories Inc, Petaluma, Calif). Chamber No. 1 contained 4% SLS and was removed after 6 hours of exposure. Chambers No. 2 through 4, containing 4% and 1% SLS, and phosphate-buffered saline, respectively, were removed 24 hours after application.

Evaluation

Participants returned for evaluation 6, 24, and 48 hours after initial application of the patch. Before all evaluations, 15 to 30 minutes were allowed for the skin to recover from chamber occlusion. During the 6-hour visit, site No. 1 and an adjacent normal skin site were evaluated. The remaining sites No. 2 through 4 were assessed at both the 24- and 48-hour follow-up.

At each follow-up session, each site was evaluated using several techniques.

Clinical evaluation. Sites were clinically graded using a visual scoring scale (Table I) and digital photographs were obtained.

Transepidermal water loss. TEWL measurements were performed using the Dermalab device (Cortex Technologies, Cyberderm Inc, Media, Pa). One probe was applied to the skin surface of the exposure site with appropriate technique and environmental controls^{17,18}; TEWL measurements were obtained during a continuous 60-second period, and expressed in units of grams per square meter per hour.

Laser Doppler velocimetry. LDV was performed using a laser Doppler flowmeter (model ALF 21, Transonic Systems Inc, Ithaca, NY). A low-power infrared diode laser was applied by a small probe constantly maintained at a fixed distance (8-10 mm) from the skin surface. The technique measures cutaneous microcirculatory flow and is on the basis of the Doppler broadening of laser light scattered by moving particles (erythrocytes). This broadened signal is detected and analyzed to give information about moving-particle density and flux (used to express blood volume flow).¹⁹ These measurements are expressed in units of milliliters per minute per 100 grams.

In vivo RCM. In vivo RCM was accomplished with a commercially available device (Vivascope 1000, Lucid Inc, Henrietta, NY) using an 830-nm diode laser with a maximum power of 15 mW. A 30x water-immersion objective lens of numeric aperture 0.95 was used. The skin contact device consists of a housing that encloses the objective lens and a ring

and template that affix to the skin. While moving the objective lens relative to the skin with X-, Y-, and Z-plane micrometer screws, images captured were in axial sections beginning with the SC, through the epidermis, and into the upper reticular dermis.

As previously reported,¹⁴ images of optimal quality are obtained by matching the refractive index (n) of immersion medium with that of the epidermis ($n = 1.34$). The immersion medium that we used for this study was water ($n = 1.33$). A coverslip was used because it produced a more stable image for reliably measuring SC thickness.

In vivo RCM was used to measure thickness of the SC and depth of the suprapapillary epidermal plate (SPP) and rete pegs in real time. "SPP depth" was defined as the distance between the top of the SC and the bottom of the cells in the uppermost portion of the stratum basale.²⁰ We also acquired sequential images of blood vessels to record the intraluminal flow. Papillary dermis and upper reticular dermis depths were noted. At each evaluation site, a minimum of 6 images of each of the aforementioned skin layers were captured.

Histology

Up to 2 confirmational skin biopsy specimens (3-mm punch) were obtained from each participant after local intradermal anesthesia (2% lidocaine with epinephrine). Biopsy specimens were obtained from the site exposed to phosphate-buffered saline and the site that demonstrated maximal evidence of ICD. The biopsy specimens were fixed in buffered 10% formalin, processed, and embedded in paraffin. Each sample was cut in half and sectioned both horizontally and vertically for hematoxylin-eosin staining and for Fontana-Masson staining.

Statistical analysis

Mean and SD values were calculated. The Student 2-tailed t test was used to determine whether there was a significant difference ($P < .05$) between the groups with black and white skin for the following parameters: (1) thickness of the SC and depth of the SPP measured by in vivo RCM for each site at each evaluation time point; (2) TEWL measurements; and (3) LDV measurements. Also, Pearson r correlation was calculated to determine correlation between SC thickness and TEWL for each site at all evaluation time points.

RESULTS

Clinical evaluation of ICD

The average clinical scores tended to be lower in participants with black skin than those with white skin at most time points assessed, for various concentrations of SLS, though this difference was not

Table II. Average clinical scores for participants with black and white skin at different time points and with different concentrations of sodium lauryl sulfate

SLS concentration and time point		Black skin	White skin
1% SLS	24 h	0.42	0.88
	48 h	0.83	1.19
4% SLS	6 h	1.08	1.06
	24 h	1	1.44
	48 h	1	1.69

SLS, Sodium lauryl sulfate.

statistically significant (Table II). Although none of the participants had severe reactions to this concentration of SLS, participants with white skin showed more mild-moderate reactions and less negative-minimal reactions to the SLS, whereas participants with black skin consistently showed more negative-minimal reactions.

Confocal images of ICD

Descriptive analysis. Differences between normal skin and skin exposed to SLS were easily appreciated in both the black and white skin groups because several key histopathologic features characteristic of ICD could be seen with in vivo RCM (Fig 1). Even skin exposed to lower SLS concentrations (1%) revealed live microscopic changes with RCM, frequently without clinical evidence of ICD. One early change seen with RCM was disruption of the SC, revealing characteristic "breaks" and gray, punched-out areas in the normally homogeneous highly refractile pattern (Fig 1, *A*). This finding was seen in all participants. Focal parakeratosis, visible as retained nuclei in the SC, and spongiosis (seen as intense intercellular brightness) were further recognized as early changes with in vivo RCM (Fig 1, *B* and *C*). Other microscopic changes such as exocytosis and acantholytic keratinocytes (Fig 1, *D*), microvesicles containing inflammatory cells and detached keratinocytes (Fig 1, *E*), and perivascular inflammatory infiltrate (Fig 1, *F*) were also seen. An interesting finding was an increase in brightness of basal keratinocytes in ICD, probably because of increased melanin content (the best endogenous contrast agent in RCM).¹⁵ This finding was supported by routine histology using the Fontana-Masson stain (Fig 2). This was typically visible at 48 hours after application of 4% SLS and was more prominent in participants with black skin.

Quantitative analysis. Participants with black and white skin did not show a significant difference between the SC thickness of control sites (Table III).

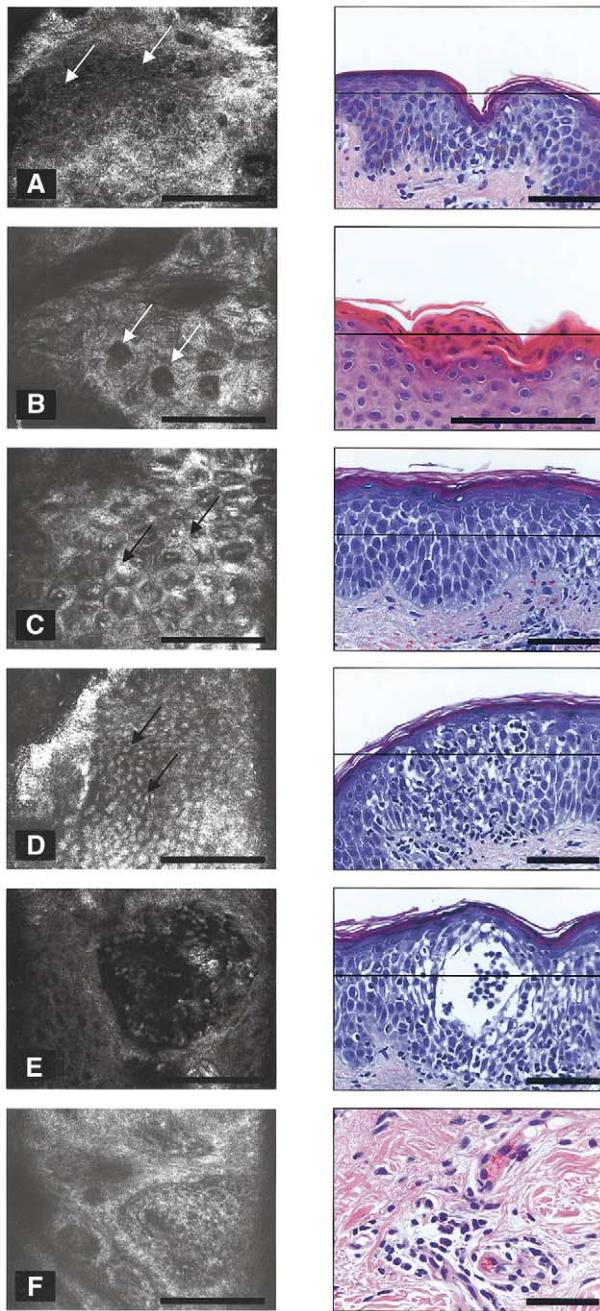


Fig 1. Histopathologic correlation: confocal microscopy images (*left*) and routine hematoxylin-eosin–stained (H&E) sections (*right*), representative from different patients. **A**, Stratum corneum (SC) disruption (*arrows*). **B**, Parakeratosis, *arrows* show retention of nuclei in SC. **C**, Spongiosis, *arrows* highlight increased intercellular brightness and edema. **D**, Exocytosis and acantholysis, confluence of dyskeratotic keratinocytes, examples shown with *arrows*. **E**, Microvesicle containing inflammatory cells and dyskeratotic keratinocytes. **F**, Note perivascular infiltrate on right lower quadrant. *Scale bars* represent 100 μ m. *Horizontal lines* on H&E sections show corresponding levels of en-face images.

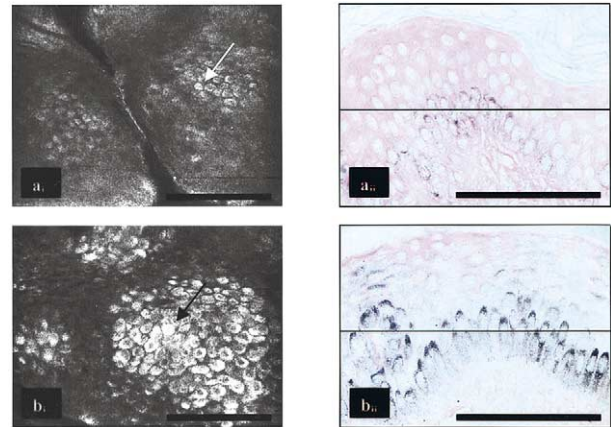


Fig 2. Histopathologic correlation: confocal microscopy images (*left*) and routine hematoxylin-eosin–stained (H&E) sections (*right*). Control: highly-refractile basal keratinocytes seen at dermoepidermal junction (**a**, left panel) and Fontana-Masson stain showing melanin in basal keratinocytes (**a**, right panel). Site exposed to 4% SLS in same participant showing brighter basal keratinocytes after 48 hours (**b**, left panel) and Fontana-Masson demonstrating increased melanin content (**b**, right panel). *Scale bars* represent 100 μ m. *Horizontal lines* on H&E sections show corresponding levels of en-face images.

However, the SC thickness was significantly less in the black skin group compared with the white skin group after exposure to 4% SLS at 48 hours ($P < .05$). No other sites revealed any additional significant differences in SC thickness at the time points assessed. Similarly, SPP depths of the control sites showed no significant difference between the 2 groups. Assessment of sites exposed to 4% SLS revealed that the SPP depth was significantly greater in participants with white skin versus those with black skin after 24 hours ($P < .05$) and 48 hours ($P < .01$) of exposure. This was also observed in sites exposed to 1% SLS at the 48-hour evaluation ($P < .01$). Other sites demonstrated no additional significant differences in SPP depth at other time points.

At all time points assessed, participants with white skin had a trend toward greater mean increases in TEWL after exposure to SLS than did participants with black skin, although the differences were not statistically significant (Fig 3). There was no statistically significant difference in LDV measurements between the participants with black and white skin. Changes in both TEWL and SC thickness between the control site and the site exposed to 4% SLS at 48 hours were negatively correlated, as shown in Fig 4, for participants with both black and white skin. Such a clear pattern was not seen at 6 or 24 hours, or with lower concentrations of SLS. Participants with white skin, however, had greater

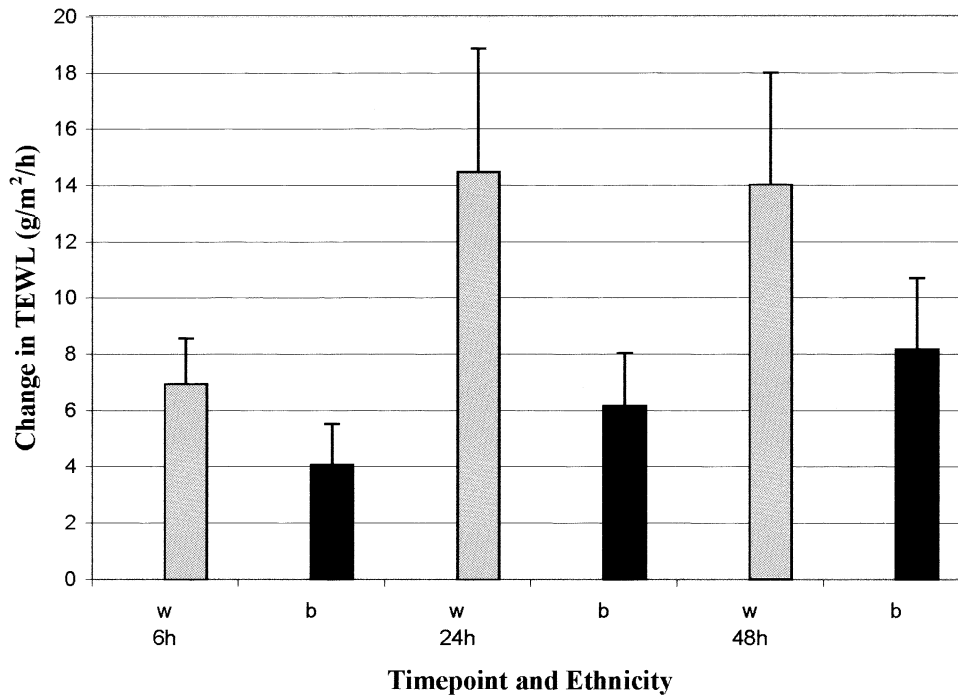


Fig 3. Change in transepidermal water loss (TEWL) with irritant exposure for participants with white (n = 7) (gray bars) and black (n = 6) (solid bars) skin. Difference in TEWL between control sites, and sites at various time points after application of 4% sodium lauryl sulfate, is shown for both groups. Data points shown are mean ± SEM.

Table III. Comparison of stratum corneum and suprapapillary epidermal plate thickness between the black skin and white skin groups after sodium lauryl sulfate application at various time points using Student *t* test

Site	Time	Histologic parameter	Mean depth (µm) for groups		<i>t</i> Value	α
			Black skin	White skin		
Control	0 h	SC	13.4 ± 2.7	13.3 ± 4.0	0.05	NS
4%	48 h	SC	10.4 ± 4.7	12.4 ± 4.6	2.35	.05
Control*	0 h	SPP	53.3 ± 12.1	53.0 ± 7.8	0.10	NS
4%*	24 h	SPP	53.2 ± 10.5	61.0 ± 17.7	2.40	.05
4%*	48 h	SPP	51.3 ± 5.5	57.8 ± 10.3	3.13	.01
1%*	48 h	SPP	50.5 ± 7.5	56.6 ± 9.9	2.82	.01

NS, Nonsignificant; SC, stratum corneum thickness; SPP, suprapapillary epidermal plate thickness.

mean increases in TEWL after exposure to 4% SLS than did participants with black skin, and this was seen at all time points assessed (Fig 4).

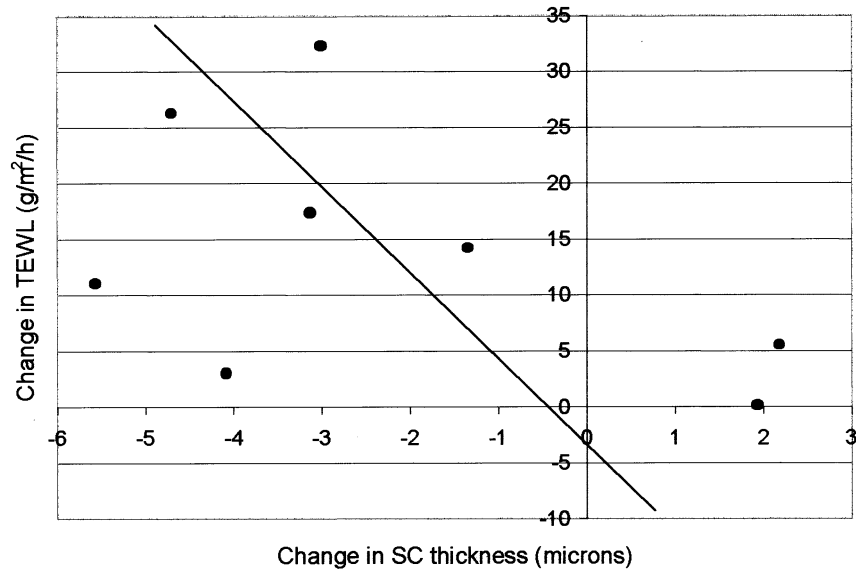
DISCUSSION

Racial or ethnic differences in skin reactivity to cutaneous irritants is an important area of research. The majority of the literature examining differences between people with black and white skin points toward a reduced susceptibility to ICD among participants with black skin, and relates this to better barrier (SC) function.^{3,5,6,12}

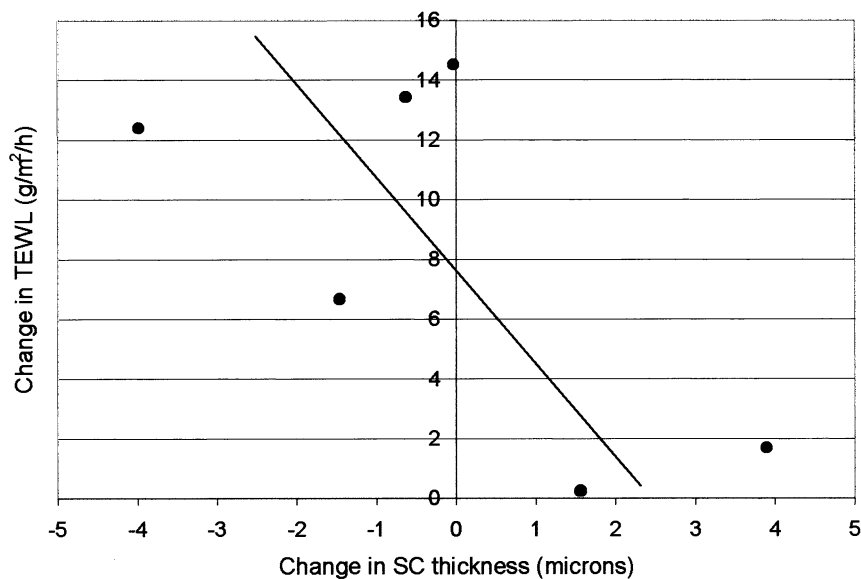
We found that participants with white skin did

tend to have more intense clinical reactions to SLS than did participants with black skin. This could be related to the pathophysiologic epidermal events like spongiosis, evidenced by significantly increased SPP depth measured by in vivo RCM in those with white skin. Skin color differences may also play a factor as darker skin tones of the participants with black skin may have prevented accurate assessment of clinical erythematous reaction, although edema and papule formation was easily appreciated in all participants.

In vivo, near-infrared RCM is a novel imaging tool that permits real-time qualitative and quantitative



A $r = -0.51$



B $r = -0.67$

Fig 4. Change in stratum corneum (SC) thickness versus change in transepidermal water loss (TEWL) at 48 hours after application of 4% sodium lauryl sulfate (SLS) for participants with white (n=8) (**A**) and black (n=6) (**B**) skin. Difference between SC thickness in site exposed to 4% SLS and control site at 48 hours was calculated and compared with difference in TEWL for same irritant concentration and time point.

study of healthy²⁰ and diseased^{21,22} human skin. It also enables monitoring of dynamic events.²³ It uses a near-infrared laser beam that is focused into the skin and detects backscattered light that passes through a pinhole. This enables “virtual sectioning” of live tissue and yields high-resolution, en-face images comparable with routine histology.¹⁶ In this study we have described the common histopatho-

logic features of ICD using this technique, including SC disruption, parakeratosis, spongiosis, acantholysis, microvesicle formation, and perivascular inflammatory infiltrate. These features have previously been demonstrated in allergic contact dermatitis using RCM.²¹ We also described a marked increase in pigmented basal keratinocytes. These features were confirmed by routine histology.

There was no difference between the black and white skin groups for the SC thickness at the control site. This supports a previous finding by Weigand, Haygood, and Gaylor,⁵ who reported that although there were more layers in the SC of those with black skin, it was more compact and the same thickness as that of people with white skin. After exposure to 4% SLS, both groups experienced a relative "thinning" of the SC as compared with control by 48 hours. Moreover, there was a significant difference between the 2 groups, with the participants with black skin having a thinner SC than the white skin group. One possible explanation is that the SC is simply thinner in the black skin group because it experienced more erosion by the irritant than the white skin group. This explanation does not support the aforementioned findings of Weigand, Haygood, and Gaylor⁵ because if the SC of people with black skin were more densely layered, one would expect that their skin would be more impenetrable to SLS.

The SPP of the white skin group was significantly deeper than that of the black skin group exposed to 4% SLS when assessed at 24 and 48 hours and also to 1% SLS assessed at 48 hours. This increased SPP depth could possibly be attributed to more severe spongiosis and vesicle formation within the epidermis, as stated previously,²¹ and is consistent with participants with white skin having a more severe clinical reaction. This appears contrary to the finding that SC thinning is greater in participants with black skin and the reason for this is incompletely understood.

The negative correlation between SC thickness and TEWL is intuitive and supports the current understanding of TEWL as a measurement of SC barrier function.²⁴ It also supports the legitimacy of *in vivo* RCM as a method for evaluating SC integrity. In addition, TEWL reflects damage of the SC in proportion to SC thickness in both the black and white skin groups. The fact that this correlation is seen at 48 hours is interesting because it suggests that although the irritant is removed at 24 hours, the damage persists and perhaps even progresses for at least 24 more hours. The mean increases in TEWL of the white skin group were consistently greater than those of the black skin group. This was not statistically significant, probably as a result of small sample size. This finding may support the concept that the SC barrier function is more durable in those with black skin than in those with white skin.

LDV was another noninvasive method used as an adjunct to *in vivo* RCM. There was no significant difference in the mean values of the black and white skin groups for any of the sites or time points tested. We found that problems arose during administration

of the flowmeter to the participant, however. As previously reported in the literature,¹⁹ any minute change in position or orientation of the probe can cause significant changes in estimation of blood flow. Furthermore, 2 measurements from different sites (or measurements from what is believed to be the same site at different points in time) cannot be directly compared, minimizing the usefulness of this technique within a person over time.¹⁹ Thus, we are not convinced of its reliability as a method for the evaluation of cutaneous blood flow for various sites at different time points.

To summarize, we found that there was a difference in the reactivity to SLS of participants with black and white skin as assessed by clinical reaction, morphometric epidermal changes as measured by *in vivo* RCM, and TEWL measurement. Our data may support the theory that people with black skin are more resistant to the development of acute ICD induced by surfactants such as SLS. Similar studies with larger sample sizes should be structured with extended evaluation time points to follow the reparative process of the SC and other epidermal layers.

REFERENCES

1. Moshell AN. Workshop on irritant contact dermatitis. *Am J Contact Dermat* 1997;8:70-105.
2. Taylor SC. Skin of color: biology, structure, function, and implications for dermatologic disease. *J Am Acad Dermatol* 2002;46: S41-62.
3. Marshall EK, Lynch V, Smith HW. On dichlorethylsulphide (mustard gas) II: variations in susceptibility of the skin to dichlorethylsulphide. *J Pharmacol Exp Ther* 1919;12:291-301.
4. Weigand DA, Gaylor JR. Irritant reaction in Negro and Caucasian skin. *South Med J* 1974;67:548-51.
5. Weigand DA, Haygood C, Gaylor JR. Cell layers and density of Negro and Caucasian stratum corneum. *J Invest Dermatol* 1974; 62:563-8.
6. Reed JT, Ghadially R, Elias PM. Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch Dermatol* 1995;131:1134-8.
7. Reinertson R, Wheatley V. Studies on the chemical composition of human epidermal lipids. *J Invest Dermatol* 1959;32:49-58.
8. La Ruche G, Cesarini JP. Histology and physiology of black skin. *Ann Dermatol Venereol* 1992;119:567-74.
9. Berardesca E, Maibach HI. Racial differences in sodium lauryl sulphate induced cutaneous irritation: black and white. *Contact Dermatitis* 1988;18:65-70.
10. Wilson D, Berardesca E, Maibach HI. *In vivo* transepidermal water loss: differences between black and white skin. *Br J Dermatol* 1988;119:647-52.
11. McFadden JP, Wakelin SH, Basketter DA. Acute irritation thresholds in subjects with type I-type VI skin. *Contact Dermatitis* 1998; 38:147-9.
12. Robinson MK. Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. *Contact Dermatitis* 1999;41:65-79.
13. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. *In vivo* confocal scanning laser microscopy of human

- skin: melanin provides strong contrast. *J Invest Dermatol* 1995;104:946-52.
14. Rajadhyaksha M, González S, Zavislan J, Anderson RR, Webb RH. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison to histology. *J Invest Dermatol* 1999;113:293-303.
 15. Pierard JE. In vivo confocal microscopy: a new paradigm in dermatology. *Dermatology* 1993;186:4-5.
 16. Rajadhyaksha M, Anderson RR, Webb RH. Video-rate confocal scanning laser microscope for imaging human tissues in vivo. *Appl Opt* 1999;38:2105-15.
 17. Grove GL, Grove MJ, Zerweck C, Pierce E. Computerized evaporimetry using the DermaLab TEWL probe. *Skin Res Technol* 1998;4:1-5.
 18. Grove GL, Grove MJ, Zerweck C, Pierce E. Comparative metrology of the evaporimeter and the DermaLab TEWL Probe. *Skin Res Technol* 1999;5:1-8.
 19. Herrick AL, Clark S. Quantifying digital vascular disease in patients with primary Raynaud's phenomenon and systemic sclerosis. *Ann Rheum Dis* 1998;57:70-8.
 20. Huzaira M, Rius F, Rajadhyaksha M, Anderson RR, González S. Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. *J Invest Dermatol* 2001;116:846-52.
 21. González S, González E, White M, Rajadhyaksha M, Anderson RR. Allergic contact dermatitis: correlation of in vivo confocal imaging to routine histology. *J Am Acad Dermatol* 1999;40:708-13.
 22. González S, Rajadhyaksha M, Rubinstein G, Anderson RR. Characterization of psoriasis in vivo by reflectance confocal microscopy. *J Med Clin Exp Mol* 1999;30:337-56.
 23. González S, Sackstein R, Anderson RR, Rajadhyaksha M. Real-time evidence of in vivo leucocyte trafficking in human skin by reflectance confocal microscopy. *J Invest Dermatol* 2001;117:384-6.
 24. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for trans-epidermal water loss measurement: a report from the standardization group of the European society of contact dermatitis. *Contact Dermatitis* 1990;22:164-78.