Non-Invasive Evaluation of the Kinetics of Allergic and Irritant Contact Dermatitis

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Reflectance confocal microscopy (RCM) allows non-invasive visualization of human skin in vivo. It has been used to describe the histopathological features of acute contact dermatitis (CD). This work was designed to investigate the kinetics of both allergic and irritant CD (ACD and ICD) in vivo. Eighteen subjects with a prior diagnosis of ACD were patch tested with the specific allergen sodium lauryl sulfate as an irritant, and appropriate controls. RCM, transepidermal water loss (TEWL), and fluorescence excitation spectroscopy (FES) were performed at several time points within 2 wk after patch removal. After removal of the Finn chambers at 48 h, superficial epidermal changes, primarily involving the stratum corneum, and increased epidermal thickness were mainly present in ICD. ACD, on the other hand, showed microvesicle formation peaking at 96 h following patch removal. Both ACD and ICD showed exocytosis and similar degrees of spongiosis on RCM. TEWL and FES demonstrated a significant difference between ACD and ICD. RCM, TEWL, and FES are valuable non-invasive tools to quantitatively study the kinetics of the pathophysiology of acute CD reactions in vivo and monitor the changes at a cellular level.

Key words: allergic contact/dermatitis/dermatitis irritant contact/occupational dermatitis/patch tests


Contact dermatitis (CD) affects approximately 20% of the population in the US and is the most common form of occupational skin disease (Mathias, 1985). Mechanistically, CD can be divided into allergic CD (ACD) and irritant CD (ICD) reactions. Despite the different pathogenesis between the two types and the distinctly different immunologic profile (Flier et al, 1999; Ulfgren et al, 2000), the clinical presentation and histopathologic features in ACD and ICD are remarkably similar (Scheynius et al, 1984; Scheynius and Fischer, 1986; Willis et al, 1986; Brasch et al, 1992; Rietschel et al, 1995). But some pathologic features can be more pronounced in ICD or ACD (Willis et al, 1986). ACD presents histologically with vesicle formation, inflammatory infiltrate, and spongiosis (Medenica and Rostenberg, 1971; Dvorak and Mihm, 1972; Dvorak et al, 1974, 1976; Gawkrodger et al, 1986) ICD, on the other hand, typically shows pronounced superficial changes involving the disruption of the corneal layer as well as intraepidermal necrosis (Willis et al, 1989). In contrast to ACD, exocytosis and spongiosis in ICD are accompanied by prominent epidermal hyperproliferation following the epidermal injury (Medenica and Rostenberg, 1971; Le et al, 1995). ICD generally has a faster onset and a shorter duration even with strong irritants, while the cutaneous changes in ACD gradually subside within 2–3 wk after elicitation.

The clinical resemblance of both forms of this disease makes the diagnosis of CD difficult to accomplish. Even the interpretation of patch tests results for mild cases of ICD and ACD is not too reliable and poses a considerable challenge to dermatological practice (Rietschel et al, 1995). Efforts, therefore, are being made to establish non-invasive evaluation modalities that would allow for in vivo, reproducible diagnosis. Previously, we have used real-time reflectance confocal microscopy (RCM) to image normal (Webb, 1996; Rajadhyaksha et al, 1995, 1999; Huzaira et al, 2001) and diseased human skin (González et al, 1999a—Psoriasis; Aghassi et al, 2000; Busam et al, 2001; González and Tannous, 2002) non-invasively in vivo. By correlating confocal histopathology with conventional histopathology, our group recently identified distinctive characteristics for ICD and ACD—the groundwork for this research study (González et al, 1999b; Hicks et al, 2003; Swindells et al, 2004).

Herein we report an in vivo human research study aimed at investigating the time course of the pathophysiologic changes in ACD and ICD, and to obtain a morphologic description and quantitative analysis of the structural and cellular changes in ACD and ICD over a 2-wk time period using RCM and other non-invasive techniques such as transepidermal water loss (TEWL) and fluorescence excitation spectroscopy (FES).

Abbreviations: ACD, allergic contact dermatitis; CD, contact dermatitis; FES, fluorescence excitation spectroscopy; ICD, irritant contact dermatitis; RCM, reflectance confocal microscope; SC, stratum corneum; SG, stratum granulosum; SLS, sodium lauryl sulfate; SS, stratum spinosum; TEWL, transepidermal water loss

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Results

Clinical scoring All 18 subjects completed the study. Clinical scores of all 18 subjects were included in the analysis. As expected, all 18 subjects reacted positively to sodium lauryl sulfate (SLS). Five of 18 patients did not react clinically to the allergen despite a prior diagnosis of ACD. In general, the average clinical scores tended to be lower in ACD when compared with ICD reactions at all time points.

Figure 1 shows the global evolution of ICD and ACD over time and the p-value is significantly different (p < 0.002, not shown in Fig 1). The value was calculated by repeated measurements MANOVA, with a within factor (time) and a between factor (ACD/ICD).

Clinical scoring values were higher for ICD than in ACD up until day 9. The difference between the two groups for individual days was calculated using a t test, indicating significance only on days 2 and 3 (p < 0.005).

In vivo RCM evaluation

Semiquantitative analysis RCM imaging at the stratum corneum (SC) level confirmed the presence of reactive superficial changes such as SC disruption, presence of individual corneocytes, parakeratosis, and superficial necrosis (Fig 2a). The SC disruption was significantly more pronounced in ICD compared with either ACD or control. In ACD, no significant changes involving the SC were found compared with control sites. Figure 2b shows a graph illustrating the kinetic evolution of SC-RCM scores of ACD versus ICD (p < 0.05 for all parameters).

At the suprabasal level (stratum granulosum and spinosum), significant differences for both ACD and ICD versus control were found at all time points (Figs 3a, b, c and 4a, b and c, respectively). Although in ICD exocytosis, spongiosis (Fig 3a and b), vesicle formation, and epidermal necrosis (Fig 4a and b) were more severe on days 2 and 3 compared with ACD, these features declined sharply on day 4. Microvesicles were more typically seen in ACD, a feature that peaked around day 4, and was persisting beyond day 9 (Fig 4b and c). Both eczematous reactions showed strong correlation between vesicle formation and epidermal necrosis; however, the presence of necrosis was more typical of ICD reactions. Overall RCM scores were higher in ICD compared with ACD reactions, except for day 9, indicating a prolonged activity of ACD reactions.

At the level of the dermoepidermal junction (DEJ), no significant differences in features between ACD and ICD were found except for the increase in epidermal thickness observed in ICD compared with ACD reactions (Fig 5).

Quantitative analysis Correlation of clinical scoring with TEWL was high for ICD (r > 0.355, p < 0.007) (Table I).

Values of TEWL measurements also correlated highly with RCM scores of superficial parameters ranging from r > 0.310–0.716, in all cases of ICD (p < 0.001). But there was no significant correlation of RCM and TEWL in allergic and control sites (r ≤ 0.249, p > 0.06) (Table I).

Correlation of clinical scoring with RCM parameters was high on days 2, 3, and 4 for both ACD and ICD reactions (r = 0.6, p < 0.05) (data not shown).

Suprapapillary epidermal thickness demonstrated significant differences between allergic versus irritant for all time points except for day 9 (Fig 5). The epidermal thickness in ACD was increased mildly but did not reach statistically significant levels compared with control.

FES evaluation Figure 6 shows the fluorescence excitation spectrum, the de-convoluted components, and the residual taken from a healthy volunteer. The averages of the 278 and 298 nm intensity bands from all volunteers exposed to allergen, irritant, and control over time are shown in Fig 7a and b, respectively. Both the 278 and 298 nm bands from the exposed irritant sites showed an increase in the fluorescence intensity peaking at day 4 and decreasing over time thereafter. On the other hand, both bands from the sites exposed to allergen did not differ from the respective bands of the control sites. Statistical analysis showed that the differences between ACD and ICD reactions were significant for both bands (p278 < 0.0001, p298 < 0.001) (ACD vs ICD). The global response over time was also found to be significant for both bands 278 and 298 nm (p278 and 298 < 0.0001). No statistical significance was found between the responses of the sites exposed to the allergen from those of the control for the 278 nm band and differences only marginally significant for the 298 nm band (p298 < 0.051). Furthermore, the global response of ACD over time was not statistically significant for both bands (p278 < 0.9, p298 < 0.4) when compared with control.

TEWL measurements As expected, there were marked differences in TEWL values when comparing ACD and ICD (Fig 8). ICD showed significantly higher values compared with ACD and control (p < 0.002) except for days 14 and 21, perhaps because of the limited number of the available subjects at these time points for follow-up. In general, TEWL increased early on in ICD reactions and slowly returned to baseline at day 6 after the patch test. There was, however, no significant difference between ACD and control skin at all evaluated time points.
Discussion

We have described the dynamics of the cutaneous changes in two forms of acute CD using non-invasive optical techniques. Previous work in this field included the measurements of TEWL, electrical conductance, electrical impedance, and Doppler flowmetry. In the majority of the studies, however, the work was aimed to facilitate the differentiation of ACD and ICD (Agner and Serup, 1989; Nyren et al., 2003). The evaluations were performed at a single time point after the removal of the chemical and only a few human studies followed CD reactions over time (Dvorak and Mihm, 1972; Dvorak et al., 1976; Lee et al., 1997). By correlating RCM results with clinical findings and histology (Hicks et al., 2003; Swindells et al., 2004), RCM has proved to be a promising tool in the distinction of ACD and ICD reactions. This study reports a non-invasive microscopic evaluation of CD longitudinally to describe the evolution of both ICD and ACD in vivo and over time.

The kinetics of the histopathologic changes that take place in acute ACD and ICD are different. The epidermal disruption can easily be visualized using RCM and the dynamics of the RCM findings parallel that of visual assessment and correlate well with the clinical scores. In concordance with previous histological studies (Medenica and Rostenberg, 1971), structural changes in the SC are a key finding in ICD and the initial presence of superficial disruption is highly indicative of irritant reactions. Although these changes are returning to baseline within 9 d in ICD, they are generally absent or develop later in the case of severe ACD reactions.

The degrees of epidermal spongiosis, vesicle formation, and exocytosis are both less distinctive and less specific in the differentiation of ICD versus ACD, which confirms the findings of previous histopathological studies (Willis et al., 1986). Intraepidermal vesicle formation was typically present more prominently in ACD and necrosis more commonly found in ICD, which confirms serial electron microscopic and histological studies by other investigators (Medenica and Rostenberg, 1971). In general, our results show that on day 9, ACD reactions show significantly higher values for all evaluated RCM parameters,
indicating the prolonged activity of ACD compared with ICD reactions.

Among the other RCM parameters, our interest has focused on the evolution of epidermal thickness. The marked increase in irritant reactions can only partially be explained by the presence of spongiosis, since increased epidermal thickness is not evident in allergic reactions with similar degrees of spongiosis. ICD and other skin diseases (Lavrijsen et al., 1995; Ghadially et al., 1996) have previously been associated with changes in epidermal growth, epidermal proliferation as well as differentiation (Le et al., 1996), often resulting in regenerative hyperplasia (Medenica and Rostenberg, 1971). In ICD increased epidermal thickness is a function of parakeratosis and hyperkeratosis (Medenica and Rostenberg, 1971; Le et al., 1995, 1998). For ACD, focal parakeratosis, hyperkeratosis, and significantly increased epidermal thickness have previously been described in late phases during follow-up of ACD (Medenica and Rostenberg, 1971), reflecting a delayed hyperproliferative response of subacute ACD reactions. RCM allows us to measure the epidermal depth in vivo repeatedly and follow the evolution of epidermal thickness over time. To our knowledge, this is a

Figure 3
Confocal features of stratum granulosum (SG). (a) Confocal images of allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) at days 2, 3, and 9 after removal of the Finn chambers. Left panel with features of ACD; days 2 and 3 with spongiosis and multichambered vesicle formation (arrows) containing small round to oval structures with a bright appearance consistent with inflammatory cells (arrowheads). Day 9 with residual spongiosis and sparse inflammatory infiltrate (arrows). Right panel with features of ICD. Split image day 2 with reflectance confocal microscopy (RCM) features of intraepidermal disruption: left shows severe spongiosis and exocytosis, right with necrosis (arrows) and inflammatory cells (arrowheads). Day 3 with microvesicle formation (arrows); on day 9 SG is almost completely recovered, with occasional inflammatory cells (arrowheads). Scale bars are 50 μm. (b) The graph illustrates the severity and evolution of selected RCM parameters (spongiosis, exocytosis) over time. The X-axis represents individual RCM parameters, the Y-axis reflects the severity of RCM scores, and the Z-axis with the evolution over time (days 2, 3, 4, 9). (c) The graph corresponds to Fig 3b and shows the level of statistical significance when comparing ACD versus ICD. *Statistical significance (p < 0.05); "NS" represents data points without significant statistical difference between ACD and ICD.
non-invasive evaluation of epidermal hyperproliferation induced by irritants and allergens over time. Our results are compatible with previous immunohistochemical and histological findings by Le et al. (1995, 1998) using involucrin and keratin 16 expression, MIB-1 as well as Ki 67 expression to describe differences of ICD and ACD over time.

Similarly, FES has previously been used to measure epidermal proliferation. But this is a study of FES on the longitudinal evolution of ACD and ICD.

FES allows the identification of excitation bands associated with specific emission bands (Kollias et al., 1998; Zonios et al., 2000; Doukas et al., 2001). They are similar to absorption spectra and thus permit identification of the individual fluorophores in complex biological systems (Labella, 1971; Young, 1997; Kollias et al., 1998).

There are two excitation bands observed in the region 260–320 nm: one with an excitation/emission at 295/345 nm and another at 280/340 nm. The spectral characteristics of

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**Figure 4**

Confocal features of stratum spinosum (SS). Images of allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) at days 2, 3, and 9 after removal of the Finn chambers. Left panel illustrates features of ACD. Days 2, 3, and 9 demonstrating the presence of intraepidermal vesicles (arrows) with cellular debris and inflammatory cells (arrowheads). Right panel illustrates the features of ICD. Split image day 2 evidences two aspects of these reactions: left shows spongiosis and bright nucleoli (arrows, left), right with intraepidermal necrosis, cellular debris, detached keratinocytes, and inflammatory cells (arrowheads). Similar features on day 3. Day 9 demonstrates a fully recovered spinous layer. Scale bars are 50 µm. (b) The graph illustrates the severity and evolution of selected reflectance confocal microscopy (RCM) parameters (vesicles, prominent nucleoli, necrosis) over time. The X-axis represents individual RCM parameters, the Y-axis reflects the severity of RCM scores, and the Z-axis with the evolution over time (days 2, 3, 4, 9). (c) The graph corresponds to Fig 4b and shows the level of statistical significance when comparing ACD versus ICD. *Statistical significance (p < 0.05); “NS” represent data points without significant statistical difference between ACD and ICD. Data of prominent nucleoli not shown (NS).
the first group are consistent with the endogenous fluorescence of tryptophan (Hoerman, 1971; Leffell et al., 1988; Kollias et al., 1998). A number of studies have shown that there is a correlation between the 295 nm excitation band and epidermal proliferation (Kollias et al., 1998; Brancaleon et al., 1999, 2001; Gillies et al., 2000) and increased cellular activity in vitro (Zhang et al., 1997). Monici et al. (1995) have observed an excitation band at 270 nm associated with inflammation. It is not known, however, whether the 278 nm band measured in this study is the same with the 270 nm band reported in the literature (Le et al., 1995, 1998).

The peak intensities in our measurements were measured at 278 and 298 nm, respectively. It should also be pointed out that the position of the peak intensity varied from subject to subject by 2–4 nm; however, we do not consider the small difference significant. Overall, the results for both bands (278 and 298 nm) showed an increase in the fluorescence intensity peaking at day 4, and a subsequent decrease to the baseline value by day 21.

TEWL has previously been used to describe and follow ACD and ICD (Serup and Staberg, 1987). It is a sensitive tool for evaluation of SC disruption, regardless of the etiology (Agner and Serup, 1990; Agner and Serup, 1989, 1990; Friebe et al., 2003). For ICD, TEWL was significantly corre-

Table I. Correlation of TEWL with (a) clinical score and (b) superficial RCM parameters, with corresponding p-values for ACD and ICD are shown

<table>
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<tr>
<th>Group</th>
<th>Correlation</th>
<th>p-value</th>
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<tr>
<td>(a) Pearson correlation of TEWL with clinical score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic</td>
<td>&lt;0.249</td>
<td>&gt;0.06</td>
</tr>
<tr>
<td>Irritant</td>
<td>&gt;0.355</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>(b) Pearson correlation of TEWL with superficial RCM features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic</td>
<td>0.150</td>
<td>&gt;0.239</td>
</tr>
<tr>
<td>Irritant</td>
<td>0.550</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TEWL, transepidermal water loss; RCM, reflectance confocal microscopy; ACD, allergic contact dermatitis; ICD, irritant contact dermatitis.

Figure 5
The evolution of the epidermal thickness over time as evaluated by reflectance confocal microscopy (RCM). The mean values of allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), and control are shown. Bars represent standard error of the mean. (days 2–14). ACD scores (○), ICD scores (▲), and control (●).

Figure 6
A typical fluorescence excitation spectrum from a volunteer of the two components produced by deconvolution (278 and 298 nm) and the residual are shown. The X-axis represents the wavelength (nm), and the Y-axis represents the relative density of signal.

Figure 7
The average peak fluorescence intensity at 278 and 298 nm intensity bands from all volunteers exposed to allergen, irritant, and control over time. Error bars represent standard error of the mean. Allergic contact dermatitis (ACD) scores (○), irritant contact dermatitis (ICD) scores (▲), and control (●).
response induced by SLS will lead to significantly increased TEWL values, ACD reactions failed to present similar changes early on in the course. The duration of follow-up in this study allowed us to demonstrate the delayed SC disruption associated with subacute ACD reactions and the results correlated well with the RCM evaluation. Our data are in concordance with previous studies, indicating a long recovery time of ACD reactions (Lee et al., 1997).

In summary, non-invasive optical techniques for longitudinal studies offer significant advantages over the conventional histology. The results are reproducible, and the procedures are painless. More importantly, these techniques preserve the live-ness of the cutaneous tissue and thus allow repeated monitoring of the dynamic cell changes implicated in CD. Our study validates previous findings in distinguishing the features of CD using confocal microscopy. Furthermore, our findings are consistent with previous reports on the evolution of CD over time and highlight and compare the longevity of severe ACD against the brisk onset of ICD.

Materials and Methods

Participants Eighteen volunteers aged between 29 and 69 y (mean 49 y) with a history of contact allergy previously confirmed by patch testing participated in the study. Written consent was obtained prior to enrollment. The research protocol was approved by the Subcommittee on Human Studies, at the Institutional Review Board at Massachusetts General Hospital. All clinical investigations were conducted according to Declaration of Helsinki Principles. All 18 subjects completed the study. Clinical scores of all 18 subjects were included in the analysis.

Protocol design and study evaluation Sites for testing were selected on the ventral forearm or thigh using a total of six 10 mm Finn Chambers (Allerderm Laboratories, Petaluma, California) and filter paper disks for the aqueous solutions (Epitest Ltd Oy, Suomi, Finland, distributed by Allerderm Laboratories). The subjects were then exposed to 3.5 mL of 4% SLS and the specific test allergen (Trolab, Hermal AG, Kurt Hermann, Reinbeck, Germany) in two duplicate chambers. Phosphate-buffered saline, and a negative control with no chemical, served as controls. Allergens used included nickel sulfate (n = 4), fragrance mix (n = 3), balsam of Peru (n = 3), quaternium-15 (n = 1), wool alcohols (n = 1), thimerosal (n = 1), paraphenylenediamine (n = 1), mercaptoxim (n = 1), 4-paratert-butyl phenol formaldehyde resin (n = 1), and imidazolidinyl urea (n = 1).

The patch test substances were applied for 48 h and individual participants returned for follow-up evaluation at three or more time points (2, 3, 4, 9, 14, and 21 d) following the patch removal. At every evaluation visit, each site was studied by using clinical judgment, RCM analysis, FES, and TEWL measurements.

Clinical evaluation Sites were clinically graded using a visual scoring scale following the guidelines of the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group (Table II) (Rietschel et al., 2001; Marks et al., 2002). Clinical photographs of the skin reactions were taken by the primary investigator (S. A.) using a digital camera (Nikon Coolpix 950, Nikon Corp., Tokyo, Japan) under standard conditions and were evaluated and scored blindly by two independent observers (E. G., S. G.).

In vivo RCM evaluation The commercially available RCM (Vivascope 1000, Lucid-Tech, Henrietta, New York) was used to image skin sites under study. A detailed description of this technique and the device used here has been published elsewhere (Webb, 1996, Rajadhyaksha et al., 1995, 1999). In each of the skin sites analyzed, systematic horizontal mapping was performed and four to six images were captured in axial sections beginning with the SC, continuing through the entire epidermis, and into the upper reticular dermis. RCM images were individually subjected to evaluation. The parameters listed in Table III were analyzed using a semi-quantitative scoring scale from 0 to 3 (0 = none to 3 = severe) (González et al., 1999a, b; Hicks et al., 2003; Swindells et al., 2004). Additionally, in vivo RCM was used to quantify the thickness of the suprapapillary epidermal plates (Huzaira et al., 2001; Hicks et al., 2003). This was accomplished with a digital micrometer attached to the Z (vertical) stage of the objective lens.

FES evaluation FES was performed by using a fiber-based fluorometer (Skin Scan, Jobin Yvon-Spix, Edison, New Jersey) (Doukas et al., 2001). The fluorescence excitation spectra were acquired (excitation 260 and 320 nm, emission 340 nm). The excitation spectra were recorded on day 0 (before the application

Table II. Clinical scoring scale

<table>
<thead>
<tr>
<th>Score</th>
<th>ACD</th>
<th>ICD</th>
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<tr>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0.5</td>
<td>Macular erythema</td>
<td>Barely perceptible macular erythema</td>
</tr>
<tr>
<td>1</td>
<td>Weak (non-vesicular) reaction, induration, possible papules</td>
<td>Mild erythema</td>
</tr>
<tr>
<td>2</td>
<td>Strong (edematous or vesicular) reaction, erythema, induration, papules, vesicles</td>
<td>Moderate-intense uniform erythema</td>
</tr>
<tr>
<td>3</td>
<td>Extreme (spreading, bullous or ulcerative) reaction</td>
<td>Intense erythema and edema, vesiculation or erosion</td>
</tr>
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</table>

The scheme follows the guidelines of the International Contact Dermatitis Research Group (ICDRG) and the North American Contact Dermatitis Group (NACDG) shown.

ACD, allergic contact dermatitis; ICD, irritant contact dermatitis.
of test substances) and on at least three varying time points there- 

Reference


