Reflectance confocal microscopy may differentiate acute allergic and irritant contact dermatitis in vivo

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Background: Acute irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) may be difficult to distinguish by clinical or histologic assessment. Reflectance confocal microscopy (RCM) enables real-time, high-resolution skin imaging in vivo.

Objective: We sought to image, characterize, and distinguish acute ACD and ICD in vivo.

Methods: Volunteers with ACD were patch tested with an allergen and the irritant, sodium lauryl sulfate. RCM imaging and transepidermal water loss measurements were performed at 24 and 72 hours. Biopsy specimens were correlated with RCM images.

Results: Spongiosis, epidermal inflammatory cell infiltrate, and vesicle formation were observed in ACD and ICD. Compared with ACD, ICD showed greater disruption of the stratum corneum, and more parakeratosis. There was a significantly greater increase in transepidermal water loss for ICD compared with ACD.

Conclusion: RCM is a promising tool for dynamic, noninvasive assessment and may help to differentiate acute ACD and sodium lauryl sulfate–induced ICD. (J Am Acad Dermatol 2004;50:220-8.)

Acute allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) are common conditions, and can be remarkably similar clinically and histologically, despite their different pathogenetic mechanisms. ICD is a heterogeneous disease for which the common feature is external damage to the epidermis, with a subsequent inflammatory reaction. The initial keratinocyte damage may be caused by damage to cell membranes (eg, organic solvents), removal of surface lipids (eg, surfactants), or direct cytotoxic effects (eg, anthralin). By contrast, ACD is mediated by memory T lymphocytes and requires previous sensitization by an allergen, producing a delayed type hypersensitivity inflammatory reaction.

Differentiation is important because different therapeutic and management strategies may be used after diagnosis. However, histopathologic changes and the nature of the inflammatory infiltrate are often surprisingly alike in both types of reaction and cytokine profiles are also very similar. Some histopathologic differences have been identified: for example, it has been demonstrated that the dermal infiltrate is deeper and more intense in ACD and the epidermal infiltrate greater in ICD. Furthermore, ACD produces a pattern of follicular spongiosis whereas ICD does not. Differences in cytokine

Abbreviations used:
ACD: allergic contact dermatitis
ICD: irritant contact dermatitis
RCM: reflectance confocal microscopy
SLS: sodium lauryl sulfate
TEWL: transepidermal water loss
expression include the demonstration that ACD results in high levels of IL-10 in the elicitation phase whereas ICD does not, and that the chemokines IP-10, Mig, and IP-9 are increased in ACD but not in ICD. However, these methods require invasive, costly, and time-consuming procedures and are not widely available for clinical application. In vitro methods also have the disadvantage of potential artifact introduced by processing, sectioning, and staining and, therefore, may not accurately reflect the in vivo situation.

The standard method for diagnosis of ACD is the patch test but this method lacks specificity and has a false-positive and false-negative rate of up to 18%. Other noninvasive tests have been used in an attempt to improve diagnosis. Among these, transepidermal water loss (TEWL) has been shown to be higher in ICD than ACD, infrared thermography has shown “hot” spots in ACD but “cold” spots in ICD, and ultrasound has demonstrated more transcutaneous edema in ACD but more superficial edema in ICD. A recent report has suggested that electrical impedance may be used to distinguish allergic and irritant reactions, with 3 of 4 impedance indices being significantly lower in irritant than in allergic reactions. Laser Doppler flowmetry, on the other hand, has not been shown to be consistently helpful in differentiating ACD from ICD. Although some of these techniques may provide useful adjuncts to diagnosis, their sensitivity and specificity have not so far been accurately defined.

In vivo near-infrared reflectance confocal microscopy (RCM) is a novel noninvasive imaging tool that produces high-resolution skin imaging in real time. We have used RCM to characterize the features of ACD in a pilot study and demonstrated the typical features of ICD in a study investigating skin color–related differences in reactivity to irritants. This study aimed to optimize RCM for imaging and characterizing contact dermatitis, and to use RCM to distinguish acute ACD and ICD in vivo.

**MATERIALS AND METHODS**

**Patients and protocol design**

In all, 22 volunteers, aged between 28 and 65 years (mean: 49 years), with a clinical history of skin allergy were recruited to participate in this study, which was approved by our institutional review board. Volunteers were patch tested on the ventral aspect of the forearm or anterior aspect of the thigh using the allergen (Pharmascience Inc, Montreal, Canada) to which they were known to be sensitive and also at a separate site with aqueous solutions of 5% sodium lauryl sulfate (SLS). All chemicals were applied using Finn Chambers (Allerderm Inc, Petaluma, Calif) and filter paper disks were used for the aqueous solutions.

To optimize parameters for RCM imaging of contact dermatitis, the first 4 patients were imaged using several different immersion media as described below. The patch test substances were applied for 48 hours, and evaluated by clinical assessment and RCM imaging 48, 72, and 96 hours after the start of the experiment. An allergen-exposed site, a 5% SLS–exposed site, and a control site of normal skin were evaluated.

The remainder of the patients had the patch-test substances applied for 12 hours and 48 hours, and were evaluated by clinical assessment, TEWL, and RCM imaging 24 and 72 hours after the start of the experiment, respectively (Table I). This methodology was to provide an assessment of early and later phases of the inflammatory response. Another site was exposed to allergen for 24 hours and evaluated at 72 hours to investigate the effect of a shorter allergen exposure period. Normal skin and a site exposed to petrolatum only were also evaluated as controls, because petrolatum was used as vehicle for the allergens.

**Clinical evaluation**

The allergic patch-test reactions were scored using the grading scale shown in Table II. This was derived from the scheme recommended by the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group, and modified to provide analogous scores for irritant reactions.

**In vivo RCM**

A detailed description of the RCM has been published. Briefly, a commercially available device (Vivascope 1000, Lucid Inc, Henrietta, NY) possessing an 830-nm diode laser and maximum power at skin surface of 25 mW, was used to produce horizontal (en face) images of all the skin sites. Water immersion (30×) and oil immersion (60×) objective lenses were used.

Images of optimal quality were obtained by matching the refractive index (n) of the immersion
medium with that of the epidermis (n = 1.34). In contact dermatitis, changes to the epidermis can cause altered refractive index, increased light scattering, and spherical aberration. Therefore, the 4 patients imaged as part of the protocol for optimization of imaging parameters were imaged using immersion media of differing refractive indices: 1.33 (water); 1.34 (5% aqueous sucrose solution); 1.35 (12% sucrose); 1.36 (18% sucrose); and 1.37 (24% sucrose).

Between 4 and 6 images were recorded from each epidermal layer and from the papillary dermis. The depth of the suprapapillary epidermal plate (defined as the distance between the surface of the stratum corneum and the bottom of the cells in the uppermost portion of the basal cells) and the depth of superficial papillary dermal capillaries were measured. This was done in real time using a digital micrometer attached to the z (vertical) stage of the objective lens. The maximum depth of imaging was also documented. Analysis was made of the presence and severity of morphologic features observed with RCM using a semiquantitative scoring scale (0 = none, 1 = mild, 2 = moderate, 3 = severe).

**Transpapillary water loss**

TEWL measurements were performed using a commercially available device (Dermalab, Cortex Technologies-Cyberderm Inc, Media, Pa), with appropriate technique and environmental controls.  

**Table II. Criteria for grading patch-test reactions**

<table>
<thead>
<tr>
<th>Score</th>
<th>Allergic contact dermatitis</th>
<th>Irritant contact dermatitis</th>
</tr>
</thead>
<tbody>
<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 (nonvesicular) reaction, induration, possible papules</td>
<td>Moderate-intense uniform erythema</td>
</tr>
<tr>
<td>2</td>
<td>Strong (edematous or vesicular) reaction, erythema, infiltration, papules, vesicles</td>
<td>Intense erythema and edema, vesiculation or erosion</td>
</tr>
<tr>
<td>3</td>
<td>Extreme (spreading, bullous, or ulcerative) reaction</td>
<td></td>
</tr>
</tbody>
</table>

**Image quality and contrast assessment**

The quality and contrast of images obtained were analyzed qualitatively (visual appearance) and quantitatively, by computer-assisted contrast calculation. This technique uses the coefficient of variation as a measure of contrast: $C = s/Im$, where $C$ is a measure of contrast, $s$ is the root-mean-square deviation, and $Im$ the arithmetic mean (average brightness) of the pixel values. Data were analyzed using software (IPLab Spectrum P, Version 3.1.1c, Scanalytics Inc, Fairfax, Va).

**Conventional histology**

We obtained 3-mm punch biopsy specimens from ACD and ICD reaction sites in 10 volunteers at the 72-hour time point. An additional biopsy was performed if microvesicles or other microscopic findings were visualized by RCM in the absence of a clinically perceptible response. Specimens were fixed in 10% buffered formalin and embedded in paraffin. They were cut in half, and sectioned horizontally and vertically before hematoxylin and eosin staining. Histopathologic evaluation was performed by the investigators and closely supervised by a dermatopathologist (M. C. M.).

**Statistical analysis**

Continuous variables were analyzed using the Student $t$ test, the Mann-Whitney $U$ test, and the Wilcoxon test depending on data distribution. Categorical data was analyzed using chi-square test. Spearman rank correlation was performed to assess strength of the relationship between variables evaluated.

**RESULTS**

**Optimization of RCM imaging by varying immersion media**

The use of sucrose solutions of differing refractive indices (1.33-1.37) did not result in better image quality or contrast, either by visual perception as-
essment or by computer-assisted contrast calculation. Maximum depth of imaging achievable was also not altered. Water (n = 1.33) proved to be as effective an immersion medium as other solutions tested, and was more convenient to use.

Clinical score

ICD reactions were generally more severe than ACD reactions (Fig 1). The characteristic delayed reaction of ACD could also be appreciated; at 24 hours, more than half of the patients had no clinically perceptible response at the ACD site. Compared with ICD, the need for prolonged allergen exposure to produce a clinical reaction in ACD was also observed; clinical reactions evaluated at 72 hours were greater in sites exposed for 48 hours compared with those exposed for 24 hours.

Fig 2. Features common to allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) observed with reflectance confocal microscopy (RCM) and correlated by routine histology. a, Spongiosis: increased intercellular brightness apparent on RCM. b, Inflammatory cell infiltrate: bright structures 12- to 15-μm size interspersed between keratinocytes. Arrows denote inflammatory cells. c, Intraepidermal vesicle (arrow) formation: dark spaces in epidermis containing inflammatory cells and necrotic keratinocytes. Scale bars = 50 μm. H&E, Hematoxylin-eosin stain.

RCM imaging

RCM features common to ACD and ICD. Several morphologic features were frequently seen in both ACD and ICD reactions using RCM, and these were later confirmed by correlating them with histologic evaluation.

Spongiosis was evident as increased intercellular brightness by RCM (Fig 2, A). It was observed to a greater extent in ICD than ACD both at 24 and 72 hours (Fig 3). However, this finding was probably a result of the greater severity of clinical reaction observed in ICD, because after grouping by clinical score, the degree of spongiosis was similar in both ACD and ICD (Fig 4, A).

The presence of epidermal inflammatory cellular infiltrate could be appreciated by visualization of bright round or oval structures 9 to 12 μm in size interspersed between keratinocytes (Fig 2, B). At 24
hours, there was no significant difference in the degree of stratum granulosum inflammatory infiltrate in ACD and ICD, which was generally mild. At 72 hours, stratum granulosum infiltrate was seen significantly more frequently in ICD than ACD (Fig 3) and this was observed for all grades of clinical reaction. The severity of stratum spinosum infiltrate was significantly greater in ICD than ACD at 24 hours, but this again appears to reflect differences in clinical reaction. At 72 hours there was no significant difference in the severity of stratum spinosum infiltrate.

Areas of necrotic epidermis, perivascular inflammatory infiltrate, and increased size and brightness of basal keratinocytes were also seen in both types of reaction.

**RCM features more typical of ICD**

Superficial epidermal changes were the most characteristic features of ICD observed with RCM, and these were seen significantly less often in ACD. These included stratum corneum disruption, which was more severe in ICD than ACD for all grades of clinical reaction (Fig 4, B); clear demarcation and separation of individual corneocytes (Fig 5, A); and striking parakeratosis (Fig 5, B). It was possible to clearly demonstrate the parakeratotic nuclei and these appeared as bright (highly refractile) oval structures centrally placed within corneocytes.

**RCM features more typical of ACD**

Overall severity of vesicle formation was not significantly different when comparing ACD and ICD. RCM features are shown in Fig 2, C. However, after grouping by clinical score it was evident that vesicle formation was more severe in ACD than ICD for all grades of clinical reaction (Fig 4, C).

**TEWL differences between ACD and ICD**

As expected, there were marked differences in TEWL when comparing ACD and ICD (Fig 6). ICD showed higher rates of TEWL for all grades of clinical reaction, in agreement with previous work. The 2 patients who had clinically negative reactions at the irritant site at 72 hours did not show marked increases in TEWL.

**Histopathologic evolution of contact dermatitis reactions over time**

Fig 7 demonstrates that RCM enabled tracking of the evolution of histopathologic features over time. With increasing duration of exposure to allergen, there was a progressive increase in the severity of the histopathologic changes. Sites exposed to 5% SLS for 48 hours also demonstrated more severe histopathology than sites exposed for 12 hours.

**Detection of clinically false-negative reactions using RCM**

In some cases of both ACD and ICD it was possible to observe typical histopathologic changes by
RCM in the absence of a marked clinical reaction. In all, 9 patients who had allergen applied for 24 hours had clinically negative reactions at 72 hours. In addition, 7 showed positive RCM changes including spongiosis, inflammatory infiltrate, vesicles in 1 patient, and 3 demonstrated increased epidermal thickness as assessed by suprapapillary plate measurement. Four patients who had allergen applied for 48 hours had clinically negative reactions but RCM-positive at 72 hours. Two of these were mild, with superficial changes including stratum corneum disruption and parakeratosis, but the other 2 demonstrated typical features of spongiosis, inflammatory infiltrate, and, in 1 case, vesicle formation. Examples of these are shown in Fig 8. No particular allergen was more frequently associated with clinically negative reactions. Two irritant patch tests were clinically negative at 72 hours and both showed stratum corneum disruption. This small number makes meaningful comparison of clinically negative allergic and irritant reactions difficult.

**RCM features correlating with clinical severity**

Spearman rank correlation demonstrated that the RCM features that showed a consistent and significant positive correlation with clinical severity scores were spongiosis and vesicle formation. Inflammatory cellular infiltrate in the stratum spinosum was also significantly correlated with clinical score except in ICD at 24 hours.

**DISCUSSION**

The results of our study using RCM for the evaluation of contact dermatitis demonstrate that it is possible to identify differences in the histopathology of acute ACD and surfactant (SLS)-induced ICD. These differences relate principally to the degree of superficial epidermal damage and disruption induced. Hence, it has been now clearly shown that acute SLS-induced ICD is characterized by disruption of the stratum corneum, individual corneocyte separation and demarcation, and often striking parakeratosis, and spongiosis and inflammatory infiltrate. Acute ACD, on the other hand, tends to show no or mild stratum corneum changes, but also produces spongiosis and an inflammatory infiltrate, with vesicle formation that is generally more severe than in acute ICD. As expected, TEWL was significantly higher in ICD than in ACD for all grades of clinical reaction. As a marker of barrier function, this presumably reflects the superficial epidermal disruption, i.e., barrier abrogation, visualized with RCM.

Interestingly, previous reports of parakeratosis observed with RCM have shown the retained nuclei as dark structures, whereas in ICD we typically saw highly refractile bright structures. We hypothesize that SLS may change the refractive properties of the retained nuclei to make them appear bright on RCM imaging, in a similar way that topical application of acetic acid causes decondensation of chromatin within nuclei, and makes the nuclei appear bright rather than dark in bright-field confocal images.

Dark oval structures in the stratum corneum consistent with nuclei were also seen in a minority of patients, more often in ACD than ICD, but the bright nuclei were exclusively seen in the ICD reactions.

The ability of RCM to show typical histopathologic features of contact dermatitis in the absence of positive clinical reactions is a notable advantage of this tech-
nique. This could potentially enable more accurate 
diagnosis of questionable clinical reactions, or better 
assessment of patients in whom it is difficult to ap- 
preciate erythema, such as those with dark skin color. 

Evaluation of contact dermatitis reactions at dif-
ferent time points and after different exposure times 
to allergen allowed assessment of the evolution of
cutaneous responses. RCM is a particularly useful 
tool for evaluation of the same skin site over time 
because it produces no tissue damage.

Fig 5. Typical features of irritant contact dermatitis visualized by reflectance confocal microscopy (RCM) and routine histology. A, Marked stratum corneum disruption with loss of normal homogeneous bright pattern and marked corneocyte demarcation and separation. B, Parakeratosis. Bright oval structures placed centrally within keratinocytes represent nuclei (arrows). H&E, Hematoxylin-eosin stain.

Differentiating ACD and ICD remains a challenge 
for clinicians and researchers. There is a consid-
erable amount of research published on this topic, but 
the majority of reports highlight similarities rather 
than differences in the histopathology and immunol-
ogy of contact dermatitis. Much of the work on the 
histopathology of ACD and ICD has concentrated on 
the nature and distribution of the inflammatory in-
filtrate in contact dermatitis and has generally shown 
that this is similar.7-10 We are not currently able to 
differentiate subtypes of inflammatory cells using 
RCM but no differences in the distribution of the 
inflammatory infiltrate were observed. As has been 
noted previously,36 one of the reasons why ACD and 
ICD are so similar likely relates to "the limited rep-
eroire of pathologic responses to skin injury," despite 
their differing initiating mechanisms. This does not 
preclude there being differences in the superficial epidermis in contact dermatitis, particularly because the primary insult in ICD is often direct damage or disruption to superficial keratinocytes, but means that the subsequent cascade of histopathologic and immunologic events triggered is likely to be very similar.

The obvious advantages of RCM over the majority 
of other research tools is that it is noninvasive, re-
peatable, and provides a more accurate picture of 
the in vivo situation because imaging is performed 
in real time. Other noninvasive tools that have pre-
viously been shown to be useful include TEWL
which we also found a reliable tool), infrared thermography, and transcutaneous ultrasound. These methods can provide meaningful information and may help differentiate ACD and ICD but do not generally have the potential to enhance understanding of underlying histopathologic events. By comparison, RCM provides high-resolution imaging in vivo in real time. However, it is limited by the depth of imaging that is possible, 250 to 300 µm in normal skin. Pathologic changes such as stratum corneum disruption, parakeratosis, and spongiosis may all further increase spherical aberration and laser light scattering and, therefore, reduce the resolution and maximum depth of imaging. The commercially available microscope used in this study is portable and can be moved between laboratory and clinic. The imaging method can be quickly learned, although some training is required to interpret and analyze images.

One of the difficulties with research studies of this design is that they do not reproduce disease observed in the clinical setting. By the time patients seek dermatologic attention, the clinical presentation is often mixed, with a combination of acute and chronic ACD and ICD. Another limitation to our study is that we have compared acute ACD with only surfactant (SLS)-induced ICD. Although this is a commonly used experimental irritant, previous data have shown that the morphology of ICD varies with different irritants. For example, the predominant features of SLS-induced ICD are parakeratosis, with spongiosis generally present. Nonanoic acid, on the other hand, produces tongues of dyskeratotic cells within the granular and spinous layers, whereas croton oil produces spongiosis and exocytosis. We have previously investigated the morphologic features of ICD produced by different irritants.

Fig 7. Evolution of histopathology of allergic contact dermatitis (ACD) over time. Data represented are mean severity scores. Column labels are hours exposed to chemical/evaluation time from exposure to chemical: light, ACD 12/24; medium, ACD 24/72; dark, ACD 48/72. RCM, Reflectance confocal microscopy.

Fig 8. Confocal histopathology in absence of clinical reaction. In same patient, intraepidermal inflammatory infiltrate (inflammatory cells denoted by arrows) (a) and intraepidermal vesicle (arrow) formation (b). c, Intraepidermal vesicle (arrow) in another patient.
using RCM, but chose to use only one in this study to minimize variability.

Future work should, therefore, evaluate not only different irritants but also provide evaluation of contact dermatitis at extended time intervals to follow the evolution of histopathologic changes, and to evaluate chronic skin reactions. In addition, the sensitivity and specificity of RCM for evaluation of contact dermatitis should be assessed.

REFERENCES