Allergic contact dermatitis: Correlation of in vivo confocal imaging to routine histology

Salvador González, MD, PhD,[a] Ernesto González, MD,[b] W. Matthew White, BS,[a] Milind Rajadhyaksha, PhD,[a,c] and R. Rox Anderson, MD[a] *Boston, Massachusetts, and Henrietta, New York*

Background: Allergic contact dermatitis (ACD) is a common and often challenging clinical problem. In vivo near-infrared confocal reflectance microscopy (CM) is a new vital microscopy technique.
Objective: CM was used to evaluate acute ACD.
Methods: Patch testing by means of Finn Chambers technique was performed in 5 subjects to induce an acute allergic skin reaction. Noninvasive CM images from normal and eczematous skin were sequentially recorded before and after removal of the Finn Chambers.
Results: The epidermis and papillary dermis were clearly seen in high resolution. Retention of nuclei in stratum corneum, epidermal edema with microvesicle formation, and transepidermal migration of inflammatory cells were observed in vivo. Isolated dendritic cells were present in the ACD sites of 2 subjects, with morphology, size, and location consistent with Langerhans cells. Dermal vasodilation was observed as well.
Conclusion: CM is a useful tool to study ACD and may be able to track Langerhans cell activation.
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Contact dermatitis (CD) is the most common form of occupational dermatosis and affects approximately 20% of the population in the United States.[1] However, its pathophysiology is only partially understood. CD is divided into irritant and allergic mechanisms, which are often indistinguishable clinically. Patch testing is an effective tool in the differential diagnosis of CD. However not all positive skin test responses are true positives[2] and may not reveal the true environmental antigen causing CD. Furthermore, patch tests frequently fail to distinguish between allergic and irritant forms of CD because of similarity of morphologic features, both at the clinical and histologic level.[3] Conventional histology is an invasive

technique that also has inherent limitations such as lack of early time data, destruction of target, and exogenous artifacts from fixation, processing, and staining. Real-time confocal microscopy (CM) of skin in vivo might overcome the limitations of patch-testing and histology.

A video-rate (real-time) CM for imaging living human tissue was developed recently.[4] Cellular-level images of noninvasive (virtual) tissue sections in vivo are obtained with resolution comparable to that of standard histology. The measured lateral resolution is 0.5 to 1 μm, and axial resolution (virtual section thickness) is 3 to 5 μm.*

Imaging is possible to a depth of 300 to 400 μm, which includes the entire epidermis, papillary dermis, and superficial reticular dermis. These are the layers most affected by acute CD. Furthermore, in vivo CM is entirely painless, noninvasive, and does not affect the tissue; thus it can be performed as many times as required without altering tissue structure.

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Table I. Concentration of tested allergens

Allergen	Concentration (%)
Nickel sulfate (hydrated)	5
Balsam of Perú	25
Fragrance mix	8

This study was conducted to evaluate the dynamics of structural and cellular changes that take place during the occurrence of allergic CD (ACD).

MATERIALS AND METHODS
Patients

Five patients, 3 female and 2 male with a clinical history of ACD, were entered after signed informed consent under a Massachusetts General Hospital Institutional Review Board–approved protocol. Patch-testing was performed with specific contact allergens by means of Finn Chambers (Epitest Ltd, Oy, Helsinki) affixed with Scanpor tape (Norgesplaster, Vennesia, Norway). Allergens (balsam of Peru, fragrance mix, and nickel sulfate) were applied to ventral forearm skin for 24 and 48 hours. Contact allergens (Trolab, Omniderm Inc, Montreal, Quebec) were tested at concentrations regularly used by the Contact Dermatitis Unit at Massachusetts General Hospital (Table I).

Confocal imaging

A detailed description of the CM has been published.[4] We used 30×, 60×, and 100× water-immersion objective lenses of numerical apertures ranging from 0.85 to 1.2, and illumination by a 1064 nm Nd:YAG laser. Laser power at the tissue level was always lower than 40 milliwatts. Immersion medium was water for 30× and 60× objective lenses and sucrose solutions (40% to 41%) for the 100× objective lens.

Real-time noninvasive imaging of the skin contact reactions was performed immediately, and at 24, 48, and 72 hours after Finn Chamber removal. Imaging of adjacent normal skin was performed as well. All of the CM images were *en face* and were recorded on a super-VHS videotape. From video tape, digital images were captured, grabbed, and enhanced by means of commercially available software (IPLab Spectrum, version 3.1, Scan Analytics, Vienna, VA; Scion Images, version 1.60c, Frederick, MD) on a Power/Macintosh 7300/200 computer. In the case of dynamic events, such as blood flow, images from the videotape are far superior to grabbed/enhanced pictures because the human visual perception "fills in" between video frames. This phenomenon makes a live videotape, a much better match to the human visual system than still images.[5]
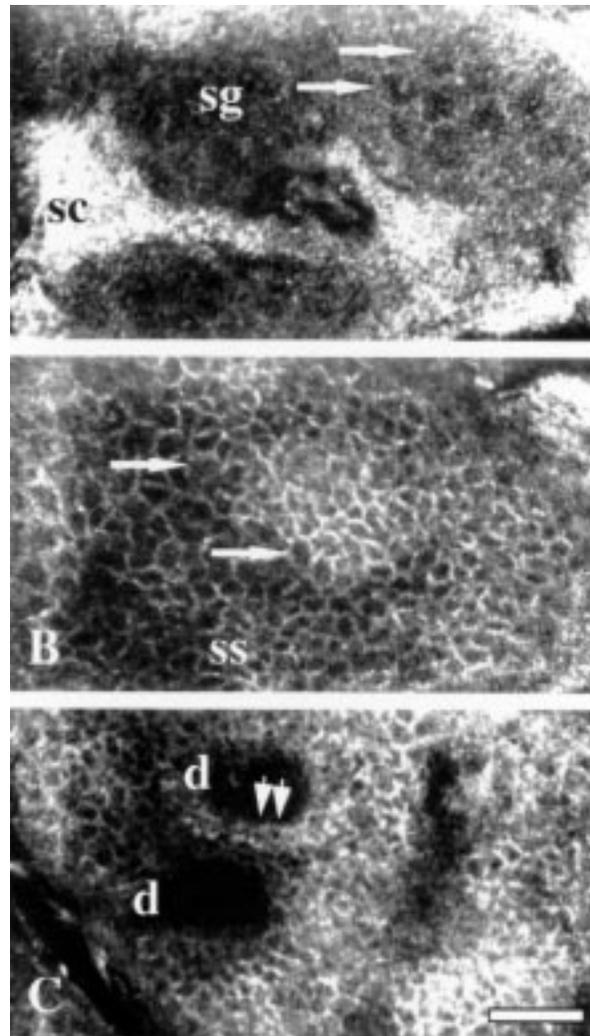

Fig 1. Confocal images of normal skin at different depths show (**A**) stratum corneum (sc), granulosum (sg), (**B**) spinosum (ss), and (**C**) dermoepidermal level. Stratum corneum produces the first image of top surface of skin because of backscattered light at water-to-stratum corneum interface. Granular cells of 25 to 35 μm (*arrows,* **A**) and spinous (*arrows,* **B**) keratinocytes of 15 to 25 μm are clearly resolved. Bright areas are cytoplasm; dark oval areas within are nuclei. **C,** Dermoepidermal view shows dermal papillae (d) surrounded by basal keratinocytes (*arrowheads*). Scale bar, 50 μm.

Morphometric analysis

Stereohistologic and morphometric study of several parameters from CM images was performed (Image Analysis software, Scion Images, version 1.60c, Frederick, Md). Morphometric parameters included cell size and shape, surface density and depth of dermal papillae, and diameter of capillary lumens. Mean and standard deviation of 15 readings per parameter from regions of interest were obtained.
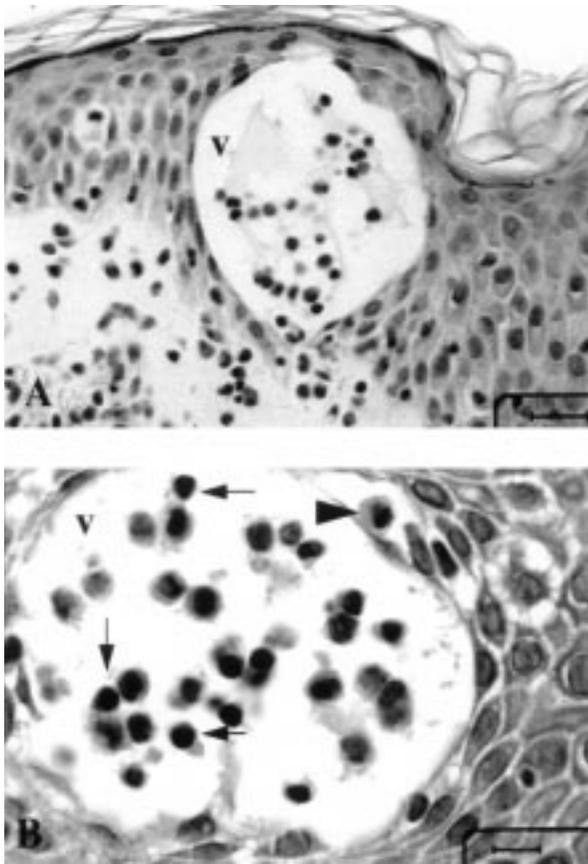
Fig 2. A, Vertical and (**B),** transverse *en face* sections of allergic skin reaction to balsam of Peru 24 hours after removal of Finn Chamber (Hematoxylin-eosin; **A,** Scale bar 25 µm; **B,** Scale bar 10 µm.) Histologic changes are those of acute spongiotic (contact) dermatitis. **B,** In spongiotic, transversally sectioned epidermis, a small intraepidermal vesicle *(v)* is visible containing shrunken, detached keratinocytes and lymphocytes (*arrows*). An eosinophil (*arrowhead*) is also identified in nearby spongiotic epidermis.

Conventional histology

Twenty-four hours after removal of the Finn Chamber, a 3 mm skin biopsy specimen was obtained from the eczematous skin for comparison with CM. The specimen was bisected and processed through formalin fixation and paraffin embedding. Half was oriented to obtain transverse (*en face*) sections parallel to skin surface and the other half was oriented for vertical routine histology. Both portions were then microtomed to 5-µm thin sections, followed by staining with hematoxylin and eosin (H&E).

RESULTS
Normal skin

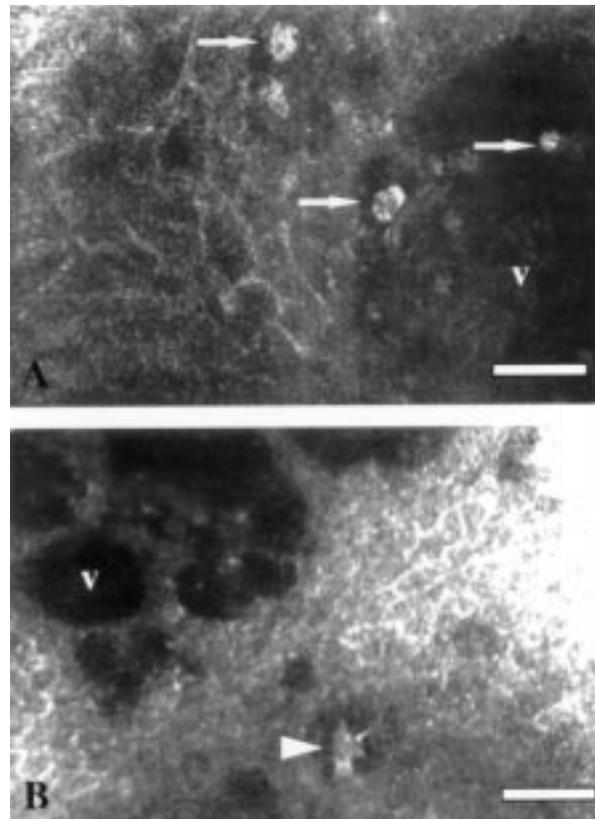In normal skin, CM showed orthokeratotic (no

Fig 3. Confocal images obtained immediately after removal of Finn Chamber containing balsam of Peru. Round cells (**A**, *arrows*) and dendritic cells (**B**, *arrowhead*) are clearly seen between keratinocytes or within spongiotic vesicles (v). These cells have a brighter cytoplasmic body than keratinocytes. Note dendritic cell in contact with surrounding spinous keratinocytes. (**A**, Scale bar 25 µm; **B**, Scale bar 50 µm.)

nuclei) and bright stratum corneum at the top of the skin surface (Fig 1, *A*). The granular and spinous cell layers of epidermis had typical morphologic features (Fig 1, *A* and *B*), with cell nuclei appearing darker than cytoplasm. Granular cells are larger in size than the polygonal and monomorphic spinous keratinocytes. Bright dots in the cytoplasm are organelles. At the dermoepidermal junction (Fig 1, *C*), because all confocal images are of transverse orientation, clusters of basal cells on the top of dermal papillae were observed. We already know that melanized basal keratinocytes in horizontal confocal images appear as bright cells. They are brighter than the surrounding spinous keratinocytes. The number of dermal papillae per *en face* square millimeter containing capillary loops ranged from 26 to 65. The capillary lumen
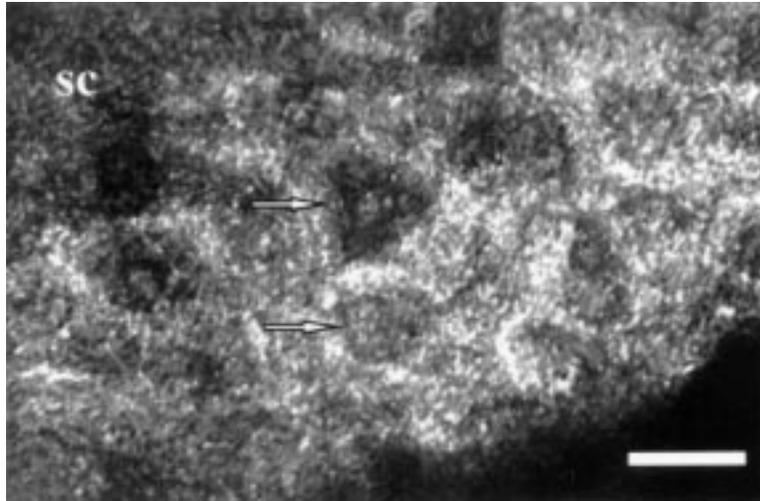
Fig 4. Confocal image of skin site challenged by nickel sulfate, obtained 24 hours after removal of Finn Chamber. Image shows no disruption, and presence of nuclei (*arrows*). (Parakeratotic stratum corneum, sc; scale bar, 25 μm.)

diameter ranged from 5 to 10 μm. Erythrocytes and nucleated cells tumbling through capillaries were easily seen on live-motion images. Deeper penetration showed the network of extracellular matrix fibers in the superficial dermis.

Allergic skin reactions

Histologic characteristics from eczematous reactions during the first 48 hours after removal of the Finn Chambers were similar. A vertical and a transverse *en face* H&E-stained skin section from an acute eczematous reaction to balsam of Peru is provided for comparison (Fig 2).

Immediately after removal of the Finn Chambers, the stratum corneum showed no disruption. In the viable epidermis, multilocular as well as individual unilocular vesicles of variable size were observed. Bright round or oval inflammatory cells were present within the spinous compartment (Fig 3). Most of these were morphometrically compatible with lymphocytes, with diameter ranging from 7 to 9 μm and a variable surface area of 51 to 83 μm². Cells with dendritic morphology (14 to 21 μm) were also observed at different depths within the malphigian compartment in 2 out of the 5 enrolled subjects. They were always seen in the margin of a microvesicle in contact with spinous keratinocytes (Fig 3). Although allergen-treated skin was imaged to a depth greater than 200 μm, we were not able to observe dermal blood vessels at this early time point after challenge.

At 24 hours after removal of the Finn Chambers, CM images showed an intact stratum corneum with patchy areas containing cell nuclei (parakeratosis; Fig 4). Round and dendritic-shaped cells were also observed. The dendritic cells were again always seen in contact with spinous keratinocytes. At the maximum depth imaged with CM, the dermal vasculature was only visualized in one of the allergic skin reactions.

At 48 and 72 hours after removal of the Finn Chambers, the most relevant CM characteristics were disruption of stratum corneum and visualization of dermal vasculature, with blood vessels increased in both number and size compared with normal skin. Dermal papillae containing capillary loops were observed from 56 μm depth below the skin surface (Fig 5). The diameter of capillary lumina in eczematous sites ranged from 10 to 22 μm. No differences in surface density of dermal papillae were found.

DISCUSSION

Evaluation of cutaneous delayed hypersensitivity response is of significant interest in dermatology. However, current methods to investigate ACD are limited by the use of indirect techniques that may be inaccurate or insensitive or by the need for multiple invasive biopsies, because it is a dynamic process. Noninvasive imaging tools for the evaluation of contact skin reactions allow the skin to be imaged as many times as required; standard nonin-
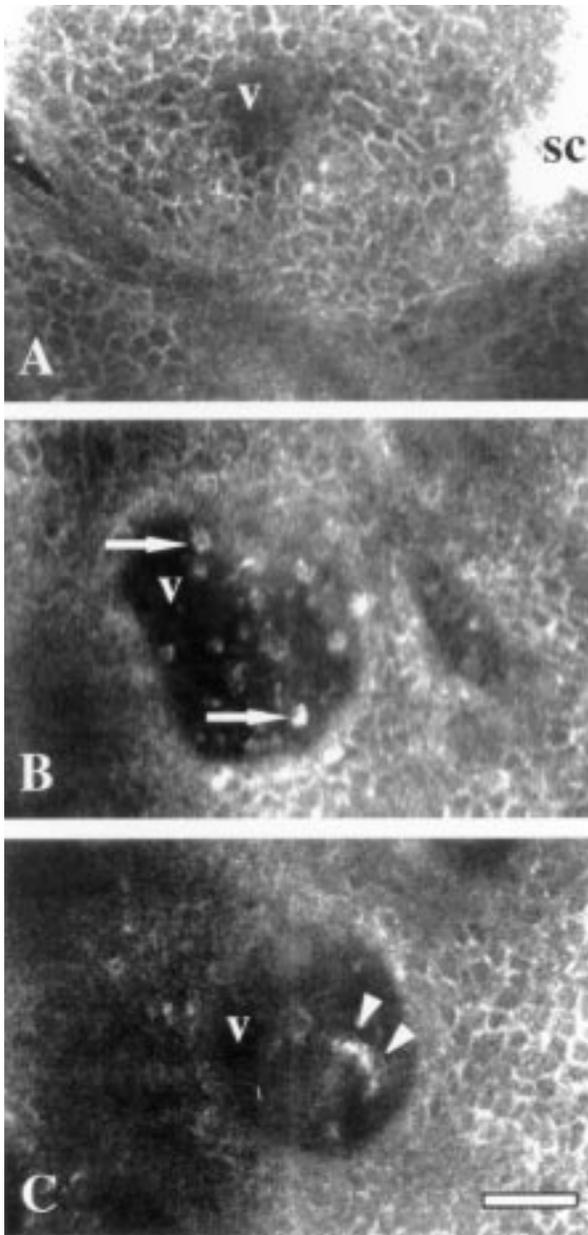
Fig 5. Confocal images from allergic skin reaction, obtained 48 hours after removal of Finn Chamber containing fragrances. **B,** Vesicle *(v)* within spongiotic epidermis contains resident bright cells *(arrows)*. **C,** Dilated blood vessels *(arrowheads)* are visualized. (Scale bar, 50 μm.)

vasive imaging tools (eg, magnetic resonance imaging, ultrasound), however, give information only at a gross level.[6,7] In contrast, in vivo CM resolves changes at the cellular and subcellular level comparable to that obtained with standard histology.

In this study, we report the first in vivo CM images of ACD. Micrographic features observed in vivo included disruption of stratum corneum, spongiosis with vesicle formation, exocytosis and infiltrating inflammatory cells within the epidermis, and vasodilation. These features showed individual variability and allergen specificity that requires further study. Dilated blood vessels were located deeper in the skin at earlier time points during the follow-up. This may be explained by the transient spongiosis-induced epidermal thickening.

Interestingly, disruption of the stratum corneum, except for the presence of nuclei within the stratum corneum (parakeratosis; Fig 4) was not observed in the early stages of the acute eczematous eruptions studied. Disruption of stratum corneum was observed in some of the positive reactions at 72 hours. Parakeratosis was observed at 48 and 72 hours after challenge, and the nuclei within living corneocytes were greater in size than nuclei seen in live parakeratotic stratum corneum of psoriatic lesions.[8] Morphometric differences in parakeratosis seem to be pathogenesis-related, whereas the parakeratotic stratum corneum of psoriatic lesions results from an incomplete epidermal differentiation and fast epidermal renewal[9,10]; in ACD it may be a consequence of the transient and acute spongiosis. As in the case of H&E-stained skin sections, multiple bright, round cells seen between keratinocytes and within the intraepidermal vesicle may be seen at the early phases of ACD (Fig 3). These cells are morphometrically consistent with lymphocytes. Additionally, we were able to distinguish dendritic cells in contact with adjacent keratinocytes, present only during ACD (not seen in normal skin). Morphometric analysis of these dendritic cells correlates well with Langerhans cells.[11] This is the first time that Langerhans cells have been viewed in vivo in human skin (Fig 3, *B*).

Reflectance CM works by capturing single-scattered photons from a thin, "virtual section" within live tissue.[12,13] Local changes in the refractive index are the cause of optical scattering; brighter objects on CM images scatter more.[4] In general, the cytoplasm of keratinocytes and other cells contains organelles, which scatter more light than the nucleus. The bright dendritic cells seen in this study by CM in ACD are morphologically and anatomically consistent with Langerhans cells, which have not been previously seen in live human

skin. Activated Langerhans cells produce a unique cytoplasm structure, the Birbeck granule,[14] which may scatter light and account for the bright CM appearance. Potentially, in vivo CM could be used to study Langerhans cell activation and migration during ACD and other processes. Future work will focus on enhancing the current knowledge and apply it to the pathogenesis of ACD and on differentiating it from irritant skin reactions.

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