

Abnormal Morphology of Blood Vessels in Erythematous Skin From Atopic Dermatitis Patients

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Abstract: Previous studies suggest that altered peripheral blood circulation might be associated with erythema or inflammation in atopic dermatitis (AD) patients. However, the overall structure of blood vessels and capillaries in AD skin is poorly understood because most studies have involved light-microscopic observation of thin skin sections. In the present study, we compared the 3-dimensional structures of peripheral blood vessels of healthy subjects and AD patients in detail by means of 2-photon microscopy. In skin from healthy subjects, superficial vascular plexus and capillaries originating from flexuous blood vessels were observed. However, skin from AD patients contained thickened, flexuous blood vessels, which might be associated with increased blood flow, in both erythematous and nonlesional areas. However, patients with lichenification did not display these morphological changes. Bifurcation of vessels was not observed in either erythematous or lichenification lesions. These results might be helpful for developing new clinical strategies to treat erythema in AD patients.

Key Words: two-photon microscopy, peripheral blood vessel, vascular plexus

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INTRODUCTION

In the dermis of normal subjects, the vascular system is organized into a deep and superficial horizontal plexus from which capillaries originate.¹ In acute inflammation, blood vascular endothelial cells are activated by multiple inflammatory mediators [such as vascular endothelial growth factor (VEGF)], leading to typical signs of inflammation as a result of both increased blood flow because of vessel dilation and edema formation because of increased vessel permeability. For example, the number and size of blood vessels are increased in inflamed skin of K14-VEGF-A transgenic mice compared with uninfamed skin.¹ Accordingly, previous reports suggest that altered peripheral blood circulation might

be associated with erythema in atopic dermatitis (AD) patients. For example, the amount of VEGF in lesional scales in AD was 25 times higher than that in normal stratum corneum.² Steinhoff et al³ suggested that the vascular system, including endothelial cells and smooth muscle cells, is intimately involved in the clinical symptoms of AD, including erythema, edema, leukocyte recruitment, and white dermographism, and they proposed that endothelial cells play a crucial role in the pathophysiology of AD.

These reports led us to hypothesize that the 3-dimensional structure of blood vessels and capillaries is altered in the erythematous skin of AD patients. To examine this question, light-microscopic observation of thin sections, as usually used, is inadequate because it cannot differentiate between increased vessel branching and increased total length of vessels. Therefore, we used 2-photon microscopy, which is a fluorescence imaging technique that allows imaging of tissue to a considerable depth to compare the 3-dimensional morphologies of blood vessels in the upper layer of dermis of healthy subjects and AD patients.

METHODS

Sample Collection

Skin samples were obtained from 10 Japanese healthy subjects and 9 AD patients with lichenification (20–37 years; mean age of 24 years; 13 men and 6 women). The study protocol was fully discussed with each patient and informed consent was obtained. The study was conducted in accordance with the guidelines of the National Institutes of Health and Declaration of Helsinki protocols and was approved by the ethics committees of Shiseido Research Center and Tohoku University Hospital. Tape stripping was performed 10–30 times under local anesthesia with lidocaine, and then a 3-mm punch biopsy was obtained from the back skin. In the case of AD patients, samples were taken from regions of erythema or lichenification and noninvolved regions. The specimens were immediately immersed in 4% paraformaldehyde overnight at 4°C.

Immunohistochemical Study

After the 4% paraformaldehyde treatment, samples were kept in 30% sucrose until they sank to the bottom. Then, one half of each sample was embedded in optimal cutting temperature compound (TissueTek; Sakura Finetek, Torrance, CA) and

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cryosectioned at 100 μm for immunostaining. The sections were treated with 10% H_2O_2 and 0.3% Triton X in phosphate-buffered saline (PBS) for 10 minutes at room temperature. Samples were immersed in blocking solution for 2 hours at room temperature before treatment with first antibodies. Samples were free-floating during the whole operation.

Mouse monoclonal anti-collagen type IV antibody (Abcam, Cambridge, United Kingdom) was diluted 1:500 with blocking solution. Sections were incubated with the antibody solution for 2–3 days at 4°C, then washed 3 times with 0.25% phosphate buffered saline with tween 20 for 30 minutes, and rinsed with PBS. Alexa Fluor 488 goat anti-mouse antibody purchased from Life Technologies was diluted 1000:1. Sections were incubated with the antibody solution for 2 hours at room temperature, then washed 3 times with 0.25% phosphate buffered saline with tween 20 for 1 hour, and rinsed with PBS.

Two-Photon Microscopic Observation

The sections were placed on glass slides and mounted with Fluoromount Plus. The sections were observed with a 2-photon laser scanning microscope (A1R MP; Nikon, Tokyo, Japan) equipped with a Ti-sapphire laser, Mai Tai DeepSee (Spectra-Physics, CA) using a $\times 40$ water immersion objective lens (numerical aperture = 1.15) (excitation wavelength, 800 nm; emission, 500–550 nm band pass filter).

RESULTS

Representative images of skin sections are shown in Figure 1. In the skin from healthy subjects, superficial vascular plexus (yellow asterisks) and capillary loops (yellow arrowheads) originating from vascular plexus were observed (Figs. 1A, B), in accordance with previous findings.¹ In the skin of AD patients who displayed erythema, flexuous complex vessels were observed in both erythematous lesional skin (white double asterisks: Figs. 1C, D) and even nonlesional skin (white double asterisks: Figs. 1E, F). Especially in the papillary region, thick flexuous blood vessels were tightly packed. Capillary loops originating from flexuous blood vessels were also observed in AD (yellow arrowheads: Figs. 1D, E). In the skin of AD patients with lichenification, a few flexuous structures were observed (white double asterisks: Figs. 1G, H). There was no obvious bifurcation of blood vessels in either area.

DISCUSSION

Our 3-dimensional structural analysis by 2-photon microscopy with thick specimens of AD skin revealed flexuous vessels without bifurcation in erythematous lesions of AD patients. The previous finding of an increased number of blood vessels in inflamed skin¹ was based on light-microscopic observation of thin sections. The apparent discrepancy can be explained by the fact that a single flexuous vessel might be cut in multiple locations in a single thin section, resulting in an apparent increase in the number of vessels in their immunostained sections.

Although we could not conclude in this study whether flexuous vessels are altered form of the superficial vascular

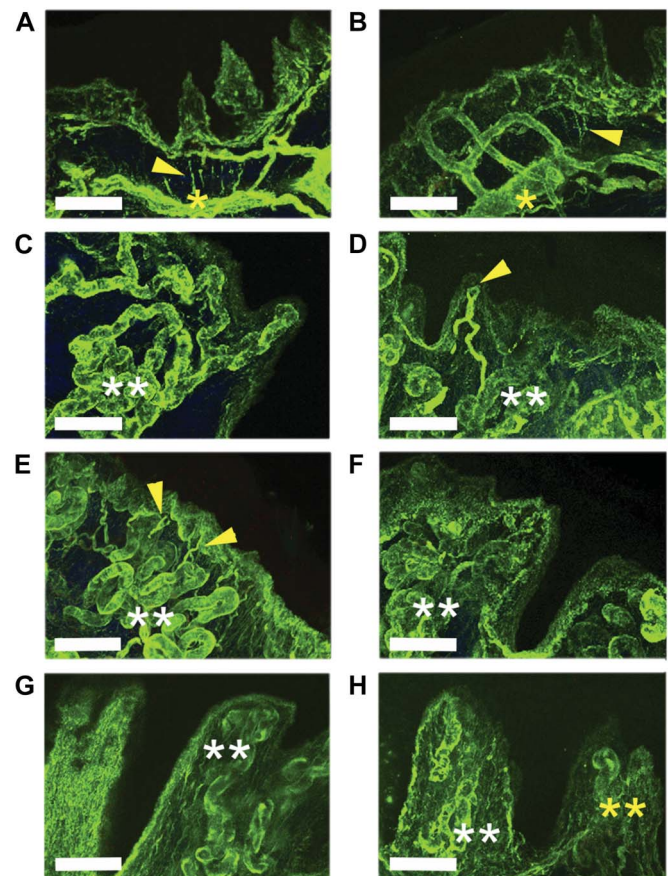


FIGURE 1. Representative images of peripheral blood vessels and capillaries in skin. A and B, Healthy subjects. Superficial vascular plexus (yellow asterisks) and capillary loops (yellow arrowheads) originating from the vascular plexus were observed. C and D, Erythematous lesional skin of AD patients. Flexuous complex structures were observed (double white asterisks). E and F, Nonlesional skin of AD patients. Flexuous complex structures were seen, similar to those in erythematous lesional skin (double white asterisks). A few capillary loops originating from flexuous blood vessels were observed in (D, E) (yellow arrowheads). G and H, Lesional skin with lichenification of AD patients. A few flexuous structures were observed (double white asterisks). Bars = 50 μm .

plexus or capillary loops, a more precise understanding of the 3-dimensional structure of blood vessels in erythematous lesions should provide clues for developing new clinical strategies to treat inflammation in AD and allergic reaction in various skin diseases.

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