

No Differences in Number and Distribution of Dermal Mast Cells between Acute and Chronic Cutaneous Lupus Erythematosus

Mikiko Uede¹, Yasushi Nakamura², Yuki Yamamoto¹ and Fukumi Furukawa^{1*}

¹Department of Dermatology Wakayama Medical University, Wakayama, Japan

²Department of Laboratory Medicine, Wakayama Medical University, Wakayama, Japan

Abstract

The appearance of mast cells at eruption sites was examined in acute (ACLE) and chronic (CCLE) cutaneous Lupus Erythematosus (LE). As shown by toluidine blue staining and c-kit immunostaining, mast cells were significantly more abundant around the sebaceous glands than in the control for both ACLE and CCLE. However, no specific difference was noted in the numbers and distribution of mast cells between ACLE and CCLE.

Keywords: Cutaneous lupus erythematosus; Mast cell; Toluidine blue; Facial lesion

Corresponding Author: Fukumi Furukawa, Department of Dermatology, Wakayama Medical University, Wakayama, Japan; E-mail: dajs@wakayama-med.ac.jp

Introduction

Systemic Lupus Erythematosus (SLE) causes systemic erythema, particularly on the face. It causes acute, subacute, and chronic eruptions. One, two and/or three types of skin eruptions are presented in the manifestation of SLE. Acute Cutaneous LE (ACLE) mainly appears as an edematous erythema in SLE. Subacute Cutaneous LE (SCLE) is presented as one of SLE skin eruptions and also is observed in SCLE as a clinical entity. Discoid LE (DLE) is mainly characterized by chronic eruption [1]. A question why such different skin manifestation occurs in

LE has arisen, but convincing information is limited. Immune and allergic reaction-related cells are involved in the onset of the eruptions [2]. Among them mast cells are involved in autoimmune diseases, as demonstrated by an experiment using a mouse model of LE [3]. Mast cells activated by ultraviolet UVB are involved in the onset of erythema due to LE [4], suggesting that mast cells play a role in the pathogenesis of eruptions due to SLE and DLE. In the present study, the appearance of mast cells of ACLE (edematous erythema in SLE) and CCLE (discoid eruption in DLE) was immunohistochemically examined.

Subjects and Methods

Subjects

Skin biopsy specimens from SLE or DLE patients who visited the Department of Dermatology, Wakayama Medical University were examined. SLE was classified according to the ACR criteria (1997 edition). Cutaneous lupus erythematosus (CLE) was diagnosed based on histopathological findings with direct immunofluorescence and classified using the "Dusseldorf Classification of Cutaneous Lupus Erythematosus 2004" [5]. For ACLE, biopsies were obtained from 26 SLE cases (facial eruption: 9 cases, non-facial eruption: 15 cases, and eruption of unknown sites: 2 cases). For CCLE, biopsies were obtained from 13 DLE cases (facial eruption: 7 cases and non-facial eruption: 6 cases). Control samples were obtained from non-lesional skin of face from 8 cases of other diseases without

facial eruption. Of these, 7 ACLE (facial biopsy: 3 cases and non-facial biopsy: 4 cases) and 3 CCLE (facial biopsy: 3 cases) patients were photosensitive.

Methods

Tissue biopsies were fixed in 10% formalin, and paraffin sections were prepared and stained with Hematoxylin and Eosin (HE) and 0.05% Toluidine Blue (TB) (pH 4.0). For immunostaining, anti-c-kit (CD117) (NICHIREI BIOSCIENCE INC., Tokyo, Japan), anti-CD4 monoclonal (NICHIREI BIOSCIENCE INC.), and anti-CD8 monoclonal (NICHIREI BIOSCIENCE INC.) antibodies were employed. Dilution of each antibody was based on the manufacturing company's recommendation. For the evaluation of dermal infiltrating mast cells, 5 microscopic fields (magnification; x400) were randomly selected, and the number of mast cells or other cells was counted. The area per field was 300 mm². The degree of mast cells infiltration was expressed as the average number of the mast cells in the 5 microscopic fields. The average numbers of positive cells were determined for four sites (papillary dermis, reticular dermis, sebaceous glands, and hair follicle). All measurements were performed without prior knowledge of the experimental procedures.

Statistical analysis was conducted using Tukey-Kramer test.

Results and Discussion

1) Numbers of mast cells

The numbers of mast cells after TB staining are shown in Figure 1, which represents the average number of all examined samples irrespective of sites. The results of For ACLE, mast cells were significantly more abundant in the reticular dermis, sebaceous glands, and hair follicle than in the papillary dermis. Similar results were obtained for CCLE. For the control, mast cells were significantly more abundant in the reticular dermis than in the papillary dermis and others, although there was no significant difference because of a small number.

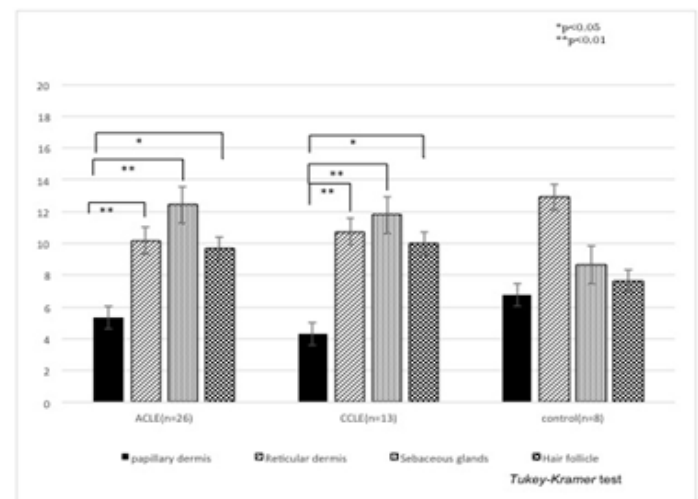


Figure 1: The number of mast cells (TB stain) from all examined samples irrespective of sites. Each bar represents the mean and one standard error.

The numbers of mast cells in the face were almost the same as those shown in Figure 2. For ACLE, mast cells were significantly more abundant in the reticular layer, sebaceous glands, and hair follicles than in the papillary dermis. Similar results were obtained for CCLE.

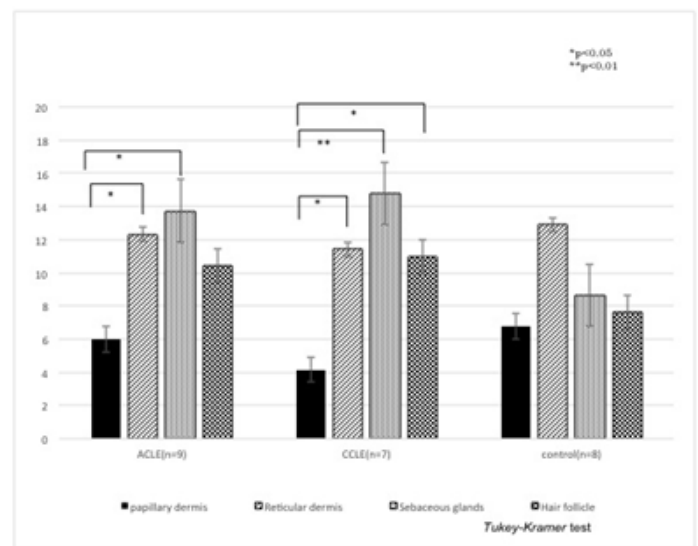


Figure 2: The number of mast cells in the facial lesions (TB stain). Each bar represents the mean and one standard error.

The numbers of mast cells at sites other than the face are shown in Figure 3. For ACLE, mast cells were significantly more abundant in sebaceous glands than in the papillary dermis among eruption sites other than the face. For CCLE, the numbers of mast cells did not vary with the eruption sites other than the face.

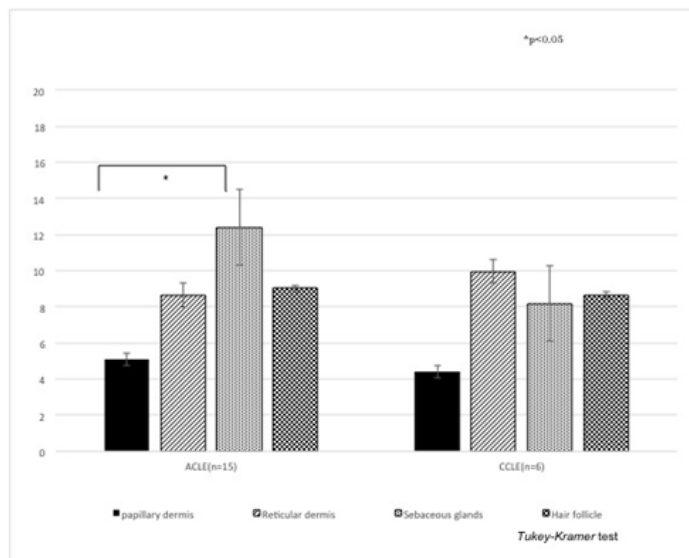


Figure 3: The number of mast cells excluding facial lesions (TB stain)

Each bar represents the mean and one standard error.

For both ACLE and CCLE, the numbers of mast cells were similar regardless of the presence or absence of photosensitivity. The mean numbers of mast cells of positive photosensitivity patients were 6.3, 11.7, 12.4 and 10.0 in the papillary dermis, reticular dermis, sebaceous glands, and hair follicle, respectively. Those of photosensitivity negative photosensitivity patients were 4.3, 10.1, 14.9 and 9.7 in the papillary dermis, reticular dermis, sebaceous glands, and hair follicle, respectively. There was no significant difference between positive and negative groups.

The numbers of mast cells by anti-c-kit immunostaining were almost the same as those by TB staining (data not shown). Staining patterns are shown in Figure 4.

As a result, no difference was noted in the numbers and distribution of mast cells of the facial site between ACLE and CCLE.

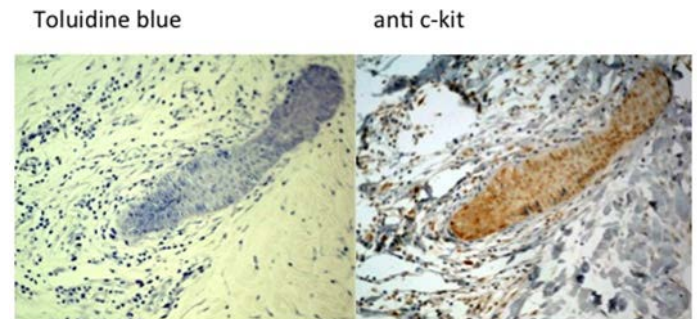


Figure 4: TB staining (left) and c-kit positive (right) cells around hair follicle of the face lesion of ACLE patient (original magnification x200)

2) Numbers of inflammatory cells

The numbers of CD4+ and CD8+ inflammatory cells were determined (data not shown). They tended to be higher in the papillary dermis and reticular dermis for both ACLE and CCLE, although no significant difference was noted because of marked individual differences. In addition, CD4+ inflammatory cells tended to be more abundant than CD8+ ones for both ACLE and CCLE. This tendency was more marked for ACLE than for CCLE, although no significant difference was noted. For CCLE, CD4+ and 8+ inflammatory cell infiltrations was observed in the sebaceous glands and hair follicles. In the control, no site-specific difference was noted, and inflammatory cell infiltration was negligible. These results are almost compatible with previous reports, which were reviewed by Mikita et al [2]. At sites with increased inflammatory cells, mast cells decreased. However, at sites with increased mast cells, inflammatory cells decreased, although no significant difference was noted. Mast cells, activated by inflammatory cell infiltration, might be markedly degranulated and therefore unstained [6].

Mast cells play various important roles in complicated immune reactions, including allergic diseases [7]. Further investigation, including the classification of eruptions, is needed to reveal how mast cells are involved in the different forms of

eruption (ACLE and CCLE) from Treg, chemokine, and other many candidate cells [8,9].

References

1. Harry W, Christine J. (2009) Connective tissue diseases. In: Elder D, Elenitsas R, Jaworsky C, Johnson Jr B (eds), Lever's Histopathology of the skin. 10th ed. 279-310.
2. Mikita N, Ikeda T, Ishiguro M, Furukawa F (2011) Recent advances in cytokines in cutaneous and systemic lupus erythematosus. *J Dermatol* 38: 839-849.
3. Yoshimasu T, Kanazawa N, Mikita N, Furukawa F (2008) The expression of histamine receptors in skin lesions of MRL/MP-lpr/lpr mice, *The Open Dermatology Journal* 2:83-86.
4. Furukawa F (2003) Photosensitivity in cutaneous lupus erythematosus: lesions from mice and men. *J Dermatol Sci* 33:81-89.
5. Kuhn A, Ruzicka T. (2004) Classification of cutaneous lupus erythematosus. In: Kuhn A, Lehmann P, Ruzicka T (eds), *Cutaneous lupus erythematosus*. 53-58.
6. Claman HN, Choi KL, Sujansky W, Vatter AE (1986) Mast cell "disappearance" in chronic murine graft-vs-host disease (GVHD)-ultrastructural demonstration of "phantom mast cells". *J Immunol* 137:2009-2013.
7. Galli SJ, Nakae S, Tsai M (2005) Mast cells in the development of adaptive immune responses. *Nat Immunol* 6:135-142.
8. Kuhn A, Beissert S, Krammer PH (2009) CD4+CD25+ regulatory T cells in human lupus erythematosus. *Arch Dermatol Res* 301:71-81.
9. Wenzel J, Zahn S, Bieber T, Tuting T (2009) Type 1 interferon-associated cytotoxic inflammation in cutaneous lupus erythematosus. *Arch Dermatol Res* 301:83-86.