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Letter to the Editor

Darier's sign in urticaria pigmentosa—significance of perivascular eosinophilic infiltration

Dear Editor,

Urticaria pigmentosa is characterized by the accumulation of mast cells in cutaneous and extracutaneous organs. Multiorgan involvement frequently causes systemic symptoms such as pruritus, flushing, abdominal pain, or headache due to mast cell degranulation releasing histamine, substance P, and other chemical mediators.^{1,2} Rubbing/stroking of the skin or other mechanical stresses induce an urticarial reaction called Darier's sign, which is a specific diagnostic sign for urticaria pigmentosa.³ However, the pathological and immunohistological features of Darier's sign have not yet been described. We investigated the histopathological features of urticaria pigmentosa in specimens presenting with or without Darier's sign.

In our department, 18 were diagnosed as urticaria pigmentosa (mean age 41.5 months, male: female ratio 12:6) (Supplementary Methods). Solitary lesions were observed in 6 cases while 12 cases showed multiple lesions. A family history of urticaria pigmentosa was positive in 3 patients and all cases had multiple lesions (Supplementary Table 1). Reportedly, all patients exhibited a positive Darier's sign at the time of their first visit. Among the 18 specimens obtained, 5 were obtained from Darier's sign areas within 30 min after scratching the site, and 13 were obtained from non-scratched lesions without Darier's sign (Supplementary Methods). Darier's sign was induced using pen friction.

Histopathologically, the non-Darier's sign group (Fig. 1A–D) showed cuboidal mast cell infiltration in the dermis to subcutis (Fig. 1A–D). These accumulated mast cells showed abundant cytoplasmic granules (Fig. 1C). Negligible perivascular lymphocytic and neutrophilic infiltration was found in all cases. Minimal perivascular eosinophilic infiltration was found in 11 of 13 specimens.

However, all cases belonging to the Darier's sign group (Fig. 1F–I) showed that most of the accumulated mast cells were degranulated with scarce intracytoplasmic granules present (Fig. 1H), and significant perivascular eosinophilic infiltration was observed in all specimens (Fig. 1I). Mild to moderate edema was noted in the papillary dermis (Fig. 1G). Only one case showed concomitant intravascular neutrophilic infiltration. The number of perivascular eosinophils was significantly higher in the Darier's sign group than in the non-Darier's sign group ($P < 0.0126$) (Fig. 2A).

Based on the results of a recent study emphasizing the role of substance P in eosinophil migration,⁴ we examined the immunohistological distribution of substance P in specimens with urticaria pigmentosa with and without Darier's sign. The immunoreactive

substance P was primarily detected in abundant quantities in the cytoplasm of mast cells in the non-Darier's sign group (Fig. 1E), while most mast cells in the Darier's sign group were seen to be degranulated (Fig. 1J), and the intensity score of cytoplasmic staining of substance P was significantly lower ($P < 0.0001$) (Fig. 2B). We found 2 cases without eosinophilia in the non-Darier's sign group and one case with neutrophilia in the Darier's sign group. There observed no specific differences in the intracytoplasmic intensity of substance P nor distribution of mast cells in these cases. Low sensitivity of immunohistological assessments and other chemotactic factors may explain the discrepancy.

We demonstrated for the first time that extensive perivascular eosinophilic infiltration is the histopathological hallmark of Darier's sign. Cases of urticaria pigmentosa do not usually exhibit eosinophilic recruitment in lesional skin.⁵ Only a few documented cases of urticaria pigmentosa have shown eosinophilic infiltration; however, the relationship between this finding and a positive Darier's sign has not been established.³ It has been observed that systemic mastocytosis is associated with abundant eosinophilic infiltration in colonic/ileal biopsies.⁶ These studies allow us to speculate that mechanical stress, such as rubbing of skin lesions (Darier's sign) and food friction against intestinal tract lesions, induces eosinophilic infiltration. Reportedly, prominent perivascular eosinophilic infiltration is a characteristic of persistent⁷ and deep pressure urticaria.⁸ Our findings also suggested that eosinophilic infiltration may be essentially involved in the urticarial reaction found in Darier's sign.

Immunohistological analysis performed in our study revealed that mast cells in urticaria pigmentosa are an abundant source of substance P. The non-Darier's sign group showed that substance P was largely compartmentalized in the cytoplasm of mast cells, whereas the Darier's sign group showed that most of the substance P-positive granules were degranulated, and therefore cytoplasmic staining of substance P was significantly lower in this group (Fig. 2B). Mast cell-derived proteases, leukotrienes and histamine can contribute to tissue inflammation and recruitment of eosinophils.⁹ It is known that substance P is produced and released from human mast cells and functions as a potent chemoattractant for eosinophils.^{4,10} Therefore, we hypothesize that substance P may be instrumental in promoting eosinophilic infiltration in cases of urticaria pigmentosa with a positive Darier's sign. But even in 2 cases without eosinophilia in the non-Darier's sign group, there is no specific difference in the intracytoplasmic intensity of substance P nor distribution of mast cells.

In conclusion, mechanical injury in patients diagnosed with urticaria pigmentosa induces a Darier's sign, which is associated with

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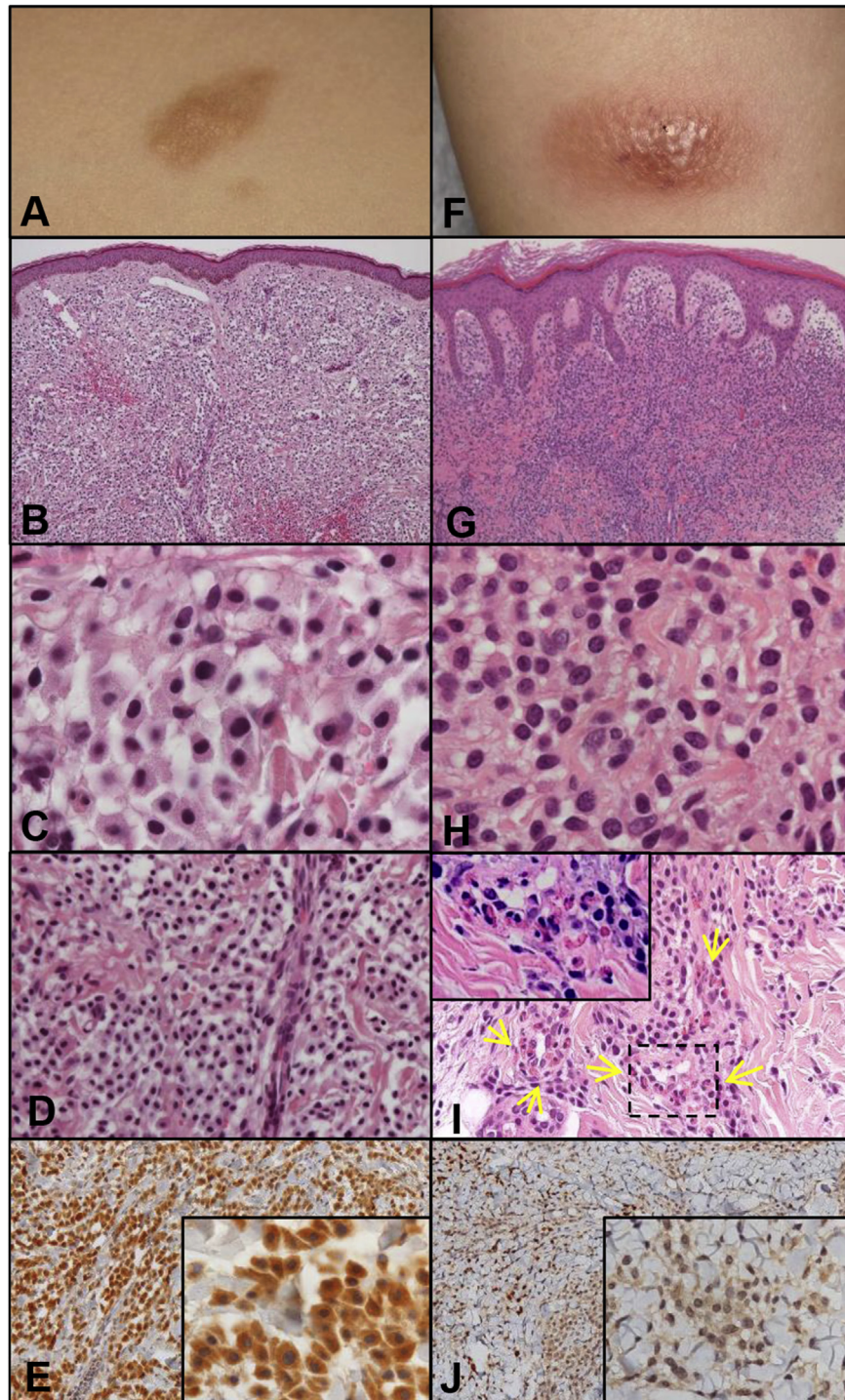


Fig. 1. Clinicopathology of urticaria pigmentosa. **A-E:** without Darier's sign. **F-J:** with Darier's sign. **E and J:** substance P immunostaining.

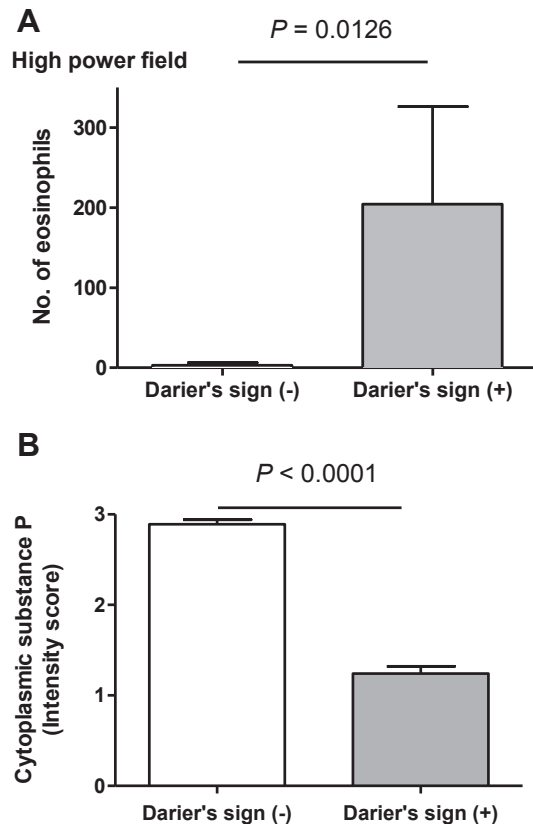


Fig. 2. A: Number of perivascular eosinophils. B: The intensity score of cytoplasmic substance P immunostaining.

significant perivascular eosinophilic infiltration and degranulation of substance P from mast cells. Targeting eosinophilic infiltration may be helpful to prevent the systemic symptoms of mastocytosis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2018.04.006>.

Conflict of interest

The authors have no conflict of interest to declare.

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Supplementary Methods:**METHODS*****Subjects and Immunohistochemistry***

In the Department of Dermatology at Kyushu University between January 1, 2003, and December 1, 2016, 18 cases were diagnosed as urticaria pigmentosa (Supplementary Table 1). Microscopic review was performed by three pathologists. All mastocytosis specimens were confirmed to be positive for toluidine blue staining.

Immunohistochemical staining was performed with anti-substance P antibody (1:100, GeneTex, GTX31105, San Antonio, TX, USA). The 3 µm-thick paraffin-embedded sections were deparaffinized and rehydrated. Antigen retrieval was performed by incubating the sections in citric acid (pH 6.0) for 40 min with microwave boiling. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min at room temperature, and washed with phosphate-buffered saline (Nichirei Histostainer, pH 7.6 ± 0.2). Sections were incubated with a primary antibody for 30 min at room temperature and washed with phosphate-buffered saline, then incubated for 30 min with MAX-PO (MULTI) at room temperature and washed with phosphate-buffered saline. Immunoreactivity was visualized using a 3,3'-diaminobenzidine chromogen solution (DAB+, Nichirei, Japan). Sections were counterstained for 1 min with Mayer's Hematoxylin and then mounted. The average of the staining intensity [negative (0), weak (1+), moderate (2+), or strong (3+)] at ten randomly selected areas was compared between the Darier's sign and non-Darier's groups (Fig 2B). Written informed consent was obtained from the patient for publication of this report. This study was approved by the ethical committee of Kyushu University Hospital (approval number: 27-157).

Statistical analysis

Statistical analyses were performed using 2-tailed t-tests using the GraphPad Prism 5.0 program. Results are expressed as mean $\pm$ standard error (SEM) and $P < 0.05$ was considered to be statistically significant.

Supplementary Table 1

Clinical information of 18 urticaria pigmentosa

No	Age / Sex	Site of biopsy	Type of urticaria pigmentosa	Family history
Specimen: Darier's sign (-)				
1	3Y / M	upper limbs	Multiple	Sibling history
2	11M / F	lower limbs	Multiple	
3	1Y / F	abdomen	Multiple	
4	1Y / M	abdomen	Multiple	
5	1Y / M	abdomen	Solitary	
6	2Y / M	chest	Multiple	
7	4M / F	abdomen	Multiple	
8	8Y / M	waist	Solitary	
9	7M / M	back	Solitary	
10	21Y / M	palpebra	Multiple	Sibling history
11	1Y / F	buttocks	Multiple	
12	8M / F	abdomen	Multiple	
13	17Y / M	forearm	Multiple	Sibling history
Specimen: Darier's sign (+)				
14	1Y / M	back	Solitary	
15	4M / M	back	Multiple	
16	1Y / M	chest	Multiple	
17	5M / M	lower limbs	Solitary	
18	2Y / F	abdomen	Solitary	

M: Month, Y: Year