SHORT COMMUNICATION

Dermal, but not Epidermal, Immune Reaction in Cutaneous Spiruroid Larva Migrans: Involvement of Basophils in Dermal Interleukin-33 and Thymic Stromal Lymphopoietin Expression

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Parasitic infections reportedly lead to activation of basophils and their infiltration into the lesion through pro-allergic cytokine interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP); they then exert anti-parasitic effects (1). Although this concept has been well-proven in animal experiments, there are few studies demonstrating basophilic infiltration in human parasitic infections. Cutaneous spiruroid larva migrans is a type of creeping eruption that arises from cutaneous infection of the larvae of a nematode parasite, Spiruria type X (*Crassicauda giliakiana*), whose intermediate hosts include firefly squids. This disease shows a regional preference in Japan, as some Japanese people have a tradition of eating raw firefly squid. This may cause per-oral infection with *Crassicauda giliakiana* larvae, which leads to cutaneous Spiruroid larva migrans (2). In this context, we report here a Japanese case of cutaneous larva migrans due to larvae of *Crassicauda giliakiana*. The patient presented massive basophilic infiltrates in skin lesions, along with dermal cells expressing IL-33 and/or TSLP.

CASE REPORT

A 49-year-old, otherwise healthy, Japanese man presented with a 5-day-history of itchy, red moving eruptions 1 week after eating raw firefly squid. Physical examination revealed serpiginous, raised tracts with vesicles on his trunk (**Fig. 1**a). Laboratory tests showed peripheral blood eosinophilia (1,150/μl) with elevated serum levels of IgE (1,300 IU/ml; normal, <170 IU/ml) and thymus

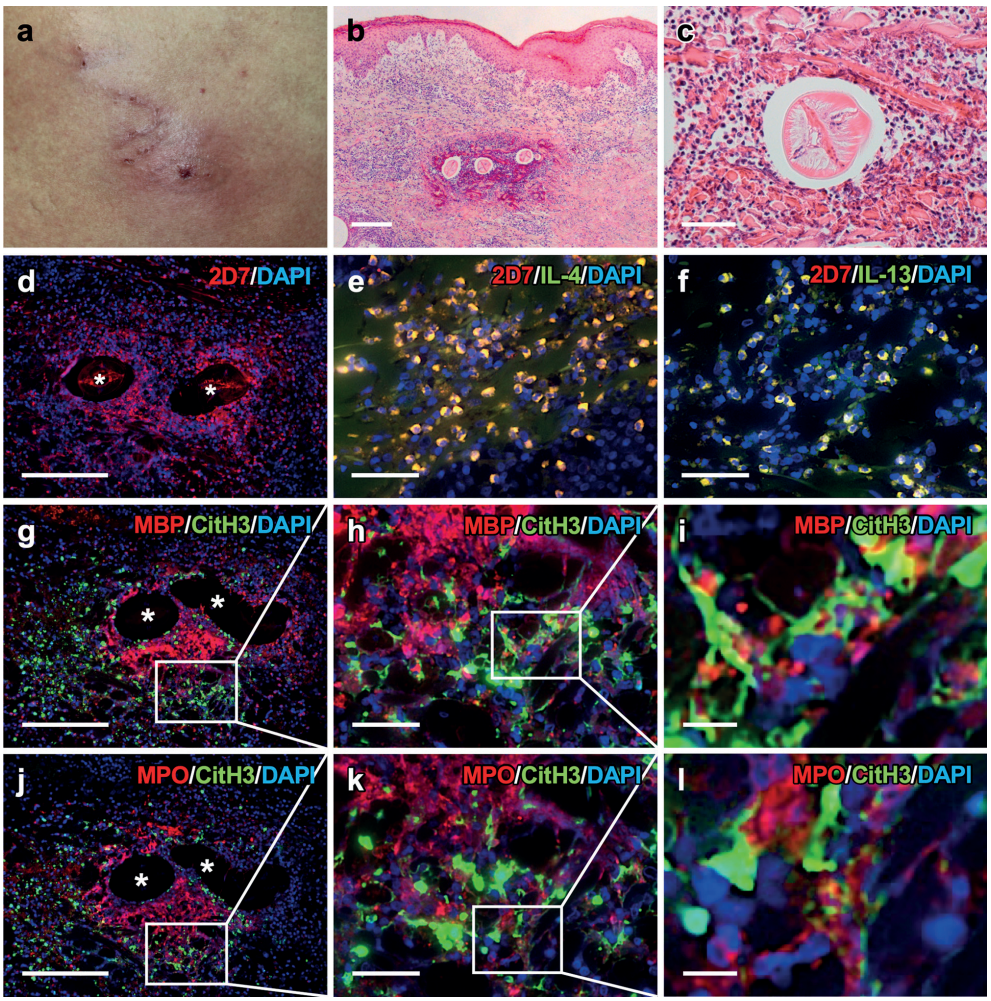

Fig. 1. Clinical, histopathological and immunofluorescence staining features. (a–c) Clinical and histopathological findings. (a) Serpiginous, raised tracts with vesicles on the trunk. (b, c) Histopathological findings (haematoxylin and eosin staining) of the lesions. Cross sections of Spiruroid type X larvae in the mid-dermis (b) surrounded by degranulated eosinophils, neutrophils and macrophages (c). (d–l) Immunofluorescence staining. (d) $2D7^+$ basophils (*red*) around the larvae. (e, f) Expression of interleukin (IL)-4 (*green*) (e) and IL-13 (*green*) (f) by $2D7^+$ basophils (*red*). (g–l) Extracellular DNA traps (ETs) identified with citrullinated histone H3 (CitH3, *green*). Major basic protein $(MBP)^+$ eosinophils (*red*) (g–i) and myeloperoxidase $(MPO)^+$ neutrophils (j–l) forming ETs. The *blue areas* indicate nuclei. *Larvae. *Bars*: 25 μm (i, l), 50 μm (c, e, f, h, k,), 100 μm (d, g, j) and 200 μm (b), respectively.

and activation-regulated chemokine (700 pg/ml; normal, <450 pg/ml), a biomarker of type 2 inflammation. Skin biopsies revealed characteristic cross-sections of Spiruroid type X larvae in the mid-dermis, with massive infiltrates of degranulated eosinophils, neutrophils and macrophages surrounding the larvae (Fig. 1b, c). Based on a diagnosis of Spiruroid larva migrans due to *Crassicauda giliakiana* larvae, the anthelmintic drug ivermectin was administered, leading to gradual resolution of symptoms.

Immunofluorescence staining showed massive dermal infiltration of 2D7$^+$ basophils, major basic protein (MBP)$^+$ eosinophils and myeloperoxidase (MPO)$^+$ neutrophils, especially around the larvae (Fig. 1d, g, j). The infiltrating basophils expressed type 2 cytokines IL-4 and IL-13 (Fig. 1e, f). In addition, since eosinophils and neutrophils are known to be protective against parasites through forming extracellular DNA traps (ETs) (3), we investigated ETs from eosinophils (EETs) and neutrophils (NETs) using an ET-marker, citrullinated histone H3 (CitH3) (4, 5). CitH3$^+$ web-like structures were found around the larvae, and were seen to co-express MBP or MPO (Fig. 1h, i, k, l), indicating the formation of EETs and NETs.

This study next investigated IL-33 and TSLP. IL-33 was expressed not by the epidermis, but by dermal infiltrating MPO$^+$ neutrophils and CD68$^+$ macrophages (**Fig. 2**a-e). CD68$^+$ macrophages expressed arginase-1, an M2 macrophage marker (Fig. 2f). TSLP was also not expressed by the epidermis (Fig. 2g), while dermal MPO$^+$ neutrophils preferentially expressed TSLP (Fig. 2h, i).

DISCUSSION

This study revealed massive lesional infiltration of basophils with eosinophils and neutrophils. Animal studies have shown that IgE-activated basophils infiltrate into the sites of parasitic infections and secrete a significant amount of IL-4/13, which leads to parasite expulsion partially through the activation of innate lymphoid cell type 2, recruitment of eosinophils, and induction of M2 macrophages secreting arginase-1 (1, 6). In humans, basophils might also have protective roles against parasites through these mechanisms.

Notably, IL-33 and TSLP were not expressed by the epidermis, even though these are generally known as "epithelial-derived" cytokines (7). Previous reports have shown that the major cellular sources of IL-33 in chronic spontaneous urticaria (8) and IgG4-related disorders (9) are neutrophils and macrophages, respectively. TSLP is also reported to be expressed by neutrophils in cutaneous allergen challenge sites in atopic subjects (10). These are diseases biased toward Th2 immunity involving basophils (1). Some of the type 2 inflammations thus seem to involve IL-33-secreting neutrophils and/or macrophages and TSLP-secretion from neutrophils, as seen in the present case. Furthermore, *Crassicauda giliakiana* larvae infect the human body per-orally, e.g. by eating raw firefly squid, and then migrate into the skin. Hence, their infection and migration do not involve the epidermis. This characteristic pathogenesis might explain the minimal epidermal expression of TSLP and IL-33 in the current case.

Dermal infiltration of basophils and dermal IL-33 and TSLP might be factors in protection against parasites. Promotion of these immunological events might be a therapeutic option for eradicating parasites.

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The patients in this study have given informed consent to publication of their case details.

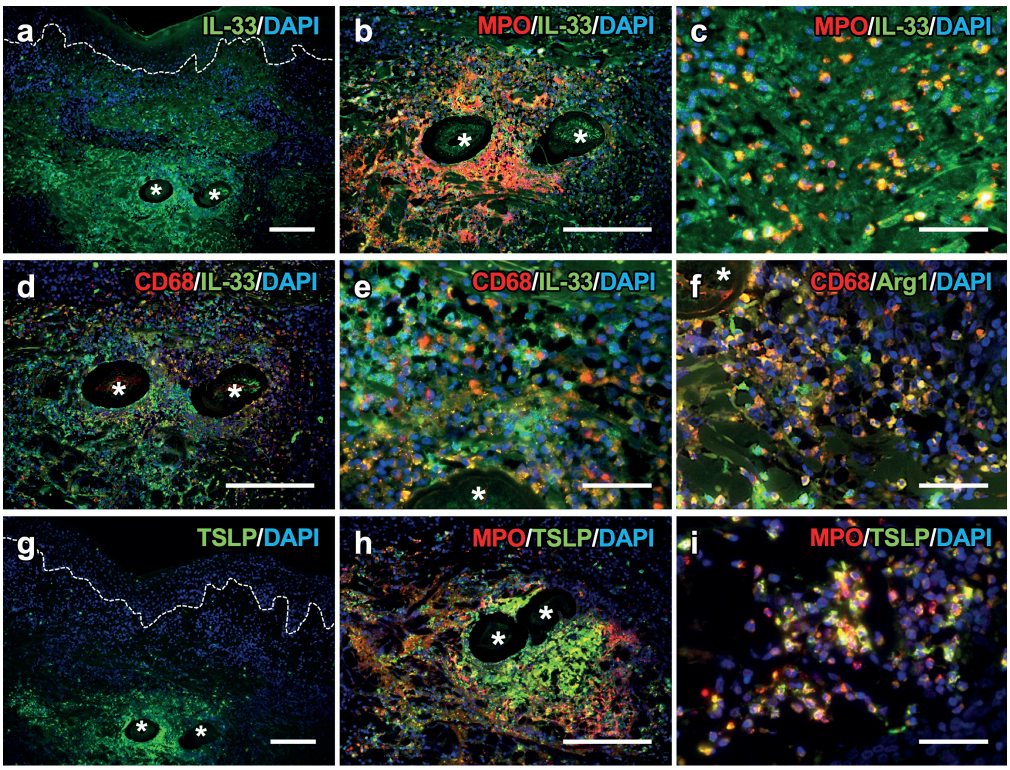

Fig. 2. Immunofluorescence staining for interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP). (a–e) IL-33 expression (*green*) in the lesion. (a) IL-33 was expressed in the infiltrated dermal cells, but only minimally in the epidermis. (b, c) Dermal infiltrating myeloperoxidase (MPO)$^+$ neutrophils (*red*) expressed IL-33. (d, e) CD68$^+$ macrophages (*red*) also expressed IL-33. (f) CD68$^+$ macrophages (*red*) expressed arginase-1 (Arg1, *green*), an M2 marker. (g–i) TSLP-expression (*green*). (g) The infiltrated dermal cells, but not the epidermis, expressed TSLP. (h, i) MPO$^+$ neutrophils (*red*) expressed TSLP. The blue areas indicate nuclei. *White dotted lines* indicate the dermo-epidermal junction. *Larvae. Bars: (c, e, f, i) 50 μm, (b, d, h) 100 μm, and (a, g) 200 μm, respectively.

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The authors have no conflicts of interest to declare.

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