

Case Report



Eosinophilic annular erythema showing eosinophil cytolytic ETosis successfully treated with benralizumab

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ABSTRACT

A 56-year-old woman presented with repeated swelling of the lips and face. She had a history of childhood asthma; she had a recurrence of asthma when she was 54 years old and was taking inhaled corticosteroids, and other antiasthma drugs. The swelling of her lips and face improved temporarily with oral corticosteroids (OCS), but recurred soon after discontinuing OCS. Her peripheral blood eosinophil count was 632/ μ L (9.3%), and her serum was negative for myeloperoxidase-anti-neutrophil cytoplasmic antibody and serine proteinase3-anti-neutrophil cytoplasmic antibody. Hematoxylin and eosin staining of her back skin revealed abundant eosinophilic infiltrate around the vascular area of the shallow dermis layer, but no evidence of vasculitis and we diagnosed her as eosinophilic annular erythema (EAE). Punctate staining of galectin-10, chromatolytic eosinophils, and net-like DNA was also evident in close proximity to the free granules, indicating extracellular vesicles and eosinophil extracellular traps (ETosis). We started daily OCS to control her asthma and skin eruption/oedema. Three months after administering daily OCS, benralizumab was initiated for withdrawal from OCS dependence and treatment of severe asthma. After initiation of benralizumab, her skin and subcutaneous tissue symptoms promptly improved. OCS was discontinued, and no edematous erythema has been observed since then. Bronchial asthma has also been well-controlled. This is the first report on the evidence of eosinophil ETosis in the dermis of EAE patients and the efficacy of benralizumab in a patient with EAE. Benralizumab may be a useful drug for treating refractory EAE with severe eosinophilic asthma.

Keywords: Eosinophil; Angioedema; Asthma; Benralizumab; ETosis

INTRODUCTION

Eosinophilic annular erythema (EAE) is a rare eosinophilic dermatosis of unknown etiology, and clinically characterized by recurrent edematous erythema [1, 2]. EAE was first reported

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Author Contributions

Conceptualization: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese. Formal analysis: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese. Investigation: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese, Sho Kanzaki, Eri Matsuki, Hidehiro Irie. Methodology: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese. Project administration: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese, Koichi Fukunaga. Writing - original draft: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese, Genta Nagao. Writing - review & editing: Katsunori Masaki, Shigeharu Ueki, Keiji Tanese, Hiroki Kabata, Jun Miyata, Ichiro Kawada.

as a pediatric disease [3, 4], but it is now known to occur in adults as well [5]. Tissue eosinophils are associated with the etiology by releasing their cytotoxic mediators [6]. However, the activation mode of eosinophils is not well understood. During the process of degranulation, a key phenomenon by which eosinophils contribute to disease, preformed contents are released via at least 3 different pathways: typical exocytosis, piecemeal degranulation, extracellular trap cell death (ETosis), or cytolytic degranulation [7]. ETosis is an active eosinophil cytolysis that releases eosinophil extracellular traps and total cellular contents. Here, we report the first case of EAE with evidence of ETosis, successfully treated with benralizumab, an anti-interleukin-5 receptor- α monoclonal antibody.

CASE REPORT

A 56-year-old woman presented with repeated swelling of the lips and face. She did not have episodes of weight gain or leg edema. She had a history of childhood asthma; she had a recurrence of asthma when she was 54 years old and was taking inhaled corticosteroids, long-acting beta-agonists, and a leukotriene receptor antagonist. Her smoking history was 10–15 cigarettes per day from the age of 20 to 50 years. The swelling of her lips and face improved temporarily with oral corticosteroids (OCS), but recurred soon after discontinuing OCS. Antihistamines had no effect on the edema. She had eosinophilic chronic rhinosinusitis but no episodes of food allergies. Her peripheral blood eosinophil, neutrophil, and lymphocyte count were 632/ μ L (9.3%), 4,148/ μ L (61.0%), and 1,850/ μ L (27.2%), respectively. Her serum was negative for myeloperoxidase-anti-neutrophil cytoplasmic antibody and serine proteinase3-anti-neutrophil cytoplasmic antibody. Her serum immunoglobulin M level was normal. Her urinalysis findings and renal function were also normal. Sputum cytology revealed the presence of eosinophils and neutrophils.

One year after the initiation of outpatient follow-up, erythematous-edematous annular plaques with central hyperpigmentation appeared from the posterior neck to the dorsal



Fig. 1. Edematous erythema appearing on the patient's back.

area with swelling of the lips and face (Fig. 1). Her peripheral eosinophil count was 840/ μ L (12.4%). Biopsy specimen was obtained from erythematous-edematous plaques on the left shoulder. Hematoxylin and eosin staining revealed abundant eosinophilic infiltrate around the vascular area of the shallow dermis layer, but no evidence of vasculitis (Fig. 2A, B). Based on these episodes and histological findings, the patient was diagnosed with EAE. To assess the activation of eosinophils in the dermis, we performed immunostaining for eosinophil-specific proteins, galectin-10, and major basic protein (MBP) (Fig. 2C, D) [8]. Double immunostaining detected cytolytic degranulation and the rupture of eosinophils, resulting in the release of intact cell-free granules. As shown in Fig. 2B and 2D (arrowheads), intact eosinophils showed cytoplasmic staining of galectin-10 and granular protein MBP. In contrast, cytolytic eosinophils showed punctate or deposition of extracellular MBP staining (arrows), indicating the presence of cell-free granules. Punctate staining of galectin-10, chromatolytic eosinophils,

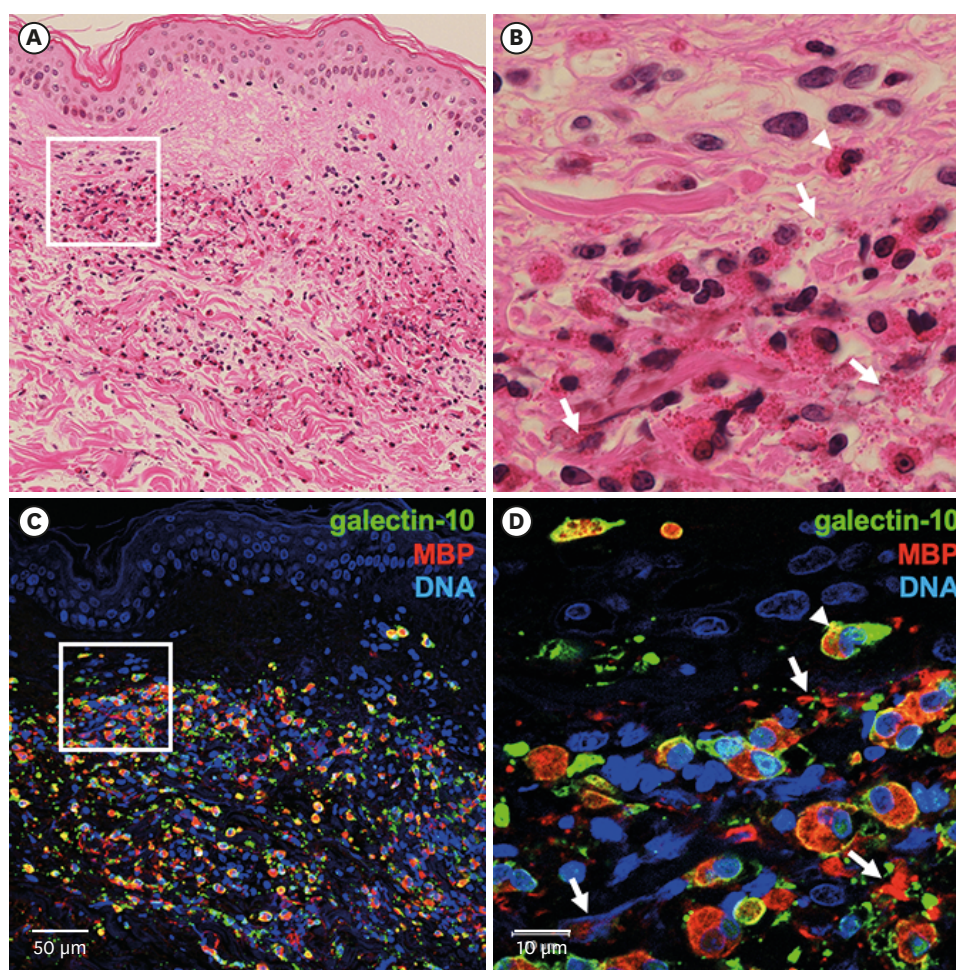


Fig. 2. Skin biopsy of the erythema. Identical sections were stained with hematoxylin and eosin (H&E) and fluorescence immunostaining. **(A)** H&E staining showed abundant eosinophilic infiltrate in the dermis layer. There was no evidence of vasculitis or flame figures. **(B)** Higher magnification of the boxed area in panel A; cell-free eosinophil granules were evident (arrows). Arrowhead indicates an intact eosinophil. **(C, D)** Immunostaining of major basic protein (MBP: red) and galectin-10 (green). The deparaffinized sample slides (2- μ m-thick sections) were incubated with 0.1% proteinase K for 6 minutes. The slides were blocked and permeabilized by 10% bovine serum albumin and Perm/Wash (BD Biosciences, San Jose, CA, USA) containing phosphate-buffered saline for 30 minutes and then incubated with primary mouse anti-galectin-10 mAb (B-F42, 1:50, Santa Cruz, CA, USA) and rabbit anti-MBP Abs (kind gift from Prof. Kita H, Mayo Clinic Arizona) for 30 minutes at 37°C. Alexa-488 conjugated Ab (goat anti-mouse IgG; Invitrogen, Carlsbad, CA, USA; 1:200) and Alexa-594 goat anti-rabbit IgG (Invitrogen) were used for secondary incubation for 30 minutes at 15°C–25°C. Control Abs and Hoechst 33342 (Invitrogen) were used for each experiment. The section was counterstained with H&E. Intact eosinophil retained galectin-10 and MBP, whereas cytolytic cells showed cell-free granules and extracellular vesicles (D, arrows). Chromatolytic and net-like DNA were also evident in close proximity to free granules. An intact eosinophil showed cytoplasmic staining of galectin-10 and granular protein MBP (D, arrowhead).

and net-like DNA was also evident in close proximity to the free granules, indicating extracellular vesicles and eosinophil extracellular traps [9], respectively.

Additional long-acting muscarinic antagonists and increased inhaled corticosteroids/long-acting beta-agonists doses (budesonide/formoterol 160/4.5 µg, from two [2 × 2] to four [4 × 2] inhalations twice a day) were administered to improve her asthma symptoms of dyspnea and sputum. Her forced expiratory volume at one second was 2.34 L (within normal limit). Even after these intensive therapies, her respiratory symptoms persisted; thus, a diagnosis of severe asthma was made. Additional daily OCS (prednisolone 20 mg/day) was effective for asthma and cutaneous symptom. We gradually tapered the dose of OCS, because interruption of OCS failed to control EAE symptoms such as annular edematous erythema, pruritic lesions with a centrifugal pattern, neck and lip swelling, and asthmatic symptoms. Three months after administering daily OCS, benralizumab was initiated for withdrawal from OCS dependence and treatment of severe asthma. Soon after initiation of benralizumab, her skin and subcutaneous tissue symptoms promptly improved. Dose and interval of benralizumab administration was 30 mg every 4 weeks for the first 3 doses, and then every 8 weeks thereafter. OCS was discontinued within 1 month, and no edematous erythema has been observed since then. Bronchial asthma has also been well-controlled.

The patient provided permission to publish the features of her case and we obtained the written informed consent for publication from her.

DISCUSSION

Diagnosis and etiology

Eosinophilic annular erythema does not usually accompany with angioedema. In our case, the skin rash was consistent with EAE, and was also complicated by elements of angioedema. Peripheral eosinophilia is usually absent in case of EAE. There was an increase in peripheral blood eosinophils because this patient had asthma and eosinophilic sinusitis. In addition, we considered the possibility of Wells syndrome (eosinophilic cellulitis), however, we diagnosed her as EAE based on the clinical course, macroscopic findings, and pathological findings.

Treatment

Patients who show refractory EAE need to take OCS, hydroxychloroquine, dapsone, doxycycline, or methotrexate to control their symptoms [10]. To prevent the side effects of these drugs, innovative treatment strategies are required. Mepolizumab, anti-IL-5 antibody, has been reported to be successful in treating EAE refractory to OCS administration [10]. Benralizumab is an anti-IL-5 receptor-α monoclonal antibody with antibody-dependent cell-mediated cytotoxicity that directly and rapidly eliminates eosinophils in circulating blood and tissues [11]. Eosinophils can be a rich source of cytotoxic mediators, growth factors, and type 2 cytokines [12]. In patients with EAE, eosinophils can primarily and directly damage the dermis in an antigen-independent manner through ETosis. Thus, it may be most effective to target eosinophils themselves as therapeutic targets, and the clinical efficacy of benralizumab in treating diseases other than asthma is promising. This is the first report on the evidence of eosinophil ETosis in the dermis of EAE patients and the efficacy of benralizumab in a patient with EAE. Benralizumab may be a useful drug for treating EAE with severe eosinophilic asthma.

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