Case report

Eosinophil ETosis and Charcot-Leyden crystals in Kimura disease

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Two cases of refractory Kimura disease that required treatment with biologic agents are reported. Their pathology suggests the involvement of eosinophil ETosis, which is active cell death producing Charcot-Leyden crystals. (J Allergy Clin Immunol Global 2025;4:100397.)

Key words: *Kimura disease, eosinophils, eosinophil ETosis, Charcot-Leyden crystal*

Abbreviations used
CLC: Charcot-Leyden crystal
EET: Eosinophil extracellular trap
EETosis: Eosinophil ETosis
KD: Kimura disease
MBP: Major basic protein

Kimura disease (KD) is a rare inflammatory disorder that affects the lymph nodes and subcutaneous tissues.[1] KD is characterized by eosinophil and histiocyte proliferation, causing nodules and mass formation in various body parts, peripheral blood eosinophilia, and elevated serum IgE levels. KD is an allergic fibromatosis in which type 2 cytokines produced by follicular T cells and activated mast cells induce eosinophil activation, forming fibrotic lesions.[2] Recently, active cell death of eosinophils, referred to as eosinophil ETosis (EETosis), was closely associated with various eosinophilic disorders. EETosis mediates rapid cytolytic cell death, releasing cell-free granules and filamentous chromatin structures called eosinophil extracellular traps (EETs), and occasionally forming Charcot-Leyden crystals (CLCs).[3] However, no studies have linked EETosis to KD pathogenesis. Herein, we report 2 cases of refractory KD that required treatment with biologic agents and whose pathology suggested EETosis involvement.

Case 1 involved an 8-year-old boy who was referred to our hospital because of the rapid growth of a subcutaneous mass with eosinophilia on his left upper arm (Fig 1, *A*), which first appeared at age 5 years. His peripheral eosinophil count was 12,455/μL and his serum IgE level was high (8,382 IU/mL). KD was diagnosed using tissue biopsy samples (Fig 1, *B*). As there was no anemia, thrombocytopenia, or presence of peripheral blood blasts indicative of hematologic malignancy, bone marrow examination was not performed. Additionally, no infections, allergies, or medications were identified as potential causes of secondary eosinophilia. After tumorectomy and administration of prednisolone (0.7 mg/kg), the patient achieved remission. However, at age 10 years, after prednisolone reduction over 16 months, the patient was evaluated for a KD relapse, which was identified through magnetic resonance imaging findings of a left ocular protrusion, polyopia, and hypertrophy of the left external rectus muscle (Fig 1, *C*). The patient was again treated with prednisolone (0.4 mg/kg) and cyclosporine (2.5 mg/kg). As the left ocular protrusion and polyopia improved, prednisolone and cyclosporine were discontinued after 12 months. At age 12 years, the patient experienced an increase in his peripheral blood eosinophil count, and at age 13 years, a mass was observed in his right elbow.

Initial treatment with mepolizumab reduced the peripheral eosinophil count from 2500/μL to approximately 200/μL; however, the mass remained unchanged, suggesting that mepolizumab had minimal impact on eosinophil infiltration or activation within KD lesions. Considering the proximity of the mass to the median nerve, a biopsy was not performed. Ten months after initiation of the mepolizumab treatment, the patient's treatment was switched to dupilumab. Magnetic resonance imaging scans obtained 1 and 3 months after commencement of dupilumab treatment showed a marked reduction in size of the subcutaneous mass. Although eosinophils were again detected in the boy's peripheral blood, the counts were elevated within the normal range.

Case 2 involved a 37-year-old man with enlarged left cervical lymph nodes and a subcutaneous mass in the posterior part of the left auricle (Fig 1, *D*) that had been present since he was approximately 20 years old. KD was diagnosed by using biopsy tissue samples; the patient had eosinophilia (3,690/μL) and intractable asthma since he was 32 years old. As in case 1, primary and secondary eosinophilia other than KD were eliminated. The enlarged left cervical lymph nodes showed resolution following administration of prednisolone (0.5 mg/kg). The prednisolone

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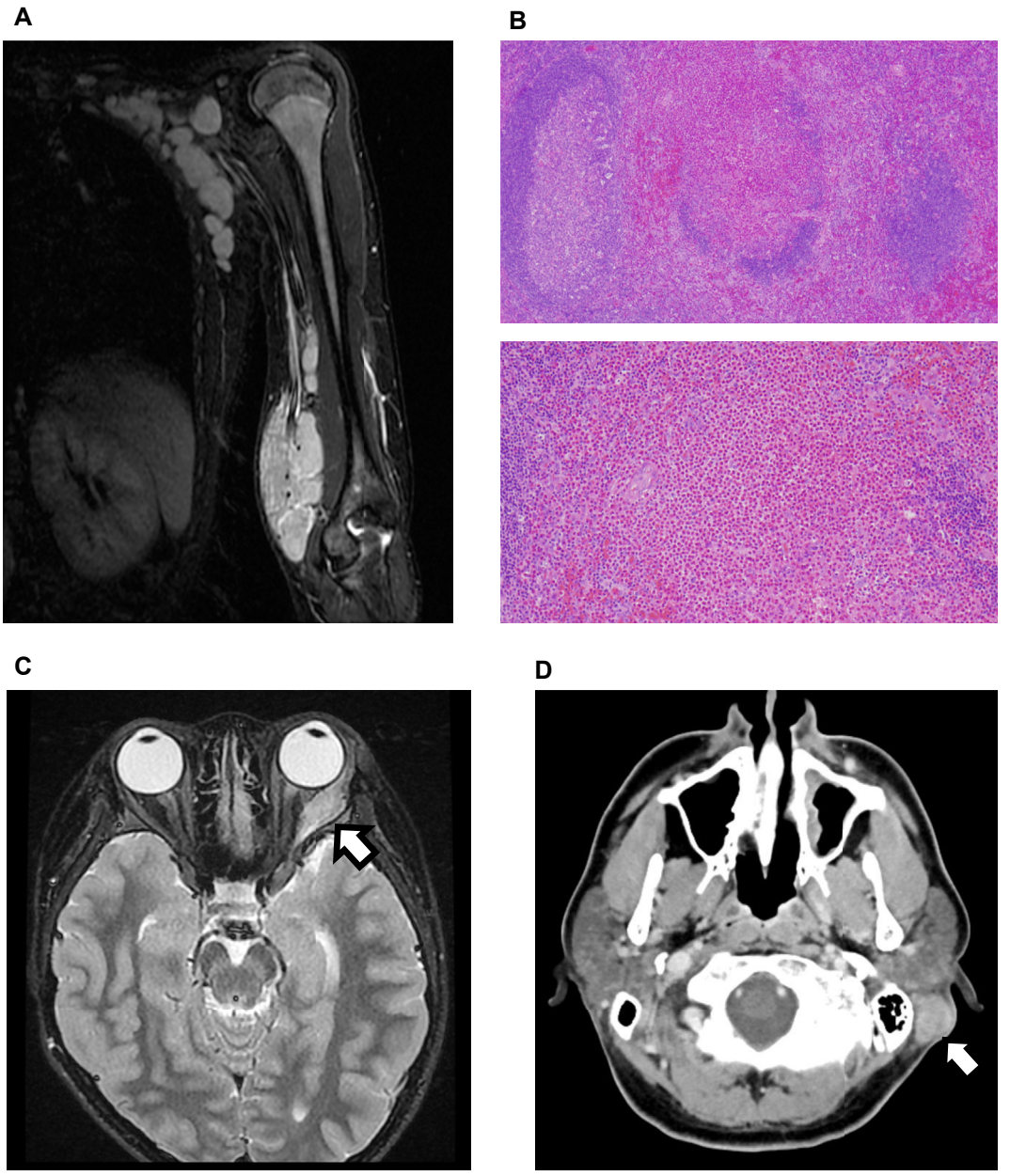

FIG 1. Image and pathologic findings of cases of Kimura disease. **A,** Coronal short TI inversion recovery magnetic resonance imaging of the left upper limb in case 1. **B,** Hematoxylin and eosin staining (*upper panel*, low magnification; *lower panel*, high magnification) of the mass in case 1. **C,** Axial short TI inversion recovery magnetic resonance imaging of the head in case 1. White arrow indicates hypertrophy of the left external rectus muscle. **D,** Axial computed tomography image of the head in case 2. White arrow indicates a subcutaneous mass in the posterior left auricle.

dosage was gradually reduced over 4 months, and although the subcutaneous mass persisted, it exhibited a size reduction. At age 38 years, the patient discontinued prednisolone after switching to mepolizumab. Further concomitant therapy with local injection of triamcinolone acetonide reduced the size of the aforesaid subcutaneous mass. Mepolizumab was discontinued when the patient was 39 years old, but it was resumed when a subcutaneous mass and enlarged cervical lymph nodes were discovered; after the mepolizumab treatment, his blood eosinophilia improved.

In both cases, a detailed pathologic evaluation of the biopsy specimens was performed at KD diagnosis. Prominent lymphoid hyperplasia and dense eosinophilic infiltration were observed in both cases. The presence of numerous lymphoid follicles with reactive germinal centers suggests an ongoing immune response to chronic inflammation in patients with KD. Eosinophils are predominantly observed in the spaces between the lymphoid follicles and surrounding tissues, including in adipose tissue. Hematoxylin and eosin staining of sections from several specimens revealed cell-free eosinophilic granules with few identifiable eosinophils. To determine whether these were cytolytic EETosis, sections were immunostained for the eosinophil granule major basic protein (MBP) and CLC-forming protein galectin-10, as previously described.[3] Intact eosinophils were stained with

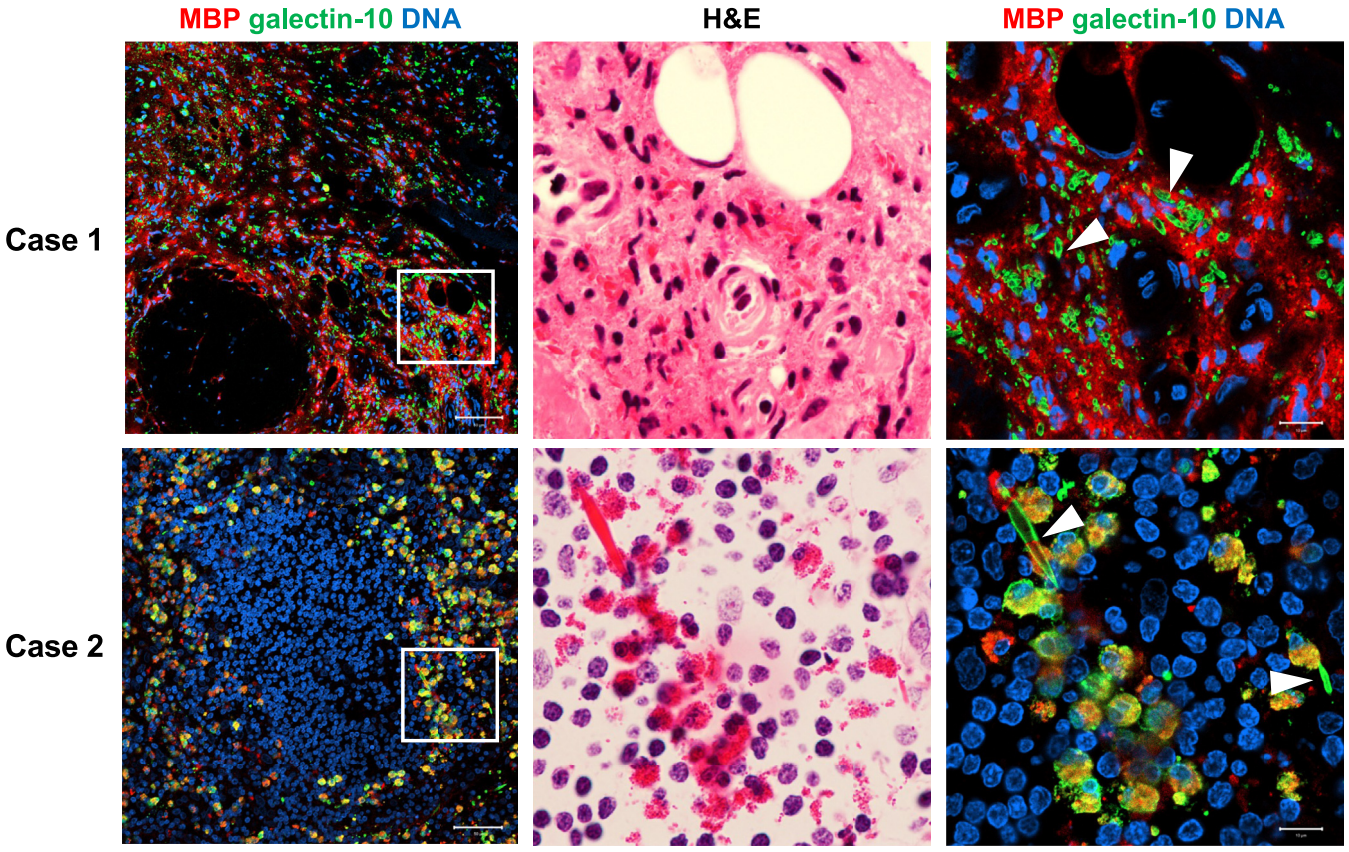

FIG 2. Cytolytic eosinophils and CLCs in the tissues. After immunofluorescence staining for galectin-10 (*green*), MBP (*red*), and DNA (*blue*), the identical sections were stained with hematoxylin and eosin (H&E). The boxed areas in the left panels (original magnification, ×200; scale bar indicates 50 μm) are shown in the middle and right panels (original magnification, ×1000; scale bar indicates 10 μm). Extracellular deposition of MBP and CLCs (*arrowheads*) was observed.

MBP and cytoplasmic galectin-10, whereas cytolysis was identified by the loss of galectin-10 and MBP presence in the tissue. Areas of dense eosinophil accumulation on hematoxylin and eosin staining were often associated with intact eosinophils that retained cytoplasmic galectin-10 on immunostaining (see Fig E1 [available in the Online Repository at www.jaci-global.org]). In contrast, eosinophilic areas were associated with cytolytic eosinophils, as evidenced by the loss of cytoplasmic galectin-10 and tissue positivity for MBP (Fig 2). Additionally, CLCs of varying sizes, occasionally forming large clusters, were observed in these areas. EETosis and CLCs were more prominently observed at the margins than at the central part of the lymph follicular structure. Because histone citrullination is essential for EET formation,[4] citrullinated histone H3 staining was performed to further investigate the presence of EETs. As shown in Fig E2 (available in the Online Repository at www.jaci-global.org), the disrupted cytolytic eosinophil nuclei stained positive for citrullinated histone H3 and DNA, indicating EET presence. Neutrophil infiltration and the presence of neutrophil extracellular traps were minimal (see Fig E3 [available in the Online Repository at www.jaci-global.org]).

EETosis releases cytotoxic granule proteins and forms CLCs, amplifying type 2 inflammation and activating inflammasomes.[5] EETs contain abundant histones that act on damage-associated molecular patterns.[6] Thus, the overproduction of type 2 cytokines at the inflammation site causes massive accumulation of eosinophils and EETosis, contributing to KD pathogenesis. In case 1, dupilumab indirectly suppressed local eosinophilic inflammation by inhibiting the IL-4 and IL-13 pathways, whereas in case 2, direct removal of eosinophils by mepolizumab may have had a positive clinical effect. The reason why mepolizumab did not have a sufficiently positive effect in case 1 is unclear, but inhibiting IL-5 did not prevent eosinophil differentiation[7] and failed to suppress eosinophilic inflammation activated by other local mediators.

To the best of our knowledge, this study is the first to identify EETosis and CLCs in patients with KD. Additional studies are necessary to elucidate the detrimental functions of EETosis and CLCs in KD and determine whether EETosis is an effective therapy for KD.

Consent for publication: Informed consent was obtained from the patients and caregivers included in this study following the principles of the Declaration of Helsinki.

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