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Letter to the Editor

Single-cell RNA sequencing of peripheral blood mononuclear cells from Kimura disease patient successfully treated with dupilumab

Dear Editor,

Kimura disease (KD) is a rare, chronic inflammatory disorder occurring predominantly in young-to middle-aged Asian males.¹ KD is characterized by recurrent subcutaneous lymphoid masses, mostly in the head and neck region, peripheral blood eosinophilia, and elevated serum IgE levels. The expression levels of interleukin (IL)-4, IL-5, and IL-13 messenger RNA were reported to be high in peripheral blood mononuclear cells (PBMC) in patients with KD.² In addition, type 2 immune cells are abundant in tissue lesions of KD.³ Based on these clinicopathologic observations, Th2 cells are thought to be important in the pathogenesis of KD; however, the etiology of KD remains elusive.

Because of its rarity, there is no consensus regarding the optimal treatment for KD. The preferred treatment for localized lesion is surgical excision and radiation therapy. Other treatment approaches for multifocal lesions include systemic immunosuppressive therapy with corticosteroids. Despite these treatment approaches, KD is often intractable. Since type 2 inflammation can be involved in the pathogenesis of KD, dupilumab, a human monoclonal antibody recognizing IL-4R α and blocking IL-4 and IL-13 signaling,⁴ could be a promising treatment. Indeed, a recent study has reported the efficacy of dupilumab for a patient with KD.⁵ However, the immunological mechanism of how dupilumab works for KD is still unknown. To dissect the complex pathologic features of KD, we performed single-cell RNA sequencing (scRNA-seq) of PBMC in a patient with KD before and after dupilumab treatment. The study procedures for sample collection and those for analyses were approved by Chiba University Ethics Committee (No. HS202101-02). The detailed materials and methods are described in the Supplementary Methods.

A 65-year-old Japanese male was referred to our hospital with a two-year history of swelling and itching in his right neck and back. At the age of 33 years, he was first diagnosed with KD based on biopsy findings and underwent surgical resection for his right subaural mass seven years after the initial diagnosis. He had other medical histories, including cardiac infarction and diabetes mellitus. On referral, he had swelling in his neck and back with tenderness and local skin redness with itching, and laboratory data showed eosinophilia (5593/mm³ [47%]) and serum IgE elevation (17817 IU/mL). His right back lesion biopsy revealed a dense inflammatory infiltrate composed of lymphocytes, plasma cells, and eosinophils. These cells exhibited a structure resembling a lymphoid follicle. Magnetic resonance imaging (MRI) revealed subcutaneous masses of the right parotid gland (Fig. 1A). Collectively, he was diagnosed with a relapse of

KD. Considering the high risk of cardiovascular disease, he was treated with dupilumab without oral corticosteroids after obtaining written informed consent. The insurer authorized this treatment upon receiving a detailed description of symptoms. We administered 600 mg of dupilumab subcutaneously as a loading dose, followed by 300 mg every two weeks. Swelling and itching in his right neck and back started to improve within the first month of dupilumab initiation. Follow-up MRI performed nine months after the initiation of treatment with dupilumab revealed a significant improvement in subcutaneous masses in his right neck (Fig. 1B). The eosinophil cell count decreased to normal level (183/mm³ [3%]), while serum IgE levels gradually decreased (4556 IU/mL) (Fig. 1C) 9 months after dupilumab initiation. No relapse was seen even 15 months after dupilumab initiation.

To explore the mechanism of action of dupilumab treatment for KD, we performed scRNA-seq of PBMC before and after dupilumab. We collected PBMC seven days before the first administration of dupilumab and 14 months after the initiation of dupilumab, respectively. Firstly, we performed scRNA-seq analysis of CD45⁺ cells. Dimensionally reduction by uniform manifold approximation and projection (UMAP) of CD45⁺ cells identified 15 distinct cell clusters composed of five major cell subtypes, including NK cells, CD4⁺ T cells, CD8⁺ T cells, B cells, and monocytes (Fig. 2A). Three subsets of CD4⁺ T cells were recognized, including memory CD4⁺ T cells (cluster 1), naïve CD4⁺ T cells (cluster 2), and GATA3⁺ CD4⁺ T cells (cluster 8). After dupilumab treatment, these three clusters decreased, especially GATA3⁺ CD4⁺ T cells (Fig. 2B). Next, we performed scRNA-seq of CD4⁺ T cells with 4 h of phorbol 12-myristate 13-acetate (PMA)/Ionomycin stimulation and found that CD4⁺ T cells were classified into 13 clusters (Fig. 2C). To identify the cluster mostly affected by dupilumab, we compared the number of differentially expressed genes (DEGs) among each cluster pre- and post-treatment. Strikingly, clusters 6 and 10 were found to be most affected by dupilumab (Fig. 2D). These two clusters were composed of GATA3⁺ Th2 cells, with cluster 6 characterized by *IL3* expression and cluster 10 characterized by *IL9* and *IL1RL1* (Supplementary Fig. 1). Notably, *IL9* expression was dramatically downregulated in cluster 10 after dupilumab treatment. Collectively, our data suggest that dupilumab might improve KD by controlling Th2 cell abundance and function.

In the present study, we found that dupilumab mainly affected Th2 cell population (Fig. 2B). This finding is consistent with a previous report demonstrating dupilumab's effects on Th2 cells in patients with atopic dermatitis.⁶ Our scRNA-seq analysis identified two clusters of Th2 cells: *IL3*^{high} clusters 6 and *IL9*^{high} cluster 10 (Fig. 2C). Seumois *et al.* have also shown the presence of Th2 cell subpopulations characterized by high expression of *IL9* and high

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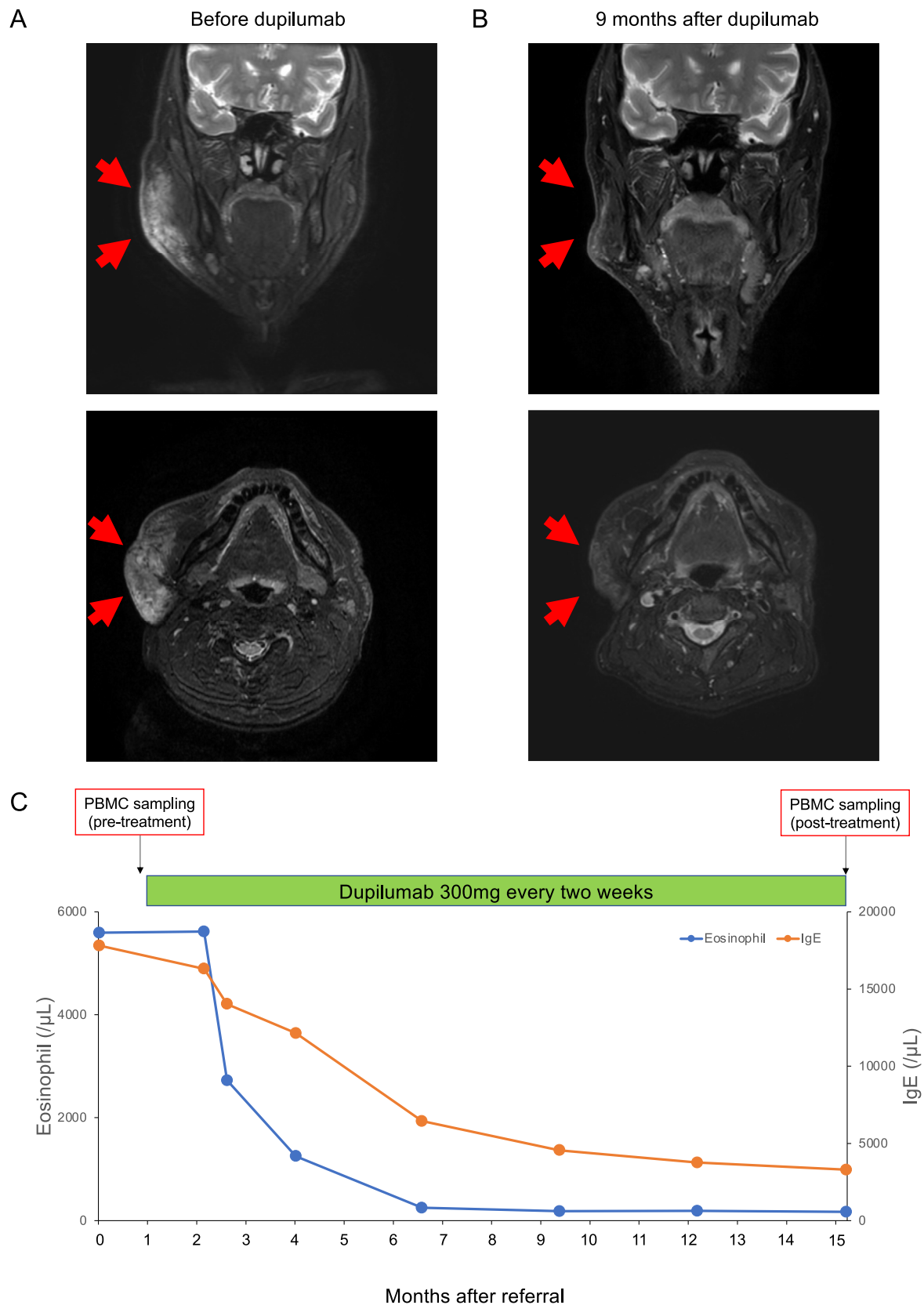


Fig. 1. Magnetic resonance imaging (MRI) and clinical course. (A, B) MRI of the head-neck before (A) and 9 months after (B) dupilumab. Upper panels show T2-weighted coronal views, and lower panels show T2-weighted axial views. Red arrows indicate subcutaneous masses of the right parotid gland. (C) Clinical course of the patient. Blue circles show the number of eosinophils, and orange circles show the levels of serum IgE. The horizontal axis represents the months after referral.

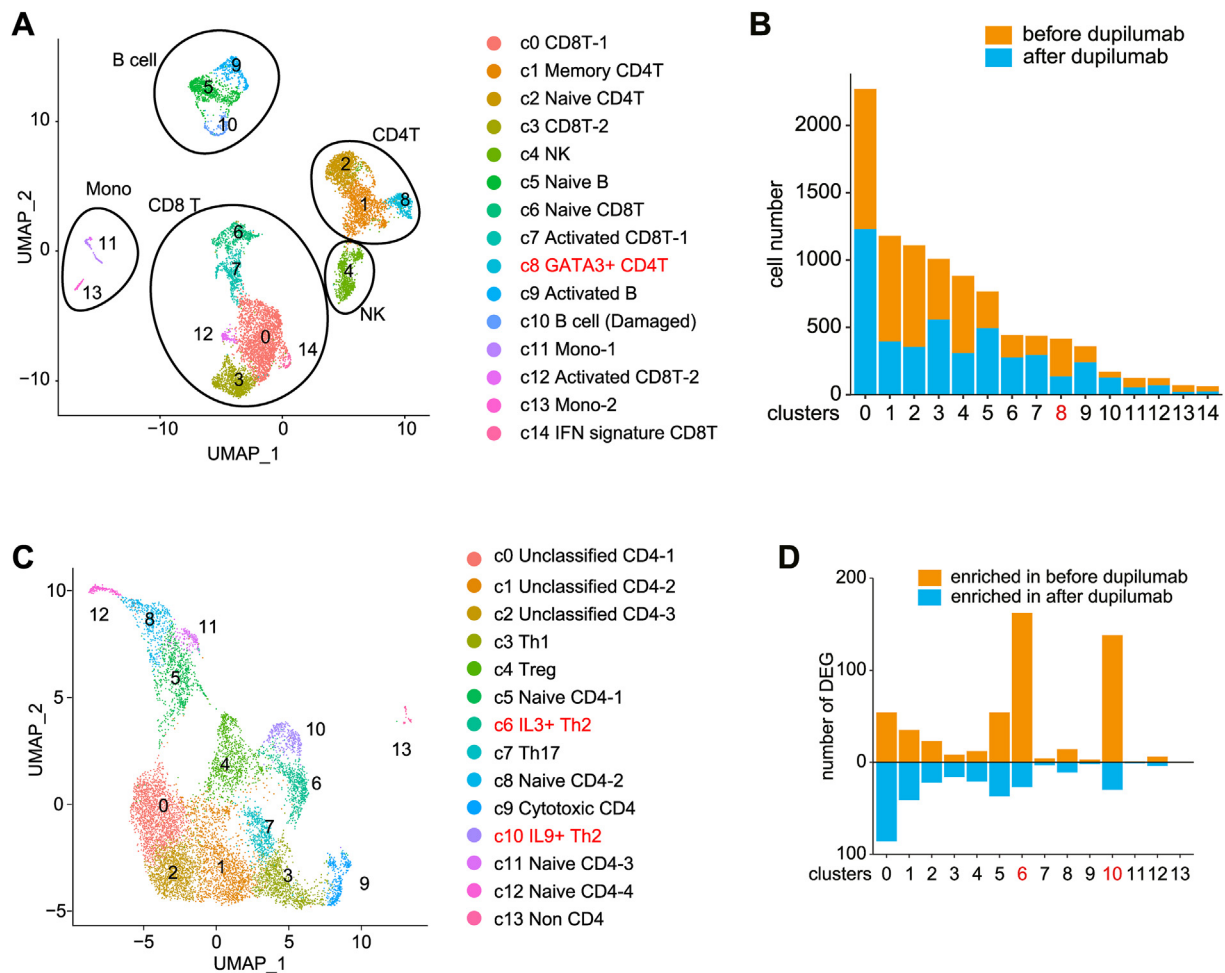


Fig. 2. scRNA-seq of CD45⁺ leukocytes and CD4⁺ T cells. **(A, B)** scRNA-seq of CD45⁺ leukocytes before and after dupilumab. **(A)** UMAP plot shows the clusters of CD45⁺ leukocytes. **(B)** The bar graph represents the cell numbers of each cluster. The subset of GATA3⁺ CD4⁺ T cells (cluster 8) was decreased after dupilumab. **(C, D)** scRNA-seq of CD4⁺ T cells before and after dupilumab. **(C)** UMAP plot shows CD4⁺ T cell subsets. **(D)** The bar graph represents the number of DEGs in each cluster. Th2 subsets (clusters 6 and 10) were dramatically decreased after dupilumab.

expression of *IL3* in asthma patients.⁷ Cluster 10, characterized by higher expression of *ILRL1* and *IL5*, showed similar characteristics to pathogenic Th2 cells.⁸ Notably, *IL9* in cluster 10 was drastically decreased after dupilumab treatment. It is well known that IL-9 enhances mast cell proliferation and survival. IL-9 has also been shown to promote germinal center development of memory B cells.⁹ Moreover, it has recently been shown that IgE-producing B cells and IgE⁺-activated mast cells are abundant in affected tissues of KD patients.³ Although there is no report analyzing the involvement of IL-9 in the pathogenesis of KD, these findings raise the possibility that IL-9 is involved in the pathogenesis of KD and that the downregulation of IL-9 might be involved in improving the symptoms by dupilumab treatment. The involvement of IL-9 in the pathogenesis of KD might explain the difference in the pathophysiology between KD and allergic diseases such as asthma.

The analysis of PBMC in this study showed the importance of Th2 cells; however, in mass lesions, not only Th2 cells but also follicular helper CD4⁺ T cells (Tfh cells) are presumed to be involved.³ Thus, analyzing affected tissues in detail may elucidate the relationship between Tfh cells and B cells. Further studies are required to analyze the mechanisms of dupilumab on other types of immune cells, including B cells and granulocytes, and non-hematopoietic cells, including fibroblasts, in the affected tissues.

In conclusion, we report a case of KD successfully treated with dupilumab. To our knowledge, this is the first report performing scRNA-seq of PBMC from a patient with KD treated with dupilumab. Dupilumab might be a promising therapeutic option for KD by controlling Th2 cell abundance and cytokine production, including IL-9.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2023.06.002>.

Conflict of interest

The authors have no conflict of interest to declare.

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