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Letter to the Editor

Two cases of dupilumab-associated eosinophilic pneumonia in asthma with eosinophilic chronic rhinosinusitis: IL-5-driven pathology?

Dear Editor,

Dupilumab is a monoclonal antibody that acts against the human interleukin (IL)-4/13 receptor. It specifically binds to the IL-4 receptor alpha subunit, which is common to both the IL-4 and IL-13 receptor complexes, and inhibits IL-4 and IL-13 signaling, thereby broadly suppressing type 2 inflammatory responses. It is expected to be clinically effective against treatment-resistant bronchial asthma and chronic rhinosinusitis in patients with nasal polyps.^{1,2} According to clinical trials, the transient hypereosinophilia typically returns to baseline after approximately six months of use without eosinophilia-associated symptoms.¹ Meanwhile, there have been reports of eosinophilic pneumonia and vasculitis, associated with dupilumab, in some patients.^{3–5} We report two cases of dupilumab-associated eosinophilic pneumonia with changes in serum cytokine levels and histological studies.

The clinical background and parameter changes of the two cases are shown in the Table. The patient in case 1 was a 37-year-old female, a never smoker who was diagnosed with asthma and eosinophil chronic rhinosinusitis (ECRS) in her 30s. Despite receiving a high dose of inhaled corticosteroid (ICS), long-acting β agonist (LABA), and a long-acting muscarinic receptor antagonist, she experienced several episodes of asthma exacerbation and nasal obstruction, that required oral prednisolone (PSL). Two years before the dupilumab induction, she also used benralizumab (anti-interleukin-5 receptor α chain monoclonal antibody). However, this was terminated after about six months because the ECRS symptoms remained uncontrolled. After dupilumab initiation, the patient's Asthma Control Test score improved from 20 to 23. In addition to the improvement of her asthma symptoms, the patient's ECRS symptoms significantly improved. The Sinus Nasal Outcome Test score improved from 28 to 3 after dupilumab initiation. The nasal polyp and Lund-Markay scores also decreased. Five months after the start of dupilumab treatment, the patient developed dry cough, dyspnea, and low-grade fever. The blood eosinophil count was markedly increased (268 cells to 9070 cells/ μ L) (Table 1). Chest computed tomography (CT) showed infiltrative shadows in the mantle region of the left and right upper lung lobes (Fig. 1 Case 1A). Transbronchial lung biopsy showed thickening of the alveolar septa and infiltration of inflammatory cells, mainly eosinophils, confirming the diagnosis of eosinophilic pneumonia (Fig. 1 Case 1Bi, ii). The biopsy samples underwent immunostaining for eosinophil-specific proteins, cytoplasmic protein galectin-10, and granular protein major basic protein (MBP).⁶ Extracellular

deposition of MBP, net-like DNA, and galectin-10-stained Charcot-Leyden crystals were observed (Fig. 1 Case 1Ci, ii). Upon confirming the diagnosis, PSL treatment (30 mg/day) was initiated, and the patient's eosinophilia and abnormal shadow were alleviated.

The patient in case 2 was a 40-year-old woman, a former smoker who was diagnosed with asthma and ECRS in her 30s. The patient had been taking oral steroids (PSL 5 mg/day), ICS/LABA, and nasal steroids for the control of asthma and nasal symptoms. She was given dupilumab to improve her asthma, nasal obstruction, nasal discharge, and olfactory disturbance. The symptoms improved quickly, and the oral steroids were discontinued two months after the start of dupilumab. Three months after

Table 1

Clinical characteristics and changes in parameters before and after dupilumab treatment.

	Patient 1	Patient 2		
Age (yr)	37	40		
Sex (F/M)	F	F		
BMI (kg/m ²)	19.0	20.2		
Smoking	Never	Ex (15-pack year)		
Atopic	Yes	Yes		
Allergic rhinitis	Seasonal and perennial	Seasonal		
Asthma exacerbation (n/yr)	4	0		
FEV ₁ (% pred)	99.9	105.6		
ICS (μ g/d, fluticasone equivalent)	800	1000		
OCS (mg/d)	0	PSL 5 mg		
Endoscopic sinus surgery	No	Yes		
JESREC score	15	17		
Eosinophil number in NPs (/HPF)	100	80		
	Pre	Post	Pre	Post
ACT	20	23	25	25
SNOT-22	28	3	28	6
NPS	2	0	5	1
LMS	14	0	21	6
Blood eosinophil (/ μ L)	276	9040	520	5150
FeNO (ppb)	165	32	212	30
Total IgE (IU/L)	674	147	369	172
Serum IL-5 (pg/mL)	0.7	84.9	ND	31.5
Serum CCL26 (pg/mL)	9.4	ND	26	11.5
Serum periostin (ng/mL)	452.4	237	296.8	49.8

yr, years; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; OCS, oral corticosteroid; PSL, prednisolone; JESREC, Japanese Epidemiologic Survey of Refractory Eosinophilic Chronic Rhinosinusitis; NPs, nasal polyps; HPF, high power field; Pre, pretreatment; Post, posttreatment of dupilumab; ACT, Asthma Control Test; SNOT-22, Sino Nasal Outcome Test-22; NPS, nasal polyp score; LMS, Lund-Markay Score; FeNO, fraction of exhaled nitric oxide; IgE, immunoglobulin E; IL-5, interleukin 5; CCL26, CC motif chemokine ligand 26; ppb, parts per billion; ND, not detectable.

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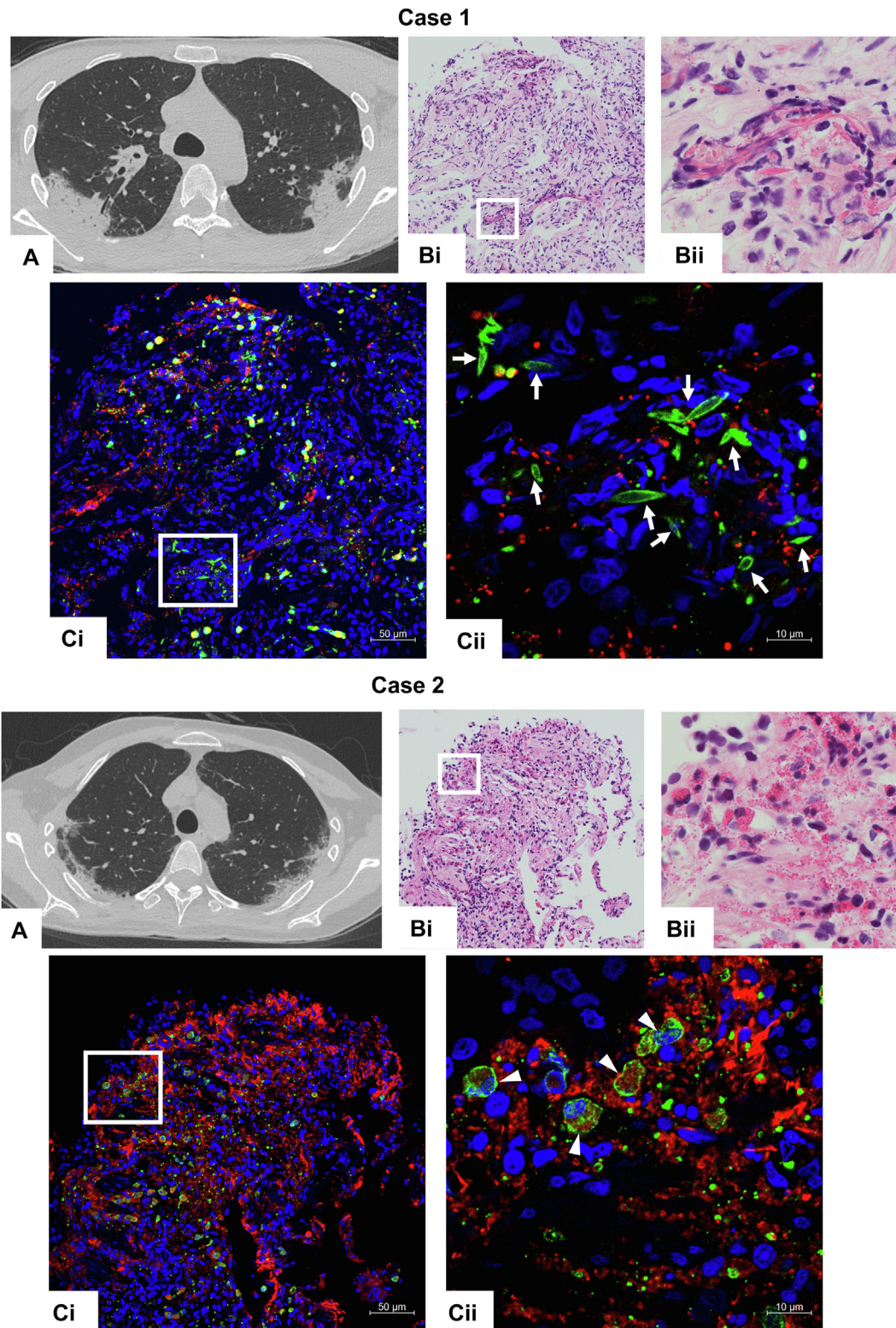


Fig. 1. Case 1 and 2 (A): Lung computed tomography images of the patients at the onset of eosinophilic pneumonia. **(Bi and Bii):** Lung histopathologic findings of the patients at the onset of eosinophilic pneumonia (original magnification, $\times 100$ and $\times 400$ [insets]). **(Ci and Cii):** Identical sections of lung histology of patients were stained for galectin-10 (green), major basic protein (MBP) (red), and DNA (blue). These were assessed using confocal microscopy. Charcot-Leyden crystals (arrows) and intact eosinophils (arrowheads) were observed in focal areas. Extracellular deposition of MBP, punctate galectin-10 staining, net-like DNA, cytolytic/chromatolytic eosinophils were also evident.

the start of dupilumab treatment, the patient developed dyspnea, chest pain, and high fever. The peripheral blood eosinophils were markedly increased (520 cells to 5150 cells/ μ L), and a chest CT scan showed mottled and infiltrative shadows in the upper lobe of both lungs (Fig. 1 Case 2A). The lung biopsy revealed a large amount of cytolytic eosinophils and free extracellular eosinophil granules in the alveolar walls, peribronchial interstitium, and intravascular spaces, leading to the diagnosis of eosinophilic pneumonia (Fig. 1 Case 2Bi, ii). Immunostaining revealed a small number of intact eosinophils (cytoplasmic galectin-10-stained cells) with marked extracellular deposition of MBP (Fig. 1 Case 2Bii, Ci, Cii). The patient's pneumonia was successfully treated with moderate doses of oral corticosteroids (PSL 30 mg/day).

One possible mechanism of action of dupilumab is reducing the expression of vascular cell adhesion protein 1 (VCAM-1), eotaxin, and periostin, which are strongly involved in the IL-4 and 13 pathways.⁷ Eosinophils that cannot infiltrate into the tissues, may be retained in the bloodstream with dupilumab administration, resulting in a temporary increase in blood eosinophils.^{1,8} To determine the mechanism of eosinophilia and eosinophilic pneumonia, we measured the serum cytokine levels. In both cases as previously reported,¹ CCL26 (eotaxin-3) and periostin significantly decreased after dupilumab administration. Meanwhile, the levels of IL-5, an eosinophil-activating cytokine, markedly increased (Table 1). The IL-5 levels in other eosinophilic pneumonia-free patients treated with dupilumab at our hospital remained almost constant (Supplementary Fig. 1). These results suggested that activated IL-5-producing cells caused blood eosinophilia and eosinophilic pneumonia.

Activated eosinophils undergo non-apoptotic cell death, namely eosinophil extracellular trap cell death (EETosis), which mediates Charcot-Leyden crystal formation and extracellular net-like chromatin structure (eosinophil extracellular traps).⁹ Histological analysis of eosinophilic pneumonia in the current cases revealed the typical signs of EETosis, including cytolysis, extracellular deposition of MBP, cell-free granules, net-like DNA, punctate staining of galectin-10 (extracellular vesicles), and Charcot-Leyden crystals.⁶ Despite this, dupilumab was biologically effective due to the decreased serum levels of CCL26 and periostin. Moreover, clinical improvement of asthma and obvious resolution of the nasal polyps in the sinus CT scan (data not shown) were observed in both patients. Interestingly, the histological findings of sinus polyps prior to dupilumab administration showed little evidence of EETosis in both cases (Supplementary Fig. 2). The difference in eosinophilic inflammation between the airways and lung parenchyma suggested a different mechanism of eosinophil infiltration, such as the expression of VCAM-1 and CCL26, or the distribution of IL-5-producing cells, specifically, T helper type 2 lymphocytes and group 2 innate lymphoid cells. Other possible explanations include the induction of VCAM-1 expression by pathways other than IL-4/13 and transmigration by eosinophil adhesion mechanisms other than VCAM-1. However, further study is required to clarify this hypothesis. Dupilumab is well-tolerated, but serious adverse events such as eosinophilic pneumonia can occur in rare cases. Measurement of serum cytokines in early phase after induction of dupilumab may be useful in predicting the onset of such events.

Finally, in both cases, anti-neutrophil cytoplasmic antibodies were negative, and there were no obvious signs of vasculitis; however, the possibility of eosinophilic pneumonia as a prodrome of vasculitis, including eosinophilic granulomatosis with polyangiitis, cannot be ruled out. Attention should be paid to the process of steroid tapering for any changes.

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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.2022.03.005>.

Conflict of interest

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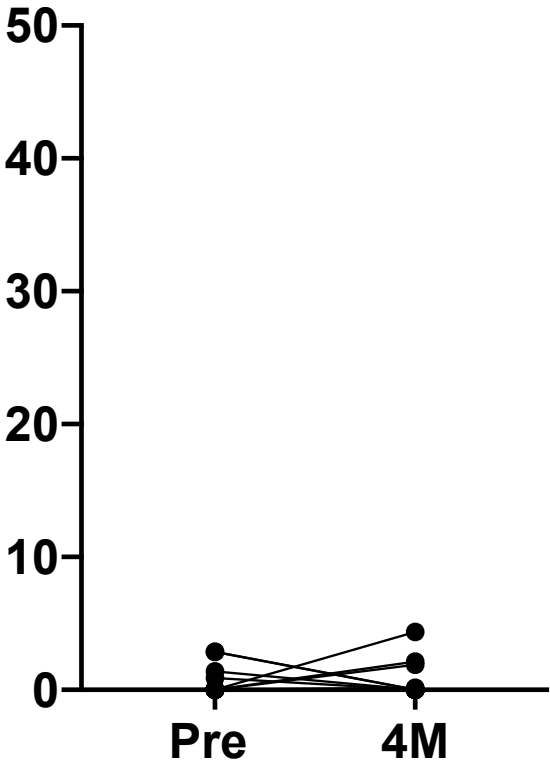
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Supplementary figure 1

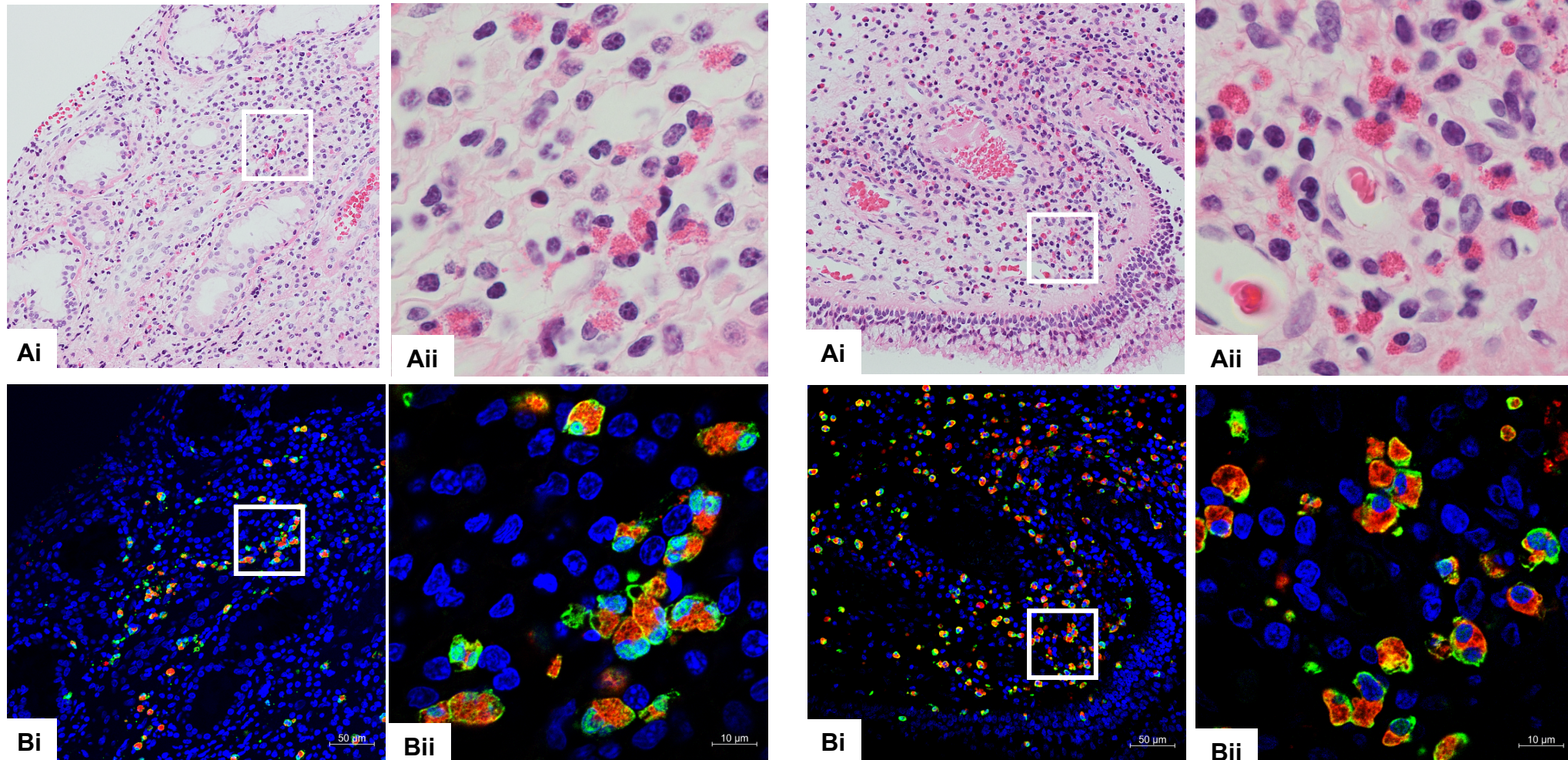


Supplementary figure 1: Serum interleukin-5 levels of the asthma patients treated with dupilumab, without eosinophilic pneumonia as an adverse event.
Pre: before dupilumab initiation. 4M: 4 months after dupilumab initiation.

Supplementary figure 2

Case 1 Nasal polyp

Case 2 Nasal polyp



Supplementary figure 2. Case 1 and 2 (Ai and Aii): Nasal polyp histopathological findings of patients before dupilumab initiation (original magnification, x100 and x400 [insets]). (Bi and Bii): Identical sections of nasal polyp histology of patients who were stained for galectin-10 (green), major basic protein (MBP) (red), and DNA (blue) and assessed using confocal microscopy. Most eosinophils were stained with cytoplasmic galectin-10 and MBP, indicating intact eosinophils.