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Blockade of IL-4Rα inhibits group 2 innate lymphoid cell responses in asthma patients

Gargi Patel[a,*], **Jiexue Pan**[b,*], **Longyun Ye**[b], **Xiaofei Shen**[b], **Daniel Rosloff**[a], **Shanti S. D'Souza**[b], **Ivan Ting Hin Fung**[b], **Jocylen Celstin**[a], **Wei Sun**[b], **Poornima Sankar**[b], **Yuanyue Zhang**[b], **Muhammad Asghar Pasha**[a,#], **Qi Yang**[a,b,#]

[a]Division of Allergy/Immunology, Department of Medicine, Albany Medical College, Albany, NY 12203

[b]Department of Immunology and Microbial Disease, Albany Medical College, Albany, NY 12208

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To the Editor:

Asthma is a complicated chronic airway inflammatory disorder. In addition to standard steroid treatment, biologic therapies targeting specific cytokines have emerged to reduce exacerbation and improve lung function. Neutralizing antibodies against IL-5, a critical growth factor for eosinophils, reduced asthma exacerbation in patients with severe eosinophilic asthma.[1] Blockade of IL4Rα, the receptor to IL-4 and IL-13, also lowered exacerbation rates and improved lung function in moderate to severe uncontrolled asthma.[2] While these biologic therapies are now used as add-on maintenance treatment of moderate to severe asthma, the effects of these biologic therapies on human immune responses are yet to be better understood.

Recent work from us and others have indicated critical roles for group-2 innate lymphoid cells (ILC2), a type of lung-resident innate effector cells, in asthma pathogenesis.[3–5] ILC2 may mediate airway inflammation and hyperresponsiveness through production of IL-5, IL-13 and VEGFA.[5, 6] They may also enhance Th2 responses by expression of MHCII and co-stimulating factors.[7] How human ILC2 may respond to biologic therapies remains

[#]Correspondence to: Qi Yang, PhD, MD., Department of Immunology & Microbial Diseases, Albany Medical College, 47 New Scotland Ave, MC-151, Albany, NY 12208. Phone: 518-264-2583, Fax: 518-262-5748, yangq@amc.edu, Muhammad Asghar Pasha, MD., Division of Allergy/Immunology, Department of Medicine, Albany Medical College, Albany, NY 12203, Phone: 518-264-2510, Fax: 518-264-2520, pasham@amc.edu.

[*]These authors contributed equally to this work

AUTHOR CONTRIBUTION

GP, MAP, JP, and QY designed the study and wrote the manuscript. GP and JP performed most of the experiments. LY, XS, SDD, ITF, JC, WS, PS, YZ helped perform some experiments, and edited the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no relevant conflicts of interest.

Data Availability

Data and protocols are available upon request from authors.

unclear. A recent study demonstrates that human ILC2 do not express IL-5R, indicating that human ILC2 are unlikely to directly respond to anti-IL-5 therapy.[8] Nevertheless, previous work with mouse models suggest that IL-4 might promote cytokine production activity of mouse ILC2, through direct interaction with IL-4Rα.[9] However, whether and how human ILC2 may respond to IL-4 remain unknown.

We hypothesize that IL-4/IL-4Rα signaling may promote human ILC2 response through both autocrine and endocrine regulation, and that blockade of IL-4Rα by dupilumab can repress pathogenic ILC2 responses in asthma patients.

To examine human ILC2 function, we isolated human blood ILC2 by fluorescence activated cell sorting (FACS), and cultured them *in vitro* in the presence of IL-2, IL-7, IL-25 and IL-33. Human ILC2 were identified as $CD45^+Lin^-IL7Ra^+CRTH2^+$ cells as previously described (Figure 1A).[5] 5×10^3 sorted ILC2 were cultured in 200 μl of alpha-MEDM medium supplemented with 20% FCS and 20ng/ml IL-2, IL-7, IL-25 and IL-33 in round bottom 96-well plates. Human ILC2 produced a large amount of IL-5 and IL-13, and also intermediate levels of IL-4 *in vitro* (Figure 1B). We next sought to understand whether IL-4 and/or IL-13 may control human ILC2 activity through autocrine regulation. We used neutralizing antibodies to block IL-4 and/or IL-13 activity in human ILC2 culture. Blockade of IL-4 signaling significantly repressed proliferation of human ILC2 *in vitro* (Figure 1C). ELISA revealed that the production of IL-13 by human ILC2 were also greatly reduced with IL-4 blockade (Figure 1D). Thus, IL-4 may promote human ILC2 proliferation and activation through autocrine regulation. IL-13 also acts through IL-4Rα signaling. However, neutralization of IL-13 did not affect the proliferation or the cytokine production of cultured human ILC2 (Figure 1C, 1E, 1F). Combined treatment with anti-IL-4 and anti-IL-13 repressed proliferation and IL-5 production of ILC2, to a similar degree as anti-IL-4 treatment alone (Figure 1C, 1F). In addition, treatment with anti-IL-4Rα had similar effects on repressing ILC2 proliferation and cytokine production as treatment with anti-IL-4 alone (Figure 1C, 1F). These data collectively indicate that IL-4, but not IL-13, may enhance ILC2 function through autocrine regulation.

Of note, several other cell types, such as Th2 cells and mast cells are also strong producers of IL-4 and therefore may enhance ILC2 through endocrine or paracrine regulation. To determine whether exogenous IL-4 may further enhance ILC2 activity, we added IL-4 to ILC2 culture. Indeed, the addition of exogenous IL-4 further enhanced the production of both IL-5 and IL-13 in ILC2 culture (Figure 1G, 1H). Thus, IL-4 might enhance ILC2 function through both autocrine and endocrine regulation.

Interestingly, although IL-13 and IL-4 both signal through IL-4Rα and Stat6 pathway, our results indicate that IL-4 but not IL-13 significantly promotes human ILC2 proliferation and cytokine production. Of note, IL-4 signaling additionally requires IL-2Rγ, whereas IL-13 signaling requires IL-13R. The predominant expression of IL-2Rγ in ILC2 might explain their preferential response to IL-4.

We next tested the hypothesis whether treatment with dupilumab, an IL-4Rα antagonist, may repress ILC2 responses in asthma patients. We recruited asthma patients with or

without dupilumab treatment and healthy controls from the Department of Allergy and Immunology of Albany Medical College (Table S1). Patients with diverse disease severity of asthma were recruited (Table S1). The disease severity is classed based on 2014 ERS/ATS guideline of severe asthma.[10] We sampled peripheral blood from each subject and examined ILC2 cellularity by flow cytometry analysis. Compared to healthy controls, asthma patients without dupilumab treatment had increased average number of ILC2 in peripheral blood, although the difference was not statistically significant (Figure 2A). Notably, dupilumab treatment significantly and consistently reduced ILC2 numbers in asthma patients (Figure 2A, 2B). Indeed, ILC2 numbers in the blood of all five asthma patients with dupilumab treatments were below the average number of ILC2 in asthma patients without dupilumab treatment, demonstrating the potency of anti-IL-4Rα treatment in repressing ILC2 responses in asthma (Figure 2A). We next sorted ILC2 and examined their expression of IL5 and IL13 by qPCR. The expression of both IL5 and IL13 was significantly reduced in asthma patients with dupilumab treatment compared to those without dupilumab treatment (Fig 2C, 2D). Together, these data provided direct evidence that pathogenic ILC2 can be targeted by biological therapy with anti-IL-4Rα antagonists.

In summary, here we describe a critical role for IL-4/IL4-Rα signaling in promoting ILC2 responses in human asthma. We show IL4 promotes human ILC2 proliferation and function. We demonstrate that blockade of IL-4Rα by dupilumab effectively suppresses ILC2 response in asthma patients. We speculate that an IL-4-driven positive feedback loop may be formed among ILC2 and other IL-4 producing cells such as Th2 cells, which might underlie the development and exacerbation of asthma. In future efforts, it would be worthwhile to investigate the association between steroid insensitivity and ILC2 responses in patients with anti-IL-4Rα therapies. Together, our results have provided evidence for the interaction between adaptive and innate type-2 immunity in human asthma and has uncovered a novel mechanism by which IL4Rα antagonists may exert anti-inflammatory effects in asthma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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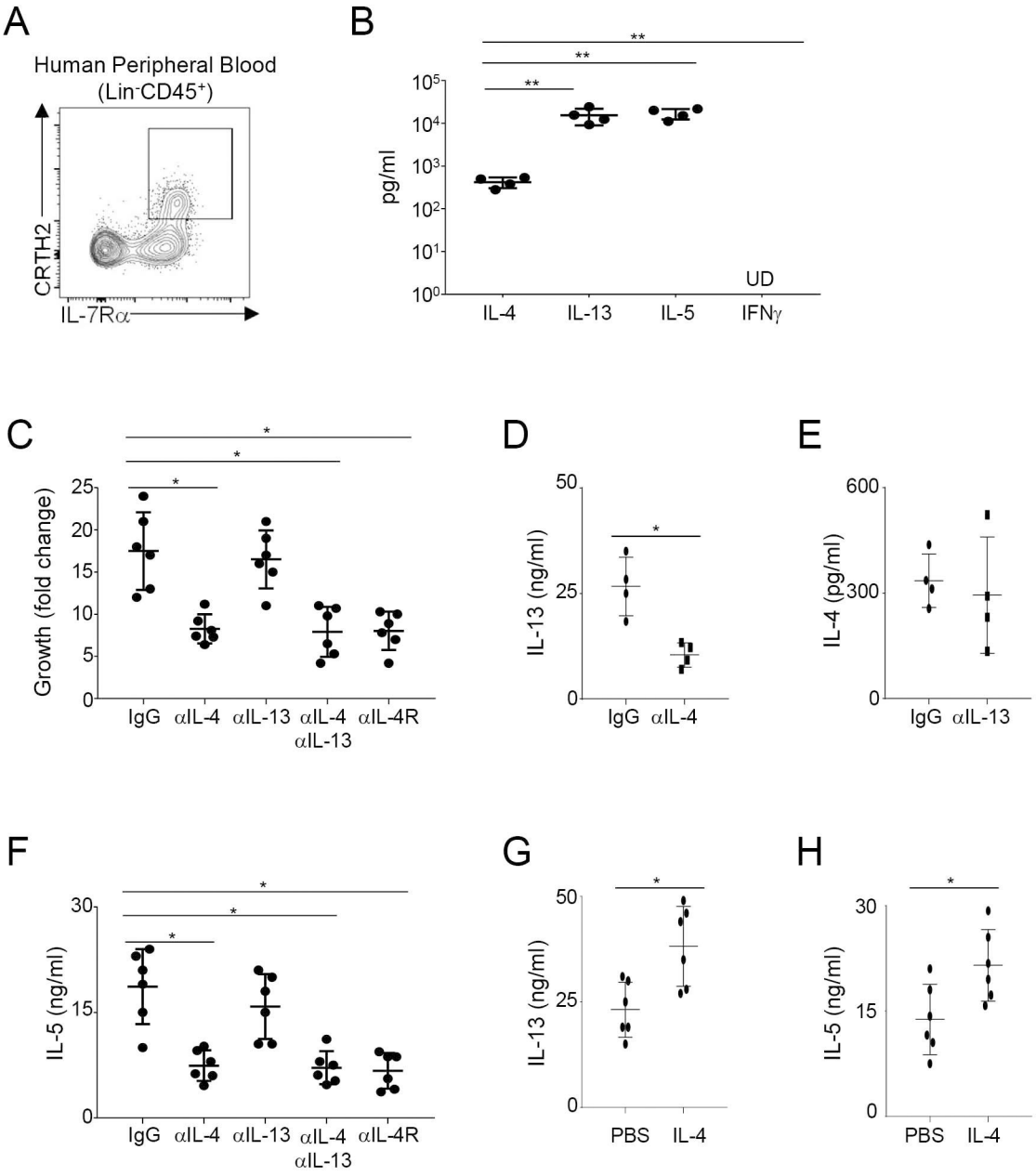

Figure 1. IL-4/IL4-Rα signaling enhances human ILC2 function.

(A) Representative flow cytometry profile of human ILC2 in peripheral blood (PB). Plots were pre-gated on $Lin^{-}CD45^{+}$ cells. Human ILC2 were identified as $CD45^{+}Lin^{-}IL7R^{+}CRTH2^{+}$ cells. (B) 5000 sorted human PB ILC2 were cultured with 20ng/ml of IL-2, IL-25, IL-7 and IL-33 for 5 days. Concentrations of IL-4, IL-13, IL-5 and IFNγ in the culture supernatant were measured after 5 days of culture. (C) Sorted human PB ILC2 were cultured with IL-2, IL-25, IL-7 and IL-33, together with the indicated neutralizing antibodies or isotype control for 5 days. Growth rates of ILC2 over 5 days of culture were shown. (D) Sorted human PB ILC2 were cultured for 5 days with IL-2, IL-25, IL-7 and IL-33, in the presence of anti-IL-4 neutralizing antibody or isotype control. IL-13 concentrations in the culture supernatant were measured. (E) Sorted human PB ILC2 were cultured for 5 days

with IL-2, IL-25, IL-7 and IL-33, in the presence of anti-IL-13 neutralizing antibody or isotype control. IL-4 concentrations in the culture supernatant were measured. (F) Sorted human PB ILC2 were cultured for 5 days with IL-2, IL-25, IL-7 and IL-33, in the presence of the indicated neutralizing antibodies or isotype control. IL-5 concentration in the culture supernatant was measured. (G) Sorted human PB ILC2 were cultured for 5 days with IL-2, IL-25, IL-7 and IL-33, in the presence or absence of IL-4. IL-13 concentration in the culture supernatant was measured. (H) Sorted human PB ILC2 were cultured for 5 days with IL-2, IL-25, IL-7 and IL-33, in the presence or absence of IL-4. IL-5 concentration in the culture supernatant was measured. Data are from 4 donors per group (B, D, E) or 6 donors per group (C, F, G, H); representative of two independent experiments (B, C, E-H) or four independent experiments (D). UD, undetectable; *$p<0.05$; Mann-Whitney test (B, D, E, G, H) or Kruskal-Wallis test (C, F).

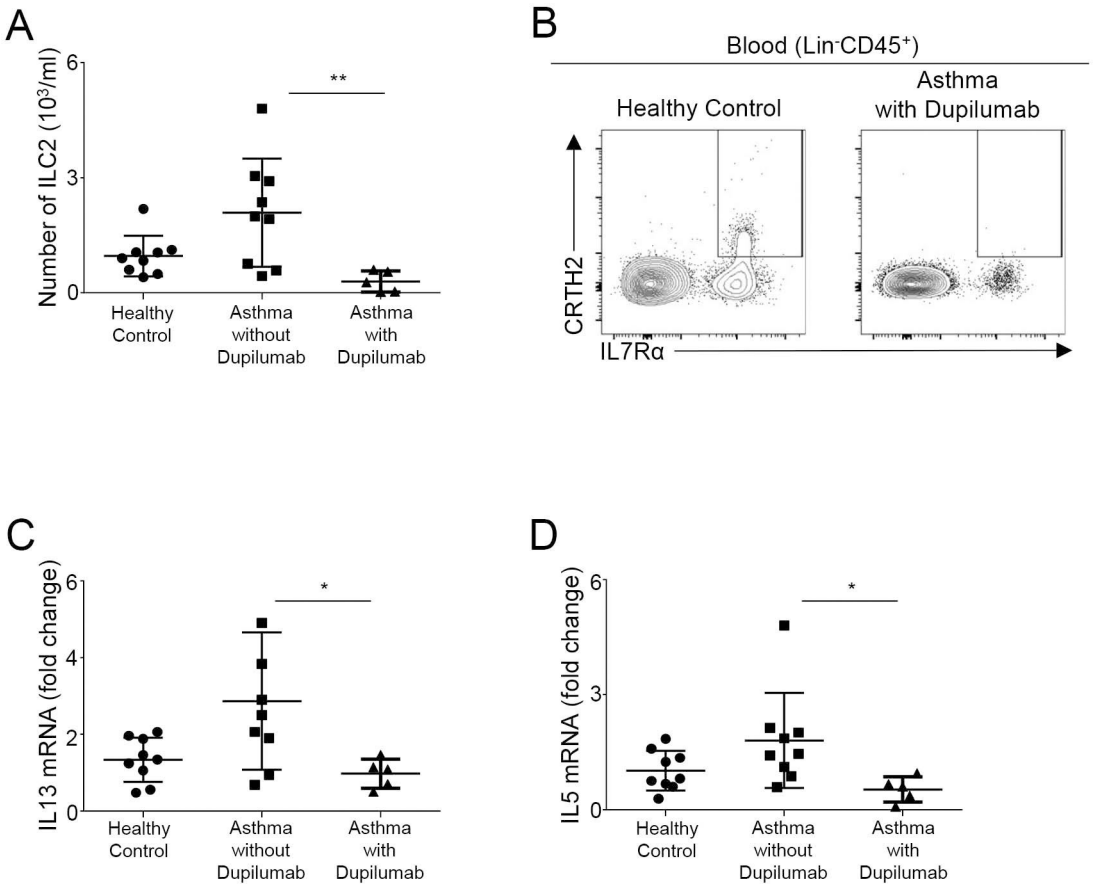

Figure 2. Blockade of IL4-Rα by dupilumab inhibits ILC2 activity in asthma patients.

(A) Peripheral blood ILC2 numbers among healthy controls and asthma patients with or without dupilumab treatment. (B) Representative flow cytometry profile of human ILC2 in healthy controls and asthma patients with dupilumab treatment. (C) IL13 mRNA levels in sorted peripheral blood ILC2 from healthy individuals or asthma patients with or without dupilumab treatment. (D) IL5 mRNA levels in sorted peripheral blood ILC2 from healthy individuals or asthma patients with or without dupilumab treatment. *p<0.05; Kruskal-Wallis test (A, C, D).