IL-4 and IL-13 act independently and synergistically to drive type 2 inflammation

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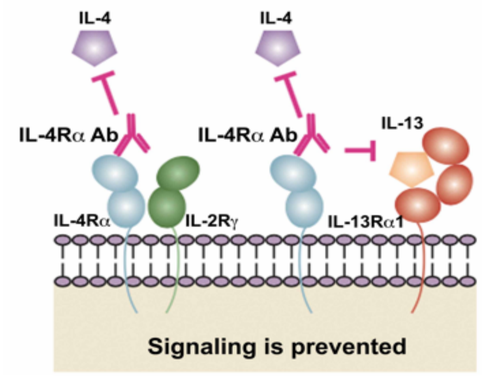
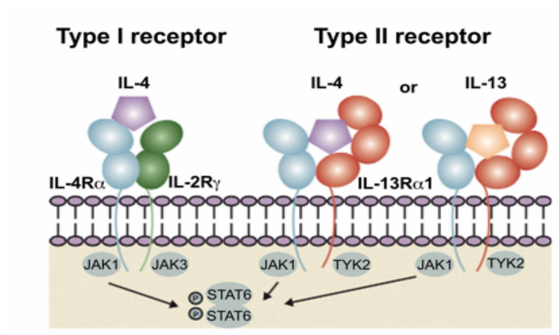
BACKGROUND

- Dupilumab is a fully human IgG4-based anti-interleukin 4 receptor α (IL-4Rα) monoclonal antibody generated using Regeneron's VelocImmune® technology
- Dupilumab inhibits both IL-4 and IL-13 signaling, key drivers of type 2 inflammation (**Figure 1**)
- Dupilumab has shown robust efficacy across multiple diseases with underlying type 2 signatures and is approved for treatment of moderate-to-severe asthma and atopic dermatitis

Figure 1: Dupilumab mechanism of action

Dupilumab binds to IL-4Rα, blocks the binding of human IL-4 and IL-13/IL-13Rα1 complex to IL-4Rα, and prevents IL-4 and IL-13 signaling through Type I and Type II receptors:





IL-13Rα1, IL-13 receptor α1; IL-2Rγ, IL-2 receptor γ; JAK, Janus kinase; STAT, signal transducer and activator of transcription; TYK, tyrosine kinase; Ab, antibody

AIM

- **To tease out the distinct or overlapping functions of IL-4 and IL-13 in type 2 inflammation, by dissecting their effects on human immune and non-immune cells and identifying their respective contributions in driving asthma-related pathologies in mice**

METHODS

Mouse models of IL-4/IL-13- and allergen-induced lung inflammation

Il4ra$^{hu/hu}$ *Il4*$^{hu/hu}$ humanized mice were either exposed to (model 1) human IL-4 or IL-13 for 12 days, or (model 2) house dust mite (HDM) for 4 weeks and received twice-weekly injections of dupilumab (dual IL-4/IL-13 blocker), IL-4 Ab, mouse IL-13Rα2 fusion protein, control Abs, or no Ab. Mice were then sacrificed, and lung tissue and serum were further analyzed by:

- ELISA (serum HDM-specific IgG1 and total IgE)
- Real-time quantitative polymerase chain reaction (qPCR)
- Flow cytometry of circulating vs lung inflammatory cell infiltrates in mice by injecting mice i.v. with CD45 Ab 5 min prior to sacrifice
- Next-Generation Sequencing (NGS) analysis
- Histology (periodic acid–Schiff [PAS] staining)

Lung function was also assessed using a FlexiVent® instrument (SCIREQ Scientific Respiratory Equipment, Inc., Montreal, Canada)

In vitro assays with human cells

Human bone marrow derived mast cells, peripheral blood eosinophils or umbilical vein endothelial cells (HUVEC) were incubated with IL-4 or IL-13 and assessed for FcεR1α expression by flow cytometry, NGS analysis, and cytokine/chemokine release by Meso Scale Discovery (MSD).

Statistical analysis

Differences were considered to be statistically significant when $P < 0.05$

RESULTS

Figure 2: Both IL-4 and IL-13 drive airway inflammation in mice

(A) Experimental design of human IL-4 and IL-13 intranasal administration. (B-E) Flow cytometry analysis of immune populations in blood and lungs; † P < 0.05; †† P < 0.01 versus mice not exposed to cytokines. (F) Fold change relative to control mice of lung tissue mRNA expression levels measured by real-time qPCR and expressed relative to β-actin (ACTB) mRNA expression.

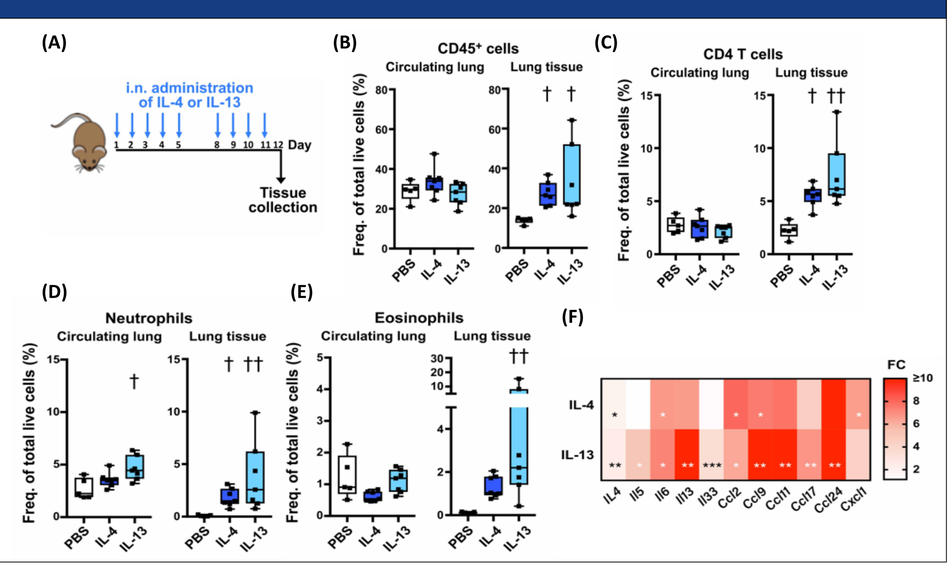


Figure 3: IL-4 drives B cell activation, class switching and pathogenic ST2+ CD4 T cell lung infiltration following lung exposure to HDM

(A) Experimental design of prophylactic use of IL-4Rα Ab, IL-4 Ab and mIL-13Rα2-Fc to treat an acute mouse model of asthma. (B-C) ELISA analysis of serum HDM-specific IgG1 and total IgE, (D-E) and flow cytometry analysis of immune populations in blood and lungs; † P < 0.05; †† P < 0.01; ††† P < 0.001 versus mice not exposed to HDM; # P < 0.05; ## P < 0.01; ### P < 0.001 versus mice exposed to HDM; * P < 0.05; ** P < 0.01; *** P < 0.001 versus corresponding isotype control;. Dotted lines: LLOQ, lower limit of quantification.

Figure 4: IL-4 dominates IgE-dependent human mast cell responses in vitro

(A) NGS analysis of the effect of IL-4 and IL-13 on cytokine/chemokine-related gene expression upon IgE crosslinking of human mast cells. (B) Effect of IL-4Rα blockade on IL-4/IL-13-induced FcεRIα surface expression. (C) mRNA expression levels measured by real-time qPCR and expressed relative to GAPDH mRNA expression; * P < 0.05; ** P < 0.01; *** P < 0.001 between indicated conditions.

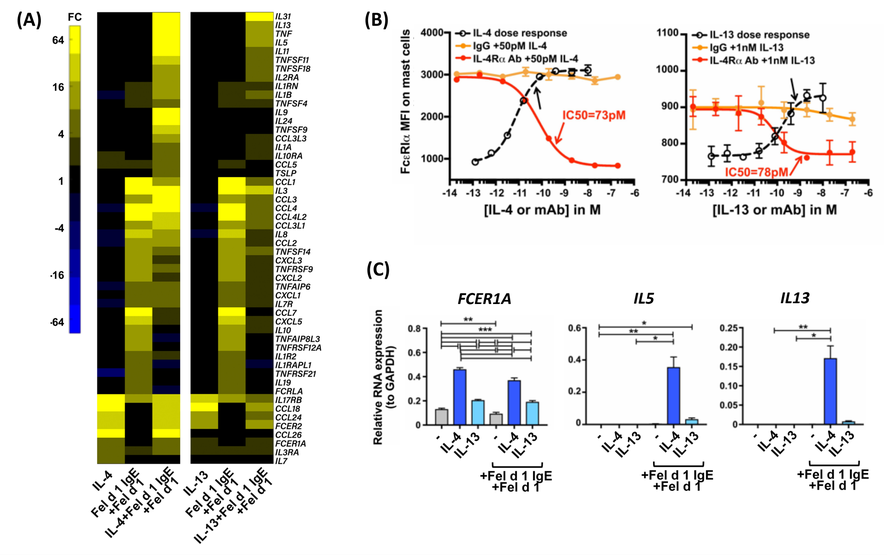
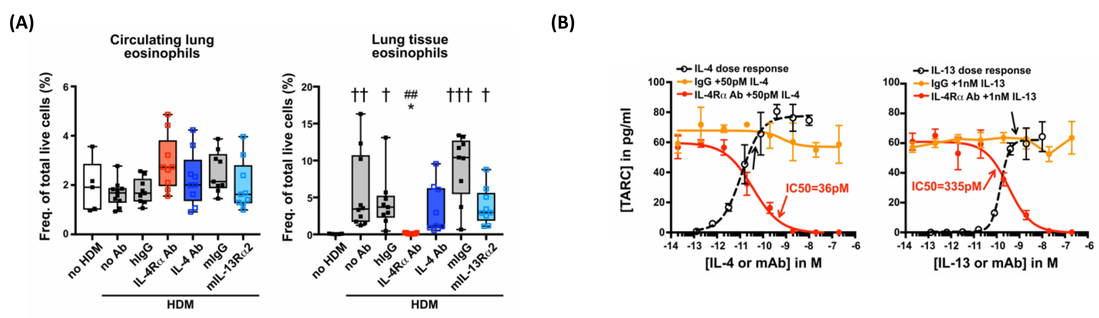


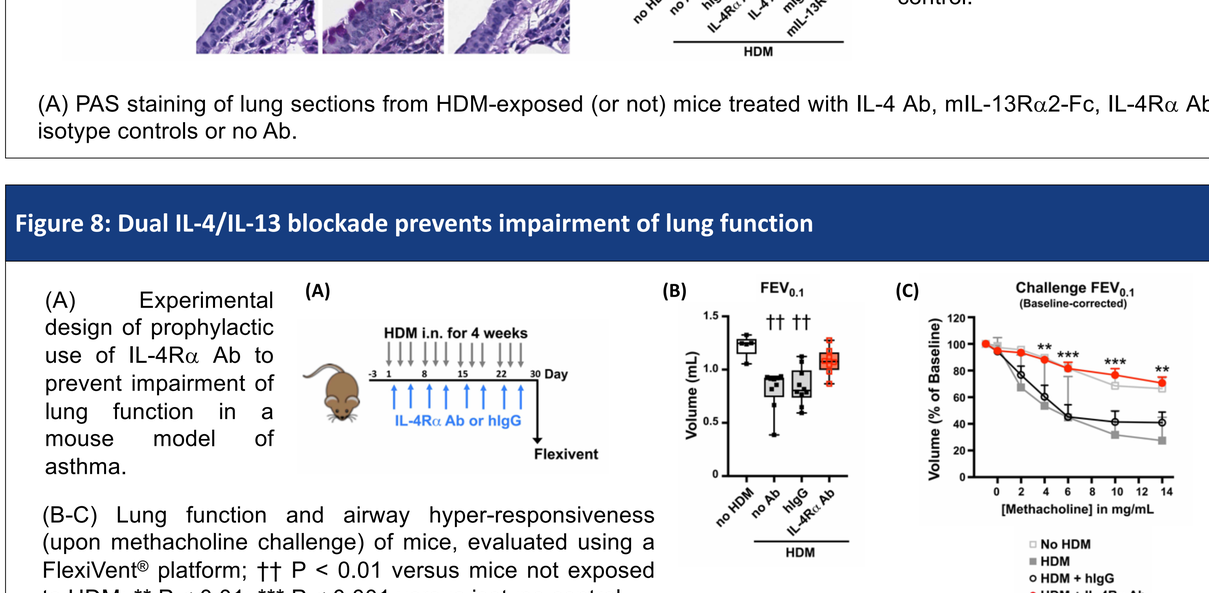
Figure 5: Both IL-4 and IL-13 promote lung eosinophilia and activate human eosinophils



(A) Flow cytometry analysis of eosinophils in blood and lungs; † P < 0.05; †† P < 0.01; ††† P < 0.001 versus mice not exposed to HDM; ## P < 0.01 versus mice exposed to HDM; * P < 0.05 versus corresponding isotype control.

(B) Effect of IL-4Rα blockade on IL-4- and IL-13-induced TARC release by human eosinophils.

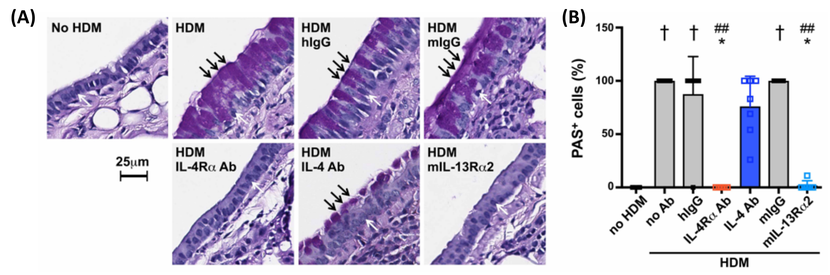
Figure 6: Dual IL-4/IL-13 blockade is required to broadly prevent chemokine and cytokine expression in the HDM-exposed lungs



(A) NGS analysis of the effect of IL-4, IL-13 and dual IL-4/IL-13 (IL-4Rα) blockade on cytokine/chemokine-related gene expression in HDM-exposed mice.

(B) IL-4- and IL-13-induced release of CCL26 and IL-6 by HUVEC.

Figure 7: IL-13 drives HDM-induced goblet cell metaplasia



(B) Goblet cell metaplasia quantification; † P < 0.05 versus mice not exposed to HDM; ## P < 0.01 versus mice exposed to HDM; * P < 0.05 versus corresponding isotype control.

(A) PAS staining of lung sections from HDM-exposed (or not) mice treated with IL-4 Ab, mIL-13Rα2-Fc, IL-4Rα Ab, isotype controls or no Ab.

Figure 8: Dual IL-4/IL-13 blockade prevents impairment of lung function

(A) Experimental design of prophylactic use of IL-4Rα Ab to prevent impairment of lung function in a mouse model of asthma.

(B-C) Lung function and airway hyper-responsiveness (upon methacholine challenge) of mice, evaluated using a FlexiVent® platform; †† P < 0.01 versus mice not exposed to HDM; ** P < 0.01; *** P < 0.001 versus isotype control.

CONCLUSIONS

Dual IL-4/IL-13 blockade with dupilumab protects against lung function decline by impacting multiple features of the type 2 response:

- IL-4 blockade prevents B cell activation, IgE production, FcεRI-expressing innate cell priming and pathogenic ST2+ CD4 T cell lung infiltration
- IL-13 inhibition is required to prevent goblet cell metaplasia and has a broad impact on cytokine/chemokine gene expression in the lungs
- Only dual IL-4/IL-13 blockade efficiently prevents lung eosinophilia and expression of inflammatory/type 2 cytokines and chemokines in the lungs

ACKNOWLEDGMENTS

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DISCLOSURES

Audrey Le Floc'h, Jeanne Allinne, Kirsten Nagashima, Wei Keat Lim, Yu Bai, George Scott, Dylan Birchard, Seblewongel Asrat, Matthew Sleeman, Andrew Murphy and Jamie Orengo: Regeneron Pharmaceuticals, Inc. – employees and shareholders