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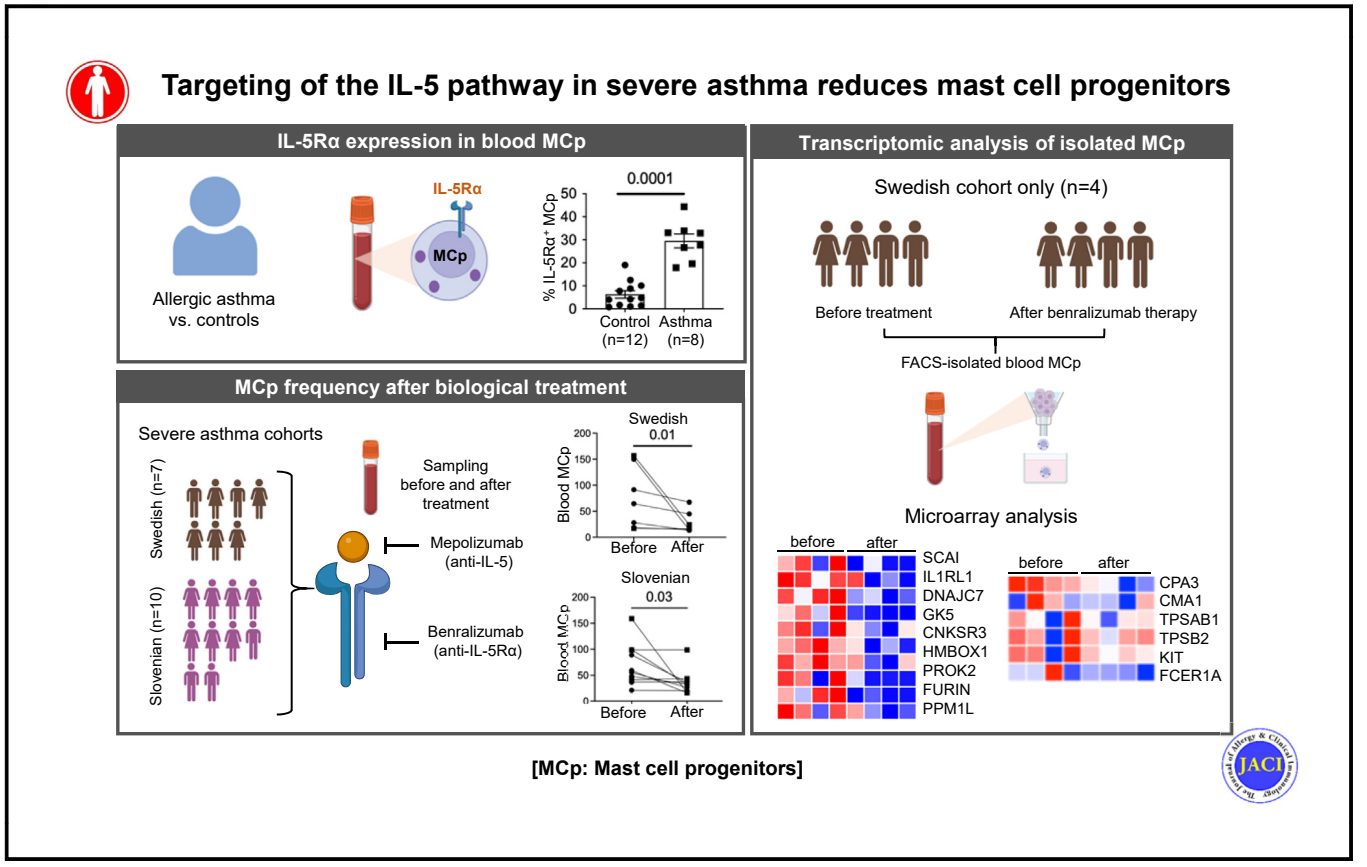
GRAPHICAL ABSTRACT



Capsule summary: A fraction of lung mast cells (MCs) and blood MC progenitors express IL-5Rα. Targeting IL-5/IL-5Rα reduces MC progenitors in severe asthma, which may reduce tissue MCs and contribute to the clinical effect.

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Targeting of the IL-5 pathway in severe asthma reduces mast cell progenitors

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Background: Therapies targeting IL-5 or its receptor (IL-5Rα) are currently used to treat patients with severe eosinophilic asthma.
Objective: We sought to investigate the impact of anti–IL-5 and anti–IL-5Rα biological therapies on mast cells (MCs) and their progenitors.
Methods: Surface IL-5Rα expression was investigated on MCs and their progenitors in mouse lungs and bone marrow and in human lungs and blood. Isolated human MC progenitors cultured in the presence or absence of IL-5 were analyzed *in vitro*. Circulating MC progenitors were quantified in patients with severe asthma before and after anti–IL-5 (mepolizumab) or anti–IL-5Rα (benralizumab) therapy. Gene expression analysis of MC progenitors was performed before and after anti–IL-5Rα therapy.
Results: Approximately 50% of the human primary lung MCs and 30% of the human MC progenitors from individuals with allergic asthma expressed IL-5Rα. In patients with mild to moderate allergic asthma and mice with acute allergic airway inflammation, the fraction of IL-5Rα$^+$ MC progenitors was elevated. In addition, IL-5 promoted the proliferation and/or survival of isolated human MC progenitors. Furthermore, patients with severe asthma from 2 independent cohorts demonstrated a reduction in blood MC progenitors after anti–IL-5 or anti–IL-5Rα treatment. This was associated with improved asthma control as well as a decline in both blood eosinophils and T_H2 cells. Finally, the blood MC progenitors remaining after anti–IL-5Rα (benralizumab) treatment exhibited a downregulated expression of genes involved in growth and proliferation.
Conclusions: This study introduces the possibility that the clinical effects of targeting IL-5/IL-5Rα in severe asthma may also involve reduction of MC populations. (J Allergy Clin Immunol 2025;155:1310-20.)

Key words: *Asthma, benralizumab, IL-5, mepolizumab, mast cells, mast cell progenitors*

Asthma is a common chronic, noncommunicable, and disabling respiratory disease characterized by chest tightness, shortness of breath, and wheezing, which can have a large negative impact on the quality of life.[1] Type 2 asthma is commonly related to allergic sensitization to aeroallergens, followed by type 2 immune responses, which are characterized by elevated levels of T_H2 cytokines (IL-4, IL-5, and IL-13), eosinophilia, and mast cell (MC) activation. However, type 2 asthma also occurs in nonatopic individuals and is associated with late-onset and chronic rhinosinusitis with nasal polyposis.[2] Despite efforts to manage asthma with corticosteroids and leukotriene modifiers, some patients fail to achieve good asthma control. In Sweden, about 4% to 10% of patients with asthma have severe asthma and struggle to control their symptoms using traditional therapies.[3,4] Importantly, severe asthma is associated with MC activation regardless of the etiology.[5,6]

It is well established that eosinophilia correlates strongly with type 2 asthma severity, implying that eosinophils contribute to the pathology.[7] Eosinophils are critically dependent on IL-5 for proliferation, survival, and activation. Hence, a strategy for managing type 2 asthma was to develop biologicals that neutralize IL-5 or block the IL-5–binding part of its receptor (IL-5Rα). Indeed, targeting of the IL-5/IL-5Rα axis has been shown to improve asthma control, and it is thus plausible that the positive effects of such treatments are attributable to effects on eosinophils.[8-10] It is also possible that anti–IL-5/IL-5Rα therapy may have direct effects on other cell types, which could also contribute to the overall clinical benefit.

MCs are innate immune cells residing in all vascularized tissues of the body and are involved in many physiological and pathological processes. In asthma, MCs typically contribute to the pathology via their activation through cross-linking of FcεRI-bound IgE molecules by allergen, resulting in the release of mediators responsible for acute respiratory symptoms related to, for example, smooth muscle constriction.[11] However, MCs have also been implicated in several non–IgE-mediated responses, as exemplified by the airway narrowing seen in response to cysteinyl leukotrienes released by ATP-

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Abbreviations used
ACT: Asthma Control Test
FACS: Fluorescence-activated cell sorting
Lin: Lineage
MC: Mast cell
SCF: Stem cell factor

activated MCs.[12] Importantly, in patients with asthma, MCs accumulate within the smooth muscle layer, thereby enhancing the probability of MC-mediated effects on smooth muscle cell contraction.[13] Furthermore, they can be found close to or within the airway epithelium,[14-16] where they are strategically situated to promptly react to aeroallergens or other inhaled stimuli.

We previously reported the existence of a rare population of MC progenitors in the blood circulation, corresponding to approximately 0.005% of enriched mononuclear cells.[17] These cells have the ability to migrate to the peripheral tissues and develop into a mature MC phenotype under the influence of the local microenvironment.[11] We showed that a high frequency of blood MC progenitors correlates with reduced lung function in patients with allergic asthma.[18] Moreover, we showed that the number of MC progenitors increases during natural allergen exposure in sensitized patients with asthma and that patients reporting poor asthma control have elevated levels of these cells.[19]

Together, our previous findings suggest that MC progenitors (or their progenies) have an impact on asthma outcomes. However, the mechanisms that regulate the expansion of MC progenitors and their progenies are only partially understood. Here, we investigated the possibility that MC progenitors may be under the influence of IL-5, thereby representing a direct link between type 2 immunity and MCs. Indeed, we show that MC progenitors and MCs express IL-5Rα. Furthermore, we demonstrate that MC progenitors in the blood of patients with severe asthma are reduced by therapeutic strategies that target the IL-5/IL-5Rα axis.

METHODS

Study participants

The study participants included birch pollen–sensitized patients with mild to moderate asthma,[19] 2 cohorts based in Sweden and Slovenia with patients diagnosed with severe asthma according to the Global INitiative for Asthma guidelines,[20] and patients who had lung resections due to lung cancer. All participants gave their written informed consent. The studies were approved by Uppsala Regional Ethics Review Board (nos. 2020-05945 and 2017/535), the Stockholm Ethics Committee (nos. 2017/832-31/1 and RBC2018/542), and the Slovenian National Medical Ethics Committee (no. 0120-189/2019/4). Anonymous blood donors at the Uppsala University Hospital were also included.

Sample collection and clinical data recording

In Uppsala, peripheral blood samples were collected in tubes containing EDTA (BD Vacutainer, BD Biosciences, Wokingham, United Kingdom), whereas in Slovenia the samples were collected in heparin-coated tubes. For the cohorts with severe asthma, a sample was collected just before the beginning of the biological treatment and a second sample was obtained after the treatment. PBMCs were isolated as previously described.[19] Briefly, the blood was diluted with PBS (pH 7.4) at a ratio of 1:1 and spun in Ficoll (ρ = 1.076 g/mL; GE Healthcare, Stockholm, Sweden). PBMCs were stored at −80°C in heat-inactivated FCS (Sigma-Aldrich, Burlington, Mass) containing 10% dimethyl sulfoxide (Sigma-Aldrich) until analysis. Pulmonary function and asthma control were recorded as previously described.[19]

Isolation and culture of human blood MC progenitors

Fresh PBMCs were stained with fluorescently labeled antibodies to detect MC progenitors and isolated using fluorescence-activated cell sorting (FACS) in a BD FACSAria III cell sorter (BD Biosciences, Franklin Lakes, NJ). Ten cells were sorted into 60-well plates (base shape conical, 83.9923.972; Sarstedt) containing 20 μL of serum-free medium StemPro-34 SFM (Thermo Fisher Scientific, Waltham, Mass) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Sigma-Aldrich). The medium was supplemented with IL-3 (30 ng/mL), IL-6 (100 ng/mL), IL-9 (20 ng/mL) stem cell factor (SCF; 100 ng/mL), GM-CSF (50 ng/mL) thrombopoietin (10 ng/mL), IL-11 (10 ng/mL), feline McDonough sarcoma (FMS)-like tyrosine kinase 3 ligand (10 ng/mL), and erythropoietin (4 U/mL) alone or with IL-5 (20 ng/mL). After 7 days of incubation at 37°C/5% CO_2, the number of cells per well was counted manually. After 10 days, the wells from each culture condition were pooled and analyzed by flow cytometry.

Induction of acute allergic airway inflammation in mice

All mouse experiments were performed in accordance with the ethical permit (Uppsala Animal Ethics Committee; nos. 5.8.18-05248/2018 and 5.8.18-21870/2022). Female mice (BALB/cBomTac) older than 6 weeks were used. Lyophilized whole-body extract of house dust mite allergen (*Dermatophagoides pteronyssius*; Stallergenes Greer, Lenoir, NC) was reconstituted in PBS and aliquoted at −80°C until use. Freshly thawed allergen (50 μg/50 μL) or an equal volume of PBS was administered (25 μL in each nostril) under isoflurane anesthesia every 3 days for 19 days. Analyses were performed the day after the last allergen administration (day 19).

Preparation of single-cell suspensions from mouse lung

Mice were euthanized by an isoflurane overdose. Excess blood was removed from the lungs by perfusing 10 mL of cold PBS through the heart. Lungs were dissected and cut into small pieces, followed by enzymatic digestion (Lung Dissociation Kit for mouse; Miltenyi Biotec, Bergish Gladbach, Germany) in the gentleMACS Octo Dissociator (Miltenyi Biotec). The enzymes were removed by washing in 2% FCS in PBS (FACS buffer). The remaining debris was removed by a 44% Percoll (Sigma-Aldrich) centrifugation step and red blood cells with lysis buffer (150 mM NH_4Cl, 9.5 mM $NaHCO_3$, and 1.2 mM EDTA).

Human lung tissue

Macroscopically healthy, tumor-free lung tissue from 3 participants who gave their informed consent was removed

during resections due to lung cancer. The lung tissue was cut into pieces and digested with the Multitissue Dissociation Kit 1 (Miltenyi Biotec) using the gentleMACS Octo Dissociator (Miltenyi Biotec). Cell suspensions were washed with 10 mL RPMI-1640 (Sigma-Aldrich) supplemented with 1% penicillin/streptomycin solution, 1% nonessential amino acids, 1% HEPES, 0.1% β-mercaptoethanol, 1% pyruvate, and 1% glutamine (all from Sigma-Aldrich). After filtering with a 70-μm strainer, residual red blood cells were removed by incubation in lysis buffer before washing and treatment with DNase (200 U/mL; 5 minutes). The cells were washed and resuspended in FACS buffer before analysis.

Flow cytometry

Frozen PBMCs were thawed in FACS buffer at 37°C. Freshly thawed PBMCs and lung-cell suspensions were washed and stained (4°C; 30 minutes) with the antibodies listed in Table E1 (in the Online Repository available at www.jacionline.org). Cells were washed to remove excess antibodies and analyzed in an LSR Fortessa cytometer (BD Biosciences) or the CytoFLEX S (Beckman Coulter, Brea, Calif). FlowJo v.10 (BD Biosciences, Ashland, Ore) was used to quantify cell populations.

Microarray analysis of isolated MC progenitors

MC progenitors (50-150 cells) were isolated (using the FACSAria III cell sorter) into 96-well plates (Sarstedt, Nümbrecht, Germany) containing 200 μL of RLT buffer (Qiagen, Hilden, Germany) and kept at −80°C until analysis. For RNA isolation, the single-cell RNA purification kit (Norgen Biotek Corp, Thorold, Ontario, Canada), and for gene expression, the Affymetrix Human Clariom S array (Thermo Fisher Scientific) were used (Bioinformatics and Expression Analysis core facility, Karolinska Institute, Stockholm). The comparison of before and after benralizumab treatment was made in the Transcriptome Analysis Console software with the implemented Bioconductor package using empirical Bayes moderated paired F tests as implemented in Limma. Heat maps were obtained using Morpheus (https://software.broadinstitute.org/morpheus), and overrepresentation analysis was performed using the gene set analysis toolkit WebGestalt (https://www.webgestalt.org). Gene expression data have been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus database (GSE263241).

Statistical analysis

Statistical analyses were performed using GraphPad Prism v.10 (GraphPad Software, Inc). Wilcoxon signed-rank test was used when comparing 2 paired groups, whereas the Mann-Whitney *U* test was used for unpaired data. *In vitro* experiments were analyzed using 1-way ANOVA followed by the Tukey *post hoc* test. A *P* value less than .05 was considered statistically significant.

RESULTS

IL-5Rα is present in lung MCs, and an increased IL-5Rα expression is observed in MC progenitors during allergic conditions

The "healthy" part of lung biopsies from patients undergoing lobectomy because of lung cancer was analyzed for IL-5Rα–expressing MCs by flow cytometry. Lung MCs were identified as $CD45^+CD4^-CD8^-CD19^-CD14^-CD117^+$ $FcεRI^+$ cells.[21] Of these, approximately 50% were positive for IL-5Rα (Fig 1, *A*; see also Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Next, we investigated the surface expression of IL-5Rα on MC progenitors from the blood of birch pollen–sensitized patients with asthma and unidentified blood donor controls (see Table E2 in this article's Online Repository at www.jacionline.org). These analyses showed that the frequency of MC progenitors (lineage $[Lin]^-CD13^-CD34^+CD117^+$ $FcεRI^+$ cells; Fig E1, *B*)[17] was higher in the patients with asthma than in the controls ($P = .0001$; Fig 1, *B*; see also Table E2). Approximately 30% of the MC progenitors from allergic patients with asthma sampled under the pollen season expressed IL-5Rα, whereas 1% to 19% of the MC progenitors in the controls were $IL\text{-}5Rα^+$ (Fig 1, *B*). To assess whether these findings can be translated to a mouse model, IL-5Rα expression was assessed in control mice versus mice with acute allergic airway inflammation.[21] Approximately 40% of the lung MC progenitors ($CD45^+Lin^-CD11b^-$ $c\text{-}kit^+$ $ST2^+$ $FcεRI^+$ integrin $β7^+$ cells; Fig E1, *D*) from mice with allergic airway inflammation were $IL\text{-}5Rα^+$, whereas it was approximately 20% in control mice (Fig 1, *C*). Given that approximately 30% of the lung MC progenitors and 5% of the bone marrow MC progenitors in mice with allergic airway inflammation express IL-5Rα on their surface (see Fig E2 in this article's Online Repository at www.jacionline.org), IL-5Rα expression does not seem to be associated with MC maturation. Hence, the proportion of MC progenitors that show surface expression of IL-5Rα seems to be increased on allergen exposure in sensitized mice and patients.

A cytokine cocktail containing IL-5 promotes more proliferation and/or survival of isolated human MC progenitors

Isolated human blood MC progenitors differentiate into MCs when cultured in a myeloerythroid cytokine cocktail, which contains IL-5.[17] To test the effect of IL-5 in this multicytokine environment, we sorted (FACS) 10 MC progenitors from fresh human blood directly into prefilled wells of Terasaki plates containing the myeloerythroid cytokines ± IL-5. After 7 days, a higher number of cells were found in wells cultured with IL-5 compared with wells without IL-5 (Fig 2, *A*). Furthermore, the progenies from IL-5–containing cultures contained larger numbers of $CD117^+$ $FcεRI^+$ cells (Fig 2, *B*; see Table E3 in this article's Online Repository at www.jacionline.org). However, the cells had a similar macroscopic cell morphology regardless of whether they were cultured with or without IL-5 (Fig 2, *B*). Collectively, these findings suggest that IL-5 is a factor that promotes better survival and/or drives the proliferation and differentiation of human MC progenitors.

Targeting the IL-5/IL-5Rα axis causes a reduction of blood MC progenitors

After demonstrating that human MC progenitors respond to IL-5, we hypothesized that anti–IL-5/IL-5Rα therapy could potentially influence them. To address this, blood samples and clinical data were obtained from 2 separate cohorts of patients with severe asthma who received mepolizumab (anti–IL-5) or benralizumab (anti–IL-5Rα). One cohort (Swedish) consisted of 5 women (3 anti–IL-5 and 2 anti–IL-5Rα) and 2 men (both anti–IL-5Rα)

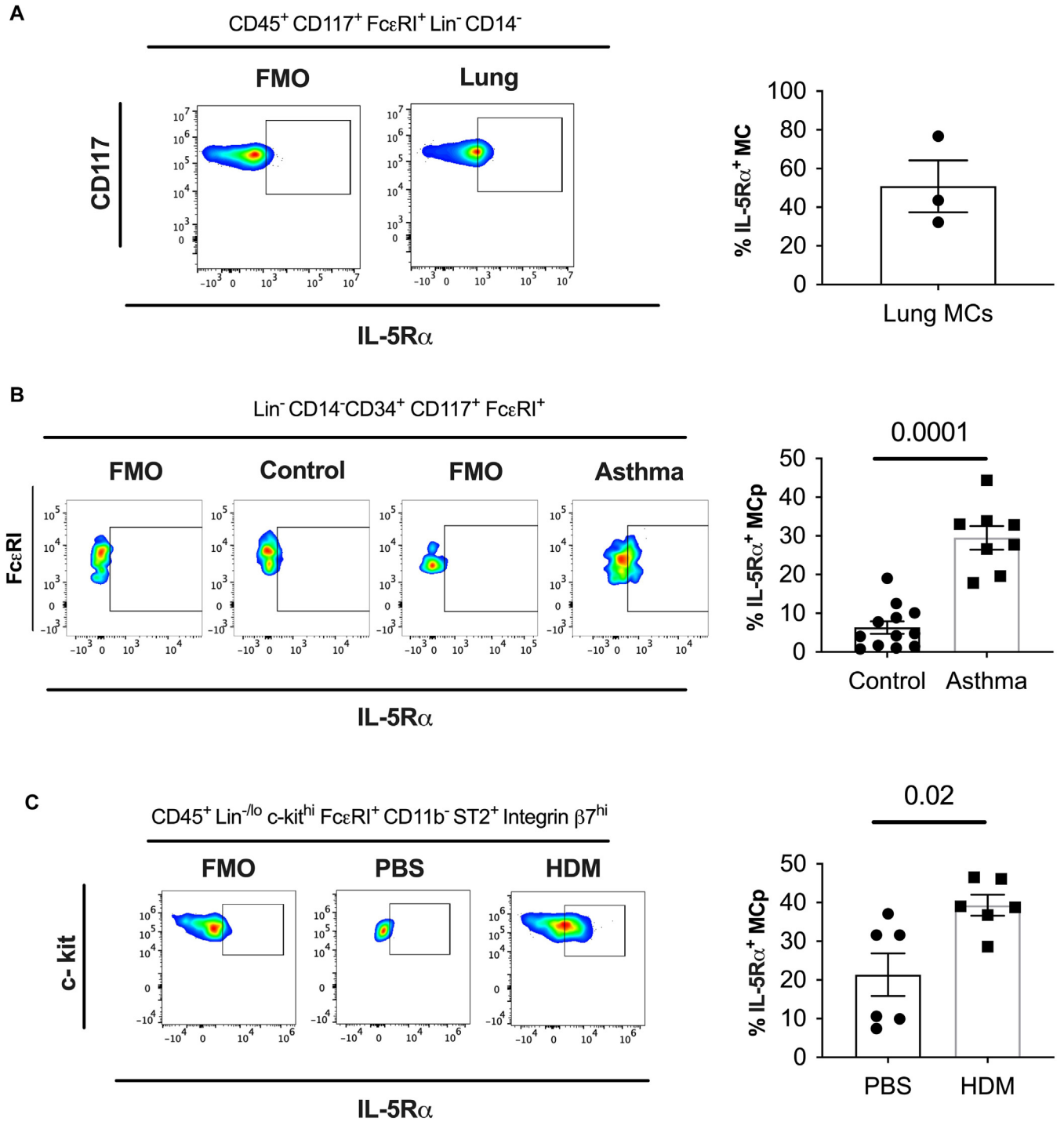

FIG 1. Elevated surface IL-5Rα expression on MC progenitors from patients with allergic asthma and mice with acute allergic airway inflammation. **A** and **B,** The expression of IL-5Rα was investigated in MCs from human lung biopsies from 3 donors (Lin = CD4CD4CD19) (Fig 1, *A*) and in peripheral blood MC progenitors from blood donors as controls and patients with allergic asthma (Lin = CD4CD8CD19) (Fig 1, *B*). **C,** The expression of mouse IL-5Rα in lung MC progenitors from mice with HDM-induced allergic airway inflammation was compared with mice that were given vehicle (PBS) as controls (Lin = CD3CD4CD8CD19B220TER-119 Gr1). The data were pooled from 2 experiments with 3 mice per group. The IL-5Rα gates were set on the basis of FMO controls. Bars represent means ± SEM of individuals or mice. Statistical analysis was performed by using the Mann-Whitney *U* test. *FMO*, Fluorescence minus one; *HDM*, house dust mice.

(Table I). Samples and clinical data were obtained before as well as 4 to 13 (6 ± 3) months after the initiation of the biological treatment. There was no significant change in FEV_1% (predicted), in fraction of inhaled nitric oxide, or in the used dose of inhaled or oral corticosteroids between pre– and post–biological treatment. However, after anti–IL-5/IL-5Rα treatment, the frequency of

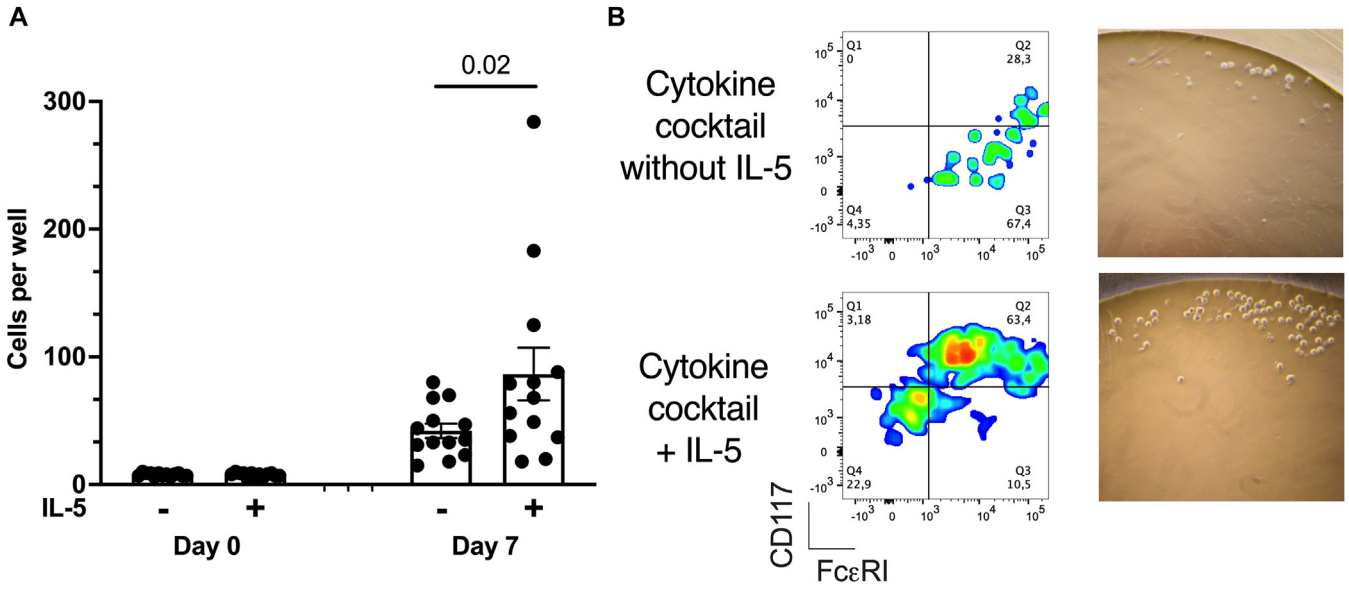

FIG 2. A cytokine cocktail containing IL-5 promotes better survival and/or proliferation of isolated human MC progenitors. Human MC progenitors from peripheral blood were isolated by FACS and cultured for days in a myeloerythroid cocktail of cytokines in the presence or absence of recombinant human IL-5. **A,** The progenies of 10 cells/well were counted manually after 7 days of culture. Bars represent means ± SEM per well in 2, 5, and 6 wells in 3 individual experiments using blood from 3 different subjects. **B,** Representative data showing surface expression of CD117 and FcεRI (*left*) and cell morphology (*right*) after 10 days of culture. Statistical analysis was performed by 1-way ANOVA followed by the Tukey *post hoc* test. *MNC,* Mononuclear cells.

blood MC progenitors and eosinophils decreased substantially (Fig 3, *A* and *B*). There was also a tendency toward improvements in asthma control (Asthma Control Test [ACT], $P = .06$; Asthma Control Questionnaire, $P = .07$) (Fig 3, *C*; see also Fig E3, *B*, in this article's Online Repository at www.jacionline.org). The anti–IL-5/IL-5Rα treatment did not cause any consistent changes in the expression of IL-5Rα in MC progenitors (Fig E3, *C*). Notably, the level of blood eosinophils before treatment varied because some of the patients were included due to frequent asthma exacerbations.

The second cohort consisted of 10 patients with severe asthma from Slovenia, which included 7 women (3 anti–IL-5 and 4 anti–IL-5Rα) and 3 men (1 anti–IL-5 and 2 anti–IL-5Rα) (Table I). The second blood sample was taken on average 32 ± 5 (20-37) months after the start of biological treatment. There was no significant change in FEV_1% (predicted), fraction of inhaled nitric oxide, or oral corticosteroids between pre– and post–biological treatment. However, the dose of inhaled corticosteroids was reduced by 50% after the biological treatment in the Slovenian cohort ($P < .007$). Combining data from both cohorts, we found that MC progenitor levels in subjects using both oral and inhaled corticosteroids were similar to those in subjects using only inhaled corticosteroids before initiation of biological treatment (Fig E3, *A*). Similar to what we found in the Swedish cohort, the frequencies of blood MC progenitors and eosinophils were decreased following biological treatment in the Slovenian cohort (Fig 4, *A* and *B*). Furthermore, when data from both cohorts were combined and analyzed on the basis of the type of biological treatment, both benralizumab and mepolizumab were shown to reduce blood MC progenitor levels (Fig E3, *D* and *E*). Interestingly, in the Slovenian cohort, the fraction of IL-5Rα$^+$ MC progenitors increased from 4%-45% to 32%-95% after the biological treatment (Fig 4, *C*). Furthermore, asthma control, as assessed by ACT, was improved by the treatment ($P < .01$; Fig 4, *D*). The proportion of T_H1 cells ($CD3^+CD4^+CCR6^-CXCR3^+CCR4^-$) and T_H2 cells ($CD3^+CD4^+CCR6^-CXCR3^-CCR4^+$) was assessed using the expression pattern of chemokine receptors (see Fig E4, *A*, in this article's Online Repository at www.jacionline.org).[22] There were no overall changes in the frequency of T_H1 and T_H2 cells after the biological treatment, although most subjects showed a decrease in T_H2 frequency and an increase in T_H1 frequency (Fig E4, *B* and *C*). However, the proportion of blood cells with a T_H2 phenotype was decreased after the biological treatment (Fig 4, *E*).

Anti–IL-5Rα treatment modulates gene expression patterns in MC progenitors

To gain further insight into how IL-5 influences MC progenitors, effects on gene expression patterns were analyzed. To this end, MC progenitors were isolated from 4 patients with severe asthma before and after 6 to 13 (8 ± 4) months of anti–IL-5Rα treatment, followed by transcriptomic analysis (Fig 5, *A*). The $\log_2$ fold- change counts with adjusted *P* values are provided in Table E4 (in the Online Repository available at www.jacionline.org). A total of 257 genes were found to be downregulated (see Table E5 in this article's Online Repository at www.jacionline.org), whereas 34 genes were upregulated after the treatment (see Table E6 in this article's Online Repository at www.jacionline.org) using a cutoff *P* value less than .05 (Fig 5, *B*). The top 10 downregulated and the top 10 upregulated genes within the entire gene set are highlighted in Fig 5, *C*. Overrepresentation and gene set analysis revealed that the anti–IL-5Rα treatment led to the downregulation of the p38 mitogen-activated protein kinase and platelet-derived growth factor

TABLE I. General characteristics of patients with severe asthma sampled in Sweden and Slovenia before and after biological treatment

Characteristics	Sweden cohort (n = 7)	Slovenia cohort (n = 10)
Age (y) (minimum-maximum)	57 ± 14 (39-74)	59 ± 8 (49-74)
Height (cm) (minimum-maximum)	166 ± 10 (152-179)	167 ± 12 (157-194)
Weight (kg) (minimum-maximum)	75 ± 24 (50-111)	83 ± 21 (54-110)
BMI (kg/m^2)		
Normal	3 (43)	2 (20)
Overweight	2 (29)	4 (40)
Obese	2 (29)	4 (40)
Sex: female	5 (71)	7 (70)
Atopy	2 (29)	3 (30)
Nasal polyps	3 (43)	7 (70)
Duration of treatment (mo)	6 ± 3 (range, 4-13)	32 ± 5 (range, 20-37)
Anti–IL-5	3 (43)	4 (40)
Anti–IL-5Rα	4 (57)	6 (60)

	Sweden cohort (n = 7)			Slovenia cohort (n = 10)		
	Before biological treatment	After biological treatment	*P* value	Before biological treatment	After biological treatment	*P* value
FEV_1% (predicted)	65 ± 25	82 ± 19	.21	76 ± 14	83 ± 19	.13
FENO (ppb)	38 ± 18	61 ± 64	.18	64 ± 40	61 ± 35	.40
ICS% (μg/d)	100% 500 ± 129	100% 400 ± 238	.25	100% 352 ± 43	100% 168 ± 16	.007*
OCS% (mg/d)	86% 8.3 ± 5.1	43% 4.1 ± 5.8	.12	30% 6.2 ± 3.3	30%† 5 ± 0	.99

All except 1 patient in the Slovenian cohort were current nonsmokers. Atopy was defined as confirmed sensitivity, indicated by a positive reaction in skin prick tests or specific IgE tests, to any of the following aeroallergens: tree pollen, grass pollen, weed pollen, house dust mites, or animal hair. Data are presented as mean ± SD or n (%). For BMI: normal, 18.5-24.99; overweight, 25-29.99; and obese, ≥30 (as determined by the World Health Organization and the number of research subjects that belonged to each BMI category). The *P* values shown for ICSs and OCSs are for testing changes in daily dose.

FENO, Fraction of inhaled nitric oxide; *ICS*, inhaled corticosteroid; *OCS*, oral corticosteroid.

*Wilcoxon signed-rank test was used to obtain the *P* values provided.

†All patients who were on OCSs before biological treatment could discontinue OCSs for asthma; however, they required OCSs because of adrenal insufficiency.

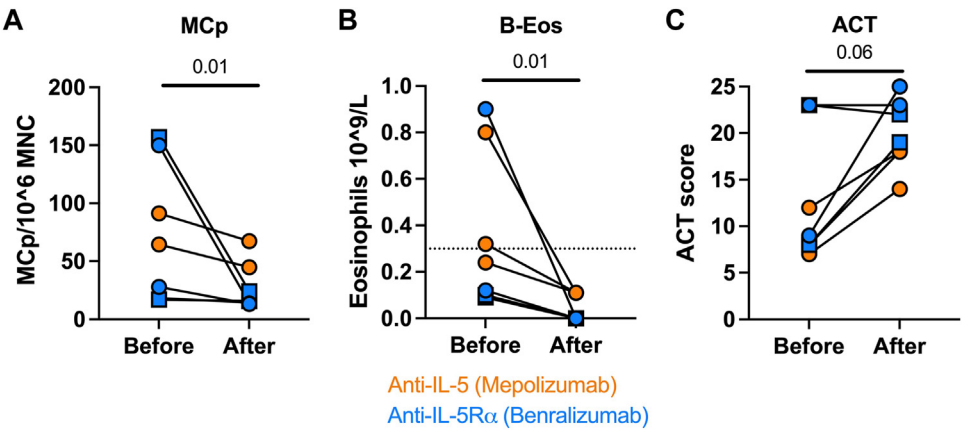

FIG 3. Anti–IL-5/IL-5Rα therapy decreases the frequency of blood MC progenitors in Swedish patients with severe asthma. **A-C,** The frequency of blood MC progenitors (MCp) (Fig 3, *A*), blood eosinophils (B-Eos) (Fig 3, *B*), and ACT scores (5, very poor; 25, excellent) (Fig 3, *C*) were quantified before and after the biological therapy in Sweden-based patients. Each *dot* represents an individual patient. The 2 male patients are indicated by *squares*, whereas the female patients are indicated by *circles*. Statistical analysis was performed using a Wilcoxon signed-rank test for paired samples. *MNC*, Mononuclear cells.

signaling pathways in MC progenitors (Table II). In contrast, 5 biosynthetic pathways (ubiquitin-mediated proteolysis, 1-carbon pool by folate, *O*-glycan biosynthesis, terpenoid backbone biosynthesis, and folate biosynthesis) were upregulated after treatment (Table II). The expression of canonical MC markers did not change after the treatment (Fig 5, *D*; see Table E7 in this article's Online Repository at www.jacionline.org). However, a consistent downregulation of genes coding for ALOX5AP, IL1RL1, and ITGA4 was observed (Fig 5, *E*; see also Table E7). Hence, in addition to promoting the proliferation and/or survival of MC progenitors, IL-5 inhibition has profound effects on their gene expression patterns.

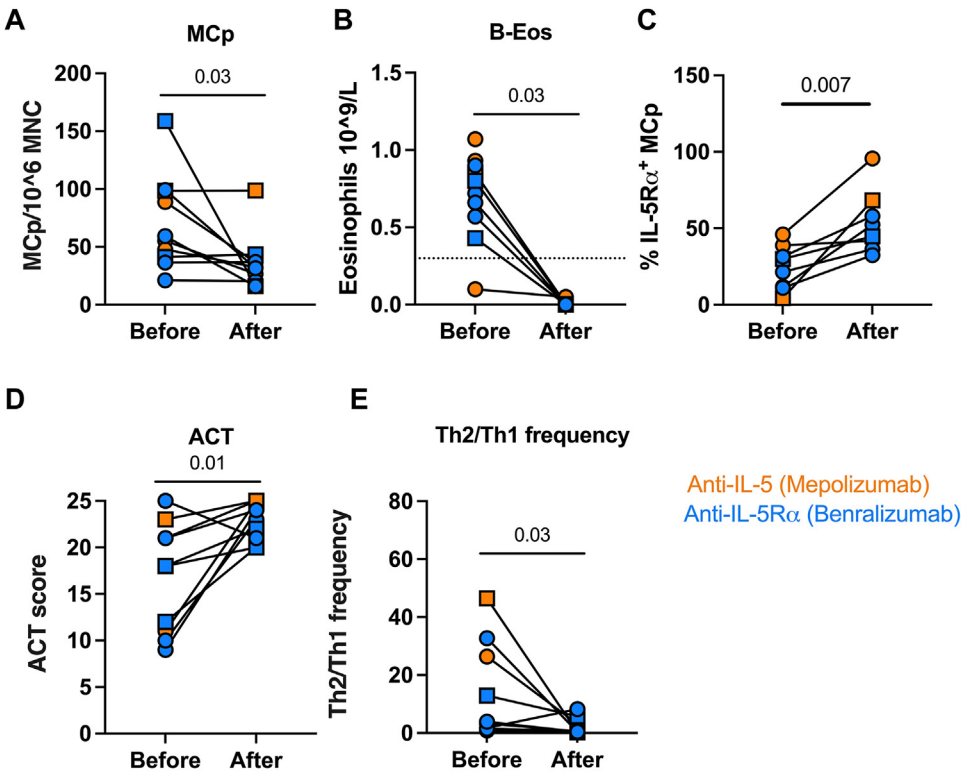

FIG 4. Anti–IL-5/IL-5Rα therapy decreases blood MC progenitor frequency in Slovenian patients with severe asthma. **A-E,** The frequency of blood MC progenitors (MCp) (Fig 4, *A*), blood eosinophils (B-Eos) (Fig 4, *B*), the proportion (%) of IL-5Rα$^+$ MCp (Fig 4, *C*), ACT scores (5, very poor; 25, excellent) (Fig 4, *D*), and the T_H2/T_H1 ratio (Fig 4, *E*) were quantified before and after the biological therapy in patients sampled and characterized in Slovenia. Each *dot* represents an individual patient. The 3 male patients are indicated by *squares*, whereas the female patients are indicated by *circles*. Note that for 1 patient in Fig 4, *B*, the after value is missing. Statistical analysis was performed using a Wilcoxon signed-rank test for paired samples. *MNC*, Mononuclear cells.

DISCUSSION

This study reveals that biological treatments targeting the IL-5 signaling pathway in patients with severe asthma reduced the MC progenitors in the blood. We speculate that this will likely lead to a gradual decrease in the number of corresponding tissue MCs. Mechanistically, we demonstrate that IL-5Rα is expressed at the protein level in a proportion of human lung MCs and blood MC progenitors. Thus, it is conceivable that MC progenitors depend on IL-5 for survival/differentiation and that the reduction of MC progenitors by anti–IL-5/IL-5Rα treatment is due to direct effects on this circulating MC population. Intriguingly, birch pollen–allergic patients with asthma were shown to have a higher proportion of IL-5Rα$^+$ MC progenitors compared with blood donors as controls. Building on our previous finding of these patients showing an increased number of MC progenitors following natural allergen exposure,[19] we suggest that this rise may be linked to elevated IL-5 levels during type 2 immune responses.

Low-level surface expression of IL-5Rα has previously been shown in cord blood–derived human MCs.[23] In addition, IL-5, in combination with SCF, exhibited a co-mitogenic effect on these cells[23] while inducing a slightly altered cytokine profile compared with SCF alone.[24] In the present study, we extend these findings by showing that IL-5 is required for an enhanced survival or proliferative response in isolated primary human MC progenitors. This is also in line with a recent study demonstrating that hematopoietic blood–derived progenitors cultured in IL-5 alone had the ability to maintain a developing population of c-kit$^+$ FcεRI$^+$ cells, although not as efficiently as IL-3 alone.[25]

Because the proportion of IL-5Rα$^+$ circulating MC progenitors was increased in patients with allergic asthma, we tested whether mouse lung MC progenitors also showed IL-5Rα surface expression on induction of allergic airway inflammation. Indeed, approximately 30% to 40% of the lung MC populations in mice with acute house dust mite–induced allergic airway inflammation showed a robust IL-5Rα surface expression. Nevertheless, having a high percentage of IL-5Rα$^+$ MC progenitors is associated not only with atopy because several of the nonatopic patients with severe asthma had more than 30% of IL-5Rα$^+$ MC progenitors. Together, IL-5Rα expression in MC progenitors seems to be induced by type 2 inflammation in general and appears to be conserved between humans and mice. IL-5Rα might thus function as a supporting survival factor for MC progenitors under such conditions. In support of this hypothesis, the proportion of IL-5Rα$^+$ MC progenitors remaining after anti–IL-5/IL-5Rα treatment for 20 months or more was even higher (up to 95%), possibly occurring in an attempt to circumvent the lost IL-5 signal.

Previous studies have suggested that anti–IL-5 treatment can cause a reduction of tissue MCs. For example, mepolizumab was shown to reduce intestinal epithelial MCs in children with eosinophilic esophagitis.[26] However, because IL-9–producing

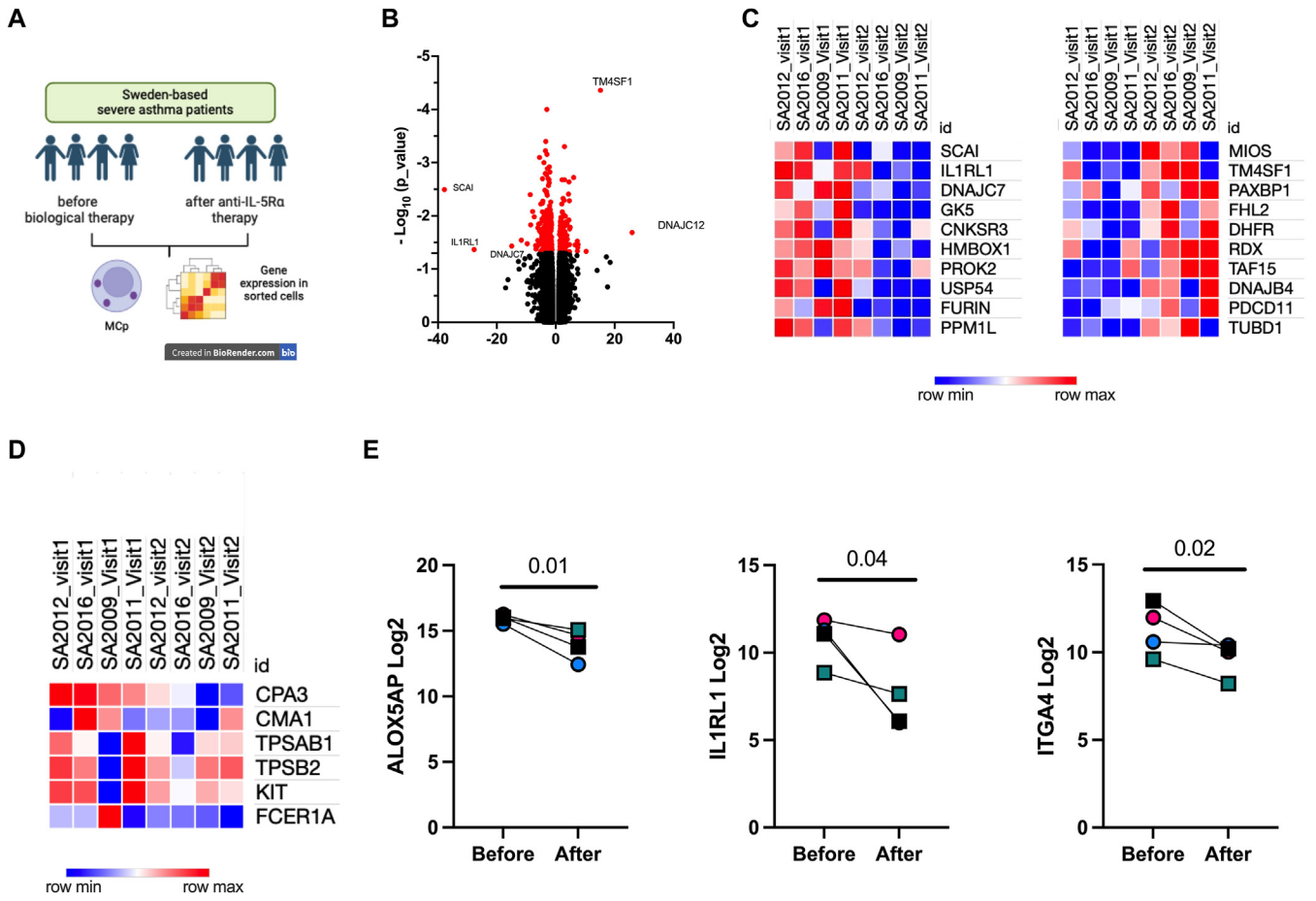

FIG 5. Transcriptomic analysis of isolated blood MC progenitors before and after anti–IL-5Rα therapy in patients with severe asthma. **A,** MC progenitors (MCp) were sorted from fresh peripheral blood before and after anti–IL-5Rα therapy in 2 male and 2 female Swedish patients with asthma. **B,** Volcano plot showing downregulated and upregulated genes (cutoff $P < .05$). **C** and **D,** Heat maps showing the expression of the top 10 downregulated (*left*) and upregulated (*right*) genes (Fig 5, *C*) or selected canonical MC markers (Fig 5, *D*) after anti–IL-5Rα treatment. **E,** Expression levels of ALOX5AP, IL1RL1, and ITGA4 in each patient. Each symbol represents an individual patient. The *P* values shown were calculated using paired F tests as implemented in Limma.

TABLE II. Overrepresentation analysis of all upregulated and downregulated genes and a description of which pathways they are involved in

Description	ID no.	Gene set size	Expected value	Enrichment ratio	*P* value
Downregulated pathways					
p38 MAPK pathway	P05918	34	0.09	22.21	.003
PDGF signaling	P00047	125	0.33	6.04	.03
Upregulated pathways					
Ubiquitin-mediated proteolysis	hsa04120	137	0.25	7.79	.02
1-carbon pool by folate	hsa00670	20	0.03	26.67	.03
Other types of *O*-glycan biosynthesis	hsa00514	22	0.04	24.25	.04
Terpenoid backbone biosynthesis	hsa00900	22	0.04	24.25	.04
Folate biosynthesis	hsa00790	26	0.04	20.51	.04

MAPK, Mitogen-activated protein kinase; *PDGF*, platelet-derived growth factor.
The table provides significantly downregulated and upregulated biological pathways after anti–IL-5Rα treatment in isolated human MC progenitors from 4 patients with severe asthma using the significantly downregulated and upregulated genes (cutoff $P < .05$). The overrepresentation analysis was performed using WebGestalt.

eosinophils were also reduced by the anti–IL-5 treatment, the decrease in MCs could be a consequence of a reduction in IL-9. Recently, a study of patients with aspirin-exacerbated respiratory disease found high levels of *IL5RA* expression in plasma cells, ciliated epithelial cells, and MCs from surgically excised sinus tissue, and the anti–IL-5 treatment caused a reduction in nasal

inflammatory eicosanoids and a decrease in blood eosinophils and basophils.[27] The authors suggested that the clinical effect of mepolizumab could be due to the targeting of multiple IL-5Rα$^+$ immune cells, including MCs.

To test whether mepolizumab and benralizumab had the ability to reduce blood circulating MC progenitors in patients with severe asthma, we analyzed 2 cohorts before and after treatment. Indeed, the blood MC progenitor frequency was reduced by the biologicals in both cohorts. Because of circumstances related to the coronavirus disease 2019 pandemic, the sampling of patients after treatment differed between the 2 cohorts (on average 6 vs 32 months). Nevertheless, our data suggested that treatment for more than 4 months reduced the frequency of blood MC progenitors and eosinophils and improved asthma control (higher ACT score). A limitation of our study is that both cohorts had low number of patient, thereby preventing subanalyses. However, when the data from the 2 cohorts were pooled and stratified on the basis of the type of biological treatment, mepolizumab or benralizumab alone was shown to also cause a reduction in blood MC progenitors. Nevertheless, because anti–IL-5 treatment reduces blood eosinophils more efficiently than it does tissue eosinophils,[28] it remains to be determined whether a decrease in blood MC progenitors also correlates with a reduction of tissue MCs. Given that MC progenitors are likely a negligible source of serum tryptase, and as a potential indication of only a minor effect on tissue MCs, 23 patients with severe asthma showed unchanged serum tryptase levels 4 to 12 months after treatment with mepolizumab.[29]

Mepolizumab acts by binding to the α chain of IL-5 and thereby prevents its interaction with IL-5Rα.[30] Benralizumab, however, prevents the binding of IL-5 to its receptor and triggers antibody-dependent cell-mediated cytotoxicity, because its Fc region binds to FcγRIII on natural killer cells.[31] Our study revealed a profound reduction in blood MC progenitors in patients treated with anti–IL-5/IL-5Rα biologicals, but not a total depletion. To gain deeper insights into the effects of these therapies on MCs, we analyzed the transcriptome of MC progenitors from 4 patients before treatment as well as the MC progenitors that remained in the blood after 6 to 13 months of benralizumab treatment. Strikingly, the MC progenitors that remained after anti–IL-5Rα treatment exhibited a downregulated expression of growth-promoting pathways (p38 mitogen-activated protein kinase and platelet-derived growth factor signaling), possibly because of initiation of apoptosis or being shifted away from a state of proliferative activity. Several other pathways were upregulated, including ubiquitin-mediated proteolysis. Numerous MC-related genes appeared consistently downregulated by benralizumab, for example, *IL1RL1* (IL-33 receptor ST2 gene) and *ALOX5AP* (FLAP gene, necessary for the synthesis of leukotrienes). Furthermore, *ITGA4* (codes for integrin α4) transcripts were reduced. Notably, we have previously shown that α4 integrins are necessary for the recruitment of mouse MC progenitors to the lung in an experimental asthma model.[32] Overall, on the basis of these transcriptional data, we propose that the MC progenitors that remain after biological treatment are less likely to be activated and recruited to the lung, thereby further limiting the expansion of MCs.

Our results lead us to speculate whether the clinical effects of benralizumab might be partly due to direct effects on MCs. In support of such a possibility, benralizumab has been shown to reduce mannitol-induced airway hyperresponsiveness in severe uncontrolled eosinophilic asthma.[33] Importantly, it has been demonstrated that mannitol-induced airway hyperresponsiveness is partly dependent on MCs,[34-37] thereby providing further evidence that the efficacy of benralizumab might be, at least partly, due to effects on MCs. However, further studies are needed to assess the *in vivo* influence of treatments targeting IL-5 or IL-5Rα on MC expansion and function.

Our study reveals that a proportion of MCs and their progenitors express surface IL-5Rα and that this is enhanced in type 2 asthma. Furthermore, we show that treatment of patients with severe asthma with mepolizumab or benralizumab reduces the blood circulating MC progenitors. Thus, we propose that treatments targeting the IL-5 pathway relieve asthma symptoms, at least partly, by limiting the number of MCs and their progenitors, thereby suppressing their detrimental role in asthma.

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Key messages

- **A significant fraction of human MC progenitors shows surface expression of the α unit of the IL-5 receptor, which is additionally increased in patients with allergic asthma.**
- **Targeting the IL-5 pathway with mepolizumab or benralizumab in patients with severe asthma reduces the frequency of blood circulating MC progenitors.**

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