# Effect of benralizumab on inflammation in skin after intradermal allergen challenge in patients with moderate-to-severe atopic dermatitis

Christiane E. Whetstone, BSc,[a] Ruth P. Cusack, MB, MPH,[a] Emma Price, MSc,[a] Karen Howie,[a] Catie Stevens, BSc,[a] Dhuha Al-Sajee, MD, PhD,[a] Sue Beaudin, BSc,[a] Jennifer Wattie,[a] Nadia Alsaji, MBChB,[a] Abbey Schlatman, BSc,[a] Vanessa Luk, BHSc,[a] Xiaotian Ju, PhD,[a] Paul O'Byrne, MB,[a,b] Mark Inman, MD, PhD,[a] Roma Sehmi, PhD,[a,b] Hermenio Lima, MD, PhD,[a] and Gail M. Gauvreau, PhD[a] *Hamilton, Ontario, Canada*

**Background: Atopic dermatitis (AD) is a skin barrier dysfunction characterized by tissue eosinophilia.**
**Objective: In patients with AD, we evaluated the effect of eosinophil depletion with benralizumab on markers of inflammation in skin after intradermal allergen challenge.**
**Methods: A total of 20 patients with moderate-to-severe AD completed a randomized, double-blind, placebo-controlled parallel-group study comparing 3 doses of benralizumab (30 mg each) administered subcutaneously every 4 weeks (n = 9) with placebo (n = 11). Allergen and saline control intradermal challenges were conducted before and after treatment, with skin biopsy samples collected 24 hours after challenge. Early and late cutaneous responses were measured by skin wheal size. Levels of eosinophils and IL-5 receptor-α–bearing cells, including eosinophil progenitor (EoP) cells, basophils, and mast cells, in papillary dermis were measured by immunofluorescence microscopy, and levels of EoP cells, hematopoietic progenitor cells, and type 2 innate lymphoid cells in the blood were measured by flow cytometry. Outcomes were compared between the placebo and benralizumab treatment groups by using the Mann-Whitney *U* test.**
**Results: Benralizumab reduced eosinophil counts in the blood ($P < .0001$) and allergen-challenged skin, as measured by hematoxylin and eosin staining and eosinophil cationic protein antibody concentration ($P < .05$). Benralizumab lowered the levels of EoP cells, mast cells, and basophils in the skin, as well as the levels of EoP cells, hematopoietic progenitor cells, and type 2 innate lymphoid cells in the blood (all $P < .05$). There was a trend toward improvement in the early cutaneous response ($P = .095$) but no effect on the late cutaneous response.**
**Conclusion: In patients with moderate-to-severe AD, benralizumab treatment significantly inhibited accumulation of eosinophils and other IL-5 receptor-α–expressing cells in the papillary dermis after intradermal allergen challenge. Targeting IL-5 receptor-α–positive cells did not modulate the size of the allergen-induced skin wheal (ClincialTrials.gov identifier NCT03563066). (J Allergy Clin Immunol Global 2024;3:100310.)**

***Key words:*** *Atopic dermatitis, benralizumab, eosinophil, CD125, intradermal allergen challenge*

Atopic dermatitis (AD) is a common skin disease that is characterized by chronic, relapsing skin inflammation and eczematous, itchy lesions caused by skin barrier dysfunction and type 2 (T2) cell-mediated immunity.[1] Eosinophil numbers as well as eosinophil granule protein levels in the blood are elevated in most patients with AD. Tissue eosinophilia, which is a feature of acute and chronic AD in addition to blood eosinophilia, appears to correlate with disease activity.[2]

IL-5 is a key cytokine involved in the differentiation and maturation of eosinophils from hematopoietic stem cells in the bone marrow, their mobilization and migration from the bone marrow to the blood, and their activation and survival in tissue.[3] The IL-5 receptor-α (IL-5Rα) is expressed on eosinophils and basophils, with expression on some neutrophils and innate lymphoid type 2 cells (ILC2s). In chronic allergic inflammatory diseases, eosinophilia may arise as a result of (1) the recruitment of mature cells from the periphery in response to locally elaborated chemoattractants such as eotaxin[4] and/or (2) the localized maturation of eosinophil lineage-committed progenitors, termed *in situ differentiation* in the presence of locally elaborated cytokines, namely, IL-5.[5,6] In this phase 2b study, we tested the hypothesis that eosinophils are crucial for the development of cutaneous response to intradermal allergen challenge.

## METHODS

### Patients

The eligible patients were men and women, aged 18 to 65 years, who had moderate-to-severe AD that was not adequately controlled with oral anti-inflammatory medications and from whom oral anti-inflammatory medications could be withheld for the duration of the study. They were required to have a positive result of skin prick testing to common aeroallergens and a positive late cutaneous response (LCR) to intradermal allergen challenge, which served as the study baseline. The protocol was approved by the institutional research ethics committee, and all patients provided written consent.

### Study design

This randomized, double-blind, parallel group, placebo-controlled study evaluated the effect of benralizumab on intradermal allergen–induced response when administered subcutaneously in 3 doses (30 mg each) every 4 weeks. Intradermal

From [a]the Department of Medicine, Division of Respirology, and [b]the Firestone Institute for Respiratory Health, McMaster University, Hamilton.

Received for publication February 14, 2024; revised May 8, 2024; accepted for publication May 16, 2024.
Available online July 22, 2024.

Corresponding author: Gail M. Gauvreau, PhD, McMaster University, HSC 3U31E, 1280 Main St West, Hamilton, ON, Canada. E-mail: gauvreau@mcmaster.ca.



2772-8293



https://doi.org/10.1016/j.jacig.2024.100310

*Abbreviations used*
AD: Atopic dermatitis
BAL: Bronchoalveolar lavage fluid
ECR: Early cutaneous response
ECP: Eosinophil cationic protein
EG2: Eosinophil cationic protein antibody
EDN/EPX: Eosinophil-derived neurotoxin
EoP: Eosinophil progenitor
HPC: Hematopoietic progenitor cell
ILC2: Type 2 innate lymphoid cell
IL-5R: IL-5 receptor
IL-5Rα: IL-5 receptor-α
LCR: Late cutaneous response
MBP: Major basic protein
T2: Type 2 inflammation

allergen and control saline challenges were performed before the start of treatment (on day 0) and after 3 doses of the drug (on day 64). At 24 hours after the intradermal challenges (on days 1 and 65), we sampled peripheral blood and obtained skin punch biopsy samples from intradermal challenge sites (Fig 1). The duration of benralizumab dosing and timing of end points was selected according to a previous study conducted in patients with mild-to-moderate asthma that demonstrated more than a 90% median reduction in airway eosinophil levels.[7] All clinical and laboratory procedures were conducted at McMaster University, Hamilton, Ontario, Canada.

## Study end points

The primary end point was the change in eosinophils per $mm^2$ of skin biopsy sample obtained from the site of intradermal allergen challenge, as measured 24 hours after challenge at baseline (day 1) and at day 65 after initiation of treatment. The secondary end points were the change in number of eosinophil progenitor (EoP) cells, basophils, and mast cells in the skin. The exploratory end points were changes in the frequency of hemopoietic cells, EoP cells, and ILC2s in the blood.

## Skin prick testing

A skin prick test using a standard panel of aeroallergen extracts identified those extracts to which each patient was sensitized. Allergen extracts, a positive control (1 mg/mL of histamine), and a negative control (0.9% saline) were applied to the patient's back by pricking the skin; the size of the wheal in the horizontal and vertical directions was then determined after 10 minutes. On the basis of the skin prick and allergen-specific IgE blood radioallergosorbent testing results, an allergen was selected for skin prick titration at dilutions from 2- to 256-fold; the dilution that resulted in a wheal size of 3 × 3 mm was selected for intradermal allergen challenge.

## Intradermal allergen challenge and skin sampling

The selected dilution of allergen was injected intradermally in a volume of 100 μL into 2 adjacent and standardized locations on the patient's back alongside a 100-μL diluent control (0.9% saline). The site chosen for intradermal challenge was a location alternate to the site of the skin prick test and did not include inflamed skin with ongoing visible AD. The size of the wheal during the early cutaneous response (ECR) was measured after 10 minutes. LCR was measured 24 hours after challenge, at which time a punch biopsy sample was taken from the center of each of the 3 challenged sites by using a sterile 4-mm skin punch.

## Immunofluorescence staining and microscopy

The skin biopsy samples from allergen- and diluent-challenged skin were fixed in formalin and embedded in paraffin blocks. Tissue sections were stained by using hematoxylin and eosin staining and indirect immunofluorescence microscopy for activated eosinophil cationic protein (ECP) (ECP antibody [EG2]), major basic protein (MBP), IL-5Rα (CD125), EoP cells (defined as $CD34^+CD125^+$ von Willebrand factor), basogranulin (2D7 antibody), and mast cells (tryptase), as detailed in the Supplementary Methods (see the Online Repository at www.jaci-global.org).

## Flow cytometry

Blood was collected for flow cytometry staining. Eosinophil counts were performed, and PBMCs were stained for the enumeration of hematopoietic progenitor cells (HPCs [$CD45^+CD34^+$]), EoP cells ($CD45^+CD34^+SSC^{low}CD125^+$), and ILC2s ($CD45^+Lin^-CD127^+CRTH2^+$) by flow cytometry, as detailed in the Supplementary Methods.

## Multiplex analysis of cytokines

One allergen-challenged punch skin biopsy sample was dissected and minced to generate fluid phase for measurement of chemoattractants, growth factors, type 2 cytokines, and alarmins by using the Meso-Scale Discovery human U-PLEX custom biomarker assay. Analyte concentrations were extrapolated from a standard curve. Missing values were assigned the group mean, and samples with analyte concentrations below the lower limit of detection were assigned a value of 50% of the lower limit of detection.

## Statistical analyses

On the basis of data from a previous study,[8] 6 patients in each treatment group were required to detect an 80% reduction in level of eosinophils with statistical significance set at $P < .05$. A sample size of 20 was chosen to allow for attrition and to increase the power for secondary and exploratory investigations. Dropout or withdrawn patients were replaced by using a new random treatment allocation. Statistical analysis was performed on all patients who completed assessments up to day 65 per protocol. The Mann-Whitney *U* test was used to compare pretreatment and posttreatment delta values between treatment groups for ECRs and LCRs as well as for cellular outcomes measured by microscopy, Meso-Scale Discovery assay, and flow cytometry.

For detailed clinical and laboratory methods, see the Supplementary Methods.

# RESULTS

## Study population

Of the 23 patients with AD who were screened, 21 met eligibility criteria and were randomized (Fig 1). One patient

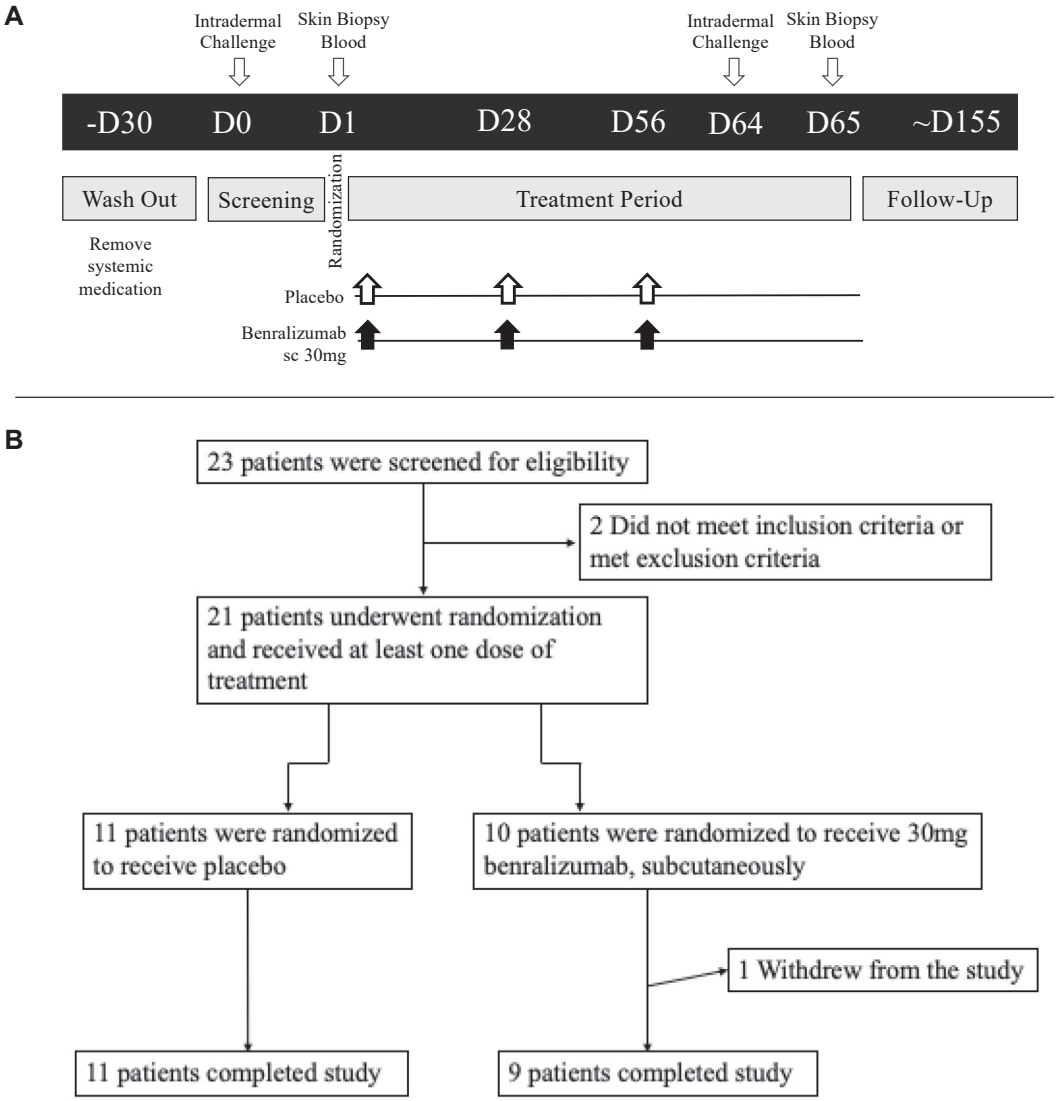


**FIG 1. A,** Study design. Patients were randomized 1:1 to receive benralizumab (30 mg subcutaneously monthly) or placebo on days 1, 28, and 56. Intradermal challenges were conducted on days 0 and 64, and samples of blood and skin were collected 24 hours after challenge on days 1 and 65. **B,** Consortium on Asthma among African-Ancestry Populations in the Americas (CONSORT) diagram summarizing the flow of patients through, randomization, treatment, and follow-up.

randomized to the benralizumab group withdrew from the study because of an increase in disease severity and was replaced, resulting in a total of 20 patients completing the study. Of those 20 patients, 11 had moderate AD and 9 had severe AD, as determined by Eczema Area and Severity Index. There were no significant differences in baseline variable demographic characteristics between the benralizumab and placebo groups (Table I). No study-related adverse or serious adverse events were reported during the study.

## Effect of intradermal allergen challenge on inflammatory cells in skin

Intradermal allergen challenge at baseline (day 1) significantly increased the numbers of eosinophils and basophils in the papillary dermis at 24 hours compared to the intradermal saline control ($P \leq .001$) (Fig 2). There was no effect of allergen on the number of mast cells.

## Effect of benralizumab treatment on eosinophil levels in the blood and skin

The blood eosinophil counts at day 1 before treatment in the placebo and benralizumab groups were similar, being 0.43 ($\pm$ 0.11) $\times$ $10^6$/mL and 0.41 ($\pm$ 0.12) $\times$ $10^6$/mL, respectively. After 65 days of treatment, there was a complete depletion of blood eosinophils in the benralizumab group versus no change in the placebo group ($P < .00001$) (Fig 2). In allergen-challenged skin on day 65, the number of eosinophils in the benralizumab group was significantly lower than in the placebo group, as assessed by hematoxylin and eosin– and EG2-stained cells ($P < .005$ in both cases) (Figs 2 and 3). Benralizumab treatment also significantly reduced the total number of allergen-induced

**TABLE I.** Baseline demographic and clinical characteristics of the patients who completed assessments up to day 65 after treatment initiation

| Characteristic | Total (N = 20) | Placebo (n = 11) | Benralizumab (n = 9) |
|---|---|---|---|
| Race, no. | | | |
| White | 14 | 9 | 5 |
| Black | 2 | 0 | 2 |
| Asian | 3 | 1 | 2 |
| South Asian | 1 | 1 | 0 |
| Age (y), mean (± SEM) | 39.5 (± 3) | 38.7 (± 4.2) | 40.9 (± 4.6) |
| Sex, no. | | | |
| Male | 9 | 4 | 5 |
| Female | 11 | 7 | 4 |
| Height (cm), mean (± SEM) | 171.3 (±2.1) | 168 (±2.9) | 175.2 (±2.8) |
| Weight (kg), mean (± SEM) | 139.3 (±49.4) | 127.9 (±15.3) | 123.3 (±16.9) |
| Age of AD diagnosis, mean (± SEM) | 11.6 (±3.8) | 11.6 (±55.3) | 11.6 (±5.7) |
| Disease severity | | | |
| EASI score, mean (± SEM) | 22.7 (±3.1) | 22 (±4.1) | 15.1 (±5) |
| Moderate AD, no. | 11 | 6 | 5 |
| Severe AD, no. | 9 | 5 | 4 |
| Blood eosinophil levels ($10^6$/mL), mean (± SEM) | 0.42 (±0.78) | 0.43 (±0.11) | 0.41 (±0.12) |

*EASI,* Eczema Area and Severity Index.

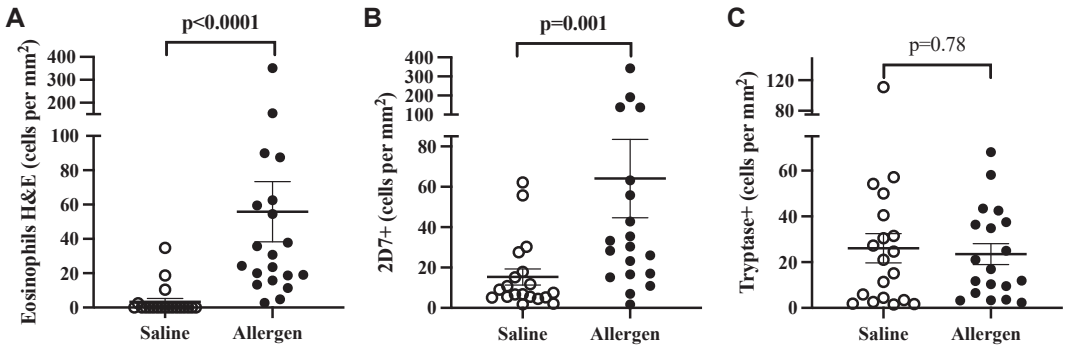


**FIG 2.** The effect of intradermal allergen challenge versus saline control challenge on the accumulation of eosinophils defined by hematoxylin and eosin (H&E) staining (**A**), basophils defined by $2D7^+$ immunofluorescence staining (**B**), and mast cells defined by $tryptase^+$ immunofluorescence staining in the papillary dermis of skin from all study patients sampled 24 hours after baseline challenge on day 1 (**C**). Individual and average (SEM) data are shown. Delta change from pretreatment to posttreatment measurements was compared between the benralizumab and placebo groups by using the Mann-Whitney *U* test.

$CD125^+$ cells ($P = .0003$) and $EG2^+CD125^+$ eosinophils ($P = .0076$) versus in the placebo group (Fig 3). The number of $MBP^+$ cells was lowered in all patients in the benralizumab group, although the number was not significantly different from that in the placebo group ($P = .32$); however, there was a significant reduction in cells double-positive for $MBP^+CD125^+$ ($P = .035$) versus in the placebo group (Fig 2). Representative staining is shown in Fig E1 (see the Online Repository at www.jaci-global.org). On day 65, benralizumab treatment had significantly reduced the numbers of EoP cells in allergen-challenged skin ($P = .0003$) and in the peripheral blood ($P = .026$) versus the numbers in the group that received placebo (Fig 2), but benralizumab did not change HPC frequency in the blood (see Fig E4 in the Online Repository at www.jaci-global.org). The low level of eosinophils present in the saline-challenged skin remained unaffected by benralizumab treatment (see Fig E2 in the Online Repository at www.jaci-global.org).

## Effect of benralizumab treatment on IL-5Rα–expressing cell populations

On day 65, benralizumab treatment significantly reduced the numbers of $2D7^+$ cells ($P = .0004$), $2D7+CD125^+$ double-positive cells ($P < .0001$), and $tryptase^+$ $CD125^+$ double-positive cells ($P = .013$), with a trend toward a significant decrease in numbers of $tryptase^+$- cells ($P = .065$) versus the numbers in the allergen-challenged skin from individuals who received placebo (Fig 4). Representative staining is shown in Fig E3 (see the Online Repository at www.jaci-global.org). In the saline-challenged skin there was no effect of benralizumab

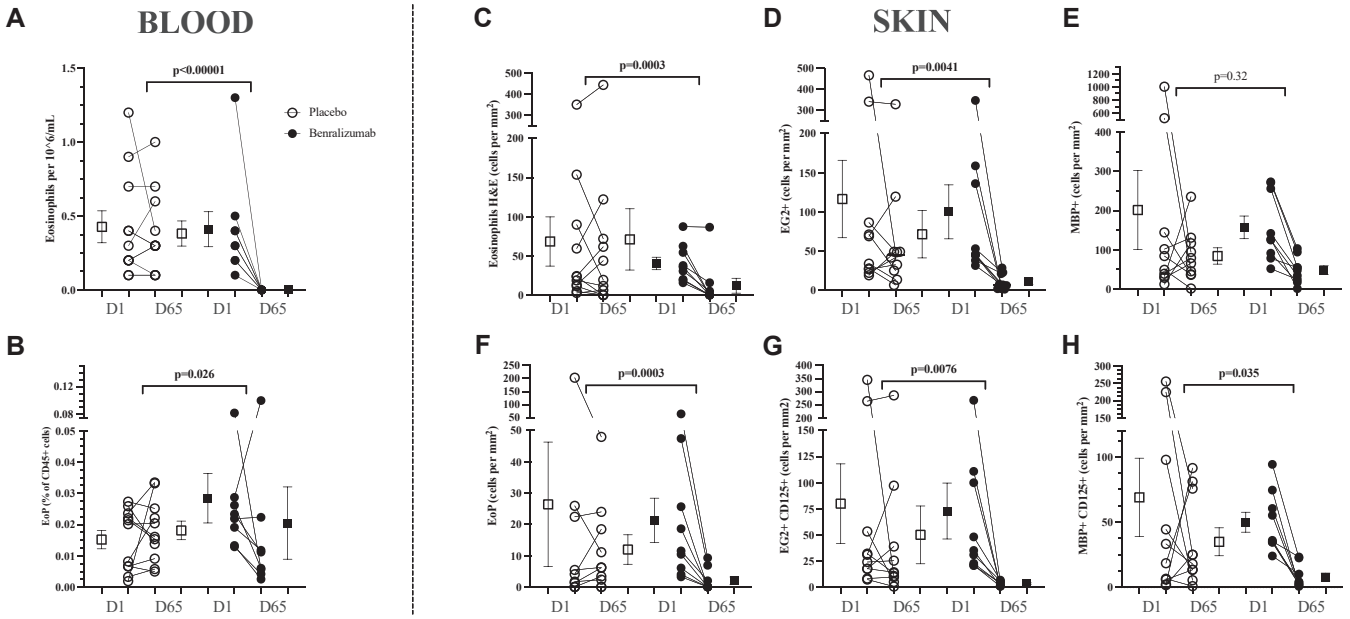


**FIG 3.** The effect of benralizumab versus placebo on blood eosinophils measured by complete blood count (**A**) and counts of EoP cells defined as $CD45^+CD34^+CD125^+$ cells according to flow cytometry staining (**B**). The effect of benralizumab versus placebo on eosinophils in the papillary dermis of skin biopsy samples, as measured by hematoxylin and eosin staining (**C**), $EG2^+$ immunofluorescence staining (**D**), $MBP^+$ immunofluorescence staining (**E**), EoP cells defined as $CD34^+CD125^+$ von Willebrand factor$^-$ immunofluorescence staining (**F**), eosinophils coexpressing $EG2^+CD125+$ immunofluorescence staining (**G**), and eosinophils coexpressing MBP+CD125 immunofluorescence staining (**H**). All samples were obtained at 24 hours after intradermal allergen challenge on day 1 before treatment and day 65 after treatment. Data are shown as individual data and means (SEMs). Delta change from pretreatment to posttreatment measurements was compared between the benralizumab and placebo groups by using the Mann-Whitey *U* test.

on the number of basophils or mast cells (see Fig E4). In blood samples on day 65, benralizumab treatment (as opposed to placebo) significantly reduced the frequency of ILC2s ($P = .0065$) and ILC2 positive for CD125 ($P = .0018$) (see Fig E4).

### Effect of treatment on cytokine measurements

In the biopsy sample supernatants from allergen-challenged skin on day 65, we observed a trend toward higher IL-5 levels in the benralizumab group than in the placebo group ($P = .067$) (see Fig E5 in the Online Repository at www.jaci-global.org), but there was no effect of benralizumab on the levels of eosinophil chemoattractants (cutaneous T-cell–attracting chemokine [CTACK] and eotaxin), growth factors (GM-CSF, IL-3, and IL-5), type 2 cytokines (IL-4 and IL-13), or alarmins (IL-25, IL-33, and TSLP).

### Effect of treatment on intradermal allergen–induced cutaneous wheal responses

Intradermal allergen challenge, compared with saline, induced a larger wheal during the ECR and LCR in both the benralizumab and placebo groups ($P < .05$ for all) (see Fig E6 in the Online Repository at www.jaci-global.org). There was a numeric, but not statistically significant, reduction in wheal size during the ECR in the benralizumab group versus in the placebo group ($P = .095$) but no effect of benralizumab on wheal size during the LCR ($P = .13$) (Fig 5). In addition, there was no effect of benralizumab on the results of skin prick testing in response to a panel of allergen extracts (see the Supplementary Methods).

## DISCUSSION

In this study, we found that treatment with the anti–IL-5Rα mAb benralizumab in patients with moderate-to-severe AD attenuates levels of eosinophils and EoP cells in the blood and inhibits allergen-induced increased levels of these cells in skin after intradermal allergen challenge. Our findings are consistent with the documented reduction of numbers of eosinophils in the circulation and tissues of patients with eosinophilic asthma after benralizumab treatment.[7,9,10] Similar to T2-driven allergic asthma, tissue eosinophilia has been shown to be a feature of both acute and chronic AD. Blood eosinophilia is present in most patients with AD and has been correlated with disease activity.[2] However, not all patients with AD have elevated levels of blood eosinophils.[2]

Eosinophils, in addition to mast cells and basophils, are thought to be one of the main effector cells activated during the allergic response. Eosinophils, EoP cells, and basophils are target cells of benralizumab due to their known expression of CD125. In skin, the proportion of eosinophils expressing CD125 was calculated to be 66% of $EG2^+$ cells and 36% of $MBP^+$ cells, whereas CD125 was expressed on 79% of basophils. Our data show that approximately 32% of mast cells in the skin and 32% of ILC2s in the blood express CD125, revealing novel targets for benralizumab. After 3 doses of benralizumab (30 mg each) every 4 weeks, the numbers of eosinophils, EoP cells,

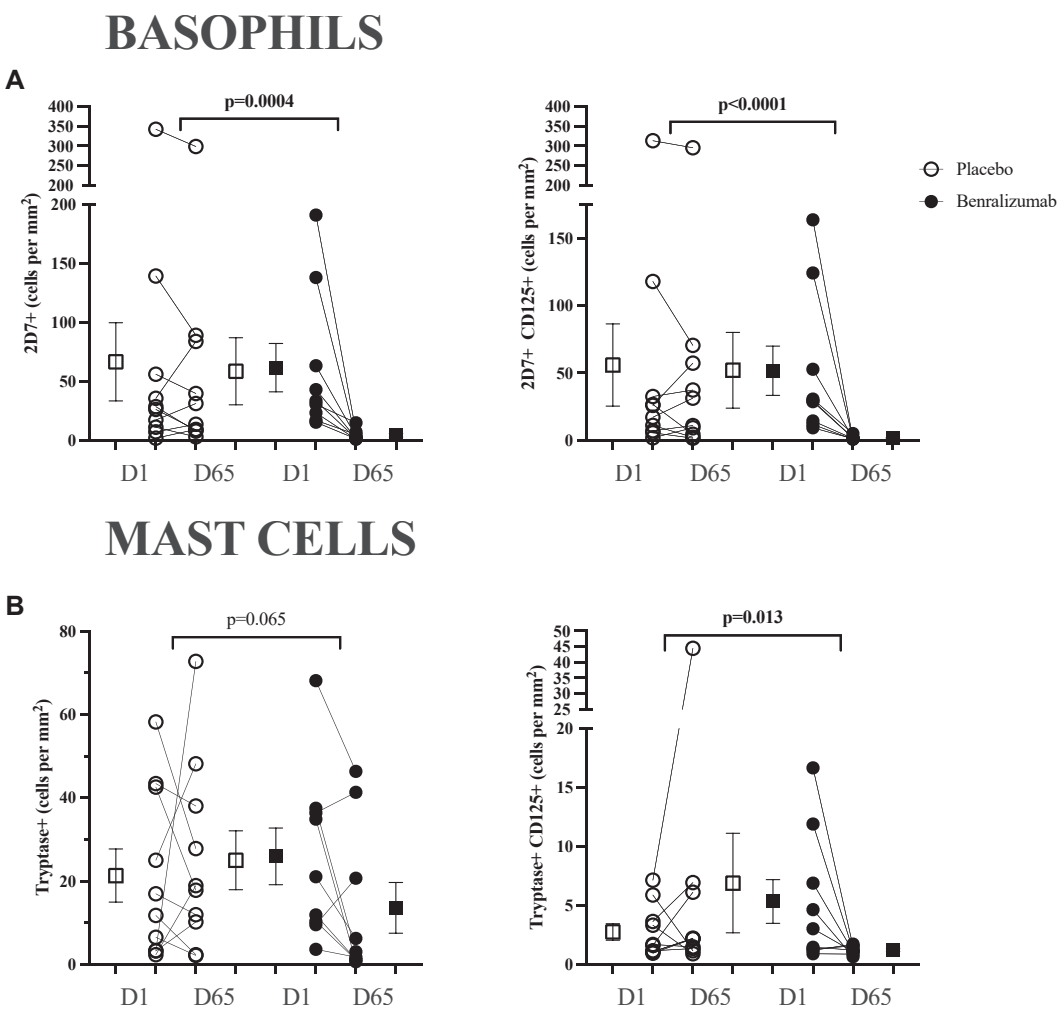


**FIG 4.** The effect of benralizumab versus placebo on basophils defined as 2D7$^+$ by immunofluorescence staining (**A**) and mast cells defined as tryptase$^+$ by immunofluorescence staining (**B**) with and without co-expression of CD125, measured in the papillary dermis of skin biopsy samples. All skin biopsy samples were obtained 24 hours after the intradermal allergen challenge on day 1 before treatment and day 65 after treatment. Individual and average (SEM) data are shown. Delta change from pretreatment to posttreatment measurements was compared between the benralizumab and placebo groups by using the Mann-Whitney *U* test.

basophils, and mast cells expressing IL-5Rα were significantly attenuated in allergen-challenged skin ($P < .05$); in addition, eosinophil, EoP cell, and ILC2 levels were significantly attenuated in blood.

The exact pathogenic role of eosinophils in skin inflammation of AD is still obscure. Eosinophils may contribute to edema through the release of granule proteins and the production of leukotrienes that have direct vasodilatory effect on blood vessel or indirect effect by stimulating mast cells and basophils.[11,12] ECP- and eosinophil-derived neurotoxin (EDN/EPX) exert cytotoxic effects on keratinocytes and cause cell matrix detachment.[13] Additionally, eosinophils may stimulate nerve cells and contribute to pruritus by releasing ECP, EDN/EPX, and MBP, as well as mediators such as substance P, vasoactive intestinal peptide, brain-derived neurotrophic factor, neurotrophin-3, nerve growth factor, and cytokines (such as IL-4, IL-13, and IL-31).[14-19]

To investigate the role of eosinophils during allergic responses in skin, we measured skin wheal size during the ECR at 10 minutes after intradermal allergen challenge and the LCR at 24 hours after intradermal allergen challenge before and after eosinophil depletion by benralizumab treatment. Although the study was underpowered for these end points, there was a trend toward a reduction in wheal size during the ECR with benralizumab treatment ($P = .0952$), which was likely an effect of the reduced mast cell and basophil numbers in the skin. There was no effect of benralizumab on the LCR to intradermal allergen challenge, suggesting that IL-5Rα–bearing cells are not critical for driving this response and aligning with results from a previous study showing that eosinophil depletion with mepolizumab in individuals with allergic asthma had no effect on the LCR to intradermal allergen challenge.[20] Our results also parallel the findings from inhaled allergen challenge studies in patients with allergic asthma, which also show no effect of eosinophil depletion by mepolizumab or benralizumab on the early or late asthmatic responses.[21,22] Curiously, despite the observed reduction in mast cell and basophil numbers in skin after benralizumab treatment,

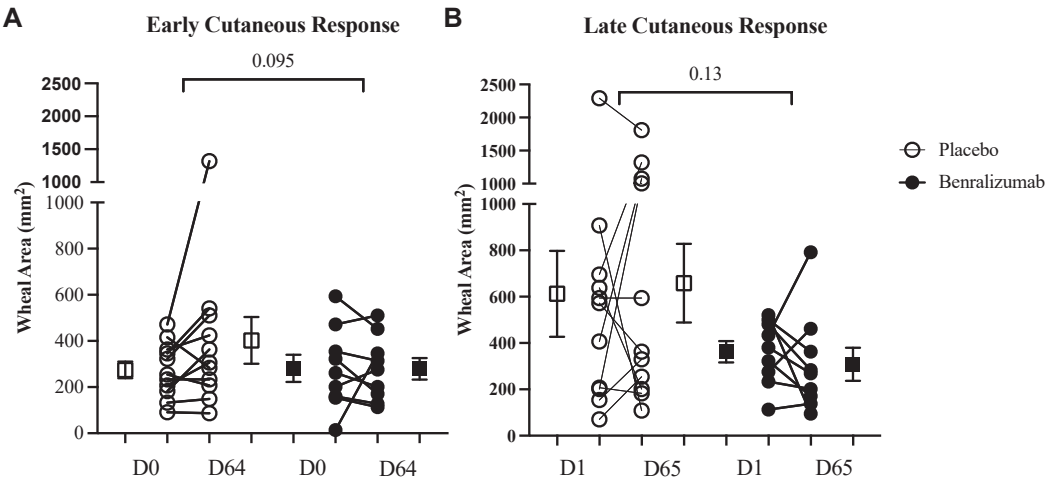


**FIG 5.** The effect of benralizumab versus placebo on skin wheal size (in $mm^2$) measured during the ECR (at 10 minutes on day 0 before treatment and day 64 after treatment) (**A**) and LCR (at 24 hours on day 1 before treatment and day 65 after treatment) (**B**) in response to intradermal allergen challenges conducted on day 0 before treatment and day 64 after treatment. Individual and average (SEM) data are shown. Delta change from pretreatment to posttreatment measurements was compared between the benralizumab and placebo groups by using the Mann-Whitney *U* test.

there was no difference in size of the wheals resulting from of the individual allergen extracts administered during skin prick testing. The lack of effect on skin wheal size suggests that either eosinophils are not critical in the pathogenesis of AD or there are IL-5–independent eosinophils (such as the $MBP^+$ population in the skin, which is not significantly reduced by benralizumab) that are contributing to the skin inflammation.

Levels of eosinophil degranulation of proteins such as MBP, ECP, and EDN/EPX are elevated in the peripheral blood of many patients with AD,[23] and dermal deposits of granular proteins in patients with AD have been demonstrated.[24] We found that the level of $EG2^+$ cells was significantly reduced with benralizumab treatment versus with placebo ($P = .0041$), whereas the level of $MBP^+$ cells was not reduced ($P = .32$). Interestingly, when $EG2^+$ cells and $MBP^+$ cells were assessed for coexpression with IL-5Rα (CD125), we observed a significant reduction in the levels of both EG2+CD125 cells ($P = .0076$) and $MBP^+CD125^+$ cells ($P = .035$) after benralizumab treatment. Our data show that not all eosinophils in the tissue express IL-5Rα (CD125), which aligns with reports of eosinophils from diseased tissue having downregulated IL-5 receptor levels. In asthmatic patients, compared with circulating eosinophils, eosinophils obtained from bronchial alveolar lavage fluid (BAL) have lower membrane IL-5Rα and CCR3 expression[25,26]; higher CCR4, CCR9, and CXCR3 expression[25]; and dampened degranulation.[26] This IL-5 independence can translate into only partial depletion of eosinophils in tissue, as observed in the airways of asthmatic patients after mepolizumab treatment,[27] and the inability to prevent activation of eosinophils in tissue, as reported by Kelly et al.[28] Specifically, Kelly et al demonstrated that BAL eosinophils after segmental allergen challenge had more surface IL-3R and GM-CSFR, more activation markers (CD69, CD44, and CD23), and less IL-5R and CCR3 than did blood eosinophils and that this activation phenotype, as well as the extensive EPX release by BAL eosinophils, was maintained after anti–IL-5 treatment.[28] The overall reduction in airway eosinophil numbers but limited effect on their activation markers demonstrated in this and other studies suggest there is a subpopulation of tissue eosinophils that are IL-5–independent. Although IL-5 has minimal effects on the properties of eosinophils in BAL, this cytokine supports formation of a heterogenous population of circulating eosinophils by partially activated $\beta_2$ , and dampening of the $\beta_2$-integrin function of eosinophils by therapies blocking IL-5 function may contribute to the decrease in airway inflammation observed after anti–IL-5 therapy.[29] Additionally, eosinophil recruitment to sites of inflammation could be regulated indirectly by other cytokines, including IL-13, through induction of the eosinophil chemoattractants eotaxin and RANTES.[30,31]

After benralizumab treatment, we observed a significant increase in IL-5 cytokine concentration within the tissue microenvironment of our patients with AD, as measured in the skin biopsy sample supernatants, which is consistent with our measurements in the blood and sputum of patients with mild allergic asthma after benralizumab treatment (Gauvreau GM et al, unpublished data, 2024), and also from measurements conducted in clinical trials of mepolizumab- and reslizumab-treated asthmatic patients.[32-34] Most of the IL-5 detected after anti–IL-5 mAb therapy is due to complexed IL-5 bound to an immunoglobulin such as mepolizumab itself and remains in circulation as a result of the prolonged half-life of the complex.[35] In contrast, benralizumab targets IL-5Rα–bearing cells through an antibody-dependent cell-mediated cytotoxicity mechanism of action; hence, we suggest that this accumulation of IL-5 is due to the reduction in cells expressing IL-5Rα and corresponding loss of potential receptor binding sites. In the skin samples, we did not observe an effect of benralizumab on the other growth factors (GM-CSF and IL-3), chemoattractants (CTACK and eotaxin), type 2 cytokines (IL-4 and IL-13), or alarmins (IL-25, IL-33, and TSLP) that we measured, suggesting that depletion of eosinophils does not regulate pathways leading to production of these cytokines.

These data have shown that benralizumab is effective at performing its designed function of targeting IL-5Rα and inducing antibody-dependent cell-mediated cytotoxicity in

IL-5Rα–bearing cells. Benralizumab was designed to target eosinophils through IL-5Rα; however, other important immune cells, including basophils, activated B cells, ILC2s, and (as shown in this study) mast cells also express IL-5Rα.[36-38] We saw a significant reduction in basophil numbers by identification of $2D7^+$ cells ($P = .0004$) and basophils expressing IL-5Rα identified as $2D7^+CD125^+$ cells ($P < .0001$) in the allergen challenge chskin. Eosinophils and mast cells have been linked together in the initiation of allergic inflammation, and the release of histamine and lipid mediators by mast cells has an important role in the recruitment of T2 lymphocytes and eosinophils to the site of inflammation and maintenance of the allergic process. Specifically, the histamine receptor H4 has been shown to mediate inflammation and pruritus in T2 skin inflammation in a murine model.[39] We observed a trend toward a reduction in mast cell numbers, as identified by $tryptase^+$ cells ($P = .065$), whereas the numbers of mast cells expressing IL-5Rα ($tryptase+CD125^+$ cells) were significantly reduced in allergen-challenged skin ($P = .013$). To our knowledge, this is the first description of mast cells expressing IL-5Rα. The magnitude of response to benralizumab could be dependent on the timing of sampling, especially for cell targets that could transiently express IL-5Rα.

Furthermore, we detected a significant reduction in the frequency of HPCs and ILC2s in the circulation, demonstrating the additional therapeutic benefit of benralizumab treatment for reducing T2 inflammation. The observed reduction in frequency of circulating ILC2s may be due not only to the direct effects of benralizumab but additionally to the downregulatory effect that depletion of eosinophils has on ILC2s. Either directly or indirectly, eosinophils promote the accumulation of activation of ILC2s, and in the absence of eosinophils, ILC2 function is diminished.[40,41]

In the current study, the overall reduction of levels of IL-5Rα–bearing cells by benralizumab, however, did not translate into a clinical effect based on skin wheal size, and this is in keeping with a larger clinical trial of patients with moderate-to-severe AD, in whom benralizumab did not improve IGA score (ClinicalTrials.gov identifier NCT04605094).[42] This lack of clinical response to benralizumab is in contrast to blockade of the IL-4/IL-13 pathway, which is efficacious for management of both AD and asthma[43] and likely reflects differences in the pathobiology of type 2 inflammation between the lung and skin, as well as the need for a broader approach for reducing inflammation in the skin of patients with AD.

In patients with moderate-to-severe AD, benralizumab was shown to effectively reduce the numbers of cells that express IL-5Rα. These patients were not required to have elevated blood eosinophil levels (≤300 μL) to be eligible for the study. Selecting patients on the basis of elevated blood eosinophil levels could provide a more favorable outcome. The lack of clinical response in skin wheal size in this patient population suggests that either those patients who would benefit most from depletion of IL-5Rα–bearing cells were not selected for this study or that IL-5R–dependent eosinophils may not play a critical role in the initiation and augmentation of allergic inflammation in the skin after intradermal allergen challenge.

## DISCLOSURE STATEMENT

Supported by AstraZeneca Canada (grant ESR-16-12430).

Disclosure of potential conflict of interest: P. O'Byrne reports research funding from AstraZeneca, Merk, and Biohaven paid to his institution; consulting fees from AstraZeneca, GSK, Sage, Teva, and Affibody; and lecture fees from AstraZeneca, Chiesi, and GSK. R. Sehmi reports research funding from AstraZeneca paid to her institution. G. M. Gauvreau reports research funding from AstraZeneca, Genentech, Third Harmonics Bio, and Biohaven paid to her institution, and lecture fees from AstraZeneca.

We would like to acknowledge funding from AstraZeneca Canada (grant ESR-16-12430). The funding source was not involved in the collection, analysis, or interpretation of the data. To ensure medical and scientific accuracy and protection of intellectual property, AstraZeneca reviewed the publications without influencing the opinions of the authors.

**Key messages**

- **Benralizumab treatment significantly attenuated the accumulation of eosinophils in the skin after intradermal allergen challenge, and it reduced levels of IL-5Rα–expressing cells, including EoP cells, basophils, and mast cells.**
- **The allergen-induced ECR measured by wheal size was numerically smaller with benralizumab treatment than with placebo, likely because of the reduction of skin mast cell and basophil levels.**
- **Incomplete depletion of eosinophils in skin suggests that the cells remaining in tissue are IL-5–independent. The IL-5Rα–expressing eosinophils that were depleted by benralizumab do not appear to play a critical role in the initiation and augmentation of allergic inflammation in the skin after intradermal allergen challenge.**

## REFERENCES

1. Bieber T. Atopic dermatitis. N Engl J Med 2008;358:1483-94.
2. Liu F-T, Goodarzi H, Chen H-Y. IgE, mast cells, and eosinophils in atopic dermatitis. Clin Rev Allergy Immunol 2011;41:298-310.
3. Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol 2009;101:81-121.
4. Wardlaw AJ. Molecular basis for selective eosinophil trafficking in asthma: a multistep paradigm. J Allergy Clin Immunol 1999;104:917-26.
5. Sehmi R, Denburg J. Differentiation of human eosinophils. Human Eosinophils 2000;76:29-44.
6. Sehmi R, Smith S, Kjarsgaard M, Radford K, Boulet LP, Lemiere C, et al. Role of local eosinophilopoietic processes in the development of airway eosinophilia in prednisone-dependent severe asthma. Clin Exp Allergy 2016;46:793-802.
7. Laviolette M, Gossage DL, Gauvreau G, Leigh R, Olivenstein R, Katial R, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. J Allergy Clin Immunol 2013;132:1086-96.e5.
8. Price E, Whetstone C, Al-Sajee D, Krisna SS, Howie K, Munoz C, et al. Prednisolone treatment reduces type 2 inflammation in skin lesions of patients with atopic dermatitis. J Allergy Clin Immunol 2021;147:AB35.
9. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet 2016;388:2115-27.
10. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2016;388:2128-41.

11. Leiferman KM, Peters MS. Eosinophil-related disease and the skin. J Allergy Clin Immunol Pract 2018;6:1462-82.e6.
12. Altman K, Chang C. Pathogenic intracellular and autoimmune mechanisms in urticaria and angioedema. Clin Rev Allergy Immunol 2013;45:47-62.
13. Amber KT, Chernyavsky A, Agnoletti AF, Cozzani E, Grando SA. Mechanisms of pathogenic effects of eosinophil cationic protein and eosinophil-derived neurotoxin on human keratinocytes. Exp Dermatol 2018;27:1322-7.
14. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol 2006;24:147-74.
15. Aliakbari J, Sreedharan SP, Turck CW, Goetzl EJ. Selective localization of vasoactive intestinal peptide and substance P in human eosinophils. Biochem Biophys Res Commun 1987;148:1440-5.
16. Johansson O, Liang Y, Marcusson JA, Reimert CM. Eosinophil cationic protein- and eosinophil-derived neurotoxin/eosinophil protein X-immunoreactive eosinophils in prurigo nodularis. Arch Dermatol Res 2000;292:371-8.
17. Lee JJ, Protheroe CA, Luo H, Ochkur SI, Scott GD, Zellner KR, et al. Eosinophil-dependent skin innervation and itching following contact toxicant exposure in mice. J Allergy Clin Immunol 2015;135:477-87.e1.
18. Numao T, Agrawal DK. Neuropeptides modulate human eosinophil chemotaxis. J Immunol 1992;149:3309-15.
19. Kroegel C, Giembycz M, Barnes P. Characterization of eosinophil cell activation by peptides. Differential effects of substance P, melittin, and FMET-Leu-Phe. J Immunol 1990;145:2581-7.
20. Phipps S, Flood-Page P, Menzies-Gow A, Ong YE, Kay A. Intravenous anti-IL-5 monoclonal antibody reduces eosinophils and tenascin deposition in allergen-challenged human atopic skin. J Investig Dermatol 2004;122:1406-12.
21. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000;356:2144-8.
22. Gauvreau G, Sehmi R, FitzGerald J, Leigh R, Cockcroft D, Davis B, et al. The effect of benralizumab on allergen-induced responses in subjects with mild allergic asthma. J Allergy Clin Immunol 2021;147:AB157.
23. Leiferman KM. A current perspective on the role of eosinophils in dermatologic diseases. J Am Acad Dermatol 1991;24:1101-12.
24. Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis: comparison with onchocerciasis. N Engl J Med 1985;313:282-5.
25. Liu LY, Jarjour NN, Busse WW, Kelly EA. Chemokine receptor expression on human eosinophils from peripheral blood and bronchoalveolar lavage fluid after segmental antigen challenge. J Allergy Clin Immunol 2003;112:556-62.
26. Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H, et al. Decreased expression of membrane IL-5 receptor α on human eosinophils: I. Loss of membrane IL-5 receptor α on airway eosinophils and increased soluble IL-5 receptor α in the airway after allergen challenge. J Immunol 2002;169:6452-8.
27. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti–interleukin-5 only partially depletes numbers in asthmatic airway. Am J Respir Crit Care Med 2003;167:199-204.
28. Kelly EA, Esnault S, Liu LY, Evans MD, Johansson MW, Mathur S, et al. Mepolizumab attenuates airway eosinophil numbers, but not their functional phenotype, in asthma. Am J Respir Crit Care Med 2017;196:1385-95.
29. Johansson MW, Gunderson KA, Kelly EA, Denlinger LC, Jarjour NN, Mosher DF. Anti-IL-5 attenuates activation and surface density of β2-integrins on circulating eosinophils after segmental antigen challenge. Clin Exp Allergy 2013;43:292-303.
30. Li L, Xia Y, Nguyen A, Lai YH, Feng L, Mosmann TR, et al. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. J Immunol 1999;162:2477-87.
31. Pope SM, Brandt EB, Mishra A, Hogan SP, Zimmermann N, Matthaei KI, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5–and eotaxin-dependent mechanism. J Allergy Clin Immunol 2001;108:594-601.
32. Pouliquen IJ, Kornmann O, Barton SV, Price JA, Ortega HG. Characterization of the relationship between dose and blood eosinophil response following subcutaneous administration of mepolizumab. Int J Clin Pharmacol Ther 2015;53:1015.
33. Tsukamoto N, Takahashi N, Itoh H, Pouliquen I. Pharmacokinetics and pharmacodynamics of mepolizumab, an anti–interleukin 5 monoclonal antibody, in healthy Japanese male subjects. Clin Pharmacol Drug Dev 2016;5:102-8.
34. Kim Y-J, Prussin C, Martin B, Law MA, Haverty TP, Nutman TB, et al. Rebound eosinophilia after treatment of hypereosinophilic syndrome and eosinophilic gastroenteritis with monoclonal anti–IL-5 antibody SCH55700. J Allergy Clin Immunol 2004;114:1449-55.
35. Roufosse FE, Kahn J-E, Gleich GJ, Schwartz LB, Singh AD, Rosenwasser LJ, et al. Long-term safety of mepolizumab for the treatment of hypereosinophilic syndromes. J Allergy Clin Immunol 2013;131:461-7.e5.
36. Smith SG, Chen R, Kjarsgaard M, Huang C, Oliveria J-P, O'Byrne PM, et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. J Allergy Clin Immunol 2016;137:75-86.e8.
37. Kolbeck R, Kozhich A, Koike M, Peng L, Andersson CK, Damschroder MM, et al. MEDI-563, a humanized anti–IL-5 receptor α mAb with enhanced antibody-dependent cell-mediated cytotoxicity function. J Allergy Clin Immunol 2010;125:1344-53.e2.
38. Sehmi R, Wood LJ, Watson R, Foley R, Hamid Q, O'Byrne PM, et al. Allergen-induced increases in IL-5 receptor alpha-subunit expression on bone marrow-derived CD34+ cells from asthmatic subjects. A novel marker of progenitor cell commitment towards eosinophilic differentiation. J Clin Invest 1997;100:2466-75.
39. Cowden JM, Zhang M, Dunford PJ, Thurmond RL. The histamine H4 receptor mediates inflammation and pruritus in Th2-dependent dermal inflammation. J Investig Dermatol 2010;130:1023-33.
40. LeSuer WE, Kienzl M, Ochkur SI, Schicho R, Doyle AD, Wright BL, et al. Eosinophils promote effector functions of lung group 2 innate lymphoid cells in allergic airway inflammation in mice. J Allergy Clin Immunol 2023;152:469-85.
41. Malik B, Bartlett NW, Upham JW, Nichol KS, Harrington J, Wark PA. Severe asthma ILC2s demonstrate enhanced proliferation that is modified by biologics. Respirology 2023;28:755-66.
42. Guttman-Yassky E, Bahadori L, Brooks L, Clark K, Grindebacke H, Ho C, et al. Lack of effect of benralizumab on signs and symptoms of moderate-to-severe atopic dermatitis: results from the phase 2 randomized, double-blind, placebo-controlled HILLIER trial. J Eur Acad Dermatol Venereol 2023;37:e1211-4.
43. Morita H, Matsumoto K, Saito H. Biologics for allergic and immunologic diseases. J Allergy Clin Immunol 2022;150:766-77.