

ORIGINAL ARTICLE

Effects of an Anti-TSLP Antibody on Allergen-Induced Asthmatic Responses

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ABSTRACT

BACKGROUND

Thymic stromal lymphopoietin (TSLP) is an epithelial-cell–derived cytokine that may be important in initiating allergic inflammation. AMG 157 is a human anti-TSLP monoclonal immunoglobulin G2 λ that binds human TSLP and prevents receptor interaction.

METHODS

In this double-blind, placebo-controlled study, we randomly assigned 31 patients with mild allergic asthma to receive three monthly doses of AMG 157 (700 mg) or placebo intravenously. We conducted allergen challenges on days 42 and 84 to evaluate the effect of AMG 157 in reducing the maximum percentage decrease in the forced expiratory volume in 1 second (FEV₁). We also measured the fraction of nitric oxide in exhaled air, blood and sputum eosinophils, and airway hyperresponsiveness. The primary end point was the late asthmatic response, as measured 3 to 7 hours after the allergen challenge.

RESULTS

AMG 157 attenuated most measures of allergen-induced early and late asthmatic responses. The maximum percentage decrease in the FEV₁ during the late response was 34.0% smaller in the AMG-157 group than in the placebo group on day 42 (P=0.09) and 45.9% smaller on day 84 (P=0.02). In addition, patients receiving AMG 157 had significant decreases in levels of blood and sputum eosinophils before and after the allergen challenge and in the fraction of exhaled nitric oxide. There were 15 adverse events in the AMG-157 group, as compared with 12 in the placebo group; there were no serious adverse events.

CONCLUSIONS

Treatment with AMG 157 reduced allergen-induced bronchoconstriction and indexes of airway inflammation before and after allergen challenge. These findings are consistent with a key role for TSLP in allergen-induced airway responses and persistent airway inflammation in patients with allergic asthma. Whether anti-TSLP therapeutics will have clinical value cannot be determined from these data. (Funded by Amgen; ClinicalTrials.gov number, NCT01405963.)

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ASTHMA IS A CHRONIC INFLAMMATORY disease of the airways that is characterized by recurrent episodes of wheezing, breathlessness, chest tightness, and cough. The cause of this disorder is multifactorial and is influenced by both genetic and environmental mechanisms,^{1,2} with environmental allergens as an important cause.^{2,3} Inhalation of allergens by patients with atopic asthma induces some of the manifestations of asthma, including reversible air-flow obstruction, airway hyperresponsiveness, and eosinophilic and basophilic airway inflammation. Allergen-inhalation challenge has become the predominant model for the evaluation of asthmalike responses in many species.^{4,5}

Thymic stromal lymphopoietin (TSLP) is an epithelial-cell–derived cytokine that is produced in response to proinflammatory stimuli and drives allergic inflammatory responses through its activity on a number of innate immune cells, including dendritic cells,^{6,7} mast cells,⁸ and CD34+ progenitor cells.⁹ Levels of human TSLP messenger RNA^{10,11} and protein¹¹ are increased in the airways of patients with asthma, as compared with controls, and the magnitude of this expression correlates with the severity of disease.^{10,11} Several studies have shown an association between a single-nucleotide polymorphism in the human TSLP locus and protection from asthma, atopic asthma, and airway hyperresponsiveness, suggesting that differential regulation of TSLP expression might influence disease susceptibility.^{1,12,13} These data suggest that targeting TSLP may inhibit multiple biologic pathways involved in asthma.

AMG 157 is a fully human anti-TSLP monoclonal immunoglobulin G2 λ that specifically binds human TSLP and prevents interaction with its receptor (Table S1 and Fig. S1 through S4 in the Supplementary Appendix, available with the full text of this article at NEJM.org). In this proof-of-concept study, we tested the hypothesis that AMG 157 would attenuate allergen-induced airway responses in patients with mild atopic asthma.

METHODS

PATIENTS

Eligible patients were nonsmoking men and women, 18 to 60 years of age, with mild, stable atopic asthma, as confirmed by positive results on a skin-prick test; a forced expiratory volume in 1 second

(FEV₁) of 70% or more of the predicted value; and airway hyperresponsiveness. For allergens with seasonal variation, patients were tested out of season for pollens affecting their asthma; all patients had no other lung disease. No asthma-controller treatments were allowed during the study, although the use of inhaled short-acting β_2 -agonists as rescue treatment administered fewer than 2 days per week was permitted. All other asthma medications were discontinued at least 4 weeks before enrollment. Patients were excluded from the study if they had worsening of asthma, respiratory-related visits to the emergency department within 6 weeks before study enrollment, previous use of AMG 157, or known sensitivity to any AMG 157 excipients.

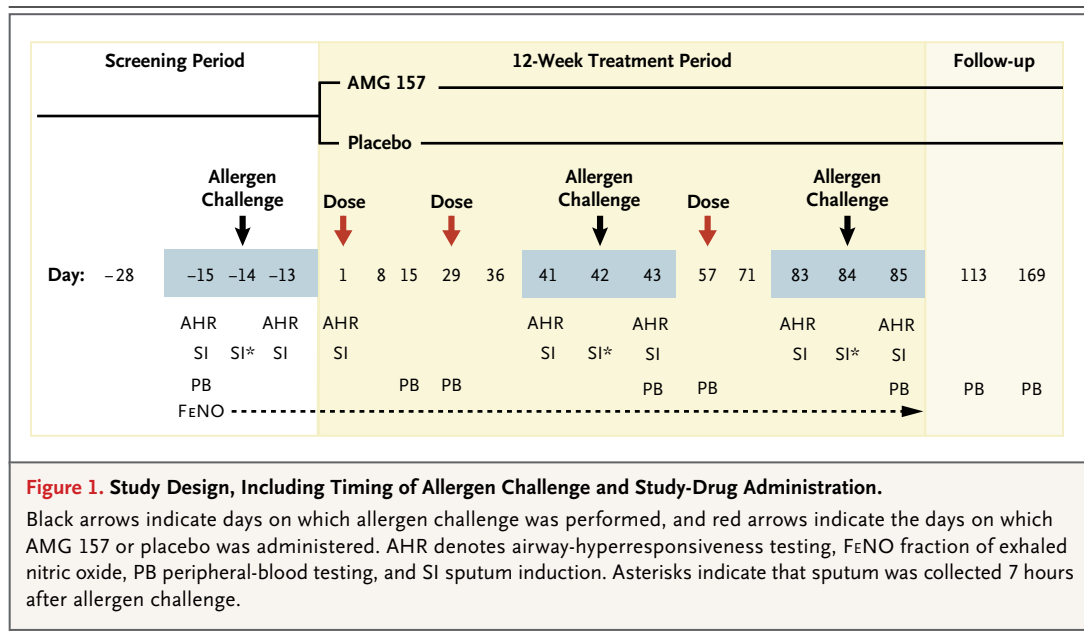
STUDY DESIGN

We conducted this proof-of-concept, randomized, double-blind, placebo-controlled study at five centers in Canada. Patients were randomly assigned, in a 1:1 ratio, by means of an interactive voice-response system to receive 700 mg of AMG 157 or placebo in a 1-hour intravenous infusion on study days 1, 29, and 57. We performed allergen and methacholine challenges and measured levels of the fraction of exhaled nitric oxide and blood and sputum eosinophils (Fig. 1).

STUDY END POINTS

The primary end point was the late asthmatic response, as measured 3 to 7 hours after the allergen challenge. The primary outcome measures that were used to evaluate the late response were the maximum percentage decrease in FEV₁ and the area under the curve (AUC) of the time-adjusted percent decrease in FEV₁.

The secondary end points were the late response, as measured by the minimum FEV₁ and the AUC of the time-adjusted minimum FEV₁; the early asthmatic response, as measured within 2 hours after the allergen challenge; and the safety, side-effect profile, and immunogenicity of AMG 157. Exploratory end points included the levels of sputum and blood eosinophils, the fraction of exhaled nitric oxide, levels of type 2 helper T (Th2) cells in the blood, the ratio of Th2 cells to type 1 helper T (Th1) cells in the blood, and total IgE levels in blood, along with the provocative concentration of methacholine required to reduce the FEV₁ by 20% (methacholine PC₂₀). Safety evaluations included the inci-



dence and severity of adverse events, changes in results on electrocardiography, laboratory profiles, vital signs, and the presence of anti-AMG 157 antibodies.

STUDY OVERSIGHT

The study protocol was approved by the institutional research ethics committee at each participating center, and all patients provided written informed consent. The study protocol, including the statistical analysis plan, is available at NEJM.org. All the authors had access to all the data and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the final protocol. The sponsor (Amgen) conducted the data analyses. The initial manuscript was drafted by the first two authors. All the authors were involved in the interpretation of the data and in the writing and editing of the manuscript with support from a professional medical writer funded by Amgen.

LABORATORY PROCEDURES

The allergen for inhalation was selected on the basis of results from skin-prick testing. The allergen challenge was performed as described previously.¹⁴ During a screening challenge conducted 14 days before the first dose of a study drug, patients inhaled allergen over a 2-minute period by means of tidal breathing from a Wright nebulizer (Roxon) filled with 2 to 3 ml of solution. This pro-

cess was repeated with doubled concentrations of allergen until there was a decrease of 20% or more in the FEV₁ at 10 minutes after the allergen challenge. We then measured the FEV₁ at regular intervals for 7 hours.

We calculated end points for the early asthmatic response (0 to 2 hours after allergen challenge) and the late asthmatic response (3 to 7 hours after allergen challenge). Selection of the allergen dose and methacholine challenges were performed as described previously.¹⁵ Venous blood was sampled for determination of levels of leukocytes, total IgE, and cytokines, and airway eosinophils were sampled from induced sputum with the use of a standard method.¹⁶ We measured the fraction of exhaled nitric oxide using American Thoracic Society guidelines.¹⁷

STATISTICAL ANALYSIS

On the basis of evidence from previous studies,¹⁸⁻²⁰ we determined that with 15 patients in each group, the study would have a power of 80% to detect an absolute between-group difference of 10 percentage points in the maximum percent decrease in the FEV₁ during the late response. We included in the analysis for each end point all patients who received at least one dose of AMG 157 or placebo. We evaluated early and late responses using a repeated-measures analysis of covariance (ANCOVA) that included study treatment and study visit as independent variables, treatment

according to study visit as an interaction term, and the corresponding baseline value (as measured 14 days before the first dose of a study drug) as a model covariate. At each study visit, we estimated and reported the mean between-group difference, the corresponding 95% confidence interval, and two-sided P values. Details of the analysis of the exploratory end points are provided in the Supplementary Appendix. The summary data are reported as means and standard errors, and log-transformed normally distributed end points are presented as geometric means with 95% confidence intervals.

RESULTS

STUDY POPULATION

From October 31, 2011, to April 5, 2013, we enrolled 31 patients (16 in the AMG-157 group and 15 in the placebo group). All the patients received at least one dose of a study drug in accordance with the randomization schedule. A total of 28 patients (90%) completed the full intervention period, and 27 (87%) completed the study (Fig. S5 in the Supplementary Appendix). Of the 4 patients who did not complete the study, 3 were lost to follow-up (1 in the AMG-157 group and 2 in the placebo group), and 1 patient in the AMG-157 group withdrew at day 34 owing to worsening of asthma. One patient in each group did not complete the allergen challenge on day 84, and one patient in the AMG-157 group withdrew from the allergen challenge before the late-response measurement. Demographic characteristics and rates of the use of inhaled allergens were similar in the two groups, and there were no significant differences between the two groups in any of the baseline variables (Table 1, and Table S2 in the Supplementary Appendix).

STUDY END POINTS

Early and Late Asthmatic Responses

Treatment with AMG 157, as compared with placebo, partially attenuated both the late response and the early response at days 42 and 84 in each of the four measures in the allergen challenge (Fig. 2, and Table S3 in the Supplementary Appendix). The maximum percentage decrease in the FEV₁ during the late response was 34.0% smaller in the AMG-157 group than in the placebo group on day 42 (P=0.09) and 45.9% smaller (a decrease of 11.7% vs. 21.6%) on day 84 (P=0.02).

Patients in the AMG-157 group, as compared

with those in the placebo group, had a significant increase in the minimum FEV₁ (P=0.01) and in the AUC of the time-adjusted minimum FEV₁ (P=0.02) during the late response on day 42 and in the minimum FEV₁ (P=0.01) on day 84. In addition, during the early response, the AUC of the time-adjusted percent decrease in the FEV₁ was significantly smaller and the AUC of the time-adjusted minimum FEV₁ significantly greater in the AMG-157 group than in the placebo group (P=0.03 for both comparisons) on day 42, and the AUC of the time-adjusted percent decrease in the FEV₁ was significantly smaller on day 84 (P=0.03) (Fig. 2, and Table S3 in the Supplementary Appendix).

Eosinophil Counts and Fraction of Exhaled Nitric Oxide

On day 29, the mean baseline blood eosinophil counts decreased from 296.5±40.2 per cubic millimeter to 121.9±14.7 per cubic millimeter in the AMG-157 group, as compared with a decrease from 281.1±57.2 per cubic millimeter to 224.1±36.5 per cubic millimeter in the placebo group (Fig. 3A). Blood eosinophil counts increased on days 43 and 85, 1 day after the allergen challenges; however, the levels were significantly lower in the AMG-157 group than in the placebo group (P=0.004).

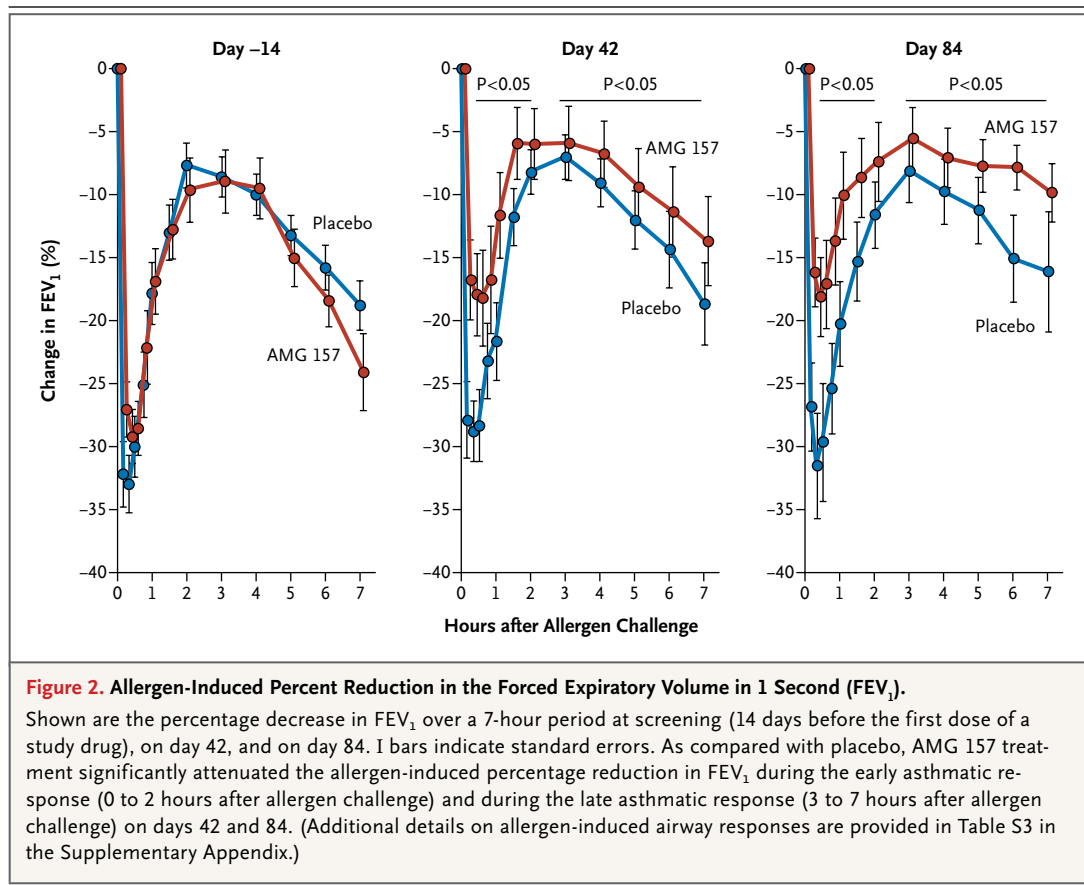
Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Placebo (N=15)	AMG 157 (N=16)
Age — yr	31.5±2.9	30.8±2.7
Female sex — no. (%)	11 (73)	10 (62)
White race — no. (%)†	13 (87)	14 (88)
Body-mass index‡	26.5±1.1	24.9±0.7
FEV ₁		
Value — liters	3.35±0.19	3.37±0.20
Percentage of the predicted value	97.6±3.9	95.4±3.3
Methacholine PC ₂₀ — mg/ml		
Geometric mean	1.87	1.31
95% CI	0.97–3.61	0.48–3.64
Fraction of exhaled nitric oxide — ppb	58.9±14.3	42.3±4.3
Sputum eosinophils — %	4.7±2.2	4.1±2.3
Blood eosinophils — per mm ³	281.1±57.2	296.5±40.2

* Plus-minus values are means ±SE. FEV₁ denotes forced expiratory volume in 1 second, and PC₂₀ provocative concentration of methacholine causing a decrease of 20% in the FEV₁.

† Race was reported by investigators, who designated three of the remaining study patients as Asian and one as “other.”

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.



Treatment with AMG 157 decreased levels of sputum eosinophils before and after allergen challenge. The mean sputum eosinophil level before allergen challenge was reduced from $4.1 \pm 2.3\%$ at baseline to $0.4 \pm 0.1\%$ on day 41 and $0.4 \pm 0.1\%$ on day 83. As compared with placebo, AMG 157 significantly reduced sputum eosinophil levels before allergen challenge over the course of the study ($P=0.02$) (Fig. 3B) and significantly attenuated allergen-induced changes 24 hours after challenge ($P=0.004$).

The fraction of exhaled nitric oxide was elevated in the two study groups under baseline conditions (Table 1). As compared with placebo, treatment with AMG 157 significantly decreased the fraction of exhaled nitric oxide throughout the study ($P=0.002$) and significantly attenuated allergen-induced changes 24 hours after challenge ($P=0.02$) (Fig. 3C).

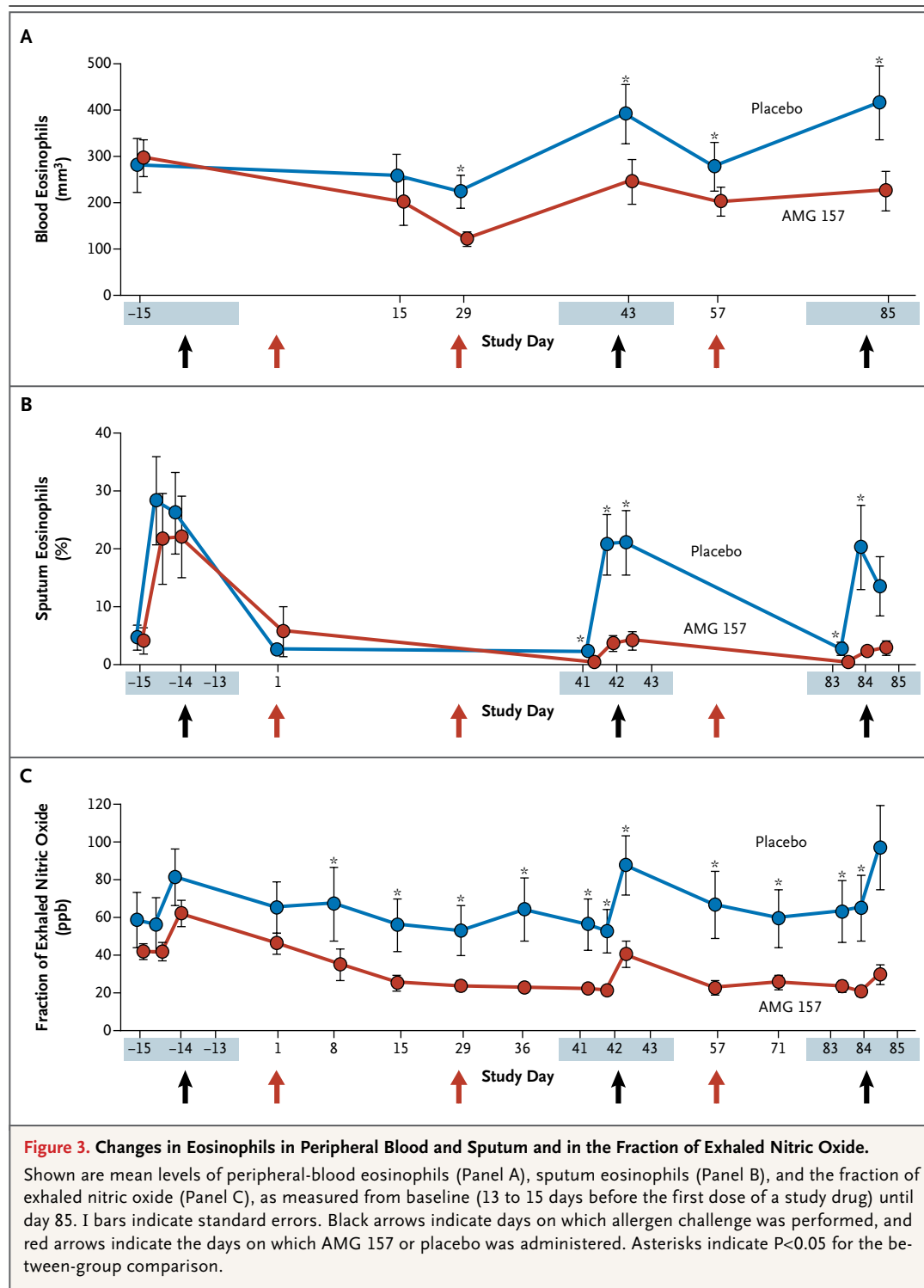
FEV₁ and Methacholine PC₂₀

Treatment with AMG 157 did not significantly change the FEV₁, as measured before allergen challenge on days 41 and 83 (Table S4 in the Supple-

mentary Appendix). There was a significant increase in methacholine PC₂₀ on day 83 in the AMG-157 group, as compared with the placebo group ($P=0.04$) (Table S5 in the Supplementary Appendix). The allergen-induced shift in methacholine PC₂₀ (as measured during the periods from day 41 to day 43 and from day 83 to day 85) was improved in the AMG 157 group, as compared with the placebo group, with a between-group treatment difference in the mean log₂ PC₂₀ of 0.76 mg per milliliter ($P=0.06$) during the former period and 0.49 mg per milliliter ($P=0.21$) during the latter period (Table S3 and Fig. S6 in the Supplementary Appendix).

Total IgE and Serum Markers

There was no significant effect of AMG 157 on levels of total IgE or the quantifiable serum markers in the HumanMAP, version 2.0, panel. Levels of interleukin-4, interleukin-5, interleukin-13, and tumor necrosis factor were below the level of quantitation in more than 95% of samples. The ratio of circulating Th2 cells to Th1 cells was numerically lower in the AMG-157 group



than in the placebo group, with the ratio 29% lower on day 41 and 13% lower on day 83, but the difference was not significant over the course of the study (Tables S6 and S7 in the Supplementary Appendix).

ADVERSE EVENTS

Treatment with AMG 157 was not associated with changes in measured laboratory values, temperature, blood pressure, pulse, or respiration. There were 15 adverse events in the AMG-157

group and 12 adverse events in the placebo group (Tables S8 and S9 in the Supplementary Appendix). There were no serious adverse events or deaths. One patient in the placebo group who had no previous exposure to AMG 157 tested positive for anti-AMG-157 antibodies on day 169.

DISCUSSION

In this study, we found that treatment with the monoclonal antibody AMG 157 in patients with stable allergic asthma attenuated measures of allergen-induced bronchoconstriction in both early and late asthmatic responses and also attenuated markers of systemic and airway inflammation. As such, our findings are consistent with the documented role of TSLP in allergen-induced airway responses in murine models.²¹ AMG 157 also reduced indexes of airway inflammation (the fraction of exhaled nitric oxide and sputum eosinophil levels), as well as levels of circulating eosinophils, for the duration of the study. It is not known whether these changes in eosinophils in sputum and blood helped to determine the subsequent changes in the FEV₁ or whether the changes were coexistent but not causal. This proof-of-concept study suggests that TSLP is a pivotal cytokine not only in allergen-induced airway responses but also in persistent airway inflammation in patients with allergic asthma.

TSLP has been identified as a “master switch” for allergic inflammation in murine models.²² Higher amounts of TSLP were produced in epithelial cells obtained from patients with asthma than in those obtained from controls,¹¹ and polymorphisms in *TSLP* have been associated with both childhood and adult allergic asthma.^{13,23} TSLP strongly induced the expression of human major histocompatibility complex I and II and costimulatory molecules such as CD40, CD80, and CD86 on myeloid dendritic cells.⁶ Induction of TSLP preceded the infiltration of dendritic cells into the skin during allergen-induced late cutaneous responses.²⁴ TSLP can also induce the production of human mast-cell Th2 cytokines.⁸ In addition, TSLP may play a role in virus-mediated processes.²⁵

TSLP is thought to cause airway and blood eosinophilia among patients with allergic asthma by activating airway dendritic cells and by increasing the numbers of Th2 cells, resulting in the production of proinflammatory cytokines, in-

cluding interleukin-5 and interleukin-13.²¹ TSLP has also been shown to influence the production of interleukin-5 and interleukin-13 from mast cells,⁸ CD34+ progenitor cells,⁹ and, most recently, type 2 innate lymphoid cells.²⁶ Inhibition of interleukin-5 has been shown to prevent allergen-induced airway eosinophilia,²⁷ a finding that supports the hypothesis regarding TSLP. Other airway epithelial-cell-derived cytokines, particularly interleukin-25 and interleukin-33, have also been implicated in allergen-induced airway inflammation in murine models,²⁸ but there is no direct evidence implicating them in allergic asthma in humans.

Epidemiologic evidence supports an important role for environmental allergens in the pathobiology of childhood asthma.²⁹ The inhalation of allergens by patients with allergic asthma results in many manifestations of asthma, including reversible airflow obstruction, airway hyperresponsiveness,³⁰ and eosinophilic and basophilic airway inflammation.³¹ Allergen inhalation challenge has been a valuable clinical model for the study of the mechanisms of allergic asthma and the evaluation of potential new treatments.^{20,32} However, allergen inhalation is not responsible for the development or persistence of asthma in many patients who have nonallergic disease or who have not been exposed to allergens. Therefore, the importance of TSLP in persistent airway inflammation in these patients cannot be extrapolated from the current study. However, pharmacologic attenuation of allergen-induced airway responses in patients with allergic asthma has been associated with effective asthma treatments, even for patients with nonallergic disease.⁵

Histamine and cysteinyl leukotrienes from airway mast cells and basophils contribute the major part of early and late asthmatic responses.³³ The late response is also caused by the allergen-induced influx of inflammatory cells, particularly basophils and eosinophils.^{31,33,34} Therefore, AMG 157 probably attenuates these responses through effects on both mast-cell activation and inflammatory-cell recruitment.

The patients in our study had stable allergic asthma, were not receiving regular maintenance treatment, and had nearly normal baseline pulmonary function. This population was chosen in order to avoid the potential modification of allergen-induced airway responses resulting from maintenance treatments (e.g., inhaled glucocor-

ticoids or leukotriene-receptor antagonists). As in other studies evaluating this population,^{20,32,33} the patients in our study had evidence of airway inflammation at the time of study enrollment, with an increased fraction of exhaled nitric oxide and levels of sputum eosinophils. The mechanism causing persistent airway inflammation in such patients is not known. Some may be regularly exposed to ubiquitous allergens, such as house-dust mites, but such exposure was identified in fewer than half the patients in our study (Table S2 in the Supplementary Appendix). Also, because the patients had nearly normal baseline FEV₁ values, it was not possible to observe improvement from the baseline FEV₁.

All currently available asthma treatments attenuate components of allergen-induced airway responses. However, only inhaled glucocorticoids attenuate baseline airway levels of the fraction of exhaled nitric oxide and eosinophils,³⁵ as well as allergen-induced increases in these measures.³⁶ This early proof-of-concept study indicates that targeting TSLP can reduce the fraction of exhaled nitric oxide and blood and airway eosinophils. Some patients with severe refractory asthma have persistent airway eosinophilia despite treatment with high-dose inhaled and oral glucocorticoids. Targeting of Th2-cell cytokines interleukin-5, interleukin-13, and interleukin-4 has improved several asthma measures. Among patients with severe refractory asthma, targeting of interleukin-5 reduced asthma exacerbations and allowed a reduction in maintenance doses of oral glucocorticoids.^{37,38}

These studies suggest that persistent airway eosinophilia is an important mechanism for some patients with severe refractory asthma. Antibodies directed against interleukin-13 have been shown to improve lung function in patients who

have asthma with a “Th2 phenotype,” as indicated by elevated levels of circulating periostin.³⁹ In addition, an antibody directed against interleukin-4 receptor α , the common component of the interleukin-4 and interleukin-13 receptors, allowed withdrawal of maintenance treatment with a combination of inhaled glucocorticoids and long-acting β_2 -agonists without a deterioration in asthma control.⁴⁰ The production of each of these proinflammatory cytokines may be a consequence of epithelial-cell production of TSLP and dendritic-cell activation, which suggests that targeting of TSLP may also provide benefit in these patient populations. Further clinical studies will be needed to evaluate this potential benefit.

In conclusion, treatment for 12 weeks with AMG 157 reduced the fraction of exhaled nitric oxide and blood and sputum eosinophils in patients with allergic asthma. This treatment also attenuated allergen-induced changes in these inflammatory measures, as well as the early and late asthmatic responses, and increased the methacholine PC₂₀. These results support further work on mechanisms of action and investigation of the clinical benefit of AMG 157 in patients with poorly controlled asthma.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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