

from concomitant staphylococcal infection that enhances their susceptibility to mite-induced disease.

The elegant demonstration of Barrett et al¹ on the increased leukotriene production induced by mites perfectly fits into the puzzle that we have been trying to solve for many years to explain the interactions between mite-induced allergy and NSAID hypersensitivity.¹² These observations might have important therapeutic implications in regard to the pharmacologic inhibition of leukotriene synthesis or blockade of leukotriene receptors. In that context it has been reported that the *LTC4S* allele is associated with a better response to leukotriene receptor antagonists,¹³ and a favorable response to such drugs was found in asthmatic children who had increased levels of urinary leukotriene E₄.¹⁴

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Nitric oxide in exhaled breath is poorly correlated to sputum eosinophils in patients with prednisone-dependent asthma

To the Editor:

Nitric oxide (NO) is a reactive molecule synthesized by NO synthases that are expressed by cells within the airway wall, and functionally, 2 isoforms exist: constitutive and inducible nitric oxide synthase (iNOS). In asthmatic patients the inducible isoform is overexpressed, leading to increased production of NO, which diffuses into the airway lumen, where it can be detected in exhaled breath. Fraction of exhaled nitric oxide (FeNO) is reported to be closely correlated to eosinophilic inflammation in the airways of patients with asthma^{1,2} and is recommended as a noninvasive marker to identify patients at risk for severe exacerbations³ and to monitor treatment that would decrease eosinophil numbers.⁴ Thus treatment algorithms for asthma have been developed to titrate the dose of inhaled corticosteroids based on FeNO levels.⁵ Such guidelines are most required for patients with asthma who require daily prednisone to control their eosinophilic bronchitis. The utility of monitoring FeNO levels in these patients is unknown. We report the lack of correlation between sputum eosinophil percentages and FeNO levels in patients with prednisone-dependent asthma and sputum eosinophilia who participated in a clinical trial of mepolizumab, a mAb against IL-5.

The study design, methods, and patient population have been described previously.⁶ Baseline characteristics were comparable (Table I). Patients with asthma and sputum eosinophil percentages of 3% or greater who required prednisone daily (median, 10 mg) to control their sputum eosinophilia were randomized to receive 5 monthly infusions of 750 mg of mepolizumab (n = 9) or matching placebo (n = 11). After the second infusion, the prednisone dose was reduced according to a predefined schedule until the predetermined dose reduction was achieved or the patient had a clinical exacerbation associated with an increase in eosinophil numbers in sputum. Quantitative cell counts in sputum and FeNO levels were measured at baseline (visit 1), after the second infusion (visit 4), and at 4 weeks after the last infusion (visit 12) or at an exacerbation when the patient was withdrawn from the study. Induced sputum selected from saliva was processed and examined as described by Pizzichini et al.⁷ NO in exhaled air was measured according to American Thoracic Society (ATS) guidelines⁸ at a flow of 50 L/min by using the NIOX analyzer (Aerocrine AB, Solna, Sweden). The correlations between eosinophil and neutrophil percentages in sputum and FeNO levels at baseline and at the end of the study were examined by using the Spearman test.

As previously reported,⁶ a single infusion of mepolizumab was associated with a reduction in the percentage of eosinophils to within normal limits in sputum (median of 16.6% reduced to a median of 0%, *P* = .005). The levels remained within normal limits after reductions in the dose of prednisone for up to 8 weeks after the last infusion of mepolizumab (median, 1.3%; *P* = .01). In comparison, a reduction in the dose of prednisone in the placebo group was associated with a significant increase in the number of eosinophils in sputum.

In contrast, mepolizumab treatment was not associated with significant changes in FeNO. The mean values at baseline, visit 4, and visit 12 were 25.1 ppb (SD, 7.6), 19.5 ppb (SD, 9.5), and 22.9 ppb (SD, 5.0), respectively. The corresponding values in the

TABLE I. Baseline subjects' characteristics

	Mepolizumab (n = 9)	Placebo (n = 11)
Atopy (n)	3	5
>10 Pack-years	2	2
Exacerbations in past 3 y (n)*	7 (3-19)	9 (4-19)
Serum IgE (IU/L)	60 (24)	78 (44)
Δ FEV ₁ after bronchodilator (%)†	28.4 (12.0)	24.6 (10.6)

Values are shown as means (SDs). Atopy was defined by a wheal 3 mm or larger than that elicited by the control to at least 1 allergen skin prick test.

*Median (minimum-maximum).

†Maximum bronchodilator response in the past 3 years.

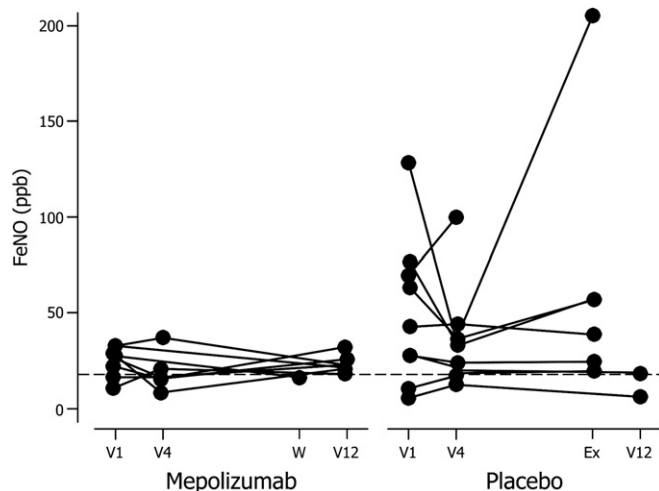


FIG 1. Change in FeNO levels after treatment with mepolizumab or placebo. Ex, Exacerbation; V, visit; W, subject withdrawn because of adverse effect.

placebo treatment arm were 50.7 ppb (SD, 36.7), 35.3 ppb (SD, 26.2), and 52.6 ppb (SD, 64.3), respectively (Fig 1). There was poor correlation between FeNO levels and sputum eosinophil and neutrophil percentages both at baseline and at visit 12 (Fig 2).

These observations illustrate that there is dissociation between the eosinophil percentage in sputum and FeNO levels in patients with asthma who require daily prednisone to control their sputum eosinophilia. This is consistent with the observations of Lemi re et al⁹ in a cross-sectional study of patients with moderate and severe asthma and those of Haldar et al¹⁰ in patients with moderate-to-severe asthma after treatment for 1 year with intravenous mepolizumab. It is possible that there might be a better correlation between FeNO level and the number of eosinophils in the airway mucosa⁹; however, luminal eosinophil numbers are reported to have better correlation to clinical asthma control than mucosal eosinophil numbers.⁹ These studies that have evaluated FeNO levels in response to a therapy specifically directed against eosinophil recruitment suggest that the previously observed response to corticosteroids^{11,12} might not be directly related to a decrease in eosinophil numbers by corticosteroids but rather an effect on epithelial iNOS activity that is a major determinant of FeNO level.¹³ It is intriguing why FeNO levels did not increase when the dose of prednisone was reduced in this study. Another

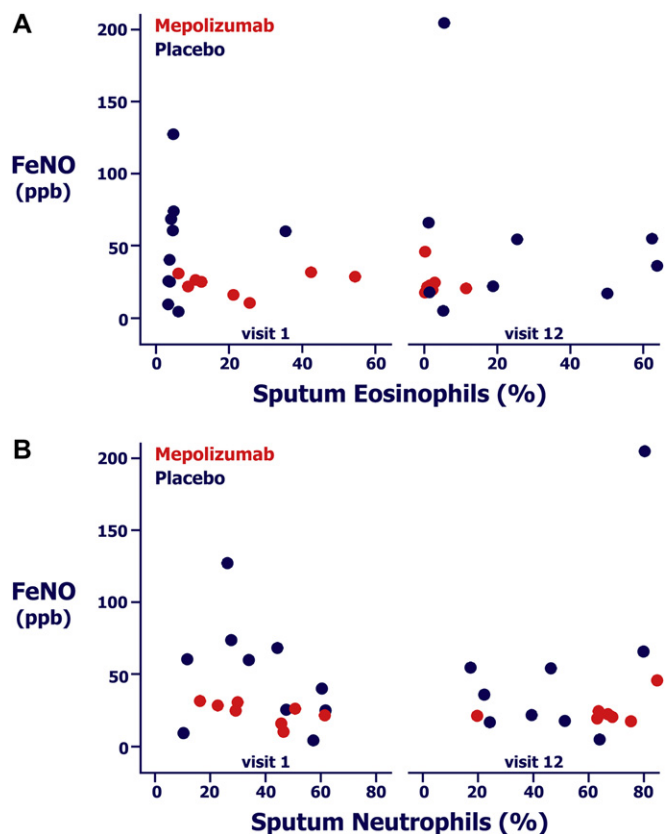


FIG 2. A, Correlation between FeNO levels and sputum eosinophil percentages. The left panel shows correlation at baseline (visit 1), and the right panel shows the correlation at visit 12. B, Correlation between FeNO levels and sputum neutrophil percentages. The left panel shows correlation at baseline (visit 1), and the right panel shows correlation at visit 12.

possible explanation for the lack of correlation between the 2 variables might be related to the source and localization of FeNO. The exhaled NO signal that is likely to be a balance between iNOS activity and S-nitrosogluthathione reductase activity¹⁴ can be partitioned into airway and alveolar components.¹⁵ It is likely that there might be better correlation between the alveolar portion of exhaled NO and airway eosinophil numbers that we were not able to identify in this study.

In summary, our observations demonstrate the limitation of using FeNO levels to evaluate and monitor therapy of eosinophilic bronchitis in patients with prednisone-dependent asthma.

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Comparison of the allergic and nonallergic CD4⁺ T-cell responses to the major dog allergen Can f 1

To the Editor:

Although it is well established that pathogenic T_H2-deviated CD4⁺ T-cell responses dominate in individuals with allergy, the nature of allergen-specific CD4⁺ T-cell responses in individuals without allergy exposed to the same allergens is still largely unclear. A number of nonexclusive mechanisms, such as immune ignorance of nonpathogenic environmental antigens, anergy, or deletion of allergen-specific T cells, and immune deviation to T_H0/T_H1 phenotype, have previously been proposed to explain the tolerance to allergens in these individuals. In recent years, however, the role of regulatory T (Treg) cells in the induction and maintenance of peripheral tolerance to allergens has been emphasized.

At least 2 distinct subsets of Treg cells exist in human beings: CD4⁺CD25⁺forkhead box protein 3 (Foxp3)⁺ Treg cells and IL-10-producing type 1 Treg (Tr1) cells. Several recent studies have

convincingly demonstrated that immune tolerance induced by specific immunotherapy is mainly mediated by Tr1 cells¹⁻³. However, whether Treg cells play a dominant role in maintaining tolerance to common respiratory allergens in healthy subjects without allergy is less clear. The seminal studies of Ling et al⁴ and Akdis et al⁵ suggested that immune tolerance to respiratory allergens is maintained by CD4⁺CD25⁺Foxp3⁺ Treg cells or Tr1 cells, respectively. However, more recent studies have not been able to corroborate these findings convincingly, challenging the dominant role of Treg cells in the healthy immune response against respiratory allergens.^{3,6-8}

In the current study, we aimed to dissect the factors associated with an allergic or nonallergic CD4⁺ T-cell response to the major dog allergen, Can f 1. To this end, we analyzed Can f 1-specific proliferative responses of peripheral blood CD4⁺ T cells of 15 subjects with dog allergy and 11 healthy nonallergic dog owners by using the carboxyfluorescein succinimidyl ester (CFSE) dilution assay (Fig 1, A; see this article's **Methods** in the Online Repository at www.jacionline.org).

The proliferative responses in the Can f 1-stimulated cultures from subjects with allergy were weak but statistically significantly stronger than those in unstimulated cultures (Fig 1, B). No proliferative responses were observed in Can f 1-stimulated cultures from subjects without allergy. Robust proliferative responses to the control antigens streptokinase and purified protein derivative (PPD) were detected in the cultures from both the subjects with and without allergy (see this article's Fig E1 in the Online Repository at www.jacionline.org; data not shown).

Next, we investigated whether suppression by CD4⁺CD25⁺Foxp3⁺ Treg cells explains the absence of proliferative responses to Can f 1 in subjects without allergy. No differences in the frequencies of CD4⁺CD25⁺Foxp3⁺ or CD4⁺CD25^{hi}Foxp3⁺ cells were detected in the PBMCs of subjects with and without allergy (see this article's Fig E2 in the Online Repository at www.jacionline.org). To evaluate the role of CD4⁺CD25⁺Foxp3⁺ Treg cells at the functional level, we used anti-CD25-magnetic beads to specifically deplete CD25^{hi} cells from PBMCs (Fig E2). After the CD25 depletion, there was a general increase in proliferative responses in the cultures. However, the proliferative responses in Can f 1-stimulated cultures from the subjects with allergy remained statistically significantly stronger than those in unstimulated cultures (Fig 1, C). In contrast, the specific proliferative responses to Can f 1 in the cultures from the subjects without allergy were not enhanced by the CD25 depletion. The specific proliferative responses to streptokinase were not affected by the CD25 depletion (Fig E1).

To evaluate whether suppression by IL-10-producing Tr1 cells contributes to the absence of proliferative responses to Can f 1 in subjects without allergy, we stimulated PBMCs with Can f 1 or streptokinase in the presence or absence of neutralizing levels of anti-IL-10 antibodies. No effect on the proliferative responses to Can f 1 (Fig 1, D) or streptokinase (Fig E1) was observed in the subject groups either with or without allergy. Moreover, only low levels of soluble IL-10 could be detected in the supernatants of either undepleted or CD25-depleted Can f 1-stimulated cultures, and these levels did not differ between subjects with and without allergy (data not shown). Collectively, our current findings do not support the notion that suppression by either CD4⁺CD25⁺Foxp3⁺ Treg cells or IL-10-producing Tr1 cells explains the absence of proliferative responses to Can f 1 in subjects without allergy.