

ORIGINAL ARTICLE

## Evidence of a Role of Tumor Necrosis Factor $\alpha$ in Refractory Asthma

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### ABSTRACT

#### BACKGROUND

The development of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) antagonists has made it feasible to investigate the role of this cytokine in refractory asthma.

#### METHODS

We measured markers of TNF- $\alpha$  activity on peripheral-blood monocytes in 10 patients with refractory asthma, 10 patients with mild-to-moderate asthma, and 10 control subjects. We also investigated the effects of treatment with the soluble TNF- $\alpha$  receptor etanercept (25 mg twice weekly) in the patients with refractory asthma in a placebo-controlled, double-blind, crossover pilot study.

#### RESULTS

As compared with patients with mild-to-moderate asthma and controls, patients with refractory asthma had increased expression of membrane-bound TNF- $\alpha$ , TNF- $\alpha$  receptor 1, and TNF- $\alpha$ -converting enzyme by peripheral-blood monocytes. In the clinical trial, as compared with placebo, 10 weeks of treatment with etanercept was associated with a significant increase in the concentration of methacholine required to provoke a 20 percent decrease in the forced expiratory volume in one second (FEV<sub>1</sub>) (mean difference in doubling concentration changes between etanercept and placebo, 3.5; 95 percent confidence interval, 0.07 to 7.0;  $P=0.05$ ), an improvement in the asthma-related quality-of-life score (by 0.85 point; 95 percent confidence interval, 0.16 to 1.54 on a 7-point scale;  $P=0.02$ ), and a 0.32-liter increase in post-bronchodilator FEV<sub>1</sub> (95 percent confidence interval, 0.08 to 0.55;  $P=0.01$ ).

#### CONCLUSIONS

Patients with refractory asthma have evidence of up-regulation of the TNF- $\alpha$  axis. (ClinicalTrials.gov number, NCT00276029.)

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THE RATES OF DEATH AND COMPLICATIONS are high among patients with refractory asthma and account for a disproportionate amount of the health resource burden attributed to asthma.<sup>1</sup> Treatment options are limited for these patients. The airway abnormality in refractory asthma differs from that in mild-to-moderate asthma in having a more heterogeneous pattern of inflammatory response,<sup>2</sup> with greater involvement of neutrophils<sup>3</sup> and the distal lung<sup>4</sup> and increased airway remodeling.<sup>5</sup> Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a pleiotropic inflammatory cytokine expressed in increased amounts by mast cells<sup>6</sup> and present in increased concentrations in bronchoalveolar fluid from the airways of patients with asthma.<sup>7</sup> It has a number of properties that might be relevant to refractory asthma.<sup>8-10</sup> Interest in the role of TNF- $\alpha$  in refractory asthma has been increased by a study showing increased concentrations of TNF- $\alpha$  in bronchoalveolar-lavage fluid from patients with more severe asthma<sup>11</sup> and by an uncontrolled study showing that treatment with the recombinant soluble TNF- $\alpha$  receptor etanercept markedly improved airway hyperresponsiveness in patients with refractory asthma.<sup>11</sup>

The biologic activity of TNF- $\alpha$  is mediated by the 26-kD transmembrane precursor protein<sup>12</sup> (membrane-bound TNF- $\alpha$ ) as well as the 17-kD cleavage product, free TNF- $\alpha$ .<sup>13</sup> This cleavage is principally mediated by TNF- $\alpha$ -converting enzyme,<sup>14</sup> and the free TNF- $\alpha$  subsequently forms highly active homotrimers,<sup>15</sup> which interact with two distinct TNF- $\alpha$  receptors on the cell surface.<sup>16</sup> The bioactivity of TNF- $\alpha$  is therefore likely to be reflected by increases in membrane-bound TNF- $\alpha$ , TNF- $\alpha$ -converting enzyme, free TNF- $\alpha$ , cell-surface receptors, and soluble receptors. We tested the hypothesis that the TNF- $\alpha$  axis is up-regulated in patients with refractory asthma by measuring the expression of membrane-bound TNF- $\alpha$ , TNF- $\alpha$  receptors, and TNF- $\alpha$ -converting enzyme by peripheral-blood monocytes. We also performed a small, randomized, double-blind, placebo-controlled, crossover pilot study of the effects of treatment with etanercept, an agent active against TNF- $\alpha$ , on airway hyperresponsiveness and the asthma-related quality of life in patients with refractory asthma.

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 METHODS
 

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**SUBJECTS**

All patients with asthma had clinical features consistent with the presence of asthma and at least one of the following objective measures of airway hyperresponsiveness and variable airflow obstruction: the concentration of methacholine required to provoke a 20 percent decrease (PC<sub>20</sub>) in the forced expiratory volume in one second (FEV<sub>1</sub>) was less than 8 mg per milliliter, the FEV<sub>1</sub> increased by at least 15 percent after the inhalation of 200  $\mu$ g of albuterol, or the variation in peak flow, expressed as a percentage of the mean, exceeded 20 percent over a period of 14 days.

Patients with refractory asthma, recruited from the difficult-asthma clinic at Glenfield Hospital, Leicester, United Kingdom, met the criteria of the American Thoracic Society for this condition,<sup>17</sup> with the exception that the daily dose of inhaled corticosteroids required to meet the definition was modified to more than 2000  $\mu$ g of beclomethasone or its equivalent to reflect European practice.<sup>4</sup> Patients met at least one major and two minor criteria for refractory asthma; all were considered to be compliant with treatment. Our assessment was based on the measurement of serum prednisolone, cortisol, and theophylline concentrations; an assessment at each patient's home by a consultant pharmacist; and an analysis of primary care records on the issuing and filling of prescriptions. We excluded patients who were thought to be symptomatic because of uncontrolled coexisting conditions such as rhinitis and gastroesophageal reflux disease. Patients were also excluded if they had any of the following: recent contact with a patient with pulmonary tuberculosis, a personal history of tuberculosis, any radiologic features suggestive of current or previous tuberculosis, or a grade III or IV tuberculin (Heaf) test.

All patients classified as having mild-to-moderate asthma met the Global Initiative for Asthma<sup>18</sup> criteria for intermittent or mild persistent asthma. All patients with refractory asthma met the Global Initiative for Asthma criteria for severe persistent asthma.

Control subjects had no respiratory symptoms,

had normal spirometric values, and had a PC<sub>20</sub> of more than 16 mg of methacholine per milliliter. All controls and patients with asthma were currently nonsmokers, with a smoking history of less than 5 pack-years, and none reported having a lower respiratory tract infection in the three months before the study. All subjects provided written informed consent to participate in the study. The protocol was approved by the Leicester-shire and Rutland regional ethics committee.

#### MEASUREMENTS

Peripheral-blood monocytes were separated from 20 ml of peripheral blood that had been treated with 500 U of heparin with the use of a density-gradient method (Histopaque 1077, Sigma). Cells were counted, incubated with antibodies labeled with fluorochrome (phycoerythrin or fluorescein isothiocyanate [FITC]) targeting CD14 (phycoerythrin), TNF receptor 1 (FITC), TNF receptor 2 (FITC), TNF- $\alpha$ -converting enzyme (FITC), or membrane-bound TNF- $\alpha$  (FITC) (all from R&D) or an equal concentration of an isotype-matched control — mouse IgG<sub>1</sub> (phycoerythrin), mouse IgG<sub>2A</sub> (FITC), or mouse IgG<sub>1</sub> (FITC) (all from R&D) — and subjected to cytometry with the use of a laser flow cytometer (FACScan, Becton Dickinson) as described previously.<sup>19</sup> The geometric mean fluorescence for membrane-bound TNF- $\alpha$ , TNF receptor 1, and TNF- $\alpha$ -converting enzyme was calculated for CD14+ cells with the use of Cellquest software (Becton Dickinson).

Single-flow exhaled nitric oxide concentrations were recorded at a rate of 50 ml per second as previously described,<sup>20</sup> and the alveolar nitric oxide concentration was derived from measurements at rates of 10, 30, 50, 100, and 200 ml per second.<sup>4</sup> FEV<sub>1</sub>, forced vital capacity (FVC), and forced expiratory flow between 25 percent and 75 percent of FVC (FEF<sub>25-75</sub>) were measured with the use of a rolling-seal spirometer (Vitalograph). The PC<sub>20</sub> was computed from the methacholine dose-response curve (the change in FEV<sub>1</sub> in relation to the methacholine concentration) by linear interpolation on a log scale. Patients initially inhaled normal saline by tidal breathing for two minutes, followed by 0.03 mg of methacholine per milliliter, and concentrations were then doubled up to a maximum of 16 mg per milliliter until the FEV<sub>1</sub>, measured at intervals of up to five minutes, decreased by at least 20 percent. Patients whose FEV<sub>1</sub> decreased by more than 20 percent after the inhalation of

normal saline were assigned a PC<sub>20</sub> value of 0.01 mg per milliliter.<sup>21</sup> Symptoms were measured with the use of three 100-mm visual-analogue scales representing cough, wheezing, and breathlessness; higher numbers denoted more symptoms.<sup>22</sup> The asthma-related quality of life was measured by means of the Juniper asthma quality-of-life scale; scores can range from 1 to 7 points, with higher values representing a better quality of life.<sup>23</sup> Sputum was induced, and samples were processed as previously described.<sup>22</sup> Details of the measurement of interleukin-8, cysteinyl leukotrienes (leukotriene C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub>), eosinophilic cationic protein, and histamine in sputum are provided in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

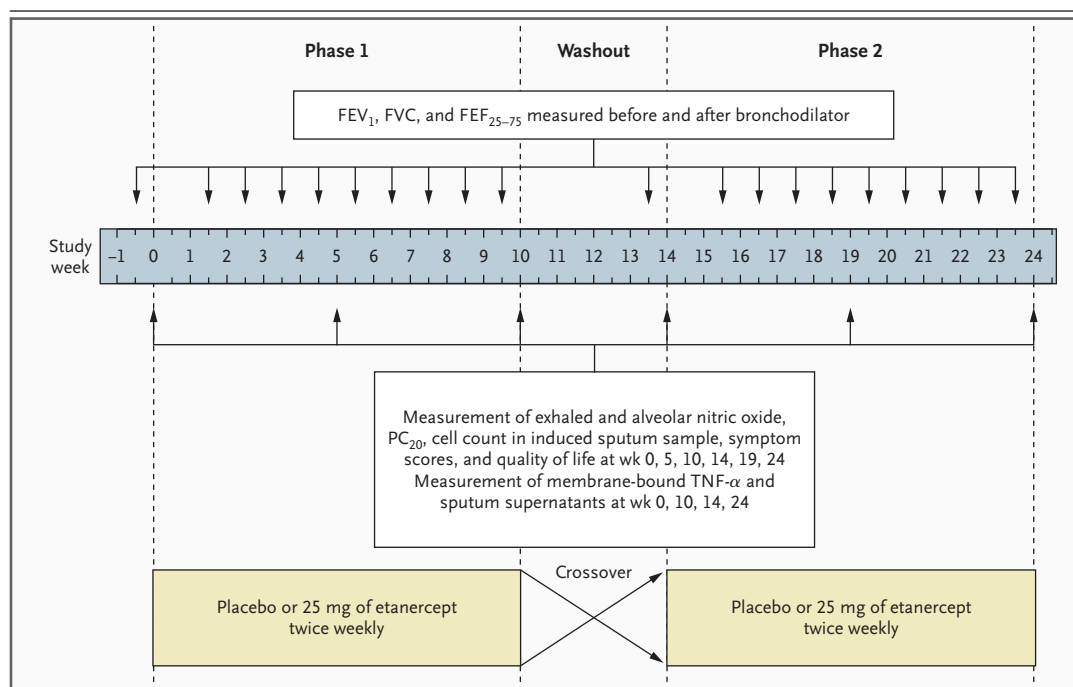
#### CROSSOVER TRIAL

Patients with refractory asthma were enrolled in a randomized, double-blind, crossover study comparing the effect of 10 weeks of treatment with etanercept at the dose used in a previously reported uncontrolled study<sup>11</sup> and placebo. An outline of the study is presented in Figure 1. Placebo (1 ml of 0.9 percent saline) or etanercept (25 mg made into a 1-ml solution with the addition of the manufacturer's diluent) was administered subcutaneously twice weekly. Etanercept was purchased by the investigators with departmental research funds, and the manufacturer had no role in the study. The order of treatment was determined with the use of a randomization sequence prepared with use of a random-number generator. Preparation and storage of the study drug were overseen by the Glenfield Hospital pharmacy. The doses of all other asthma medications were kept constant during the study.

#### STATISTICAL ANALYSIS

We compared the geometric mean fluorescence of membrane-bound TNF- $\alpha$ , TNF- $\alpha$ -converting enzyme, TNF- $\alpha$  receptor 1, and TNF- $\alpha$  receptor 2 with that of isotype-matched controls and expressed the results as a ratio. Comparisons between groups were made with use of one-way analysis of variance, with Tukey's post hoc test for the three individual-group differences.

The prespecified primary outcome measures for the etanercept trial were the difference in the change in the PC<sub>20</sub> from 0 to 10 weeks between the placebo and etanercept treatment phases<sup>21</sup> and the difference in the change in the asthma



**Figure 1. Study Plan.**

Treatment periods lasted 10 weeks and were separated by a 4-week washout period. The washout phase was chosen because the half-life of etanercept is 70 hours and, according to information provided by the manufacturer, clinical experience in patients with rheumatoid arthritis suggested that symptoms return within one month after treatment is stopped. Assessments were made at the same time of day at the times and in the order listed. Short-acting  $\beta_2$ -agonist treatment was withheld for more than 6 hours and treatment with long-acting  $\beta_2$ -agonists and ipratropium bromide withheld for more than 12 hours before each visit. PC<sub>20</sub> denotes the concentration of inhaled methacholine required to induce a 20 percent decrease in the forced expiratory volume in one second (FEV<sub>1</sub>), FVC forced vital capacity, and FEF<sub>25-75</sub> forced expiratory flow between 25 and 75 percent of FVC.

quality-of-life score from 0 to 10 weeks between the treatment phases. The change in PC<sub>20</sub> was expressed as a doubling concentration, calculated as the difference in the log PC<sub>20</sub> after and before treatment divided by log 2. Differences between and within treatment phases were normally distributed, as assessed by the Kolmogorov–Smirnov test; they were compared with the use of paired t-tests. The effect of treatment period and order was analyzed by means of analysis of covariance. The analysis was conducted according to the intention to treat: patients who withdrew during a treatment phase for asthma-related reasons were assigned a value equal to the worst net outcome for that treatment phase; patients who withdrew for reasons unrelated to asthma were assigned the last recorded spirometric values and, for other measures, a value equal to that measured at their five-week assessment if they had completed five weeks of treatment or no change if they had not.

Patients who withdrew during the washout phase were assigned baseline values from the previous treatment phase and were assumed to have had no net change during the second treatment phase. We also performed a post hoc analysis of the net change in the population that completed both phases of the study (per-protocol population). An earlier, uncontrolled study estimated a treatment effect on the PC<sub>20</sub> of 2.5 doubling concentrations.<sup>11</sup> Our study had a statistical power of 90 percent to detect a change of two doubling concentrations in the PC<sub>20</sub>, assuming a standard deviation of one doubling concentration within subjects.<sup>21</sup>

Prespecified secondary outcome measures were the net change in post-bronchodilator FEV<sub>1</sub>, FEF<sub>25-75</sub>, and FVC; symptom scores; exhaled nitric oxide concentrations; computed alveolar nitric oxide concentrations; differential inflammatory-cell counts in sputum; and mediator concentrations in sputum supernatant.

Multiple independent linear regression analysis was used to explore the relationship between the baseline expression of membrane-bound TNF- $\alpha$  by peripheral-blood monocytes and the net change in primary outcomes.

## RESULTS

### BASELINE CHARACTERISTICS

The baseline characteristics of the subjects are provided in Tables 1 and 2. Patients with refrac-

tory asthma had a significantly lower FEV<sub>1</sub> and FEV<sub>1</sub> as a percentage of the predicted value, as well as FEV<sub>1</sub>:FVC ratio, after receipt of a bronchodilator than did controls or patients with mild-to-moderate asthma. There was a trend toward an increased neutrophil count in sputum from patients with refractory asthma (P=0.10). The alveolar nitric oxide concentration was significantly higher in patients with refractory asthma than in those with mild-to-moderate asthma or controls (Table 1).

**Table 1. Baseline Characteristics of the Subjects.\***

Characteristic	Controls (N=10)	Patients with Mild-to-Moderate Asthma (N=10)	Patients with Refractory Asthma (N=10)
Sex (no. of subjects)			
Male	4	5	4
Female	6	5	6
Age (yr)			
Median	38	42	49
Range	23–49	18–72	25–59
Age at onset of symptoms (yr)			
Median	—	14	16
Range	—	1–36	1–58
FEV <sub>1</sub>			
Liters	3.8±0.4	3.7±0.9	2.4±0.7†
% of predicted value	97±18	94±23	62±21†
FEV <sub>1</sub> :FVC ratio (%)	80±25	78±21	65±17†
Nitric oxide — ppb			
Exhaled‡	16.5±0.2	34.2±0.3	41.8±0.2†
Alveolar	3.4±1.2	3.4±1.6	10.2±15†
Sputum measurements			
Eosinophils (%)‡	0.3±0.2	4.1±0.7	5.6±0.8†
Neutrophils (%)	57±25	55±26	62±27
Total cells (×10 <sup>-3</sup> /mg)‡	0.6±0.2	0.9±0.4	1.6±0.2
Plasma IgE (IU/ml)‡	16±0.6	172±1.1	77±0.9
PC <sub>20</sub> (mg of methacholine/ml)‡	>16	0.4±0.6	0.14±0.1
Continuous oral corticosteroids (no. of subjects)	0	0	7
TNF- $\alpha$ receptor 1 — ratio§	2.3±0.3	3.1±0.2	5.5±0.6¶
TNF- $\alpha$ -converting enzyme — ratio§	2.8±0.4	3.5±0.5	6.5±0.5¶
TNF- $\alpha$ receptor 2 — ratio§	3.9±0.3	3.9±0.4	3.5±0.2

\* Unless otherwise noted, plus-minus values are means  $\pm$ SD. FEV<sub>1</sub> denotes post-bronchodilator forced expiratory volume in one second, FVC post-bronchodilator forced vital capacity, and PC<sub>20</sub> concentration of inhaled methacholine required to induce a 20 percent decrease in FEV<sub>1</sub>.

† P<0.05 for the comparison among the groups by analysis of variance.

‡ Plus-minus values are geometric means  $\pm$ log SD.

§ Values are the ratio of fluorescence as compared with that for the isotype-matched control.

¶ P<0.001 for the comparison among the groups by analysis of variance.

**Table 2. Baseline Characteristics of 10 Patients with Refractory Asthma.**

Patient No.	Age (yr)/Sex	Age at Onset yr	Atopy	Plasma IgE IU/ml	Inhaled Fluticasone Dose µg/day	Systemic Prednisolone Dose mg/day	Long-Acting β <sub>2</sub> -Agonist	Other Treatment	ATS Criteria*
1†	58/M	58	No	19	1000	20	Salmeterol	—	1, 2, 3, 4, 6
2‡	25/F	6	Yes	99	2000	—	Salmeterol	Aminophylline, 200 mg twice daily	2, 3, 4, 5, 6, 7, 8
3†	57/F	27	No	32	500	10	Salmeterol	Montelukast, 10 mg/day orally	1, 3, 4, 5, 6, 7, 9
4‡	40/F	12	No	75	1000	30	Formoterol	Ipratropium	1, 2, 3, 4, 5, 6, 7
5†	30/F	2	Yes	4298	2000	—§	Salmeterol	—	1, 2, 3, 4, 5, 6, 7, 9
6‡	59/F	19	Yes	11	2000	—	Salmeterol	Aminophylline, 300 mg twice daily	2, 3, 4, 5, 6, 7
7‡	33/M	2	Yes	2066	2000	15	Salmeterol	Montelukast, 10 mg/day orally	1, 2, 3, 4, 5, 6, 7, 9
8‡	49/M	2	Yes	131	2000	—	Salmeterol	Montelukast, 10 mg/day orally	2, 3, 5, 6, 7
9‡	47/F	29	Yes	18	1000	20	Salmeterol	Aminophylline, 375 mg twice daily	1, 2, 3, 4, 5, 6, 7
10‡	50/M	1	No	7	500	7.5	Salmeterol	—	1, 3, 4, 5, 6, 8

\* The American Thoracic Society (ATS) criteria for refractory asthma are as follows. One or both of the following major criteria: 1, continuous treatment (more than 50 percent of the year) with oral corticosteroids; 2, required treatment with high-dose inhaled corticosteroids; and two or more of the following minor criteria: 3, the need for daily “reliever” medication; 4, the presence of symptoms requiring daily treatment; 5, persistent airflow obstruction (defined by a forced expiratory volume in one second [FEV<sub>1</sub>] value that is less than 80 percent of the predicted value); 6, a history of one or more urgent care visits for asthma; 7, the need for three or more bursts of oral corticosteroids per year; 8, prompt deterioration in clinical condition after a reduction in the corticosteroid dose by less than 25 percent; and 9, a history of near-fatal asthma.

† This patient received etanercept first in the crossover trial.

‡ This patient received placebo first in the crossover trial.

§ This patient received 80 mg of triamcinolone per month.

**TNF- $\alpha$  EXPRESSION**

The mean ( $\pm$ SE) ratio of fluorescence for antibody against membrane-bound TNF- $\alpha$  to that for the isotype-matched control was  $8.9\pm 0.9$  in patients with refractory asthma,  $3.8\pm 0.7$  in control subjects (mean difference between groups, 5.1; 95 percent confidence interval, 2.5 to 7.7;  $P<0.001$ ) (Fig. 2), and  $3.3\pm 0.4$  in patients with mild-to-moderate asthma (mean difference between this group and the group with refractory asthma, 5.5; 95 percent confidence interval, 2.7 to 8.4;  $P<0.001$ ). The ratio of the expression of TNF- $\alpha$  receptor 1 ( $P<0.001$ ) and TNF- $\alpha$ -converting enzyme ( $P<0.001$ ), but not of TNF- $\alpha$  receptor 2 ( $P=0.48$ ), was also significantly higher in the group with refractory asthma than in the other groups (Table 1).

**CROSSOVER TRIAL OF ETANERCEPT**

One patient withdrew for personal reasons during the washout phase after etanercept treatment owing to a change in employment, and one patient withdrew during week 4 of the second treatment phase (etanercept) owing to the death of a relative and the development of a cough productive of sputum associated with repeated isolation of *Haemophilus influenzae* despite a course of oral antibiotics (Table 2 and Fig. 3). Data from the latter patient were treated as an asthma-related withdrawal. There were no other adverse effects. Neither the treatment period nor the order of treatment influenced values before treatment or the change in primary outcome measures (Table 2 and Fig. 3).

**PRIMARY OUTCOMES**

As compared with placebo, etanercept significantly reduced the ratio of fluorescence of peripheral-blood monocytes for membrane-bound TNF- $\alpha$  (geometric mean change,  $-6.9$  vs.  $-0.1$ ; mean difference between groups, 6.8; 95 percent confidence interval, 0.5 to 13.1;  $P=0.04$ ). Etanercept treatment was associated with a progressive improvement in the PC<sub>20</sub>, as reflected by a doubling concentration of methacholine of 2.3 at 10 weeks, as compared with  $-1.2$  after 10 weeks of placebo (mean difference, 3.5 doubling concentrations; 95 percent confidence interval, 0.07 to 7.0;  $P=0.05$ ) (Fig. 3). The Juniper asthma quality-of-life score improved by 0.84 with etanercept, as compared with a decrease of 0.02 with placebo (mean difference, 0.85; 95 percent confidence interval, 0.16

to 1.54;  $P=0.02$ ) (Fig. 3). The net changes in the PC<sub>20</sub> and the asthma quality-of-life score with etanercept treatment, as compared with placebo, were both independently associated with the baseline expression of membrane-bound TNF- $\alpha$  by peripheral-blood monocytes (adjusted R<sup>2</sup>, 0.73;  $P=0.004$ ) and the treatment-associated change in expression from baseline (adjusted R<sup>2</sup>, 0.56;  $P=0.02$ ). The net changes in the PC<sub>20</sub> and the asthma quality-of-life score remained significant when the analysis was restricted to the per-protocol population ( $P=0.04$  for both comparisons).

**SECONDARY OUTCOMES**

Secondary outcomes are shown in Table 3 and the Supplementary Appendix; the changes in FEV<sub>1</sub> during the 10 weeks of etanercept and placebo administration are shown in Figure 3C. There was a greater improvement in post-bronchodilator FEV<sub>1</sub> in the etanercept group than in the placebo group, and this difference became significant by week 5 and was 0.32 liter (95 percent confidence interval, 0.08 to 0.55;  $P=0.01$ ) by week 10 (Table 3 and Fig. 3). The total symptom score at 10 weeks decreased by 48 mm (out of a possible 300 mm) after treatment with etanercept, as compared with 9 mm with placebo (mean difference, 39; 95 percent confidence interval, 7 to 71;  $P=0.01$ ). There were no significant differences between groups in single-flow nitric oxide concentration, calculated alveolar nitric oxide concentration, sputum total or differential cell counts, or sputum eosinophilic cationic protein, interleukin-8, or cysteinyl leukotriene concentrations (see the Supplementary Appendix). The mean histamine concentration in sputum decreased from 36.1 to 14.0 ng per milliliter with etanercept and increased from 37.0 to 41.3 ng per milliliter with placebo (mean difference in the change in histamine concentration, 26 ng per milliliter; 95 percent confidence interval, 5 to 48;  $P=0.02$ ) (Table 3).

**DISCUSSION**

We have demonstrated that the TNF- $\alpha$  axis is up-regulated in patients with refractory asthma, as evidenced by the increased expression of membrane-bound TNF- $\alpha$ , TNF receptor 1, and TNF- $\alpha$ -converting enzyme by peripheral-blood monocytes. Antagonism of TNF- $\alpha$  with 10 weeks of etanercept therapy significantly reduced the expression of membrane-bound TNF- $\alpha$  by periph-

eral-blood monocytes and improved the PC<sub>20</sub>, the asthma-related quality of life, FEV<sub>1</sub>, and symptom scores, as compared with placebo. The baseline expression of membrane-bound TNF- $\alpha$  by peripheral-blood monocytes and the extent to which it was reduced by etanercept treatment were independently associated with the net improvement in both primary outcome measures.

We chose to assess TNF- $\alpha$  activity by measuring the expression of membrane-bound TNF- $\alpha$ , cell-surface receptor, and TNF- $\alpha$ -converting enzyme by peripheral-blood monocytes because monocytes and macrophages are an important source of TNF- $\alpha$  and the technique is noninvasive and suitable for repeated measurements after etanercept treatment. Membrane-bound TNF- $\alpha$  was used to assess the response to treatment, since there is evidence that it is more closely associated with biologic activity<sup>19</sup> and the clinical outcome of septic shock<sup>24</sup> than are other markers. The extent to which measurements made in peripheral-blood monocytes relate to the up-regulation of TNF- $\alpha$  in the airway is unclear. The relationship between the expression of membrane-bound TNF- $\alpha$  and the response to etanercept suggests that the effects in peripheral-blood monocytes are relevant to those in more biologically relevant sites. More work is required to determine the main source of TNF- $\alpha$  in the airway in patients with refractory asthma and how this relates to markers of TNF- $\alpha$  activity on peripheral-blood monocytes.

Potential explanations for the increased TNF- $\alpha$  activity in peripheral-blood monocytes include the coexistence of asthma with other inflammatory conditions associated with increased TNF- $\alpha$  activity and genetic differences in the TNF- $\alpha$  gene or genes associated with the regulation of TNF- $\alpha$  production. The increased TNF- $\alpha$  activity is unlikely to reflect the effects of corticosteroid treatment, since *in vitro* studies show that corticosteroids reduce the production of TNF- $\alpha$  by monocytes,<sup>25</sup> although this effect may be diminished in patients who have refractory asthma as a result of resistance to corticosteroids. An effect of other treatments cannot be excluded, although there is no strong biologic rationale to provide support for such an effect.

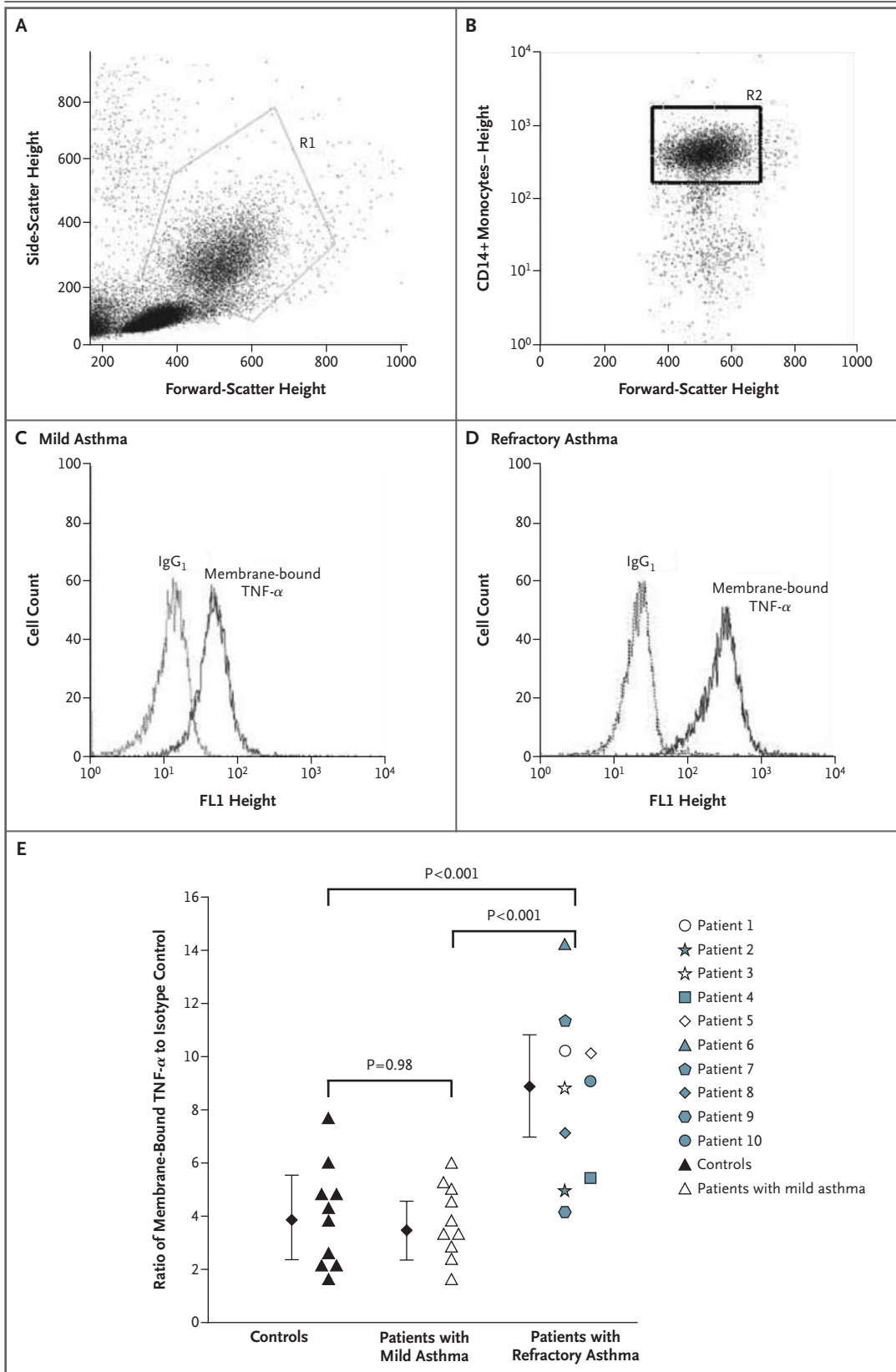
The beneficial effects of etanercept-induced antagonism of TNF- $\alpha$  on markers of asthma control support the view that TNF- $\alpha$  contributes to the pathogenesis of refractory asthma. Our pilot study involved small numbers of patients, and the

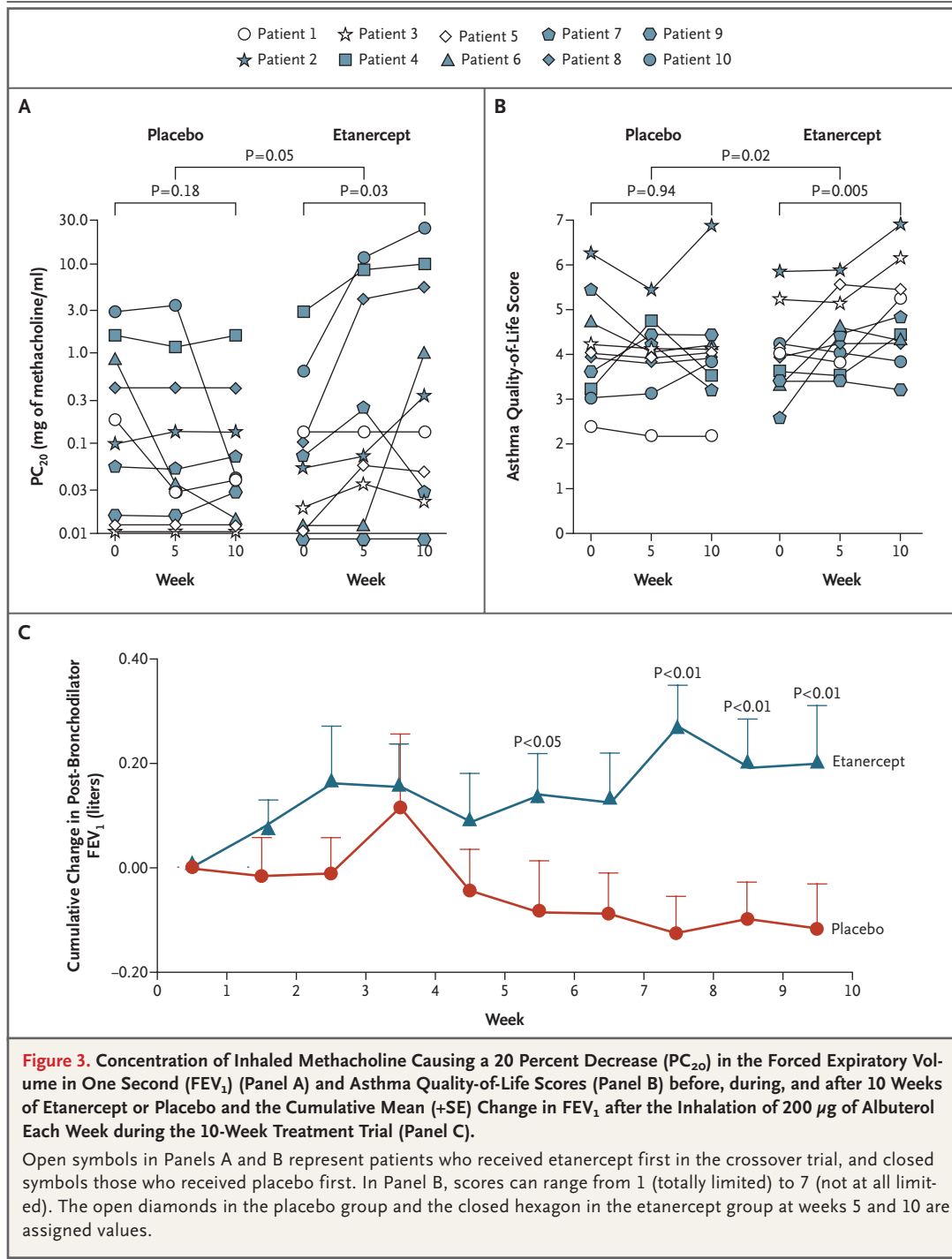
**Figure 2 (facing page).** Representative Flow-Cytometric Plots (Panels A and B), Representative Histograms from a Patient with Mild Asthma (Panel C) and a Patient with Refractory Asthma (Panel D), and the Ratios of Membrane-Bound TNF- $\alpha$  in the Three Groups (Panel E).

Panel A shows a representative flow-cytometric plot with forward and side scatter; monocytes are identified on the basis of their typical pattern of size and granularity (R1). Panel B demonstrates further selection of monocytes (shown as R2) on the basis of gating on the cells staining for CD14. Panel C shows a representative histogram from a patient with mild asthma, and Panel D a representative histogram from a patient with refractory asthma. The ratio of fluorescence for membrane TNF- $\alpha$  as compared with that for an isotype-matched control is calculated by dividing the geometric mean fluorescence for membrane TNF- $\alpha$  (FL1) by that of the isotype-matched control. Panel E shows the ratio of fluorescence for membrane TNF- $\alpha$  as compared with that for the isotype-matched control in subjects from each group. Open symbols in Panel E represent patients who received etanercept first in the crossover trial, and closed symbols those who received placebo first. The characteristics of the individual patients are described in Table 2. Diamonds with I bars represent means  $\pm$  SE.

results could have been compromised by missing data, the crossover design, or the imbalance in the treatment order. Thus, the clinical findings cannot be regarded as a directive for treatment. The large effect of etanercept on measures of airway function in our study is consistent with the findings of an uncontrolled study.<sup>11</sup> We found a significant decrease in histamine concentrations in sputum supernatant but no effects on other markers of airway inflammation, suggesting that the effect of etanercept is mediated primarily by an effect on airway smooth-muscle and mast cells.<sup>6,26,27</sup>

Our findings suggest that the beneficial effects of etanercept may be confined to patients with refractory asthma, since patients with mild-to-moderate asthma had no evidence of increased markers of TNF- $\alpha$  activity on peripheral-blood monocytes. For this reason and because treatment with an expensive agent with potential side effects could not be justified clinically, we did not study the effects of etanercept in patients with mild-to-moderate asthma. The view that systemic dysregulation of the TNF- $\alpha$  axis is peculiar to patients with refractory asthma is supported by a study showing that TNF- $\alpha$  production by peripheral-blood monocytes in response to lipopolysaccharide and other stimuli was increased in patients with severe asthma but not in those whose asthma was controlled by low-dose inhaled cor-





ticosteroids.<sup>28</sup> In addition, there is preliminary evidence that two weeks of treatment with etanercept has no effect on airway responsiveness or the bronchoconstrictor and inflammatory response to endobronchial allergen challenge in subjects

with mild atopic asthma.<sup>29</sup> However, these findings are limited by the short duration of treatment and small numbers of subjects, and further study of patients with less-severe asthma is required.

**Table 3.** Measurements before, during, and after Placebo and Etanercept Treatment in 10 Patients with Refractory Asthma.\*

Variable	Placebo			Etanercept			Between-Group Difference (95% CI)
	Wk 0	Wk 5	Wk 10	Wk 0	Wk 5	Wk 10	
Fluorescence for membrane-bound TNF- $\alpha$ ratio†	8.3±5.1	—	8.2±5.4	11.5±7.2	—	4.6±2.2	-6.8 (-13 to -0.5)‡
Asthma quality-of-life score§	4.2±1.0	4.3±1.0	4.1±1.2	4.1±0.9	4.6±1.0	4.9±1.1	0.85 (0.2 to 1.5)¶
PC <sub>20</sub> (mg of methacholine/ml)**	0.17±0.3	0.08±0.3	0.07±0.2	0.14±0.1	0.21±0.3	0.44±0.4	2.3 (0.6 to 4.0)‡
FEV <sub>1</sub> (liters)	2.36±0.9	2.27±0.9	2.22±0.8	2.30±0.8	2.37±0.7	2.48±0.6	0.18 (-0.04 to 0.4)
FVC (liters)	3.12±0.9	2.9±0.8	3.12±0.9	2.94±1.0	3.08±1.0	3.23±1.1	0.29 (0.05 to 0.52)‡
FEF <sub>25-75</sub> (liters/sec)	1.87±0.7	1.88±0.7	1.87±0.8	1.98±0.8	2.08±0.8	2.24±0.8	0.26 (0.02 to 0.5)‡
Symptom score (mm)††	146±79	136±72	137±70	125±50	92±44	77±54	-48 (-90 to -7)‡‡
Exhaled nitric oxide concentration (ppb)**	40.9±0.5	43.3±0.4	46.2±0.4	44±0.5	32.5±0.4	37.2±0.4	0.84 (0.5 to 1.4)
Sputum measurements							
Eosinophils (%)**	4.3±0.8	2.5±0.9	4.1±0.8	5.9±0.7	3.9±0.7	4.8±0.9	0.80 (0.4 to 3.6)¶
Neutrophils (%)	65.4±22.6	68.7±25.5	64.7±31.8	55.8±27.2	61.6±23.2	59.8±34.8	3.9 (-5.7 to 13.5)
Total cells (×10 <sup>-3</sup> /mg)**	1.7±0.1	2.6±0.1	2.4±0.1	1.2±0.1	1.5±0.1	2.1±0.1	1.7 (1.1 to 2.7)§
Histamine (ng/ml)	37.0±11.9	—	41.3±11.5	36.1±12.0	—	14.0±11.7	-22 (-41.2 to -2.7)§

\* Unless otherwise indicated, plus-minus values are means ±SD. CI denotes confidence interval, FEV<sub>1</sub> post-bronchodilator forced expiratory volume in one second, FVC post-bronchodilator forced vital capacity, FEF<sub>25-75</sub> the post-bronchodilator forced expiratory flow between 25 percent and 75 percent of FVC, and PC<sub>20</sub> the concentration of inhaled methacholine required to induce a 20 percent decrease in FEV<sub>1</sub>.

† Values are the ratio of fluorescence as compared with that for the isotype-matched control.

‡ P<0.05.

§ Scores can range from 1 (totally limited) to 7 (not at all limited).

¶ P<0.01.

|| P=0.02.

\*\* Plus-minus values are geometric means ±log SD. Differences in the PC<sub>20</sub> are expressed as doubling concentrations. Otherwise, differences are expressed as a ratio.

†† The sum of three 100-mm visual-analogue scales was used; higher scores indicate more severe symptoms.

‡‡ P=0.01.

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