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Effect of tumor necrosis factor antagonism on allergen-mediated asthmatic airway inflammation

Farshid N. Rouhani[a], Catherine A. Meitin[b], Maryann Kaler[a], Dianne Miskinis-Hilligoss[a], Mario Stylianou[c], Stewart J. Levine[a,*]

[a]*Pulmonary-Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 10, Room 6D03, MSC 1590, Bethesda, MD 20892-1590, USA*
[b]*Critical Care Medicine Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA*
[c]*Office of Biostatistics Research, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA*

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KEYWORDS
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Summary
Objective: To assess whether tumor necrosis factor (TNF) antagonism can attenuate eosinophilic airway inflammation in patients with mild-to-moderate allergic asthma.
Design: Randomized, double-blind, placebo-controlled trial.
Setting: National Institutes of Health (NIH) Clinical Center.
Patients: Twenty-six patients with mild-to-moderate allergic asthma, receiving only inhaled β-2-agonists, who demonstrated both an early and late phase response to inhalational allergen challenge.
Intervention: Injection of a soluble TNF receptor (TNFR:Fc, etanercept, Enbrel) or placebo, 25 mg subcutaneously, twice weekly for 2 weeks, followed by a bronchoscopic segmental allergen challenge.
Measurements: The primary outcome measure was whether TNFR:Fc can access the lung and inhibit TNF bioactivity. Secondary outcome measures included pulmonary eosinophilia, Th2-type cytokines, and airway hyperresponsiveness.
Results: Anti-TNF therapy was associated with transient hemiplegia in one patient, which resulted in suspension of the study. Data from the 21 participants who completed the study were analyzed. Following treatment, patients receiving anti-TNF therapy had significantly increased TNFR2 levels in epithelial lining fluid (ELF) ($P<0.001$), consistent with delivery of TNFR:Fc to the lung. TNF antagonism did not attenuate pulmonary eosinophilia and was associated with an increase in ELF IL-4

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; ELF, epithelial lining fluid; FEV_1, forced expiratory volume in 1 s, PD_{20}, provocative dose inducing a 20% decrease in FEV_1; TNF, tumor necrosis factor; TNFR2, 75-kDa, type II tumor necrosis factor receptor; TNFR2:Fc, etanercept, Enbrel

*Corresponding author. Tel.:+1 301 402 1448; fax: +1 301 496 2363.
E-mail address: levines@nhlbi.nih.gov (S.J. Levine).

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levels ($P = 0.033$) at 24 h following segmental allergen challenge. TNF antagonism was not associated with a change in airway hyperresponsiveness to methacholine.
Conclusions: TNF antagonism may not be effective for preventing allergen-mediated eosinophilic airway inflammation in mild-to-moderate asthmatics. Transient hemiplegia, which may mimic an evolving stroke, may be a potential toxicity of anti-TNF therapy.
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Introduction

Tumor necrosis factor (TNF), a pro-inflammatory cytokine, plays a key role in disease pathogenesis.[1] Further, anti-TNF therapies are effective treatments for rheumatoid arthritis and inflammatory bowel disease.[2,3] Multiple lines of evidence also support a role for TNF in the pathogenesis of asthmatic airway inflammation. For example, elevated TNF levels have been found in bronchoalveolar lavage fluid (BALF) from asthmatic patients and up-regulated TNF expression has been detected in alveolar macrophages, mast cells, and bronchial epithelial cells.[4–9] TNF induces the expression of multiple airway epithelial cell genes, including cytokines (IL-5, IL-6, IL-8, G-CSF, GM-CSF), chemokines (eotaxin, MCP-1, RANTES), adhesion molecules (ICAM-1), extracellular matrix glycoproteins (tenascin), neuropeptides (endothelin-1), mucins (MUC-1, MUC-2, MUC-5AC), and cytosolic phospholipase A_2.[10–21] TNF increases the adhesion of activated eosinophils to respiratory epithelial cell cultures and promotes neutrophil chemotaxis, adherence, and transendothelial and transepithelial migration.[22–24] IgE receptor activation induces TNF release from human lung tissue and up-regulates eosinophil TNF mRNA levels.[25] An association between asthma and polymorphisms in the TNF locus that correlate with increased TNF secretion has been proposed.[26–28] Further, administration of aerosolized TNF to human subjects and animal models induces airway hyperreactivity and neutrophilia.[29–31] Lastly, anti-TNF strategies have successfully reduced airway inflammation in animal models of allergic asthma.[32–36]

Since clinical and experimental evidence support a role for TNF in asthma pathogenesis, we reasoned that anti-TNF therapy might attenuate airway inflammation in allergic asthmatics. To test this hypothesis, we administered a dimeric fusion protein comprising the extracellular ligand-binding domain of the human 75-kDa, type II TNF receptor (TNFR2), linked to the Fc portion of human IgG1 (TNFR:Fc, etanercept, Enbrel), to mild-to-moderate allergic asthmatics who demonstrated both early and late phase responses to inhalational allergen challenge and assessed its effect on airway inflammation following a bronchoscopic segmental allergen challenge.

Methods

Study design

This randomized, double-blind, placebo-controlled study was approved by the National Heart, Lung, and Blood Institute Institutional Review Board and conducted between August 1999 and February 2002 at the National Institutes of Health (NIH) Clinical Center. All study participants provided informed consent. This trial was designed prospectively to enroll participants until both the TNFR:Fc and placebo groups had 13 completers. Allergy was defined by skin test reactivity to cat dander, *Dermatophagoides farinae*, short ragweed, or Timothy grass using a Multi-Test II® applicator (generously provided by Lincoln Diagnostics, Decatur, Illinois). Allergic asthma was established by reversible airflow obstruction, forced expiratory volume in 1 s (FEV_1) $\geqslant$ 70% of predicted, medications limited to inhaled beta-agonists, and both early and late phase responses to inhalational allergen challenge. Patients had mild intermittent or mild/moderate persistent asthma.[37] The early phase response to inhalational allergen challenge was defined by a 20% decrease in the FEV_1 and was utilized to calculate the antigen PD_{20}. The late phase response was defined by a 20% decrease in FEV_1 from the baseline established following the early phase response.

One month following the inhalation allergen challenge, a baseline bronchoalveolar lavage (BAL) was performed. Participants were then randomized in a double-blind fashion to receive four doses of either TNFR:Fc or placebo (generously provided by Immunex Corporation, Seattle, Washington), 25 mg subcutaneously, twice per week for 2 weeks.

Pulmonary function and methacholine challenge testing were performed following the fourth dose. A post-drug BAL was performed 24 h after the final dose, followed by a segmental allergen challenge in

the right middle lobe, utilizing 20% of the antigen PD_{20}, and a control saline challenge in the right upper lobe.[38–40] This technique produces a physiologically relevant and comparable allergic response in each participant, as measured by the change in FEV_1 after allergen challenge.[39] BAL was performed after 5 min, to assess the early phase response, and after 24 h, to assess the late phase response.

Bronchoalveolar lavage fluid (BALF) analysis

BALF cell differentials were performed on Diff-Quik-stained cytospin slides. BALF cytokine measurements were performed by ELISA (R&D Systems, Minneapolis, MN). All BALF volumes were normalized to epithelial lining fluid (ELF) volume.[41]

Outcome measures

The primary outcome was whether TNFR:Fc could access the lung and inhibit TNF bioactivity. The secondary outcomes were pulmonary eosinophilia, Th2-type cytokines, and airway hyperresponsiveness to methacholine.

Statistical analysis

We estimated a sample size of 13 in each group to have an 85% power to detect a difference between BALF TNF protein levels of 300 pg/ml with an α error of 0.05.[38] The baseline characteristics were compared utilizing the Wilcoxon–Mann–Whitney rank-sum test for continuous variables and the Fisher's exact test for binary variables. Comparisons between the TNFR:Fc and placebo groups were made at three time points; (i) post-treatment, (ii) early phase response (e.g., 5 min following segmental allergen challenge), and (iii) late phase response (e.g., 24 h following segmental allergen challenge). The differences between the TNFR:Fc and placebo groups were calculated after adjusting for the baseline measurement in (i) (e.g., post-treatment minus baseline) and after adjusting for the saline challenge in (ii) and (iii) to control for the non-specific effects of the bronchoscopy (e.g., allergen challenge minus saline challenge) utilizing the Wilcoxon–Mann–Whitney exact test. Two-sided P values and 95% confidence intervals are reported. It was prospectively determined that a P value of <0.05 would be significant for ELF eosinophils, Th2-type cytokines, and the methacholine PD_{20}, while a P value of <0.01 was utilized for all other analyses.

Results

Patient characteristics

There were no statistically significant differences in baseline characteristics between the TNFR:Fc and placebo groups (Table 1). Three participants developed serious adverse events, one of which resulted in the suspension of the study. This serious adverse event was a transient left-sided hemiplegia of 36-h duration, which developed following the third dose of TNFR:Fc in a patient with no prior history of neurologic disease. No causal pathology was identified by CT and MRI imaging. A mild transient neutropenia was also noted. Two other participants experienced palpitations and transient neutropenia while receiving placebo. All serious adverse events resolved completely without intervention. Eighteen of the 21 participants returned 1-month following the completion of the study and reported no interval change in asthma symptoms. The data from the 21 participants who completed the study were analyzed.

Pulmonary delivery of TNFR:Fc

After the treatment phase, there was a significant increase in ELF TNFR2 in the TNFR:Fc group ($P<0.001$, 95% confidence interval 9, 33 ng/ml), consistent with pulmonary delivery of TNFR:Fc (Fig. 1A). There was also a trend toward increased ELF TNF in the TNFR:Fc group after the treatment phase ($P=0.06$, 95% confidence interval 0.0, 20.2 pg/ml), consistent with TNF binding to TNFR2 (Fig. 1B). BALF TNF bioactivity assays did not yield reproducible results, therefore, an analysis of TNF bioactivity could not be performed.

Effect of segmental allergen challenge on ELF cell counts

There were significantly more ELF eosinophils in the allergen-challenged segment, than in the saline-challenged segment, at the 24-h time point in both the TNFR:Fc ($P<0.001$, 95% confidence interval 8.0×10^7, 48.2×10^7 cells) and placebo groups ($P=0.016$, 95% confidence interval 1.3×10^7, 39.4×10^7 cells), consistent with the successful induction of a late phase allergic airway inflammatory response by segmental allergen challenge (Fig. 2A). There was no difference between ELF neutrophils (Fig. 2B) in the allergen-challenged segment and the saline-challenged segment at 24 h in either the TNFR:Fc or placebo groups.

Table 1 Characteristics of patients who completed the study.

	Placebo ($N = 9$)	TNFR:Fc ($N = 12$)
Baseline characteristics		
Age (years)		
Median	23	27
Range	21–40	20–54
Male	2	8
Female	7	4
FEV_1 (l)		
Median	3.06	3.29
Range	2.13–4.17	2.67–4.71
FEV_1 (% of predicted)		
Median	96	92
Range	79–116	70–114
Methacholine PD_{20} (mg)		
Median	0.06	0.07
Range	0.001–0.49	0.006–0.31
Blood eosinophils (k/μl)		
Median	0.19	0.25
Range	0.05–1.07	0.04–0.54
ELF eosinophils ($\times 10^7$ cells)		
Median	1.34	0.50
Range	0–4.94	0–8.19
Allergen		
Cat dander	2	2
D. farinae	4	7
Short ragweed	2	1
Timothy grass	1	2
Adverse events		
Bronchospasm	3	1
Dermatitis	1	0
Headache	1	2
Hemiplegia (transient)*	0	1
Injection site reaction	4	3
Nausea	0	1
Neutropenia*	1	1
Palpitations*	1	0
Throat dryness	0	1
Viral upper respiratory tract Infection	2	0

*Denotes serious adverse event.

Effect of TNF antagonism on ELF cell counts

There was no significant difference in ELF eosinophils, neutrophils, lymphocytes, or macrophages (Fig. 2) between the TNFR:Fc and placebo groups following the completion of therapy, or during the early and late phase responses.

Effect of TNF antagonism on ELF Th2-type cytokine levels

The TNFR:Fc group had significantly greater ELF interleukin-4, measured in pg/ml, during the late phase response to segmental allergen challenge ($P = 0.033$, 95% confidence interval 1.2, 9.8 pg/ml) than did the placebo group (Fig. 3A). ELF IL-5 was only detected in the allergen-challenged segment at 24 h and there was no significant difference between the placebo and TNFR:Fc groups ($P = 0.111$, 95% confidence interval −96, 1180 pg/ml) (Fig. 3B).

Effect of TNF antagonism on airway hyperresponsiveness and airflow obstruction

There was no difference in airflow obstruction (Fig. 4A), as determined by the FEV_1 in liters ($P = 0.9$, 95% confidence interval −0.23, 0.25 L), or airway hyperresponsiveness (Fig. 4B), as measured by the methacholine PD_{20} in milligrams ($P = 0.4$, 95% confidence interval −0.202, 0.053 mg), between the TNFR:Fc and placebo groups following TNFR:Fc administration.

Discussion

Administration of a recombinant soluble human TNF receptor for 2 weeks to subjects with mild-to-moderate allergic asthma did not prevent pulmonary eosinophilia during the late phase response to bronchoscopic segmental allergen challenge. Since eosinophils are key effector inflammatory cells in allergic asthma, our results suggest that TNF antagonism may not ameliorate allergen-mediated airway inflammation in mild-to-moderate asthmatics. Further, TNF antagonism increased pulmonary IL-4 levels, which suggests that TNF signaling pathways may play a counter-regulatory role by down-regulating the function of Th2-type T cells.

The ability of TNF to attenuate allergen-mediated airway inflammation may occur at two levels. First, chronic TNF stimulation has been reported to suppress T cell receptor signaling, proliferative responses, and cytokine production,

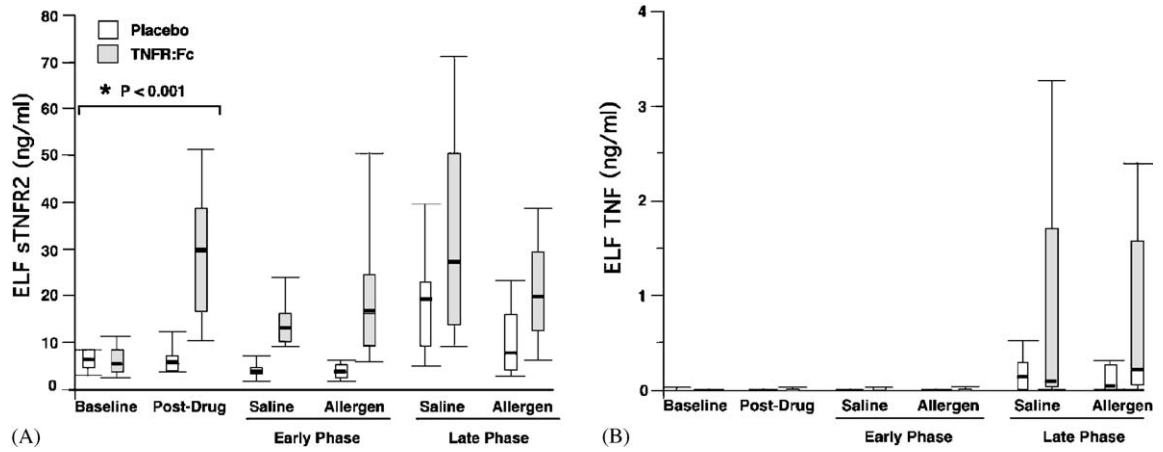

Figure 1 The effect of TNF antagonism on sTNFR2 and TNF in epithelial lining fluid. Data are presented as medians, with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (I bars). ELF levels of the soluble type II, 75-kDa tumor necrosis factor receptor (sTNFR2) are presented in panel A and levels of ELF TNF are presented in panel B. The asterisk in panel A indicates that after the treatment phase, there was a significant increase in ELF TNFR2 levels, measured in ng/ml, in the TNFR:Fc group ($P<0.001$, 95% confidence interval 9, 33 ng/ml), consistent with pulmonary delivery of TNFR:Fc.

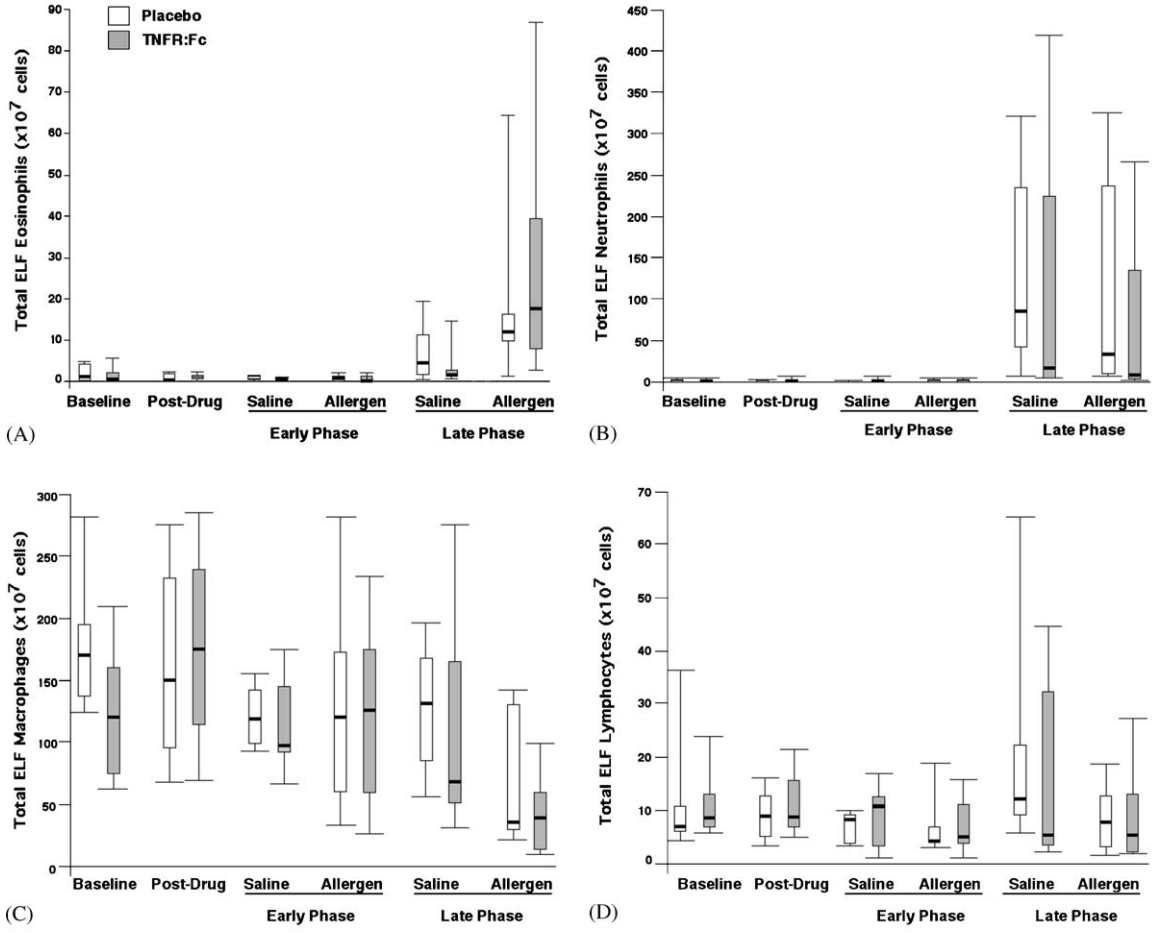

Figure 2 The effect of TNF antagonism on cell counts in epithelial lining fluid (ELF). Data are presented as medians, with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (I bars). ELF cell counts are presented for eosinophils (panel A), neutrophils (panel B), macrophages (panel C), and lymphocytes (panel D). There was no significant difference in ELF eosinophil, neutrophil, lymphocyte, or macrophage cell counts between the TNFR:Fc and placebo groups following the completion of therapy, or during the early and late phase responses.

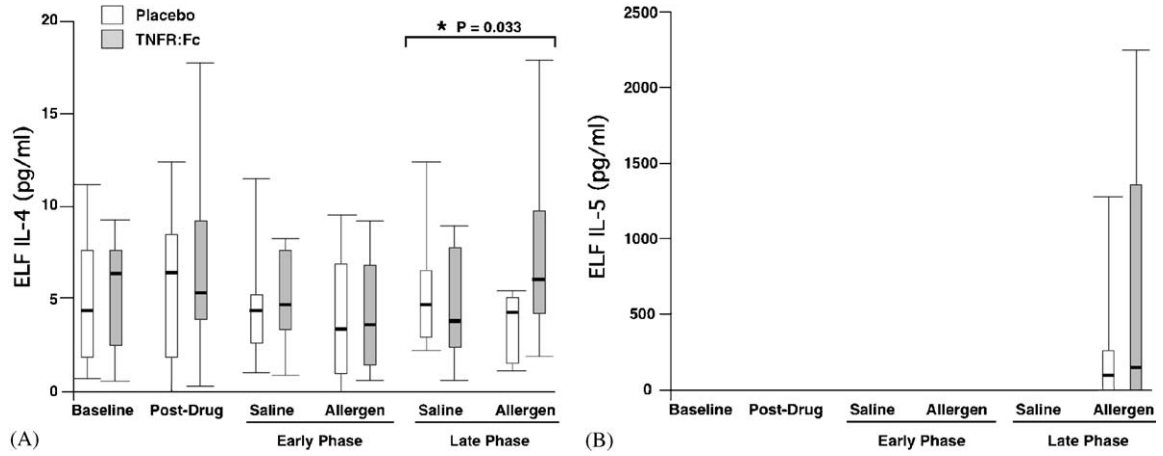

Figure 3 The effect of TNF antagonism on IL-4 and IL-5 levels in epithelial lining fluid (ELF). Data are presented as medians, with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (I bars). ELF fluid levels of interleukin-4 (IL-4) are presented in Panel A and ELF IL-5 levels are presented in panel B. The asterisk in panel A indicates that TNF antagonism resulted in significantly greater ELF IL-4 levels, measured in pg/ml, in the TNFR:Fc group during the late phase response to the bronchoscopic segmental allergen challenge ($P = 0.033$, 95% confidence interval 1.2, 9.8 pg/ml).

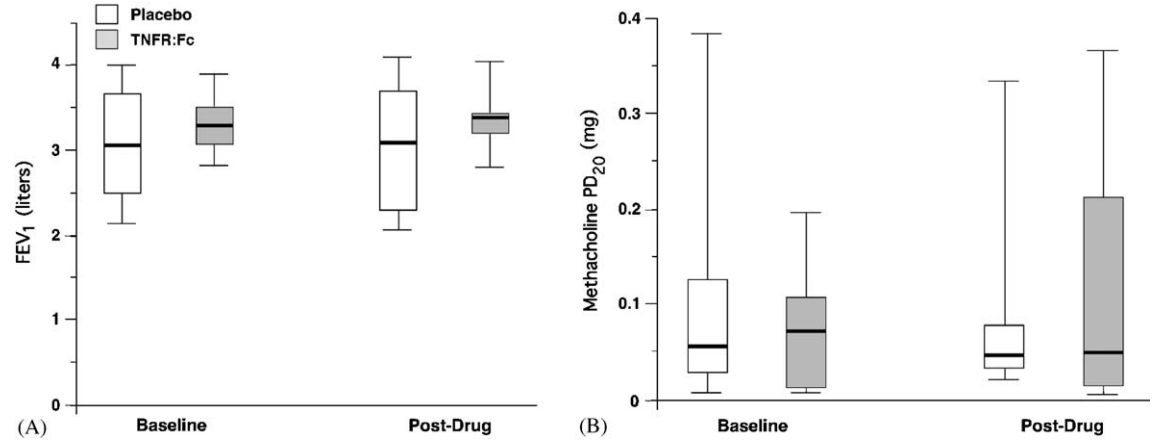

Figure 4 The effect of TNF antagonism on airflow obstruction and hyperresponsiveness. Data are presented as medians, with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (I bars). TNF antagonism had no effect on either airflow obstruction (Panel A), as measured by the FEV_1 in liters ($P = 0.9$, 95% confidence interval −0.23, 0.25 L), or airway hyperresponsiveness (panel B), as measured by the methacholine PD_{20} in milligrams ($P = 0.4$, 95% confidence interval −0.202, 0.053 mg).

thereby negatively regulating the function of both Th1- and Th2-type T cells.[42,43] Conversely, TNF inhibition enhances T cell proliferation and cytokine production. Second, TNF signaling has been proposed to repress IL-4 gene expression in Th2-type T cells via binding of the receptor-associated factor (TRAF2) to the nuclear factor of activated T cells (NFAT)-interacting protein (NIP45).[44] The TRAF2-NIP45 interaction suppresses IL-4 production by inhibiting NIP45/NFATc2/c-maf transactivation of the IL-4 promoter.[44] Therefore, our finding that anti-TNF therapy amplifies allergen-mediated pulmonary IL-4 levels is consistent with a potential counter-regulatory role for TNF in modulating the function of Th2-type T cells in allergic asthma.

TNF antagonism may be associated with significant neurological effects in a subset of patients, including case reports of new-onset central nervous system demyelinating lesions in patients with inflammatory arthritides and increased disease activity in multiple sclerosis.[45–48] This information was included in the informed consent document for our trial, which was suspended following a serious adverse event of transient hemiplegia. The hemiplegia resolved completely following discontinuation of TNFR:Fc and no radiographic or laboratory

abnormality was identified as being causal for this episode. The patient was not re-challenged with TNFR:Fc. No evidence of central nervous system demyelination was detected. Although it is not possible to determine definitively whether this severe neurologic dysfunction was related to TNFR:Fc, it raises the concern that transient hemiplegia, which may closely mimic an evolving stroke, may be a toxicity related to anti-TNF therapy.

We conclude that TNF antagonism did not prevent the development of pulmonary eosinophilia in a segmental allergen challenge model of mild-to-moderate allergic asthma. Our results also suggest that TNF may play an important counter-regulatory role in allergen-mediated airway inflammation. Further, the possible association between TNF antagonism and transient hemiplegia should be considered in patients who develop new-onset muscular weakness while receiving anti-TNF therapy.

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