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Letter

Effect of inhalation exposure to wheat flour on sputum levels of brain-derived neurotrophic factor

In the physiopathogenesis of occupational asthma (OA), neurogenic mechanisms may be involved, although they remain poorly understood. Currently, there is increasing interest in the potential role of neuroeffector mechanisms in asthma. The neurotrophins family, comprising nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin 3, and neurotrophin 4, appears to have a role in features of asthma, including airway hyperresponsiveness and inflammation, neuronal plasticity, and airway remodelling.¹ BDNF levels have been found to be enhanced in serum from asthmatic patients.² In addition, BDNF levels are increased in bronchoalveolar lavage fluid (BALF) from asthmatic patients after allergen challenge.³ This finding has been corroborated in mouse models of allergen challenge.⁴ BDNF also appears to play a role in the eosinophilic inflammation observed in asthma. This effect is exerted by direct interaction with the neurotrophin receptors on the surface of eosinophils.⁵ In addition, human peripheral eosinophils are capable of producing BDNF.⁶ There are no data on changes in BDNF levels in induced sputum (IS) after challenge with occupational allergens. We assessed the effect of specific inhalation challenge (SIC) with flour on sputum levels of BDNF.

Eight men (27–49 years old) with a known diagnosis of OA due to wheat flour were studied (eTable 1). The diagnosis of OA was made in all cases by SIC in a specialized hospital laboratory. The men had a positive skin prick test result to one or more common aeroallergens and to wheat flour. Forced expiratory volume in 1 second (FEV₁) was 70% or greater in all study participants. Most participants were nonsmokers or ex-smokers ($n = 7$ of 8), with a mean duration of exposure to causal agent of 12.7 years. After the diagnosis of OA, all participants were no longer exposed to causal agent and asthma symptoms were stable. Informed consent was obtained from all participants.

The study participants underwent standardized SIC as previously described,⁷ with monitoring of bronchial responses on consecutive control and active challenge days. FEV₁ was measured before and serially (every 10 minutes for 1 hour, every 30 minutes for 2 hours, and then hourly) for 6 hours after exposure. IS examination and measurement of bronchial responsiveness to methacholine were performed at the end of the day on the control and flour challenge days, according to standardized procedures.⁸ BDNF was measured in IS samples using the RayBio Human BDNF enzyme-linked immunosorbent assay kit (Lucerna-Chem AG, Luzern, Switzerland) according to the manufacturer's instructions.

Disclosures: Authors have nothing to disclose.

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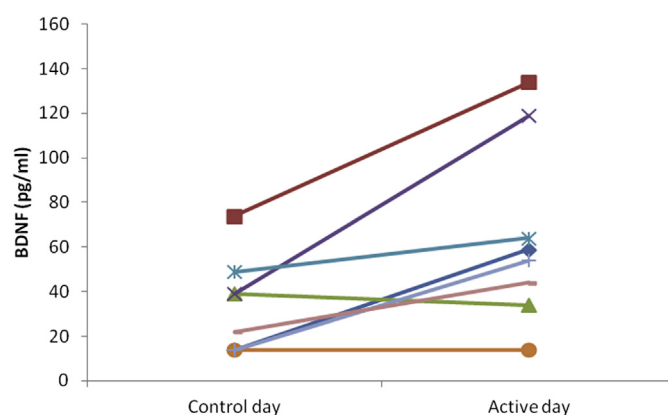


Figure 1. Levels of brain-derived neurotrophic factor (BDNF) in induced sputum after control and active challenge.

Differences within sets of paired data were analyzed using the Wilcoxon signed rank test. Spearman rank correlation coefficient was used to analyze the correlation between BDNF levels and clinical and inflammatory parameters.

FEV₁ did not significantly change after control challenge. In contrast, the challenge with flour induced an early-phase reaction in all patients defined as a decrease in FEV₁ of more than 20% after challenge.⁷ During the control day, the mean (SD) maximum percentage decrease in FEV₁ from baseline was 3.9% (4.0%), whereas after flour challenge it was 17.7% (7.9%).

There was a significant increase in the number of eosinophils ($P = .04$) and nonsignificant changes in the number of neutrophils ($P = .60$), macrophages ($P = .60$), and lymphocytes ($P = .20$) when comparing levels in IS collected 6 hours after control and active challenge (eTable 2). BDNF levels were significantly greater after challenge with flour compared with those after control challenge ($P = .02$) (Fig 1). A significant negative correlation between the maximal decrease in FEV₁ and BDNF levels was observed ($r = -0.71$; $P = .04$) after allergen challenge (eFig 1). A moderate nonsignificant negative correlation between BDNF and eosinophils levels ($r = -0.47$; $P = .20$) was observed after allergen challenge.

We found that BDNF levels in IS from allergic OA patients increased after SIC with flour. This finding was associated with elicitation of asthma symptoms and bronchial eosinophilic inflammation. These findings add to a increasing body of research that suggests a role of BDNF in asthma. To our knowledge, only one study has assessed changes in levels of BDNF in the lower airways after allergen challenge. The study found increased levels of BDNF, nerve growth factor, and neurotrophin 3 in BALF from asthmatic

patients after segmental allergen challenge.³ Our study differs from the above-referred study in that participants were challenged with an occupational allergen using the SIC method and BDNF levels were measured in IS. However, our findings concur with this study that found increased levels of BDNF in IS in the late phase of the asthmatic response to challenge. Moreover, this observation is also consistent with animal models that found increased levels of BDNF in allergen-sensitized and challenged mice.⁴

Contrary to the expected pattern, we found that BDNF levels were inversely correlated with postchallenge changes in FEV₁. This finding may be explained by a plausible association of BDNF effects with disease severity, with the maximum of BDNF levels and BDNF effects occurring in patients with less severe disease and/or reaction after challenge. A study has found that BDNF serum level was inversely correlated with FEV₁ in a group of pediatric patients with asthma.⁹ However, our finding requires further investigation because this pattern was not clearly evidenced in our patients.

Eosinophils appear to play a role in allergic OA because they increase in the sputum of patients with OA after SIC with occupational allergens.⁸ There is evidence supporting a role of neurotrophins contributing to eosinophilia in asthma. Neurotrophins may exert immunomodulatory functions on eosinophils by influencing the activation and survival of these cells in the lungs.¹ Particularly, BDNF has a functional role on eosinophil viability and activation.⁵ In addition, recent data indicate that eosinophils can produce BDNF.⁶ As expected, we observed a significant increase in sputum eosinophils after SIC with flour; however, the correlation between eosinophils counts and BDNF levels after challenge with flour was moderate and nonsignificant. Thus, we cannot speculate on a potential functional relationship between the observed increase in BDNF and eosinophils levels in our patients.

Our study found interesting results despite some limitations (ie, small sample size, lack of additional laboratory analyses, and lack of data on other neurotrophins). The results support the hypothesis that BDNF may contribute to the physiopathogenesis of asthma. We also believe that they extend findings on a potential role of BDNF in nonoccupational asthma to work-related asthma.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.01.018>.

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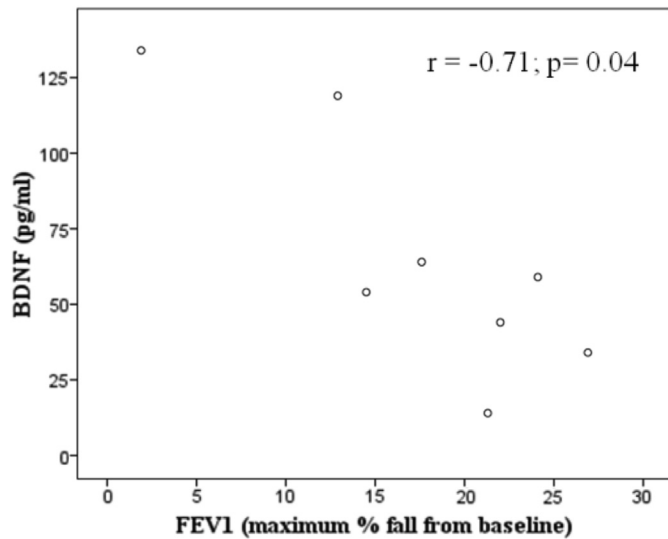
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**eTable 2**

Cell analysis of induced sputum after control and active challenge

Cell type	After control challenge, % (IQR)	After active challenge, % (IQR)
Neutrophils	40.1 (38.4)	53.1 (33.8)
Eosinophils	1.0 (6.2)	13.8 (15.1) ^a
Macrophages	30.8 (47.9)	19.8 (38.9)
Lymphocytes	1.5 (2.1)	0.6 (1.4)

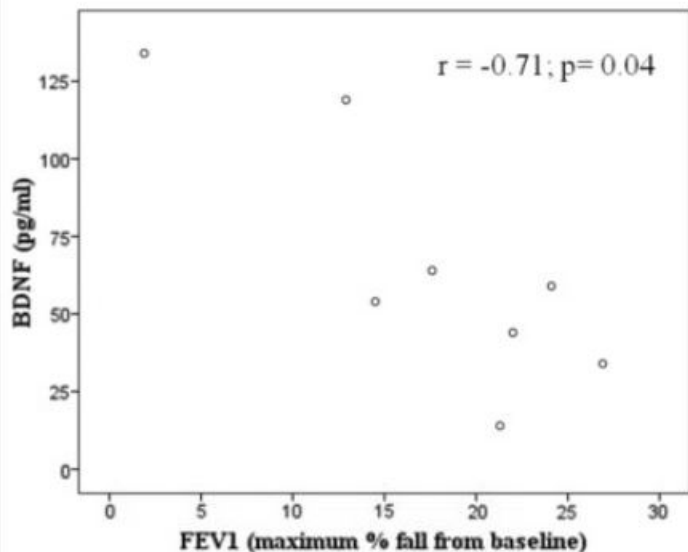
Abbreviation: IQR, interquartile range.

^a*P* < .05.**eFigure 1.** Correlation between the sputum levels of brain-derived neurotrophic factor (BDNF) and the maximal decrease in forced expiratory volume in 1 second (FEV₁), expressed as the percentage decrease from baseline after challenge.**eTable 1**

Clinical characteristics of the study participants

Characteristic	Finding
Participants, No.	8
Sex, No. M/F	8/0
Age, mean (SD), y	40.2 (9.0)
Atopy, No. present/absent	8/0
Smoking, S/ES/NS	1/4/3
Duration of exposure at work, mean (SD), y	12.7 (7.1)
FEV ₁ , mean (SD), % predicted	97.6 (15.2)
PC ₂₀ ≤16 mg/ml, No./total No.	5/8

Abbreviations: ES, ex-smoker; FEV₁, forced expiratory volume in 1 second; NS, nonsmoker; PC₂₀, concentration of methacholine that caused a 20% decrease in FEV₁; S, smoker.



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