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Effect of influenza vaccinations on immune response and serum eotaxin level in patients with allergic bronchial asthma

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***Background*: One of the most promising markers of allergic inflammation is eotaxin, which has a selective influence on the migration of eosinophils. Its serum content significantly correlates with the intensity of allergic symptoms, so it might be interesting to know whether vaccination has any influence on serum expression of this chemokine.**

***Aims*: Comparison of the humoral response to influenza vaccine and post-vaccination changes in the serum eotaxin level in patients with allergic bronchial asthma and healthy controls.**

***Methods*: Forty-two asthmatics and 45 healthy individuals were vaccinated with a single dose of influenza subunit vaccine (Influvac). The serum eotaxin level and the antibody response to haemagglutinin (HI) and neuraminidase (NI) glycoproteins were measured before and after vaccination.**

***Results*: A significant increase of geometric mean titres of HI and NI was observed in both groups. There were no significant differences between the groups in meanfold increase of HI and NI titres, response rate and protective level of HI. After vaccination, a significant decrease of the mean serum eotaxin value was observed in patients with asthma (149.4 ± 71.0 versus 125.1 ± 67.0, $p = 0.0017$), while no similar effect was present in healthy individuals (153.4 ± 56.9 versus 159.3 ± 54.4, $p = 0.5$).**

***Conclusions*: The results indicate that in patients with allergic bronchial asthma influenza vaccinations assure efficient protective antibody level and modulate the serum level of eotaxin.**

Key words: Influenza vaccination, Eotaxin, Asthma, Allergy

Introduction

Influenza is one of the most common respiratory tract diseases. Infections with influenza virus can cause exacerbations of bronchial asthma and, in consequence, can lead to life-quality impairment and higher mortality in patients with asthma.[1–3] Although vaccinations are considered the most efficacious method of providing protection against influenza in most people, there are some data suggesting that influenza vaccinations do not bring about effective anti-virus immunization.[4–6] On the other hand, it might be interesting to know whether vaccination has any influence on serum expression of inflammation cytokines. One of the most promising markers of allergic inflammation is eotaxin, which has a selective influence on migration of eosinophils.[7–9] Many authors have revealed that the concentration of eotaxin is higher in patients with allergic bronchial asthma.[10,11] In this study we assess humoral antibody response and changes in serum eotaxin level after influenza vaccinations in healthy people and inpatients with allergic bronchial asthma.

Materials and methods

The study population comprised 42 subjects (22 females and 20 males) with stable allergic bronchial asthma with a mean age of 46.6 years (standard deviation, 18.0), and 45 healthy controls (32 females and 13 males) with a mean age of 44.5 years (standard deviation, 13.5).

Subjects with bronchial asthma had a history of intermittent wheeze, chest tightness, cough and sputum production either spontaneously, after allergen exposure, or after exercise, together with reversible airflow limitation documented within 6 months preceding the study.[12] Respiratory symptoms were controlled with β_2-adrenergic drugs and inhaled steroids in an equivalent dose of less than 800 μg of budesonide per day. The allergic origin of the

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complaints has been proven by positive results of the skin prick tests and a significant serum level of immunoglobulin E specific to common aeroallergens.

Each subject from the healthy control group had a normal value of pulmonary function tests, a negative history of allergic diseases and negative results of the skin prick tests.

People with a smoking habit, hypersensitivity to eggs, chicken, influenzal proteins or evidence of neoplasmatic disease, leukaemia and any other significant immunocompromised status were not included into the study. Also, individuals who had been vaccinated against influenza during the 2 years before the study, or had had symptoms of any infection or had received antivirals or immunosupressive drugs during 30 days before the recruitment were not registered.

Each subject received an intramuscular single dose of influenza subunit vaccine (Influvac; Solvay Pharmaceuticals GmbH, Brussels, Belgium). The vaccine contained 15 μg of haemagglutinin (HI) of each of the following virus strains: A/New Caledonia/20/99 (H1N1), A/Moscow/10/99 (H3N2) and B/Sichuan/379/99. The antibody response to HI and neuraminidase (NI) glycoproteins of influenza virus and the eotaxin level were measured in serum collected during 24 h before and 6 weeks after vaccination. All samples were stored at -20°C until use. An enzyme-linked immunosorbent assay method was used for serum eotaxin measurements (ELISA R&D kits, USA).[13] After non-specific serum inhibitor inactivation (by heating and treatment with receptor-destroying enzyme), anti-haemagglutinin and anti-neuraminidase levels were determined by the standardized inhibition tests and were expressed as the last serum dilution showing complete inhibition.[14,15] For expression of humoral response in each studied group the following parameters were used:

- geometric mean titres (GMT) of HI and NI antibodies (titre $<1{:}10$ was arbitrarily set at 5);
- meanfold increase of GMT and response rate (i.e. the proportion of subjects showing at least a fourfold increase in HI antibody titre); and
- protection rate (i.e. the proportion of subjects showing HI antibody level $\geq 1{:}40$).

Quantitative, normally distributed data were analysed by *t*-test. Chi-squared and Wilcoxon's signed-rank sum tests were used for comparative analysis of non-paired, non-normally distributed variables. Correlation coefficients were calculated for assessment of the relationship between humoral response and serum eotaxin concentration. $p<0.05$ was considered significant.

The study protocol was approved by the ethics committee of the Military School of Medicine in Warsaw (Poland).

Results

The results of humoral response are summarized in Tables 1 and 2. Six weeks after immunization, there was a significant increase of the GMT of HI and NI antibodies to the three virus antigens in both studied groups (Wilcoxon's rank-sum test, $p<0.05$). The meanfold increase of HI and anti-neuraminidase titres ranged accordingly: 5.2–23.7 and 4.3–11.1 for the subjects with asthma; and 8.2–14.1 and 7.1–10.2 for the healthy controls. There were no significant differences between the asthmatics and normals in response rate to vaccination and the proportion of subjects with the protection level of HI before and after vaccine administration (chi-squared test, $p>0.05$).

After vaccination a significant decrease of the mean serum eotaxin value was observed in the patients with asthma (149.4 ± 71.0 versus 125.1 ± 67.0, $p=0.0017$), while no similar effect was present in the healthy individuals (153.4 ± 56.9 versus 159.3 ± 54.4, $p=0.5$) (Fig. 1). No significant linear correlation was found between changes in serum eotaxin content and meanfold increase of HI and NI antibody titres after vaccination ($p>0.05$).

Discussion

Respiratory tract infections, especially infection with influenza virus, can trigger exacerbations of bronchial asthma, and can lead to a higher frequency of illness-related hospitalizations and higher death rate.[16–18] Therefore, annual vaccinations against influenza in patients with chronic pulmonary diseases are recommended by The Advisory Committee on Immunization Practices.[1] Safety and good tolerability of vaccines has been proven in many studies;[19–22] however, there are still scarce data on whether immune response after vaccination in patients with bronchial asthma is comparable with that in healthy people. The main protective effect of influenza vaccination is related to antibody response to HI antigen of virus.[23] Depending on their concentration, HI antibodies inhibit effectively the attachment of virus to target cell membrane receptors and provide prevention against serious illness. Protection studies have shown that anti-haemagglutinin antibody titres ≥ 40 can be considered as the safety threshold beyond which serious influenza infection is rather unlikely.[24] According to the settlement of the Commmittee for Proprietary Medicinal Products and

Table 1. Anti-haemagglutinin antibodies kinetics before and after vaccinations against influenza in healthy subjects and patients with bronchial asthma

Antigen	GMT (minimal–maximal level)		Meanfold increase	Proportion of protected subjects		Response rate
	Before vaccination	6 weeks after vaccination		Before vaccination	6 weeks after vaccination	
Asthma patients						
A/New Caledonia/20/99 (H1N1)	8.5 (5.0–40.0)	201.6 (20.0–640.0)	23.7	10/42 (23.8%)	40/42 (95.2%)	42/42 (100%)
A/Moscow/10/99 (H3N2)	21.4 (5.0–320.0)	111.3 (20.0–320.0)	5.2	12/42 (28.6%)	38/42 (90.5%)	26/42 (62%)
B/Sichuan/379/99	7.7 (5.0–40.0)	63.5 (20.0–160.0)	8.3	4/42 (9.5%)	32/42 (76.2%)	38/42 (90.5%)
Healthy subjects						
A/New Caledonia/20/99 (H1N1)	10.8 (5.0–160.0)	152.8 (20.0–640.0)	14.1	9/45 (20.0%)	43/45 (95.6%)	42/45 (93.3%)
A/Moscow/10/99 (H3N2)	9.7 (5.0–80.0)	80.0 (10.0–640.0)	8.2	6/45 (13.3%)	36/45 (80.0%)	35/45 (77.8%)
B/Sichuan/379/99	6.7 (5.0–40.0)	69.6 (20.0–320.0)	10.4	3/45 (6.7%)	43/45 (95.6%)	3/45 (93.3%)

the Commission of the European Community, after influenza vaccination the protection rate should amount to at least 70% (60% in patients older than 60 years) of vaccinees and the response rate should be at least 40% (30% in people older than 60 years) of vaccinees.[25,26]

In the present study no significant differences in the proportion of subjects protected were observed between the healthy individuals and the patients with allergic bronchial asthma after influenza vaccination. Moreover, the protection rate reached the recommended level in each tested group. The response rate value was also comparable in these groups and reached the recommended value. Significant increase of antineuraminidase antibodies level, responsible for inhibition of the release of mature viral particles from infected cells,[27] was present after influenza vaccination in both groups.

It is noteworthy that the serum eotaxin level significantly decreased after vaccination in the patients with bronchial asthma. An increased expression of eotaxin within the peripheral airways of lungs of the asthmatic subjects was shown, suggesting that this chemokine contributes to the small airways and peripheral lung inflammation in asthma.[11] Some studies indicate that the plasma eotaxin concentration significantly rises in the case of exacerbation of allergic symptoms.[28,29] Thus, decreasing the serum eotaxin content can be an additional health benefit of vaccination for patients with allergic asthma. It seems probable that the mechanism of serum eotaxin depletion after vaccination is associated with stimulation of different subsets of T-helper (Th) lymphocytes.[30] One type of $CD4^+$ clone (Th1) produces, among others, interferon (IFN)-γ; a second type (Th2) produces interleukin (IL)-4 and IL-5.[31,32] It is well known that IFN-γ plays a pivotal role in the defence against viruses and its expression significantly increases during viral infections or after antiviral vaccinations.[33,34] *In vitro* analysis of the mechanisms of eotaxin generation by Th1-/Th2-derived cytokines revealed that Th2-type cytokine (i.e. IL-4) induced eotaxin production, while Th1-type cytokine (IFN) inhibited eotaxin generation.[35] This mechanism seems to be a good explanation for downregulation of the serum eotaxin level in subjects with asthma, but should be proved in following studies. We did not observe any significant correlation between humoral response and serum eotaxin changes in asthmatics and healthy individuals. However, some data indicate also that there is no relationship between antibody response to influenza vaccine and IFN-γ.[34]

Table 2. Anti-neuraminidase antibodies titres before and after vaccinations against influenza in healthy subjects and patients with bronchial asthma

Antigen	GMT (minimal–maximal level)		Meanfold increase
	Before vaccination	6 weeks after vaccination	
Asthma patients			
A/New Caledonia/20/99 (H1N1)	7.2 (5.0–20.0)	80.0 (40.0–160.0)	11.1
A/Moscow/10/99 (H3N2)	11.4 (5.0–160.0)	48.8 (10.0–160.0)	4.3
B/Sichuan/379/99	6.3 (5.0–20.0)	45.6 (20.0–160.0)	7.2
Healthy subjects			
A/New Caledonia/20/99 (H1N1)	7.5 (5.0–80.0)	72.9 (20.0–160.0)	9.7
A/Moscow/10/99 (H3N2)	8.1 (5.0–40.0)	57.9 (10.0–160.0)	7.1
B/Sichuan/379/99	6.2 (5.0–20.0)	63.5 (20.0–160.0)	10.2

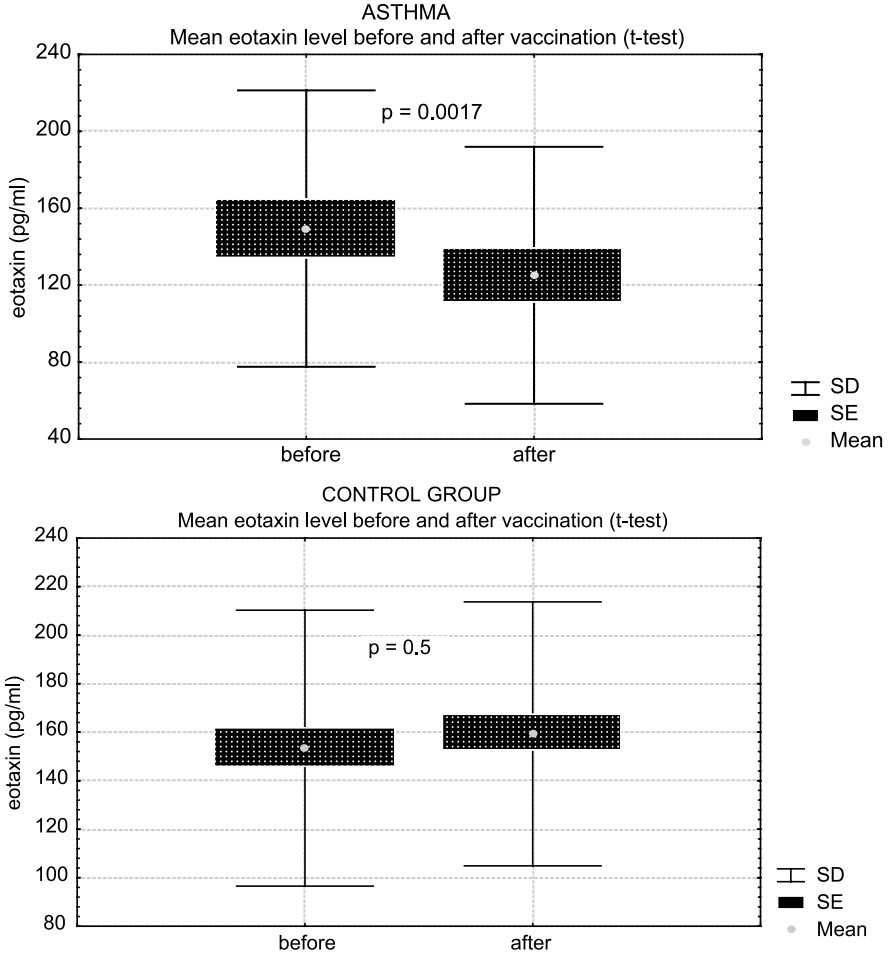

FIG. 1. Serum eotaxin level before and after vaccinations against influenza in both studied groups. SD, standard deviation; SE, standard error.

Conclusions

The results of this trial indicate that the immune response to influenza vaccine in asthmatics is comparable with those in healthy individuals. Moreover, in patients with allergic asthma, vaccinations can cause depletion of the serum level of eotaxin — a strong primary mediator of tissue eosinophilia. The mechanism that makes influenza vaccination have different influence on serum eotaxin level in healthy people and subjects with allergic asthma is unclear, and requires further study.

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