Advances in Medical Sciences 63 (2018) 79–86

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

A similar pro/anti-inflammatory cytokine balance is present in the airways of competitive athletes and non-exercising asthmatics

Marcin Kurowski[a], Janusz Jurczyk[b], Agnieszka Olszewska-Ziąber[a], Marzanna Jarzębska[a], Hubert Krysztofiak[b,c], Marek L. Kowalski[a,*]

[a] Department of Immunology, Rheumatology and Allergy, Healthy Ageing Research Centre, Medical University of Łódź, Łódź, Poland
[b] National Centre for Sports Medicine (COMS), Warsaw, Poland
[c] Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

ARTICLE INFO

Article history:
Received 14 February 2017
Accepted 18 July 2017
Available online 17 August 2017

Keywords:
Airway inflammation
Innate immunity
Exercise
Asthma
Competitive athletes

ABSTRACT

Purpose: Intensive exercise modifies airway inflammation and infection susceptibility. We aimed to determine the effect of exercise on pro- and anti-inflammatory cytokine (TNF-α, IL-1ra, IL-10) and innate immunity protein (HSPA1, sCD14) levels in exhaled breath condensate (EBC) and nasal secretions of competitive athletes, non-exercising asthmatics and healthy controls (HC).

Material and methods: The study group consisted of 15 competitive athletes (five speed skaters and ten swimmers) aged 15–25. The control groups comprised 10 mild-to-moderate asthmatics (AC) and seven HC. Athletes were assessed in- and off-training while asthmatics and controls at one time point. Nasal lavages and EBC were collected before and after a treadmill exercise challenge. Protein levels were assessed using ELISA.

Results: TNF-α levels in EBC were significantly higher in athletes than HC, but similar to asthmatic patients. In contrast, IL-1ra EBC concentrations were significantly lower in athletes than in HC, but again similar to asthmatics. Significant positive correlations were seen between baseline concentrations of TNF-α in EBC and fall in FEV1 following exercise challenge in athletes during training period ($R = 0.74$, $p < 0.01$) and in asthmatics ($R = 0.64$, $p < 0.05$). In nasal secretions, baseline IL-1ra levels were significantly higher in athletes and asthmatics than in HC. Exercise caused a slight, yet significant, increase in EBC HSPA1 in athletes ($p = 0.02$). The exercise challenge did not considerably influence TNF-α, IL-1ra, HSPA1 and sCD14 in EBC or nasal secretions.

Conclusions: Dysregulation of the TNF-α/IL-1ra balance in EBC and nasal secretions from athletes may reflect the presence of airway inflammation induced by repeated strenuous exercise.

© 2017 Medical University of Bialystok. Published by Elsevier B.V. All rights reserved.

1. Introduction

Regular physical exercise modifies the immune response depending on training level and frequency [1]. Strenuous exercise is believed to increase the prevalence of symptoms of respiratory tract infections (RTI) in regularly training subjects [2,3]. Nevertheless, microbial pathogens could be identified in less than one-third of cases of RTI-like symptoms [4] suggesting that non-infectious inflammatory factors could be responsible [1]. This airway inflammation may be further enhanced by unfavorable ambient conditions during exercise [5–8]. Exercise-induced bronchoconstriction (EIB) is almost an inherent feature of asthma, although it may occur as an isolated phenomenon in otherwise asthma-free subjects following exercise [9]. Local and systemic inflammation is important in the temporary narrowing of the airway associated with exercise [10–14]. The influence of exercise on the respiratory mucosa has been studied in a number of studies testing the concentrations of multiple mediators in exhaled breath condensate (EBC) or nasal lavage fluid (NLF) as a reflection of innate and inflammatory response [12,14–17]. Changes in the serum levels of innate immunity proteins are known to occur in professional athletes during the training season, in response to exercise load and ambient training conditions; similarly, serum levels of interleukin-1 receptor antagonist (IL-1ra) and heat shock protein HSPA1 increase in outdoor speed skaters during winter training, and serum IL-1ra and airway hyperreactivity are influenced on a long-term basis by the weather in the training area [6].

* Corresponding author at: Department of Immunology, Rheumatology and Allergy, Medical University of Lodz, Pomorska 251 bldg C-5, 92-213 Łódź, Poland.
E-mail addresses: marcin.kurowski@umed.lodz.pl (M. Kurowski), janusz.jurczyk@coms.pl (J. Jurczyk), aoz@csk.umed.lodz.pl (A. Olszewska-Ziąber), marzanna.jarzebska@umed.lodz.pl (M. Jarzębska), hubert.krysztofiak@coms.pl (H. Krysztofiak), marek.kowalski@csk.umed.lodz.pl (M.L. Kowalski).

http://dx.doi.org/10.1016/j.advms.2017.07.004
1896-1126/© 2017 Medical University of Bialystok. Published by Elsevier B.V. All rights reserved.

Data concerning the influence of exercise on mediators of inflammation and innate immunity in the upper and lower airways is limited. TNF-α is a well-known pleiotropic pro-inflammatory cytokine released by a wide spectrum of cells. Its mRNA and protein levels are increased in asthmatic airways. Moreover, mast cell-derived TNF-α has been postulated as playing a role in the pathophysiology of airway smooth muscle contraction (as reviewed in [18,19]). A bout of exercise induces a peripheral increase of TNF-α, followed shortly by a secondary release of interleukin 10 (IL-10) and IL-1ra. These two interleukins are believed to have anti-inflammatory and immunomodulatory properties, and their plasma concentrations have been found to decrease in infection-prone subjects following exercise [2]. CD14 is a receptor for bacterial lipopolysaccharide (LPS) and is regarded as a marker of monocyte activation. HSPA1 is a "danger signal" for innate immunity mechanisms associated with natural killer (NK) cells and monocyte activation. Acute exercise has been described as a stimulus used to increase circulating heat shock protein levels [20].

The current study hypothesizes that the profile of inflammatory and innate immunity cytokines present locally in the upper and lower respiratory mucosae of competitive athletes resembles that present in the airways of non-exercising asthmatics. Therefore, the study compares the levels of three selected pro- and anti-inflammatory cytokines, i.e. tumor necrosis factor- α (TNF-α), interleukin 1 receptor antagonist (IL-1ra) and interleukin 10 (IL-10), and those of two innate immunity proteins, i.e. heat shock protein A1 (HSPA1) and soluble form of CD14 particle (sCD14), in the EBC and nasal secretions of competitive athletes, non-exercising asthmatics and healthy controls. In addition, the influence of acute exercise on the above parameters was studied with an aim to correlate the baseline levels of proteins with lung function parameters and exercise load in training.

2. Methods

2.1. Participants and study design

The study group consisted of 15 competitive athletes (five speed skaters and 10 swimmers) aged 15–25 years. The control groups comprised 10 mild-to-moderate asthmatics (asthma controls, AC) aged 19–39 and seven healthy, non-smoking subjects aged 21–27 (healthy controls, HC). The asthmatic subjects were recruited from the patients treated at the outpatient clinic of the Department of Allergology. The healthy control (HC) group comprised volunteering medical students and doctors. The exclusion criteria comprised any signs or symptoms of respiratory infection on assessment days and/or within four weeks beforehand, assessed according to the patient's reported history.

The clinical characteristics and exercise load data of the participating subjects are presented in Table 1. Median asthma duration in AC subjects was 4.5 years (range: 1–28 years). All asthmatics were fully controlled on inhaled corticosteroids (428 ± 66 μg budesonide equivalent, mean ± SE). Among the athletes, 11 (73.3%) had never been diagnosed with allergy, four (26.7%) had allergic rhinitis and one (6.67%) had been diagnosed with asthma. Symptoms of respiratory discomfort were self-reported by 66.7% (chest tightness/wheeze) and 53.3% (shortness of breath/cough) of athletes. Five athletes (33.3%) reported symptoms of rhinitis and one (6.67%) conjunctivitis.

Speed skaters and swimmers were competitive athletes performing at national level in their respective disciplines. They had been training on a regular basis for at least three consecutive seasons at the moment of recruitment into the study. To avoid possible selection bias, all athletes from local speed skating club and academic swimming section were invited to the study without presenting any incentive for those self-suspecting asthma or allergy or having been diagnosed with either condition. The athletes were assessed at two time points: Firstly, during a period of more intensive training and participation in competitions (training period, TP), and secondly, when the exercise performed was less typical for a given discipline and no participation in competition tournaments was required (off-training period, OTP). However, the one-week and four-week periods preceding the assessment had similar overall exercise loads (Table 1). The TPs for skaters lasted from January through March, and for swimmers from June through to the beginning of October. OTPs were from May through July for speed skaters and late autumn and winter for swimmers. The usual training regimen for both speed skaters and swimmers, irrespective of season, typically included a daily three-hour session, six days a week. Assessments were carried out at both time points (i.e., TP and OTP) during normal training activity and no abstention from exercise was required. In most cases, previous training activity took place in the afternoon of the preceding day. Ten athletes (three skaters and seven swimmers) were assessed during both periods. One skater and three swimmers were seen during OTP and one skater during TP only. AC and HC were assessed at one time point during late summer or early autumn 2014; in addition, a lifestyle assessment in terms of regular physical activity was also performed based on declarations by the subjects.

Table 1
Clinical and exercise load characteristics of the studied groups. Medians with interquartile ranges in parentheses are given unless indicated otherwise. *$p < 0.05$ versus HC; ND, not done.

	Athletes training period (TP)	Athletes off-training period (OTP)	Asthmatic controls (AC)	Healthy controls (HC)	P (Kruskal-Wallis ANOVA)
No. subjects (males/females)	11 (6/5)	14 (10/4)	10 (6/4)	7 (4/3)	ND
Age [years]	18 (15–20)	19 (16.5–20)	26 (21.5–34)	24 (23–26)	<0.0001
FEV1 [%predicted]	112 (107–125)	115 (109.5–123.3)	103.5 (96.75–111)	109 (107–113)	0.08
FEV1/FVC	0.84 (0.71–0.89)	0.83 (0.72–0.90)	0.82 (0.77–0.89)	0.84 (0.80–0.90)	0.97
IPAQ 1 week [MET-h/week]	8166 (4692–11679) *	9443 (5340–12980) *	3212 (1496–6645)	1236 (676–1920)	0.0067
Mean IPAQ 4 weeks [MET-h/week]	7566 (5724–11071) *	9657 (4875–12085) *	3786 (1686–7105)	1236 (1052–3252)	0.0027
No. atopics (%)	2 (18.2)	3 (21.4)	10 (100)	0 (0)	ND
No. positive exercise challenges (%)	2 (18.2)	1 (7.14)	5 (50)	0 (0)	ND
Percent FEV1 decrease during exercise challenge [geometric mean with range]	4.11 (1.49–19.35)	3.56 (1.02–17.28)	4.18 (0.38–22.68)	2.89 (0.39–7.65)	0.93

2.2. Questionnaires

Data regarding the history of allergic diseases, exercise-associated symptoms and demography was acquired using the Allergy Questionnaire in Athletes (AQUA) [21] The subjects also completed the Polish version of the standard short form of the International Physical Activity Questionnaire (IPAQ) providing information on exercise load as metabolic equivalents (MET)-h/week [22]. Since the training regime may vary over a longer period, the subjects completed four separate IPAQ sheets providing retrospective data on exercise load during the four preceding weeks.

2.3. Exercise challenge testing

A non-specific exercise challenge test was performed in accordance with current guidelines [23]. In athletes, exercise challenge was performed twice: once during the TP and once during the OTP. In asthmatic controls and in healthy controls, the exercise challenge was performed once between June and October. The challenge was performed on a Track Performance treadmill (Heinz Kettler GmbH und Co. KG, Ense-Parsit, Germany). After a two-minute warm-up, the subjects ran for six minutes with a target heart rate maintained between 80 and 90% predicted value calculated as: HR_{pred} = 220-[age in years]. Treadmill inclination was automatically adjusted to allow the heart rate to stay within the target value range. Heart rate was monitored using a wireless Cardio Pulse Set compatible with the treadmill (Heinz Kettler GmbH und Co. KG, Ense-Parsit, Germany), consisting of a chest belt and plug-in receiver. Spirometry was performed using a Lungtest 1000 device (MES, Kraków, Poland) at 5, 10, 15, 20 and 30 min after exercise. A fall in FEV1 greater than or equal to 10% was used as a criterion for positive challenge. The assessment of lung function parameters was based on reference equations previously published by Zapletal et al. [24] for children and adolescents and the European Respiratory Society [25] for adults.

2.4. Exhaled breath condensate (EBC)

EBC samples were obtained using a Turbo DECCS (Disposable Exhaled Condensate Collection System) 09 device (Medivac di Romei Vanna, Parma, Italy) immediately before and 30 min after exercise challenge in accordance with the current recommendations [26,27] as described previously [11,13]. All measurements were performed at the same time (between 8.00 and 10.00 AM) to avoid circadian fluctuations of EBC mediator concentrations. Patients were asked to refrain from eating and drinking before EBC collection. Aliquots of condensate were immediately frozen at −80 °C for further storage. EBC collection was performed immediately before and 30 min after the exercise challenge test. Samples were not concentrated prior to measurement. Because no marker has been identified which can be used to correct for the difference in the degree of dilution, the study makes no attempt to assess the dilution of airway lining fluid (ALF) in EBC. Regarding exercise-induced changes of protein concentrations in EBC, the lowest inter-subject variability was seen for TNF-α in athletes during TP (c_v[coefficient of variation] = 12.7%) while the highest c_v was observed for IL-1ra in athletes during OTP (215.9%).

2.5. Nasal lavage

Nasal lavage fluid (NLF) samples were obtained immediately before and 20 min after exercise through lavage of the nasal cavity using 0.9% saline solution. With the head bent forward and the face held horizontally, the nasal cavity was filled with saline using a 5 ml syringe connected to the nostril via a short tube and nasal olive. Saline was kept in the nostril for 10 min and afterwards recovered by aspiration. The mean recovered volume (±SD) was 2.98 ± 0.69 ml (range 1.8–4.4 ml). The samples were centrifuged for 10 min at 700*g* to remove cellular elements, and the supernatants were aliquoted and stored at − 80 °C for further use. The opposite nostril was used for the lavage procedure performed after exercise testing.

The dilution of nasal secretion by lavage fluid was estimated according to the previously published formula:

dilution factor (DF) = [urea serum] × 1.2/[urea in NLF],

while the protein concentration in nasal secretion (NS) was calculated by multiplying DF by NLF concentration [28]. Urea levels in serum and NLF were measured spectrophotometrically using a Urea Assay Kit (Cell Biolabs, Inc., San Diego, CA, USA). No significant exercise-induced changes in urea concentrations were observed in serum or NLF.

In serum, the coefficient of variation of urea levels ranged from 32.3% in pre-exercise HC to 93.5% post-exercise in athletes during TP, while in NLF, the coefficient of variation ranged from 41.7% in post-exercise HC to 71.9% post-exercise in athletes during TP. No significant differences were observed between baseline and post-exercise DF during TP, OTP or AC. In HC, the dilution factor of nasal secretions was significantly higher in samples acquired before exercise challenge (11.25[8.43–21.64] vs 6.65[3.44–10.63]; p = 0.03; medians[IQR]). The inter-subject variability of exercise-induced fold-change of protein levels in nasal secretions was lowest for IL-1ra in athletes during the off-training period (c_v = 47.3%) and highest for TNF-α in HC (c_v = 227.5%).

2.6. Enzyme-linked immunosorbent assays (ELISA)

TNF-α, IL-10 and sCD14 levels were measured using a Gen Probe kit (Diaclone SAS, Besançon, France) while IL-1RA and HSPA1 were measured using kits from USCN Life Science Inc. (Wuhan, People's Republic of China) according to the manufacturer's instructions.

2.7. Statistics

Statistical analyses were carried out using STATISTICA 12 (StatSoft, Tulsa, OK, USA) and GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA, USA). The data was tested for a normal distribution using the Shapiro-Wilk normality test. The Mann-Whitney *U* test and Wilcoxon signed rank test were used for comparisons of continuous variables between unpaired and paired observations, respectively. The Kruskal-Wallis test and *post hoc* Dunn's multiple comparisons test were used for comparisons between more than two groups. Associations between variables were evaluated using the Spearman's rank correlation test. A *p*-value less than 0.05 was considered statistically significant.

2.8. Ethics

The study protocol was approved by Bioethics Commission of the Medical University of Łódź. All subjects or their legal guardians gave written informed consent for participation, study procedures and biological material sampling and storage.

3. Results

3.1. Cytokines in EBC

EBC TNF-α levels were significantly higher in athletes than in healthy controls, and were similar to those found in the asthmatic

subjects. In contrast, the IL-1ra levels of athletes were significantly lower than those of the HCs during both training periods, but were similar to those of the asthmatic subjects. IL-10 levels were below the lower limit of detection (5 pg/ml) in all EBC samples. (Fig. 1)

Exercise challenge did not induce any significant change in TNF-α nor IL-1ra level in EBC in any group (Table 2). However, significant positive correlations between baseline EBC TNF-α levels and percentage drop of FEV1 following exercise challenge were seen in athletes during the training period ($R = 0.74$, $p < 0.01$) (Fig. 2**A**) and in asthmatics ($R = 0.64$, $p < 0.05$) (Fig. 2**B**). Regression analysis showed that in athletes during TP, a 1 pg/ml increase of baseline EBC TNF-α level lead to 1.75 percent-point greater fall in FEV1 after the treadmill exercise challenge, and that 47% of the variability of the fall in FEV1% can be explained by changes in baseline EBC TNF- α ($b = 1.7483$; corrected $R^2 = 0.47$; $p = 0.01$). No relationships were revealed through regression analysis regarding remaining TNF- α or IL-1ra EBC levels in other training periods or study groups. No correlations between baseline TNF- α or IL-1ra in EBC and IPAQ-assessed exercise load were ascertained.

3.2. Cytokines in nasal secretions

Baseline IL-1ra levels in nasal secretions of athletes in both training periods were significantly higher than those of healthy controls but similar to asthma patients. No differences were observed regarding baseline levels of TNF-α in nasal secretions (Fig. 3). The levels of IL-10 were below the lower limit of detection (5 pg/ml) in all nasal secretion samples. Exercise challenge did not induce significant change in the levels of TNF-α of IL-1ra in nasal secretions in any group (Table 3).

3.3. Innate immunity proteins

No differences were observed regarding baseline HSPA1 levels in EBC. The sCD14 levels were below the lower limit of detection (6 ng/ml) in all EBC samples. No differences were observed in the case of baseline levels of HSPA1 and sCD14 in nasal secretions. (Fig. 3).

The bout of exercise induced a significant increase only in HSPA1 levels in EBC in athletes during training period ($p = 0.02$) (Table 2); however, this difference was only significant due to the presence of a several-fold change in individual subjects (Fig. 4). The exercise challenge did not induce significant change in any investigated protein level in nasal secretions (Table 3). No uniform pattern of exercise-associated changes in innate protein levels was observed regarding both EBC and nasal secretions. The fold changes of protein levels post-exercise did not differ between the groups (Fig. 5).

The athletes outside training season demonstrated a significant positive correlation between baseline HSPA1 level in nasal secretions and exercise load over seven days before assessment as determined by IPAQ ($R = 0.60$, $p = 0.039$; Spearman's rank correlation) **(Fig. S1 in the Supplementary material)**. Outside the training season, significant positive correlations were observed between fold change in nasal secretion sCD14 content and exercise load over the period of seven days ($R = 0.83$, $p = 0.002$) and 28 days ($R = 0.74$, $p < 0.01$) before assessment **(Fig. S2 in the Supplementary material)**.

4. Discussion

The present study indicates that the baseline pattern of proinflammatory cytokine TNF-α and anti-inflammatory IL-1ra concentrations in the lower airways appear to be similar in top level athletes and asthma patients, but different in healthy controls. However, some general factors restricting the interpretation of data concerning airway inflammation should be considered. Firstly, it must be noted that airway inflammation may, at least in part, be influenced by exposure to unfavorable ambient conditions in swimmers and speed skaters, resulting in damage to the airway epithelium [5,6,29]. Our study was not making provisions for separate influence of exercise or environment, therefore, changes observed in athletes should be regarded as resulting from joint influence of exercise and unfavorable conditions. However, since both factors are inseparable and act concomitantly, findings from the athletes' airways reflect their actual inflammatory status and can be put in comparison with findings from airways of asthmatics. Although ambient conditions have been reported as considerable and partially independent modifiers of local and systemic immune response in athletes [6], the extent of their contribution to airway

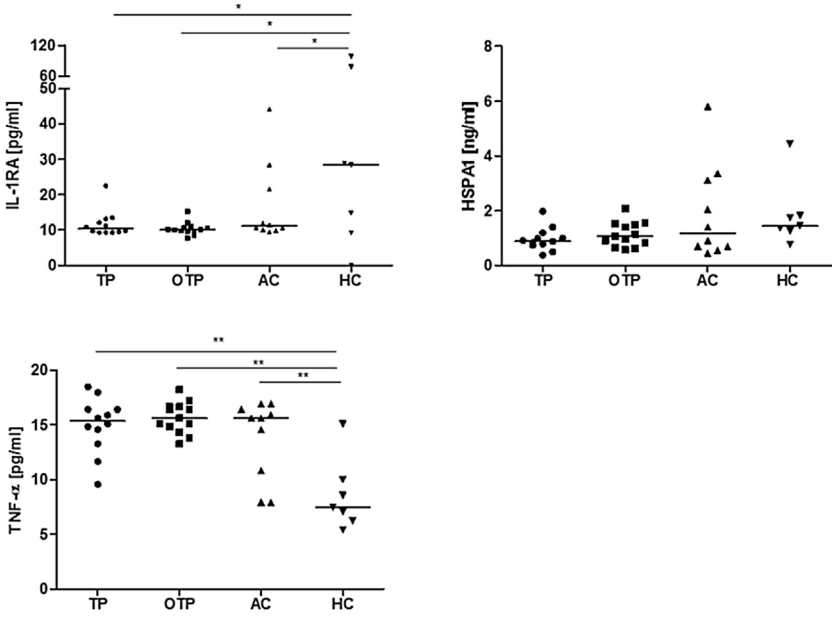

Fig. 1. Baseline (pre-exercise) levels of IL-1ra, HSPA1 and TNF-α in exhaled breath condensate. TP, athletes in-training period; OTP, athletes off-training period; AC, asthmatic controls; HC, healthy controls. Horizontal lines denote median values. * $p < 0.05$; ** $p < 0.01$.

Table 2
IL-1ra, HSPA1 and TNF-α levels in exhaled breath condensate before and after treadmill exercise challenge. Significant exercise-induced changes are highlighted with bold italics. Median values with interquartile ranges in brackets are presented. TP, athletes in-training period; OTP, athletes off-training period; AC, astmatic controls; HC, healthy controls. p values indicate level of significance between pre- and post-exercise values within a given subject group.

	TP			OTP			AC			HC		
	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P
IL-1ra [pg/ml]	10.88 [9.3–13.15]	11.45 [10.09–13.83]	0.55	10.09 [9.7–10.83]	10.09 [9.41–11.85]	0.75	11.11 [10.03–23.36]	10.66 [9.86–18.56]	0.39	28.48 [9.18–78.13]	20.8 [8.0–24.21]	0.06
HSPA1 [ng/ml]	0.89 [0.75–1.14]	1.13 [0.96–4.37]	***0.02***	1.07 [0.75–1.5]	1.31 [0.89–1.87]	0.38	1.16 [0.67–3.17]	2.81 [0.95–6.16]	0.06	1.45 [1.27-1.82]	1.56 [0.87–4.11]	0.81
TNF-α [pg/ml]	15.37 [13.61–16.41]	16.8 [15.17–19.01]	0.07	15.63 [14.58–6.67]	15.36 [14.32–17.58]	0.97	15.63 [10.1–16.54]	15.24 [11.14–16.93]	0.68	7.5 [6.25-10.0]	7.92 [6.67–10.5]	0.15

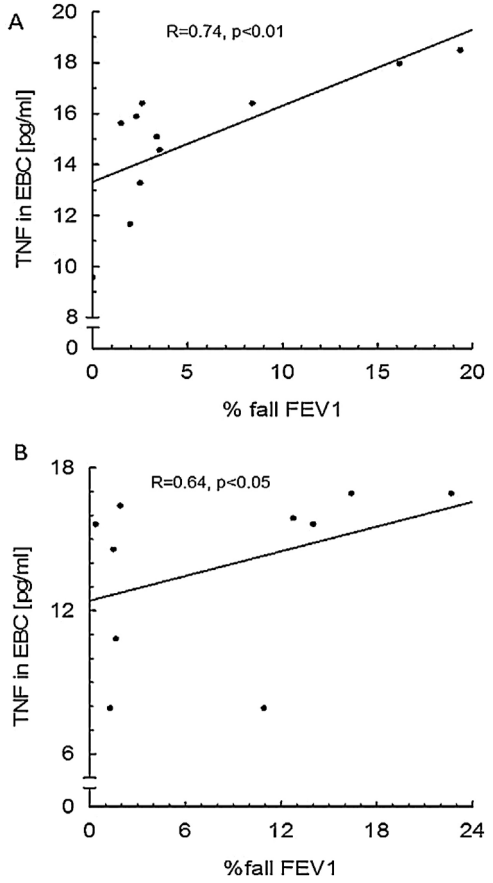

Fig. 2. Correlations between baseline EBC TNF-α levels and percent fall of FEV1 following exercise challenge in athletes during training period (panel A) and in asthmatics (panel B).

inflammation cannot be determined with high degree of accuracy unless specifically designed study is carried out.

In addition, aeroallergens and pollutants also contribute to airway inflammation, especially in outdoor exercisers [30,31]. Although our population was relatively homogenous in terms of residence area, and the influence of pollutants on airway inflammation can be regarded as evenly affecting all participants, fluctuations in the presence of aeroallergens may have influenced airway inflammation in atopic subjects. However, due to the small number of recruited atopic athletes it was not possible to perform a statistical sub-analysis of these groups.

The exercise challenge test protocol employed in this study is a generally-accepted method of assessing non-specific bronchial hyperresponsiveness. It is designed to be used in the general population, not only athletes, and the exercise load that it exerts is not nearly sufficient to imitate actual training conditions. However, this protocol was chosen to provide uniform exercise conditions throughout the study group, which included non-exercisers.

Baseline TNF-α EBC levels in athletes, both during in- and off-training periods, were significantly higher than in controls and comparable to those found in asthmatics. This finding is remarkable, since only one of the athletes had been previously diagnosed with asthma. However, two-thirds of athletes noted respiratory discomfort in the AQUA questionnaire and two of them hadpositive exercise provocationtest. This observation suggests that increased EBC TNF-α levels may reflect the presence of airway inflammation in athletes that is similar to the inflammatory process occurring in asthmatics. Exercising at a competitive level stimulates airway inflammation and causes epithelial damage [9]. Considering that our study was performed in athletes performing disciplines typified with a high risk of airway inflammation [32], it can be assumed that their high EBC TNF-α levels, which are similar to those seen in asthmatics, reflect chronic inflammation induced by repeated exercise. Moreover, the fact that a considerable number of athletes self-reported asthma-like symptoms without having been diagnosed with asthma further confirms the implication that TNF-α plays a role in EIB pathogenesis [19]. Increased plasma concentrations and cellular expression of TNF-α have been described in subjects with moderate-to-severe asthma refractory to intensive corticosteroid therapy [33,34]. Previous studies do not note any significant differences between mild, moderate and refractory asthmatics regarding TNF-α release from sputum and blood cells [35]. As the nature of the observations regarding local TNF-α behavior in the airways remains unclear, the presence of an elevated level in the airways of our subjects should not be considered the sole indication of ongoing airway inflammation.

Furthermore, as TNF-α concentrations in EBC were not increased by the acute exercise challenge, it is possible that they may reflect chronic stimulation and not acute exposure to hyperventilation. However, significant positive correlations were observed between baseline TNF-α concentration in EBC and percentage fall in FEV1 after exercise in both training athletes and in asthmatics, suggesting again that TNF-α plays an important role in the development of bronchial hyperreactivity in the airways of both athletes and asthmatics. Notably, the least inter-subject exercise-induced fold-change variability was seen with regard to EBC TNF-α (Fig. 5), which may add further weight to the hypothesis that TNF-α is a stable marker of chronic airway inflammation not directly related to EIB. It also suggests that TNF-α may not act as an exercise-inducible cytokine in the serum [36] or in the lower airways. The TNF-α concentrations observed in the EBCs of all groups in the present study were generally higher than those observed previously in other asthmatics [37–39]; however, the pleiotropic action of TNF-α and the complex pathogenesis of airway and systemic inflammation in which TNF-α is implicated

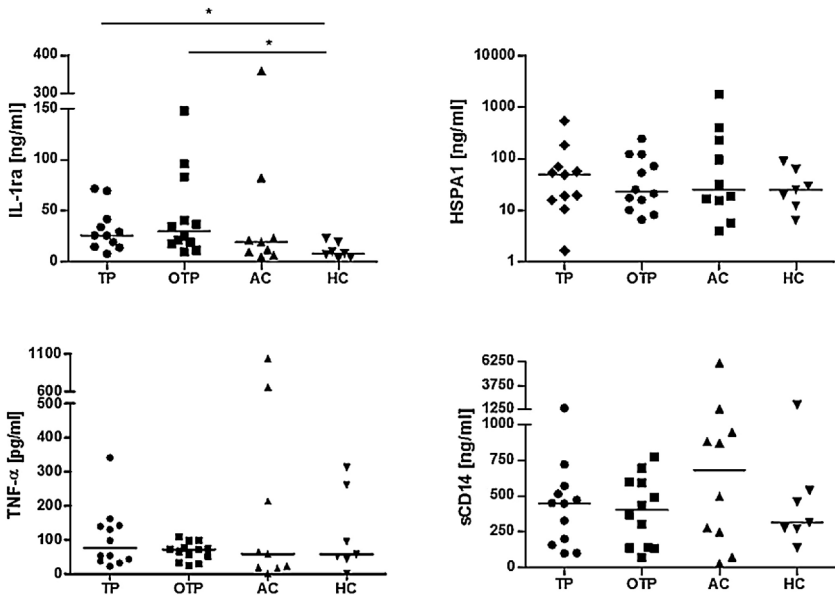

Fig. 3. Baseline (pre-exercise) levels of IL-1ra, HSPA1, TNF-α and sCD14 in nasal secretions. TP, athletes in-training period; OTP, athletes off-training period; AC, asthmatic controls; HC, healthy controls. Horizontal lines denote median values. Log scale for HSPA1 concentrations. * $p < 0.01$.

Table 3
IL-1ra, HSPA1 TNF-α and sCD14 levels in nasal secretions before and after treadmill exercise challenge. Median values with interquartile ranges in brackets are presented. TP, athletes in-training period; OTP, athletes off-training period; AC, astmatic controls; HC, healthy controls. p values indicate level of significance between pre- and post-exercise values within a given subject group.

	TP			OTP			AC			HC		
	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P	Pre-exercise	Post-exercise	P
IL-1ra [ng/ml]	25.81 [14.55–41.71]	38.42 [15.2–71.61]	0.12	29.76 [17.66–72.63]	18.36 [13.65–59.87]	0.47	19.09 [7.65–52.57]	72.68 [22.76–133.4]	0.20	7.52 [3.83–19.29]	4.35 [3.04–9.27]	0.58
HSPA1 [ng/ml]	49.43 [15.82–69.29]	50.89 [16.96–196.1]	0.37	23.26 [11.6–109.7]	29.77 [11.72–53.78]	0.79	25.39 [13.15–273]	64.75 [12.55–120.1]	0.28	25.31 [12.39–63.7]	13.92 [5.98–27.64]	0.47
TNF-α [pg/ml]	75.5 [38.94–141.4]	104.6 [34.28–289.9]	0.73	70.9 [42.08–86.25	81.88 [47.95–109.6]	0.25	58.36 [17.16–434.3]	65.86 [24.06–240.7]	0.73	57.15 [43.51–260.7]	46.42 [24.15–96.41]	0.20
sCD14 [ng/ml]	450 [168–558]	501 [107–1404]	0.34	401 [136–597]	251 [125–626]	0.57	684 [204–1038]	465 [165–2052]	0.77	313 [272–539]	297 [238–376]	0.47

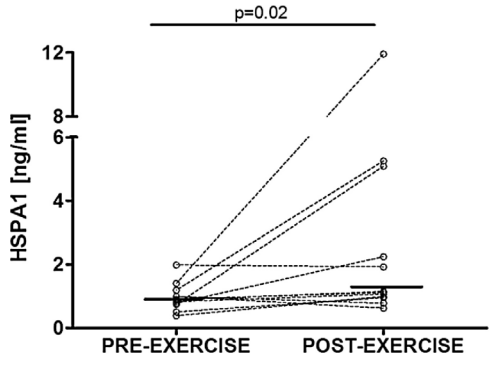

Fig. 4. Baseline and post-exercise levels of HSPA1 levels in athletes during training period. One open circle represents one subject. * p = 0.02.

underline the need for caution when assessing such findings, particularly those obtained from small, selected groups.

The EBC levels of the anti-inflammatory cytokine IL-1ra were found to be decreased in athletes and in asthmatics, suggesting that chronic exercise incurs similar inflammatory changes in the airways of athletes and asthmatics with regard to their character and intensity. The action of IL-1ra in EBC has not been studied extensively; however, it has been previously suggested that elevated IL-1ra may be one of the markers of poor asthma control [40]. Unfortunately, as the asthmatic subjects tested in the present study were well controlled on inhaled steroids, this is impossible to confirm based on the present findings. Decreased levels of IL-1ra have been described in the sputum of neutrophilic asthma subjects [41], indicating that dysregulation of the pro/anti-inflammatory mediator balance in the lower airway plays an important role in asthma.

In contrast to our findings in the lower airways, the TNF-α concentrations in nasal secretions did not differ between athletes, asthmatics and healthy controls. However, IL-1ra levels in upper airways were higher in athletes and asthmatics than healthy subjects. This contradicts earlier results showing decreased IL-1ra in nasal fluids in rhinitic [42] and asthmatic [43] subjects. Such differences might reflect differences in the cytokine response of the upper airways to exercise or other inflammatory stimuli and could be a sign of a local anti-inflammatory counter-response; however, this was not accompanied by shifts in other mediators, especially TNF-α. In addition, unlike the experiments cited above, the present study applied a correction for the dilution of the nasal secretion by the fluid used for the lavage procedure. Finally, our results are influenced to a degree by the small number of subjects in each group and within-group dispersion of protein concentrations (Fig. 3).

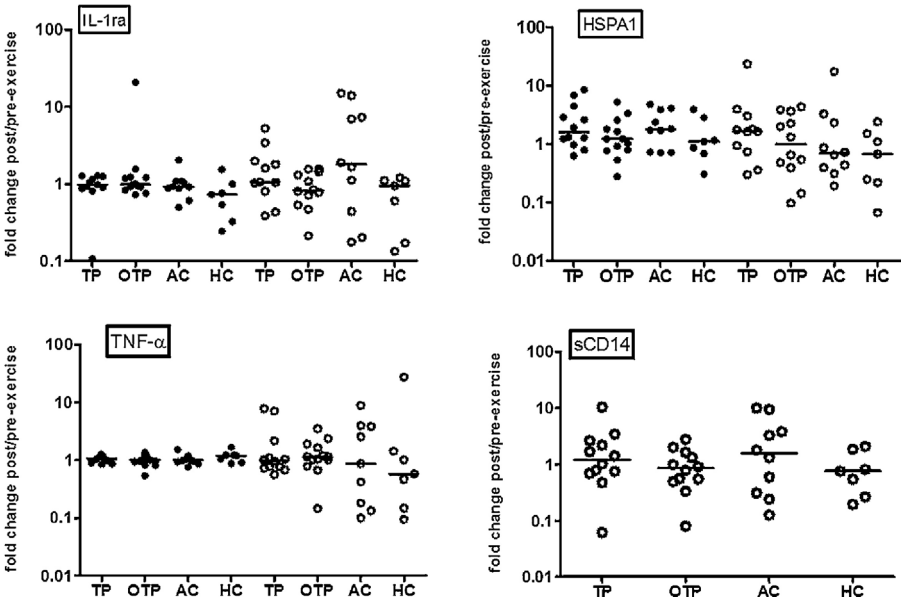

Fig. 5. Fold change in IL-1ra, HSPA1, TNF-α and sCD14 levels in EBC (black dots) and nasal secretions (open circles). Medians marked with horizontal lines. Log scale. TP, athletes in-training period; OTP, athletes off-training period; AC, asthmatic controls; HC, healthy controls.

Our findings demonstrate that a single acute single exercise bout is not a triggering factor for significant changes in TNF-α or IL-1ra in the EBC or nasal secretions, regardless of the studied group, as no significant pattern of influence was found regarding the level of mediators. However, considerable within-group variability was observed regarding post-exercise fold-change in protein levels. Due to the small size of the sample, it is not possible to describe the factors that determine whether exercise increases or decreases the level of a given protein in EBC or nasal secretion.

As no studies have so far examined the presence of heat shock proteins and sCD14 in EBC, our findings suggesting that the HSPA1 and sCD14 present in lower airway mucosa do not reflect acute or chronic inflammatory stimulation, represent a novel addition. They also are the first indication that neither exercise or chronic inflammation have any significant influence on the presence of HSPA1 in nasal secretions. A study by Besançon-Watelet et al. [44] reported lower sCD14 in the nasal lavages of subjects with local hypereosinophilia. Our findings confirm the presence of sCD14 in upper airway mucosa but do not provide proof of its role in the local response to exercise stimulus.

5. Conclusions

Similar dysregulations in the pro/anti-inflammatory TNF-α/IL-1ra balance in exhaled breath condensate were observed in competitive athletes and asthmatics, which may reflect the presence of chronic lower airway inflammation induced by competitive exercise. Although the study presents novel findings about the presence of innate proteins in EBC and nasal secretions and their behavior after exercise stimulation, disease-focused studies involving larger cohorts are required to further elucidate the role of innate mediators in airway mucosal inflammation.

Conflict of interests

The authors declare no conflict of interests with regard to this article.

Financial disclosure

This study was financed by National Science Center of the Republic of Poland grant no. 5981/B/P01/2011/40.

Acknowledgements

The authors wish to acknowledge the contribution of Ms. Dorota Żaromińska, RN, to questionnaire data collection, performing treadmill exercise challenges and nasal lavages.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.advms.2017.07.004.

References

[1] Walsh N, Gleeson M, Shephard R, Gleeson M, Woods J, Bishop N, et al. Position statement. Part one: immune function and exercise. Exerc Immunol Rev 2011;17:6–63.
[2] Cox A, Pyne D, Saunders P, Callister R, Gleeson M. Cytokine responses to treadmill running in healthy and illness-prone athletes. Med Sci Sports Exerc 2007;39(11):1918–26.
[3] Robson-Ansley P, Howatson G, Tallent J, Mitcheson K, Walshe I, Toms C, et al. Prevalence of allergy and upper respiratory tract symptoms in runners of London Marathon. Med Sci Sports Exerc 2012;44(6):999–1004.
[4] Spence L, Brown W, Pyne D, Nissen M, Sloots T, McCormack J, et al. Incidence: etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 2007;39(4):577–86.
[5] Seys SF, Daenen M, Dilissen E, Van Thienen R, Bullens DMA, Hespel P, et al. Effects of high altitude and cold air exposure on airway inflammation in patients with asthma. Thorax 2013;68(10):906–13.
[6] Kurowski M, Jurczyk J, Moskwa S, Jarzębska M, Krysztofiak H, Kowalski ML. Winter ambient training conditions are associated with increased bronchial hyperreactivity and with shifts in serum innate immunity proteins in young competitive speed skaters. Arch Med Sci 2017 in press.
[7] Seys SF, Hox V, Van Gerven L, Dilissen E, Marijsse G, Peeters E, et al. Damage-associated molecular pattern and innate cytokine release in the airways of competitive swimmers. Allergy 2015;70(2):187–94.
[8] Kim K, Suzuki K, Peake J, Ahn N, Ogawa K, Hong C, et al. Physiological and leukocyte subset responses to exercise and cold exposure in cold-acclimatized skaters. Biol Sport 2014;31(1):39–48.

[9] Del Giacco SR, Firinu D, Bjermer L, Carlsen K-H. Exercise and asthma: an overview. Eur Clin Respir J 20152:, doi:http://dx.doi.org/10.3402/ecrj.v2.27984.
[10] Hallstrand TS, Moody MW, Wurfel MM, Schwartz LB, Henderson WR, Aitken ML. Inflammatory basis of exercise-induced bronchoconstriction. Am J Resp Crit Care Med 2005;172(6):679–86.
[11] Zietkowski Z, Skiepko R, Tomasiak-Lozowska MM, Mroczko B, Szmitkowski M, Bodzenta-Lukaszyk A. RANTES in exhaled breath condensate of allergic asthma patients with exercise-induced bronchoconstriction. Respiration 2010;80(6):463–71.
[12] Zietkowski Z, Skiepko R, Tomasiak-Lozowska MM, Mroczko B, Szmitkowski M, Bodzenta-Lukaszyk A. Changes in high-sensitivity C-reactive protein in serum and exhaled breath condensate after intensive exercise in patients with allergic asthma. Int Arch Allergy Immunol 2010;153(1):75–85.
[13] Zietkowski Z, Skiepko R, Tomasiak-Lozowska MM, Zietkowska E, Bodzenta-Lukaszyk A. Eotaxin in exhaled breath condensate of allergic asthma patients with exercise-induced bronchoconstriction. Respiration 2011;82(2):169–76.
[14] Bikov A, Gajdócsi R, Huszár É, Szili B, Lázár Z, Antus B, et al. Exercise increases exhaled breath condensate cysteinyl leukotriene concentration in asthmatic patients. J Asthma 2010;47(9):1057–62.
[15] Kiwata J, Anouseyan R, Desharnais R, Cornwell A, Khodiguian N, Porter E. Effects of aerobic exercise on lipid-Effector molecules of the innate immune response. Med Sci Sports Exerc 2014;46(3):506–12.
[16] Morissette MC, Murray N, Turmel J, Milot J, Boulet L-P, Bougault V. Increased exhaled breath condensate 8-isoprostane after a swimming session in competitive swimmers. Eur J Sport Sci 2016;16(5):569–76.
[17] West NP, Pyne DB, Kyd JM, Renshaw GM, Fricker PA, Cripps AW. The effect of exercise on innate mucosal immunity. Br J Sports Med 2010;44(4):227–31.
[18] Brightling C, Berry M, Amrani Y. Targeting TNF-α: A novel therapeutic approach for asthma. J Allergy Clin Immunol 2008;121(1):5–12.
[19] Lauzon A-M, Martin JG. Airway hyperresponsiveness; smooth muscle as the principal actor. F1000Research 2016; 5: (F1000 Faculty Rev)306. doi: 10.12688/f1000research.7422.1.
[20] Fehrenbach E, Niess A, Voelker K, Northoff H, Mooren F. Exercise intensity and duration affect blood soluble HSP72. Int J Sports Med 2005;26(7):552–7.
[21] Bonini M, Braido F, Baiardini I, Del Giacco S, Gramiccioni C, Manara M, et al. AQUA: allergy questionnaire for athletes: development and validation. Med Sci Sports Exerc 2009;41(5):1034–41.
[22] Craig C, Marshall A, Sjöström M, Bauman A, Booth M, Ainsworth B, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35(8):1381–95.
[23] Parsons JP, Hallstrand TS, Mastronarde JG, Kaminsky DA, Rundell KW, Hull JH, et al. An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction. Am J Resp Crit Care Med 2013;187:1016–27.
[24] Zapletal A, Samanek M, Paul T. Lung Function in Children and Adolescents. Basel: Karger; 1987.
[25] Quanjer P, Tammeling G, Cotes J, Pedersen O, Peslin R, Yernault J. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993;6(Suppl. 16):5–40.
[26] Horváth I, Hunt J, Barnes PJ. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J 2005;26(3):523–48.
[27] Mutlu G, Garey K, Robbins R, Danziger L, Rubinstein I. Collection and analysis of exhaled breath condensate in humans. Am J Resp Crit Care Med 2001;164(5):731–7.
[28] Puts E, Lammerts L, Bast A, van Dieijen-Visser M, Kremer B. Ureum als Verdünnungsmarker von Nasensekret in nasaler Lavageflüssigkeit. Allergologie 2006;29(5):184–93.
[29] Bernard A, Carbonnelle S, Dumont X, Nickmilder M. Infant swimming practice, pulmonary epiuthelium integrity, and the risk of allergic and respiratory diseases later in childhood. Pediatrics 2007;119:1095–103.
[30] Rundell KW, Sue-Chu M. Air quality and exercise-induced bronchoconstriction in elite athletes. Immunol Allergy Clin North Am 2013;33(3):409–21.
[31] Helenius I, Tikkanen H, Haahtela T. Occurrence of exercise induced bronchospasm in elite runners: dependence on atopy and exposure to cold air and pollen. Br J Sports Med 1998;32(2):125–9.
[32] Wuestenfeld JC, Wolfarth B. Special considerations for adolescent athletic and asthmatic patients. Open Access J Sports Med 2013;4:1–7.
[33] Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE. Green RH, et al. Evidence of a role of tumor necrosis factor α in refractory asthma. N Engl J Med 2006;354(7):697–708.
[34] Brown SD, Brown LA, Stephenson S, Dodds JC, Douglas SL. Qu H, et al. Characterization of a high TNF-α phenotype in moderate-to-severe asthmatic children. J Allergy Clin Immunol 2015;135(6):1651–4.
[35] Manise M, Schleich F, Gusbin N, Godinas L, Henket M, Antoine N, et al. Cytokine production from sputum cells and blood leukocytes in asthmatics according to disease severity. Allergy 2010;65(7):889–96.
[36] Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 2005;98(4):1154–62.
[37] Grzela K, Zagorska W, Krejner A, Litwiniuk M, Zawadzka-Krajewska A, Banaszkiewicz A, et al. Prolonged treatment with inhaled corticosteroids does not normalize high activity of matrix metalloproteinase-9 in exhaled breath condensates of children with asthma. Arch Immunol Ther Exp (Warsz) 2015;63(3):231–7.
[38] Liu H-C, Lu M-C, Lin Y-C, Wu T-C, Hsu J-Y, Jan M-S, et al. Differences in IL-8 in serum and exhaled breath condensate from patients with exacerbated COPD or asthma attacks. J Formos Med Assoc 2014;113(12):908–14.
[39] Warwick G, Thomas PS, Yates DH. Non-invasive biomarkers in exacerbations of obstructive lung disease. Respirology 2013;18(5):874–84.
[40] Hara A. The relationships between the levels of cytokines and chemokines in exhaled breath condensate and the control status of asthma. Teikyo Med J 2010;33(1):39–50.
[41] Gao P, Gibson PG, Baines KJ, Yang IA, Upham JW, Reynolds PN, et al. Anti-inflammatory deficiencies in neutrophilic asthma: reduced galectin-3 and IL-1RA/IL-1β. Respir Res 2015;16(1):1–10.
[42] Benson M, Wennergren G, Fransson M, Cardell LO. Altered levels of the soluble IL-1: IL-4 and TNF receptors, as well as the IL-1 receptor antagonist, in intermittent allergic rhinitis. Int Arch Allergy Immunol 2004;134(3):227–32.
[43] De Kluijver J, Grünberg K, Pons D, De Klerk EPA, Dick CR. Sterk PJ, et al. Interleukin-1β and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects. Clin Exp Allergy 2003;33(10):1415–8.
[44] Besançon-Watelet C, Béné MC, Montagne P, Faure GC, Jankowski R. Eosinophilia and cell activation mediators in nasal secretions. Laryngoscope 2002;112(1):43–6.