

Expression of *SCGB1C1* gene as a potential marker of susceptibility to upper respiratory tract infections in elite athletes – a pilot study

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ABSTRACT: High levels of exercise in athletes result in temporary immunosuppression, which could increase the susceptibility to upper respiratory tract infections. Understanding of immunological mechanisms responsible for this phenomenon could enable optimization of training schemes for elite athletes and avoidance of infection-related episodes of absence during sports championships. The aim of this study was to detect genes that may be responsible for modulation of individual susceptibility to infections. The blood and saliva samples were collected from 10 healthy, medically examined kayakers (4 females and 6 males) aged 24.7 ± 2.3 years. All samples were taken in the morning, after overnight fasting, in a seated position. The ELISA method was used to determine the levels of secretory immunoglobulin A (sIgA) and interleukin 5 (IL-5). Whole genome expression in blood was assessed using microarrays. The study did not reveal any significant correlation between genome expression and sIgA concentration. However, low expression of a gene involved in protection against the common cold – secretoglobin 1C1 (*SCGB1C1*) – was detected in athletes with high IL-5 concentrations (corrected $p=0.00065$; fold change=3.17). Our results suggest that blood expression of the *SCGB1C1* gene might be a marker of susceptibility to upper respiratory tract infections in athletes.

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INTRODUCTION

Respiratory infections are the most common reason for seeking medical advice, responsible for approximately 50-60% of all community-acquired infections [1]. High-intensity endurance training increases the risk of upper respiratory tract infections (URTI) by impairing the immune function in athletes [2]. Thus, elite athletes have a higher number of infectious episodes than recreational athletes and the general population [3]. It is worth noting that the highest relative incidence of URTI was noted in elite athletes at the Winter Olympics, the Summer Olympics and the World Championships [4-6]. Since URTI is a major problem in athletes, it is important to find immune markers which are linked (directly or indirectly) to these infections.

So far it is not known which elements of the immune system contribute to the increased incidence of the above-mentioned infections. One of them could be secretory immunoglobulin A (sIgA). sIgA plays a key role in the mucosa-associated lymphoid tissue (MALT), which forms the first line of defence against infections of the upper respiratory tract [7]. Previous research has shown that exercise influences the concentration/secretion rate of sIgA [8, 9], and that a low level of sIgA could be associated with increased frequency of

upper respiratory tract infections in athletes [10]. The concentration of sIgA in saliva depends on the synthesis of this immunoglobulin and the efficiency of its transport across the mucosa.

Local plasma cells produce sIgA at mucosal sites. This process is regulated by cytokines [7]. Interleukin-5 (IL-5) is one of the most important cytokines responsible for triggering the production of sIgA [11, 12]. IL-5 is produced by Th2 cells, mast cells and eosinophils and modulates inflammatory, innate and acquired immune responses [12]. It is well known that intense exercise produces a rapid, transient increase in cytokine production (for example IL-6, IL-10, IL-1ra and IFN- γ) [13, 14]. In contrast, IL-5 production decreases after resistance exercise [15].

No specific gene or genetic mechanism has yet been suggested which could link the metabolic effects of intensive endurance training with the secretion of sIgA and blood concentration of IL-5. Nevertheless, it seems probable that the activity of some important genomic pathways, which maintain the metabolic homeostasis in blood lymphocytes, corresponds with the sIgA or IL-5 levels. Therefore we aimed to assess the whole genome expression in blood of athletes in the hope of detecting genes that may be responsible for

modulation of sIgA or IL-5 levels. Identifying such genes would contribute to the understanding of immunological mechanisms responsible for decreased immunity in elite athletes and could contribute to optimization of their training schemes to avoid infections and episodes of absence during sports championships.

MATERIALS AND METHODS

Participants. A group of 10 international-class kayakers (six men aged 23.3 ± 0.4 and four women aged 26.7 ± 2.6 years) agreed to participate in this pilot study. The potential correlation of sIgA and IL-5 concentrations and of genome expression in white blood cells (in particular of the genes important for the immunologic response to viral infections) was analysed in study participants. The protocol of the study was approved by the Ethical Research Committee at the Institute of Sport in Warsaw. Information about the study was given to the participants, who signed an informed consent form.

In order to exclude participants potentially suffering from a chronic infection, physical examination of each athlete was performed with additional assessment of CRP concentrations and white blood cell counts in blood.

Blood and saliva collection and analysis

The blood and saliva were collected from healthy, medically examined athletes, who had returned from a training camp. Sample collection was performed on the same day in the morning, after overnight fasting and in a seated position.

The blood samples were collected from the antecubital vein. White blood cell (WBC) counts were measured using the ADVIA 120 haematology analyser (Siemens, Germany). Concentration of C-reactive protein (CRP) in serum was determined using the immunoturbidimetric method (Pentra, Japan). The concentration of IL-5 in serum was measured using a specific ELISA kit (SunRed, Shanghai). All serum samples were frozen at -80°C until analysis. All assays were performed in duplicate.

Approximately 5 minutes before saliva collection all athletes were required to rinse the mouth with plain water. The unstimulated saliva samples were collected by passive dribble into a sterile collection tube. After centrifugation for 15 min at 3000 rpm, saliva samples were stored frozen at -20°C prior to analysis. Salivary sIgA was analysed using commercially available ELISA kits (DIAMETRA, Italy) according to the manufacturer's protocol. All assays were performed in duplicate.

The study participants were stratified into subgroups with above-mean and below-mean concentrations of sIgA and IL-5. Subsequently, the genome expression profiles were compared between athletes with above-mean concentrations of sIgA or IL-5 and those in whom below-mean concentrations of sIgA or IL-5, respectively, were detected (Table 1).

A sample of 2.5 ml of blood was obtained from every person in order to extract total RNA (the PAXgene system manufactured by PreAnalytiX was used). Subsequently, the whole genome expression was assessed using SurePrint Human Gene Expression 8x60K v2 Microarrays (Agilent, USA) according to the manufacturer's protocol. The microarrays were scanned on the Agilent SureScan G2600D scanner.

SurePrint G3 Human Gene Expression Microarrays provide comprehensive coverage of the entire human genome (50,599 genes and transcripts) with high specificity of target detection and a very wide dynamic range allowing for detection of biological features both with very high and with very low expression.

Statistical analysis

GeneSpring software (www.genespring.com) was used to perform data analysis. The single colour expression technology designed for Agilent microarrays was applied. A standard statistical procedure allowing for analysis of small sample sets was chosen. The moderated t-test with the Westfall-Young permutative correction for multiple testing was used to detect statistically significant differences

TABLE 1. Characteristics of study participants and results of genomic analysis.

Participant ID	Gender	sIgA concentration [$\mu\text{l}\cdot\text{ml}^{-1}$]	IL-5 concentration [$\text{pg}\cdot\text{ml}^{-1}$]	Differently expressed transcripts	
				Comparison of groups with sIgA above vs below mean (136.6)	Comparison of groups with IL-5 above vs below mean (18.25)
1	F	72.07	8.28		
2	M	91.39	13.05	Up-regulated:	Up-regulated:
3	M	92.92	8.83	LOC101928738, RFX3, Inc-	FAM106A, Inc-DHX37-10,
4	M	100.38	12.78	TMEM132D-2, SLC15A2,	MIAT, LOC283788, LINC01395,
5	F	136.39	15.83	ACTR3C, HOMER1, TMTC1,	PARPBP, TGFB3L, Inc-PIK3R1-1
6	F	154.20	11.93	C7orf61, H6PD	
7	F	156.43	32.81	Down-regulated:	Down-regulated:
8	M	162.25	23.97	XLOC_I2_002477,	Inc-TP53I11-1, FAM208B,
9	M	186.13	20.42	LOC101927895, ADTRP, DDTL,	ZNF606, XLOC_I2_009182,
10	M	214.22	34.61	ELOVL6, ZNF606, LINC00476,	SCGB1C1, LOC221946, Inc-
				LOC100507250, LCE3C, CELA1	RP11-736N17.6.1-1, RWDD4,
					TRIM23

between the two subgroups of study participants. The corrected value of $p < 0.05$ was considered significant. The transcripts revealing a fold change value (FC) > 2 were further analysed.

RESULTS

Table 1 presents the results of the initial blood and saliva tests performed in study participants. Blood leukocyte counts and CRP concentrations were within the normal range in all of them. The mean concentration of sIgA was $136.6 \mu\text{l}\cdot\text{ml}^{-1}$ and the mean concentration of IL-5 was 18.25 pg/ml . The group of athletes with higher ($N=5$) and with lower ($N=5$) concentrations of sIgA differed significantly with regard to 19 transcripts. None of them seemed to be involved in the pathophysiology of upper respiratory tract infections. The genomic analysis also identified 17 transcripts expressed differently between the athletes with higher ($N=4$) and with lower ($N=6$) concentrations of IL-5. Interestingly, one of these transcripts represents a gene involved in development of the common cold – secretoglobin 1C1 (*SCGB1C1*, ligand binding protein RYD5). The above transcript was down-regulated in athletes with higher IL-5 concentrations (corrected $p=0.00065$; fold change=3.17).

DISCUSSION

The prevalence of upper respiratory tract infections is influenced by environmental, immunological (adaptive and innate) and genetic factors. The heritability of the genetic component for recurrent URTI was estimated at approximately 60% based on a twin study [16]. However, little is known about the role of specific genes [17]. Recent work showed that the *IFITM3* and *Mx1* genes are important in host susceptibility to influenza [18]. Some studies have suggested that cytokine gene polymorphisms (for example for IL-10 and IL-6) may contribute to the variable risk of development of URTI in highly trained athletes [19, 20]. Previous studies have also demonstrated that low levels of sIgA or of sIgA fractions may be associated with upper respiratory tract infections in athletes [10], but the role of IL-5 in URTI is not clear [19, 21, 22].

The main finding of our study is the correlation of high IL-5 concentration with decreased activity of the secretoglobin 1C1 gene (*SCGB1C1*). Secretoglobins are found at high concentrations in many secretions, including uterine, prostatic, pulmonary, lacrimal and salivary glands. The expression of *SCGB1C1* was also found in human upper respiratory airway mucosa, where it is regulated by different cytokines – down-regulated by $\text{IFN-}\gamma$ and up-regulated by IL-4 and IL-13 [23]. The *SCGB1C1* protein plays a role in primary defence mechanisms by recognizing and removing pathogenic microorganisms from the mucosa and also by protecting lung epithelial cells [24, 25]. Moreover, the *SCGB1C1* protein may be involved in development of the common cold [25; Gene Ontology, GO:0005576]. On the other hand, it is well known that Th1/Th2 balance is important in maintaining host immune response. T lymphocytes produce interleukins, which play a role in host protection against infection. Th1 cells produce predominantly $\text{IFN-}\gamma$, $\text{TNF-}\alpha$ and

IL-2, whereas Th2 cells are responsible for the production of IL-4, IL-5, IL-6 and IL-10 [26]. It was found that strenuous exercise could affect expression of genes encoding transcription factors associated with cytokine production, which induces a shift in the Th1/Th2 balance to Th2 predominance [27]. Th1/Th2 imbalance may contribute to increased URTI [27]. Gleeson et al. (2012) [21] demonstrated that high IL-10 production in response to antigen challenge in illness-prone athletes may be a risk factor for development of URTI. They also support the hypothesis that decreased Th1 responses are responsible for higher incidence of URTI. It was also demonstrated that 83% of children with 2009 H1N1 pneumonia had an elevated serum level of IL-5 in the first 24 hours after the onset of fever, independently of the presence of underlying allergies [28]. Elevation of IL-5 concentration in the blood was also observed in children with rhinovirus-induced wheezing [29]. The above-mentioned data may suggest that an increased concentration of IL-5 may impair the protective role of *SCGB1C1* in respiratory airway mucosa, which in turn may increase the frequency of developing URTI. Animal studies support this hypothesis, as IL-5 may suppress expression of one of the anti-inflammatory proteins from the secretoglobin family (*SCGB3A2*) in murine lung tissue [30]. Thus, the relationship between IL-5 and *SCGB1C1* which was observed in our study may reflect an important biological phenomenon.

Several limitations of this preliminary study should be taken into account. First of all, the whole genome assessment was performed only in a few samples, and application of multi-stage statistical methods with correction for multiple comparisons was necessary in this analysis. Gene expression profiling studies are highly complex. The large amount of genomic data results in a high probability for errors. The search for differentially expressed genes includes data normalization and interpretation, before the biological question can be addressed. However, the final list of differently expressed genes depends not only on the statistical methods, but also on the potential selection bias of the sample sets to be tested. The small sample size increases the risk of incidental, false-positive results and the risk of selection bias. In addition, various inter-individual biological differences and several environmental factors which were not addressed in our analysis may influence immunity in athletes. Thus, further, prospective studies and larger sample sets are necessary to confirm the potential role of the *SCGB1C1* gene expression level as a potential marker of resistance to upper respiratory tract infections.

CONCLUSIONS

In conclusion, we observed an inverse correlation between blood expression of the *SCGB1C1* gene (which probably closely reflects mucosal concentration of *SCGB1C1* protein) and the blood expression of the *IL-5* gene. This mechanism could provide one explanation of the frequently observed sudden episodes of immunological dysfunction resulting in development of the common cold in high-endurance athletes. However, the role of IL-5 and sIgA in the host response to URTI needs further clarification [28, 31].

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