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Differential Expression of Inflammation-related Genes after Intense Exercise

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Abstract: The present study focused on the identification of the difference in expression of inflammation-related genes after intense exercise by oligonucleotide microarray methods. This may finally lead to an improved understanding of underlying cellular and molecular mechanism of the immunological alterations in response to exercises. The study group consisted of three healthy road cyclists. Peripheral blood mononuclear cells (PBMCs) were collected pre-exercise, immediately post-exercise and after 15 min of recovery. The analysis of the expression profile of genes related to the inflammation was performed in PBMCs using HG-U133A oligonucleotide microarrays. 4 genes were found to be regulated by more than 2.0-fold (IL1R2, IL2RB, IL8, IL8RB). Venn diagram indicated that only one of differentially expressed genes (TXLNA) remains the same in each comparison. The balance of both pro- and anti-inflammatory cytokines after exercise seems to be important for athletes. Optimal inflammatory and immune response may help optimize exercise regimes, link physical activity with health and diagnose or prevent athletes from overtraining.

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Introduction

Several different systems are activated or regulated after exercises. Changes in response to physical exertion are related to various aspects of metabolism, mitochondrial biogenesis, oxidant stress, electrolyte balance, stress management and inflammation process (Marin et al., 2011). For immune system activity after exercise may have influence several factors, i.e. personal characteristics (gender, age), intensity and duration of exercises, training status and environmental conditions. There is known that endurance exercise e.g. marathon running can induce a greater inflammatory cytokine response than more concentric or intermittent in intensity exercises such as cycling, tennis or rowing (Nieman et al., 2001). Ultra-endurance exercise, such as an Ironman triathlon, induced low-grade systemic inflammation, which persisted until at least 5 days post-exercise. Additionally, chronic inflammation may be a risk factor for several chronic diseases (Beavers et al., 2010). It is also noteworthy that cytokine responses after endurance exercises differ from resistance training.

Many previous studies have shown that exercise elicits changes in inflammatory process, but most researches determine only the cytokine protein level using immunoenzymatic methods (Nieman et al., 2001; Shojaei et al., 2011). Oligonucleotide microarray technique enables analysis of tens of thousands of genes simultaneously. Therefore, it can become a useful tool to identify genes involved in many physiological or pathological processes including inflammatory response.

The present study focused on the identification of the difference in expression of inflammation-related genes after intense exercise by oligonucleotide microarray methods. This may finally lead to an improved understanding of underlying cellular and molecular mechanism of the immunological alterations in response to exercises.

Material and Methods

Subjects

The study group comprised 3 healthy road cyclists (males, mean age 26.7 ± 7.8 years, mean height 175.7 ± 4.6 cm; mean weight 65.6 ± 4.8 kg; mean Body Mass Index 21.2 ± 0.6 kg/m^2). All cyclists had been training for more than seven years on the same cycling team. All athletes underwent medical evaluations at the same time of the pre-season training process, which included clinical history and physical examination. For three days before experiment, the subjects were on a standardized normocaloric (37 kcal/kg/day) diet with 50–60% carbohydrate, 15–20% protein, and 20–30% fat. They were also obliged to abstain from strenuous exercise. The cyclists were non-smokers, and none had taken any medication or drugs.

All subjects performed a graded cardiopulmonary exercise test on Lode Excalibure Cycle Ergometer (MEDGRAPHICS, USA). The exercise started with unloaded cycling for 5 min, and then intensity was increased by 40 W every 3 min up to maximal exercise intensity and 60–70 rpms were maintained.

Oxygen uptake (VO_2) was measured continuously from the 6th min prior to exercise and throughout each stage of the exercise load using the Oxycon Alpha Apparatus (Jaeger, Germany). The mean values of VO_2max for all participants were 63.00 ± 3.00 ml/kg/min.

The study was approved by the Bioethics Committee of the Medical University of Silesia, in Katowice (KNW/0022/KD1/122/I/09) in accordance with the Declaration of Helsinki regarding medical research involving human subjects. The study and its purpose were explained to each participant, who gave informed written consent.

Venous blood samples of each subject were collected into EDTA-containing tubes at three points of time: pre-exercise, immediately post-exercise, and after 15 min of recovery. The samples were centrifuged on a Ficoll-Conray gradient (specific gravity 1.077; Immunobiological Co., Gumma, Japan) immediately after blood collection.

Ribonucleic acid extraction

Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA extracts were treated with DNase I (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The quality of extracts was checked electrophoretically using 0.8% agarose gel stained with ethidium bromide. Total RNA concentration was determined by spectrophotometric measurement using the Gene Quant II RNA/DNA Calculator (Pharmacia Biotech, Cambridge, UK).

Oligonucleotide microarray analysis

The analysis of expression profile of genes related to inflammation was performed using commercially available HG-U133A oligonucleotide microarrays (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations and described previously by Rostkowska-Nadolska et al. (2009).

Statistical analysis

The data from all arrays were analyzed using GeneSpring 12.0 platform (Agilent Technologies UK Limited, South Queensferry, UK) to identify transcriptome differences. The normalized microarray data were used to compile a list of selected inflammation-related genes whose expression appeared to be up- or down-regulated by a cut-off of at least 1.1-fold change. The one-way ANOVA test and the Tukey's post hoc test were applied to detect differentially expressed genes. Significant differential gene expression was identified by 1.1-fold change at $p<0.05$. Venn diagram was used to show the logical relationships between groups of differentially expressed genes. Gene Ontology analysis was carried out with the PANTHER 8.0 Classification System database (Protein Analysis Through Evolutionary Relationships; http://www.pantherdb.org) to classify genes based on their biological process, molecular function and pathways.

Results

The microarray analysis was performed in three road cyclists. Three transcriptome groups (pre-exercise, immediate post-exercise and 15 min post-exercise groups) were chosen for a comparative analysis aimed at finding transcripts of inflammation-related genes differentiating post-exercise and pre-exercise samples.

Differential expression of inflammation-related genes

Typing of differentiating genes was performed in a panel of 150 transcripts of 105 genes encoding proteins involved in inflammation process. Among these genes, 10 expressed changes in the post-exercise in relation to pre-exercise samples at $p<0.05$ (Table 1). Six differentially expressed genes were found when the immediate post-exercise were compared to the pre-exercise samples. Among these genes, 4 (IL2RB, IL17B, IL18R1, TXLNA) were up-regulated, whereas 2 (IL5RA, IL6) were down-regulated. When the pre-exercise group was compared to the 15 min post-exercise group, six differentially expressed genes were found. The up-regulated transcripts were recorded for 3 genes (IL1B, IL8, IL18R1) and the down-regulated transcripts were found for 3 genes (IL1RL1, CSF2, TXLNA). Three differentially expressed genes were found when the immediate post-exercise was compared to the 15 min post-exercise samples. Among these genes, 1 (IL5RA) were up-regulated, whereas 2 (IL2RB, TXLNA) were down-regulated. Among the analyzed genes, 4 (IL1R2, IL2RB, IL8, IL8RB) expressed ≥ 2-fold change (Table 1).

Table 1 – Characteristics of inflammation-related genes which exhibit differential expression in the pre-exercise, immediate post-exercise and 15 min post-exercise recovery period groups

Probe set	Gene symbol	Gene name	FC[a]		
			Te/Ts	Tr/Ts	Tr/Te
205291_at	**IL2RB**	**interleukin 2 receptor, beta**	**1.58 ↑[b]***	**1.33 ↓**	**2.11 ↓***
212300_at	TXLNA	taxilin alpha	1.20 ↑*	1.30 ↓*	1.55 ↓*
220273_at	IL17B	interleukin 17B	1.13 ↑*	1.05 ↑	1.08 ↓
205067_at	IL1B	interleukin 1, beta	1.21 ↑	1.50 ↑*	1.24 ↓
206618_at	IL18R1	interleukin 18 receptor 1	1.29 ↑*	1.31 ↑*	1.01 ↑
202859_x_at	**IL8**	**interleukin 8**	**1.39 ↑**	**2.18 ↑***	**2.00 ↑**
205403_at	**IL1R2**	**interleukin 1 receptor, type II**	**1.11 ↑**	**2.63 ↑**	**2.37 ↑**
207008_at	**IL8RB**	**interleukin 8 receptor type B**	**1.42 ↑**	**2.62 ↑**	**1.85 ↑**
207526_s_at	IL1RL1	interleukin 1 receptor-like 1	1.06 ↓	1.13 ↓*	1.07 ↓
210229_s_at	CSF2	colony stimulating factor 2 (granulocyte-macrophage)	1.04 ↓	1.11 ↓*	1.06 ↓
205207_at	IL6	interleukin 6	1.15 ↓*	1.07 ↓	1.07 ↑
207902_at	IL5RA	interleukin 5 receptor, alpha	1.19 ↓*	1.08 ↓	1.10 ↑*

*$p<0.05$; [a]FC – fold change; [b]↑, ↓ higher and lower expression in the pre-exercise (Ts), immediate post-exercise (Te) and 15 min post-exercise recovery period groups (Tr); in bold – genes with FC ≥ 2.0

Further analysis of these differentially detected genes by plotting using similarity overlapping in a Venn diagram, indicate that only one of differentially expressed genes (TXLNA) remain the same in the overlap region of each comparison.

Functional classification of inflammation-related genes altered by intense exercises
To classify genes into biological categories, there were analyzed the Gene Ontology annotations of the differentially expressed inflammation-related genes in PBMCs

Table 2 – PANTHER classification of biological processes, molecular function and pathways significantly enriched in the set of 12 differentially expressed inflammation-related genes

PANTHER classification	p-value	Genes
Biological process		
cytokine-mediated signaling pathway	<0.001	IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
immune system process	<0.001	TXLNA, IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
cell surface receptor linked signal transduction	<0.001	IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
signal transduction	<0.001	IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
cell communication	<0.001	IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
immune response	<0.001	IL8RB, IL1B, CSF2, IL2RB, IL5RA, IL8
cellular process	0.005	IL8RB, IL1R2, IL18R1, IL1RL1, IL1B, CSF2, IL2RB, IL17B, IL5RA, IL6, IL8
response to stimulus	0.007	TXLNA, IL8RB, IL1B, CSF2, IL2RB, IL5RA, IL8
cell-cell signaling	0.015	IL1B, CSF2, IL17B, IL5RA, IL6, IL8
JNK cascade	0.016	IL17B, IL6, IL8
MAPKKK cascade	0.023	IL17B, IL5RA, IL6, IL8
intracellular signaling cascade	0.028	IL8RB, CSF2, IL17B, IL5RA, IL6, IL8
JAK-STAT cascade	0.048	CSF2, IL5RA, IL6
Molecular function		
cytokine activity	<0.001	IL1B, CSF2, IL17B, IL5RA, IL6, IL8
cytokine receptor activity	<0.001	IL1R2, IL18R1, IL1RL1, IL2RB, IL5RA
hematopoietin/interferon-class (D200-domain) cytokine receptor binding	0.001	IL1B, IL17B, IL6
receptor binding	0.006	IL1B, CSF2, IL17B, IL5RA, IL6, IL8
Pathway		
interleukin signaling pathway	<0.001	IL8RB, IL2RB, IL17B, IL5RA, IL6, IL8
inflammation mediated by chemokine and cytokine signaling pathway	<0.001	IL8RB, IL1B, IL17B, IL6, IL8

obtained from healthy cyclists PANTHER found several functional categories that were significantly enriched in this gene set compared to the entire NCBI reference list of human genome. Categories showing a $p<0.05$ were considered as potentially interesting, as determined by the binomial statistic. The 12 genes of the dataset were significantly classified by the PANTHER System in 13 biological processes, i.e. processes in which genes participate, 4 molecular functions, i.e. biological functions of gene products and 2 transduction signal pathways (Table 2).

Discussion

Widely used oligonucleotide microarray technology was applied to detect of inflammation gene expression change. Only a few studies have evaluated the underlying molecular mechanisms triggered by exercise using this technique (Radom-Aizik et al., 2009). Our study revealed that expression of 10 inflammation-related genes (IL2RB, IL18R1, TXLNA, IL17B, IL6, IL5RA, IL1B, IL8, IL1RL1, CSF2) were significantly changed after intense exercise in relation to pre-exercise state. It can be suggested that intense exercise greatly altered expression of genes related to the interleukin signalling pathway and inflammation mediated by chemokine and cytokine signalling pathway. In turn, Radom-Aizik et al. (2009) detected expression changes of genes related to inflammatory, stress, apoptotic pathways and natural killer cell-mediated cytotoxicity pathway in leukocytes of girls and boys after exercises. Similarly to our study, Connolly et al. (2004) also showed that the majority of upregulated genes after physical exertion can be classified as proinflammatory. There was found only one research focused on inflammatory markers of half marathon runners using a cDNA microarray (Zieker et al., 2005).

Our results revealed that among the analyzed genes, 4 (IL8, IL8RB, IL1R2, IL2RB) expressed > 2-fold change. Interleukin 1 family, included IL1α, IL1β and interleukin 1 receptor antagonist protein (IRAP), is primary regulator of inflammatory response. There were identified two types of IL1 receptors. The type I of IL1 receptor (IL1R1) transduces the IL1 signal and is widely expressed, whereas the type II receptor IL1R2 was only found on B cells, neutrophils and monocytes, preferentially binds IL1β and it can act as a decoy inhibitor of IL1. In our study, there was revealed that IL1β and IL1R2 expression significantly increased immediate after exercise, as well as 15 min of recovery after exercise in comparison to pre-exercise level. Our observations are supported by previous research, which revealed increase of IL1β and IL1α level after different type of exercises (Ostrowski et al., 2001). However, Suzuki et al. (2000) and Nieman et al. (2001) revealed that post-exercise plasma levels of IL1β and other cytokines such as TNF-a, IL2, IL4, IL12, and INFγ remained near pre-exercise levels in marathon runners.

In our study, there was revealed also that expression of IL2RB was significantly changed after cycle ergometer exercises. Nevertheless, IL2RB mRNA level strongly increased immediate after exercise, but 15 min of recovery after physical exertion it was lower than before starting of exercises. Previous research indicated

significant decrease in IL2 concentrations after exercises (Suzuki et al., 2000). Other authors suggested that the decrease of IL2 concentration after exercise might reflect a concomitant increase in the number of immune cells expressing the IL2 receptor. These observations are partially in line with our results, which showed increase of IL2RB immediate after exercises.

In many cell types, the synthesis of IL8 is strongly stimulated by IL1 and TNFα (Qazi et al., 2011). Our study revealed significantly increase of IL1β ad both IL8 and IL8RB after intense exercise. In turn, Büttner et al. (2007) found the IL8RA to be significantly upregulated. Likewise, Ostrowski et al. (2001) showed that protein concentration of IL8 was markedly elevated after the run. Whereas, Akerstrom et al. (2005) found increase in IL8 mRNA in muscle biopsy samples obtained after exercises, but without changes in plasma concentration of IL8. Frydelund-Larsen et al. (2007) also showed that the IL8 receptor is overexpressed in skeletal muscle after exercises. These authors suggest that IL8 may stimulate locally angiogenesis through IL8RB signaling. On the other hand, there was revealed that incremental bicycle ergometer exercises to exhaustion resulted in a small increase in plasma IL8 concentration, whereas it was remained unaltered during bicycle exercises for 1 h (Chan et al., 2004).

Similarly, Donges et al. (2014) studied expression of inflammation-related genes after intense exercise, but in sedentary middle-aged men. Muscle of these men was obtained pre-exercise, and at 1 h and 4 h post-exercise, and analyzed for changes of glycogen concentration, tumor necrosis factor, TNF receptor 1 and 2, IL6, IL6R, IL1β and IL1 receptor antagonist (IL1RA). These authors showed that exercise upregulated cytokine mRNA expression at 1 h post-exercise. In turn, Random-Aizik et al. (2014) suggested that exercise can limit monocyte pro-inflammatory function through regulation of the expression of inflammatory genes, gene pathways and microRNAs. These authors also revealed that exercise significantly altered expression of a number of genes or microRNAs that can influence monocytes involvement in vascular health. Moreover, reprogramming of gene expression can play an important role in organism responses and adaptations to endurance training. Neubauer et al. (2014) investigated the time course-dependent changes in the muscular transcriptome after an endurance exercise and showed that genes related with leukocyte migration, immune and chaperone activation, cyclic AMP responsive element binding protein 1 signalling, actin cytoskeleton remodelling, chemokine signalling, cell stress management and extracellular matrix remodelling were upregulated.

Associated with increased expression in specific pro-inflammatory genes was the concurrent up-regulation of the anti-inflammatory genes such as TXLNA immediate post-exercise. This gene is important in reducing pro-inflammatory cytokine synthesis. Moreover, decrease of it expression after the rest was observed, which suggest role of TXLNA in modulation of inflammatory process after intense exercise.

Summarizing, our findings may provide further insights into the molecular mechanisms involved in acute stress, recovery and adaptive organism responses to exercise. The balance of both pro- and anti-inflammatory cytokines after exercise seems to be important for athletes. Optimal inflammatory and immune response may help optimize exercise regimes, link physical activity with health and diagnose or prevent athletes from overtraining.

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