SPORTS MEDICINE

Expression of Early Immune Response Genes during Physical Exercise

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Study by means of expression profiling with Human GeneChip ST1.0 microchips (Affymetrix) revealed 10 early immune response genes, whose expression was modified after short-term intense physical exercise. They include genes for immunoglobulin-like receptors of natural killer cell, genes for IL-2Rβ and IL-18RAP receptors, and two genes for functional proteins (perforin 1 and granzyme B) that provide the cytotoxic potential of killer cells. Possible mechanisms of stimulation, activation of the receptor apparatus, and cytotoxic effect on natural killer cells are evaluated under conditions of physical exercise.

Key Words: *immune system; gene expression; natural killer cells; physical exercise*

Studying the state of immune defense during physical exercises is an important fields of sport medicine. The immune system plays a role in adaptive reactions to physical factors [12]. Evaluation of primary immune parameters under conditions of physical exercise is one the main problems of sport medicine and rehabilitation.

Physical activity is followed by significant changes in blood cells. Previous studies revealed an increase in the number of circulating mononuclear cells (lymphocytes, monocytes, and macrophages) and changes in the ratio of lymphocyte subpopulations [11]. Natural killer cells (NK cells) are most sensitive to physical exercise. The number of these cells in the blood increases, which contributes to improvement of immune defense in sportsmen [14].

Molecular and genetic mechanisms of regulation of the primary immune response to intense physical activity are poorly understood. Studying the expression of genes regulating functional activity of innate immune cells is important for evaluation of the mechanisms of human adaptation to physical stress.

Here we studied the expression of genes for proteins involved in activation of NK cells and determining the cytotoxic function of these cells.

MATERIALS AND METHODS

We examined 11 highly skilled sportsmen (age 19.3±0.7 years, height 175.0±7.0 cm, weight 68.0±4.6 kg, maximum oxygen consumption [MOC] 59.8±1.5 ml/kg/min). The sportsmen signed the informed consent for participation in this trial. The study was approved by the Ethics Committee of the All-Russian Institute of Physical Culture and Sport.

The incremental test-to-failure was used as a model exercise. Testing was performed on a Venus treadmill (h.p.Cosmos). The mean time of activity was 15 min. Blood samples were taken before and immediately after the test.

Serum interleukin-2 (IL-2) concentration was measured using a commercial test system (Interleukin-

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2-EIA-BEST, Vector-Best). RNA was isolated with PaxGene Blood RNA kit. The yield of purified RNA was 100 ng/μl. The tubes contained a reagent, which prevented RNA degradation with RNases and blocked the expression of genes. The pellet was obtained after centrifugation of tubes. Protein kinase K was added to remove the protein fraction. RNA was washed from admixtures of other nucleic acids and oligonucleotides. This procedure was performed on PaxGene sorbent-packed columns. The yield of purified RNA was 100 ng/μl.

Gene expression was studied with Affymetrix chips. The isolated RNA was used as a matrix to synthesize complementary DNA chain. A part of this chain was used for the synthesis of biotin-labeled cDNA. After fragmentation, biotinylated DNA was put on a HuGene 1.0 ST Array chip (Affimetrix). The chip was incubated in a hybridization oven at 45°C for 16 h. The chips were washed from unbound cRNA and stained with streptavidin-phycoerythrin on a Fluidics Station 450 (Affymetrix). The stained chips were scanned on a GeneChip Scanner 3000 (Affymetrix).

The data on sample intensity were imported into medium R and analyzed with the xps library (C. Stratova; http://www.bioconductor.org). The expression was evaluated by the RMA algorithm (xps) [7].

RESULTS

The I/NI algorithm allowed us to select 761 of 28,869 mRNA from the chip. Variations in mRNA were related to biological causes. The moderated *t*-statistics was computed with the limma library [1] to search for differentially expressed genes. We revealed the existence of 104 differentially expressed mRNA (FDR=0.05 [Benjamini and Hochberg correction]; change in luminescence by at least 1.3 times).

According to the RTGG database (http://www.genome.jp/kegg/pathway6.html), we selected 10 differentially expressed immune genes that are responsible for the cytotoxic effect of the nonspecific immune system (Table 1). Table 1 shows that they include the genes for immunoglobulin-like receptors of NK cells (killer cell immunoglobulin-like receptors, KIR: KIR2DL1, 2DL2, 2DL3, 3DL1, 2DS4, and 2DS1), genes for receptor subunits of IL-2 (IL-2Rβ, interleukin-2 receptor β chain) and IL-18 (IL-18RAP, interleukin-18 receptor accessory protein), and genes

TABLE 1. Immune Gene Expression during Physical Exercise (FDR=0.05)

Gene	Full name of the gene	Change in expression	Function in the NK cell
Stimulation of NK cell activation			
IL-2Rβ	IL-2 receptor, β chain	1.41	Stimulatory signal transduction
IL-18RAP	IL-18 receptor, accessory protein	1.35	Stimulatory signal transduction
Interaction with the target cell			
KIR2DL3	Two-domain Ig-like receptor, long cytoplasmic domain	2.2	Inhibitory
KIR2DL1	Two-domain Ig-like receptor, long cytoplasmic domain 1	2.07	Inhibitory
KIR2DL2	Two-domain Ig-like receptor, long cytoplasmic domain 2	1.93	Inhibitory
KIR2DS4	Two-domain Ig-like receptor, short cytoplasmic domain 4	1.9	Activating
KIR2DS1	Two-domain Ig-like receptor, short cytoplasmic domain	1.9	Activating
KIR3DL1	Three-domain Ig-like receptor, long cytoplasmic domain 1	1.84	Inhibitory
Cytotoxic action			
PRF1	Perforin 1	1.81	Induction of the killer response
GZMB	Granzyme B	1.65	Induction of the killer response

for functional proteins granzyme B (GZMB) and perforin 1 (PRF1).

The most significant changes were observed in gene expression of KIR that play a role in activation of NK cells. Depending on the length of the cytoplasmic domain, KIR exhibit the inhibitory (long cytoplasmic domain; KIR2DL1, 2DL3, 2DL2, and 3DL1) or activating properties (short domain; KIR2DS4 and 2DS1) [3,9,10]. The expression of both receptors is characterized by similar changes, which probably results from the influence of various stimulating factors on activation of NK cells.

Various cytokines, including IL-2, IL-12, IL-15, and IL-18, are the major triggering factors for activation of NK cells [2]. They stimulate proliferation and maturation of cells and increase their cytotoxic function. We revealed an increase in transcription activity of receptor genes for IL-2Rβ and IL-18RAP (Table 1). Published data show that their expression increases significantly under the influence of IL-2 [5]. We measured the concentration of IL-2 in serum samples from sportsmen. Physical exercise was followed by the increase in serum IL-2 concentration (p=0.008, Mann–Whitney test; Fig. 1).

We conclude that an increase in the expression of genes for interleukin receptors and KIR serve as one of the criteria for activation of NK cells.

Stimulation of KIR (inhibitory or activating receptors) is mediated by some factors and provides the functional response of NK cells. It is manifested in the secretion of cytokines (INF-γ, TNF-α, and GM-CSF) [3] that activate the cellular immune response. These changes are also accompanied by the synthesis of cytotoxic molecules, granzymes and perforins [4].

The molecule of SLP-76 (Src homolog 2 with 76-kDa leukocyte protein) is one of the major intermediates for cytokine formation in NK cells. This molecule triggers the mechanism of cytokine gene transcription, which is mediated by phosphorylation. The expression of SLP-76 mRNA is also regulated by IL-2 [6]. However, we did not find any changes in transcription of the *SLP-76* gene [15].

The synthesis of cytotoxic molecules is activated due to the interaction of activating receptors with the DAP12 molecule (DNAX-activated protein, 12 kDa), which binds to ZAP-70 kinase (zeta-chain, 70-kDa binding protein). These changes are followed by phosphorylation of the tyrosine residue in the effector molecule of LAT (linker for activation of T lymphocytes), which can activate the release of perforins and granzymes [15]. No changes were found in the expression of genes *DAP12*, *ZAP-70*, and *LAT*. However, transcription activity of cytotoxic molecules (granzymes and perforins) was elevated under these conditions (Table 1). It was probably related to activation with

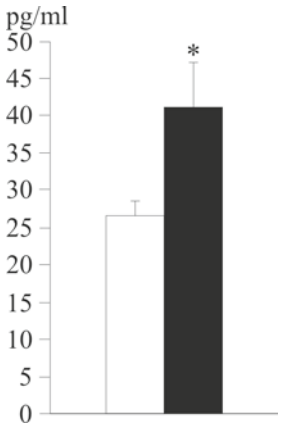

Fig. 1. IL-2 concentration before (light bar) and after short-term intense physical exercise (dark bar). *p<0.01 compared to the initial level.

IL-2 [8]. The main functions of granzymes are induction of apoptosis and lysis of the target cell due to rapid fragmentation of cellular DNA [13]. Activity of granzymes is realized after their incorporation into the target cell [4]. Perforins are present in granules of NK cells and have a key role in this process. The release of perforins is followed by their incorporation into the target cell membrane and polymerization into the transmembrane channel. It was hypothesized that granzymes enter the cell through this channel and provide lysis of the target cell.

It should be emphasized that the increased expression of KIR and cytotoxic molecules can result from an increase in the number of NK cells after physical exercise. However, NK cells are not characterized by variations in transcription activity of secondary messengers. These features can serve as a marker of increased expression of receptors and functional proteins.

Our results demonstrate an increase in the expression of receptor genes under the influence of various triggering factors (*e.g.*, IL-2). These data illustrate early activation of the immune system in response to short-term physical exercise.

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REFERENCES

1. *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, Eds. R. Gentlemen, V. J. Carey, W. Huber, R. A. Irizarry, and S. Dudoit, New York (2005), pp. 397-420.
2. M. A. Caligiuri, *Blood*, **112**, No. 3, 461-469 (2008).
3. K. Cheent and S. I. Khakoo, *Immunology*, **126**, No. 4, 449-457 (2009).

4. S. P. Cullen and S. J. Martin, *Cell Death Differ*., **15**, No. 2, 251-262 (2008).
5. K. Dybkaer, J. Iqbal, G. Zhou, *et al*., *BMC Genomics*, **8**, 230 (2007).
6. D. L. Hodge, W. B. Schill, J. M. Wang, *et al*., *J. Immunol*., **168**, No. 12, 6090-6098 (2002).
7. R. A. Irizarry, B. Hobbs, F. Collin, *et al*., *Biostatistics*, **4**, No. 2, 249-264 (2003).
8. M. L. Janas, P. Grooves, N. Kienzle, and A. Kelso, *J. Immunol*., **175**, No. 12, 8003-8010 (2005).
9. G. Kroemer, *Cell Death Differ*., **5**, No. 6, 547 (1998).
10. D. Middleton, M. Curran, and L. Maxwell, *Transpl. Immunol*., **10**, Nos. 2-3, 147-164 (2002).
11. F. Mignini, E. Traini, D. Tomassoni, *et al*., *Clin. Exp. Hypertens*., **30**, No. 8, 720-731 (2008).
12. B. K. Pedersen, T. Rohde, and M. Zacho, *J. Sports Med. Phys. Fitness*, **36**, No. 4, 236-245 (1996).
13. A. Rabson, I. Roitt, and P. Delves, *Really Essential Medical Immunology*, Oxford (2004).
14. B. W. Timmons and T. Cieslak, *Exerc. Immunol. Rev*., **14**, 8-23 (2008).
15. E. Vivier, J. A. Nunes, and F. Ve'ly, *Science*, **306**, 1517-1519 (2004).