SPORTS MEDICINE

Expression of KIR2DL3 and KIR2DS2 Natural Killer Receptors in Exercise

V. A. Shleptsova, E. S. Grebenyuk, S. A. Khaustova, N. P. Obraztsova, M. Yu. Shkurnikov, D. A. Sakharov, and E. A. Tonevitsky

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 6, pp. 695-698, June, 2010
Original article submitted February 8, 2010

The mechanisms of early activation of natural killers were studied in volunteers of different athletic qualification. The initial levels of expression of KIR2DL2, PRF1, and GZMB genes regulating the functions of natural killer differ in athletes and untrained subjects. Moderate exercise stimulates transcription activities of these genes. In athletes, the expression increases more intensely than in controls. Stimulation of inhibitory (KIR2DL3) and activation (KIR2DS2) receptors was revealed. This indicated nonspecific stimulation of natural killers, probably mediated by an increase in serum concentration of heat shock protein with a molecular weight of 70 kDa.

Key Words: *immune system; gene expression; natural killers; exercise*

Long-term training sessions lead to restructuring of a wide spectrum of physiological processes in humans. For example, training exercises modulate the function of nonspecific immunity [14]. Natural killers (NK cells) are most sensitive to exercise [14]. These cells are a heterogeneous population consisting of two groups differing by the intensity of CD56 receptors expression on the cell surface: $CD56^{dim}$ and $CD56^{bright}$ [3]. The main function of $CD56^{dim}$ is destruction of damaged or dead cells by producing perforins (PRF) and granzymes (GZM), while $CD56^{bright}$ provide mainly the production of cytokines responsible for specific immune response [6].

Recent studies demonstrated quantitative redistribution of NK subpopulations (an increase in $CD56^{dim}$/$CD56^{bright}$ ratio) in response to short-term exercise [14].

The main regulatory mechanism of $CD56^{dim}$ stimulation is modification of ratio of Ig receptors (KIR) on the cell surface [7]. Two KIR variants differing by the number of extracellular domains are distinguished: two-domain KIR2D and three-domain KIR3D. The length of the cytoplasmic tail of the receptor indicates its function: inhibition (long cytoplasmic tail) KIR2DL or KIR3DL (KIR2DL1, 2DL3, 2DL2, 3DL1) or stimulation (short cytoplasmic tail) KIR2DS, or KIR3DS (KIR2DS4, 2DS1, 3DS1) [3].

We previously showed that short-term highly intense exercise leads to more intense expression of *KIR* genes and PRF1 and GZMB functional proteins in athletes [1].

Here we compare the effects of moderate exercise on activity of *KIR* and functional proteins PRF1 and GZMB expression in individuals with different physical training status.

MATERIALS AND METHODS

The study was carried out in 12 athletes of high qualification specialized in cyclic sports for more than 5 years. Control group consisted of 7 young men with

Institute of Physical Culture and Sports, Moscow, Russia. ***Address for correspondence:*** varja.shlepzova@gmail.com. V. A. Shleptsova

0007-4888/10/1496-0755

TABLE 1. Anthropometric and Physiological Values of Volunteers

Characteristic	Group	
	control	athletes
Age, years	23.5±1.5	22.4±0.7
Body length, cm	174.3±5.6	172.8±3.8
Body weight, kg	76.4±2.7	73.1±1.6
Body weight index, kg/m^2	24.4±4.5	23.1±5.8
VO_{2max}, ml/min×kg	48.9±8.8*	68.8±4.4

Note. *p<0.001 compared to the control.

common level of physical activity. Anthropometric and physiological values of the volunteers are presented in Table 1. All volunteers gave informed consent to participation in the study. The protocol of experiment was approved by the Ethic Committee of Institute of Physical Culture and Sports.

The readiness to exercise was evaluated by measuring the maximum oxygen consumption (VO_{2max}) in each volunteer. One week before the experiment, incremental cycle ergometry test to volitional exhaustion was carried on a Venus treadmill (Cosmos) [14]. The groups differed significantly (p<0.001) by VO_{2max} values (Table 1).

Treadmill running (30 min) at a certain preset rate served as the model exercise (at 80% VO_{2max}). Blood was collected before exercise (T1), directly after the test (T2), and after 1-h rest (T3).

Serum concentrations of IL-2, IL-15, and IL-18 were measured using commercial test systems Interleukin-2-EIA-BEST, Interleukin-18-EIA-BEST (Vector-Best) and IL-15 human ELISA kit (Invitrogen), respectively.

The RNA was isolated using PaxGene Blood RNA kit (PaxGene) according to manufacturer's instruction. The yield of purified nonfragmented RNA (RIN>8) was about 100 ng/μl.

Reverse transcription was carried out using reverse transcription kit (DNA-Technology). Total RNA (40 ng) was added to the reverse transcription mixture

TABLE 2. Primer Sequences

Protein	Primers
KIR2DL3	forward: 5'-AAC-AGT-GAA-CAG-GGA-GGA-CTC-TG-3'
	reverse: 5'-AAG-GGC-GAG-TGA-TTT-TTC-TCT-G-3'
	sample: 5'-FAM-CTC-AGG-AGG-TGA-CAT-ATG-CAC-AGT-TGA-ATC-A-BHQ2-3'
KIR2DL2	forward: 5'-AGA-GGC-CCA-AGA-CAC-CCC-3'
	reverse: 5'-CAA-GGC-CTG-ACT-GTG-GTG-C-3'
	sample: 5'-FAM-AGA-TAT-CAT-CGT-GTA-CGC-GGA-ACT-TCC-AAA-TGC-T-BHQ2-3'
KIR2DS2	forward: 5'-TGG-TCA-GAT-GTC-AGG-TTT-GAG-C-3'
	reverse: 5'-TGG-CCT-TGG-AGA-CCC-CAT-3'
	sample: 5'-FAM-AGG-GGA-AGT-ATA-AGG-ACA-CTT-TGC-ACC-TCA-TTG-GAG-AGC-BHQ2-3'
PRF	forward: 5'-TGG-AGT-GCC-GCT-TCT-ACA-GTT-3'
	reverse: 5'-GCC-CTC-TTG-AAG-TCA-GGG-TG-3'
	sample: 5'-FAM-CCA-TGT-GGT-ACA-CAC-TCC-CCC-GC-BHQ1-3'
GZMB	forward: 5'-AAT-CCT-AAG-AAC-TTC-TCC-AAT-GAC-ATC-3'
	reverse: 5'-GCA-CAG-CTC-TGG-TCC-GCT-3'
	sample: 5'-FAM-GGC-CTT-TCT-CTC-CAG-CTG-CAG-TAG-CA-BHQ1-3'
B2M	forward: 5'-AGC-GTA-CTC-CAA-AGA-TTC-AGG-TT-3'
	reverse: 5'-ATG-ATG-CTG-CTT-ACA-TGT-CTC-GAT-3'
	sample: 5'-BHQ1-TCC-ATC-CGA-CAT-TGA-AGT-TGA-CTT-ACT-G-FAM-3'
HPRT	forward: 5'-CTC-AAC-TTT-TAA-CTG-GAA-AGA-ATG-TC-3'
	reverse: 5'-TCC-TTT-TCA-CCA-GCA-AGC-T-3'
	sample: 5'-BHQ1-TTG-CTT-TCC-TTG-GTC-AGG-CAG-TAT-AAT-C-FAM-3'

with nonspecific primers. The reaction was carried out at 40°C for 30 min followed by reverse transcriptase inactivation at 95°C for 5 min. The resultant cDNA was diluted 100-fold and used in real-time PCR.

The expression of RNA was evaluated by semi-quantitative real-time PCR (5 min at 95°C, 1 cycle; 20 sec at 94°C, 1 min at 60°C, 50 cycles; storage at 10°C). cDNA samples were analyzed in three replicates. Real-time PCR was carried out using specific primers to the studied genes and two housekeeping genes: β_2-microglobulin (β_2-M) and hypoxanthine phosphoribosyl transferase 1 (HPRT) [13]. The expression of β_2-M and HPRT is constant in various cell types and does not depend on external factors, but the number of copies per cell varied. β_2-M is a highly presented gene, HPRT a low presented one. The expression of the studied genes in leukocytes can be quantitatively evaluated after standardization for these two genes. The results are presented in relatively units (rel. units) after standardization by β_2-M and HPRT [10].

The real time PCR was carried out by the TaqMan method with 6-carboxyfluorescein (FAM) fluorescent stain and Black Hole Quencher 1, 2 (BHQ1, 2). The primers (Sintol) to the studied genes are presented in Table 2.

RESULTS

Since lasting highly intense training has a suppressive effect on the immune system [8], it can be hypothesized that activation capacity of NK cells can be modified in athletes. In order to verify this hypothesis, we studied changes in the expression of genes regulating activity of NK cells in athletes and young people with common physical activity levels, differing by the readiness to exercise (that is, by VO_{2max} values).

A previous screening study making use of Human GeneChip ST1.0 microchips (Affymetrix) has detected and selected the immunological genes whose expression is changing significantly in response to intense exercise: KIR, PRF1, and GZMB [1].

The effects of moderate exercise on changes in the expression of KIR2DL3, KIR2DL2, KIR2DS2, PRF1, and GZMB in individuals with different training status were described.

Differences in the initial levels of expression were detected for genes KIR2DL2, PRF1, and GZMB (Fig. 1). Transcription activity in the athletes is lower than in controls. Transcription activity differed 3.9 times between the groups ($p<0.001$) for KIR2DL2 gene, 7.8 times ($p<0.001$) for PRF1 gene, and 4.8 times ($p<0.001$) for gene GZMB (Fig. 1). This can be due to athletes' adaptation to intense exercise. It is known that DNA methylation inhibiting transcription stimulation is the main regulator of transcription of KIR genes

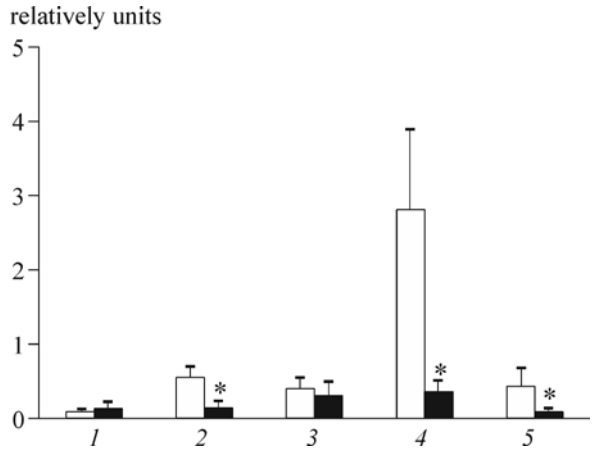

Fig. 1. Gene expression at rest (before exercise). Here and in Fig. 2: *1*) KIR2DL3; *2*) KIR2DL2; *3*) KIR2DS2; *4*) PRF1; *5*) GZMB. Light bars: young men with common level of physical activity (control); dark bars: highly qualified athletes. *$p<0.01$ compared to the control.

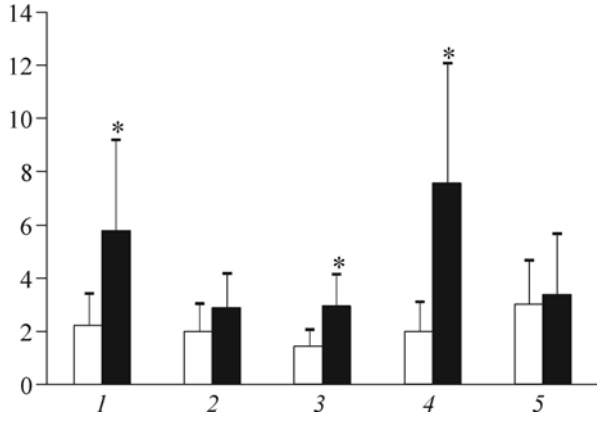

Fig. 2. Changes in gene expression under the effect of exercise. Ordinate: multiplicity of changes in expression.

[5]. Exercise exhausts body reserve potential and promotes the development of oxidative stress (a factor stimulating DNA methylation) [4]. Presumably, lower expression of KIR2DL2 in athletes is caused by more intense methylation of its promoter in comparison with other KIR genes. Reduced expression of PRF1 and GZMB can indicate reduction of $CD56^{dim}$ cytotoxic activity in athletes as a result of physical work [2,11].

Moderate exercise leads to greater expression of KIR, PRF1, and GZMB genes. The level of all these genes' expression decreases within 1 h of rest after exercise (T3) and tends to the initial level (T1).

Gene expression in athletes increases more significantly than in controls (Fig. 2). The differences are significant ($p<0.01$) for genes KIR2DL3, KIR2DS2, PRF1. Changes in the expression of KIR2DL2 and GZMB genes are about the same in the groups, with a trend to increase of expression in the athletes. The greatest changes in the expression after exercise were

noted for genes KIR2DL3 and PRF1 in athletes, which can cause more intense mobilization of NK cells in response to exercise in athletes compared to controls.

An increase in the expression of inhibitory (KIR2DL3) and stimulation (KIR2DS2) receptors of NK cells in response to nonspecific stimulation was revealed. The main factors stimulating NK cells are IL-2, -15, and -18 cytokines [3] and the changes in the serum concentration of heat shock protein (HSP) with a molecular weight of 70 kDa (HSP-70) [9]. However studies of the serum cytokine status of athletes revealed trace levels of these IL at rest and after exercise (data not presented). Exercise promotes an increase in blood concentration of HSP-70 [12]. Hence, it seems that the increase of serum concentration of HSP-70 is a mechanism stimulating NK cells during exercise.

Hence, the lower initial expression of KIR2DL2, PRF1, and GZMB genes in athletes compared to controls is a manifestation of adaptation changes in the immune system as a result of athletic exercises. Similarly as intense exercises, moderate ones lead to an increase of KIR, PRF1, and GZMB genes expression. Changes in gene expression in athletes are significantly more pronounced than in controls; this attests to more intense stimulation of NK cells in athletes in response to exercise. These shifts are paralleled by more active expression of inhibitory and stimulation receptors, presumably due to nonspecific stimulation of NK cells at the expense of increased concentration of serum HSP-70.

The study was supported by the Federal for Special Program “Research and Development of the Priority Trends of Scientific and Technological Complex of Russia for 2007-2012” and State Contracts (Nos. 02.512.1.2015 and 02.512.11.2318).

The authors are grateful to O. A. Bystrykh, E. B. Akimov, R. S. Andreev, and V. V. Sar’yants for participation in the work at the paper.

REFERENCES

1. V. A. Shleptsova, E. V. Trushkin, Ya. I. Davydov, *et al.*, *Byull. Eksp. Biol. Med.*, **148**, No. 1, 97-100 (2010).
2. W. A. Braun, M. G. Flynn, D. E. Jacks, *et al.*, *J. Appl. Physiol.*, **87**, No. 6, 2237-2243 (1999).
3. M. A. Caligiuri, *Blood*, **112**, No. 3, 461-469 (2008).
4. A. C. Campos, F. Molognoni, F. H. Melo, *et al.*, *Neoplasia*, **9**, No. 12, 1111-1121 (2007).
5. H. W. Chan, J. S. Miller, M. B. Moore, and C. T. Lutz, *J. Immunol.*, **175**, No. 9, 5966-5974 (2005).
6. K. Cheent and S. I. Khakoo, *Immunology*, **126**, No. 4, 449-457 (2009).
7. C. M. Gardiner, *Int. J. Immunogenet.*, **35**, No. 1, 1-8 (2008).
8. M. Gleeson, *Curr. Opin. Clin. Nutr. Metab. Care*, **9**, No. 6, 659-665 (2006).
9. G. Multhoff, *Int. J. Hyperthermia*, **25**, No. 3, 169-175 (2009).
10. D. V. Rebrikov and D. Iu. Trofimov, *Prikl. Biokhim Mikrobiol.*, **42**, No. 5, 520-528 (2006).
11. S. G. Rhind, G. A. Gannon, M. Suzui, *et al.*, *Am. J. Physiol.*, **276**, No. 5, Pt. 2, R1496-R1505 (1999).
12. D. A. Sakharov, A. V. Stepanov, M. Y. Shkurnikov, and A. G. Tonevitskii, *Bull. Exp. Biol. Med.*, **147**, No. 3, 361-365 (2009).
13. N. Silver, S. Best, J. Jiang, and S. L. Thein, *BMC Mol. Biol.*, **7**, 33 (2006).
14. B. W. Timmons and T. Cieslak, *Exerc. Immunol. Rev.*, **14**, 8-23 (2008).