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Natural killer cell lytic activity and CD56^{dim} and CD56^{bright} cell distributions during and after intensive training.

Masatoshi Suzui[1], Takeshi Kawai[2], Hiroko Kimura[3], Kazuyoshi Takeda[3],

Hideo Yagita[3], Ko Okumura[3], Pang N. Shek[4, 5], Roy J. Shephard[6]

[1]School of Business Administration, Meiji University, Suginami, Tokyo, Japan, 168-8555

[2]School of Health and Sports Science, Juntendo University, Inba, Chiba, Japan, 270-1695

[3]School of Medicine, Juntendo University, Bunkyo, Tokyo, Japan, 113-8421

[4]Defence Research and Development Canada-Toronto, Toronto, Ontario, Canada, M3M 3B9

[5]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada, M5G 1L5

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[6]Faculty of Physical Education & Health, Department of Public Health Sciences, University of Toronto, Toronto, Ontario, Canada, M5S 1A1

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Contact information: Masatoshi Suzui

Meiji University

1-9-1 Eifuku, Suginami, Tokyo, Japan, 168-8555

Phone +81-3-5300-1121

Fax +81-3-5300-1203

E-mail suzui@isc.meiji.ac.jp

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Abstract

The purpose of this study was to examine the impact of intensive training for competitive sports on natural killer (NK) cell lytic activity and subset distribution. Eight female college-level volleyball players undertook one-month of heavy pre-season training. Volleyball drills were performed 5 hours per day, 6 days per week. Morning resting blood samples were collected prior to training (PRE), on the 10th day of training (DURING), one day before the end of training (END) and one week after intensive training had ceased (POST). $CD3^-CD16^{bright}CD56^{dim}$ ($CD56^{dim}$ NK), $CD3^-CD16^{dim/-}CD56^{bright}$ NK ($CD56^{bright}$ NK) cells and $CD3^+CD16^-CD56^{dim}$ ($CD56^{dim}$ T) cells in peripheral blood were determined by flow cytometry. The circulating count of $CD56^{dim}$ NK cells (the predominant population, with a high cytotoxicity) did not change, nor did the counts for other leukocyte subsets. However, counts for $CD56^{bright}$ NK and $CD56^{dim}$ T cells (subsets with a lower cytotoxicity) increased significantly ($p<0.01$) in response to the heavy training. Overall

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NK cell cytotoxicity decreased from PRE to END ($p=0.002$), with a return to initial values at POST. Lytic units per NK cell followed a similar pattern ($p=0.008$). Circulating levels of interleukin-6, interferon-β and tumor necrosis factor-β remained unchanged. These results suggest that heavy training can decrease total NK cell cytotoxicity as well as lytic units per NK cell. Such effects may reflect in part an increase in the proportion of circulating NK cells with a low cytotoxicity.

Key words

catecholamines, creatine kinase, CD44, CD62L, cytokines

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Introduction

Natural killer (NK) cells are important components of the innate immune system, owing to their cytokine production and cytolytic activity against target cells (5). NK cells are also one of the leukocyte subsets which is most responsive to physiological and psychological stress (15, 38). The effects of acute exercise on NK cells are already well documented (31, 38); cytolytic activity increases during exercise, but decreases post exercise, usually for no more than a few hours. These changes reflect mainly an altered NK cell distribution, with little change in per cell cytotoxicity (3, 15, 37). The effects of chronic exercise on resting NK cell cytolytic activity and cell distribution remain less clear (37). Cross-sectional studies have related habitual physical activity to NK cell cytotoxicity. Thus, Nieman et al. reported that NK cell cytolytic activity was greater in marathon runners (29), rowers (28) and active elderly (30) than in untrained individuals, although there were no inter-group differences in NK cell count. Such results suggest that chronic exercise

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increases cytotoxicity per NK cell. On the other hand, many studies have failed to establish positive relationships between NK cell cytolytic activity and chronic exercise (2, 36, 37). A few longitudinal studies have shown increased cytotoxicity after relatively low intensity training (7, 23), but most researchers have not observed any changes in cell counts or cytotoxicity (4, 30, 37). Watson et al. reported that 15 weeks of training at 75-85%$\dot{V}O_2$max decreased NK cell cytolytic activity without altering NK cell counts (44). This implied a negative effect of chronic exercise on NK cell function. Any influence of training on NK cell counts and cytotoxicity seems complex. A substantial number of reviews have proposed that low to moderate intensities of training have beneficial effects on immune functions including NK cell cytotoxicity, but that high intensity training downregulates NK function (37, 45). These results seem to support the “open window” theory, whereby some athletes become susceptible to upper respiratory infections for a brief period following very heavy exercise (31).

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NK cells can be divided into two subsets, based on their cell surface density of CD56 (5). The majority of NK cells are $CD56^{dim}$ cells, but approximately 10% of NK cells are $CD56^{bright}$ cells. The $CD56^{dim}$ NK cells have a greater cytolytic activity (21) and express higher levels of CD16 than the $CD56^{bright}$ subset. Therefore, a redistribution of the NK cell subsets could explain the changes in total and calculated per cell cytotoxicity induced by chronic exercise. Motivation to achieve high levels of performance may cause participants in competitive sports to engage in a high intensity training, sometimes inducing the overtraining syndrome including immunosuppression (20). We have thus examined the impact of intensive sports training on NK cell cytotoxicity and subset distribution.

Methods

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Subjects. Fifteen female university students participated in this study, which was approved by the institutional committee on human experimentation. Eight female members of a college volleyball team served as the training group, and seven age-matched healthy female students formed the control group. Each participant signed a consent form following a detailed description of procedures and possible risks. Baseline characteristics of the training and control groups were, respectively: age 20.1±0.4 and 20.6±0.3 (SE) years, height 1.69±0.01 and 1.59±0.01m, body mass 62.6±2.3 and 52.6±1.1 kg; body fat 27.2±1.4 and 24.3±0.6%; resting heart rate 60±2 and 64±1 beats•min^{-1}; resting systolic blood pressure 112±4 and 109±1.6 mmHg; and resting diastolic blood pressure 70±3 and 70±2mmHg.

Experimental design. Subjects in the training group undertook one-month of heavy pre-season training. Volleyball drills were performed 5 hours per day, 6 days per week. Control subjects were instructed to avoid vigorous exercise during this period. Four blood

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samples were collected prior to training (PRE), on the 10th day of training (DURING), one day before the end of training (END) and one week after training (POST). On each occasion, subjects came to the laboratory at 0800 h, after an overnight fast. Following 30 minutes of rest, we measured heart rate (Polar Vantage heart rate monitor, USA), blood pressure (hemodynamomometer, HEM-906, OMRON, Kyoto, Japan) and the percentage of body fat (an electrical impedance technique, TBF-102, Tanita, Tokyo, Japan). Then 20 ml of blood was drawn from the median ante-cubital vein, with the subject in the supine position. All procedures were performed in an air-conditioned room, maintained at 20-25 °C and 50-60% relative humidity.

Leukocyte subsets. Total circulating counts for leukocytes, lymphocytes, monocytes, and neutrophils were determined on 2 ml of ethylenediamine tetra-acetate (EDTA) treated blood, using an automated hematology analyzer (Sysmex NE8000, Toa Medical Electronics Corp., Kobe, Japan).

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NK cell subsets. Peripheral blood mononuclear cells (PBMC) were separated from 10 ml of heparinized whole blood by density gradient centrifugation (30 min, 20 ˚C, 400 g) using Separate-L (Muto Pure Chem., Tokyo, Japan). They were then stored in RPMI-1640 (GIBCO, BRL, Life Technologies, NY) with 10% fetal calf serum (FCS), penicillin (100 $IU\bullet ml^{-1}$) and streptomycin (100 $\mu g\bullet ml^{-1}$) at 4 °C until analysis. NK cells were enumerated by 3-color immunophenotyping, using appropriate combinations of monoclonal antibodies (PharMingen, San Diego, CA) conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin-chlorophyll protein (PerCP). Briefly, a sample of $1\bullet10^6$ of PBMC was mixed with saturating amounts of monoclonal antibody conjugated with FITC (CD16), PE (CD56) and PerCP (CD3). After 20 minutes of incubation on ice, the cells were washed twice with phosphate-buffered saline (PBS) and stored in the dark at 4 °C until measurement. Stained cells were passed through a flow-cytometer (FACScan, Becton Dickinson, CA). Results were expressed as the percentage of cells in a gated lymphocyte

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region (CELLQuest 3.1, Becton Dickinson, CA). NK cells were divided into two subsets, based on the surface density of CD56. $CD3^{-}CD16^{bright}CD56^{dim}$ ($CD56^{dim}$ NK) and $CD3^{-}CD16^{dim/-}CD56^{bright}$ NK ($CD56^{bright}$ NK) cell subsets were distinguished by gated flow cytometric analyses (Figure 1). $CD3^{+}CD16^{-}CD56^{dim}$ ($CD56^{dim}$ T) cells were also determined, because these cells show some cytotoxicity against the NK sensitive tumor cell K562 (21). Absolute cell counts were derived by multiplying the percentage of a given cell subset by the total lymphocyte concentration found in peripheral blood.

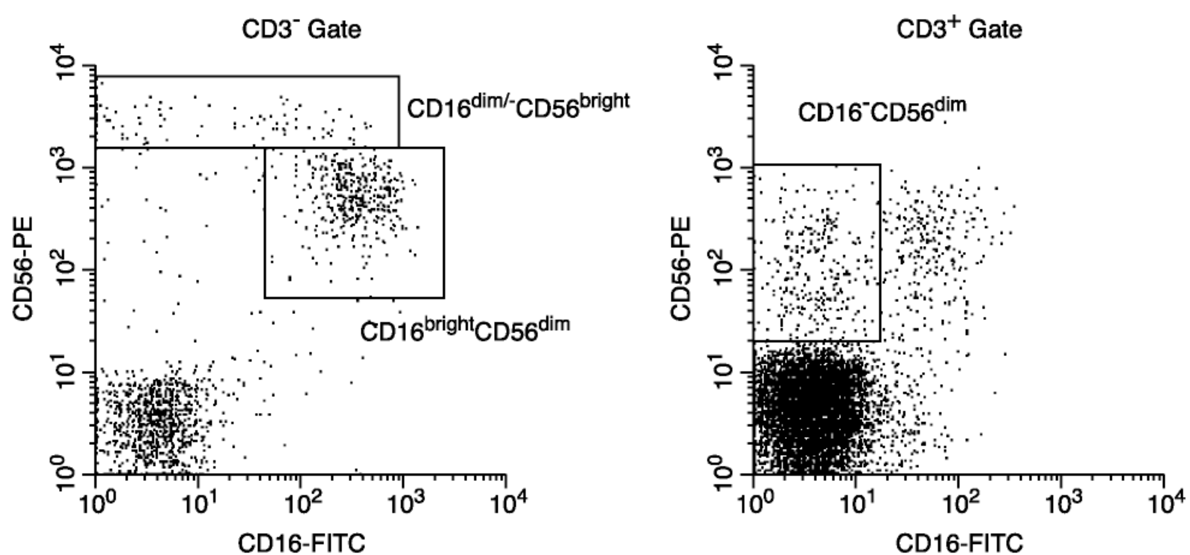

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Figure 1. Flow cytometric analysis of CD5^{6dim} NK and CD56^{bright} NK cells and CD56^{dim} T cells.

The left lower box shows the CD5^{6dim} NK cell subset, the left upper box the CD56^{bright} NK cell subset, and the right box the CD56^{dim} T cell subset.

Adhesion molecules on NK cells. The fluorescence intensities of expression of adhesion molecules, such as lymphocyte function associated antigen-1alpha (LFA-1β, CD11a), beta-2 integrin (CD18), homing cell adhesion molecule (H-CAM, CD44) and leukocyte endothelial cell adhesion molecule-1 (LECAM-1, CD62L) on CD56^{dim} NK and CD56^{bright} NK cells were measured by flow cytometry. Samples of $1 \cdot 10^6$ of PBMC were stained with FITC (CD11a, CD18, CD44 or CD62L), PE (CD56) and PerCP (CD3). Monoclonal antibodies were obtained from PharMingen (San Diego, CA). The staining procedure was as described above. The mean fluorescence intensities (MFI) of adhesion

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molecule expression in the NK cell subset regions ($CD3^{-}CD56^{dim}$ and $CD3^{-}CD56^{bright}$) were analyzed using logarithmic frequency distribution graphs to indicate the mean surface density for all adhesion molecules of interest.

NK cell cytotoxicity assay. NK cell cytotoxicity was assessed by a non-radioactive europium (Eu) release assay (27). The NK-sensitive K562 tumor cell line served as target cells. Before labeling, the K562 target cells were washed twice with 5 ml of filtered buffer A (50mM Hepes, 93mM NaCl, 5mM KCl, 2mM $MgCl_2$). The K562 cells were then incubated in a labeling buffer [40mM Eu, 125mM diethylene-triaminepentaacetate , 250mM dextran sulfate] for 20 min at 4°C. After labeling, the cells were washed 7 times with buffer B (50mM Hepes, 93mM NaCl, 5mM KCl, 2mM $CaCl_2$, 10mM dextrose) and 2 times with RPMI-1640 complete medium. They were then resuspended in the medium at a concentration of $1 \cdot 10^5$ cells•ml^{-1}. Ten thousand Eu-labeled K562 target cells were added to each well of a 96-well round bottom plate. Effector cells were then added to the wells to

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yield 20:1, 10:1 and 5:1 effector-to-target (E:T) ratios. The plate was centrifuged for 1 minute (20°C, 1000 rpm) to promote optimal E:T cell conjugation and was then incubated for 2 hours at 37°C in 5% CO_2. After incubation, a 20µl aliquot of supernatant was transferred to a 96-well flat bottom plate (Immunoassay plate, Nalge Nunc International) and 100 µl of enhancement solution (Delphia ™, Pharmacia LKB, Uppsala, Sweden) was added. The release of Eu was detected by a time-resolved fluorometer (Arcus [1232]Delphia fluorometer, Pharmacia). The spontaneous release of Eu was determined by incubation of control wells with 100 µl of plain medium and the maximum release was estimated by incubation of $1 \cdot 10^4$ target cells plus 10µl of 10% Triron X. NK cell cytotoxicity was calculated, using the mean value of triplicate determinations for each E:T ratio. It was expressed as a percentage lysis, calculated as:

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$$\text{NK cell cytotoxicity (\%)} = (\text{Experimental Eu release} - \text{Spontaneous Eu release}) \bullet (\text{Maximal Eu release} - \text{Spontaneous Eu release})^{-1} \bullet 100$$

As a second index of cytotoxicity, lytic units were calculated as the number of effector cells required to lyse 15% of $1 \bullet 10^4$ target cells, results being expressed as the number of lytic units contained in $1 \bullet 10^6$ PBMC. The lytic units were then adjusted on a per NK cell basis according to the formula:

$$\text{Lytic Units (15\%)} \bullet \text{NK}^{-1} \bullet 10^{-5} = \text{Lytic Units} \bullet [\%\text{NK cells} \bullet (1 \bullet 10^6 \text{ PBMC} - \text{Monocytes})]^{-1}$$

Plasma cytokine assays. Plasma concentrations of interleukin (IL)-6, interferon (INF)-gamma (β) and tumor necrosis factor (TNF)- alpha (β) were determined on 0.5 ml samples of plasma, using a previously reported time-resolved fluoroimmunoassay (47). Monoclonal

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anti-IL-6, IFN-β and TNF-β (PharMingen, San Diego, CA) were diluted with 0.1 M carbonate-bicarbonate buffer (pH 9.3) to 2 μg•ml^{-1}. The diluted antibody to each cytokine (200 μg•100ml^{-1}) was used to coat wells of the microtiter plates (Fluoro Nunc module plate, Nalge Nunc International). After washing the wells twice with 0.05 M Tris-HCl buffer (pH 7.8) containing 0.05% Tween 20 and once with Tris-HCl buffer, the plates were stored at –20 ºC until use. 50 μl of diluted standard cytokine (recombinant human IL-6, human INF-β and human TNF-β, , San Diego, CA) solutions or plasma samples (diluted 2-5 times with Tris-HCl buffer) were added to the coated wells, and these were then incubated for 1 h at room temperature. 50 μl of the respective biotinylated antibody (1 μg•ml^{-1}) was then added. After incubation for 1 h at room temperature, the wells were washed 3 times with Tris-HCl buffer containing Tween 20, and then 50 μl of streptavidin-bovine serum albumin-4,4'-bis (1",1", 1",2",2",3",3"-heptafluoro-4",6"-hexanedion-6"-yl)-chlorosulfo-*o*-terphenyl (BHHCT)-Eu was added. After incubation for a further 2 h at 37

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ºC, the wells were washed 4 times with 0.05M Tris-HCl buffer (pH 9.1), and the solid-phase fluorescence was measured by Wallac ARVO sx multi label counter (Amersham Biosciences, Uppsala, Sweden).

Hormones and creatine kinase (CK) Analyses. Venous blood samples for adrenaline, noradrenaline and cortisol determinations were drawn into 5 ml vacutainer (Terumo, Tokyo, Japan) containing EDTA. 1.5 ml of plasma was used to determine plasma adrenaline and noradrenaline concentrations by high-performance liquid chromatography (18). Plasma cortisol concentrations were determined radioimmunologically (INCSTAR Corp., MN), using 0.2 ml of plasma. Plasma CK concentrations were measured by ultraviolet-N-acetyl cysteine spectrophotometry (39), using 0.5 ml of plasma. All plasma samples were frozen at –80 °C until analysis. Internal controls have shown less than 5% change in resting samples from untrained subjects over the normal storage period.

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Data analysis. Results are expressed as means ± SE. Statistical analyses were performed using a StatView-J5.0 (Abacus Concepts Inc., CA, USA) microcomputer software package, with statistical significance set at $p<0.05$. Possible interactions were analyzed using 2 (control and training groups) x 4 (PRE, DURING, END and POST time points), repeated measures ANOVA. When significant F ratios were observed, differences among control and training groups at each time point were examined using Bonferroni's *post hoc* tests. One-factor ANOVA was also applied to compare the responses from pre-training values.

Results

Table 1. Changes in circulating leukocyte, lymphocyte, monocyte, neutrophil eosinophil and basophil counts during and post training.

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Time	PRE	DURING	END	POST
Leukocyte (cells•μL^{-1})				
Control	6,050 ± 424	5,358 ± 296	5,273 ± 472	5,933 ± 709
Training	5,025 ± 393	5,015 ± 418	4,874 ± 317	4,984 ± 437
Lymphocyte (cells•μL^{-1})				
Control	2,104 ± 364	1,894 ± 228	2,279 ± 139	1,971 ± 218
Training	1,710 ± 128	1,726 ± 137	1,950 ± 170	1,749 ± 180
Monocyte (cells•μL^{-1})				
Control	422 ± 49	284 ± 61	332 ± 70	336 ± 52
Training	322 ± 42	361 ± 37	317 ± 21	297 ± 27
Neutrophil (cells•μL^{-1})				
Control	2,881 ± 476	2,322 ± 386	2,358 ± 360	2,977 ± 690
Training	2,772 ± 251	2,726 ± 259	2,284 ± 128	2,623 ± 312

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Eosinophil (cells•μL^{-1})				
Control	239 ± 54	238 ± 73	199 ± 81	201 ± 68
Training	173 ± 49	172 ± 52	213 ± 50	229 ± 75
Basophil (cells•μL^{-1})				
Control	51 ± 19	46 ± 9	44 ± 10	37 ± 21
Training	48 ± 5	29 ± 13	110 ± 61	86 ± 49

Values are means ± SE. There were no statistically significant changes.

Physiological changes. Neither group of subjects showed significant changes in resting HR, BP, body mass and body fat over the period of observation. There were also no reported upper respiratory or other infections during the study.

NK cell and leukocyte subsets distributions. Total circulating leukocyte, lymphocyte, neutrophil, monocyte, eosinophil and basophil counts did not change from initial values

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(Table 1). The majority of NK cells were of the $CD3^{-}CD16^{bright}CD56^{dim}$ phenotype; circulating counts for this cell population remained unchanged throughout the experiment (Figure 2). However there were significant repeated-measures ANOVA interactions in $CD56^{bright}$ NK (p=0.011) and $CD56^{dim}$ T cell (p=0.021) counts. Relative to control subjects, the training group showed higher cell counts of $CD56^{bright}$ NK cell during (p<0.001) and at the end of training (p=0.002). By the end of training, counts in the training group had increased significantly relative to initial values (one-factor ANOVA, p<0.001), with a subsequent return to normal levels. The $CD56^{dim}$ T cell counts were also increased at the end (p=0.005) and post-training (p=0.017).

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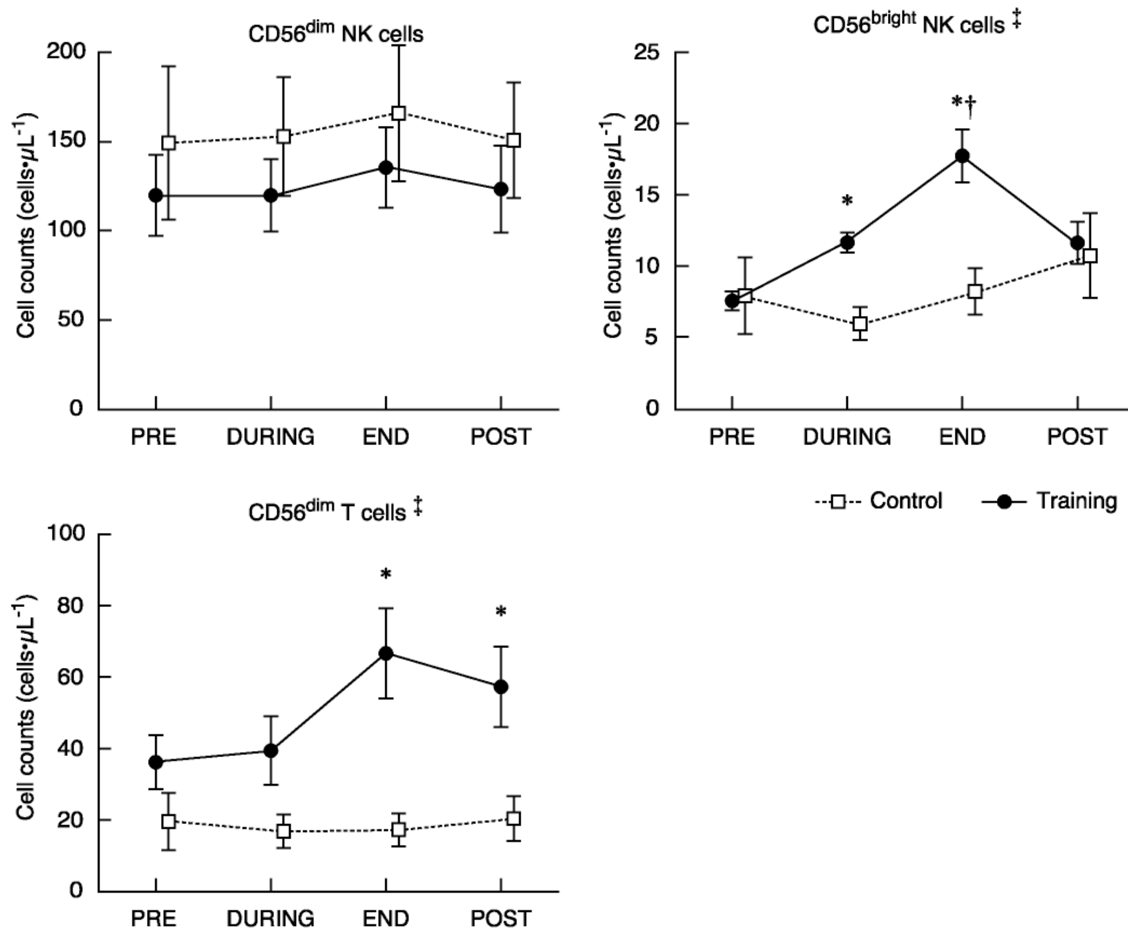

Figure 2. $CD56^{dim}$ NK and $CD56^{bright}$ NK cell counts and $CD56^{dim}$ T cell counts during and post training.

Values are means ± SE. * Significant difference between control and training groups, † significant difference from pre-training value, ‡ significant repeated-measures ANOVA interactions: $p<0.05$.

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NK cell cytotoxicity. Total NK cell cytotoxicity decreased significantly by the end of training (p=0.002), with recovery post-training (Figure 3). Lytic units per NK cell followed a similar pattern (p=0.008).

Plasma cytokine levels. Circulating IL-6, INF-β and TNF-β levels remained unchanged throughout (Table 2).

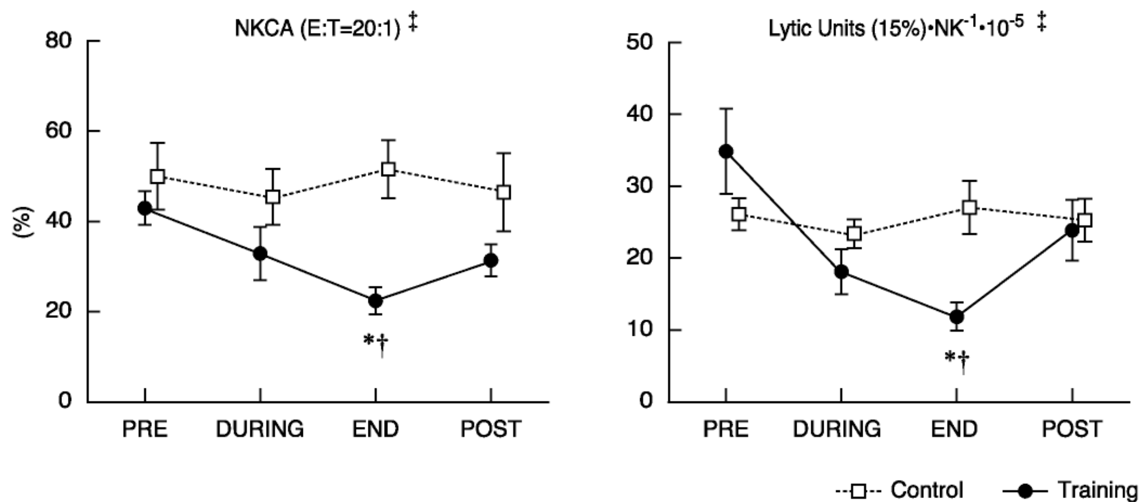

Figure 3. NK cell cytolytic activities during and post training.

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Values are means ± SE. * Significant difference between control and training groups, † significant difference from pre-training value, ‡ significant repeated-measures ANOVA interactions: $p<0.05$.

Table 2. Changes in plasma IL-6, INF-β and TNF-β levels during and post training.

Time	PRE	DURING	END	POST
IL-6 ($pgmL^{-1}$)	0.28 ± 0.17	0.79 ± 0.46	0.47 ± 0.21	0.38 ± 0.20
INF-β ($pgmL^{-1}$)	34.9 ± 21.1	59.0 ± 20.2	37.8 ± 20.1	9.5 ± 6.5
TNF-β ($pgmL^{-1}$)	11.0 ± 0.8	12.1 ± 0.7	13.2 ± 0.8	13.6 ± 0.8

Values are means ± SE. There were no statistically significant changes and no control data in cytokine concentrations.

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Expression of adhesion molecules on CD56^{dim} NK cells and CD56^{bright} NK cells.

Intensities of expression of adhesion molecules differed between CD56^{dim} NK cells and CD56^{bright} NK cells (Figure 4). Higher expressions of CD11a and CD18 were found in CD56^{dim} NK cells. On the other hand, CD56^{bright} NK cells showed a higher expression of CD44 and CD62L.

The control group showed no changes in the expression of CD11a, CD18, CD44 and CD62L on either CD56^{dim} or CD56^{bright} NK cells throughout the experiments. In the training group, the expression of CD11a on CD56^{dim} NK cells decreased significantly post-training ($p=0.008$), but there were no significant interactions between groups. The expression of CD18 on both cell subsets decreased post-training (both, $p<0.001$). CD56^{dim} NK cells and CD56^{bright} NK cells also showed an increased expression of CD44. The higher expression of CD44 on CD56^{dim} NK cells was seen in the training group during, at the end and post training with significant repeated-measures interactions ($p=0.001$). The

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expression of CD44 on CD56^{bright} NK cells also increased by the end of training (one-factor ANOVA, p<0.001), with a return to initial values post-training. The expression of CD62L remained unchanged on both CD56^{dim} and CD56^{bright} NK cells. The majority of NK cells did not express the CD62L molecule (Figure 5). The CD56^{dim} NK cells. showed no change in circulating cell counts of CD62L negative and CD62L positive cells In contrast, CD62L negative CD56^{bright} NK cells increased during training (p=0.003) and CD62L positive CD56^{bright} NK cells had increased by the end of training (p=0.002).

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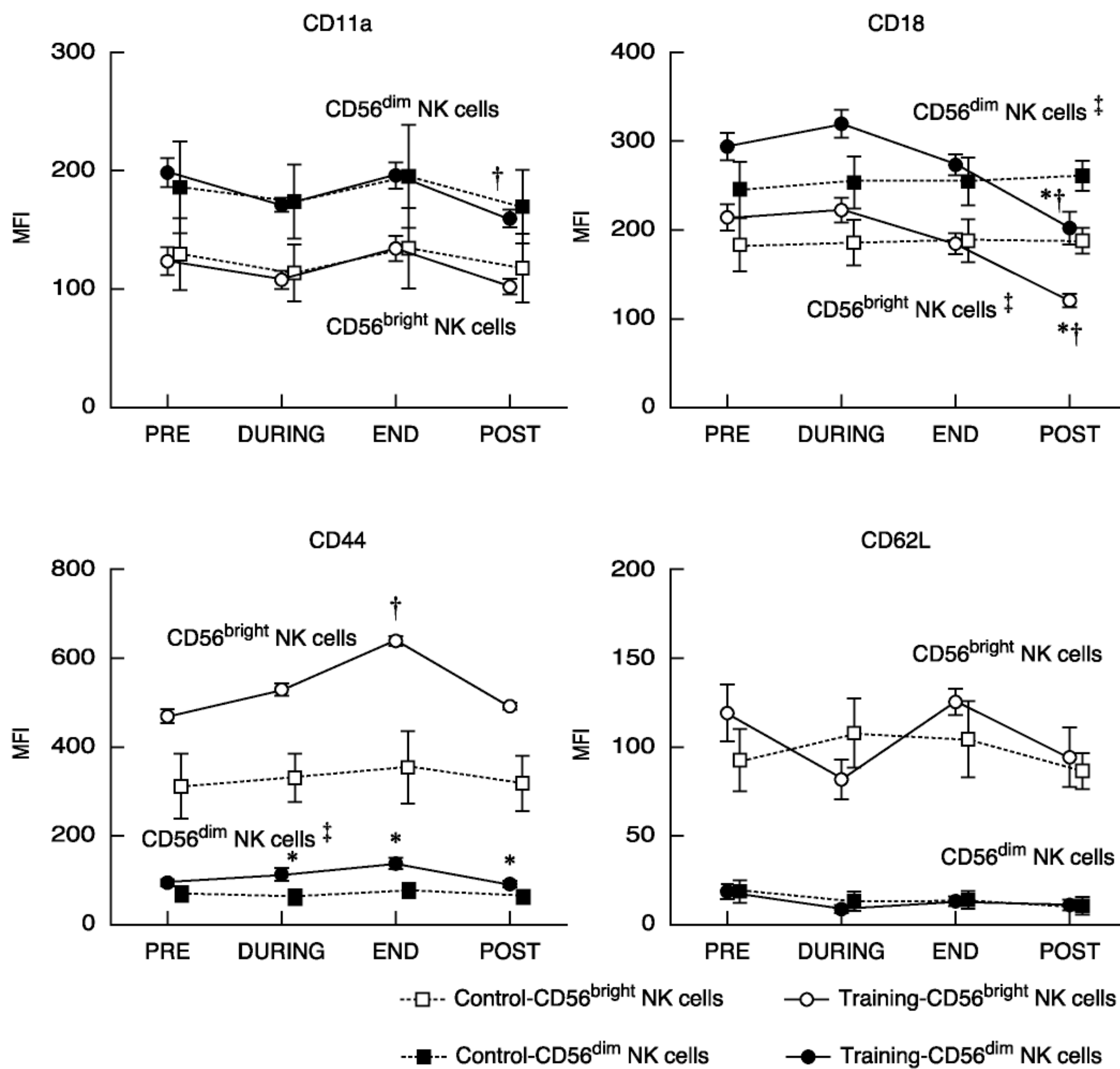

Figure 4. Expression of adhesion molecules on $CD3^-CD56^{dim}$ and $CD3^-CD56^{bright}$ NK cells during and post training.

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Values are means ± SE. * Significant difference between control and training groups, † significant difference from pre-training value, ‡ significant repeated-measures ANOVA interactions: $p<0.05$.

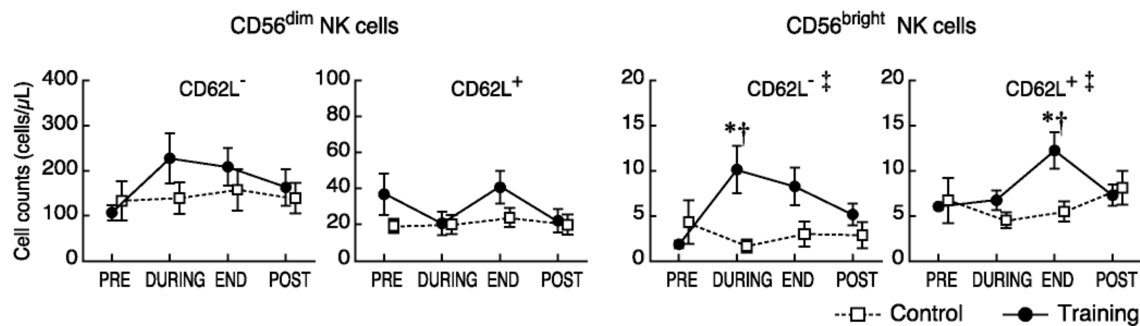

Figure 5. CD62L negative and positive cell counts in $CD3^{-}CD56^{dim}$ and $CD3^{-}$ $CD56^{bright}$ NK cell subsets during and post training.

Values are means ± SE. * Significant difference between control and training groups, † significant difference from pre-training value, ‡ significant repeated-measures ANOVA interactions: $p<0.05$.

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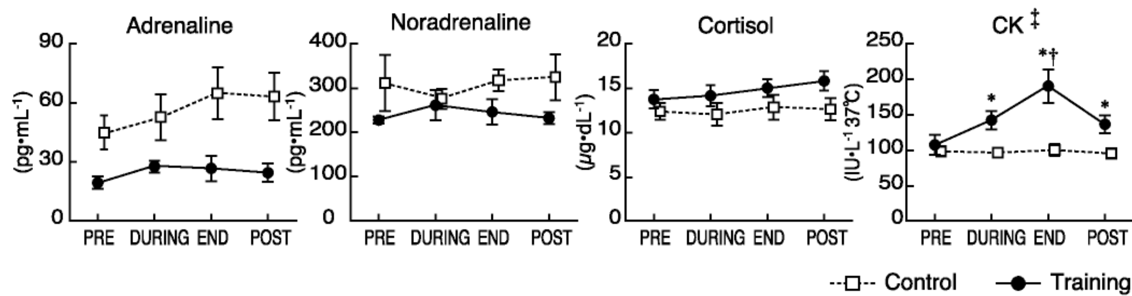

Figure 6. Plasma adrenaline, noradrenaline, cortisol and CK concentrations during and post training.

Values are means ± SE. * Significant difference between control and training groups, † significant difference from pre-training value, ‡ significant repeated-measures ANOVA interactions: $p<0.05$.

Plasma concentrations of hormones and CK. Circulating levels of adrenaline, noradrenaline and cortisol remained unchanged (Figure 6), but a significant increase of plasma CK concentration was found during ($p=0.010$), at the end ($p=0.005$) and post training ($p=0.017$) in the training group.

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Discussion

One month of intensive competitive sports training induced decreases in NK cell cytotoxicity. These results are in keeping with the observation that heavily trained athletes sometimes become more susceptible to infections. However, no infections were reported during our experiments, and pre-training cytotoxicities were restored within one week. This suggests that the adverse impact of heavy training on the immune status of young athletes is quite short-lived and infections can be avoided by an appropriate recovery period.

In regard to the changes in NK cell cytotoxicity and subset distributions, it was initially suggested that $CD56^{bright}$ cells were precursors of $CD56^{dim}$ NK cells. But more recent investigations have demonstrated that $CD56^{bright}$ NK cells are a functionally distinct subset of mature NK cells, which are primarily responsible for cytokine production in response to

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monokines (6). Differing intensities of expression of adhesion molecules have been noted on this NK cell subset (22). Our results indicate a differential redistribution of NK cell subsets during intensive training. There are decreases in total cytotoxicity without any changes in $CD56^{dim}$ NK cell counts. As we had hypothesized, the numbers of the $CD56^{bright}$ NK cell subset increased in response to training, as did the $CD56^{dim}$ T cell counts. An increase in the proportion of cells with a low cytolytic activity seems to contribute to decreases in both total cytolytic activity and the calculated lytic units per NK cell. However, there is only a small increase in the proportion of cells with a low cytolytic activity. It is thus is likely that other factors also contribute to the observed change, and further research is needed to examine whether the affinity of the target cells of these subsets is altered by training. Cytokines and hormones can each modify NK cell cytotoxicity (34, 35, 37, 45). INF-β, TNF-β, IL-2, IL-12, IL-15 and IL-18 upregulate the cytolytic activity of NK cells (9), whereas IL-4 and IL-10 downregulate it. Catecholamines (40), cortisol

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(48) β-endorphin (24) and prostaglandin E_2 (1) also modulate NK cell cytotoxicity. We found no changes in plasma INF-β, TNF-β, adrenaline, noradrenaline or cortisol levels during the experiments. Nevertheless, other humoral factors, including the plasma glutamine concentration that is essential to lymphocyte metabolism could modulate NK cell cytolytic activities during training. Cytokines also increase as a part of the inflammatory response that follows muscle damage. Increased plasma IL-6 levels have often been reported following exercise (35, 36). However, we found no changes in IL-6 levels despite increased CK levels. Further research is needed to clarify the underlying mechanisms.

Changes in the expression of adhesion molecules modulate the circulating number of lymphocytes (34). A decreased expression of molecules such as CD11a (11, 17), CD18 (17), CD49d (12) and CD62L (12, 13, 19, 25, 43) has been observed during acute exercise. Such reports support the idea that a reduced surface expression of adhesion molecules is one of the mechanisms regulating the influx of cells into the circulating blood. Our

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previous reports have demonstrated that increased cell counts during acute exercise were associated with decreased NK cell expression of CD18 and CD44 (26). In contrast, the present results noted an elevated CD44 expression on CD56^{dim} and CD56^{bright} cells despite no change or an increase in the number of these cells at the end of training. CD44 is a widely expressed cell adhesion molecule that has been implicated in a variety of biological processes, including lymphopoiesis, angiogenesis, wound healing and tumor metastasis (16). CD44 is also involved in leukocyte attachment to and rolling on endothelial cells, homing to peripheral lymphoid organs, extravasation to sites of inflammation, and leukocyte aggregation. Nevertheless, the observed change seems opposite to the response anticipated when recruiting cells to the peripheral blood. Uksila et al. previously reported that the expression of CD44 on CD16^{+} cells increased after a 3-day incubation with recombinant IL-2 (42), this treatment leading to decreased binding at the peripheral lymph nodes, but increased adherence to the mucosal high endothelium. This seems to support the

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possibility of an association between an increased expression of CD44 and increased CD56^{bright} cell counts in peripheral blood, and it also indicates the possibility that plasma IL-2 levels may have been augmented during training. IL-2 augments cytotoxicity (14), proliferation, and IFN-β production in NK cells, increases the expression of CD11a/CD18 (33) on CD56^{+} cells and decreases CD62L expression on CD56^{bright} cells (10). However, the reduced cytotoxicity, with no changes in plasma IFN-β levels and constant intensities of expression of CD11a, CD18 and CD62L on CD56^{bright} NK cells from PRE to END are difficult to reconcile with an increased concentration of IL-2. Moreover, Rhind et al. showed that phytohemagglutinin stimulated production of IL-2 by PBMC was unchanged by 12-weeks of moderate endurance training (32). The expression of CD18 and CD44 are also modulated by catecholamines (26); there was no changes in resting adrenaline or noradrenaline concentrations, but conceivably concentrations were increased repeatedly

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during the bouts of volleyball drill, and this may have affected the expression of adhesion molecules.

CD62L is an l-selectin and is another important molecule for leukocyte-endothelial cell adhesion and subsequent responses (10). CD62L is expressed on the surfaces of B cells, T cells, monocytes, granulocytes and some NK cells (41). Both CD44 and CD62L molecules mediate the initial binding of leukocytes to endothelial cells at an inflammatory site (16). As exercise decreased the expression of these molecules (12, 13, 19, 25, 43), there was a preferential increase of CD62L negative NK cells in the circulation during exercise (12) and psychological stress (8). Our results showed selective increases in CD62L positive $CD56^{bright}$ NK cells at the end of training. Frey et al. reported that CD62L expression was modulated by various cytokines, including IL-2, IL-10, IL-12, IL-15, IFN-β and transforming growth factor-β (10). They also reported that IL-12 caused a marked increase

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in CD62L expression on both CD56^{dim} and CD56^{bright} cells, and increased the frequency of CD62L positive cells.

Cell maturation is another possible cause of altered adhesion molecule expression (34). CD62L may be shed from T cells when they assume their activated memory phenotype (46). Chronic exercise may facilitate turnover and cell generation, with the appearance of more immature cells. However, the development from CD56^{bright} NK cell to CD56^{dim} NK cells is probably not an important factor, because CD56^{dim} NK cells have never been generated from CD56^{bright} NK cells in vitro (5). Additionally, circulating leukocytes represent only a very small fraction (1-2%) of total leukocytes. Therefore, a selective circulatory recruitment of CD56^{bright} NK cells may occur, depending on changes in the internal environment.

Conclusions

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One month of intensive, competitive sports training decreases both total NK cell cytotoxicity and lytic units per NK cell, with no change in the numbers of $CD56^{dim}$ NK cells, but an increase in numbers of the $CD56^{bright}$ NK cell and $CD56^{dim}$ T cell subsets. The increased proportion of cells with a low cytotoxicity partially accounts for the observed decreases in total cytolytic activity and lytic units per NK cell. These results might affect the immune response of athletes. However, changes in adhesion molecule expression did not lead to selective increases in $CD56^{bright}$ NK cells. Further research is needed to clarify the mechanisms of NK cell subset redistribution and changes of subset cytotoxicity in response to chronic exercise.

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