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

Masatoshi Suzui,\textsuperscript{1} Takeshi Kawai,\textsuperscript{2} Hiroko Kimura,\textsuperscript{3} Kazuyoshi Takeda,\textsuperscript{3} Hideo Yagita,\textsuperscript{3} Ko Okumura,\textsuperscript{3} Pang N. Shek,\textsuperscript{5,6} and Roy J. Shephard\textsuperscript{6}

\textsuperscript{1}School of Business Administration, Meiji University, Suginami, Tokyo 168-8555; \textsuperscript{2}School of Health and Sports Science, Juntendo University, Inba, Chiba 270-1695; \textsuperscript{3}School of Medicine, Juntendo University, Bunkyo, Tokyo, Japan 113-8421; \textsuperscript{4}Defence Research and Development Canada-Toronto, Toronto M3M 3B9; \textsuperscript{5}Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto M5G 1L5; and \textsuperscript{6}Faculty of Physical Education & Health, Department of Public Health Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Submitted 14 May 2003; accepted in final form 27 January 2004

Natural killer cell lytic activity and CD56\textsuperscript{dim} and CD56\textsuperscript{bright} cell distributions during and after intensive training. \textit{J Appl Physiol} 96: 2167–2173, 2004. First published January 29, 2004; 10.1152/japplphysiol.00513.2003.—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 1 mo of heavy preseason season. Volleyball drills were performed 5 h/day, 6 days/wk. Morning resting blood samples were collected before training (Pre), on the 10th day of training (During), 1 day before the end of training (End), and 1 wk after intensive training had ceased (Post). CD3\textsuperscript{+}CD16\textsuperscript{bright}CD56\textsuperscript{dim} (CD56\textsuperscript{dim} NK), CD3\textsuperscript{+}CD16\textsuperscript{dim}/−CD56\textsuperscript{bright} NK (CD56\textsuperscript{bright} NK), and CD3\textsuperscript{+}CD16\textsuperscript{bright}CD56\textsuperscript{dim} (CD56\textsuperscript{dim} T) cells in peripheral blood were determined by flow cytometry. The circulating count of CD56\textsuperscript{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\textsuperscript{bright} NK and CD56\textsuperscript{dim} T cells (subsets with a lower cytotoxicity) increased significantly (P < 0.01) in response to the heavy training. Overall 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-γ, and tumor necrosis factor-α 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.

catecholamines; creatine kinase; CD44; CD62l; cytokines

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 that is most responsive to physiological and psychological stress (15, 38). The effects of acute exercise on NK cells are already well documented (31, 38); cytotoxic activity increases during exercise but decreases after 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 and coworkers 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 intergroup differences in NK cell count. Such results suggest that chronic exercise 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. (44) reported that 15 wk of training at 75–85% maximal O\textsubscript{2} uptake decreased NK cell cytolytic activity without altering NK cell counts. 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 after very heavy exercise (31).

NK cells can be divided into two subsets, on the basis of their cell surface density of CD56 (5). The majority of NK cells are CD56\textsuperscript{dim} cells, but ~10% of NK cells are CD56\textsuperscript{bright} cells. The CD56\textsuperscript{dim} NK cells have a greater cytolytic activity (21) and express higher levels of CD16 than the CD56\textsuperscript{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

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 agreement for reprint requests and other correspondence: M. Suzui, Meiji Univ., 1-9-1 Eifuku, Suginami, Tokyo, Japan, 168-8555 (E-mail suzui@isc.meiji.ac.jp).

http://www.jap.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Copyright © 2004 the American Physiological Society
form after 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 (means ± SE) yr, height 1.69 ± 0.01 and 1.59 ± 0.01 m, body mass 52.6 ± 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; resting systolic blood pressure 112 ± 4 and 109 ± 1.6 mmHg; and resting diastolic blood pressure 70 ± 3 and 70 ± 2 mmHg.

**Experimental design.** Subjects in the training group undertook 1 mo of heavy preseason training. Volleyball drills were performed 5 h/day, 6 days/wk. Control subjects were instructed to avoid vigorous exercise during this period. Four blood samples were collected before training (Pre), on the 10th day of training (During), 1 day before the end of training (End), and 1 wk after training (Post). On each occasion, subjects came to the laboratory at 0800, after an overnight fast. After 30 min of rest, we measured heart rate (Polar Vantage heart rate monitor), blood pressure (hemodynamometer, HEM-906, OMRON) and the percentage of body fat (an electrical impedance technique, TBF-102, Tanita). Then 20 ml of blood were drawn from the median antecubital 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 EDTA-treated blood, by use of an automated hematology analyzer (Sysmex NE8000, Toa Medical Electronics, Kobe, Japan).

**NK cell subsets.** Peripheral blood mononuclear cells (PBMC) were separated from 10 ml of heparinized whole blood by density gradient separation from 10 ml of heparinized whole blood by density gradient centrifugation (30 min, 20°C, 400 g) using Separate-L (Muto Pure Chemical). They were then stored in RPMI-1640 (GIBCO, Invitrogen) with 10% fetal calf serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 4°C until analysis. NK cells were enumerated by three-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 × 10^6 of PBMC was mixed with saturating amounts of monoclonal antibody conjugated with FITC (CD16), PE (CD56), and PerCP (CD3). After 20 min of incubation on ice, the cells were washed twice with PBS and stored in the dark at 4°C until measurement. Stained cells were passed through a flow cytometer (FACScan, Becton Dickinson). Results were expressed as the percentage of cells in a gated lymphocyte region (CELLQuest 3.1, Becton Dickinson). NK cells were divided into two subsets, on the basis of the surface density of CD56. CD3^−CD16^{dim}/CD56^{dim} NK and CD3^−CD16^{dim}/CD56^{bright} NK (CD56^{bright} NK) cell subsets were distinguished by gated flow cytometric analyses (Fig. 1). CD3^−CD16^{bright}/CD56^{bright} (CD56^{bright} 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.

**Adhesion molecules on NK cells.** The fluorescence intensities of expression of adhesion molecules, such as lymphocyte function-associated antigen-1 (CD11a), β-2 integrin (CD18), homing cell adhesion molecule (CD44) and leukocyte endothelial cell adhesion molecule-1 (CD62L) on CD56^{dim} NK and CD56^{bright} NK cells were measured by flow cytometry. Samples of 1 × 10^6 of PBMC were stained with FITC (CD11a, CD18, CD44, or CD62L), PE (CD56), and PerCP (CD3). Monoclonal antibodies were obtained from PharMingen. The staining procedure was as described above. The mean fluorescence intensities of adhesion molecule expression in the NK cell subset regions (CD3^−CD56^{dim} and CD3^−CD56^{bright}) were analyzed via 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 nonradioactive 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 (50 mM HEPES, 93 mM NaCl, 5 mM KCl, 2 mM MgCl2). The K562 cells were then incubated in a labeling buffer (40 mM Eu, 125 mM diethylene-triaminepentaacetate, 250 mM dextran sulfate) for 20 min at 4°C. After labeling, the cells were washed seven times with buffer.

![Flow cytometric analysis of CD56^{dim} natural killer (NK) and CD56^{bright} NK cells and CD56^{dim} T cells. Left, lower box: CD56^{dim} NK cell subset. Left, upper box: CD56^{bright} NK cell subset. Right box: CD56^{dim} T cell subset. PE, phycoerythrin.](image-url)

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

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>During</th>
<th>End</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte, cells/µl</td>
<td>6,050±424</td>
<td>5,358±296</td>
<td>5,273±472</td>
<td>5,933±709</td>
</tr>
<tr>
<td>Control</td>
<td>5,025±393</td>
<td>5,015±418</td>
<td>4,874±317</td>
<td>4,984±437</td>
</tr>
<tr>
<td>Training</td>
<td>2,104±364</td>
<td>1,894±228</td>
<td>2,279±139</td>
<td>1,971±218</td>
</tr>
<tr>
<td>Lymphocyte, cells/µl</td>
<td>1,710±128</td>
<td>1,726±137</td>
<td>1,950±170</td>
<td>1,749±180</td>
</tr>
<tr>
<td>Control</td>
<td>422±49</td>
<td>284±61</td>
<td>332±70</td>
<td>336±52</td>
</tr>
<tr>
<td>Training</td>
<td>322±42</td>
<td>361±37</td>
<td>317±21</td>
<td>297±27</td>
</tr>
<tr>
<td>Monocyte, cells/µl</td>
<td>2,881±476</td>
<td>2,322±386</td>
<td>2,358±360</td>
<td>2,977±690</td>
</tr>
<tr>
<td>Control</td>
<td>2,772±251</td>
<td>2,726±259</td>
<td>2,284±128</td>
<td>2,623±312</td>
</tr>
<tr>
<td>Neutrophil, cells/µl</td>
<td>239±54</td>
<td>258±73</td>
<td>199±81</td>
<td>201±68</td>
</tr>
<tr>
<td>Control</td>
<td>173±49</td>
<td>172±52</td>
<td>213±50</td>
<td>229±75</td>
</tr>
<tr>
<td>Basophil, cells/µl</td>
<td>51±19</td>
<td>46±9</td>
<td>44±10</td>
<td>37±21</td>
</tr>
<tr>
<td>Training</td>
<td>48±5</td>
<td>29±13</td>
<td>110±61</td>
<td>86±49</td>
</tr>
</tbody>
</table>

*Table values are means ± SE. Pre, before training; During, 10th day of training; End, 1 day before the end of training; Post, 1 wk after intensive training had ceased. There were no statistically significant changes.*
NK cell cytotoxicity (%) = (Experimental Eu release − Spontaneous Eu release) × (Maximal Eu release − Spontaneous Eu release)^−1

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

Lytic Units (15%)-NK⁻¹×10⁻⁵ = Lytic Units×[%NK cells(1×10⁶ PBMC − Monocytes)]⁻¹

Plasma cytokine assays. Plasma concentrations of IL-6, IFN-γ, and TNF-α were determined on 0.5-ml samples of plasma by using a previously reported time-resolved fluoroimmunoassay (47). Monoclonal anti-IL-6, IFN-γ, and TNF-α (PharMingen) were diluted with 0.1 M carbonate-bicarbonate buffer (pH 9.3) to 2 μg/ml. The diluted antibody to each cytokine (200 μg × 100 ml⁻¹) was used to coat
Table 2. Changes in plasma IL-6, INF-γ and TNF-α levels during and post training

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>During</th>
<th>End</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/ml</td>
<td>0.28±0.17</td>
<td>0.79±0.46</td>
<td>0.47±0.21</td>
<td>0.38±0.20</td>
</tr>
<tr>
<td>INF-γ, pg/ml</td>
<td>34.9±21.1</td>
<td>59.0±20.2</td>
<td>37.8±20.1</td>
<td>9.5±6.5</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>11.0±0.8</td>
<td>12.1±0.7</td>
<td>13.2±0.8</td>
<td>13.6±0.8</td>
</tr>
</tbody>
</table>

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

...nephrine concentrations by high-performance liquid chromatography in sample volumes of plasma were used to determine plasma epinephrine and norepinephrine, norepinephrine, and cortisol determinations were drawn into a well of the microtiter plates (Fluoro Nunc module plate, Nalge Nunc International). After the wells were washed 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°C until use. Fifty microliters of diluted standard cytokine (recombinant human IL-6, human IFN-γ and human TNF-α) 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. Fifty microliters of the respective biotinylated antibody (1 µg/ml) were then added. After incubation for 1 h at room temperature, the wells were washed three times with Tris-HCl buffer containing Tween 20, and once with 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 CK analyses. Venous blood samples for epinephrine, norepinephrine, and cortisol determinations were drawn into a 5-ml vacutainer (Terumo, Tokyo, Japan) containing EDTA, and 1.5 ml of plasma were used to determine plasma epinephrine and norepinephrine concentrations by high-performance liquid chromatography (18). Plasma cortisol concentrations were determined radioimmunologically (INCSTAR), by using 0.2 ml of plasma. Plasma creatine kinase (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 <5% change in resting samples from untrained subjects over the normal storage period.

Data analysis. Results are expressed as means ± SE. Statistical analyses were performed using a StatView-J5.0 (SAS) microcomputer software package, with statistical significance set at P < 0.05. Possible interactions were analyzed using 2 (control and training groups) × 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 pretraining values.

RESULTS

Physiological changes. Neither group of subjects showed significant changes in resting heart rate, blood pressure, 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 (Table 1). The majority of NK cells were of the CD3−CD16brightCD56dim phenotype; circulating counts for this cell population remained unchanged throughout the experiment (Fig. 2). However, there were significant repeated-measures ANOVA interactions in CD56bright NK (P = 0.011) and CD56dim T cell (P = 0.021) counts. Relative to control subjects, the training group showed higher cell counts of CD56bright 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 (1-factor ANOVA, $P < 0.001$), with a subsequent return to normal levels. The CD56$^{\text{dim}}$ T cell counts were also increased at the end ($P = 0.005$) and posttraining ($P = 0.017$).

**NK cell cytotoxicity.** Total NK cell cytotoxicity decreased significantly by the end of training ($P = 0.002$), with recovery posttraining (Fig. 3). Lytic units per NK cell followed a similar pattern ($P = 0.008$).

**Plasma cytokine levels.** Circulating IL-6, IFN-$\gamma$, and TNF-$\alpha$ levels remained unchanged throughout (Table 2).

**Expression of adhesion molecules on CD56$^{\text{dim}}$ NK cells and CD56$^{\text{bright}}$ NK cells.** Intensities of expression of adhesion molecules differed between CD56$^{\text{dim}}$ NK cells and CD56$^{\text{bright}}$ NK cells (Fig. 4). Higher expressions of CD11a and CD18 were found in CD56$^{\text{dim}}$ NK cells. On the other hand, CD56$^{\text{bright}}$ 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$^{\text{dim}}$ or CD56$^{\text{bright}}$ NK cells throughout the experiments. In the training group, the expression of CD11a on CD56$^{\text{dim}}$ NK cells decreased significantly posttraining ($P = 0.008$), but there were no significant interactions between groups. The expression of CD11a on both cell subsets decreased posttraining (both $P < 0.001$). CD56$^{\text{dim}}$ NK cells and CD56$^{\text{bright}}$ NK cells also showed an increased expression of CD44. The higher expression of CD44 on CD56$^{\text{dim}}$ NK cells was seen in the training group during, at the end of, and after training with significant repeated-measures interactions ($P = 0.001$). The expression of CD44 on CD56$^{\text{bright}}$ NK cells also increased by the end of training (1-factor ANOVA, $P < 0.001$), with a return to initial values posttraining. The expression of CD62L remained unchanged on both CD56$^{\text{dim}}$ and CD56$^{\text{bright}}$ NK cells. The majority of NK cells did not express the CD62L molecule (Fig. 5). The CD56$^{\text{dim}}$ NK cells showed no change in circulating cell counts of CD62L-negative and CD62L-positive cells. In contrast, CD62L-negative CD56$^{\text{bright}}$ NK cells increased during training ($P = 0.003$) and CD62L-positive CD56$^{\text{bright}}$ NK cells had increased by the end of training ($P = 0.002$).

**Plasma concentrations of hormones and CK.** Circulating levels of epinephrine, norepinephrine, and cortisol remained unchanged (Fig. 6), but a significant increase of plasma CK concentration was found during ($P = 0.010$), at the end of ($P = 0.005$), and after training ($P = 0.017$) in the training group.

**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 pretraining cytotoxicities were restored within 1 wk. 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$^{\text{bright}}$ cells were precursors of CD56$^{\text{dim}}$ NK cells. However, more recent investigations have demonstrated that CD56$^{\text{bright}}$ NK cells are a functionally distinct subset of mature NK cells, which are primarily responsible for cytokine production in response to 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$^{\text{dim}}$ NK cell counts. As we had hypothesized, the numbers of the CD56$^{\text{bright}}$ NK cell subset increased in response to training, as did the CD56$^{\text{dim}}$ 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 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). IFN-$\gamma$, TNF-$\alpha$, 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 (48), $\beta$-endorphin (24), and prostaglandin $E_2$ (1) also modulate NK cell cytotoxicity. We found no changes in plasma IFN-$\gamma$, TNF-$\alpha$,
epinephrine, norepinephrine, or cortisol levels during the experiments. Nevertheless, other humoral factors, including the plasma glucose 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 after 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 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 CD56dim and CD56bright 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. (42) previously reported that the expression of CD44 on CD16+ cells increased after a 3-day incubation with recombinant IL-2, this treatment leading to decreased binding at the peripheral lymph nodes but increased adherence to the mucosal high endothelium. This seems to support the possibility of an association between an increased expression of CD44 and increased CD56bright 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-γ production in NK cells, increases the expression of CD11a/CD18 (33) on CD56+ cells, and decreases CD62L expression on CD56bright cells (10). However, the reduced cytotoxicity, with no changes in plasma IFN-γ levels and constant intensities of expression of CD11a, CD18, and CD62L on CD56bright NK cells from Pre to End, is difficult to reconcile with an increased concentration of IL-2. Moreover, Rhind et al. (32) showed that phytohemagglutinin stimulated production of IL-2 by PBMC was unchanged by 12-wk of moderate endurance training. The expression of CD18 and CD44 is also modulated by catecholamines (26); there were no changes in resting epinephrine or norepinephrine concentrations, but conceivably concentrations were increased repeatedly 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 CD56bright NK cells at the end of training. Frey et al. (10) reported that CD62L expression was modulated by various cytokines, including IL-2, IL-10, IL-12, IL-15, IFN-γ, and transforming growth factor-β. They also reported that IL-12 caused a marked increase in CD62L expression on both CD56dim and CD56bright 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 CD56bright NK cell to CD56dim NK cells is probably not an important factor, because CD56dim NK cells have never been generated from CD56bright 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 CD56bright NK cells may occur, depending on changes in the internal environment.

In conclusion, 1 mo of intensive, competitive sports training decreases both total NK cell cytotoxicity and lytic units per NK cell, with no change in the numbers of CD56dim NK cells but an increase in numbers of the CD56bright NK cell and CD56dim 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 CD56bright 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.

ACKNOWLEDGMENTS

We especially thank the late Dr. Fumiko Nagao for expert techniques throughout the experimental procedure and the constructive suggestions. We also gratefully acknowledge the assistance of Hatsue Takahashi, Health Care Center, Juntendo University.

GRANTS

This study was supported by the Grant-in-Aid for Scientific Research, Japan Society for the Promotion of Science, No. 11680058.

REFERENCES


