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Exercise-induced change in type 1 cytokine-producing CD8$^+$ T cells is related to a decrease in memory T cells

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Ibfelt, Tobias, Emil Wolsk Petersen, Helle Bruunsgaard, Marie Sandmand, and Bente Klarlund Pedersen. Exercise-induced change in type 1 cytokine-producing CD8$^+$ T cells is related to a decrease in memory T cells. J Appl Physiol 93: 645–648, 2002. First published May 3, 2002; 10.1152/japplphysiol.01214.2001.—In response to exercise, both CD4$^+$ and CD8$^+$ T cells are mobilized to the blood, but the levels of these cells decline below preexercise values in the postexercise period. T cells are functionally polarized, depending on the cytokines they produce. Type 1 cells produce, e.g., interferon (INF)-γ, whereas type 2 produce, e.g., interleukin (IL)-4. It was recently demonstrated that exercise induces a decrease in the percentage of type 1 T cells. The present study further investigated the mechanisms underlying the exercise-induced shift in the balance between type 1 and type 2 cytokine-producing cells. Seven healthy men performed 1.5 h of treadmill running with blood samples drawn before exercise, at the end of exercise, and 2 h after exercise. Intracellular expression of IFN-γ, IL-2, and IL-4 was detected in CD4$^+$ and CD8$^+$ T cells after stimulation with phorbol 12-myristate 13-acetate and ionomycin. Intracellular expression of IFN-γ within CD8$^+$ cells was decreased in the postexercise period compared with values obtained immediately after exercise, whereas the expression of IL-2 and IL-4 did not change within the CD4$^+$ and CD8$^+$ cell populations. The decrease in IFN-γ-producing CD8$^+$ T cells postexercise was negatively correlated with a decrease in percentage of memory T cells within the CD8$^+$ cells ($r = -0.94; P \leq 0.002$). In conclusion, this study demonstrates that the exercise-induced change in type 1 cytokine-producing T cells is related to a decline in memory cells.

Intracellular cytokines; type 1/type 2; memory cells

STRENУOUS EXERCISE INDUCES recruitment of lymphocytes to the circulation, whereas the lymphocyte concentration declines below the preexercise value in the postexercise period (13, 14). It has been shown that CD8$^+$ T cells are mobilized more than CD4$^+$ T cells (14). Furthermore, recent studies suggest that more memory T cells (CD45RO$^+$), relative to naive T cells (CD45RA$^+$CD62L$^+$), are recruited to the blood (2, 3). Considerable evidence has accumulated to suggest the existence of functionally polarized responses by both CD4$^+$ and CD8$^+$ T cells that depend on the cytokines they produce (16, 17). Cells with a type 1 cytokine profile produce, e.g., interferon (IFN)-γ, whereas cells with a type 2 cytokine profile produce, e.g., interleukin (IL)-4 (15). Production of other cytokines is not as tightly restricted to a single subset in humans, although IL-2 is normally classified as a type 1 cytokine (12). The balance between type 1 and type 2 cytokines is important in the protection against both intra- and extracellular infections (16, 17). Type 1 cytokines provide protection mainly against intracellular infections (13), whereas the percentage of IL-4-producing T cells does not change. However, the mechanisms responsible for the exercise-induced shift in the type 1/type 2 cytokine profile balance are not known. However, a strong link between CD45RO$^+$ expression and IFN-γ-producing T cells has previously been demonstrated (4). Furthermore, the CD45RO$^+$ T-cell subpopulation is highly sensitive to exercise stimuli (2, 3). Thus we hypothesized that the exercise-induced decline in IFN-γ-producing T cells was related to a decline in CD45RO expression. Flow cytometry with intracellular cytokine staining was used because this method has the advantage of determining type 1/type 2 cytokine balance on a single-cell level.

MATERIALS AND METHODS

The exercise consisted of 1.5 h of 5% downhill running at 75% of maximum oxygen consumption ($V_{O2, max}$). Exercise was performed on a running belt with 5% downhill incline (Technogym, Cesena, Italy). $V_{O2, max}$ and maximum heart rate (HR) were obtained in a test performed at least 7 days preexercise. To ensure easy water intake throughout the exercise, the speed was reduced, corresponding to 50% of $V_{O2, max}$ and maximum heart rate (HR) were obtained in a test performed at least 7 days preexercise. To ensure easy water intake throughout the exercise, the speed was reduced, corresponding to 50% of $V_{O2, max}$ for 3 min every 30 min. HR and $V_{O2, max}$ were monitored throughout the exercise (Polar Vantage TV and MedGraphics CPF-S and CPX). Blood samples were all collected from an antecubital vein at the following time points: $T = 0$ h (exercise start), $T = 1.5$ h (end of exercise), and $T =$...
4 h (2 h postexercise). Venous blood (20 ml) was sampled into heparinized (25 IU heparin/ml blood) blood containers (Becton Dickinson, San Jose, CA). The blood mononuclear cells (BMNC) were isolated by density gradient centrifugation (Lymphoprep, Nyegaard, Oslo, Norway) on LeuconSep tubes (Greiner, Frickenhausen, Germany). The BMNC were then frozen in freezing medium (50% medium 1640 RPMI, 30% normal human serum, and 20% DMSO) and stored in liquid nitrogen until thawed for analysis. The samples were subsequently processed by flow cytometry by using a fluorescence-activated cell sorter (Epics XL-MCL, Coulter). All of the flow cytometry data analysis was made by using Winlist 3D version 4.0 software (Verity Software House). The study was approved by the ethics committee of the Copenhagen and Frederiksberg communities.

Subjects. Seven healthy men, aged 28 yr (mean) (range 20–39 yr) with an average \( \text{VO}_2 \text{max} \) of 5.1 l/min (range 3.7–5.6 l/min), participated in the study. The subjects’ maximum HR was an average of 193 beats/min (range 181–207 beats/min).

Measurement of intracellular cytokines. The frozen cells were washed twice in RPMI, and the BMNC concentration was set to 10⁹ cells/l. The cells were then stimulated for 4 h with phorbol 12-myristate 13-acetate (1 µg/ml), monensin (0.2 µmol/l), and ionomycin (0.1 µmol/l). The cells were harvested, and the following anti-human antibodies were added for surface cytokine detection: carboxyvin-5-conjugated anti-CD8 (clone D525) and mouse anti-IgG1 (DAKO, Glostrup, Denmark); and phycoerythrin (PE) Texas red ethyl cytosine dimer-conjugated anti-CD3 (clone UCHT1) and mouse anti-IgG1 (Immunotech, Marseille, France). The cells were then fixed with a 4% formaldehyde buffer solution (Lilies Liquid; Bie & Bernsten, Redovre, Denmark) and then permeabilized with a buffer solution containing 0.1 g saponine, 100 ml PBS, and 1 ml FCS. The following anti-human antibodies were added for intracellular cytokine detection: FITC-conjugated anti-IL-4 (clone MP4-25D2) and rat anti-IgG1; and PE-conjugated anti-IL-2 (clone MQ1-1712), anti-INF-γ (clone 4S.B3), rat anti-IgG2a, and mouse anti-IgG1 (Becton Dickinson). The cells were then analyzed by a flow cytometer (Epics XL-MCL).

Lymphocyte subpopulations. The following anti-human antibodies were used to detect surface cell markers: FITC-conjugated antibodies included mouse anti-IgG1, mouse anti-IgG2a, anti-CD45 (clone T29/33), and anti-CD45RO (clone UCHL1) from DAKO; and anti-CD16 (clone NKP15) and anti-CD45RA (clone L48) from Becton Dickinson. R-PE conjugates included mouse anti-IgG1 and anti-CD14 (clone TuK4) which were both from DAKO; and anti-CD56 (clone MY51) and anti-CD62L (clone SK11) from Becton Dickinson. Finally, anti-CD3 (clone SK7), anti-CD4 (clone SK3), and anti-CD8 (clone SK1) were conjugated with peridinin chlorophyll protein, which were all supplied from Becton Dickinson. Mouse IgG1 and IgG2a served as controls.

Tubes containing antibody were each filled with 10⁹ cells suspended in 100 µl PBS (J. T. Baker, Deventer, Holland) with 3% FCS (GIBCO). The samples were incubated for 30 min at 4°C and then washed twice in the above-mentioned solution. After being resuspended in 300 µl PBS, the samples were subsequently processed by flow cytometry with a fluorescence-activated cell sorter (Epics XL-MCL). Winlist 3D version 4.0 software (Verity Software House) was used to analyze the data.

RESULTS

The lymphocyte concentration was increased at the end of exercise (twofold), whereas the neutrophil count increased during exercise (twofold) and continued to increase after exercise (fourfold) (Table 1). The CD3⁺ T-cell concentration rose at the end of exercise and then returned to baseline levels 2 h postexercise. The CD4⁺ and the CD8⁺ lymphocytes followed the same pattern. At the end of exercise, the CD4⁺ and CD8⁺ populations increased and then returned to baseline levels 2 h after exercise. Cells expressing CD45RO showed an increase within both CD4⁺ and CD8⁺ cells but only significantly within those cells not coexpressing CD62L (CD45RO⁺CD62L⁻). The true naïve (CD45RA⁺CD62L⁺) and the CD45RA⁺CD62L⁻ subset did not change. The natural killer cell subpopulations (CD3⁺CD16⁻CD56⁺, CD3⁻CD16⁺CD56⁻, CD3⁻CD16⁻CD56⁺) were all increased in response to exercise and then returned to baseline values 2 h postexercise (except for the CD3⁻CD16⁺CD56⁺ population, which decreased significantly compared with preexercise values).

Table 1. Effect of exercise on the lymphocyte subpopulations

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>T = 0 h</th>
<th>T = 1.5 h</th>
<th>T = 2 h Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>2.20(1.70–2.75)</td>
<td>5.31(4.37–6.35)</td>
<td>9.76(8.9–21)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.60(1.40–2.05)</td>
<td>3.14(2.51–4.42)</td>
<td>1.37(1.01–1.56)</td>
</tr>
<tr>
<td>CD3⁺</td>
<td>0.91(0.71–1.34)</td>
<td>1.25(1.18–2.23)</td>
<td>0.87(0.57–1.01)</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>0.55(0.43–0.85)</td>
<td>0.78(0.58–1.12)</td>
<td>0.55(0.30–0.66)</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>0.25(0.22–0.36)</td>
<td>0.41(0.36–0.81)</td>
<td>0.24(0.17–0.27)</td>
</tr>
<tr>
<td>CD4⁺CD56RA⁻CD62L⁻</td>
<td>0.13(0.05–0.16)</td>
<td>0.20(0.08–0.26)</td>
<td>0.11(0.03–0.12)</td>
</tr>
<tr>
<td>CD4⁺CD56RA⁻CD62L⁺</td>
<td>0.07(0.01–0.09)</td>
<td>0.08(0.03–0.14)</td>
<td>0.04(0.02–0.11)</td>
</tr>
<tr>
<td>CD4⁺CD5RA⁻CD62L⁻</td>
<td>0.09(0.01–0.30)</td>
<td>0.14(0.03–0.40)</td>
<td>0.08(0.01–0.16)</td>
</tr>
<tr>
<td>CD4⁺CD5RA⁻CD62L⁺</td>
<td>0.12(0.05–0.18)</td>
<td>0.16(0.06–0.21)</td>
<td>0.12(0.06–0.19)</td>
</tr>
<tr>
<td>CD8⁺CD56RA⁻CD62L⁻</td>
<td>0.04(0.04–0.05)</td>
<td>0.10(0.08–0.15)</td>
<td>0.03(0.03–0.03)</td>
</tr>
<tr>
<td>CD8⁺CD56RA⁻CD62L⁺</td>
<td>0.86(0.29–1.80)</td>
<td>1.12(0.55–3.50)</td>
<td>0.36(0.23–1.64)</td>
</tr>
<tr>
<td>CD8⁺CD5RA⁻CD62L⁻</td>
<td>0.11(0.05–0.20)</td>
<td>0.24(0.11–0.38)</td>
<td>0.10(0.03–0.16)</td>
</tr>
<tr>
<td>CD8⁺CD5RA⁻CD62L⁺</td>
<td>0.02(0.01–0.12)</td>
<td>0.08(0.08–0.15)</td>
<td>0.05(0.02–0.15)</td>
</tr>
<tr>
<td>CD3⁻CD16⁺CD56⁺</td>
<td>0.02(0.02–0.02)</td>
<td>0.07(0.05–0.07)</td>
<td>0.01(0.01–0.01)</td>
</tr>
<tr>
<td>CD3⁻CD16⁺CD56⁻</td>
<td>0.10(0.09–0.13)</td>
<td>0.53(0.41–0.57)</td>
<td>0.03(0.03–0.04)</td>
</tr>
<tr>
<td>CD3⁻CD16⁻CD56⁺</td>
<td>0.03(0.02–0.03)</td>
<td>0.08(0.04–0.11)</td>
<td>0.01(0.01–0.02)</td>
</tr>
</tbody>
</table>

Values are medians with quartiles in parentheses. Concentrations are expressed as 10⁹ cells/l; n = 7 subjects. Lymphocyte subpopulations are shown before, at the end of 1.5 h of treadmill running, and 2 h postexercise. Significantly different from T = 0 h: *P < 0.05, †P ≤ 0.01, and §P ≤ 0.001.

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The percentage of cells expressing IFN-γ was significantly decreased 2 h postexercise within both CD4⁺ and CD8⁺ T cells compared with values just after exercise (Fig. 1). Intracellular expression of IL-2 showed no significant change within CD4⁺ or CD8⁺ cells compared with preexercise values (Fig. 2). Neither did the IL-4 expression change in the CD4⁺ or CD8⁺ population (Fig. 3). The IFN-γ-to-IL-4 ratio was calculated as an expression of T helper type 1/T helper type 2 balance (Fig. 4). The T helper type 1-to-T helper type 2 ratio declined in the postexercise period. The decrease in expression of IFN-γ was strongly negatively correlated to the increase in percentage of memory cells defined as T cells, excluding true naïve cells ($r = -0.94; P = 0.002$), but only within the CD8⁺ cell population.

**DISCUSSION**

The major finding in the present study was that exercise induces a shift in the balance between type 1 and type 2 cytokine-producing CD8⁺ T cells. In the present study, a decline in IFN-γ-producing CD8⁺ cells was found in the postexercise period compared with values obtained immediately after exercise, whereas this decline was not significantly different from preexercise values. Other studies have demonstrated a true postexercise decline in IFN-γ-producing CD8⁺ T cells (19, 20), and Baum et al. (1) found a decrease in IFN-γ in whole blood culture supernatants after strenuous exercise. The decrease in IFN-γ-producing CD8⁺ T cells was associated with a decrease in the percentage of CD8⁺ memory T cells. In general, exercise influences CD8⁺ cells more than CD4⁺ cells because of the fact that the exercise-induced redistribution of lymphocytes is mediated by epinephrine, and CD8⁺ T cells have more β₂-adrenergic receptors on the surface compared with CD4⁺ cells (14). The finding of a correlational relationship between IFN-γ-producing cells and memory cells is in accordance with studies demonstrating a progressive increase in IFN-γ-producing T cells in aging with a strong correlation to CD45RO expression (4, 9). Epinephrine suppresses the type 1 T cells, both at the level of antigen-presenting cells and directly on T-cell receptors (11, 18). In accordance with this, Steensberg et al. (20) found an inverse relationship between epinephrine level and percentage of circulat-
ing CD8+ T cells producing IL-2, indicating that adrenergic stimulation might play a mechanistic role. In contrast, Starkie et al. (19) demonstrated that β-adrenoceptor blockade did not influence exercise-induced suppression of cytokine production. Glucocorticoids are powerful stimulators of type 2 outcomes and powerful inhibitors of type 1 outcomes, directly influencing IL-4 and IL-10 production from lymphocytes (18). However, in a previous study, our laboratory found no correlation between plasma cortisol levels and type 1/type 2 cells in relation to exercise (20).

In conclusion, the present study adds to two previous studies (19, 20), demonstrating a postexercise decline in the circulating number of INF-γ-producing CD8+ T cells by sharing a tight correlation between the latter cells and the number of CD8+ memory cells. Thus this study suggests an overlap between these two subpopulations. The present study did not allow us to determine whether the low number of IFN-γ-producing/memory CD8+ T cells was due to apoptosis of these cells or redistribution of mature T cells to other compartments. The decrease in type 1 cytokine-producing T cells results in a shift in the type 1/type 2 cytokine balance toward a relative type 2 cytokine profile dominance. However, because the number of type 2 cytokine-producing T cells did not change, it is not likely that strenuous exercise thereby predisposes to type 2 cytokine-related diseases, such as increased risk of infections by extracellular microorganisms (17, 20).

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REFERENCES