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Strenuous exercise decreases the percentage of type 1 T cells in the circulation

ADAM STEENSBERG,1,2 ANDERS DYHR TOFT,1,2 HELLE BRUUNSGAARD,1 MARIE SANDMAND,1 JENS HALKKJÆR-KRISTENSEN,3 AND BENTE KLARLUND PEDERSEN1,2

1Department of Infectious Diseases, 2Copenhagen Muscle Research Centre, and 3Department of Orthopedic Medicine and Rehabilitation, Rigshospitalet, University of Copenhagen, DK-2100 Copenhagen, Denmark

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Strenuous exercise decreases the percentage of type 1 T cells in the circulation. J Appl Physiol 91: 1708–1712, 2001.—Prolonged strenuous exercise is followed by a temporary functional immune impairment. Low numbers of CD4⁺ T helper (Th) and CD8⁺ T cytotoxic (Tc) cells are found in the circulation. These cells can be divided according to their cytokine profile into type 1 (Th1 and Tc1), which produce interferon-γ and interleukin (IL)-2, and type 2 (Th2 and Tc2) cells, which produce IL-4. The question addressed in the present study was whether exercise affected the relative balance between the circulating levels of these cytokine-producing T cells. Nine male runners performed treadmill running for 2.5 h at 75% of maximal oxygen consumption. The intracellular expression of cytokines was detected following stimulation with ionomycin and phorbol 12-myristate 13-acetate in blood obtained before, during, and after exercise. The percentage of type 1 T cells in the circulation was suppressed at the end of exercise and 2 h after exercise, whereas no changes were found in the percentage of type 2 T cells. Plasma epinephrine correlated negatively with the percentage of circulating CD8⁺ T cells producing IL-2, whereas peak IL-6 correlated with the percentage of CD8⁺ IL-4-producing T cells in the circulation. Peak plasma IL-6 correlated with plasma cortisol postrunning. In conclusion, the postexercise decrease in T lymphocyte number is accompanied by a more pronounced decrease in type 1 T cells, which may be linked to high plasma epinephrine. Furthermore, IL-6 may stimulate type 2 T cells, thereby maintaining a relatively unaltered percentage of these cells in the circulation compared with total circulating lymphocyte number.

interleukin-4; interleukin-2; interleukin-6; interleukin-12; interferon-γ

PROLONGED STRENUEOUS EXERCISE is followed by a temporary functional immune impairment (10) and an increased sensitivity to upper respiratory tract infections (URTI) (8). In the postexercise period, the number of circulating T lymphocytes is low (10). However, it is not known whether all T lymphocyte subsets are affected to the same extent. The CD4⁺ T helper (Th) and the CD8⁺ T cytotoxic (Tc) cells can be divided into type 1 (Th1 and Tc1) and type 2 (Th2 and Tc2) cells according to their cytokine profile. Type 1 T cells produce interferon (IFN)-γ and interleukin (IL)-2, whereas type 2 T cells produce IL-4, IL-5, IL-6, and IL-10 (6). Type 1 T cell responses are stimulated by IL-12 (6) and have been shown to protect against intracellular pathogens such as several viruses (5). IL-6 has been shown to induce Th2 polarization by stimulating the initial production of IL-4. Type 2 T cells are important in the defense against extracellular parasites such as several helminths and schistosomiasis (5). Studies suggest that both cortisol and epinephrine inhibit the production of type 1 T cell cytokines (3, 4). However, little is known about the physiological regulation of type 1 and type 2 T cell balances. Exercise has been accepted as a model to study interactions between endocrine and immune systems during physical stress (10). Intense and prolonged exercise induces significant changes in a number of immune parameters (10). Thus, during exercise, lymphocytes are recruited to the blood. However, in the postexercise period, the number of circulating lymphocytes declines below preexercise values. Concomitantly, the plasma levels of proinflammatory cytokines are elevated. Thus the level of plasma IL-6 may be enhanced more than 100-fold (9), and plasma IL-12 has been demonstrated to increase (1). To understand how the type 1 and type 2 T cell balances are regulated, it is of interest to investigate the effect of exercise. The observation of an increased risk of URTI after an acute bout of heavy exercise may be a consequence of an impaired type 1 T cell response. Viruses often cause URTI, and type 1 T cells are crucial in the defense against intracellular pathogens.

The hypothesis tested in this study is whether changes in stress hormones and cytokines during prolonged strenuous exercise induce a suppression of type 1 T cells compared with type 2 T cells. Thus the present
study investigates how 2.5 h of treadmill running affects the number of circulating type 1 and type 2 T cells using flow cytometry. This method allows detection of intracellular cytokines within CD4^+ and CD8^+ T cells (14). Furthermore, a possible relation between IL-6, IL-12, and stress hormones on one hand and circulating type 1 and type 2 T cells on the other is investigated by using correlations.

METHODS

Subjects. Nine endurance-trained male runners aged 25–50 yr (median = 30 yr) with a maximal pulmonary oxygen consumption (\(\dot{V}O_2\) max) of 3.61–5.20 l/min (median = 4.48 l/min), corresponding to 52.2–68.3 ml·kg\(^{-1}\)·min\(^{-1}\) (median = 60.1 ml·kg\(^{-1}\)·min\(^{-1}\)), were included. The subjects did not take any medication. The study was approved by the local ethical committee for Copenhagen and Frederiksberg Communities (no. 01-111/97). Subjects were informed of the risks of the experiment before their voluntary written consent was obtained.

Exercise protocol. For each subject, \(\dot{V}O_2\) max was determined approximately 1 wk before the experiment by an incremental exercise test on the same treadmill (Technogym, HC1200) and CPX express (MedGraphics) as used in the experiment. In the experiment, subjects ran for 2.5 h at a speed determined in the \(\dot{V}O_2\) max test to give an oxygen consumption of 75% \(\dot{V}O_2\) max. Actual oxygen consumption was sampled during the experiment as a control.

Experimental protocol. At 8:00 AM, subjects reported to the laboratory after an overnight fast, during which they were allowed to drink water ad libitum. They were instructed to arrive well rested and to abstain from any extraordinary training in the week before and no training at all for 2 days before the experiment. Blood samples were drawn from the antecubital vein of both arms. Blood was obtained before, after 0.5 and 1.5 h of running, and at the end of running (2.5 h). When sampling was done during exercise, the speed of the treadmill was lowered to walking speed (average duration = 3 min (range = 2–4 min)). For the next 2 h, the subjects stayed at the laboratory, and blood was sampled at 0.5, 1, 1.5, and 2 h postrunning. The next day, subjects reported back to the laboratory for the last blood sample. In the recovery period, subjects were allowed to eat and drink.

Lymphocyte number. This measurement was performed at the Central Laboratory, University Hospital of Copenhagen, Rigshospitalet, using standard laboratory procedures.

Isolation of blood mononuclear cells. Blood mononuclear cells (BMNC) were isolated by density-gradient centrifugation (Lymphoprep Nyegaard, Oslo, Norway) on LeucoSep tubes (Greiner, Freikhausen, Germany) and washed three times in medium RPMI. Analyses were performed on nonfrozen cells.

Flow cytometry analyses of intracellular cytokines. We stimulated 10^6 BMNC with 20 µl of ionomycin (0.1 µmol/l) and 50 µl of phorbol 12-myristate 13-acetate (1 µg/ml) for 4 h at 37°C in the presence of 10 µl of monensin (0.2 µmol/l). Stimulated cells were harvested, washed in staining buffer, and incubated with surface antibodies for 20 min at 4°C. After wash and fixation in 4% paraformaldehyde for 10 min in a dark place, cells were spun down and resuspended in PBS with 1% FCS. The next day, cells were incubated with 200 µl of saponin buffer (0.1 g of saponin in 100 ml PBS) for 10 min at room temperature to make the cell membranes permeable. The cytokine antibodies were then added and incubated in the presence of saponin buffer for 30 min at 4°C and subsequently washed twice in saponin buffer. Labeled cells were analyzed by flow cytometry using a fluorescence-activated cell sorter analyzer (Epics XL-MCL, Coulter, Miami, FL). The data were analyzed using WinList PC software (Verity Software House). Dead cells, platelets, and monocytes were excluded by forward and side-scattered light angle. Monocytes were also excluded by being CD14^+ CD3^- and CD8^- cells were accepted as CD4^-Th cells because stimulation with phorbol 12-myristate 13-acetate induces downregulation of the CD4 receptor (11). The following antibodies were used: FITC-conjugated CD45 (clone T29/31, DAKO), rlgG1 FITC (clone MOPC-21, PharMingen), tumor necrosis factor-α FITC (clone Mab11, PharMingen), rlgG1 FITC (clone R3-34, PharMingen), IL-4 FITC (clone MP4-25D2, PharMingen), R-phycocerythrin (PE)-conjugated CD14 (clone TUK4), rlgG2a PE (clone R35–95, PharMingen), IL-2 PE (clone MQ1-17H12, PharMingen), rlgG1 (clone TC647, DAKO), and IFN-γ PE (clone 4S.B3, PharMingen). PE covalently linked to Texas red ethyl cytochrome dimer (ECD)-conjugated IgG1 (clone 679.1 MC7 Coulter) and CD3 ECD (clone UCHT1, Coulter). PE covalently linked to Cy5 (Cy5)-conjugated IgG1 (clone DAK-G01, DAKO) and CD8 Cy5 (clone DK25, DAKO).

Extracellular cytokine measurements. Blood samples for cytokine measurements (IL-6, IL-12) were drawn into precooled glass tubes containing EDTA. The tubes were spun immediately at 2,200 g for 15 min at 4°C. The plasma was stored at −80°C until analyses were performed. Enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN) were used. According to R&D Systems, the IL-6 ELISA kit is insensitive to the addition of the recombinant forms of the soluble receptor (sIL-6R), and the measurements, therefore, correspond to both soluble and receptor-bound cytokine. The intra-assay coefficient of variation (CV) for the IL-6 kit was 5.9%.

Measurements of epinephrine. Blood samples for measurements of epinephrine were drawn into ice-cold glass tubes containing glutathione (1.3 mg/ml blood) and EGTA (1.5 mg/ml blood) with a pH of 6–7 and spun immediately. Plasma was stored at −80°C until analyzed by high-performance liquid chromatography (Hewlett-Packard HPLC, Waldbronn, Germany) with electrochemical detection.

Measurements of cortisol. Blood samples for cortisol measurement were drawn into precooled glass tubes containing EDTA. The tubes were spun immediately at 2,200 g for 15 min at 4°C. The plasma was stored at −80°C until analyses were performed. For cortisol measurement, ELISA kits from Diagnostic Laboratories (Webster, TX) were used (DSL-10-2000). The intra-assay CV was 5%.

Statistics. None of the intracellular cytokine data were distributed normally; therefore, these data are presented as medians and quartiles. Changes over time were tested using a nonparametric Friedman test; if this was significant, a pairwise comparison was done using a nonparametric Wilcoxon test.

Data showing a normal distribution, i.e., lymphocyte concentrations and log plasma IL-6, log plasma epinephrine, and log plasma cortisol, are shown as means and SE. The normally distributed data were tested for changes over time using repeated-measurements ANOVA. Pairwise comparisons were done using a paired t-test with Bonferroni correction. A Spearman rank data correlation analysis was performed to test for correlations. \(P < 0.05\) was accepted as significant.
RESULTS

The median running speed was 12.6 km/h (range = 10–15.1 km/h) at 75 ± 1% (SE) \( \text{VO}_{2} \) max. The total number of circulating lymphocytes, \( \text{CD}^{+} \), and \( \text{CD}^{8+} \) T cells increased in response to exercise and declined below preexercise values in the recovery period (Fig. 1). The percentage of circulating \( \text{CD}^{+} \) and \( \text{CD}^{8+} \) T cells producing IFN-\( \gamma \) decreased by almost 50% after exercise and remained low 2 h postexercise compared with preexercise values. Twenty-four hours later, the percentage of the IFN-\( \gamma \)-producing \( \text{CD}^{8+} \) T cells was back to preexercise values, whereas the percentage of the IFN-\( \gamma \)-producing \( \text{CD}^{+} \) T cells remained low (Fig. 2A). The percentage of IL-2-producing \( \text{CD}^{+} \) T cells in the circulation was slightly increased after 30 min of exercise. The percentage of both \( \text{CD}^{+} \) and \( \text{CD}^{8+} \) T cells producing IL-2 decreased by almost 50% at the end of exercise and remained low, compared with preexercise values, 2 h after exercise but had returned to preexercise values 24 h later (Fig. 2B). The percentage of \( \text{CD}^{+} \) and \( \text{CD}^{8+} \) T cells in the circulation-producing IL-4 did not change in response to exercise (Fig. 3). Plasma IL-6 was increased by ~30-fold at the end of exercise and declined toward preexercise values in the recovery period (Fig. 4). Plasma IL-12 was below detection limits (\( n = 4 \)). Plasma cortisol increased ~1.7-fold in response to running and declined toward preexercise values in the recovery period (Fig. 5). Plasma epinephrine increased approximately threefold during exercise, and plasma epinephrine measured after 0.5, 1.5, and 2.5 h of running was 0.76 ± 0.1 nmol/l (Fig. 6).

Correlations. Mean plasma epinephrine during running correlated negatively with the percentage of circulating IL-2-producing \( \text{CD}^{8+} \) T cells 2 h postexercise (\( r = -0.717; P < 0.05 \)). Peak plasma IL-6 correlated with the percentage of IL-4 producing \( \text{CD}^{8+} \) T cells in the circulation immediately after exercise (\( r = 0.686; P < 0.05 \)) and 2 h postrunning (\( r = 0.783; P < 0.05 \)). Plasma cortisol did not correlate with the percentage of type 1 T cells in the circulation. Peak plasma IL-6 positively correlated with plasma cortisol 1 h postrunning (\( r = 0.703; P < 0.05 \)).
Epinephrine and percentage of IL-2-producing CD8 T cells 

The major finding of this study was that the percentage of circulating type 1 T cells decreased after prolonged exercise, whereas the percentage of type 2 T cells did not change, and concomitantly the total number of circulating T cells declined. Moreover, the postexercise decrease in circulating T lymphocytes is accompanied by a more pronounced decrease in the type 1 T cell subpopulation.

It has previously been shown that the immune response to strenuous exercise has some similarities to that of other physical stress conditions such as surgery, trauma, sepsis, and burn (10). Regarding Th1 and Th2 responses, it has recently been demonstrated that major surgery suppresses maximal production of Th1 cytokines without influencing the Th2 cytokines (2). Thus surgery and strenuous exercise influence the Th1 and Th2 responses in a similar manner.

It has been demonstrated both in animal and in human studies, in vivo and in vitro, that T cells producing IFN-γ and IL-2 are suppressed by cortisol and epinephrine (3, 4). These hormones increase during and in response to exercise. Cortisol probably works through inhibiting the antigen-presenting cells' production of IL-12 (3) and also by decreasing the ability of T cells to respond to IL-12 (4). Epinephrine also suppresses the type 1 T cells both at the level of antigen-presenting cells and directly on T cell receptors. In this study, a negative correlation between mean plasma epinephrine and percentage of IL-2-producing CD8+ T cells was found. CD8+ T cells express more β2-adrenoceptors on the surface compared with CD4+ T cells (10), which may explain that only the percentage of CD8+ T cells correlated with epinephrine during exercise. In regard to plasma cortisol, no correlation with type 1 T cells was found.

Rincon et al. (13) showed that IL-6 stimulates the production of IL-4 by naive CD4+ T cells. It is well established that plasma IL-6 increases enormously in response to exercise (9) and that contracting skeletal muscles release IL-6 (15). Even the 30-fold increase in plasma IL-6 found in this study did not increase the percentage of IL-4-producing CD4+ or CD8+ T cells either at the end of exercise or in the recovery period. However, the correlation between plasma IL-6 and percentage of IL-4-producing CD8+ T cells may indicate that the increase in plasma IL-6 during exercise contributes to maintain an unaltered percentage of type 2 T cells and, thereby, influences the balance between the type 1 and type 2 T cells. It should be noted that all subjects were exercising in a fasting state to eliminate a possible effect of nutrition. During exercise, muscle glycogen is reduced. This contributes to enhancing the production of IL-6 and, thereby, the effect of IL-6 on cortisol secretion. Furthermore, the level of catecholamines in plasma increases more when subjects exercise under fasting conditions. Thus exercising in a carbohydrate-loaded state might have induced a less pronounced effect on the type 1 or type 2 T cell balance.

Local immune responses have been shown to induce the endothelial expression of P- and E-selectins, which specifically adhere to Th1 cells (12). It is generally accepted that strenuous exercise causes a local inflammatory response in muscles (7). Therefore, a larger increase in type 1 T cell immigration into the muscles postexercise compared with other CD3+ T cells might explain the decrease in the percentage of type 1 T cells.

Tsigos et al. (16) demonstrated that infusion of recombinant human IL-6 into humans increases the levels of glucocorticoids. IL-6 probably functions through the hypothalamic-pituitary-adrenal axis, thereby increasing secretion of cortisol. In accordance, this study demonstrated a correlation between peak plasma IL-6 and plasma cortisol 1 h postrunning.

The relatively more pronounced decrease in type 1 compared with type 2 T cells in the recovery period may explain the increased sensitivity to URTI following strenuous exercise, as these infections are often caused by viruses.

In conclusion, the present study demonstrates that the postexercise decrease in T lymphocyte number is accompanied by a more pronounced decrease in type 1 T cells. Although the correlational relationship should be taken with some caution (the r values are high, but the n values are only 9), our data suggest that the increase in plasma epinephrine during exercise contributes to the suppression of IL-2-producing T cells and that high plasma IL-6 helps to maintain the IL-4-producing T cells in the circulation. Thus epinephrine and IL-6 may participate in the exercise-induced shift toward a relative type 2 T cell dominance. However, other physiological changes, such as local inflammation, may also play a role. In addition,
the present study supports the idea that an exercise-induced increase in plasma IL-6 induces elevated levels of plasma cortisol.

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