Circulating Levels of Peripheral Blood Leucocytes and Cytokines Following Competitive Cycling

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Catalogue Data

Key words: prolonged exercise, cyclists, immunity, cell recruitment and activation, interleukins, tumour necrosis factor, interferon

Mots-clés: effort prolongé, cyclistes, immunité, mobilisation et activation des cellules, interleukine, facteur de nécrose des tumeurs, interferon

Abstract/Résumé

The objective of the study was to determine if prolonged and strenuous cycling leads to a polarized cytokine response, and/or unique mobilization of circulating leucocyte populations. Resting venous blood samples were collected from 6 amateur cyclists, 24 hr before, and at 10-25 min and 150 min after completion of a 250-km road race (race time: 404 ± 3.5 min). Total leucocyte counts were significantly elevated following competition. Cell counts of CD3+CD8+ T lymphocytes were depressed by 50% 150 min after competition. A significant increase in CD4+ cells expressing the IL-2Ra chain was evident 150 min after competition. IL-6 concentrations were greatly increased, both at 10-25 min and 150 min after competition. Resting TNF-α concentrations were approximately doubled at both time points after competition. Plasma levels of IFN-γ, IL-10 and IL-12 were below detection thresholds at all time points. These results suggest that performance of a 6.5 h competitive cycle-race does not induce a Type-1- or Type-2-dominated cytokine response, but one that is typical of a proinflammatory cytokine response.

Le but de l’étude est de déterminer si une longue et dure épreuve à bicyclette déclenche une réaction polarisée des cytokines et/ou une mobilisation particulière des populations de leucocytes dans la circulation. De échantillons de sang veineux de 6 cyclistes amateurs au
repos sont prélevés 24 hr avant et 10 à 25 min ainsi que 150 min après une course sur route de 250 km d'une durée de 404 ± 3.5 min. Le nombre de leucocytes demeure significativement plus élevé après la course et cela. Les nombres des lymphocytes CD3+CD8+ étaient diminués de 50%, 150 min après la compétition. À ce moment, l'augmentation du nombre de cellules CD4+ qui représentent la chaîne IL-2Rx, est évidente. La concentration des IL-6 est fortement accrue 10 à 25 min après et 150 min après la course; aux mêmes moments, les concentrations de repos de TNF-α sont presque doublées. Les concentrations plasmatiques de IFN-γ, de IL-10, et de IL-12 sont sous le seuil de détection dans chacun des échantillons veineux. Les résultats indiquent qu'une épreuve de 6,5 hr à bicyclette n'entraîne pas de réaction de Type 1 ou de Type 2 des cytokines mais plutôt une réaction pro-inflammatoire des cytokines.

Introduction

Following periods of intense athletic competition, endurance athletes have an increased risk of infection, particularly of the respiratory tract (Shephard and Shek, 1993). Current theories suggest that the increased susceptibility to infection results from a neuroendocrine-mediated immunosuppression induced by physical and psychological stress (Cohen, 1995; Pedersen & Bruunsgaard, 1995) and/or a posttraumatic immunosuppression resulting from a counterregulatory control of inflammation (Northoff et al., 1995). Such immunosuppression is most likely in the hours or days immediately following strenuous athletic competition, when immune homeostasis is in a state of imbalance. Characterized by a circulating leucocytosis and lymphopenia, changes in the proportion, number, and proliferation of circulating lymphocyte subsets (Shephard et al., 1991), the activation of specific leucocyte subsets (Gabriel et al., 1993) and altered levels of circulating cytokines (Rhind et al., 1995).

Cytokines may be classified as either Type 1 or Type 2, depending upon their immunoregulatory function (Mosmann and Coffman, 1989). A Type 1 cytokine response is characterized by normal or increased plasma levels of interleukin (IL)-2, IL-12, interferon (IFN)-γ, or a combination of these. Together, these cytokines promote a strong cellular immune response and a heightened level of immunosurveillance. In contrast, a Type 2 response is characterized by increased plasma levels of IL-4, IL-5, IL-10, IL-13, or a combination of these, with a downregulation of cell-mediated immunity and stimulation of the humoral responses.

Two of the more notable cytokines released during exercise are the proinflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α). Elevated plasma levels of IL-6 have been shown consistently following strenuous exercise (Northoff and Berg, 1991), and although detection of TNF-α in plasma specimens has been inconsistent (Smith et al., 1992), increased concentrations have been reported after running (Espersen et al., 1990). Of the Type 1 cytokines, circulating levels of plasma IL-2 are reduced in the hours following strenuous exercise (Rhind et al., 1995), while changes in circulating levels of plasma IFN-γ have not been detected. No studies have yet examined the possible influence of exercise on IL-12
levels. Similarly, there have been no published reports concerning the exercise response of the Type 2 cytokines IL-4, IL-5, IL-10 or IL-13.

The mechanisms by which strenuous endurance competition culminates in an "open window" of immunosuppression are not well understood. Hypothetically, exercise-induced, neuroendocrine-mediated shifts in effector cell populations might inhibit an individual’s ability to mount an effective immune response since the elimination of foreign pathogens requires a robust and cooperative involvement of these cells (Fleshner et al., 1992). Alternatively, strenuous exercise may induce a shift from a Type 1 to a Type 2 directed cytokine response (Rhind et al., 1995). The ability of the immune response to become dominated by either a Type 1 or a Type 2 directed cytokine response is well documented for various clinical syndromes (Swain, 1993). Systemic levels of the inflammatory cytokines (IL-6 and TNF-α) are elevated following strenuous running events. To prevent tissue damage and to minimize systemic effects associated with high plasma levels of these inflammatory cytokines, production of these cytokines must be regulated. In theory, downregulation of this inflammatory cytokine network would involve the counterregulatory production of Type 2 anti-inflammatory cytokines, such as IL-4 or IL-10 (de Waal Malefyt et al., 1991). Recent work by Daftarian et al. (1996) demonstrates an upregulated production of IL-10 by CD4+/CD8+ T cells and monocytes in the presence of interleukins-6 and -12 and TNF-α, respectively, suggestive of an autoregulatory control of these cytokines by the self-induction of IL-10. It is thus reasonable to question whether this same counterregulatory mechanism plays a role in the control of cytokine production during exercise. If so, such mechanisms may have immunosuppressive side effects, as demonstrated for IL-10 (Northoff et al., 1995).

To determine if prolonged and strenuous exercise leads to a polarized cytokine response, unique mobilization of circulating leucocyte populations, or both, we decided to make detailed immunologic observations on 6 young male cyclists before and after a 250-km road-cycling competition, the longest road-race of their competitive season. Observations were made on total leucocytes, specific leucocyte subsets, CD4+ lymphocyte expression of interleukin-2 receptor (IL-2R) alpha (α), and plasma levels of the cytokines IL-6, IL-10, IL-12, TNF-α and IFN-γ.

**Methods**

**SUBJECTS**

Following the approval of the Human Subjects Research Committee of the University of Toronto, written informed consent was obtained from 6 males (24.5 ± 4.6 yr, 1.80 ± 0.07 m, 70.2 ± 5.9 kg) who volunteered to participate in the study. All subjects were Canadian Cycling Association Level-1 competitive cyclists, recruited during the midseason of their competitive phase of training. All had 5 or more years of amateur racing experience, and each covered more than 15,000 km per year in training. They had remained free of infection during the 2 weeks before the study, and none of the group was taking any current medication.
THE EVENT

The annual one-day classic covered a paved and relatively flat highway route between Québec City and Montréal, Canada, a total distance of 250.5 km. The mass start competition, with a field of 103 competitors, began at 9:00 a.m. on July 31, 1994. During the course of the race, which was held on a sunny day, the ambient temperature increased from 19.1 °C to 26.8 °C. The barometric pressure ranged from 758 to 762 torr, the relative humidity ranged from 60 to 90%, and subjects had a tail or side wind (north to northeast) at a speed of 8 to 13 knots (15 to 24 km · hr⁻¹). During the race, all competitors had free access to fluids and food; however, following the race consumption was restricted to fluids ad libitum.

EXPERIMENTAL PROTOCOL

Participation in heavy training or competition was restricted for at least 48 hr prior to the event. Serial blood samples were obtained from all 6 subjects 24 hr before competition (8:00 a.m.), and at 10–25 min (17.0 ± 5.9) and 2.5 hr after completion of the race (Pre⁻二十四, Post⁻十七, and Post⁻五百, respectively). Three of the 6 subjects were randomly selected for assessment of heart rate response during competition. Assessment of peak oxygen intake, ventilatory threshold, and body composition, occurred 1–2 weeks later in our laboratory.

PHYSICAL AND PHYSIOLOGICAL MEASURES

Heart rates were recorded at 60-s intervals throughout the race by Polar Vantage XL heart rate monitor (Polar USA, Inc., NY). For the determination of peak oxygen intake (\(\dot{V}O_2\)peak), subjects performed a 30 W · min⁻¹ incremental exercise test on a friction-braked cycle ergometer at a cadence of 80 rpm (Monark Ergomedic 818, Stockholm). The test began at a loading of 50 W and continued until subjective exhaustion, reached in 8–12 min. Expired gas was collected breath by breath; analyses for respiratory minute volume and oxygen consumption were made using a metabolic measurement cart (SensorMedics 2900C, Yorba Linda, CA). The apparatus was calibrated by a 3-L syringe and precision-analyzed cylinder gases. The ventilatory threshold was determined as the first upward break in a plot of \(\dot{V}E/\dot{V}CO_2\) against work-rate, without a concomitant increase of \(\dot{V}E/\dot{V}CO_2\). Body fat content was assessed using Harpenden skinfold calipers. Measurements were made at the four sites (biceps, triceps, subscapular, and suprailliac), as recommended by the International Biological Programme (Weiner and Lourie, 1981). Body fat was predicted by the equations of Durnin and Womersley (1974) and Siri (1961).

BLOOD SAMPLING

Venous blood samples (3 × 12 ml in total) were obtained from an antecubital vein with subjects sitting. For standard hematology and lymphocyte subset analysis, 3-ml sterile liquid ethylene-diaminetetra-acetic acid (EDTA) glass vacutainers (Becton-Dickinson, Oakville, ON) were used. For cytokine analysis, blood was drawn into chilled, sterile 10-ml vacutainers containing the anticoagulant EDTA.
For plasma separation, these specimens were immediately transferred to frozen 1.5-ml microcentrifuge tubes and spun at 1,000 g for 20 min in a microcentrifuge (Beckman Instruments, Mississauga, ON). Plasma separation was completed within 30 min of blood collection. The separated plasma was stored in 2-ml sterile cryovials (Nalgene Cryoware, Rochester, NY) and kept on dry ice (for ~5 hours) until transferred to a freezer (~80 °C).

STANDARD HEMATOLOGY

Total blood cell counts, white blood cell (WBC) differential counts (granulocytes, monocytes, and lymphocytes), hemoglobin, and hematocrit determinations were performed using an automated counter (Model JT Coulter Counter, Coulter Electronics, Hialeah, FL). Blood cell counts following the race were corrected to resting (Pre2hr) blood volumes, using the formulae of Dill and Costill (1974).

LYMPHOCYTE SUBSETS

Two-colour immunophenotyping, using dual-combinations of monoclonal antibodies (mAb) conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE), was used to enumerate the following subsets of peripheral blood lymphocytes: total T cells (CD3+), T helper/inducer cells (CD3+CD4+), T suppressor/cytotoxic cells (CD3+CD8bright), B cells (CD19-), natural killer cells (CD3-CD56-, CD3-CD16-CD56+, CD3-CD16-CD56bright), and CD4+ cell expression of the interleukin-2-α receptor (CD4+CD25+). Briefly, 10 μl aliquots of each mouse, antihuman, mAb combination were added to a 100 μl volume of EDTA whole blood and gently vortexed. After 30-min incubation at 4 °C, red blood cells were lysed with 2-ml of 1 X FACS lysing solution (Becton Dickinson). Residual nonlysed cells were collected by centrifugation (6 min at 4 °C and 1,100 rpm), washed and stored in 2 ml of cold phosphate-buffered saline (PBS) containing 0.1% sodium azide. Whole blood samples with counts greater than 9.8 × 10⁸ WBC·L⁻¹ were diluted with 1 X PBS containing 0.1% sodium azide prior to staining. Stained cell suspensions were analysed for fluorescence using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems Inc., Mountain View, CA). List-mode data from 10,000 ungated events were acquired for each sample. Lymphocytes were gated out from other leucocyte populations based on their forward- versus side-scatter characteristics on dot plots. Absolute subset counts (cells·L⁻¹) were calculated by multiplying the percentage of cells yielding a specific fluorescence in a gated lymphocyte region by the total number of lymphocytes in a unit volume of peripheral blood.

CYTOKINE DETERMINATIONS

The plasma concentrations of IL-6, IL-10, IL-12, TNF-α, and IFN-γ were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits that employ a quantitative "sandwich" technique (R & D Systems, Minneapolis, MN). Each kit provides a 96-well polystyrene microtiter plate, precoated with cytokine-specific murine monoclonal antibodies. Briefly, aliquots (200 μL) of
standard, control, or sample were incubated in the wells, allowing the natural ligand to be bound by the immobilized antibody. In a second incubation, enzyme-linked polyclonal antibodies were added to sandwich the natural ligand. Finally, a substrate solution was added. Colour thus developed in proportion to the amount of cytokine that was bound during the initial step of the analysis.

The optical densities were read by an automated spectrophotometer microplate reader set to 490 nm (Model EL340, Bio-Tek Instruments, Inc., Winooski, VT). All assays were performed in duplicate. Cytokine concentrations were corrected to resting (Pr24hr) plasma volumes, using the formulae of Dill and Costill (1974). The sensitivity of the ELISA kits were 0.094 pg·ml⁻¹ for IL-6, 2.0 pg·ml⁻¹ for IL-10, 5.0 pg·ml⁻¹ for IL-12, 0.120 pg·ml⁻¹ for TNF-α, and 3.0 pg·ml⁻¹ for IFN-γ. The inter- and intra-assay coefficients of variation were <10% for all cytokines.

NORMAL VALUES

Reference ranges for normal resting peripheral blood cell counts of endurance-trained athletes were taken from Rowbottom et al. (1995). Reference values for IL-2Rα and IL-2Rβ expression were taken from Rhind et al. (1994), while normal values for cytokines were taken from R & D Systems (Minneapolis, MN).

STATISTICAL ANALYSIS

Means and standard deviations (SD) were calculated, and statistical analysis was performed using a one-way ANOVA with repeated measures. The Student-Newman-Keuls post hoc test was used to identify specific mean differences when a significant F ratio was observed for dependent measures. A p < .05 was considered significant. Data are expressed as mean ± SD.

Results

EXERCISE DURATION AND INTENSITY

The 6 subjects we examined completed the race at an average pace of 37.1 ± 0.3 km·h⁻¹, in a time of 404.8 ± 3.5 min (range = 401.1 to 410.9 min), falling on average 3.7 ± 3.5 min behind the winner of the race (range = 0 to 5.5 min). Heart rate profiles for the 3 subjects who were tested averaged 157.7 ± 16.7 beats·min⁻¹, corresponding to 94.4 ± 0.33% of the subjects' individual ventilatory thresholds and 80.9 ± 4.0% of their peak heart rates.

PHYSICAL AND PHYSIOLOGICAL MEASURES

As expected, the cyclists were relatively thin, with 11.4 ± 0.5% body fat and a lean body mass of 58.5 ± 5.5 kg (32.5 kg·m⁻²). Peak oxygen intake was 4.22 ± 0.02 L·min⁻¹ (64.9 ± 1.3 ml·kg⁻¹·min⁻¹), with a peak heart rate of 195 ± 13 beats·min⁻¹ and a peak respiratory gas exchange ratio of 1.16 ± 0.09. The ventilatory threshold
was reached at a heart rate of 167 ± 11 beats·min⁻¹, and an oxygen consumption of 3.37 ± 0.23 L·min⁻¹, providing a relatively high 79.9 ± 5.7% of peak oxygen intake.

**Red Cell Counts.** The red cell counts prior to competition (Pre₂₄hr) were all within normal limits, with a red cell count of 4.81 ± 0.32 × 10¹² cells·L⁻¹, a hemoglobin level of 147.5 ± 10.0 g·L⁻¹, and a hematocrit of 43.8 ± 3.0%. A minor hemococoncentration occurred over the race, with an immediate post-race (Post₁₇) red cell count of 5.03 ± 0.02 × 10⁹ cells·L⁻¹, a hemoglobin of 152.2 ± 6.5 g·L⁻¹, and a hematocrit of 45.9 ± 2.0%. Application of the formula of Dill and Costill (1974) suggested a 3.1% decrease of blood volume and a 6.8% decrease of plasma volume from Pre₂₄hr to Post₁₇. However, recovery was rapid, the corresponding hematological values at 2.5 hr postrace (Post₁₅₀) being 4.89 ± 0.2 × 10¹² cells·L⁻¹, 150 ± 8.4 g·L⁻¹, and 43.9 ± 2.0%. At this stage the blood volume had returned to 98.3% of its original value, and the plasma volume was 98.0% of the initial reading.

**Total Leucocyte and Differential Counts.** Prior to the competition (Pre₂₄hr), individual and mean counts of total leucocytes, granulocytes, monocytes, and lymphocytes were all within normal limits. However, shortly after competition (Post₁₇), total leucocyte counts had increased dramatically to 17.5 × 10⁹ cells·L⁻¹ (p < .05), with the increment persisting to the final (Post₁₅₀) sampling (p < .05). The leucocytosis was reflected by a 356% (p < .05) and 396% (p < .05) increase in polymorphonuclear granulocytes, at Post₁₇ and Post₁₅₀ respectively, well beyond the normal resting range of 2.0–8.2 × 10⁹ cells·L⁻¹. A monocytes was also observed at both Post₁₇ and Post₁₅₀ sampling times, as reflected by increases of 78% and 127%, respectively. Due to the small sample size and large interindividual variation, increases in monocyte count were not significantly greater than the Pre₂₄hr value. However, at 2.5 hr postrace, 2 of the subjects had monocyte levels that exceeded the normally accepted reference range (Table 1) and were elevated by over 160% as compared to rest (Pre₂₄hr). Counts of circulating lymphocytes were unchanged at Post₁₇, although a nonsignificant lymphopenia was observed at Post₁₅₀ (Table 1).

**Lymphocyte Subset Counts.** Individual and mean resting lymphocyte subset counts were initially within normal limits. Numbers of circulating T cells (CD3⁺) were unchanged at Post₁₇, but had decreased sharply by Post₁₅₀ (p < .05). A nonsignificant trend to a decrease in the CD3⁺CD4⁺ cell count apparently contributed to the decrease observed at Post₁₅₀, however, counts for the CD4⁺ subset remained within the expected normal range of 0.4–1.4 × 10⁹ cells·L⁻¹ throughout (Table 1). Similarly, the CD3⁺CD8⁺ cell count fell below the resting level (p < .05), although remaining within the normal range of 0.2–1.2 × 10⁹ cells·L⁻¹ at Post₁₅₀.

Numbers of circulating B cells (CD3⁻CD19⁺) were elevated at Post₁₇, with counts rising to an average of 0.52 × 10⁹ cells·L⁻¹, just above the expected norm of 0.1–0.5 × 10⁹ cells·L⁻¹. B cell numbers had returned to normal limits at Post₁₅₀. Natural killer (NK) cell counts remained within the expected normal range (0.1–0.5 × 10⁹ cells·L⁻¹) at all sampling times, although counts of CD3 CD56⁺ cells were consistently greater than counts of CD3 CD16⁺CD56⁺ NK cells at all time points (Table 1). After competition, circulating NK cell numbers tended to decrease
### Table 1  Leucocyte and Lymphocyte Subset Concentrations, CD4/CD8 Ratio, and IL-2Rα Expression Before and After Competition

<table>
<thead>
<tr>
<th>Blood cell (× 10⁶·L⁻¹)</th>
<th>Pre_24hr</th>
<th>Post_7</th>
<th>Post_150</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leucocyte subsets</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leukocytes</td>
<td>6.00 ± 0.8 (100%)</td>
<td>17.45 ± 3.3 (100%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>17.28 ± 2.5 (100%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.3 – 11.0</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>2.93 ± 0.7 (49.1%)</td>
<td>13.35 ± 0.8 (81.1%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>14.52 ± 2.1 (84.2%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.0 – 8.2</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>2.69 ± 0.3 (45.1%)</td>
<td>2.78 ± 0.9 (15.6%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.00 ± 0.5 (11.4%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.2 – 4.0</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.37 ± 0.2 (6.0%)</td>
<td>0.66 ± 0.3 (4.0%)</td>
<td>0.84 ± 0.6 (4.9%)</td>
<td>0.2 – 1.0</td>
</tr>
<tr>
<td><strong>Lymphocyte subsets</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CD3⁺</td>
<td>1.88 ± 0.3 (69.9%)</td>
<td>1.70 ± 0.5 (61.2%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.25 ± 0.3 (62.5%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.6 – 2.3</td>
</tr>
<tr>
<td>CD3⁺CD4⁺</td>
<td>1.06 ± 0.2 (39.4%)</td>
<td>0.79 ± 0.3 (28.4%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.79 ± 0.2 (39.5%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.4 – 1.4</td>
</tr>
<tr>
<td>CD3⁺CD8⁺bright⁺</td>
<td>0.59 ± 0.1 (21.9%)</td>
<td>0.54 ± 0.1 (19.4%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.36 ± 0.1 (18.0%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.2 – 1.2</td>
</tr>
<tr>
<td>CD3⁺CD56⁺</td>
<td>0.50 ± 0.2 (18.6%)</td>
<td>0.39 ± 0.2 (14.0%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.31 ± 0.1 (15.5%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.1 – 0.5</td>
</tr>
<tr>
<td>CD3⁺CD16⁺CD56⁺</td>
<td>0.34 ± 0.2 (12.6%)</td>
<td>0.30 ± 0.2 (10.8%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.21 ± 0.1 (10.5%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.1 – 0.5</td>
</tr>
<tr>
<td>CD3⁺CD16⁺CD56⁺bright⁺</td>
<td>0.15 ± 0.1 (5.6%)</td>
<td>0.10 ± 0.07 (3.6%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.10 ± 0.03 (5.0%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.1 – 0.5</td>
</tr>
<tr>
<td>CD3⁺CD19⁺</td>
<td>0.33 ± 0.1 (12.3%)</td>
<td>0.52 ± 0.3 (18.7%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.32 ± 0.2 (16.0%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.1 – 0.5</td>
</tr>
<tr>
<td>CD4⁺ / CD8⁺bright⁺ ratio</td>
<td>1.86 ± 0.5</td>
<td>1.47 ± 0.3</td>
<td>2.16 ± 0.2</td>
<td>0.6 – 1.9</td>
</tr>
<tr>
<td>CD4⁺CD25⁺ / CD4⁺</td>
<td>0.37 ± 0.03 (13.8%)</td>
<td>0.54 ± 0.4 (19.4%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.43 ± 0.3 (21.5%)&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>%CD4⁺ that express CD25</td>
<td>36.1 ± 7.6%</td>
<td>38.5 ± 8.3%</td>
<td>43.4 ± 8.1%</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Note.* Numbers in parentheses represent the percentage of total leucocytes or total lymphocytes. NA = not available.

<sup>*</sup>p < .05 as compared with Pre_24hr rest value. <sup>+</sup>p < .05 as compared with Post_7 value.
12–22% (Post17) and 38% (Post150), although the reduction was not statistically significant. Counts of the CD3-CD16-CD56bright subset remained unchanged at all sampling times.

**L-2Rα Expression.** The number of circulating IL-2Rα+ T-helper/inducer cells (CD4+CD25+), at 0.37 ± 0.03 × 10⁶ cells · L⁻¹, was below the expected reference range prior to competition. After competition, counts had increased by 50% at Post17 and 16% at Post150 (Table 1). Similarly, T-helper cell (CD4) expression of IL-2Rα increased significantly (p < .05) from 36.1% of CD4+ cells at Pre24hr to 43.4% at Post150.

**Cytokines.** Cytokine results represent the mean of 5 of the 6 subjects, due to technical difficulties encountered during blood collection from one subject. Prior to competition, the mean resting plasma IL-6 concentration was 0.9 ± 0.4 pg · ml⁻¹. After competition, levels had increased to 41.2 ± 28.2 pg · ml⁻¹ (45-fold) at Post17 (p < .05), and 23.5 ± 19.9 pg · ml⁻¹ (25-fold) at Post150 (Figure 1). The plasma concentrations of TNF-α were also increased following competition. The mean resting concentration was 0.7 ± 0.5 pg · ml⁻¹ (Figure 1), increasing to 1.6 ± 0.9 pg · ml⁻¹ (1.3-fold) at Post17 (p < .05) and to 1.2 ± 0.8 pg · ml⁻¹ (0.7-fold) at Post150 (p < .05). Plasma levels of IL-10, IL-12 and IFN-γ were below the detection thresholds of 2.0 pg · ml⁻¹, 5.0 pg · ml⁻¹ and 3.0 pg · ml⁻¹, respectively, for our ELISA kits in all subjects and samples.

**Discussion**

The present investigation indicates that a 6.5-hr competitive cycling race results in persistent changes in the numbers of circulating granulocyte, monocyte and lymphocyte populations, and in the plasma levels of IL-6 and TNF-α. Responses that are consistent with a proinflammatory cytokine response and the notion that prolonged competitive cycling is a formidable immunological stressor.
A review of exercise immunological studies suggests that the magnitude, direction, and time course of exercise-induced cellular mobilization depends on the intensity and duration of exercise (Gannon et al., 1995). Strenuous, unaccustomed, and prolonged exercise typically induces the largest cellular changes through a combination of physical and psychological stress. Our data demonstrate a substantial postexercise mobilization of both the phagocytic (granulocyte and monocyte) and cytotoxic lymphocyte (CD3<sup>+</sup>CD16<sup>+</sup>CD56<sup>+</sup> and CD3<sup>+</sup>CD16<sup>-</sup>CD56<sup>+</sup>) cell populations. The >350% increase in circulating granulocytes induced by the present competition is similar to that induced by marathon running (Hao et al., 1993), but has not been seen in shorter or less intense bouts of cycling. Furthermore, low- to moderate-intensity cycling exercise does not induce a postexercise monocytosis (Pedersen, 1991), whereas the present competition increased a 127% increase in circulating monocytes 2.5 hr postexercise, a response typical of prolonged and strenuous running (Nieman et al., 1989). Numbers of circulating lymphocytes also exhibit an intensity-specific response, such that only highly strenuous exercise leads to a postexercise lymphopenia (Nieman and Nehlsen-Cannarella, 1992). Total CD3<sup>+</sup> and CD3<sup>+</sup>CD4<sup>+</sup> lymphocyte counts were depressed by 35%, whereas cytotoxic lymphocytes (i.e., CD3<sup>+</sup>CD8<sup>+</sup> and CD3<sup>+</sup>CD16<sup>-</sup>CD56<sup>+</sup>) were the most highly responsive populations, as demonstrated by the >60% decrease in the circulating numbers of these cells at 2.5 hr postexercise.

Resting counts for the CD3<sup>+</sup>CD16<sup>-</sup>CD56<sup>+</sup> subset of the NK cell population were apparently increased in these highly trained athletes. CD3<sup>+</sup> killer cells contain distinct subpopulations with CD16<sup>hi</sup>CD56<sup>di</sup> and CD16<sup>lo</sup>CD56<sup>hi</sup> phenotypes, with the CD3<sup>+</sup>CD16<sup>-</sup>CD56<sup>hi</sup> subset accounting for approximately 10% of the total population in normal healthy untrained individuals (Lanier et al., 1986). In the athletes studied, resting levels of the CD16<sup>-</sup>CD56<sup>hi</sup> subset accounted for 30% of their CD3<sup>+</sup> killer cells. Although the CD16<sup>-</sup> NK cells constitutively express high-affinity IL-2 receptors (i.e., both IL-2Rα and IL-2Rβ), permitting activation at low physiological concentrations of IL-2 (Nagler et al., 1990), they are less efficient cytotoxic effector cells than the CD16<sup>+</sup> NK cell subset (Nagler et al., 1989). Furthermore, these cells may represent a progenitor population for mature NK cells (Nagler et al., 1990). To suggest that the chronic stimulus of strenuous training and competition led to the expansion of this population would be premature. However, the greater prevalence of the CD16<sup>-</sup> cytolytic cell population indicates a potential disturbance of cell-mediated cytolysis within this well-trained group of competitive cyclists.

Whether the immediate exercise-induced cellular changes influence immunosurveillance is unclear. We were unable to collect data beyond 2.5 hours of recovery. However, most, if not all, of the cellular shifts induced by prolonged exercise are transient, and normal baseline values are reestablished within 24 hr postexercise (Nieman and Nehlsen-Cannarella, 1992). It is thus questionable how far such changes can have an impact upon immunological defence mechanisms.

Under resting conditions, the low affinity IL-2Rα (p55) is expressed constitutively on ~30–50% of peripheral blood CD4<sup>+</sup> lymphocytes and on more than
50% of CD4+ lymphocytes from aerobically trained individuals (Rhind et al., 1994). In this study, the resting CD4+ expression of IL-2Rα fell within the range for untrained subjects (Table 1). This finding was unexpected, given that the subjects were well documented as highly trained individuals. Following competition (Post$_{150}$), the CD4+ lymphocyte expression of IL-2Rα (CD25), had increased by 20% ($p < .05$); this could indicate an increased activation of the CD4+ T-cell subset or, as proposed by Chiappelli et al. (1992), a preferential clearance of naive CD4+ lymphocytes (i.e., typically CD25+) from the peripheral circulation. The CD4+CD25+ lymphocyte count continued to increase over the postexercise period in spite of a stable CD4+ lymphocyte count (Table 1). Therefore we favour the explanation of T cell activation, as previously reported for a 240-km competitive cycling event (Gabriel et al., 1993). Furthermore, such a hypothesis is consistent with reports of systemic endotoxemia following prolonged strenuous exercise, as gram-negative bacteria are capable of inducing T-cell activation (Bosenberg et al., 1988).

In accordance with Haahr et al. (1991), we were unable to measure any effect of strenuous cycling exercise on the plasma levels of IFN-γ. Historically, there has been difficulty in detecting IFN in plasma specimens, possibly due to its short half-life, its rapid uptake and utilization, or both. Similarly, we found no evidence that plasma levels of IL-10 or IL-12 were increased following the competition. Considering that cytokines are active at extremely low concentrations (picomolar and femtomolar levels), the local production of these cytokines may have been sufficient to induce a response without a measurable “spillover” into the general circulation, given the detection limits of our commercially purchased ELISA kits. Alternatively, if these cytokines are indeed produced in response to heavy exercise, secretion may have either preceded or followed our sampling schedule.

The increase of plasma TNF-α, both immediately and 2.5 hr after the competition, is the first such report following cycling exercise. Previous studies of lower intensity and shorter duration cycling have not reported significant increases (Smith et al., 1992; Ullum et al., 1994). However, strenuous running competition is known to increase plasma TNF-α, possibly in response to muscle injury (Espersen et al., 1990). TNF-α is known to induce IL-6, and in the present study, postexercise plasma concentrations of IL-6 increased in parallel with TNF-α. The elevated concentrations were consistent with strenuous, prolonged bouts of running (Drent et al., 1995). However, previous studies involving 1 hr of cycling exercise (75% VO$_{2\text{max}}$) have demonstrated a smaller increase in postexercise plasma IL-6 levels (Haahr et al., 1991; Ullum et al., 1994), likely because of the shorter exercise duration.

The stimuli and mechanisms of IL-6 and TNF-α release are not well-understood. Gut-associated endotoxin, which passes from the intestine to the general circulation during prolonged strenuous exercise (Bosenberg et al., 1988), could increase systemic levels of IL-6 and TNF-α (Fong et al., 1990), although other Type-2 cytokines would also be expected to increase. Alternatively, the production of these cytokines could be stimulated by cell debris from exercise-induced muscle injury. Tissue levels of IL-6 and TNF-α are reportedly elevated following muscle
inflammation or trauma (Pullicinio et al., 1990), and plasma concentrations of IL-6 and muscle-specific CK-MM, are positively correlated during prolonged endurance runs (Northoff et al., 1995). Furthermore, activated macrophages have been demonstrated in muscle tissue following strenuous exercise (Michna, 1988), and these cells are well-known producers of IL-6 and TNF-α (Hagiwara et al., 1995). However, complaints of muscle soreness among the cyclists were minimal, and the team was able to complete a criterium race of some 80 km at an average speed of over 40 km · h⁻¹ the day following competition. Furthermore, cycling is predominately a concentric form of exercise, and it is therefore unlikely that the muscle damage during the 6-hr race was as great as during a run of similar duration (Drent et al., 1995). While Drent et al. (1995) demonstrated a magnitude increase of plasma IL-6 similar to our present study, they were unable to detect an elevated plasma TNF-α concentration; it might thus be argued that muscle injury is not necessary for the induction of these inflammatory mediators.

At low levels, proinflammatory cytokines provide the necessary signals for optimal functioning of our defense and repair systems. At higher levels, however, the same cytokines are potential mediators of severe and harmful immunoinflammatory reactions. As we have demonstrated, prolonged competitive cycling induces a substantial postexercise increase in the proinflammatory cytokines, IL-6 and TNF-α. We had theorized that this inflammatory-like reaction could cause a shift to a Type-2 directed peripheral blood cytokine profile, led by IL-10, an important anti-inflammatory cytokine. However, plasma levels of this cytokine were not elevated in the immediate hours following 6 hr of competitive cycling. Possible explanations for this finding may be that (a) IL-10 is not involved in downregulating the production of IL-6 and TNF-α, as these proinflammatory cytokines may regulate their own production via autocrine pathways; (b) local production of IL-10 may be insufficient to increase plasma concentrations of this cytokine, or (c) our choice of sampling times may have missed the response of this cytokine to exercise, since the production of IL-10 is known to be delayed in comparison to IL-6 and TNF-α (de Waal Malefyt et al., 1991). Further study is thus required to determine if IL-10 is an important regulator of the exercise-induced proinflammatory cytokine response.

In summary, prolonged competitive cycling does not appear to induce either a Type-1- or Type-2-dominated cytokine response; rather, findings are typical of a proinflammatory cytokine response to injury. The substantial increase in plasma IL-6 and TNF-α, the mobilization of phagocytic and cytotoxic cell populations and the up-regulated expression of IL-2Rα indicate that prolonged competitive cycling activates several components of the immune system. Whether this modulates the risk of acute viral infection remains to be determined.

References


Acknowledgments

Our special thanks are due to the 6 members of the Toronto Italia cycling team for their cooperation in this study and to Canadian Tire Acceptance Limited for financial support of R.J. Shephard.

Received August 1, 1996; accepted in final form November 18, 1996.