IL-17, neutrophil activation and muscle damage following endurance exercise

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ABSTRACT

The T-cell subset Th17 is induced partly by interleukin (IL)-6 and activated by IL-23, and produces a proinflammatory cytokine IL-17. Since IL-6 increases dramatically following long-lasting endurance exercise, this response may also stimulate the induction of IL-17 and IL-23 after exercise. The aim of this study was to clarify the dynamics of IL-17 in association with endurance exercise-induced muscle damage and inflammatory responses. Fourteen male triathletes participated in a duathlon race consisting of 5 km of running, 40 km of cycling and 5 km of running. Venous blood and urine samples were collected before, immediately after, 1.5 h and 3 h after the race. Plasma and urine were analyzed using enzyme-linked immunosorbent assays (ELISA). Haematological and biochemical variables such as neutrophil activation marker (myeloperoxidase: MPO), muscle damage marker (myoglobin: Mb) and soluble receptor activator of nuclear factor (NF)-κB ligand (sRANKL) were also determined to estimate the biological and pathological significance. Plasma concentrations of IL-6 (+26.0×), MPO (+3.2×) and Mb (+4.9×) increased significantly immediately after the race and IL-17 and IL-23 tended to increase. Furthermore, plasma concentrations of IL-12p40 and sRANKL increased significantly after the race. The measured parameters related to Th17 cytokines in the urinary output were closely correlated with each other and muscle damage marker. These findings suggest that IL-17 induced by IL-6 and activated by IL-23 or other IL-17 producing-cells and IL-23 might promote neutrophil activation and muscle damage following prolonged endurance exercise.

Key words: interleukin (IL)-17, helper T (Th) cells cytokine balance, neutrophil activation, muscle damage, urinary excretion

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INTRODUCTION

Exercise induces peripheral blood neutrophilia (28, 29), and enhances the capacity of neutrophils and monocytes to produce reactive oxygen species (ROS) (22, 29). Furthermore, exercise induces neutrophil and cytokine accumulation in damaged muscle (25), which then releases myocellular proteins such as creatine kinase (CK) and myoglobin (Mb) into the circulation in a delayed-onset manner (30, 31). The involvement of neutrophils in muscle damage has also been demonstrated in animal experiments in which administration of anti-neutrophil antibody to deplete circulating neutrophils prevented post-exercise muscle proteolysis due to neutrophil infiltration (17). It was also observed that in myeloperoxidase (MPO) knockout mice, soleus muscles showed a significant 52% reduction in membrane lysis compared with wild-type mice (18), suggesting that MPO-containing neutrophils and their activation factors such as proinflammatory cytokines facilitate muscle damage following exercise.

Exhaustive exercise induces a systemic inflammatory response syndrome (SIRS), characterized by hypercytokinaemia (19, 22, 23, 32). It is apparent that the cytokine response to exercise differs from that elicited by severe infections. In sepsis, tumour necrosis factor (TNF)-α and interleukin (IL)-1 stimulate the production of IL-6, whereas IL-6 is the first cytokine present in the circulation during exercise (15, 20). The level of circulating IL-6 increases in an exponential fashion (up to 100-fold) in response to exercise (24). In these circumstances, it is suggested that stimulated production of proinflammatory cytokines by increased IL-6 might be related to muscle damage and neutrophil activation following exhaustive endurance exercise, but which proinflammatory cytokines are involved has not been determined yet.

IL-17 and IL-23 are also classified as proinflammatory cytokines. A subset of CD4+ T cells, helper T (Th) 17 cells and innate immune cells such as γδ T cells are major producers of IL-17 (4, 5, 37). It is clear that the Th17 subset has a role as a Th cell with a unique function distinct from Th1 and Th2 (7, 8, 12, 27, 35). IL-17 is believed to act primarily on parenchymal cells such as fibroblasts, epithelial cells, and endothelial cells. Signaling by IL-17 increases matrix metalloproteinase and proinflammatory cytokine expression (12, 36). IL-17 also acts to recruit neutrophils to peripheral sites through the induction of chemokines such as IL-8 (12). In addition, it has been reported that IL-17 promotes osteoclastogenesis through the induction of receptor activator of nuclear factor (NF)-κB ligand (RANKL) on osteoblasts (13). On the other hand, IL-23 is made by both dendritic cells (DCs) and macrophages (10). The receptor for IL-23 is expressed on activated/memory T cells (11). IL-23 has an important role in the regulation of the innate immune response, and also could serve to expand and stabilize Th17 responses (16, 34). The mRNA expression of RANKL correlates with that of IL-23 in the synovial tissues of patients with rheumatoid arthritis which develops by involvement of Th17 cells (26). Based on these findings, it might be hypothesized that neutrophil activation and inflammatory reactions via NF-κB induced by IL-17 and IL-23 are related to muscle damage following exhaustive endurance exercise.

As described above, Th17 cells are induced party by IL-6 and activated by IL-23, resulting in the production of the proinflammatory cytokine IL-17 (1, 2).

Since IL-6 increases dramatically during long-lasting endurance exercise, this response may also stimulate the induction of IL-17 and IL-23 after exercise. The aim of this study was to clarify the dynamics of IL-17 in association with endurance exercise-induced IL-6 release, neutrophil activation and muscle damage.

METHODS

Subjects

Fourteen male triathletes (age 28.7 ± 7.9 (mean ± SD) yr and body mass 63.2 ± 6.0 kg), volunteered to take part in this study. The participants were seven professional triathletes and seven amateur triathletes. All subjects completed a medical questionnaire and gave written informed consent prior to the study. None of them had been ill in the previous month. The experimental procedure was approved by the institutional ethics committee of Waseda University.

Duathlon race

The present investigation was conducted at the 19th Kikunotsuyu duathlon race on March 16th, 2008. It was held on the road course of Miyako Island, Okinawa, Japan. This race consisted of 5 km of running, 40 km of cycling, and 5 km of running, and began at 14:00. The weather was fair, and the ambient temperature was 24.6 °C.

Research design

All participants agreed to avoid the use of vitamin/mineral supplements, herbs and medications from the previous day until after the last sampling point. All participants ate the same breakfast at 08:30. The breakfast contained 574 kcal, with 22.1 g protein, 13.7 g fat and 88.8 g carbohydrate. With the subjects resting quietly, the pre-race blood and urine samples (Pre) were collected at 10:30. They did not exercise for approximately 18 h before the pre-race blood and urine sampling. The post-race blood and urine samples were collected immediately (0 h), 1.5 h (1.5 h) and 3 h (3 h) after the race. Peripheral blood samples were drawn by antecubital venepuncture with the subjects in the sitting position. They ate the same lunch at 11:00. The lunch contained 211 kcal, with 9.3 g protein, 2.4 g fat and 38.6 g carbohydrate. All participants drank the same quantity of fluid during exercise. After a warm-up, they each drank 600 ml of fluid before the race. During the race, they each drank 1400 ml of fluid. Therefore, the total fluid intake for each individual was 2000 ml. They each drank 1500 ml of water after the race until 3 h after the race.

Haematological and biochemical parameters

Approximately 7 ml of blood was drawn by a standard venepuncture technique from the antecubital vein using vacutainers containing no additive or disodium EDTA as an anticoagulant to obtain serum and plasma samples, respectively. Collected blood samples containing no additives were allowed to clot at room temperature for one hour before centrifugation at 1000 *g* for 10 min for serum preparation, whereas blood samples containing disodium EDTA were centrifuged

immediately for plasma preparation. Plasma was stored at -80 °C until the day of analysis. Complete blood cell counts, haemoglobin and haematocrit were determined on EDTA-treated venous blood using an automatic blood cell counter (pocH-100i, Sysmex, Kobe, Japan). Serum concentrations of creatinine (Cre), Mb and CK activity were measured using an automated analyzer (Model 747-400, Hitachi, Tokyo, Japan).

Urine samples were centrifuged immediately at 1000 *g* for 10 min to remove sediments, and the supernatants were stored at -80 °C until the day of analysis. Urinary concentrations of Cre and Mb were measured using an automated analyzer (Model 747-400, Hitachi, Tokyo, Japan).

Assays for inflammatory substances

Inflammation-related substances were measured in serum, EDTA-plasma and urine samples with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. We chose to measure the concentrations of IL-17 and IL-23 (Quantikine, R&D Systems, Minneapolis, MN, USA), IL-6 (Quantikine HS, R&D Systems, Minneapolis, MN, USA), IL-12p40 (OptEIA, Beckton Dickinson Biosciences, San Diego, CA, USA), MPO (Hbt ELISA test kit, Hycult biotechnology, Uden, The Netherlands), and sRANKL (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria) using ELISA. These concentrations were determined by comparison to a standard curve established in the same set of measurements using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA).

Statistical analyses

Data are presented as means ± SD. Statistical validation was made using Friedman's test. If significance was detected, the Scheffe method was used for multiple comparisons. Associations among measured variables were determined by Spearman's rank correlation coefficient (r). Statistical significance was evaluated at $p < 0.05$ or $p < 0.01$.

RESULTS

Haematological data

Total neutrophil counts were significantly elevated immediately (+4.9×), 1.5 h (+4.4×) and 3 h (+4.1×) after exercise as compared to the pre-exercise values. Total lymphocyte counts were also elevated immediately after the race (+1.7×), but decreased 1.5 h and 3 h post-exercise compared with the pre-exercise values. Haemoglobin (+1.1×) and haematocrit (+1.1×) values increased significantly immediately after the race; thereafter both haemoglobin and haematocrit returned to pre-exercise levels, indicating that haemoconcentration occurred during exercise. Therefore, the post-exercise raw data were adjusted for alterations in plasma volume (Table 1).

Biochemical data

Serum concentrations of Cre (+1.4×) and Mb (+4.9×) and serum CK (+1.2×) activity increased significantly immediately after the race. Thereafter, CK

Table 1. Changes of haematological parameters following the duathlon race.

	Unit	Pre	0 h	1.5 h	3 h	Friedman test	Scheffe test
leucocyte	$\times 10^2/\mu l$	47.4±13.6	147.9±37.5	136.2±35.7	124.9±36.5	**	Pre-0 h**, Pre-1.5 h**, Pre-3 h*
neutrophil	$\times 10^2/\mu l$	25.4±10.9	113.0±33.0	113.4±31.3	102.6±32.9	**	Pre-0 h**, Pre-1.5 h**
lymphocyte	$\times 10^2/\mu ll$	16.9±4.8	25.6±8.2	13.6±5.0	14.6±5.1	**	Pre-0 h*, 0 h-1.5 h**, 0 h-3 h**
Hb	g/dl	14.3±1.2	15.1±1.2	14.3±1.1	14.5±1.1	**	Pre-0 h*, 0 h-1.5 h**
Hct	%	42.6±3.3	45.1±3.2	42.4±3.0	42.9±3.1	**	Pre-0 h**, 0 h-1.5 h**, 0 h-3 h*

Values: means ± SD (n=14). Statistics: * $p < 0.05$ and ** $p < 0.01$.
leucocyte: leucocyte count, neutrophil: neutrophil count, lymphocyte: lymphocyte count, Hb: Haemoglobin concentration, Hct: haematocrit in the peripheral venous blood.

remained elevated by 3 h post-exercise; Cre and Mb decreased, but remained above pre-exercise values at 1.5 h (Cre: +1.3×, Mb: +5.7×) and 3 h (Cre: +1.2×, Mb: +4.9×) post-exercise.

The amount of urinary Mb decreased significantly immediately after the race and thereafter increased. Urinary Cre concentration was elevated significantly 1.5 h post-exercise (+2.2×) and then decreased. Because Cre clearance changed following exercise (3), the urinary concentrations of cytokines and other markers are reported as the gross amount (Table 2).

Cytokines, MPO and sRANKL

The plasma concentrations of IL-6 (+26.0×), IL-12p40 (+1.3×) and MPO (+3.2×) increased significantly immediately after the race. Plasma concentrations of IL-6

Table 2. Changes of biochemical parameters following the duathlon race.

	Unit	Pre	0 h	1.5 h	3 h	Friedman test	Scheffe test
Cre-S	mg/dl	0.81±0.08	1.11±0.21	1.08±0.19	1.00±0.16	**	Pre-0 h**, Pre-1.5 h**, 0 h-3 h*
Cre-U	g/l	1.1±0.3	2.0±1.4	2.4±1.5	1.3±0.8	**	Pre-1.5 h*
Mb-S	ng/ml	42.9±10.8	210.1±111.4	245.1±135.6	212.1±125.7	**	Pre-0 h*, Pre-1.5 h**, Pre-3 h*
Mb-U	ng	2462.7±1187.1	681.2±490.0	1114.0±632.3	2018.5±2118.6	**	Pre-0 h**
CK-S	U/l	357.9±264.8	437.7±290.9	495.4±291.6	528.3±299.1	**	Pre-1.5**, Pre-3 h**, 0 h-3 h*

Values: means ± SD (n=14). Statistics: * $p < 0.05$ and ** $p < 0.01$.
Cre-S: serum creatinine concentration, Cre-U: urinary creatinine concentration, Mb-S: serum myoglobin concentration, Mb-U: urinary myoglobin amount, CK-S: serum creatine kinase activity.

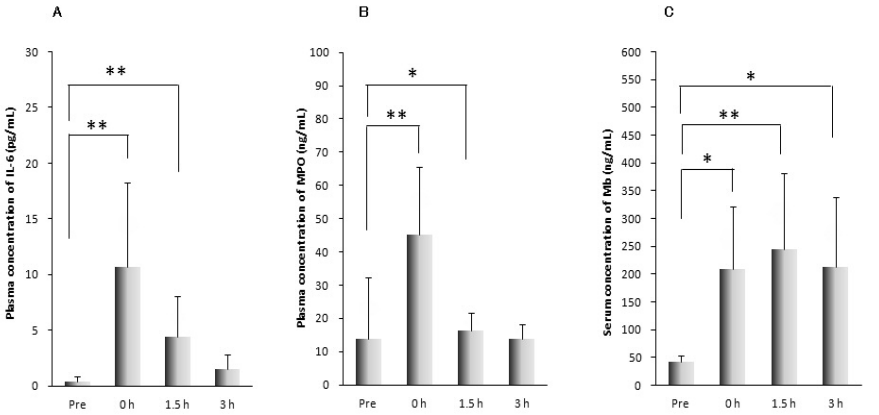

Figure 1. Changes in plasma concentrations of interleukin 6 (IL-6) and myeloperoxidase (MPO) and serum concentration of myoglobin (Mb) following the duathlon race.
A: plasma concentration of IL-6
B: plasma concentration of MPO
C: serum concentration of Mb
Values: means + SD.
Statistics: *$p < 0.05$ and **$p < 0.01$.

(+10.9×) and MPO (+1.2×) were also significantly higher 1.5 h after the race compared with the pre-exercise values. Plasma concentrations of IL-17 and IL-23 decreased significantly immediately after the race, but were significantly higher at 1.5 h and 3 h compared with values at 0 h post-exercise. There was a trend for serum sRANKL concentration to increase following exercise.

In contrast, the urinary amounts of IL-6, IL-17, IL-23 and IL-12p40 decreased significantly immediately after the race. Thereafter, the urinary amounts of IL-6 (+3.4×), IL-17 (+2.6×) and IL-12p40 (+5.3×) increased significantly at 3 h after the race compared with immediately after the race. The urinary amount of sRANKL changed significantly following exercise. There was a trend for urinary MPO to change following exercise (Fig. 1) (Table 3).

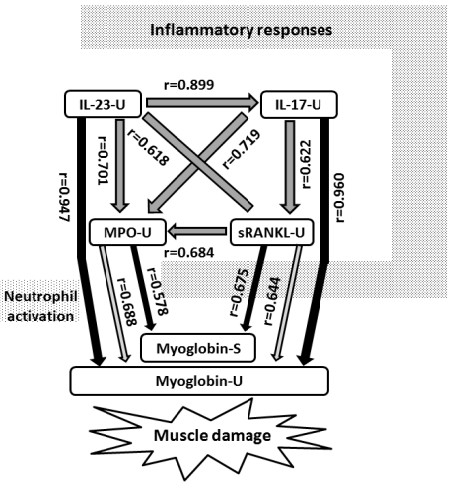

Figure 2. Correlations of inflammatory responses, neutrophil activation and muscle damage markers.
MPO: neutrophil activation marker
sRANKL: NF-κB activation factor
IL-17: proinflammatory cytokine
IL-23: promoter of the differentiation of Th17 cells

Associations between measured parameters

The area under the curve (AUC) for

Table 3. Changes in plasma and urinary cytokines following the duathlon race.

	Unit	Pre	0 h	1.5 h	3 h	Friedman test	Scheffe test
MPO-P	ng/ml	13.9±18.5	45.2±20.5	16.3±5.2	14.0±4.4	**	Pre-0 h**, Pre-1.5 h*, 0 h-3 h**
MPO-U	ng	33.3±15.6	14.7±10.1	20.6±12.1	28.8±29.0	†	-
IL-17-P	pg/ml	2.4±1.0	2.0±0.7	2.5±0.8	2.6±0.8	**	Pre-0 h**, 0 h-1.5 h**, 0 h-3 h**
IL-17-U	pg	845.9±349.7	280.0±171.6	340.8±189.4	735.3±751.2	**	Pre-0 h**, 0 h-3 h*
IL-23-P	pg/ml	5.0±2.9	4.1±2.3	4.9±3.0	5.4±3.2	**	Pre-0 h*, 0 h-1.5 h*, 0 h-3 h**
IL-23-U	pg	966.4±463.9	319.8±209.1	407.5±247.3	1169.0±1204.2	**	Pre-0 h**
sRANKL-S	pmol/l	0.14±0.10	0.15±0.11	0.16±0.13	0.18±0.14	†	-
sRANKL-U	pmol	0.22±0.16	0.07±0.07	0.08±0.07	0.20±0.23	*	-
IL-12p40-P	pg/ml	91.0±43.9	118.7±51.4	118.4±53.1	98.7±35.7	**	Pre-0 h*
IL-12p40-U	pg	18815.5±14747.8	5494.1±4524.7	16808.3±16851.0	28872.4±14574.4	**	Pre-0 h**, 0 h-3 h**

Values: means ± SD (n=14). Statistics: * $p < 0.05$, ** $p < 0.01$, † $p < 0.1$.
- P & -S: Data are adjusted for alterations in plasma volume.
- U: Data are the gross amount in the volume of urinary excretion.
Abbreviations: interleukin (IL)-17, 23, 12p40, myeloperoxidase (MPO), soluble receptor activator of nuclear factor (NF)-κB ligand (sRANKL).

Pre-, 0 h, 1.5 h and 3 h of plasma concentrations of IL-17 (IL-17-P) was correlated with that of IL-12p40-P ($r = 0.613$, $p < 0.05$). The AUC of urinary amounts of IL-17 (IL-17-U) was correlated with MPO-U ($r = 0.719$, $p < 0.01$), sRANKL-U ($r = 0.622$, $p < 0.05$), Mb-U ($r = 0.960$, $p < 0.01$), IL-23-U ($r = 0.899$, $p < 0.01$) and that of serum CK (CK-S) activity ($r = 0.543$, $p < 0.05$). The AUC of IL-23-P was correlated with IL-12p40-P ($r = 0.622$, $p < 0.05$). The AUC of IL-23-U was correlated with MPO-U ($r = 0.701$, $p < 0.01$), sRANKL-U ($r = 0.618$, $p < 0.05$), Mb-U ($r = 0.947$, $p < 0.01$) and CK-S ($r = 0.587$, $p < 0.05$). The AUC of sRANKL-U was correlated with MPO-U ($r = 0.684$, $p < 0.01$), Mb-U ($r = 0.644$, $p < 0.05$) and Mb-S ($r = 0.675$, $p < 0.01$). The AUC of Mb-S was correlated with that of MPO-U ($r = 0.578$, $p < 0.05$). The AUC in Mb-U was positively correlated with MPO-U ($r = 0.688$, $p < 0.01$) and CK-S ($r = 0.582$, $p < 0.05$) (Table 4) (Fig. 2).

DISCUSSION

The Th1/Th2 cytokine balance is an important paradigm from an immunomodulatory viewpoint, and the balance of cellular and humoral immunity regulated by Th1 (IL-12, IL-2, interferon (IFN)-γ and TNF-α) and Th2 (IL-4 and IL-10) cytokines is increasingly recognized to be important in the maintenance of health

Table 4. Spearman's rank correlation coefficient matrix of inflammatory mediators, neutrophil activation and muscle damage markers.

	IL-17-P	IL-17-U	IL-23-P	IL-23-U	sRANKL-P	sRANKL-U	Mb-S	Mb-U	CK	MPO-P	MPO-U	IL-12p40-P
IL-17-P		0.015	0.288	0.068	0.064	−0.275	−0.090	0.046	0.336	−0.222	−0.336	0.613*
IL-17-U	0.015		0.262	0.899**	−0.077	0.622*	0.451	0.960**	0.543*	−0.121	0.719**	0.143
IL-23-P	0.288	0.262		0.051	0.213	0.147	0.112	0.138	0.429	−0.169	0.130	0.622*
IL-23-U	0.068	0.899**	0.051		−0.138	0.618*	0.587*	0.947**	0.587*	−0.160	0.701**	0.156
sRANKL-P	0.064	−0.077	0.213	−0.138		−0.033	0.099	−0.112	0.130	0.398	−0.244	−0.112
sRANKL-U	−0.275	0.622*	0.147	0.618*	−0.033		0.675**	0.644*	0.297	0.143	0.684**	−0.077
Mb-S	−0.090	0.451	0.112	0.587*	0.099	0.675**		0.534*	0.125	0.262	0.578*	0.213
Mb-U	0.046	0.960**	0.138	0.947**	−0.112	0.644*	0.534*		0.582*	−0.095	0.688**	0.152
CK	0.336	0.543*	0.429	0.587*	0.130	0.297	0.125	0.582*		−0.147	0.196	0.455
MPO-P	−0.222	−0.121	−0.169	−0.160	0.398	0.143	0.262	−0.095	−0.147		−0.073	−0.327
MPO-U	−0.336	0.719**	0.130	0.701**	−0.244	0.684**	0.578*	0.688**	0.196	−0.073		0.051
IL-12p40-P	0.613*	0.143	0.622*	0.156	−0.112	−0.077	0.213	0.152	0.455	−0.327	0.051	

All data were calculated as area under the curve (AUC).
Statistics: * $p < 0.05$, ** $p < 0.01$.
AUC: total value of Pre, 0 h, 1.5 h and 3 h.
– P & -S: plasma and serum data were adjusted for alteration in plasma volume.
– U: urinary data were the gross amount into the volume of urinary excretion.
Myoglobin (Mb), creatine kinase (CK), interleukin (IL)-17, 23, 12p40, myeloperoxidase (MPO), soluble receptor activator of nuclear factor (NF)-κB ligand (sRANKL).

and the development of immune-based diseases including infections, autoimmune, allergic and asthmatic diseases (21). Several studies have reported that blood concentrations of Th1 cytokines show no change or decrease, and that peripheral blood production of Th1 cytokines by lymphocytes decreases following exhaustive exercise (32, 36). In this study, the plasma concentrations of the Th1 cytokines IL-12 (0.27 ± 0.16 pg/ml), IL-2 (0.24 ± 0.27 pg/ml), IFN-γ (0.12 ± 0.23 pg/ml) and TNF-α (0.09 ± 0.04 pg/ml) were low immediately after the race. On the other hand, several studies have reported that blood concentrations of Th2 cytokines (IL-4 and IL-10) and anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1ra) increase following exhaustive exercise (13, 19, 26). In the present study, the plasma concentration of IL-10 increased significantly immediately after the race and IL-1ra also increased markedly 1.5 h after the race. It has been reported that plasma IL-4 increased several hours after exhaustive exercise (32). In the present study, the plasma concentration of IL-4 was low immediately (0.33 ± 0.15 pg/ml), 1.5 h (0.30 ± 0.10 pg/ml) and 3 h (0.24 ± 0.08pg/ml) after the race. Th1 and Th2 cell differentiations depend on their respective effector cytokines (i.e., IFN-γ and IL-4, respectively) (4). Therefore, it might be difficult to state that Th2 cytokine responses occurred during exercise at least in the circulation.

Aside from Th1 and Th2 cytokines, a third family of effector T-cells, Th17 cells are also important to induce inflammation. Th17 cell development can occur in the presence of IL-6 and moreover in the absence of IFN-γ and IL-4 (1, 36). IL-23 is not involved in the initial differentiation of Th17 cells (14), however, IL-23 promotes the production of IL-17 from Th17 cells (16). In this study, the plasma concentrations of IFN-γ (0.12 ± 0.23 pg/ml) and IL-4 (0.33 ± 0.15 pg/ml) were lower than IL-17 (2.0 ± 0.7 pg/ml) immediately after the race, whereas the plasma concentration of IL-6 was markedly elevated immediately after the race (+26.0×), and this response occurred earlier than the IL-1β and TNF-α responses. Moreover, IL-12 (IL-12p70) is a heterodimeric molecule formed by the subunits p35 and p40 and classified as a major immunomodulatory cytokine promot-

ing the differentiation of Th1 cells, whereas IL-12p40 is a homodimer and acts as an antagonist of IL-12 (6) and has recently been recognized as having some homology with IL-23 (8). IL-23 is a heterodimeric molecule formed by subunits p40 and p19, and IL-12p40 is a monodimeric molecule formed by subunit p40. Hence, IL-12p40 and IL-23 might share p40 to induce production of IL-17. The plasma concentration of IL-12 (0.27 ± 0.16 pg/ml) was much lower than IL-12p40 (118.7 ± 51.4 pg/ml) immediately after the race in this study. Therefore, we examined the associations between these cytokines. Positive correlations were found for changes in the plasma concentrations of IL-12p40 and IL-17 and IL-23. That is, it might be possible that released IL-6 induced IL-17, IL-23 and IL-12p40, activated neutrophils and/or monocytes and was related to inflammation.

We observed more close associations in the urinary analyses. IL-17 was correlated with IL-23, MPO, Mb and sRANKL. IL-23 was also correlated with MPO, Mb and sRANKL. MPO was correlated with Mb and sRANKL. Mb was correlated with sRANKL. MPO is an activation marker of neutrophils, Mb is a marker of muscle damage and sRANKL is a factor of activated NF-κB, and these markers were closely correlated. Taken together, these findings suggest that IL-17 induced by IL-6 and IL-23 activates sRANKL. Moreover, our results suggest that IL-17 and IL-23 might promote neutrophil activation and muscle damage following prolonged endurance exercise (Fig. 2).

As mentioned earlier, we observed that plasma concentrations of IFN-γ and IL-4 were low, whereas the urinary excretion of these cytokines were large following exhaustive exercise in this study which implies that IFN-γ and IL-4 production was increased during exercise. Therefore, it might be possible that the differentiation of naive CD4+ T cells to Th17 cells was suppressed in the presence of IFN-γ and IL-4 during and following exhaustive endurance exercise (9, 36). Moreover, because there were trends for plasma IL-17 and IL-23 concentrations to increase following exercise and urinary excretion of IL-6 was not correlated with IL-17, IL-23, MPO, Mb and sRANKL, it might be possible that IL-17 was produced by either Th17 cells or by other cells (2, 4, 5, 37). Further research is needed to determine the mechanisms influencing the plasma concentrations and urinary excretion of each cytokine 3 h after exhaustive endurance exercise, and to clarify which cells produce IL-17 in relation to neutrophil activation and muscle damage during and following prolonged endurance exercise.

In conclusion, it is suggested that IL-17 induced by IL-6 and activated by IL-23 might promote neutrophil activation and muscle damage in a different way from the classical proinflammatory cytokines IL-1β and TNF-α following prolonged endurance exercise. However, further research is needed to clarify the cells that produce IL-17 in relation to neutrophil activation and muscle damage during and following prolonged endurance exercise.

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