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ORIGINAL ARTICLE

An ultra-endurance event leads to changes in circulating regulatory T-cells, CD4+ naïve and CD8+ effector memory T-cells in the 48 h post-race recovery period

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Abstract

Purpose Exercise is known to acutely affect T-lymphocyte populations in the peripheral blood, which is intensity- and duration-dependent. However, effects of longer duration endurance exercise (>5 h) on T-cells in the days following are unknown. The aim of this study was to investigate the circulating T-cell changes that occur in response to an ultra-endurance event, which may provide insight into the inflammatory response to ultra-endurance exercise.

Methods Ten individuals (m = 7, f = 3) completing an Ironman 70.3 event volunteered for the study. Peripheral blood samples were taken 1–2 days pre-race (PRE-RACE), and 1 day (RACE + 1) and 2 days (RACE + 2) post-race, with circulating T-cells enumerated by flow cytometry (total CD3+, CD4+ and CD8+ T-cells, regulatory T-cells [CD4+CD25+CD127−; T_{REG}], naïve [CD27+CD45RA+; NA], central memory [CD27+CD45RA−; CM], effector memory [CD27−CD45RA−; EM], and effector memory CD45RA+ [CD27−CD45RA+; EMRA]).

Results There were no changes in total CD3+, CD4+ and CD8+ T-cells. T_{REG} RACE + 1 was significantly higher compared to PRE-RACE, as were the proportion of CD4+ NA cells and CD8+ CM cells at RACE + 2; CD8+ EM cells fell at RACE + 2 (absolute counts and proportion).

Conclusion In conclusion, the ultra-endurance event evoked T-cell changes over the 48 h recovery period, with an increase in T-cells that regulate the immune response, and a reduction in circulating EM T-cells, most likely trafficked to sites of tissue damage and inflammation.

Keywords Endurance · Lymphocytes · Exercise · Adaptive immunity · T-cells

Abbreviations

ANOVA	Analysis of variance
BLa	Blood lactate
CM	Central memory T-cells
CRP	C-reactive protein
EM	Effector memory T-cells
HR	Heart rate
IM70.3	Half ironman triathlon (70.3 miles)

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NA	Naïve T-cells
TEMRA	Terminally differentiated T-cells
T_{REG}	Regulatory T-cells
PBMC	Peripheral blood mononuclear cells
URTI	Upper respiratory tract infection
$\dot{V}O_2$max	Maximum oxygen uptake

Introduction

It is well established that long duration (>1.5 h) strenuous exercise can modulate immune function (Nieman 2007). Such bouts of exercise may affect immune function via modulating neutrophils (Quindry et al. 2003), monocyte/macrophage (Slusher et al. 2018) and/or lymphocyte function (Shaw et al. 2018). Indeed, we consistently observe drastic changes in T-cell populations in response to acute exercise bouts, with a dramatic rise in circulating T-cells immediately post-exercise (lymphocytosis), returning to baseline, or even below baseline (lymphocytopenia) within 30–60 min after the cessation of exercise (Ross et al. 2016; Turner et al. 2010). These effects are largely due to exercise-induced catecholamine release, for example, increased β_2 adrenergic signalling (Dimitrov et al. 2010; Kruger et al. 2008) as a result of elevated circulating epinephrine and norepinephrine (Anane et al. 2009).

It is unlikely that the lymphocytopenia observed in the 30–60 min post-exercise period is reflective of depressed immune function (Campbell and Turner 2018, 2019), as cells are most likely redistributed to lymph tissues, lung and gut for immune surveillance (Kruger and Mooren 2007) or skeletal muscle to help coordinate muscle repair (Deyhle and Hyldahl 2018). We observed increased circulating T-cell subsets (namely CD4+ T-helper cells and regulatory T-cells [T_{REG}]) 24 h post-marathon (Clifford et al. 2017), potentially indicative of greater immune surveillance, and regulation of the immune response to tissue damage and inflammation. However, due to the T-cell pool consisting of a wide variety of subsets, and the fact that these subsets respond differently to exercise (Simpson et al. 2007), it is likely that a long-duration, endurance exercise bout stimulates divergent responses across T-cell phenotypes. Therefore, the aim of the current study was to investigate the influence of a strenuous, long-duration endurance event (Ironman 70.3 race) on a wide range of circulating T-cell subsets (including total CD3+, CD4+, CD8+ T-cells, T_{REG}, and naïve [NA], central memory [CM], effector memory [EM], and effector memory CD45RA+ [EMRA] cells). It was hypothesised that the ultra-endurance event would lead to significant elevations in cytotoxic and effector T-cells in the 48 h post-event.

Materials and methods

Ethical approval

The authors confirm that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Ethical approval was granted by the Edinburgh Napier University Research and Ethics Governance Committee. Written informed consent was obtained from all participants prior to commencement of the study.

Participants

Ten (m = 7, f = 3) participants, aged 22–48 years, non-obese (<28 kg m^2), normotensive (blood pressure < 140/90 mmHg) volunteered to take part in the study. All participants were already enrolled in an Ironman 70.3 (IM70.3) event prior to volunteering for the study. Participants visited the Human Performance Laboratory 1–2 days prior to the race for blood sampling (PRE-RACE), as well as the following 2 mornings after the race (RACE + 1, RACE + 2, respectively). Participants visited the lab between 7:30 a.m. and 9:00 a.m. on each day in a fasted state for peripheral blood sampling and other laboratory measures. Baseline characteristics are shown in Table 1.

Assessment of peak oxygen consumption and lactate threshold

Within 3 weeks of the race, but no closer than 1 week of the race, participants underwent an incremental cycling exercise test on a magnetically braked cycle ergometer (Velotron, RacerMate, USA) to volitional exhaustion to quantify lactate

Table 1 Participant characteristics and exercise trial data

	Participants (n = 10, 7 = m, 3 = f)
Age (years)	40 ± 9
Body mass index (BMI; kg m^2)	22.2 ± 2.0
Systolic blood pressure (mmHg)	120 ± 7
Diastolic blood pressure (mmHg)	71 ± 2
$\dot{V}O_2$peak (mL kg min^{-1})	56.5 ± 5.3
Power output @ $\dot{V}O_2$peak (W)	347 ± 44
Power output @ 4 mmol L^{-1} BLa (W)	244 ± 46
$\dot{V}O_2$ @ 4 mmol L^{-1} BLa (% of $\dot{V}O_2$peak)	78.7 ± 8.4
Race time (hh:mm:ss) [range]	5:53:44 [05:30:23–6:20:28]

Values shown are mean ± standard deviation

BLa blood lactate

threshold and maximum oxygen consumption ($\dot{V}O_2$max). The test began at 100 W for males, and 75 W for females, and increased by 25 W every 3 min to quantify lactate threshold (Messias et al. 2018), and conducted in line with recommendations from Bentley et al. (2007). Blood lactate (BLa) was measured using capillary finger prick blood samples using a portable lactate analyser (Lactate Pro 2; Arkray Inc., Japan) at the end of each stage. Once the participant reached or surpassed BLa of 4 mmol L^{-1}, the intensity of exercise was increased by 25 W every minute to exhaustion. The intensity (%$\dot{V}O_2$max) at which the participant exhibited a BLa of 4 mmol L^{-1} was recorded. Heart rate (HR) was monitored using HR telemetry (Polar, Finland).

Blood sampling and T-cell phenotyping

Fasting blood samples were taken from participants 1–2 days prior to the race (PRE-RACE), and the two mornings after the race (RACE + 1, RACE + 2) by a trained phlebotomist using venepuncture. Peripheral blood was drawn into 6 mL vacutainers spray coated with EDTA anti-coagulant (BD Biosciences, UK), with the first 3 mL of peripheral blood discarded. Total blood differential leukocyte counts were determined using an automated haematology analyser (XS 1000i, Sysmex, UK). Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation as described elsewhere (Ross et al. 2016). To quantify T_{REG} cells, cells were analysed on the day of blood collection. For the remainder of T-cell subsets, cells were frozen in RPMI and 10% dimethyl sulfoxide at −80 °C until batch analysis.

For T_{REG} cell analysis, PBMCs were stained with monoclonal antibodies anti-CD3, anti-CD4-BV650, anti-CD25-BV510, and anti-CD127-FITC (all BD Biosciences, UK) and left to incubate at 4 °C in the dark for 30 min prior to enumeration by flow cytometry (BD FACS Celesta, BD Biosciences, UK). T_{REG} cells were defined as CD3+CD4+CD25+CD127− cells (see Fig. 1 for flow cytometry gating strategy).

For CD4+ and CD8+ NA, CM, EM and EMRA phenotyping, these were performed in batch analysis on stored PBMCs. Frozen PBMCs were thawed on ice and subsequently

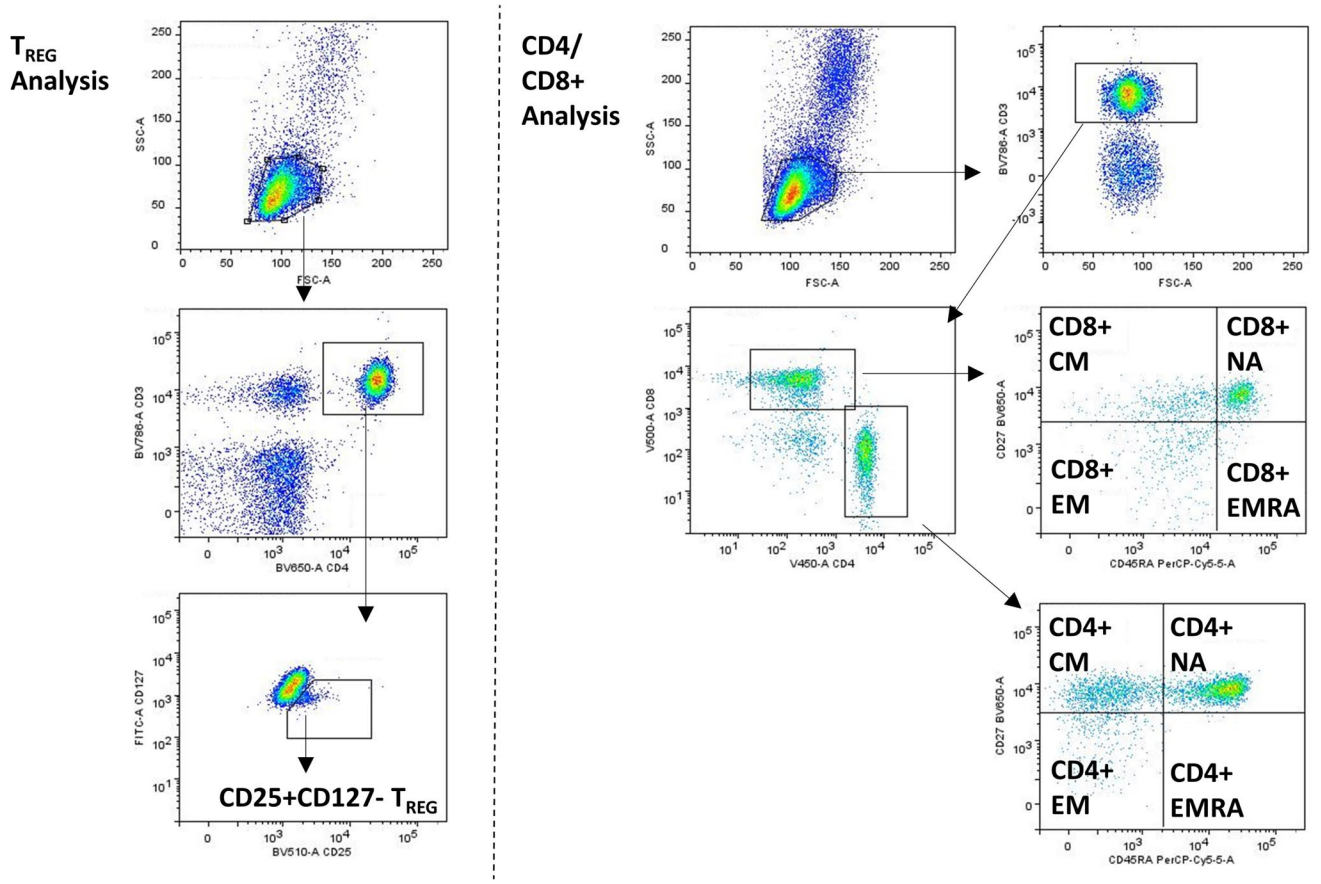

Fig. 1 Flow cytometric quantification of T-lymphocyte populations. Side scatter vs. forward scatter for identification of lymphocyte gate, followed by gating CD3+ events. Subsequent gating for CD4+ T_{REG} are shown (CD25+CD127−), and CD4+ and CD8+ NA (CD27+CD45RA+), CM (CD27+CD45RA−), EM (CD27−CD45RA−), and EMRA (CD27−CD45RA+) events are shown

Table 2 T-cell population phenotyping

T-cell population	Phenotype
Total T-cells	CD3+
CD4+ T-cells	CD3+CD4+
CD8+ T-cells	CD3+CD8+
T_{REG}	CD3+CD4+CD25+CD127−
CD4+ NA	CD3+CD4+CD27+CD45RA+
CD4+ CM	CD3+CD4+CD27+CD45RA−
CD4+ EM	CD3+CD4+CD27−CD45RA−
CD4+ EMRA	CD3+CD4+CD27−CD45RA+
CD8+ NA	CD3+CD8+CD27+CD45RA+
CD8+ CM	CD3+CD8+CD27+CD45RA−
CD8+ EM	CD3+CD8+CD27−CD45RA−
CD8+ EMRA	CD3+CD8+CD27−CD45RA+

T_{REG} regulatory T-cells, *CM* central memory, *EM* effector memory

stained with monoclonal antibodies against anti-CD3 BV786, anti-CD4 V450, anti-CD8 V500, anti-CD27 BV650 and anti-CD45RA PerCP Cy5.5 (all BD Biosciences, UK). NA, CM, EM and EMRA cells were defined as follows: CD27+CD45RA+, CD27+CD45RA−, CD27−CD45RA−, CD27−CD45RA+, respectively (Table 2). Cells were incubated with antibodies for 30 min at 4 °C prior to enumeration by flow cytometry. A minimum of 100,000 mononuclear cells were enumerated per sample for each T-cell panel. Flow cytometric gating strategy is shown in Fig. 1.

Statistical analysis

All data were assessed for normality using the Shapiro–Wilk test for normality. All data were deemed to be normal for subsequent analyses. For comparisons between PRE-RACE, RACE + 1, and RACE + 2 for all cell populations, several one-way repeated measures analyses of variance (ANOVA) were performed. Main effects of time were determined, and where there were significant main effects and where there were significant main effects, Tukey's multiple comparisons tests were performed to detect specific differences across the different visits (PRE-RACE, RACE + 1, RACE + 2). Data was analysed using SPSS Statistics for Windows (SPSS v26, IBM, Corp, New York, USA) and figures designed using GraphPad (GraphPad Prism 6.4.1, Dotmatics, USA). Significance alpha was set at $p < 0.05$. All data are presented as mean ± SD unless otherwise stated.

Results

Influence of IM70.3 race on peripheral blood mononuclear cells

Our data show that there were significant elevations in circulating neutrophils and monocytes 1 day post-race compared to pre-race (neutrophils: PRE-RACE 2200 ± 777 cells μL^{-1} vs. RACE + 1 3249 ± 875 cells μL^{-1}, $p = 0.001$; monocytes: PRE-RACE 422 ± 145 cells μL^{-1} vs. RACE + 1 585 ± 174 cells μL^{-1}, $p = 0.002$). Both neutrophils and monocytes returned to similar to baseline levels after 48 h post-race. Total lymphocyte numbers did not change across the 3 days (see Table 3).

Influence of IM70.3 race on T-lymphocyte subpopulations

There was no effect of the ultra-endurance event on absolute counts (cells μL^{-1}) of peripheral blood CD3+ T-cell ($F = 2.582$, $p = 0.103$), CD4+ T-cells ($F = 3.266$, $p = 0.062$), or CD8+ T-cells ($F = 0.209$, $p = 0.814$). There were no significant changes in proportion of CD4+ cells (% of CD3+) ($F = 2.191$, $p = 0.141$), however, there was an increase in proportion of CD8+ T-cells (% of CD3) from RACE + 1 to RACE + 2 (main effect $F = 5.462$, $p = 0.014$, RACE + 1: 28.1 ± 6.7%, RACE + 2: 31.9 ± 8.1%, $p = 0.011$). Data are shown in Table 4.

There were no significant changes in absolute counts of CD4+ NA ($F = 1.041$, $p = 0.373$), CD4+ CM ($F = 2.626$, $p = 0.100$), CD4+ EM ($F = 3.414$, $p = 0.055$), or CD4+ EMRA cells ($F = 1.735$, $p = 0.205$). Likewise, there were no significant changes in absolute counts of CD8+ NA ($F = 1.013$, $p = 0.383$), CD8+ CM ($F = 3.375$, $p = 0.057$), or CD8+ EMRA cells ($F = 1.459$, $p = 0.259$). There was a

Table 3 Changes in circulating leukocyte number in response to ultra-endurance event

	PRE-RACE	RACE + 1	RACE + 2	Main effects (*F* value, *p* value)
Neutrophils	2200 ± 777	3249 ± 875$^{\delta,\gamma}$	2399 ± 707	10.590, 0.001**
Monocytes	422 ± 145	585 ± 174$^{\delta,\gamma}$	466 ± 123	9.007, 0.002**
Lymphocytes	1668 ± 363	1836 ± 506	1684 ± 478	2.190, 0.141

Values shown are mean ± SD

** $p < 0.001$ main effect

$^{\delta}$Significantly different from PRE-RACE

$^{\gamma}$Significantly different from RACE + 2

Table 4 Changes in circulating T-cell populations in response to ultra-endurance event

	PRE-RACE	RACE + 1	RACE + 2	Main effect (*F* value, *p* value)
CD3+ T-cells				
Cells μL^{-1}	1184 ± 310	1325 ± 463	1160 ± 460	2.582, 0.103
CD4+ T-cells				
Cells μL^{-1}	731 ± 195	851 ± 299	707 ± 273	3.266, 0.062
% of CD3+	63 ± 11	65 ± 9	62 ± 10	2.191, 0.141
CD8+ T-cells				
Cells μL^{-1}	365 ± 154	381 ± 174	372 ± 174	0.209, 0.814
% of CD3+	30 ± 9	28 ± 7$^{\gamma}$	32 ± 8	5.462, 0.014*

Values shown are mean ± SD

*$p < 0.005$, main effect

$^{\gamma}$Significantly different from RACE + 2

significant decline in absolute counts of CD8+ EM cells from PRE-RACE to RACE + 2 (main effect $F = 3.929$, $p = 0.038$; PRE-RACE: 110 ± 77 cells μL^{-1}, RACE + 2: 75 ± 29 cells μL^{-1}, $p = 0.040$).

There were largely no significant changes in proportional data (cells as % of parent cell, e.g. % of CD4+ or % of CD8+). There were no changes in CD4+ CM ($F = 0.229$, $p = 0.799$), EM ($F = 1.444$, $p = 0.262$), EMRA ($F = 1.29$, $p = 0.299$), or CD8+ NA ($F = 0.733$, $p = 0.494$), or EMRA ($F = 2.902$, $p = 0.081$) cells, but noted increases in proportion of CD4+ NA and CD8+ CM from RACE + 1 to RACE + 2 (CD4+ NA: main effect $F = 3.978$, $p = 0.037$; RACE + 1: 52.0 ± 9.6%, RACE + 2: 57.0 ± 11.8%, $p = 0.041$; CD8+ CM: main effect $F = 5.453$, $p = 0.014$; RACE + 1: 13.0 ± 10.8%, RACE + 2: 20.7 ± 8.4%, $p = 0.016$), with a significant drop in proportion of CD8+ EM cells from PRE-RACE to RACE + 2 (main effect $F = 4.041$, $p = 0.036$; PRE-RACE: 28.8 ± 11.0%, RACE + 2: 21.9 ± 6.9%, $p = 0.046$).

CD4+ and CD8+ T-cell data are shown in Figs. 2 and 3.

Despite no changes in total CD4+ cell number, circulating T_{REG} cells were significantly elevated on RACE + 1 compared to PRE-RACE (absolute counts: main effect $F = 41.730$, $p < 0.001$; PRE-RACE: 16 ± 6 cells μL^{-1}, RACE + 1: 55 ± 20 cells μL^{-1}, $p < 0.001$; proportional data as % of CD4+: main effect $F = 61.230$, $p < 0.001$; PRE-RACE: 2.3 ± 0.5%, RACE + 1: 6.9 ± 1.8%, $p < 0.001$). These values returned to baseline levels at RACE + 2. T_{REG} data are shown in Fig. 4.

Discussion

Our data show that an ultra-endurance event (IM 70.3), significantly altered circulating leukocytes in the 2 days after the event. Namely, there were elevations in neutrophils and monocytes (RACE + 1 vs. PRE-RACE), possibly reflective of inflammatory response to extreme exercise (Comassi et al. 2015; Shin and Lee 2013; Stelzer et al. 2015), but also alterations in specific T-lymphocyte subsets, with elevations in T_{REG}, CD4+ NA, CD8+ CM, and a drop in CD8+ EM cells, with no other alterations in other T-lymphocyte subsets (CD4+ CM, EM, EMRA, CD8+ NA, EMRA).

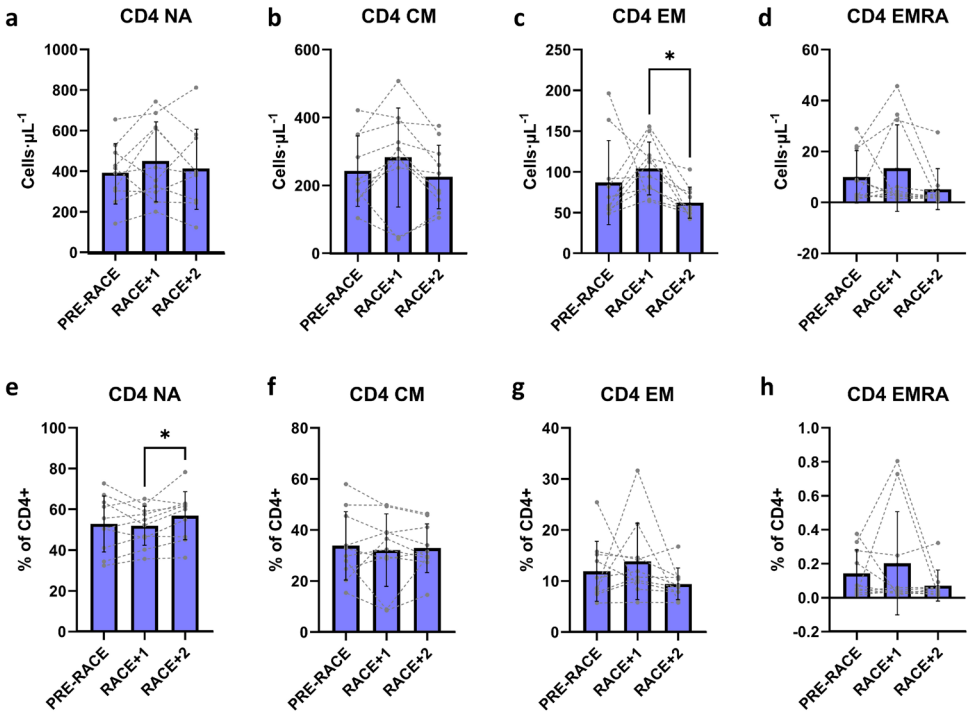

Fig. 2 CD4+ T-cell changes in 48-h post-exercise period in response to ultra-endurance race ($n = 10$). CD4+ naïve (CD4+ NA, **a**), central memory (CD4+ CM, **b**), effector memory (CD4+ EM, **c**) and effector memory CD45RA+ (CD4+ EMRA, **d**) absolute counts over 3 days (PRE-RACE, RACE + 1, RACE + 2). Corresponding proportional data are shown in **e**–**h**. Values shown are mean ± SD and individual datapoints, * $p < 0.05$

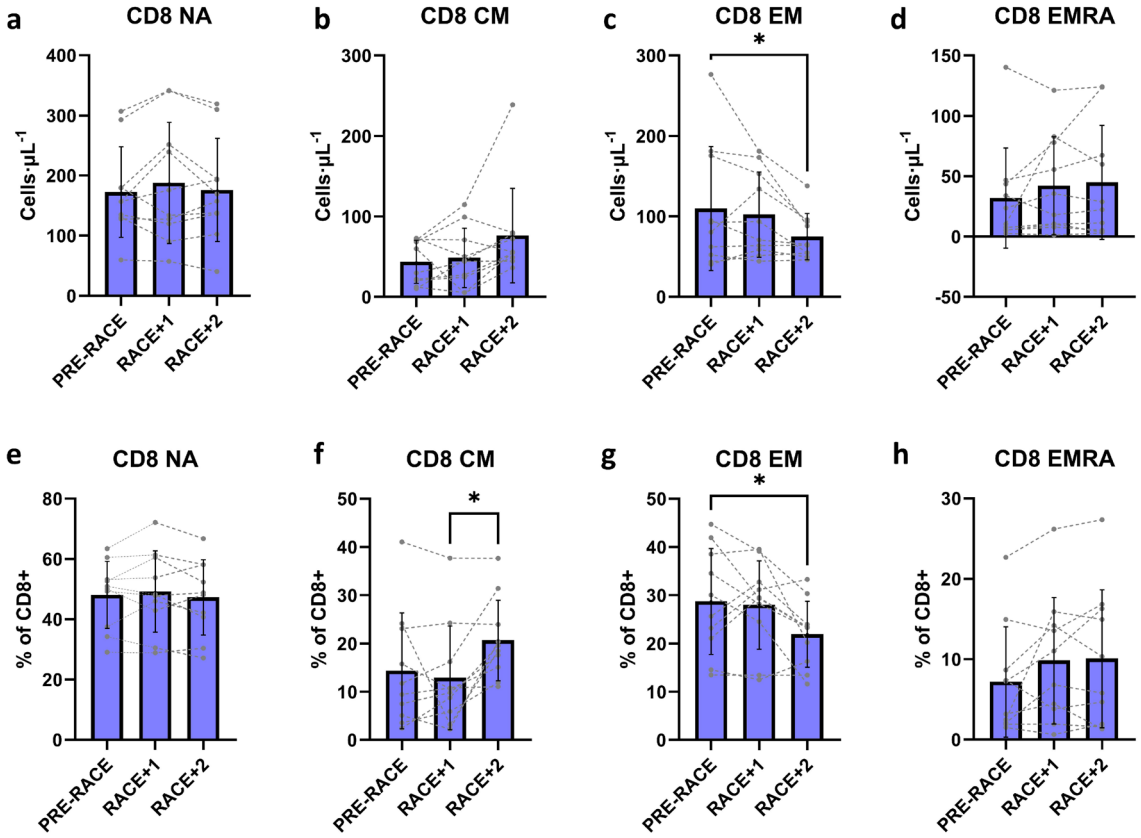

Fig. 3 CD8+ T-cell changes in 48-h post-exercise period in response to ultra-endurance race ($n = 10$). CD8+ naïve (CD8+ NA, **a**), central memory (CD8+ CM, **b**), effector memory (CD8+ EM, **c**) and effector memory CD45RA+ (CD8+ EMRA, **d**) absolute counts over 3 days (PRE-RACE, RACE + 1, RACE + 2). Corresponding proportional data are shown in **e**–**h**. Values shown are mean ± SD and individual datapoints, * $p < 0.05$

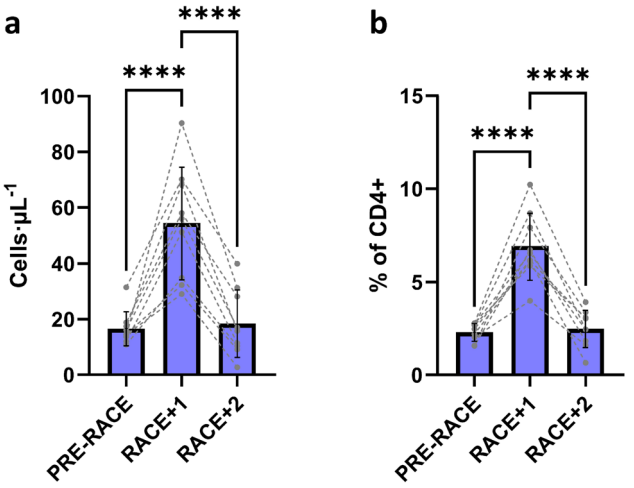

Fig. 4 CD4+ regulatory T-cell (CD4+ T_{REG}) changes in 48-h post-exercise period in response to ultra-endurance race ($n = 10$). **a** absolute counts over 3 days (PRE-RACE, RACE + 1, RACE + 2), **b** corresponding proportional data. Values shown are mean ± SD and individual datapoints, **** $p < 0.001$

The neutrophil and monocyte data suggest a strong inflammatory response to the ultra-endurance bout. Acute exercise is known to increase neutrophils and monocytes, which can be elevated for up to 6–24 h post-exercise (Peake et al. 2017; Walsh et al. 2011). In this study both neutrophils and monocytes were elevated at 24 h post-race (RACE + 1), which returned to near baseline (PRE-RACE) levels by 48 h post-race (RACE + 2). The neutrophilia may be due to cortisol-stimulated bone marrow release (McCarthy and Dale 1988), or other inflammatory factors which are also responsible for mobilisation of cells from the bone marrow, such as interleukin-6 (IL-6), glucocorticoids, and granulocyte colony stimulating factor (Suzuki et al. 2003). These neutrophils, once in the circulation, can be attracted to muscle damage by chemoattractants (Tsivitse et al. 2005), and subsequently the cells migrate into the affected muscle tissue (McLoughlin et al. 2003). Monocyte elevations are also likely due to increased bone marrow production and release (Shi and Pamer 2011), which also infiltrate skeletal muscle after tissue damaging exercise (Marklund et al. 2013; McLoughlin et al. 2003) such as ultra-endurance bouts (Marklund et al. 2013), subsequently transitioning into

macrophages. These tissue infiltrating macrophages contribute to tissue repair and regeneration, and without this process of immune cell infiltration, recovery from tissue damaging exercise is limited (Tidball and Wehling-Henricks 2007). Studies have demonstrated that monocytes/macrophages can contribute to tissue repair via clearing debris (Arnold et al. 2007), stimulating muscle satellite cell differentiation (Tidball and Wehling-Henricks 2007), and promoting angiogenesis (Latroche et al. 2017; Ochoa et al. 2007)thus, these cells are a key player in the recovery from ultra-endurance exercise, where tissue damage is extensive (Rubio-Arias et al. 2019).

This is the first study to enumerate specific circulating T-cell subsets in the days after an ultra-endurance event. Previous work has demonstrated that exercise results in an acute lymphocytosis during exercise followed by lymphocytopenia in the minutes post-exercise (Rooney et al. 2018), which is likely to have occurred in this study. The longer-term changes (days post-race vs. minutes/hours post-race) are likely reflecting the chronic inflammatory processes taking place in muscle, lung and/or other peripheral tissues that result from such exercise. Turner et al. (2013) observed elevated C-reactive protein (CRP) after a single-stage, multi-day 233 km running event (100-fold for 24 h, eight-fold after 7 days post-race), and Rubio-Arias et al. (2019), whilst also observing elevations in CRP over 72 h post-ultra race, observed significant muscle damage (plasma creatine kinase) over the same timepoints. These studies and ours indicate that an ultra-endurance event represents a significant inflammatory stimulus, which could be contributing to the peripheral blood immune cell components, due possibly to trafficking of key immune cell subsets into inflamed/damaged tissues.

Significant elevations in T_{REG} absolute counts and proportions were observed RACE + 1 vs. PRE-RACE. The function of these cells is primarily to regulate the immune response to infection and inflammation (Littringer et al. 2018; Lei et al. 2015), and the elevation of these cells in the peripheral blood 24 h post-race may indicate upregulated production of these cells to control inflammatory processes, or an active transport of these cells from lymph stores into the blood for re-direction to inflamed tissue (such as muscle and lungs). An alternate role for these cells in the context of recovery from extreme exercise, could be a contribution to repair and regeneration (Li et al. 2018). Recent evidence shows that these cells contain potent regenerative proteins, such as amphiregulin (Liu et al. 2022; Zaiss et al. 2015) which can promote tissue repair through epidermal growth factor signalling (Zaiss et al. 2015) and have been implicated in myocardial muscle repair post-myocardial infarction (Zhuang et al. 2022) as well as wound healing (Zaiss et al. 2019). Therefore, T_{REG} elevations within 24 h post-event could be contributing to a muscle tissue remodelling process, as well as suppressing macrophage- and other T-cell mediated inflammatory responses. Recently, Langston et al. (2023) demonstrated the role of T_{REG} in muscle post-exercise, with T_{REG} infiltration into skeletal muscle post-exercise promoting the long-term exercise training aerobic adaptations. However, this study was performed in mice, and thus human studies should now be undertaken to elucidate the role of T_{REG} changes with exercise in muscle adaptation. It must be noted that in this study, T_{REG} were measured as CD3+CD4+CD25+CD127−, and we did not include FoxP3 in our flow cytometry assay. CD127(−) was used to enumerate T_{REG} cells in our sample, as CD127 is downregulated in these cells and correlates well with T_{REG} suppressor functions (Liu et al. 2006; Yu et al. 2012), and CD4+CD25+CD127− cells were found to have greater suppressive function than broadly CD4+CD25+ T-cells (Yu et al. 2012). However, some CD127+ T-cells may also express FoxP3 (Klein et al. 2010), and therefore, the CD127low/− phenotype may be excluding a small proportion of T_{REG} cells in our study.

CD4+ and CD8+ EM absolute counts were reduced 48 h post-race, resulting in proportional increases in CD4+ NA cells. This drop in CD4+ and CD8+ EM absolute count could be explained by (1) selective apoptosis of these cells, or (2) egress of these cells into peripheral tissues at this timepoint. It is known that a small proportion of T-cells acutely express pro-apoptotic markers (Navalta et al. 2013; Kruger et al. 2016), with high intensity exercise preferentially promoting apoptosis in highly differentiated subsets, such as CD4+ and CD8+ EM and EMRA cells (Kruger et al. 2016). However, as we did not observe declines in CD4+ or CD8+ EMRA cells in this study, apoptosis may not be the only reason we observed changes in T-cells.

Upon exercise cessation, T-cells egress from the circulation into peripheral tissues (Kruger and Mooren 2007), with highly differentiated subsets displaying preferential egress (Graff et al. 2018), likely mediated by greater β_2 adrenergic receptor expression on such subsets (Graff et al. 2018). Whilst we observed a reduction in CD4+ and CD8+ EM subsets 48 h post-exercise, it is likely that the reason for this reduction differs to that observed minutes post-exercise. The reduction of these cells in the circulation 48 h post-exercise is most likely due to trafficking to sites of muscle damage (Deyhle et al. 2020), with evidence suggesting an accumulation of CD4+, CD8+ T-cells with an effector phenotype in damaged skeletal muscle tissue in male Lewis rats (Deyhle et al. 2020), as well as in ultra-endurance athletes after a 24 h endurance bout of exercise (Marklund et al. 2013). Both studies documented elevations in skeletal muscle infiltration of CD8+ T-cells, with the former demonstrating a greater infiltration of CD4+ T-cells than CD8+ T-cells (Deyhle et al. 2020).

There is some argument for the reduction in CD4+ and CD8+ EM cells in the circulation to reflect suppressed immune function. However, this has been debated extensively (Campbell and Turner 2018; Simpson et al. 2020). The participants in the current study completed a 28-day upper respiratory tract infection (URTI) symptom questionnaire after the event (data not shown; Wisconsin Upper Respiratory Symptom Survey WURSS-11) (Obasi et al. 2014). Out of ten participants, 3 reported feeling sick within the 28 days, and this was unrelated to extent of changes within the T-cell phenotypes. This study was not designed to assess immune function and infection risk in these individuals, and thus more robust measures of URTI infections/symptoms should be incorporated into larger studies of this sort, as well as including appropriate controls. As a result, we cannot conclude whether the changes in EM absolute counts and proportions were resulting in elevated infection risk.

Limitations

In this study, dietary behaviours post-race were not controlled, however, participants were encouraged to keep the same evening and morning routine for each blood sampling timepoint. Due to the event being a race in nature, intensity of the exercise (swim, cycle, run) could not be controlled, therefore the high variability in the T-cell data may be due to differences in finishing time and/or relative intensity. Inflammatory biomarkers, including markers of tissue damage, were not evaluated in this study, and therefore we can only speculate that the immunological response observed stems from tissue damage and inflammation. However, as exercise-induced inflammation and muscle damage is documented extensively elsewhere (Rubio-Arias et al. 2019; Turner et al. 2013; Marklund et al. 2013), we are confident these are related.

Conclusion

A half ironman ultra-endurance event increased circulating T_{REG} populations and reduced circulating differentiated T-cells (EM subsets). These data reflect possible T-cell specific inflammatory processes, including trafficking of key cells to damaged and inflamed tissue, and immunoregulatory pathways, with T_{REG} subset elevations as a potential means to regulate inflammatory activity.

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Author contributions The study was conceived and designed by Mark Ross, Tom Clifford and Lesley Ingram-Sills. Data collection was performed by Mark Ross, Laura Gibson, Hannah Lithgow, Lesley Ingram-Sills, Neil Guthrie and Russell Wilson. Analysis was performed by Mark Ross, Hannah Lithgow and Lesley Ingram-Sills. The first draft of the manuscript was written by Mark Ross and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Upon acceptance of this manuscript, data will be deposited open access and freely available via Heriot-Watt University.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Anane LH, Edwards KM, Burns VE, Drayson MT, Riddell NE, van Zanten JJ, Wallace GR, Mills PJ, Bosch JA (2009) Mobilization of gammadelta T lymphocytes in response to psychological stress, exercise, and beta-agonist infusion. Brain Behav Immun 23(6):823–829. https://doi.org/10.1016/j.bbi.2009.03.003

Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, Gherardi RK, Chazaud B (2007) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 204(5):1057–1069. https://doi.org/10.1084/jem.20070075

Bentley DJ, Newell J, Bishop D (2007) Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. Sports Med 37(7):575–586. https://doi.org/10.2165/00007256-200737070-00002

Campbell JP, Turner JE (2018) Debunking the myth of exercise-induced immune suppression: redefining the impact of exercise on immunological health across the lifespan. Front Immunol 9:648. https://doi.org/10.3389/fimmu.2018.00648

Campbell JP, Turner JE (2019) There is limited existing evidence to support the common assumption that strenuous endurance exercise bouts impair immune competency. Expert Rev Clin Immunol 15(2):105–109. https://doi.org/10.1080/1744666X.2019.1548933

Clifford T, Wood MJ, Stocks P, Howatson G, Stevenson EJ, Hilkens CMU (2017) T-regulatory cells exhibit a biphasic response to prolonged endurance exercise in humans. Eur J Appl Physiol 117(8):1727–1737. https://doi.org/10.1007/s00421-017-3667-0

Comassi M, Vitolo E, Pratali L, Del Turco S, Dellanoce C, Rossi C, Santini E, Solini A (2015) Acute effects of different degrees of ultra-endurance exercise on systemic inflammatory responses. Intern Med J 45(1):74–79. https://doi.org/10.1111/imj.12625

Deyhle MR, Hyldahl RD (2018) The role of T lymphocytes in skeletal muscle repair from traumatic and contraction-induced injury. Front Physiol 9:768. https://doi.org/10.3389/fphys.2018.00768

Deyhle MR, Carlisle M, Sorensen JR, Hafen PS, Jesperson K, Ahmadi M, Hancock CR, Hyldahl RD (2020) Accumulation of skeletal muscle T cells and the repeated bout effect in rats. Med Sci Sports Exerc 52(6):1280–1293. https://doi.org/10.1249/MSS.0000000000002256

Dimitrov S, Lange T, Born J (2010) Selective mobilization of cytotoxic leukocytes by epinephrine. J Immunol 184(1):503–511. https://doi.org/10.4049/jimmunol.0902189

Graff RM, Kunz HE, Agha NH, Baker FL, Laughlin M, Bigley AB, Markofski MM, LaVoy EC, Katsanis E, Bond RA, Bollard CM, Simpson RJ (2018) beta(2)-Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of CD8+ T-cells, NK-cells and non-classical monocytes in response to acute exercise in humans. Brain Behav Immun 74:143–153. https://doi.org/10.1016/j.bbi.2018.08.017

Klein S, Kretz CC, Krammer PH, Kuhn A (2010) CD127(low/−) and FoxP3(+) expression levels characterize different regulatory T-cell populations in human peripheral blood. J Invest Dermatol 130(2):492–499. https://doi.org/10.1038/jid.2009.313

Kruger K, Mooren FC (2007) T cell homing and exercise. Exerc Immunol Rev 13:37–54

Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC (2008) Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun 22(3):324–338. https://doi.org/10.1016/j.bbi.2007.08.008

Kruger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, Gindler K, Kimmelmann L, Walscheid R, Muders K, Frech T, Eder K, Mooren FC (2016) Apoptosis of T-cell subsets after acute high-intensity interval exercise. Med Sci Sports Exerc 48(10):2021–2029. https://doi.org/10.1249/MSS.0000000000000979

Langston PK, Sun Y, Ryback BA, Mueller AL, Spiegelman BM, Benoist C, Mathis D (2023) Regulatory T cells shield muscle mitochondria from interferon-gamma-mediated damage to promote the beneficial effects of exercise. Sci Immunol 8(89):eadi5377. https://doi.org/10.1126/sciimmunol.adi5377

Latroche C, Weiss-Gayet M, Muller L, Gitiaux C, Leblanc P, Liot S, Ben-Larbi S, Abou-Khalil R, Verger N, Bardot P, Magnan M, Chretien F, Mounier R, Germain S, Chazaud B (2017) Coupling between myogenesis and angiogenesis during skeletal muscle regeneration is stimulated by restorative macrophages. Stem Cell Rep 9(6):2018–2033. https://doi.org/10.1016/j.stemcr.2017.10.027

Lei H, Schmidt-Bleek K, Dienelt A, Reinke P, Volk HD (2015) Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners. Front Pharmacol 6:184. https://doi.org/10.3389/fphar.2015.00184

Li J, Tan J, Martino MM, Lui KO (2018) Regulatory T-cells: potential regulator of tissue repair and regeneration. Front Immunol 9:585. https://doi.org/10.3389/fimmu.2018.00585

Littringer K, Moresi C, Rakebrandt N, Zhou X, Schorer M, Dolowschiak T, Kirchner F, Rost F, Keller CW, McHugh D, LeibundGut-Landmann S, Robinson MD, Joller N (2018) Common features of regulatory T cell specialization during Th1 responses. Front Immunol 9:1344. https://doi.org/10.3389/fimmu.2018.01344

Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, Gottlieb PA, Kapranov P, Gingeras TR, de St F, Groth B, Clayberger C, Soper DM, Ziegler SF, Bluestone JA (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 203(7):1701–1711. https://doi.org/10.1084/jem.20060772

Liu J, Pan L, Hong W, Chen S, Bai P, Luo W, Sun X, He F, Jia X, Cai J, Chen Y, Hu K, Song Z, Ge J, Sun A (2022) GPR174 knockdown enhances blood flow recovery in hindlimb ischemia mice model by upregulating AREG expression. Nat Commun 13(1):7519. https://doi.org/10.1038/s41467-022-35159-8

Marklund P, Mattsson CM, Wahlin-Larsson B, Ponsot E, Lindvall B, Lindvall L, Ekblom B, Kadi F (2013) Extensive inflammatory cell infiltration in human skeletal muscle in response to an ultraendurance exercise bout in experienced athletes. J Appl Physiol 114(1):66–72. https://doi.org/10.1152/japplphysiol.01538.2011

McCarthy DA, Dale MM (1988) The leucocytosis of exercise. A review and model. Sports Med 6(6):333–363. https://doi.org/10.2165/00007256-198806060-00002

McLoughlin TJ, Mylona E, Hornberger TA, Esser KA, Pizza FX (2003) Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions. J Appl Physiol 94(3):876–882. https://doi.org/10.1152/japplphysiol.00766.2002

Messias LHD, Polisel EEC, Manchado-Gobatto FB (2018) Advances of the reverse lactate threshold test: non-invasive proposal based on heart rate and effect of previous cycling experience. PLoS ONE 13(3):e0194313. https://doi.org/10.1371/journal.pone.0194313

Navalta JW, Lyons S, Prestes J, Arnett SW, Schafer M, Sobrero GL (2013) Exercise intensity and lymphocyte subset apoptosis. Int J Sports Med 34(3):268–273. https://doi.org/10.1055/s-0032-1312581

Nieman DC (2007) Marathon training and immune function. Sports Med 37(4–5):412–415. https://doi.org/10.2165/00007256-200737040-00036

Obasi CN, Brown RL, Barrett BP (2014) Item reduction of the Wisconsin upper respiratory symptom survey (WURSS-21) leads to the WURSS-11. Qual Life Res 23(4):1293–1298. https://doi.org/10.1007/s11136-013-0561-z

Ochoa O, Sun D, Reyes-Reyna SM, Waite LL, Michalek JE, McManus LM, Shireman PK (2007) Delayed angiogenesis and VEGF production in CCR2−/− mice during impaired skeletal muscle regeneration. Am J Physiol Regul Integr Comp Physiol 293(2):R651–R661. https://doi.org/10.1152/ajpregu.00069.2007

Peake JM, Neubauer O, Walsh NP, Simpson RJ (2017) Recovery of the immune system after exercise. J Appl Physiol 122(5):1077–1087. https://doi.org/10.1152/japplphysiol.00622.2016

Quindry JC, Stone WL, King J, Broeder CE (2003) The effects of acute exercise on neutrophils and plasma oxidative stress. Med Sci Sports Exerc 35(7):1139–1145. https://doi.org/10.1249/01.MSS.0000074568.82597.0B

Rooney BV, Bigley AB, LaVoy EC, Laughlin M, Pedlar C, Simpson RJ (2018) Lymphocytes and monocytes egress peripheral blood within minutes after cessation of steady state exercise: a detailed temporal analysis of leukocyte extravasation. Physiol Behav 194:260–267. https://doi.org/10.1016/j.physbeh.2018.06.008

Ross M, Tormey P, Ingram L, Simpson R, Malone E, Florida-James G (2016) A 10 km time trial running bout acutely increases the number of angiogenic T cells in the peripheral blood compartment of healthy males. Exp Physiol 101(10):1253–1264. https://doi.org/10.1113/EP085771

Rubio-Arias JA, Avila-Gandia V, Lopez-Roman FJ, Soto-Mendez F, Alcaraz PE, Ramos-Campo DJ (2019) Muscle damage and inflammation biomarkers after two ultra-endurance mountain races of different distances: 54 km vs. 111 km. Physiol Behav 205:51–57. https://doi.org/10.1016/j.physbeh.2018.10.002

Shaw DM, Merien F, Braakhuis A, Dulson D (2018) T-cells and their cytokine production: the anti-inflammatory and immunosuppressive effects of strenuous exercise. Cytokine 104:136–142. https://doi.org/10.1016/j.cyto.2017.10.001

Shi C, Pamer EG (2011) Monocyte recruitment during infection and inflammation. Nat Rev Immunol 11(11):762–774. https://doi.org/10.1038/nri3070

Shin YO, Lee JB (2013) Leukocyte chemotactic cytokine and leukocyte subset responses during ultra-marathon running. Cytokine 61(2):364–369. https://doi.org/10.1016/j.cyto.2012.11.019

Simpson RJ, Florida-James GD, Cosgrove C, Whyte GP, Macrae S, Pircher H, Guy K (2007) High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. J Appl Physiol 103(1):396–401. https://doi.org/10.1152/japplphysiol.00007.2007

Simpson RJ, Campbell JP, Gleeson M, Krüger K, Nieman DC, Pyne DB, Turner JE, Walsh NP (2020) Can exercise affect immune function to increase susceptibility to infection? Exerc Immunol Rev 26:8–22

Slusher AL, Zuniga TM, Acevedo EO (2018) Maximal exercise alters the inflammatory phenotype and response of mononuclear cells. Med Sci Sports Exerc 50(4):675–683. https://doi.org/10.1249/MSS.0000000000001480

Stelzer I, Kropfl JM, Fuchs R, Pekovits K, Mangge H, Raggam RB, Gruber HJ, Pruller F, Hofmann P, Truschnig-Wilders M, Obermayer-Pietsch B, Haushofer AC, Kessler HH, Machler P (2015) Ultra-endurance exercise induces stress and inflammation and affects circulating hematopoietic progenitor cell function. Scand J Med Sci Sports 25(5):e442–e450. https://doi.org/10.1111/sms.12347

Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, Sugawara K (2003) Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 35(2):348–355. https://doi.org/10.1249/01.MSS.0000048861.57899.04

Tidball JG, Wehling-Henricks M (2007) Macrophages promote muscle membrane repair and muscle fibre growth and regeneration during modified muscle loading in mice in vivo. J Physiol 578(Pt 1):327–336. https://doi.org/10.1113/jphysiol.2006.118265

Tsivitse SK, Mylona E, Peterson JM, Gunning WT, Pizza FX (2005) Mechanical loading and injury induce human myotubes to release neutrophil chemoattractants. Am J Physiol Cell Physiol 288(3):C721–C729. https://doi.org/10.1152/ajpcell.00237.2004

Turner JE, Aldred S, Witard OC, Drayson MT, Moss PM, Bosch JA (2010) Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. Brain Behav Immun 24(8):1362–1370. https://doi.org/10.1016/j.bbi.2010.07.239

Turner JE, Bennett SJ, Campbell JP, Bosch JA, Aldred S, Griffiths HR (2013) The antioxidant enzyme peroxiredoxin-2 is depleted in lymphocytes 7 days after ultra-endurance exercise. Free Radic Res 47(10):821–828. https://doi.org/10.3109/10715762.2013.828836

Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P (2011) Position statement. Part one: immune function and exercise. Exerc Immunol Rev 17:6–63

Yu N, Li X, Song W, Li D, Yu D, Zeng X, Li M, Leng X, Li X (2012) CD4(+)CD25 (+)CD127(low/−) T cells: a more specific Treg population in human peripheral blood. Inflammation 35(6):1773–1780. https://doi.org/10.1007/s10753-012-9496-8

Zaiss DMW, Gause WC, Osborne LC, Artis D (2015) Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. Immunity 42(2):216–226. https://doi.org/10.1016/j.immuni.2015.01.020

Zaiss DM, Minutti CM, Knipper JA (2019) Immune- and non-immune-mediated roles of regulatory T-cells during wound healing. Immunology 157(3):190–197. https://doi.org/10.1111/imm.13057

Zhuang R, Meng Q, Ma X, Shi S, Gong S, Liu J, Li M, Gu W, Li D, Zhang X, Wang Z, Ge X, Tang J, Lin F, Liang X, Zheng L, Liu Z, Zhou X (2022) CD4(+)FoxP3(+)CD73(+) regulatory T cell promotes cardiac healing post-myocardial infarction. Theranostics 12(6):2707–2721. https://doi.org/10.7150/thno.68437