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Acute aerobic exercise induces a preferential mobilisation of plasmacytoid dendritic cells into the peripheral blood in man.

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Abstract

Dendritic cells (DCs) are important sentinel cells of the immune system responsible for presenting antigen to T cells. Exercise is known to cause an acute and transient increase in the frequency of DCs in the bloodstream in humans, yet there are contradictory findings in the literature regarding the phenotypic composition of DCs mobilised during exercise, which may have implications for immune regulation and health. Accordingly, we sought to investigate the composition of DC sub-populations mobilised in response to acute aerobic exercise. Nine healthy males (age, 21.9 ± 3.6 years; height, 177.8 ± 5.4 cm; body mass, 78.9 ± 10.8 kg; body mass index, 24.9 ± 3.3 kg.m²; $\dot{V}O_{2\text{ MAX}}$, 41.5 ± 5.1 mL.kg.min⁻¹) cycled for 20 minutes at 80% $\dot{V}O_{2\text{ MAX}}$. Blood was sampled at baseline, during the final minute of exercise and 30 minutes later. Using flow cytometry, total DCs were defined as Lineage⁻ (CD3, CD19, CD20, CD14, CD56) HLA-DR⁺ and subsequently identified as plasmacytoid DCs (CD303⁺) and myeloid DCs (CD303⁻). Myeloid DCs were analysed for expression of CD1c and CD141 to yield four sub-populations; CD1c⁻CD141⁺; CD1c⁺CD141⁺; CD1c⁺CD141⁻ and CD1c⁻CD141⁻. Expression of CD205 was also analysed on all DC sub-populations to identify DCs capable of recognising apoptotic and necrotic cells. Total DCs increased by 150% during exercise ($F_{(1,10)}=60$; $p<0.05$, $\eta^2=0.9$). Plasmacytoid DCs mobilised to a greater magnitude than myeloid DCs (195 ± 131 % vs. 131 ± 100 %; $p<.05$). Among myeloid DCs, CD1c⁻CD141⁻ cells showed the largest exercise-induced mobilisation (167 ± 122 %), with a stepwise pattern observed among the remaining sub-populations: CD1c⁺CD141⁻ (79 ± 50 %), followed by CD1c⁺CD141⁺ (44 ± 41 %), with the smallest response shown by CD1c⁻CD141⁺ cells (23 ± 54 %) ($p<.05$). Among myeloid DCs, CD205⁻ cells were the most exercise responsive. All DC subsets returned to resting levels within 30 minutes of exercise cessation. These results show that there is a preferential mobilisation of

47 plasmacytoid DCs during exercise. Given the functional repertoire of plasmacytoid DCs, which
48 includes the production of interferons against viral and bacterial pathogens, these findings
49 indicate that exercise may augment immune-surveillance by preferentially mobilising effector
50 cells; these findings have general implications for the promotion of exercise for health, and
51 specifically for the optimisation of DC harvest for cancer immunotherapy.

52

1. Introduction

Acute aerobic exercise causes profound alterations to the cellular composition of peripheral blood, whereby the frequency of many leukocyte subsets increases during exercise, followed by a decline in the hours after [1]. For many types of immune cell subsets, the magnitude of change in response to exercise is usually largest among cells with the strongest effector potential [2-4]. Accordingly, this exercise-induced effect is considered a conserved evolutionary response which causes the redistribution of effector cells to peripheral tissues to conduct immune-surveillance [5]. Cells of a lymphoid lineage, such as T cells [2, 6] and natural killer (NK) cells [7], are the most widely researched. Cells with myeloid characteristics have received less attention in the exercise literature, except for a limited number of studies which have examined monocytes [8-10]. For example, it has been shown that alternatively-activated M2-like monocytes preferentially mobilise into blood during exercise [8, 9], whereas other work has shown that the most exercise responsive cells are classically-activated M1-like monocytes [10]. Studies examining the mobilisation patterns of dendritic cell (DC) subsets in response to exercise have provided equivocal evidence, despite the critical role DCs play in initiating and directing immune responses.

DCs are often considered tissue resident cells, but these sentinels of the immune system, consist of multiple sub-populations with unique functions, and many DC subsets are found transmigrating between peripheral blood and the lymphatic system [11]. The central function of these professional antigen-presenting cells (APCs) is to ingest pathogens or debris from apoptotic or necrotic cells, and subsequently process and present antigen to lymphocytes [11]. DCs also help to regulate the immune response through co-stimulatory or co-inhibitory molecules [11, 12]. The two major sub-populations of DCs are myeloid DCs and plasmacytoid

DCs [13]. Some studies have shown that immediately after 15-20 minutes of moderate intensity exercise, total DC numbers increase in blood [14, 15] with a preferential increase in plasmacytoid DCs [16]. However, other studies have shown that after more prolonged exercise, such as a marathon, myeloid DCs increase but plasmacytoid DCs may decrease immediately post-exercise [17, 18]. In light of these contradictory findings, further investigation of the DC response to exercise is warranted. In addition, greater clarity on the phenotypic composition of plasmacytoid and myeloid DCs mobilised during exercise in healthy adults is needed to provide insight into the functional and homing characteristics of exercise-responsive DCs.

DCs express high levels of MHC class II (HLA-DR) and do not express other lineage markers expressed on monocytes, T cells, B cells and NK cells, and are therefore referred to as being Lineage⁻ (CD3, CD19, CD20, CD14, CD56) HLA-DR⁺ [13, 19]. Expression of the cell surface protein CD303 enables further differentiation of plasmacytoid (CD303⁺) and myeloid DCs (CD303⁻) [20]. Among myeloid cells, four sub-populations can be identified based on CD1c and CD141 expression [21-24] (Table 1). Other cell-surface proteins, such as co-stimulatory or co-inhibitory molecules, can indicate the functional characteristics of DCs, for example receptors such as CD205 (also known as DEC-205) [25] which enables recognition of apoptotic or necrotic cells [26]. Another commonly assessed cell-surface receptor expressed on activated DCs is CD209 (also known as DC-SIGN) which recognises a wide array of ligands from viruses and bacteria, and is also involved in adhesion, migration, signalling and antigen presentation [27]. To date, the effect of exercise on DCs that express these functional markers is not known.

Clarifying the exercise-induced kinetics of DCs is important because it has been proposed that acute bouts of vigorous steady state exercise may be a strategy to optimise immune

99 competency, for example, by enhancing vaccination responses [28-31]. Additionally, it has
100 recently been proposed that exercise could be a powerful means of increasing peripheral blood
101 mononuclear cell yields for the purposes of immunotherapy [32, 33]. To date, the most targeted
102 malignancies for DC immunotherapies are melanoma, prostate cancer, glioblastoma and renal
103 cell carcinoma, but trials are being conducted with many other cancers [34, 35]. A common
104 approach is to isolate peripheral blood mononuclear cells from patients to generate monocyte-
105 derived DCs *ex vivo* with growth factors and antigen stimulation, before re-administering the cell
106 preparations [34, 36]. There are several examples of clinically effective DC immunotherapy
107 regimens, but methodologies continue to be adapted and improved, with recent emphasis on
108 harvesting DC sub-populations directly from blood, with a particular focus on either
109 plasmacytoid DCs due to their effector potential, or CD1c+ and/or CD141+ myeloid subsets for
110 their ability to cross present antigen to cytotoxic CD8+ T cells [34, 36]. Thus, if adjunctive
111 strategies such as exercise are employed to improve cell yields for DC immunotherapy, it is
112 important to understand how naturally occurring DC sub-populations respond to exercise-
113 induced stimulation. Therefore, the aim of this study was to conduct a detailed immuno-
114 phenotypic analyses of DC sub-populations present in peripheral blood before, during and after
115 an acute bout of vigorous steady state aerobic exercise.

2. Methods

2.1. Participants

Nine healthy men were included in the present analyses (age, 21.9 ± 3.6 years; height, 177.8 ± 5.4 cm; body mass, 78.9 ± 10.8 kg; body mass index, 24.9 ± 3.3 kg.m²; $\dot{V}O_{2\text{ MAX}}$, 41.5 ± 5.1 mL.kg.min⁻¹) (ethical approval reference: ERN_12-0830; University of Birmingham, UK).

These nine participants represent a sub-group from a total of ten men who took part in other investigations [37-40] with peripheral blood mononuclear cells (PBMCs) that were available for analysis following cryopreservation.

2.2. Pre-experimental procedures

Height and body mass were assessed using standard methods and cardiorespiratory fitness ($\dot{V}O_{2\text{ MAX}}$) was measured on a cycle ergometer. Expired air samples were assessed for oxygen consumption and carbon dioxide production using breath-by-breath analysis, with heart rate monitored via telemetry, and ratings of perceived exertion recorded using the Borg scale [37-40].

2.3. Exercise trial and blood sampling

At least seven days after preliminary measurements, and following an overnight fast, participants reported to the laboratory in the morning, and a blood sample was collected from a cannulated forearm vein after a 15-minute seated rest (baseline). The exercise trial consisted of steady state cycling at 80% $\dot{V}O_{2\text{ MAX}}$ for 20 minutes, at a power output determined from the $\dot{V}O_{2\text{ MAX}}$ test. Exercise intensity was monitored with breath-by-breath measurements. Heart rate and ratings of perceived exertion were recorded throughout the exercise trial. A second blood sample was

collected in the final minute of exercise at 80% $\dot{V}O_{2\text{ MAX}}$ (exercise) and a third blood sample collected post-exercise, after 30 minutes of seated rest (+30 minutes) [37-40].

2.4. Peripheral blood mononuclear cell (PBMC) isolation

Blood with potassium ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant was diluted 1:1 with Roswell Park Memorial Institute Media (RPMI), and layered on top of Ficoll paque PLUS (GE Healthcare) (2 blood:1 Ficoll), before centrifuging at $400 \times g$ for 30 minutes at 21°C. PBMCs were aspirated and washed three times in RPMI by centrifuging at $200 \times g$ for 5 minutes. Cells were re-suspended in freezing mixture (70% RPMI, 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO)) and frozen at $-1^\circ\text{C}/\text{min}$ using a freezing container (Nalgene 'Mr Frosty' Thermoscientific). Cells were stored at -80°C and analysed within six months [39, 40].

2.5. Flow cytometry

PBMCs were thawed rapidly at 37°C and washed twice in phosphate buffered saline (PBS) containing 2% FCS and 2mM EDTA by centrifuging at $400 \times g$ for 5 minutes. PBMCs were counted using a haemocytometer and approximately 300,000 cells were added to tubes for incubation with fluorescently conjugated antibodies to identify DCs and sub-populations using eight-colour flow cytometry (FACS-CANTO, Becton-Dickenson, San Jose, USA). The following monoclonal antibodies were used: FITC-conjugated anti-Lineage 2 cocktail (CD3 clone # SK7, CD19 clone # SJ25C1, CD20 clone # L27, CD14 clone # M ϕ P9, CD56 clone # NCAM 16.2), V500-conjugated anti-HLA-DR clone # G46-6, V450-conjugated anti-CD209 clone # DCN46 (BD Biosciences, San Diego, USA), APC-conjugated anti-CD303 clone # 201A,

PE-Cy7-conjugated anti-CD141 clone # M80, APC-Cy7-conjugated anti-CD1c clone # L161 (BioLegend, San Diego, USA), PE-conjugated anti-CD205 clone # MG38 (BD Pharmingen, San Diego, USA). In addition, 7-aminoactinomycin D (7-AAD) (BD Pharmingen, San Diego, USA) was used to exclude necrotic and apoptotic cells. Fluorescence-minus-one (FMO) tubes established negative and positive gating strategies for CD205 and CD209 expression (data not shown).

2.6. Flow cytometry analysis

Data were analysed using FlowJo version Xv 0.7 (Tree Star, Inc., Ashland, OR). Doublets were excluded by gating forward *versus* forward-scatter. PBMCs were gated on the forward *versus* side-scatter. Dead cells were excluded by gating 7AAD *versus* side-scatter. Total DCs were identified as being Lineage–HLA-DR+, and analysed for expression of CD303 to identify plasmacytoid DCs (Lineage–HLA-DR+CD303+) and myeloid DCs (Lineage–HLA-DR+CD303–). Myeloid DCs were analysed for expression of CD1c and CD141 to yield four sub-populations: CD1c–CD141–; CD1c+CD141–; CD1c+CD141+; CD1c–CD141+ (Table 1 and Figure 1). All cell populations were examined for expression of CD205 and CD209. The absolute number of DCs and sub-populations was determined from the PBMC count (Coulter ACT^{diff} haematology analyser, Beckman-Coulter, High Wycombe, UK).

2.7. Statistical analyses

Data were inspected for normal distribution using the Shapiro-Wilk test. Non-normally distributed data were transformed logarithmically. Responses to exercise were examined using repeated-measures Analyses of Variance (ANOVA). When data violated sphericity, a

184 Greenhouse-Geisser correction was applied. Differences between individual time points were
185 examined using post-hoc paired samples *t*-tests. Statistical significance was accepted at the $p < .05$
186 level. Data are presented as means \pm standard deviation (SD) unless otherwise stated. Data were
187 analysed using SPSS statistical package version 22 (SPSS Inc, USA).

3. Results

3.1. Exercise trial

All participants completed the exercise trial [38]. The mean intensity of exercise was $80 \pm 6\%$ of $\dot{V}O_{2\text{ MAX}}$, average ratings of perceived exertion were 16 ± 1 , and the mean heart rate throughout the exercise task was 176 ± 7 beats per minute representing $91 \pm 3\%$ of measured maximum heart rate.

3.2. DCs

Total leukocytes, lymphocytes and monocytes exhibited the characteristic exercise-induced changes as reported previously [39, 40]. DCs (Lineage–HLA-DR+) increased in numbers in peripheral blood with exercise by approximately 150%, returning to baseline levels within 30 minutes (main effect of time; $F_{(1,10)} = 60$; $p < 0.05$ $\eta^2 = 0.9$) (Figure 2a and 2b).

3.3. DC sub-populations

All DC sub-populations exhibited a statistically significant increase in cell numbers during exercise, except for CD1c–CD141+ myeloid DCs, and returned to pre-exercise levels within 30 minutes of exercise cessation (Table 2). Overall, plasmacytoid DCs mobilised to a greater magnitude than myeloid DCs ($195 \pm 131\%$ vs. $131 \pm 100\%$; $p < .05$; Figure 2b). Among myeloid DCs, CD1c–CD141– showed the largest magnitude of exercise-induced change ($167 \pm 122\%$) with a stepwise mobilisation pattern among remaining sub-populations: CD1c+ CD141– ($79 \pm 50\%$), followed by CD1c+ CD141+ ($44 \pm 41\%$) with the smallest response shown by CD1c–CD141+ cells ($23 \pm 54\%$) $p < .05$.

211 *3.4. DCs and sub-populations expressing CD205 and analysis of CD205 cell-surface expression*
212 *density*

213 The majority of DCs and sub-populations were CD205+ (mean \pm SD; $97.8 \pm 3.6\%$; across all
214 sub-populations and participants) [13]. There were no differences in the proportion of DCs
215 expressing CD205+ between the different sub-populations (data not shown). Generally, CD205+
216 and CD205- cells among all sub-populations mobilised into blood during exercise, returning to
217 baseline levels within 30 minutes of exercise (Table 2 and Figure 3). However, there was a trend
218 for a larger mobilisation of CD205- cells in the majority of sub-populations (Figure 3).

219 Compared to CD205+ cells, a larger and statistically significant mobilisation of CD205- cells
220 was observed among CD1c-CD141+ cells and the CD1c+CD141+ cells (p 's $< .05$; Figure 3e
221 and 3f). For example, CD1c-CD141+CD205- cells and CD1c+CD141+CD205- cells exhibited
222 a mobilisation that was 80% and 70% greater than their CD205+ counterparts. Different to other
223 cells, plasmacytoid DCs exhibited a trend for a larger mobilisation of CD205+ cells (Figure 3c).
224 We also examined whether exercise altered the cell-surface expression density of CD205. At
225 baseline, the cell-surface expression density of CD205 was greater in the three myeloid sub-
226 populations; CD1c-CD141+ and CD1c+CD141+ and CD1c+CD141- compared to
227 CD1c-CD141- and plasmacytoid DCs (data not shown). In addition, CD205 expression density
228 did not change in response to exercise (data not shown).

229
230 *3.5. DCs and sub-populations expressing CD209 and analysis of CD209 cell-surface expression*
231 *density*

232 DCs and their sub-populations did not express CD209 and there were no changes in the numbers
233 or proportions of CD209+ cells, or alterations in the cell-surface expression density of CD209 in
234 response to exercise (data not shown).

4. Discussion

The present study demonstrates that the total number of DCs increased in peripheral blood during exercise by 150% and among the major DC sub-populations, plasmacytoid DCs mobilised by 195% whereas myeloid DCs exhibited a smaller increase of 131%. We show for the first time, that among the four sub-populations of myeloid DCs, there was a stepwise mobilisation pattern: a 167% increase with CD1c–CD141– cells, a 79% increase with CD1c+CD141– cells, a 44% increase with CD1c+ CD141+ cells and a 23% increase with CD1c–CD141+ cells.

To date, the phenotypic characteristics of DC kinetics during exercise remains unclear as only a limited number of studies have investigated the mobilisation of DC sub-populations in response to physical stressors, and these studies have produced seemingly contradictory findings. In the study herein, we show that both major DC subsets increase during exercise, with a greater mobilisation response observed among plasmacytoid DCs compared to myeloid DCs. In agreement with these results, a large and preferential exercise-induced mobilisation of plasmacytoid DCs (200% increase) compared to myeloid DCs (100% increase) has also been reported by a study that collected blood samples after vigorous ice hockey [16]. Contradicting these findings, in two studies it has been shown that plasmacytoid DCs may decrease immediately after long-duration exercise [17, 18]. However, these latter findings may be an artefact, because DC sub-populations were analysed as a proportion of total leukocytes, and thus DCs may artificially appear to be reduced because of a larger relative influx of NK cells, T cells and other highly exercise-responsive leukocyte subsets. In a separate study, plasmacytoid and myeloid DCs were examined before and after a combined protocol of moderate aerobic and intermittent resistance exercise undertaken by patients with multiple sclerosis, and healthy

participants [15]. In the aforementioned work, it was shown that myeloid cells increased by 75% and plasmacytoid cells increased by 50% and there were no differences between patients and healthy controls [15]. Thus, sustained vigorous steady state exercise appears to at least mobilise both myeloid and plasmacytoid DCs, yet we and others [16] have found higher mobilisation responses among plasmacytoid DCs.

A preferential mobilisation of plasmacytoid DCs likely represents an adaptive process, in which cells capable of mounting effector responses against infections or cancerous cells are redistributed. Indeed, plasmacytoid DCs are major effector cells in the context of viral infection due to their robust production of type 1 interferons [41, 42]. In addition, these cells express high levels of the toll like receptors TLR7 and TLR9, which transduce signals from virus or self-nucleic acids leading to rapid identification and robust eradication of pathogens [43, 44]. In comparison, myeloid cells are specialised in producing IL-12 that is critical for T cell activation and differentiation [42, 45, 46]. Thus, in an evolutionary context, given that plasmacytoid DCs have a greater inflammatory and migratory potential compared to myeloid DCs [42, 47], it is perhaps unsurprising that these effector cells are preferentially mobilised by exercise. Mechanistically, the magnitude of this mobilisation response is – akin to other effector immune cells preferentially mobilised by acute exercise – likely to be intensity-dependent and driven, in a dose-dependent fashion, by the density of adrenergic receptors on the surface of DCs [48, 49]. Indeed, it has been demonstrated that the degree of DC mobilisation during strenuous exercise appears to correlate positively with the concentration of catecholamines released into the peripheral blood [16].

Extending previous investigations, we show for the first time that among myeloid DCs, there is a stepwise mobilisation pattern, with the largest responses exhibited by CD1c–CD141–

cells, followed by a decreasing magnitude of response from CD1c+CD141⁻ cells, then CD1c+CD141⁺ cells, with the smallest exercise-induced change exhibited by CD1c⁻CD141⁺ cells. The least exercise responsive CD1c⁻CD141⁺ cells identified in the present study, represent a small sub-population of myeloid DCs that have a strong capacity to phagocytose apoptotic and necrotic cells or their debris, cross-presenting antigen to CD8⁺ cytotoxic T cells [24, 50, 51]. In the present study, the two DC sub-populations that mobilised moderately with exercise (CD1c+CD141⁺ and CD1c+CD141⁻) both expressed CD1c, and DCs with this characteristic, are potent stimulators of CD4⁺ T cells [24, 51]. Recently, two additional sub-populations of CD1c⁺ DCs have been established, referred to as CD1c+_A and CD1c+_B, which exhibit non-inflammatory and inflammatory characteristics respectively [23]. However, as these new sub-populations must be identified by uniquely expressed cell-surface proteins (CD32B for CD1c+_A, and CD163 and CD36 for CD1c+_B), in the present study, we are unable to infer whether the CD1c-expressing cells mobilised by exercise exhibit inflammatory potential [23]. Importantly, the present study provides novel information about the least well-characterised myeloid DC sub-population [23]: we show that DCs with a CD1c⁻CD141⁻ phenotype, are the most exercise-responsive myeloid subset. In addition, future studies may seek to investigate the functional characteristics and homing properties of these cells to better infer the clinical implications of CD1c⁻CD141⁻ mobilisation during exercise.

We also show for the first time, that among myeloid DCs, the most exercise responsive cells are CD205⁻. For example, CD1c⁻CD141⁺ and CD1c+CD141⁺ cells which did not express CD205, exhibited an exercise-induced mobilisation that was 80% and 70% greater than their CD205⁺ counterparts, respectively. The cell surface protein CD205 (also known as DEC-205) is upregulated upon DC maturation [25] and facilitates recognition of apoptotic or necrotic cells

[26] by having a critical role in receptor-mediated antigen uptake [52]. Thus, these CD205– cells, which are not specialised in recognising apoptotic or necrotic cells, may have other functions, such as targeting viral infection [53]. In the present study, we also examined DC expression of CD209 (also known as DC-SIGN), a multifunctional receptor which recognises glycans from viruses and bacteria, and is involved in adhesion, migration, signalling and antigen presentation [27]. In agreement with prior research, we showed that DCs in peripheral blood do not express CD209 [13, 54] but we extend these findings by showing that exercise does not stimulate an upregulation of CD209, or at least, does not preferentially mobilise a sub-population of DCs that already express CD209. Circulating DCs become primed to acquire antigens when ‘activating stimuli’ such as cytokines interact with a variety of cell surface receptors [11]. Upon activation, DCs upregulate chemokine receptors, adhesion molecules and co-stimulatory molecules, including CD209 [11]. Indeed, it is well established that developing DCs *in vitro* for several days with granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4 leads to an increase in CD209 expression [32, 55]. In addition, inflammatory stimuli such as TNF- α , IFN- γ , and lipopolysaccharide, which also increase with vigorous exercise, upregulate cell surface expression of CD209 [56]. In the present study, it seems that the inflammatory stimulus of exercise was too short, or of insufficient magnitude, to elicit an upregulation of CD209, and thus, these signals might most likely be encountered post-exercise in peripheral tissues, where DCs have been shown to strongly express CD209 [55].

The findings presented in this study improve our understanding of how exercise could be used to bolster cell yields for DC immunotherapy [32, 33]. Our results are timely, given the recent focus of harvesting DC sub-populations directly from blood [34, 36, 51, 57]. For example, a feasibility study isolated peripheral blood plasmacytoid DCs from 15 patients with stage IV

melanoma for expansion *ex vivo* with cytokines and antigen, before re-infusing the cell preparations [58]. The results showed that 7 out of 15 patients were alive two years after plasmacytoid DC administration, compared with 6 of 72 patients treated with standard chemotherapy [58]. Using similar methodology in another feasibility study of 14 patients with stage IV melanoma, immunotherapy using CD1c+ myeloid DCs resulted in long-term progression free survival for 12-35 months [59]. Furthermore, CD1c+ DC immunotherapies are being trialled with other cancers, including prostate cancer [60]. The results of the present study show that if peripheral blood DCs could be harvested during exercise, the total DC yield might increase by 150%, with a potential increased cell yield among plasmacytoid and myeloid dendritic cells by 195% and 131% respectively. Depending on the subset of myeloid cells being targeted, exercise could improve cell yields by 23-167%. Future studies are needed to establish whether exercise can increase the yield of peripheral blood DCs in patients with different forms of cancer, and in addition, whether these changes, which might improve the preparation of DC immunotherapy products, leads to better clinical outcomes.

The findings of the present study also provide some support for mechanisms underlying vaccine-enhancing effects of acute psychological stress [61] and acute bouts of exercise [29]. Indeed, both stressors cause a substantial leukocytosis, and it has been suggested that as part of this response, DCs are mobilised into peripheral blood, later homing to the site of vaccine administration in skeletal muscle, facilitating antigen processing and presentation [62]. Further, the most robust and consistent interventions that enhance vaccine responses induce damage and local inflammation in the muscle selected for vaccine administration [28]. Our study confirms that as part of exercise-induced leukocytosis, DCs are mobilised into peripheral blood, with a preferential response from plasmacytoid cells. Other human studies have shown that leukocytes

appear in muscles damaged by exercise within four to six hours [63, 64]. In support, animal studies have shown that DCs accumulate in damaged muscle within 24 hours [65] perhaps in response to myoblast-derived cytokines and chemokines [66] or heat shock proteins, uric acid and cell debris from necrotic cells [67]. Thus, given the results of the present study and those discussed herein, it is likely that Matzinger's 'danger model', which proposes that antigen presenting cells are attracted to distressed and injured cells, and subsequently activated by endogenous cellular alarm signals, could be a mechanism for improved vaccine responses following muscle-damaging exercise [68]. Further support for this idea is provided by the observation that DCs, and in particular plasmacytoid DCs, are a common feature of lesions in inflammatory myopathies [69, 70].

When interpreting the results from the present study it should be considered that DCs did not fall below resting levels 30 minutes after exercise. It is likely that the intensity and/or duration of exercise was insufficient to stimulate a post-exercise extravasation of DCs to peripheral tissues [5]. It is well established that a dose-response relationship between exercise duration and the magnitude of lymphocyte trafficking exists, but relationships have not been investigated among DCs [71]. The extravasation of cells out of the bloodstream is likely to be driven by catecholamines and cortisol, and the magnitude of this neuroendocrine response is positively correlated with exercise intensity and duration [72]. In support, a strong positive correlation between adrenergic activity and the exercise-induced increase of plasmacytoid DCs has been reported [16], suggesting an adrenergic dependent mechanism of DC mobilisation, as with other cell populations [73]. However, if in the present study, exercise did indeed invoke a neuroendocrine response of sufficient magnitude, extended blood sampling may have enabled assessment of DC extravasation, given that the post-exercise nadir among lymphocytes is

typically 1-2 hours after the stimulus [3]. To better determine the DC kinetics in response to exercise, future studies should investigate different durations and intensities of exercise with extended post-exercise blood sampling.

In summary, acute exercise increased the number of DCs in peripheral blood by 150% with a preferential mobilisation of plasmacytoid DCs (195%) compared to myeloid DCs (131%). Among myeloid DCs, there was a stepwise mobilisation pattern: 167% increase with CD1c⁺CD141⁺ cells, a 79% increase with CD1c⁺CD141⁺, a 44% increase with CD1c⁺CD141⁺ cells and a 23% increase with CD1c⁺CD141⁺ cells. The most exercise responsive myeloid DCs did not express CD205, suggesting that immature cells, unspecialised in recognising apoptotic or necrotic cells, are preferentially mobilised.

Figure legends

Figure 1

Flow cytometry gating strategy. Doublets were removed by gating forward *versus* forward-scatter (a). Mononuclear cells were gated on the forward *versus* side-scatter (b) dead cells were excluded gating 7AAD *versus* side-scatter (c) followed by subsequent gating of Lineage⁺HLADR⁺ dendritic cells (d), which were analysed for expression of CD303 (e). Plasmacytoid dendritic cells were identified as Lineage⁺HLA-DR⁺ CD303⁺ and myeloid dendritic cells identified as Lineage⁺HLA-DR⁺CD303⁺ (e). Myeloid dendritic cells Lineage⁺HLADR⁺CD303⁺ were analysed for expression of CD141 and CD1c (f) to yield four sub-populations CD1c⁺CD141⁺; CD1c⁺CD141⁺; CD1c⁺CD141⁺; CD1c⁺CD141⁺ (g). CD205 gating was determined using fluorescence-minus-one (FMO) tubes and applied to all cell populations (h).

Figure 2

Mobilisation of total dendritic cells and subpopulations during exercise. (a) Exercise-induced kinetics of dendritic cells. Main effect of time: $F_{(1,10)} = 60.0$; $p < 0.05$ $\eta^2 = 0.9$. * Indicates a significant difference from baseline $p < 0.05$ (t -test between baseline and exercise; $t_{(8)} = -6.9$, $p < 0.05$) and a significant difference from +30min (t -test between exercise and +30min; $t_{(8)} = 14.2$, $p < 0.05$). Data are expressed as cell/ μ L (mean \pm SEM). (b) Percentage change from baseline for major dendritic cell subsets in response to exercise. * Indicates a significant difference between subsets $p < 0.05$ (t -test; Plasmacytoid vs. Myeloid, $t_{(8)} = -2.9$, $p < 0.05$). Data are expressed as percentage change from baseline (mean \pm SEM). (c) Percentage change for myeloid dendritic cell sub-populations in response to exercise. * Indicates a significant difference between subsets $p < 0.05$ (t -test; CD1c-CD141+ vs CD1c-CD141-, $t_{(8)} = -3.0$, $p < 0.05$; CD1c+CD141+ vs CD1c-CD141- $t_{(8)} = -3.1$, $p < 0.05$; CD1c+CD141- vs CD1c-CD141-, $t_{(8)} = -3.9$, $p < 0.05$). No other significant differences were observed between cell types. Data are expressed as percentage change from baseline (mean \pm SEM).

Figure 3

Differential magnitude of dendritic cell and subpopulation mobilisation on the basis of CD205 expression. Percentage change from baseline for major dendritic cell subsets and the myeloid dendritic cell sub-populations in response to exercise. * Indicates a significant difference between CD205+ and CD205- $p < 0.05$ t -test. CD1c-CD141+, $t_{(8)} = -2.5$, $p < 0.05$; CD1c+CD141+, $t_{(8)} = -3.1$, $p < 0.05$. Data are expressed as percentage change from baseline (mean \pm SEM).

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Table 1. Dendritic cell sub-population identification

Sub-population name	Cell surface markers	Functional properties	Reference
DCs	Lineage– HLADR+	Presentation of ingested pathogens or cell debris to T-cells.	Ziegler Heitbrock (2010) Merad (2013)
Plasmacytoid DCs	Lineage– HLADR+ CD303+	Major effector sub-population of DCs. Produce type 1 interferons in response to viral infection.	Dzionek (2000) Liu (2005)
Myeloid DCs	Lineage– HLADR+ CD303–	Regulatory DC sub-populations. Produce IL-12 for T-cell activation and differentiation.	Dzionek (2000) Heufler (1996)
CD1c–CD141+	Lineage– HLADR+ CD303– CD1c– CD141+	Cross presentation of antigen to CD8+ T -cells for anti-tumour immunity.	Penna (2002) Ding (2014)
CD1c+CD141+	Lineage– HLADR+ CD303– CD1c+ CD141+	Cross presentation of antigen to CD8+ T cells for anti-tumour immunity and stimulate CD4+ T-cells.	Villani (2017) Ding (2014)
CD1c+CD141–	Lineage– HLADR+ CD303– CD1c+ CD141–	Stimulate CD4+ T-cells.	Villani (2017) Ding (2014)
CD1c–CD141–	Lineage– HLADR+ CD303– CD1c– CD141–	Unknown	Villani (2017)

Legend: Indentation indicates a sub-population of parent cells (i.e., Myeloid Dendritic Cells are a sub-population of Dendritic Cells, and CD1c+CD141– Dendritic Cells are a sub-population of Myeloid Dendritic Cells). Lineage cocktail = CD3, CD19, CD20, CD14, CD56. HLADR = marker for major histocompatibility complex MHC class II. CD = cluster of differentiation. In addition CD205 (DEC-205) a cell surface marker that enables recognition of apoptotic or necrotic cells (Cao et al 2015) and CD209 (DC-SIGN) a cell surface marker that recognises a wide variety of ligands, is involved in adhesion, migration and antigen presentation (Garcia-Vallejo and van Kooyk 2013) were examined on all dendritic cells and sub-populations.

Table 2. Total DCs, and DC sub-populations differentiated on CD205 expression (mean \pm SD)

Cells / μ L		Baseline	Exercise	+30 min	Main effect of time
DCs		79 \pm 38	196 \pm 126 ^{***}	76 \pm 34†	$F_{(1,10)} = 59.9$; $p < 0.05$ $\eta^2 = 0.9$
	CD205+	78 \pm 37	192 \pm 123 ^{***}	74 \pm 33†	$F_{(1,10)} = 59.9$; $p < 0.05$ $\eta^2 = 0.9$
	CD205–	1.4 \pm 1.4	4.3 \pm 4.8 ^{***}	1.5 \pm 1.5†	$F_{(2,16)} = 27.8$; $p < 0.05$ $\eta^2 = 0.8$
Plasmacytoid DCs		19 \pm 11	55 \pm 42 ^{***}	22 \pm 19†	$F_{(2,16)} = 45.9$; $p < 0.05$ $\eta^2 = 0.9$
	CD205+	19 \pm 11	55 \pm 42 ^{***}	22 \pm 19†	$F_{(2,16)} = 45.9$; $p < 0.05$ $\eta^2 = 0.9$
	CD205–	0.07 \pm 0.07	0.1 \pm 0.09 ^{**}	0.06 \pm 0.03	$F_{(2,16)} = 5.0$; $p < 0.05$ $\eta^2 = 0.4$
Myeloid DCs		60 \pm 30	139 \pm 88 ^{***}	53 \pm 22†	$F_{(2,16)} = 43.6$; $p < 0.05$ $\eta^2 = 0.8$
	CD205+	58 \pm 30	135 \pm 85 ^{***}	52 \pm 21†	$F_{(2,16)} = 43.4$; $p < 0.05$ $\eta^2 = 0.8$
	CD205–	1.4 \pm 1.3	4.2 \pm 4.7 ^{***}	1.4 \pm 1.5†	$F_{(2,16)} = 28.1$; $p < 0.05$ $\eta^2 = 0.8$
CD1c– CD141+		2.1 \pm 1.3	2.4 \pm 1.5	1.7 \pm 0.9	$F_{(2,16)} = 1.4$; $p > 0.05$ $\eta^2 = 0.2$
	CD205+	2.0 \pm 1.3	2.2 \pm 1.5	1.6 \pm 0.9	$F_{(2,16)} = 1.5$; $p > 0.05$ $\eta^2 = 0.2$
	CD205–	0.08 \pm 0.1	0.1 \pm 0.2	0.09 \pm 0.1	$F_{(2,16)} = 3.6$; $p > 0.05$ $\eta^2 = 0.3$
CD1c+ CD141+		0.6 \pm 0.2	0.8 \pm 0.4 ^{**}	0.6 \pm 0.2	$F_{(2,16)} = 5.1$; $p < 0.05$ $\eta^2 = 0.4$
	CD205+	0.54 \pm 0.2	0.78 \pm 0.38 [*]	0.54 \pm 0.2	$F_{(2,16)} = 4.6$; $p < 0.05$ $\eta^2 = 0.4$
	CD205–	0.01 \pm 0.01	0.03 \pm 0.01 ^{***}	0.01 \pm 0.01†	$F_{(2,16)} = 21.3$; $p < 0.05$ $\eta^2 = 0.7$
CD1c+ CD141–		20 \pm 12	34 \pm 18 ^{***}	16 \pm 6†	$F_{(2,16)} = 31.2$; $p < 0.05$ $\eta^2 = 0.8$
	CD205+	20 \pm 12	33.7 \pm 17.4 ^{***}	16 \pm 6†	$F_{(2,16)} = 31.1$; $p < 0.05$ $\eta^2 = 0.8$
	CD205–	0.07 \pm 0.08	0.14 \pm 0.16	0.04 \pm 6†	$F_{(2,16)} = 3.9$; $p < 0.05$ $\eta^2 = 0.3$
CD1c– CD141–		37 \pm 22	102 \pm 78 ^{***}	35 \pm 21†	$F_{(2,16)} = 45.2$; $p < 0.05$ $\eta^2 = 0.9$
	CD205+	35.7 \pm 21.3	98.1 \pm 74 ^{***}	33.3 \pm 19.6†	$F_{(2,16)} = 45.2$; $p < 0.05$ $\eta^2 = 0.9$
	CD205–	1.3 \pm 1.1	4 \pm 4.4 ^{***}	1.3 \pm 1.3†	$F_{(2,16)} = 27.1$; $p < 0.05$ $\eta^2 = 0.8$

Legend: * <0.05 ** <0.01 *** <0.001 indicates a significant difference (paired samples t-test from baseline). † indicates a significant difference <0.05 (paired samples t-test from exercise to +30 min).

Figure 1. Flow cytometry gating strategy

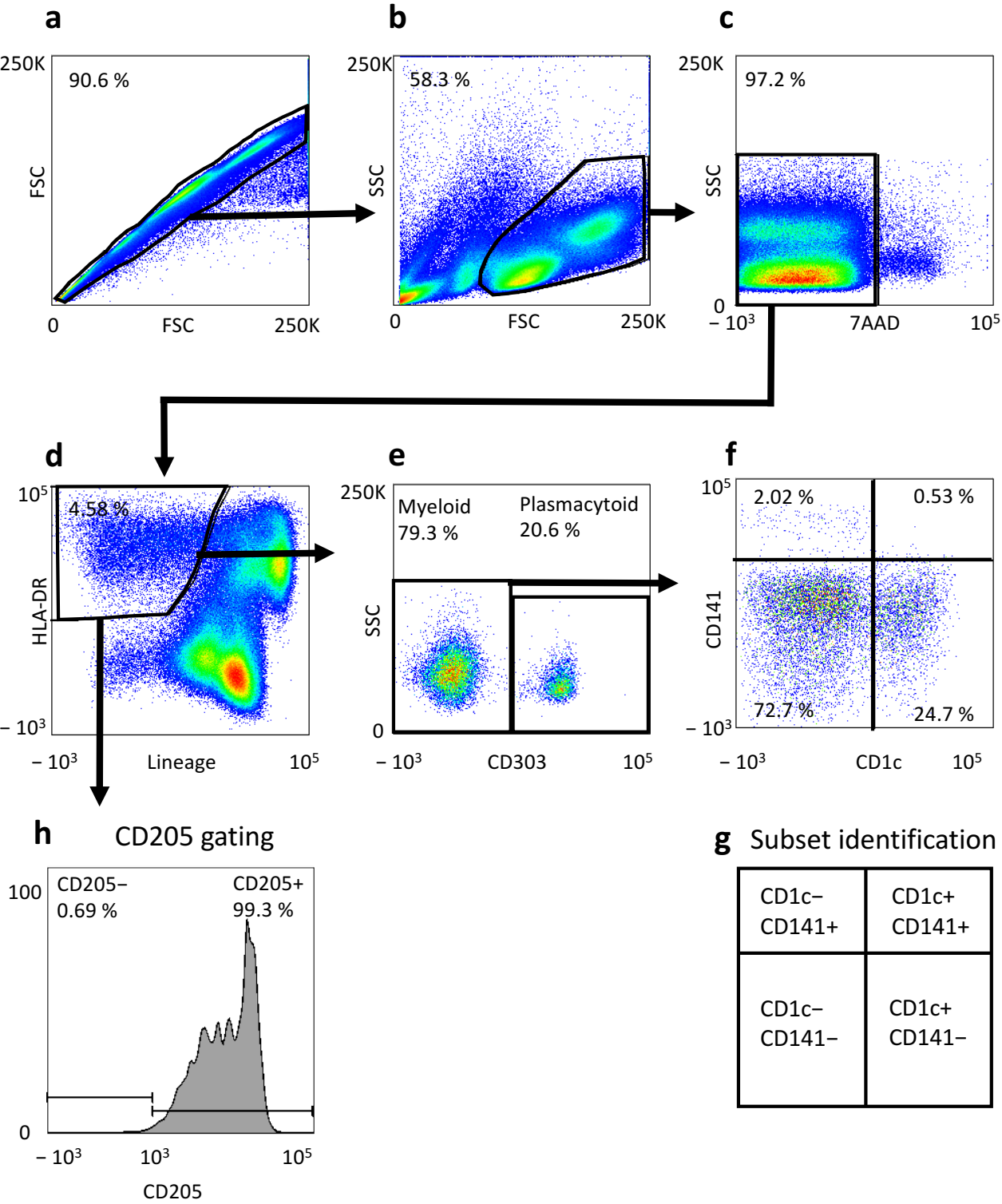


Figure 2. Mobilisation of total dendritic cells and sub-populations during exercise

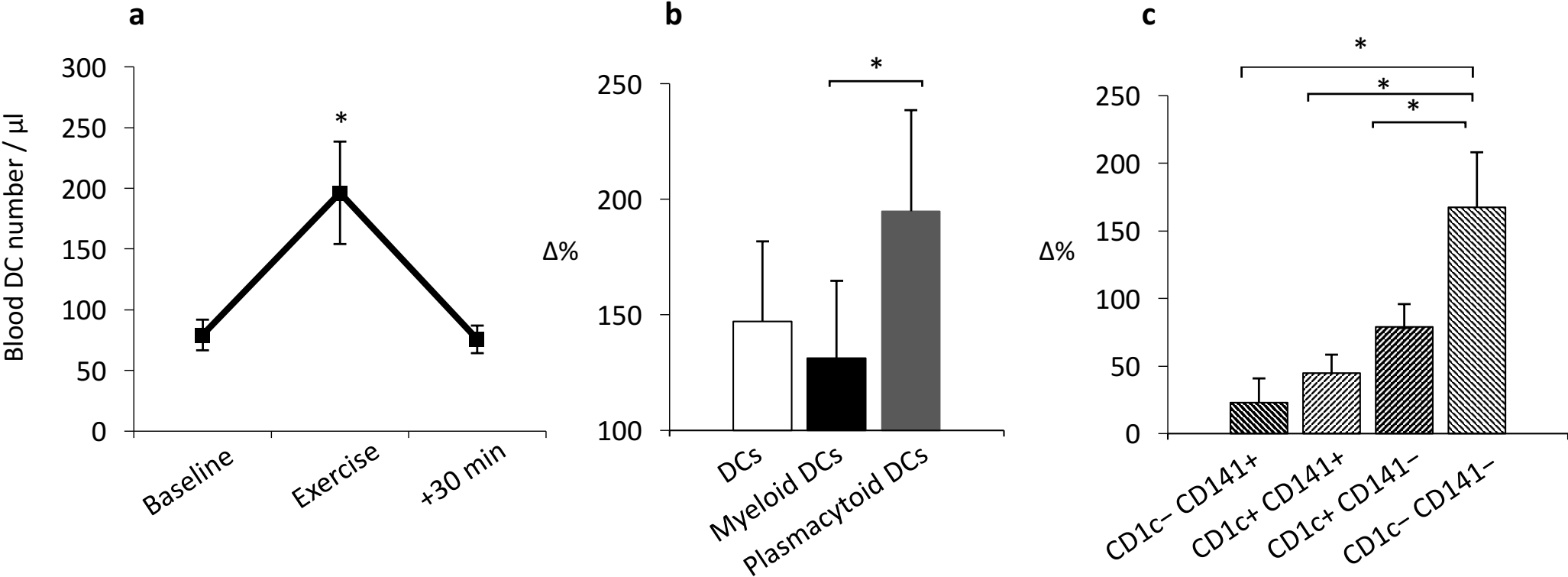


Figure 3. Differential magnitude of dendritic cell and sub-population mobilisation on the basis of CD205 expression

