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

3

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22 **Key words:** dendritic cells, myeloid, plasmacytoid, exercise, humans

23

24 **Abstract**

25 Dendritic cells (DCs) are important sentinel cells of the immune system responsible for
26 presenting antigen to T cells. Exercise is known to cause an acute and transient increase in the
27 frequency of DCs in the bloodstream in humans, yet there are contradictory findings in the
28 literature regarding the phenotypic composition of DCs mobilised during exercise, which may
29 have implications for immune regulation and health. Accordingly, we sought to investigate the
30 composition of DC sub-populations mobilised in response to acute aerobic exercise. Nine
31 healthy males (age, 21.9 ± 3.6 years; height, 177.8 ± 5.4 cm; body mass, 78.9 ± 10.8 kg; body
32 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%
33 $\dot{V}O_{2\text{MAX}}$. Blood was sampled at baseline, during the final minute of exercise and 30 minutes
34 later. Using flow cytometry, total DCs were defined as Lineage⁻ (CD3, CD19, CD20, CD14,
35 CD56) HLA-DR⁺ and subsequently identified as plasmacytoid DCs (CD303⁺) and myeloid DCs
36 (CD303⁻). Myeloid DCs were analysed for expression of CD1c and CD141 to yield four sub-
37 populations; CD1c⁻CD141⁺; CD1c⁺CD141⁺; CD1c⁺CD141⁻ and CD1c⁻CD141⁻. Expression
38 of CD205 was also analysed on all DC sub-populations to identify DCs capable of recognising
39 apoptotic and necrotic cells. Total DCs increased by 150% during exercise ($F_{(1,10)}=60$; $p<0.05$,
40 $\eta^2=0.9$). Plasmacytoid DCs mobilised to a greater magnitude than myeloid DCs (195 ± 131 % vs.
41 131 ± 100 %; $p<.05$). Among myeloid DCs, CD1c⁻CD141⁻ cells showed the largest exercise-
42 induced mobilisation (167 ± 122 %), with a stepwise pattern observed among the remaining sub-
43 populations: CD1c⁺CD141⁻ (79 ± 50 %), followed by CD1c⁺CD141⁺ (44 ± 41 %), with the
44 smallest response shown by CD1c⁻CD141⁺ cells (23 ± 54 %) ($p<.05$). Among myeloid DCs,
45 CD205⁻ cells were the most exercise responsive. All DC subsets returned to resting levels within
46 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

53 **1. Introduction**

54 Acute aerobic exercise causes profound alterations to the cellular composition of
55 peripheral blood, whereby the frequency of many leukocyte subsets increases during exercise,
56 followed by a decline in the hours after [1]. For many types of immune cell subsets, the
57 magnitude of change in response to exercise is usually largest among cells with the strongest
58 effector potential [2-4]. Accordingly, this exercise-induced effect is considered a conserved
59 evolutionary response which causes the redistribution of effector cells to peripheral tissues to
60 conduct immune-surveillance [5]. Cells of a lymphoid lineage, such as T cells [2, 6] and natural
61 killer (NK) cells [7], are the most widely researched. Cells with myeloid characteristics have
62 received less attention in the exercise literature, except for a limited number of studies which
63 have examined monocytes [8-10]. For example, it has been shown that alternatively-activated
64 M2-like monocytes preferentially mobilise into blood during exercise [8, 9], whereas other work
65 has shown that the most exercise responsive cells are classically-activated M1-like monocytes
66 [10]. Studies examining the mobilisation patterns of dendritic cell (DC) subsets in response to
67 exercise have provided equivocal evidence, despite the critical role DCs play in initiating and
68 directing immune responses.

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

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

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

97 Clarifying the exercise-induced kinetics of DCs is important because it has been proposed
98 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.

116 **2. Methods**

117 *2.1. Participants*

118 Nine healthy men were included in the present analyses (age, 21.9 ± 3.6 years; height, $177.8 \pm$
119 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
120 mL.kg.min⁻¹) (ethical approval reference: ERN_12-0830; University of Birmingham, UK).
121 These nine participants represent a sub-group from a total of ten men who took part in other
122 investigations [37-40] with peripheral blood mononuclear cells (PBMCs) that were available for
123 analysis following cryopreservation.

124

125 *2.2. Pre-experimental procedures*

126 Height and body mass were assessed using standard methods and cardiorespiratory fitness ($\dot{V}O_2$
127 MAX) was measured on a cycle ergometer. Expired air samples were assessed for oxygen
128 consumption and carbon dioxide production using breath-by-breath analysis, with heart rate
129 monitored via telemetry, and ratings of perceived exertion recorded using the Borg scale [37-40].

130

131 *2.3. Exercise trial and blood sampling*

132 At least seven days after preliminary measurements, and following an overnight fast, participants
133 reported to the laboratory in the morning, and a blood sample was collected from a cannulated
134 forearm vein after a 15-minute seated rest (baseline). The exercise trial consisted of steady state
135 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.
136 Exercise intensity was monitored with breath-by-breath measurements. Heart rate and ratings of
137 perceived exertion were recorded throughout the exercise trial. A second blood sample was

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

140

141 *2.4. Peripheral blood mononuclear cell (PBMC) isolation*

142 Blood with potassium ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant was diluted
143 1:1 with Roswell Park Memorial Institute Media (RPMI), and layered on top of Ficoll paque
144 PLUS (GE Healthcare) (2 blood:1 Ficoll), before centrifuging at $400 \times g$ for 30 minutes at 21°C.
145 PBMCs were aspirated and washed three times in RPMI by centrifuging at $200 \times g$ for 5
146 minutes. Cells were re-suspended in freezing mixture (70% RPMI, 20% fetal calf serum (FCS)
147 and 10% dimethyl sulfoxide (DMSO)) and frozen at $-1^\circ\text{C}/\text{min}$ using a freezing container
148 (Nalgene 'Mr Frosty' ThermoScientific). Cells were stored at -80°C and analysed within six
149 months [39, 40].

150

151 *2.5. Flow cytometry*

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

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

167

168 2.6. Flow cytometry analysis

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

179

180 2.7. Statistical analyses

181 Data were inspected for normal distribution using the Shapiro-Wilk test. Non-normally
182 distributed data were transformed logarithmically. Responses to exercise were examined using
183 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).

188 3. Results

189 3.1. Exercise trial

190 All participants completed the exercise trial [38]. The mean intensity of exercise was $80 \pm 6\%$ of
191 $\dot{V}O_{2\text{ MAX}}$, average ratings of perceived exertion were 16 ± 1 , and the mean heart rate throughout
192 the exercise task was 176 ± 7 beats per minute representing $91 \pm 3\%$ of measured maximum
193 heart rate.

194

195 3.2. DCs

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

200

201 3.3. DC sub-populations

202 All DC sub-populations exhibited a statistically significant increase in cell numbers during
203 exercise, except for CD1c–CD141+ myeloid DCs, and returned to pre-exercise levels within 30
204 minutes of exercise cessation (Table 2). Overall, plasmacytoid DCs mobilised to a greater
205 magnitude than myeloid DCs ($195 \pm 131\%$ vs. $131 \pm 100\%$; $p < .05$; Figure 2b). Among
206 myeloid DCs, CD1c–CD141– showed the largest magnitude of exercise-induced change ($167 \pm$
207 122%) with a stepwise mobilisation pattern among remaining sub-populations: CD1c+ CD141–
208 ($79 \pm 50\%$), followed by CD1c+ CD141+ ($44 \pm 41\%$) with the smallest response shown by
209 CD1c–CD141+ cells ($23 \pm 54\%$) $p < .05$.

210

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).

235 **4. Discussion**

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

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

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

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

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

281 cells, followed by a decreasing magnitude of response from CD1c+CD141- cells, then
282 CD1c+CD141+ cells, with the smallest exercise-induced change exhibited by CD1c-CD141+
283 cells. The least exercise responsive CD1c-CD141+ cells identified in the present study,
284 represent a small sub-population of myeloid DCs that have a strong capacity to phagocytose
285 apoptotic and necrotic cells or their debris, cross-presenting antigen to CD8+ cytotoxic T cells
286 [24, 50, 51]. In the present study, the two DC sub-populations that mobilised moderately with
287 exercise (CD1c+CD141+ and CD1c+CD141-) both expressed CD1c, and DCs with this
288 characteristic, are potent stimulators of CD4+ T cells [24, 51]. Recently, two additional sub-
289 populations of CD1c+ DCs have been established, referred to as CD1c+_A and CD1c+_B, which
290 exhibit non-inflammatory and inflammatory characteristics respectively [23]. However, as these
291 new sub-populations must be identified by uniquely expressed cell-surface proteins (CD32B for
292 CD1c+_A, and CD163 and CD36 for CD1c+_B), in the present study, we are unable to infer
293 whether the CD1c-expressing cells mobilised by exercise exhibit inflammatory potential [23].
294 Importantly, the present study provides novel information about the least well-characterised
295 myeloid DC sub-population [23]: we show that DCs with a CD1c-CD141- phenotype, are the
296 most exercise-responsive myeloid subset. In addition, future studies may seek to investigate the
297 functional characteristics and homing properties of these cells to better infer the clinical
298 implications of CD1c-CD141- mobilisation during exercise.

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

304 [26] by having a critical role in receptor-mediated antigen uptake [52]. Thus, these CD205–
305 cells, which are not specialised in recognising apoptotic or necrotic cells, may have other
306 functions, such as targeting viral infection [53]. In the present study, we also examined DC
307 expression of CD209 (also known as DC-SIGN), a multifunctional receptor which recognises
308 glycans from viruses and bacteria, and is involved in adhesion, migration, signalling and antigen
309 presentation [27]. In agreement with prior research, we showed that DCs in peripheral blood do
310 not express CD209 [13, 54] but we extend these findings by showing that exercise does not
311 stimulate an upregulation of CD209, or at least, does not preferentially mobilise a sub-population
312 of DCs that already express CD209. Circulating DCs become primed to acquire antigens when
313 ‘activating stimuli’ such as cytokines interact with a variety of cell surface receptors [11]. Upon
314 activation, DCs upregulate chemokine receptors, adhesion molecules and co-stimulatory
315 molecules, including CD209 [11]. Indeed, it is well established that developing DCs *in vitro* for
316 several days with granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4 leads
317 to an increase in CD209 expression [32, 55]. In addition, inflammatory stimuli such as TNF-
318 alpha, IFN-gamma, and lipopolysaccharide, which also increase with vigorous exercise,
319 upregulate cell surface expression of CD209 [56]. In the present study, it seems that the
320 inflammatory stimulus of exercise was too short, or of insufficient magnitude, to elicit an
321 upregulation of CD209, and thus, these signals might most likely be encountered post-exercise in
322 peripheral tissues, where DCs have been shown to strongly express CD209 [55].

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

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

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

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

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

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

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

383

384 **Figure legends**

385 **Figure 1**

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

396

397 **Figure 2**

398 Mobilisation of total dendritic cells and subpopulations during exercise. (a) Exercise-induced
399 kinetics of dendritic cells. Main effect of time: $F_{(1,10)} = 60.0$; $p < 0.05$ $\eta^2 = 0.9$. * Indicates a
400 significant difference from baseline $p < 0.05$ (t -test between baseline and exercise; $t_{(8)} = -6.9$,
401 $p < 0.05$) and a significant difference from +30min (t -test between exercise and +30min; $t_{(8)} =$
402 14.2, $p < 0.05$). Data are expressed as cell/ μ L (mean \pm SEM). (b) Percentage change from baseline
403 for major dendritic cell subsets in response to exercise. * Indicates a significant difference
404 between subsets $p < 0.05$ (t -test; Plasmacytoid vs. Myeloid, $t_{(8)} = -2.9$, $p < 0.05$). Data are
405 expressed as percentage change from baseline (mean \pm SEM). (c) Percentage change for myeloid
406 dendritic cell sub-populations in response to exercise. * Indicates a significant difference
407 between subsets $p < 0.05$ (t -test; CD1c-CD141+ vs CD1c-CD141-, $t_{(8)} = -3.0$, $p < 0.05$;
408 CD1c+CD141+ vs CD1c-CD141- $t_{(8)} = -3.1$, $p < 0.05$; CD1c+CD141- vs CD1c-CD141-, $t_{(8)} =$
409 -3.9 , $p < 0.05$). No other significant differences were observed between cell types. Data are
410 expressed as percentage change from baseline (mean \pm SEM).

411

412 **Figure 3**

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

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581

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 ± SD)

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

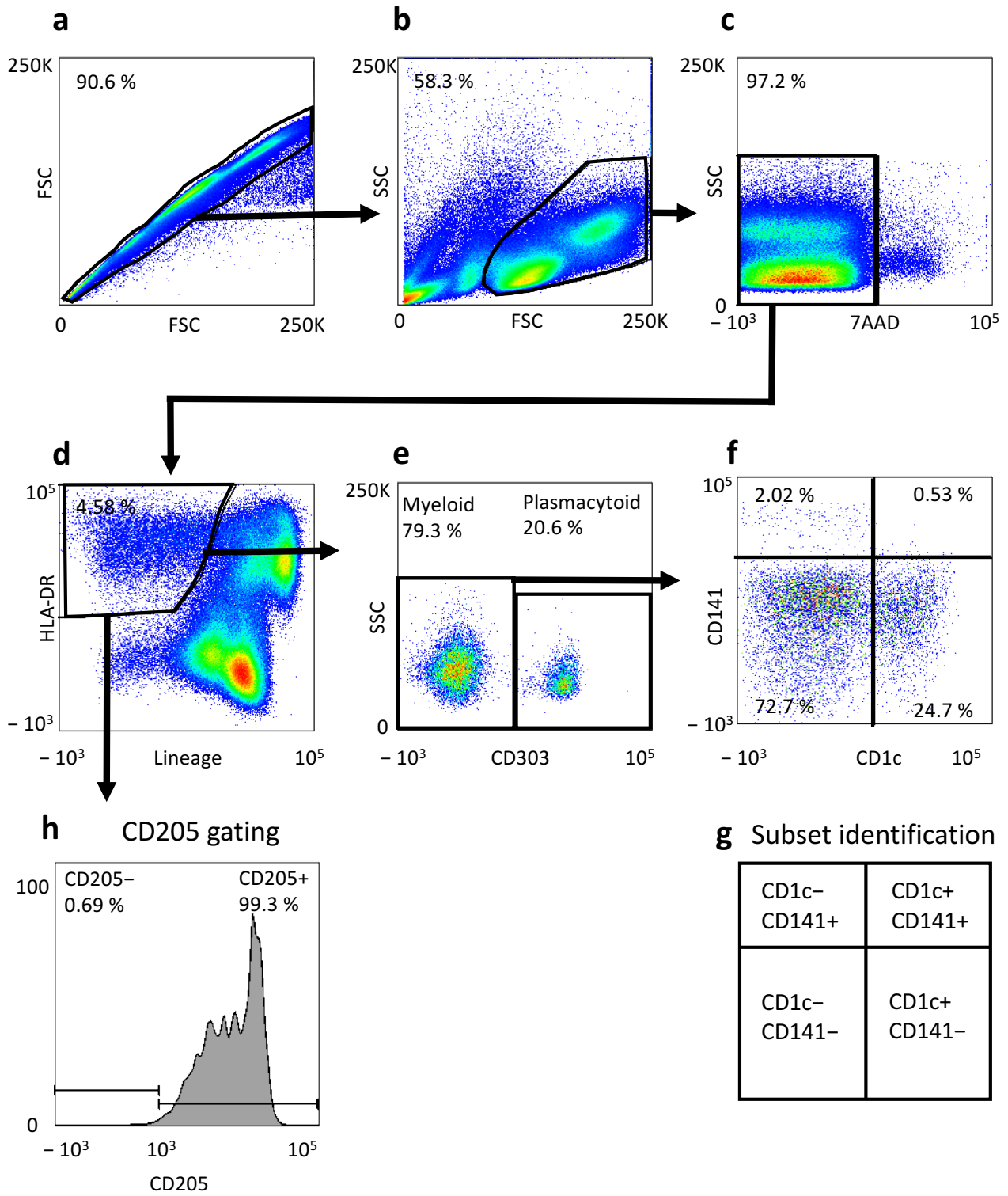


Figure 2. Mobilisation of total dendritic cells and sub-populations during exercise

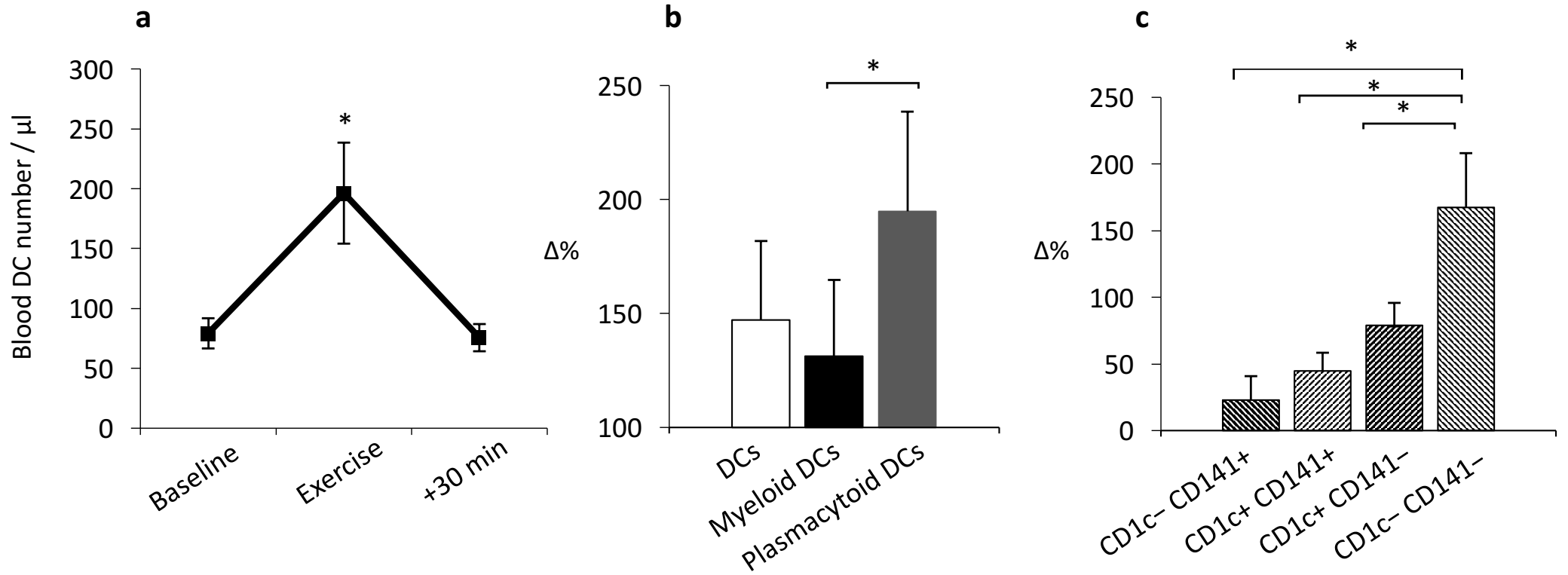


Figure 3. Differential magnitude of dendritic cell and sub-population mobilisation on the basis of CD205 expression

