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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·m^{2}; $\dot{V}O_{2\ MAX}$, 41.5 ± 5.1 mL·kg·min^{-1}) cycled for 20 min at 80% $\dot{V}O_{2\ MAX}$. Blood was sampled at baseline, during the final minute of exercise and 30 min 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%; $p < 0.05$). 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%) ($p < 0.05$). Among myeloid DCs, CD205− cells were the most exercise responsive. All DC subsets returned to resting levels within 30 min of exercise cessation. These results show that there is a preferential mobilisation of plasmacytoid DCs during exercise. Given the functional repertoire of plasmacytoid DCs, which includes the production of interferons against viral and bacterial pathogens, these findings indicate that exercise may augment immune-surveillance by preferentially mobilising effector cells; these findings have general implications for the promotion of exercise for health, and specifically for the optimisation of DC harvest for cancer immunotherapy.

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

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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 min 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 competency, for example, by enhancing vaccination responses [28–31]. Additionally, it has recently been proposed that exercise could be a powerful means of increasing peripheral blood mononuclear cell yields for the purposes of immunotherapy [32,33]. To date, the most targeted malignancies for DC immunotherapies are melanoma, prostate cancer, glioblastoma and renal cell carcinoma, but trials are being conducted with many other cancers [34,35]. A common approach is to isolate peripheral blood mononuclear cells from patients to generate monocyte-derived DCs *ex vivo* with growth factors and antigen stimulation, before re-administering the cell preparations [34,36]. There are several examples of clinically effective DC immunotherapy regimens, but methodologies continue to be adapted and improved, with recent emphasis on harvesting DC sub-populations directly from blood, with a particular focus on either plasmacytoid DCs due to their effector potential, or CD1c+ and/or CD141+ myeloid subsets for their ability to cross present antigen to cytotoxic CD8+ T cells [34,36]. Thus, if adjunctive strategies such as exercise are employed to improve cell yields for DC immunotherapy, it is important to understand how naturally occurring DC sub-populations respond to exercise-induced stimulation. Therefore, the aim of this study was to conduct a detailed immuno-phenotypic analyses of DC sub-populations present in peripheral blood before, during and after 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 $kg \cdot m^2$; $\dot{V}O_{2\ MAX}$, 41.5 ± 5.1 $mL \cdot kg \cdot min^{-1}$) (ethical approval reference: ERN_12-0830; University of Birmingham, UK). These nine participants represent a subgroup 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 ($\dot{V}O_{2\ MAX}$) was measured on a cycle ergometer. Expired air samples were assessed for oxygen consumption and carbon

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 et al. [19] Merad et al. [11]
Plasmacytoid DCs	Lineage− HLA-DR+CD303+	Major effector sub-population of DCs. Produce type 1 interferons in response to viral infection.	Dzionek et al. [20] Liu [42]
Myeloid DCs	Lineage− HLA-DR+CD303−	Regulatory DC sub-populations. Produce IL-12 for T-cell activation and differentiation.	Dzionek et al. [20] Heufler et al. [45]
CD1c−CD141+	Lineage− HLA-DR+CD303− CD1c− CD141+	Cross presentation of antigen to CD8+ T -cells for anti-tumour immunity.	Penna et al. [21] Ding et al. [24]
CD1c+CD141+	Lineage− HLA-DR+CD303− CD1c+ CD141+	Cross presentation of antigen to CD8+ T cells for anti-tumour immunity and stimulate CD4+ T-cells.	Villani et al. [23] Ding et al. [24]
CD1c+CD141−	Lineage− HLA-DR+CD303− CD1c+CD141−	Stimulate CD4+ T-cells.	Villani et al. [23] Ding et al. [24]
CD1c−CD141−	Lineage− HLA-DR+CD303− CD1c−CD141−	Unknown	Villani et al. [23]

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. HLA-DR = 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 [26] and CD209 (DC-SIGN) a cell surface marker that recognises a wide variety of ligands, is involved in adhesion, migration and antigen presentation [27] were examined on all dendritic cells and sub-populations.