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A single bout of dynamic exercise by healthy adults enhances the generation of monocyte-derived-dendritic cells

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ABSTRACT

The *ex vivo* generation of monocyte-derived-dendritic cells (mo-DCs) has facilitated the use of DCs in immunotherapy research. However, low blood monocyte numbers frequently limit the manufacture of sufficient numbers of mo-DCs for subsequent experimental and clinical procedures. Because exercise mobilizes monocytes to the blood, we tested if acute dynamic exercise by healthy adults would augment the generation of mo-DCs without compromising their differentiation or function. We compared mo-DC generation from before- and after-exercise blood over 8-days of culture. Function was assessed by FITC-dextran uptake and the stimulation of autologous cytomegalovirus (pp65)-specific-T-cells. Supporting the hypothesis, we found a near fourfold increase in number of mo-DCs generated after-exercise. Furthermore, relative FITC-dextran uptake, differentiation rate, and stimulation of pp65-specific-T-cells did not differ between before- and after-exercise mo-DCs. We conclude that exercise enhances the *ex vivo* generation of mo-DCs without compromising their function, and so may overcome some limitations associated with manufacturing these cells for immunotherapy.

1. Introduction

Dendritic cells (DCs) play a crucial role in initiating adaptive immune responses. Immature DCs reside in peripheral tissues where they capture and process antigens. Following activation through microbial components or inflammatory mediators, DCs assume a mature phenotype and migrate to lymphoid organs, where they present antigens and co-stimulatory molecules to B-cells and T-cells [1]. The ability of DCs to orchestrate various arms of the immune response, as well as their unique ability to cross-present viral and tumor antigens [2], has led to great interest in the use of DCs in the development of cancer vaccines and other immunotherapy products [3].

While very few DCs are found in the peripheral blood, DCs can be generated *ex vivo* by culturing blood monocytes in the presence of interleukin 4 (IL-4) and granulocyte–macrophage colony-stimulating factor (GM–CSF) for 6–8 days [4]. This process yields relatively pure populations of monocyte-derived DCs (mo-DCs) [4–7]. The differentiation of monocytes into mo-DCs results in morphological changes such as an increase in size and dendritic processes [6] and the decreased expression of CD14 and increased expression of CD209 (DC-SIGN) on the cell surface [8,9]. Maturing the immature mo-DC product, a necessary step to yield cells with the full ability to stimulate T-cells, is often achieved by adding LPS to cultures for 24–48 h [10,11]. Mature mo-DCs can be identified by the increased surface expression of several molecules, including CD83, HLA-DR, and CCR7 [7].

The manufacture of mo-DCs from normal blood donors has greatly facilitated the use of DCs in immunotherapy research. However, due to relatively low blood monocyte numbers, the number of mo-DCs manufactured *in vitro* is often low as well, which can limit subsequent experimental assays and clinical procedures that require large numbers of mo-DCs (i.e. the expansion of autologous

Abbreviations: APC, allophycocyanin; CMV, cytomegalovirus; DCs, dendritic cells; ELISA, enzyme linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-4, interleukin-4; LPS, lipopolysaccharide; mo-DCs, monocyte-derived dendritic cells; PBMCs, peripheral blood mononuclear cells; PE, phycoerythrin; PE-CY5, phycoerythrin cyanin 5.0.

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cytotoxic T-cells). Monocyte-derived DC yield from peripheral blood mononuclear cells (PBMCs) ranges from 2.7% to 20% of input cells [7], and the yield from 40 ml blood ranges from 0.8 to 8×10^6 DCs [6], or $1–3 \times 10^6$ mature DCs [5]. Current clinical approaches such as stem cell mobilizing therapy and apheresis can increase the number of generated mo-DCs [12,13], but this is a highly invasive technique which created an interest to explore other methods of improving cell yield.

A brief bout of physical exercise leads to a well-characterized increase in the number of mononuclear cells in peripheral blood, including monocytes [14–16]. We wondered therefore whether exercise could be a physiological way to increase the number of monocytes found in the peripheral blood of a healthy participant, and thus increase the number of mo-DCs generated from a single blood donation. However, one possible complication is the fact that exercise not only alters cell numbers but also cellular composition and phenotype. For example, exercise mobilizes 'pro-inflammatory' CD16+ monocytes to a greater extent than 'classical' CD16-monocytes [15,16], and also impacts surface expression of pathogen recognition receptors and activation molecules (e.g., increased TLR4 and HLADR) [16–18]. Moreover, exercise is known to alter the phenotypes and function of other mononuclear cells, including T-cells and NK-cells [19]. It is relevant, therefore, to ensure that mo-DCs generated from blood donated after exercise do not result in phenotypic or functional alterations that would negatively impact downstream experimental assays and clinical procedures.

To this end, we recruited healthy men and women to donate blood before and after a brief bout of maximal exercise, and generated mo-DCs from each donation. We then compared the expression of various mo-DC markers, antigen uptake, and the ability to expand autologous cytotoxic T-cells between mo-DCs generated before- and after-exercise. We hypothesized that a single bout of dynamic exercise would yield a greater number of mo-DCs, and further tested if these enumerative changes would compromise their function or rate of differentiation. The results support this hypothesis, as blood donated after exercise yielded a significant increase in the number of mo-DCs, without altering the resulting cell phenotype or function.

2. Materials and methods

2.1. Participants

Twelve physically active men (6) and women (6) between the ages of 21 and 44 volunteered for this study. Research was carried out in accordance with the Declaration of Helsinki. Written informed consent and medical history were obtained from each participant after the procedures, benefits, and risks were explained verbally and provided in writing. The Committee for the Protection of Human Subjects at the University of Houston granted ethical approval for the study. Participants were instructed to avoid alcohol, nonprescription drugs, and strenuous exercise 24 h prior to each laboratory visit.

2.2. Exercise trials and blood sampling

Participants visited the laboratory between 6 am and 10 am following an overnight fast, and were screened to ensure exercise-readiness according to American College of Sports Medicine guidelines [20]. If passed, they completed the Bruce Maximal Exercise Test, in which they walked and ran on a treadmill (Woodway Desmo, Woodway USA Inc., Waukesha, WI) at increasing intensity until volitional exhaustion [21]. Participants wore a heart rate monitor and face-mask for the continuous measurement of heart rate and respiratory gasses (Quark PFT, COSMED Srl, Italy). A 3 ml venous blood sample was collected before and immediately after exercise in vacutainer tubes treated with ethylene-diamine-tetra-acetic acid (EDTA) (Becton–Dickinson, USA) for differential leukocyte cell counts (BC3200, Mindray North America, Mahwah, NJ). A 40 ml venous blood sample was collected before and after exercise in vacutainer tubes treated with sodium heparin (Becton–Dickenson) for subsequent mo-DC generation assays. An additional blood sample was taken before exercise into a 6 ml serum gel tube (Becton–Dickenson). Serum was obtained by centrifugation and stored at −80 °C until analysis. Two participants also donated 20 ml resting blood collected in vacutainer tubes treated with sodium heparin eight days after the exercise trial for the expansion of CMV-specific T-cells. Blood was processed within 1 h of being drawn for cell counting, mononuclear cell separation, and subsequent assays.

2.3. Generation of dendritic cells from blood monocytes

Mononuclear cells were isolated from venous blood samples obtained before and after exercise using density gradient centrifugation (Histopaque-1077, Sigma Aldrich, USA). Mononuclear cells were washed twice and plated at 5×10^6 cells/well in dendritic cell (DC) medium (CellGro GMP serum-free DC medium; CellGenix, Freiburg Germany) in a 12-well plate (Costar) for 2 h at 37° C in a humidified CO_2 incubator. Equal numbers of cells were used from before- and after-exercise. Nonadherent cells were removed by rinsing wells twice with PBS (Sigma Aldrich). The adherent cell fraction, containing the monocytes, was cultured in DC media with 800 U/ml granulocyte–macrophage colony-stimulating factor (GM–CSF (Sigma Aldrich) and 1000U/ml interleukin-4 (IL-4) (R&D Systems) for 6 days at 37° C in a humidified CO_2 incubator. This adherence method of monocyte isolation does not differ from other methods (such as immunomagnetic separation) in the resulting DC phenotype and function [7]. On day 3, half of the media was removed and replaced with media containing 400 U/ml GM–CSF and 500 U/ml IL-4. On day 6, half of the media was removed and replaced with fresh media containing 400 U/ml GM–CSF, 500 U/ml IL-4 and 100 ng/ml LPS (Sigma Aldrich).

2.4. Phenotypic analysis

Loosely adherent cells were harvested from sample wells at day 0, day 3, day 6, and day 8; these were cells that detached from the tissue culture plastic after rinsing the wells twice with PBS. Cell phenotype was assessed by flow cytometry at each day; non-adherent cells were also analyzed at day 0. Harvested cells were labeled with FITC-conjugated anti-CD14 (clone 61D3), PE-conjugated anti-CD16 (clone eBioCB16), anti-CD209 (clone eB-h209), or anti-CCR7 (IgG2a, clone 3D12), PerCP-Cy5.5-conjugated anti-CD56 (IgG1, Clone CMSSB) or anti-CD11c (clone 3.9), and APC-conjugated anti-CD3 (IgG1, clone UCHT1), anti-CD83 (clone HB1se), or anti-HLADR (clone LN3) in a four-color direct immunofluorescence procedure. All monoclonal antibodies (mAb) were previously titrated to determine optimal dilutions for flow cytometry. Cells were incubated with 50 µl of each pre-diluted mAb for 30 min in the dark at room temperature. Antibodies were purchased from eBioscience Inc. (San Diego, CA, USA).

Cell phenotypes were assessed on a BD Accuri C6 flow cytometer (BD Accuri, Ann Arbor, MI, USA) equipped with a blue laser emitting light at a fixed wavelength of 488 nm and a red laser emitting light at a fixed wavelength of 640 nm. Lymphocytes, monocytes and mo-DC were identified by forward and side scatter characteristics and gated electronically using Accuri C6 (CFlow software v1). Single color tubes were used for compensation. A minimum of 20,000 gated events were collected.