SRTM Student Research in Translational Medicine

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The Differentiation of Monocytes Into DCs: A Morphologically Multi- Step Phenomenon

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ABSTRACT

Dendritic cells (DCs) are remarkable professional antigen- presenting cells (APCs) that are pivotal in bridging the gap between innate and adaptive immunity (1). Given this unique ability, these cells are a promising target for immunotherapy of various diseases (2). Monocytes serve as a valuable resource for generating DCs in vitro. To generate monocyte-derived DCs and investigate morphological changes during the differentiation phenomenon, following the reception of written informed consent, peripheral blood samples were obtained from healthy donors using falcon tubes containing heparin. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll solution with a density of 1.077 g/ ml and density gradient centrifugation. Monocytes were subsequently extracted from PBMCs through the magnetic-activated cell sorting method (MACS) following the instructions provided with the kit. The culture of monocytes was carried out in a concentration of 1.5×10^6/mL in a 6-well plate using complete culture media (RPMI-1640 supplemented with 15% FBS, 100 µg/ mL streptomycin, 100 IU/ mL penicillin, and 2 mM L-glutamine), in conjunction with 50 µM 2-mercaptoethanol solution and recombinant human GM-CSF and IL-4 cytokines at concentrations of 40 and 25 ng/ mL, respectively. The plate was incubated at 37°C with 5% CO2 and humidification. After three days, half of the culture media was substituted with a new mixture containing GM-CSF and IL-4 cytokines. Immature DC (iDCs) cells were collected on the fifth day. Following this, iDCs were treated with 100 ng/ mL of lipopolysaccharide (LPS) and incubated for 24 hours to induce the maturation of iDCs. On the sixth day, mature DCs were harvested. Monocytes and DCs were examined for their morphology, with images captured utilizing an inverted light microscope. In this regard, microscopic analysis revealed morphological alterations between monocytes derived from PBMCs at the initiation of culture and differentiated mature DCs acquired on day 6. Monocytes appeared as round cells with no visible dendrites, in contrast to DCs which were distinguishable by their visible dendrites (Figure1).

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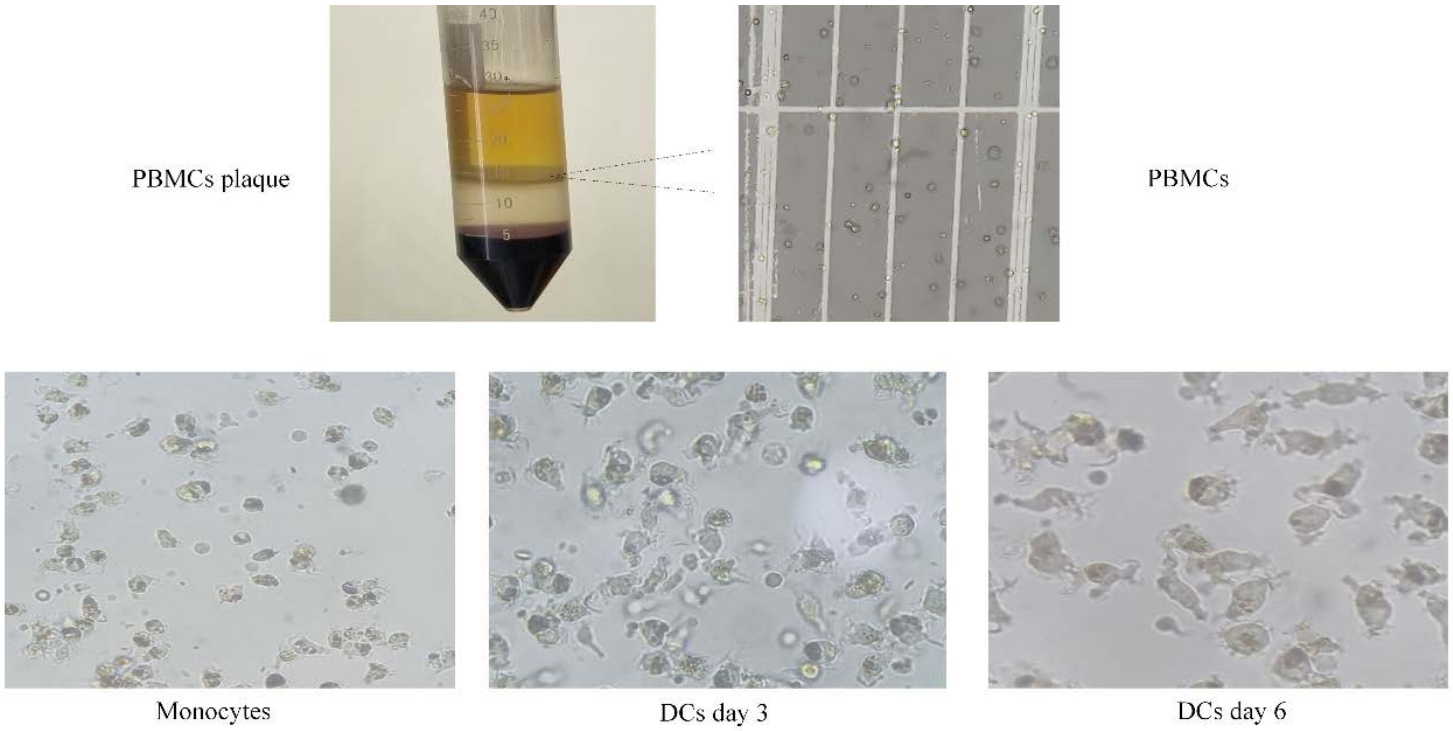

FIGURE 1. Morphological characterization of monocytes and DCs.
PBMCs: peripheral blood mononuclear cells, DCs: dendritic cells.

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