Cytotherapy, 2017; 19: 486–499

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Human platelet lysate is a successful alternative serum supplement for propagation of monocyte-derived dendritic cells

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Abstract

Background aims. Clinical protocols for dendritic cell (DC) generation from monocytes require the use of animal serum-free supplements. Serum-free media can also require up to 1% of serum supplementation. In addition, recommendations based on the 3Rs (Refinement, Reduction, Replacement) principle also recommend the use of non-animal sera in *in vitro* studies. The aim of this study was to explore the potential use of platelet lysate (PL) for generation of optimally differentiated DCs from monocytes. *Methods.* Cells were isolated from buffy coats from healthy volunteers using immunomagnetic selection. DCs were differentiated in RPMI1640 supplemented with either 10% fetal bovine serum (FBS), 10% AB serum or 10% PL with the addition of granulocyte monocyte colony stimulating factor and interleukin-4. Generated DCs were assessed for their morphology, viability, endocytotic capacity, surface phenotype (immature, mature and tolerogenic DCs) and activation of important signaling pathways. DC function was evaluated on the basis of their allostimulatory capacity, cytokine profile and ability to induce different T-helper subsets. *Results.* DCs generated with PL displayed normal viability, morphology and endocytotic capacity. Their differentiation and maturation phenotype was comparable to FBS-cultured DCs. They showed functional plasticity and up-regulated tolerogenic markers in response to their environment. PL-cultured mature DCs displayed unhindered allostimulatory potential and the capacity to induce Th1 responses. The use of PL allowed for activation of crucial signaling proteins associated with DC differentiation and maturation. *Discussion.* This study demonstrates for the first time that human PL represents a successful alternative to FBS in differentiation of DCs from monocytes. DCs display the major phenotypic and functional characteristics compared with existing culture protocols.

Key Words: *dendritic cells, differentiation, monocytes, platelet lysate*

Introduction

The differentiation of human dendritic cells (DCs) from peripheral blood monocytes represents one of the most widely used approaches for generation of DCs for therapeutic purposes. The basic methodology of this approach relies on the use of granulocyte-monocyte colony stimulating factor (GM-CSF) and interleukin (IL)-4, which drive the differentiation of monocytes toward antigen-presenting cells (APCs) with general phenotypic and functional characteristics of native DCs [1]. Such monocyte-derived DCs have increasingly been used for immunotherapy of cancer since mid-1990s. DC-based immunotherapy is safe and can improve patient survival and antitumor immunity [2]. In addition, due to numerous recent discoveries highlighting the role of DCs in immune tolerance induction, there is a rising trend in use of tolerogenic DCs (TolDCs) as negative vaccines for immunosuppressive cell therapy. Such TolDCs can be used in various clinical settings such as transplantation, autoimmune and chronic inflammatory diseases [3–5]. For the present clinical purposes, which must correspond to Good Manufacturing Practice (GMP) criteria, the DCs are generated under animal serum–free conditions in various serum-free media (AIM-V, X-VIVO15, CellGro, etc.) or in other basal media substituted using human AB serum, avoiding the use of animal sera such as fetal bovine serum (FBS).

In addition, slowly but surely, the use of FBS is increasingly being avoided in basic research studies, and it is likely we will see this trend in the future. The major concern in this context is of course the existing method of FBS collection, which may cause suffering of the animals, in particular, the fetuses [6]. The technical reason for avoiding FBS is the potential nonspecific immunomodulatory effect of FBS compared with human serum. The great number of heterologous proteins present in FBS compared with human sera can serve as an important antigenic (Ag) load, which is taken up and presented by the DCs and can later influence the outcome of functional study experiments involving Ag presentation [7].

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(Received 22 November 2016; accepted 12 January 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.01.005

The main purpose of serum supplementation in cell culture is the promotion of cellular growth and differentiation [8]. Animal serum is a rich source of various constituents, biomolecules with different molecular weight and different effects on both growth promotion or growth inhibition. When present in culture media, serum provides hormonal factors that can stimulate growth and proliferation. Additionally, serum can provide various transport proteins, lipids, trace elements, minerals, attachment and spreading factors, as well as stabilizing and detoxifying factors that can stabilize pH levels and serve as protease inhibitors (e.g., α-antitrypsin) [9].

A number of experimental groups platelet lysate (PL) have recognized as a valuable, non-animal alternative to the use of FBS in cell culture [10–16]. It is usually prepared from outdated human donor platelets from apheresis products, which are treated by repeated freeze-thawing of platelet suspension to achieve the release of growth factors. After final centrifugation, which removes the debris, PL can be used as cell media supplement. Although PL has been successfully implemented in culture of various cell lines, its uses for *ex vivo* cell culturing protocols have been mostly limited to propagation of mesenchymal stem cells (MSCs).

According to Clinicaltrials.gov, there are now more than 700 registered clinical trials using DCs in cell therapy studies, most of which are using or have used monocyte-derived DCs for either positive or negative cell vaccination purposes. Until now, there have been no reports on whether PL could be used successfully in this context. The aim of this study was to evaluate the potential of PL as a non-xenogenous cell media supplement for the generation of optimally differentiated human DCs from peripheral blood monocytes.

Methods

Preparation of human PL

Human PL was prepared at the Blood Transfusion Centre of Slovenia using the Platelet Lysate Preparation Kit (Macopharma) according to manufacturer's protocol. For the preparation of PL, we used platelets collected during apheresis procedure. All selected donors signed an informed consent agreement. The platelet apheresis bags were properly labeled, indicating the collection number, product volume, date of collection, date of freezing and date of centrifugation. The platelet apheresis bag was then welded to the freezing bag in a sterile manner and the bag content transferred to the freezing bag. The platelets were then placed in a –80°C freezer and left to freeze. In the next step, frozen platelets are placed inside 2–8°C refrigerator and left to thaw for approximately 12–15 h. When the product was completely thawed, it was centrifuged at 3500*g* for 30 min at room temperature. After centrifugation, the bag was carefully removed from the centrifuge and the pellet separated from the supernatant with a clamp. The bag was then sterilely connected to the filtration set and hung to allow for gravitation-assisted filtration. The filtration set contained nine smaller bags in which the end product was stored at –80°C for future use. This study was performed using platelet lysate obtained from three donors.

Cell culture and isolation

Buffy coats from venous blood of normal healthy volunteers were obtained by the Blood Transfusion Centre of Slovenia according to institutional guidelines. Peripheral blood mononuclear cells (PBMCs) were isolated using Lympholyte-H (Cedarlane Laboratories). The cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS), counted and used as a source for immunomagnetic isolation of $CD14^+$ cells (Miltenyi Biotec). The purity of monocytes was always >90%, as determined by flow cytometry. For monocyte to DC differentiation, we used RPMI 1640 (Lonza) supplemented with 10% FBS, 10% human AB serum or 10% PL. Additionally, each medium preparation was always supplemented with gentamicin (50 mg/mL; Gibco) and GlutaMAX (Gibco). In all cases, 800 U/mL of recombinant human (rh)GM-CSF and 1000 U/mL of rhIL-4 (both Peprotech) were added to cell culture. On days 2 and 4, half of the medium was exchanged with starting quantities of rhGM-CSF and IL-4. On day 6, the differentiated monocytes (DCs) were harvested, washed twice with DPBS and counted using Vi-Cell XR cell viability analyzer (Beckman Coulter). To obtain mature DCs, the cells were activated using lipopolysaccharide (LPS; 20 ng/mL). In some experiments, the cells were treated with the activated form of vitamin D (1,25-dihydroxyvitamin D_3; 10 nmol/L), which is a known inducer of tolerogenic DCs [17], to induce tolerogenic DCs.

T cells were purified from human buffy coats. Whole $CD4^+$ T cells were obtained by positive selection using CD4 microbeads (Miltenyi Biotec). The purity of $CD4^+$ cells was always >95% as determined by flow cytometry. Naive $CD4^+CD45RA^+$ T cells were isolated using the naive $CD4^+$ T-cell isolation kit (Miltenyi Biotec), strictly following the manufacturer's protocol. The purity of isolated naive $CD4^+$ T cells was always >98%.

Morphological cell analysis

To analyze the cellular morphology of variously differentiated DCs, we performed microscopy analysis