Culture and Differentiation of Monocyte Derived Macrophages Using Human Serum: An Optimized Method

Article · June 2016

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Introduction

Macrophages are professional scavenger immune cells which regulate innate and adaptive immunity responses [1, 2]. Interestingly, the proper function of macrophages is related to immune regulation in some infectious, autoimmune and inflammatory diseases [2]. To study the function of macrophages in vitro, the monocyte derived macrophage (MDM) is an acceptable and cost effective model [3-5]. Macrophages in vitro, the monocyte derived macrophage (MDM) is an acceptable and cost effective model [3-5].

Current protocols for MDM preparation require expensive supplementations. Different variables such as incubation time and serum concentration affect this simple MDM preparation method. Here we represent an optimized simple method for MDM preparation from peripheral blood mononuclear cells (PBMC).

Materials and Methods

To introduce an optimized method we accomplished the present descriptive study. After PBMC isolation and monocyte enrichment in complete RPMI 1640 growth media, macrophages were cultured and differentiated using human serum. Efficient phagocytosis was evaluated using heat-inactivated Escherichia coli followed by SYBR staining. Geimsa staining of macrophages was accomplished to visualize the typical morphology under light microscopy.

Results: The derived macrophages have the typical morphology of differentiated macrophages and are able to phagocyte the heat inactivated SYBR stained E. coli.

Conclusion: This optimized method is a simple and cost effective method to prepare MDM with typical morphology representations able to phagocytosis efficiently.

Abstract

Background: Monocyte derived macrophages (MDMs) are appropriate in vitro models to study the function of macrophages in immune related diseases. Not only most of the methods in literature for efficient MDM culture and differentiation are expensive but also they require specific equipment. However, there are some reports indicating that monocyte enrichment is possible through attachment. Purification and differentiation of macrophages occurs in media containing human serum or platelet depleted plasma without extra supplantations. Different variables such as incubation time and serum concentration affect this simple MDM preparation method. Here we represent an optimized simple method for MDM preparation from peripheral blood mononuclear cells (PBMC).

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Results

Peripheral blood mononuclear cells consist of a heterogeneous population of mononuclear cells including lymphocytes, natural killer cells and monocytes [13]. Monocytes distinguishable ability for attachment makes it possible to purify them from PBMC simply [14, 15]. Different variables could affect the efficiency of purification [5, 8, 9, 11, 14, 16]. To achieve the most favorable attachment condition for monocytes, we tested non gelatin-treated, gelatin (2%) coated, plasma treated or both gelatin and plasma treated tissue culture plates. Approximately, $2.5 \times 10^6$ PBMCs were settled down in RPMI 1640 (0.5% AB positive human serum, 1% penicillin-streptomycin) in 5% CO$_2$ incubator for 3 h. Lymphocytes were aspirated afterwards and monocytes were washed to remove all the remaining floated or loosed cells. Finally, monocytes were visualized by light microscopy and then detached to count by the use of Trypan blue 0.4% and hemocytometer. A total number of $1.5 \times 10^6$ monocytes were acquired from a 10 mL blood volume ($1.5 \times 10^5$ per blood mL). As shown in figure 1 the percentage of monocyte to PBMCs is about 5% which is in normal range. There were no obvious differences of enriched monocyte populations in different plates coated with gelatin, plasma or gelatin and plasma together as attachment enhancers with monocytes enriched in non-treated tissue culture plates (not shown).

Attachment triggers the differentiation of monocytes to macrophages [14] and some cytokines like granulocyte and granulocyte-monocyte colony stimulation factors (GCSf, GMCSf) enhance this process [17]. There are some evidences indicating FBS or human serum can alternatively supply the necessary cytokines and colony stimulation factors (CSFs) for the maturation of macrophages [8, 11].

![Figure 1. Enriched peripheral blood monocytes. Before (A) and after (B) attachment mediated enrichment process. (Magnification 40x). The table describes cell counts calculated by Trypan blue viable staining method](image-url)
Geimsa staining was used to observe the morphological changes of differentiation in macrophages in details. The macrophages were stained and studied by light microscopy with higher magnification after 12 days of differentiation in 10% human AB positive human serum. A normal and distinct feature of macrophage morphology was observed including increased cytoplasmic ratio, pseudopodia and vacuolar system indicating phagocytic activity (Fig. 3). Interestingly, all macrophages displayed similar phenotypic characteristics and were morphologically homogenous.

**Figure 2.** Macrophages differentiation monitoring by light microscopy. Early at day 3 the spindled shape macrophages were observed although non differentiated monocytes were noticeable (A). As shown by Geimsa staining, by the end of day 12, almost all of cells were differentiated to macrophage morphology (B and C), although culture in human AB positive serum (C) provided more better results (B). (Magnification 40x)

**Figure 3.** Distinct morphology of differentiated macrophages stained by Geimsa. The increased cytoplasmic ratio, pseudopodia (arrow head) and extended vacuolar system (arrow) are shown. (Magnification 400x)

Phagocytosis is the most important function of macrophages as a scavenger cell. We were interested to study if the resulted differentiated macrophages were able to accomplish the phagocytosis process successfully. Non-opsonized SYBR stained heat inactivated *E. coli* was used for phagocytosis assay according the method described previously. With fluorescent microscopy imaging, we noticed not all but a number of macrophages engulfed green colored bacteria successfully during phagocytosis assay (Fig. 4).

**Figure 4.** Phagocytosis assay, macrophages engulfed green colored bacteria (marked by Arrows) successfully (A), B shows blue stained nucleus of same section with DAPI. Merged A and B indicates cytoplasmic position of green colored phagocytes (C). The heat inactivated SYBR stained *E. coli* is imaged by fluorescent microscopy as a control (D). (Magnification 100x)

**Discussion**

Here, we presented an optimized method to culture and differentiate PBMCs into MDMs and obtaining functional macrophages which are able to function and phagocyte efficiently. Culture and differentiation of monocyte derived macrophages can provide an applicatory in vitro tool for studies related to innate immunity and inflammatory diseases [18].

Monocytes constitute about 2-8% of WBC population in peripheral blood [3]. Utilizing specific surface markers in magnetic activated cell sorting (MACS) selection to purify monocytes from PBMC is feasible but this requires more facilities, indeed a remarkable proportion of monocytes might be missed due to restriction factors such as antigen-antibody cross reaction [6]. According to literature, monocyte purification based on their distinctive affinity of attachment can yield 95% purity [8, 15]. Some researchers have reported gelatin coating or fibronectin coating [19] and plasma treatment the surface of culture plates can facilitate the attachment [12] while we found no difference between monocyte attachment enrichment in gelatin or plasma treated and non-treated tissue culture plates.

Differentiation of macrophages is a regulated process depending on conditions which can result in classical M1 or alternative M2 macrophages [4]. Different populations of macrophages can exhibit different features and functions [3]. While G-CSF enhances M1 differentiation, GM-CSF promotes M2 phenotypes [20]. Some researchers supply both GCSF and GMCSF to avoid this bias, but it actually disturbs the plasticity of macrophages differentiation [21, 22]. This method is unprofitable when the differentiation plasticity or M1/M2 ratio is the main subject of study. Some older protocols have used human
serum instead for macrophages differentiation [11], although less macrophage proliferation will occur in this case. Here, we showed AB positive blood type human serum is the best choice for this purpose, since it is preferred by human cells in comparison to FBS and indeed has no cross reaction with probable RBCs contamination. The resulted macrophages present typical morphology with increased cytoplasmic ratio, pseudopodia and vacuolar system.

In conclusion, this optimized method is a simple and cost effective method to prepare monocyte derived macrophages with typical morphology representations able to phagocytosis efficiently; applicable for in vitro functional studies of macrophages.

Acknowledgements

References


This article is extracted from a research study financially supported by Golestan University of Medical Sciences (grant number: 930618118). The authors also would like to thank personnel of cell culture Lab and Molecular Medicine Lab in faculty of Advanced Medical Technologies specially Mrs. M. Haydari, Dr S. Zhand and Dr Ayoob Khosravi for being great supports.

Authors’ Contributions

All authors are contributed equally to this work.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding/Support

Golestan University of Medical Sciences.