Journal of Immunological Methods xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Facilitating THP-1 macrophage studies by differentiating and investigating cell functions in polystyrene test tubes

Annabelle Small[a,b,c], Nikki Lansdown[a,b,c], Marwah Al-Baghdadi[a,b,c], Alex Quach[b,c], Antonio Ferrante[a,b,c,*]

[a] Department of Molecular and Cellular Biology, School of Biological Sciences, University of Adelaide, South Australia, Australia
[b] Robinson Research Institute, Disciplines of Paediatrics and Obstetrics and Gynaecology, School of Medicine, University of Adelaide, South Australia, Australia
[c] Department of Immunopathology, SA Pathology, Women's and Children's Hospital, South Australia, Australia

ARTICLE INFO

Keywords:
Macrophage
Differentiation
THP-1 cell line
Complement receptors
Phagocytosis

ABSTRACT

Macrophage cell lines are a useful model to explore the properties of primary macrophages. However, a major limitation in the use of these cells is that when they are differentiated, they become adherent and hence present with the same limitation as natural macrophages. The cells need to be detached and are often subjected to detachment techniques such as detachment buffers containing proteolytic enzymes or scraping with a rubber 'policeman'. These steps are time-consuming, reduce cell yields as well as cell viability and function. We have therefore investigated the possibility of differentiating the human macrophage THP-1 cell line in polystyrene FACS tubes to enable cells to be directly used for investigations by flow cytometry. Here we demonstrate that when the human macrophage cell line THP-1 are cultured in FACS tubes with phorbol myristate acetate added, they undergo differentiation into macrophages, assessed morphologically and by autofluorescence expression, in a similar manner to those cultured in tissue culture dishes. The cells can be readily washed and adjusted in concentration by centrifugation in the same tubes and can be directly tested for expression of cell surface markers and function by flow cytometry. This avoids the use of either detachment reagents or physical cell scraping. Consequently, we showed that the tube culture method results in increased cell yield and viability compared to those subjected to detachment procedures. The tube method generated functional macrophages which expressed the complement receptors, CR3 and CR4, and effectively phagocytosed complement opsonised *Staphylococcus aureus* via these receptors.

1. Introduction

THP-1 cells are a human monocytic cell line derived from a childhood M5 subtype of acute monocytic leukaemia (Tsuchiya et al., 1980). Since their establishment, these cells have been extensively used as a model to study monocyte and macrophage function (Bosshart and Heinzelmann, 2016). However, like primary macrophages, THP-1 differentiated cells are highly adherent (Lund et al., 2016), a characteristic which hinders the ability to use the cells in investigations. In order to overcome this issue, multiple techniques to detach these cells have been established, including the use of buffers containing trypsin, commercial buffers such as Accutase, EDTA containing buffers, and physically scraping cells using rubber cell scrapers (Chen et al., 2015). However, these buffers can be expensive, and the use of trypsin has been known to cleave cell surface proteins (Zhang et al., 2012), resulting in changes to cell function. In addition, cell scraping and the use of EDTA buffers often result in low cell yields with low viability due to damage to the cell structure (Van Veldhoven and Bell, 1988). Thus, there remains a need for new approaches to handling adherent cell types such as THP-1 derived macrophages.

Here, we describe a method for differentiating functional THP-1 macrophages using 5 mL polystyrene FACS tubes; a method which is cheaper, provides higher cell yields, and higher cell viability compared to the classical culture dishes.

2. Materials and methods

2.1. Reagents and antibodies

RPMI 1640 tissue culture medium, foetal calf serum (FCS) and L-glutamine were purchased from SAFC Biosciences (Lenexa, KS). Phorbol myristate acetate (PMA) was purchased from Sigma Aldrich (St. Louis, MO). PE-conjugated CD11b (clone 2LPM19c) and FITC-conjugated CD11c (clone KB90) antibodies were purchased from Dako

* Corresponding author at: Department of Immunology, SA Pathology, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia.
E-mail address: antonio.ferrante@adelaide.edu.au (A. Ferrante).

https://doi.org/10.1016/j.jim.2018.06.019
Received 16 May 2018; Received in revised form 13 June 2018; Accepted 28 June 2018

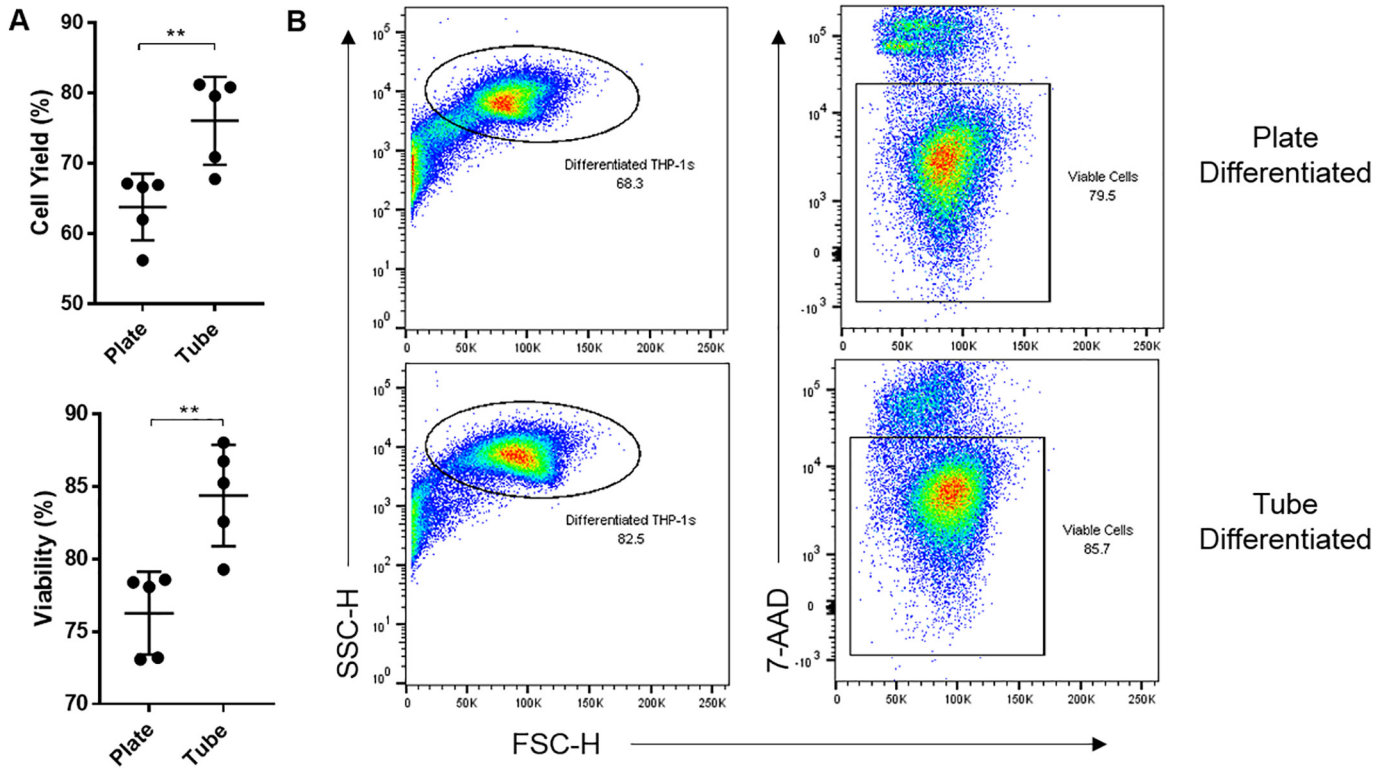

Fig. 1. Differentiating THP-1s in polystyrene FACS tubes yields higher cell numbers and higher viability than those differentiated in tissue culture plates (A). (B) Gating strategy for differentiated cells is based on side vs. forward scatter (left panel) and viable cells are gated based on their ability to exclude 7-AAD (right panel). Data are presented as percentage viable cells of the gated differentiated population ± SD, and is representative of five experiments. $^{**}p < .01$, unpaired student's *t*-test.

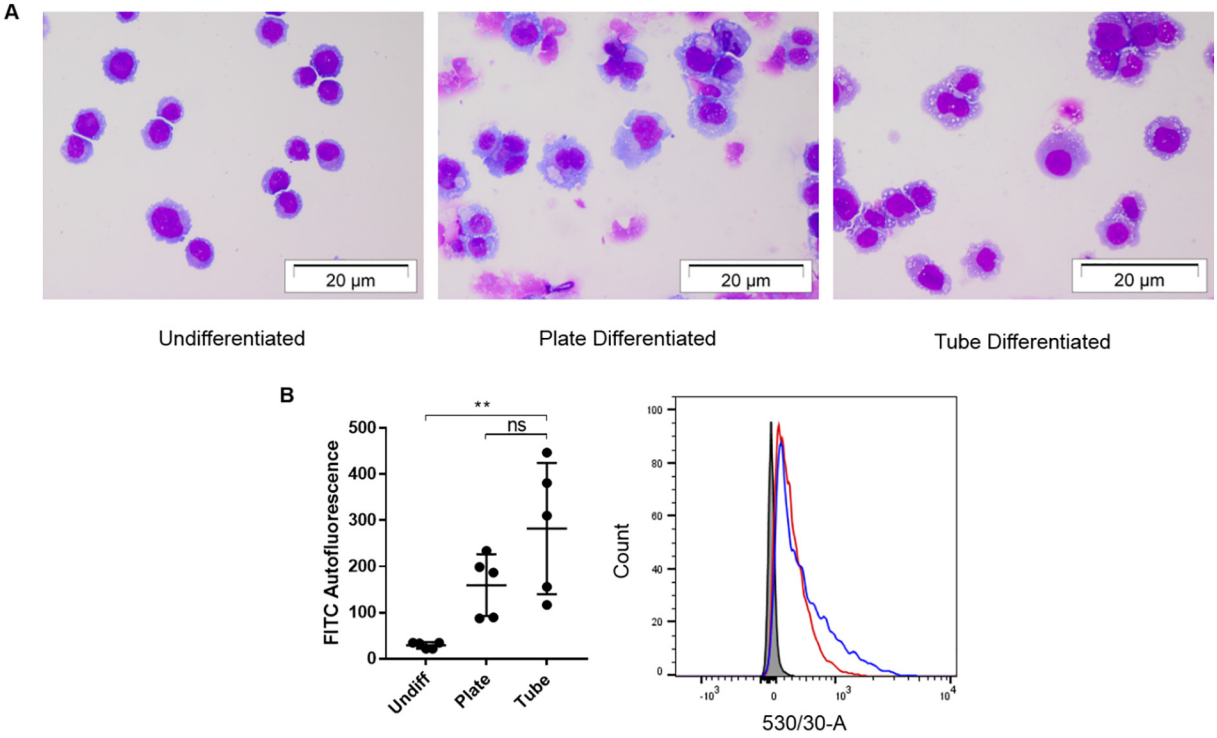

Fig. 2. Differentiated THP-1 macrophages produced in either FACS tubes or tissue culture dishes show similar morphological changes and equal amounts of autofluorescence. (A) Giemsa stained smears comparing undifferentiated THP-1s (left panel), with pDMs (centre panel) and tDMs (right panel). (B) Autofluorescence of undifferentiated THP-1s, pDMs, and tDMs detected by blue (488 nm) excited green fluorescence (530 nm, 30 nm bandwidth), with representative autofluorescence histogram shown (bottom right). Undifferentiated cells show close to no fluorescence (shaded), compared with increased levels observed in pDMs (red), and tDMs (blue). Data are presented as median fluorescent intensity of gated macrophages ± SD, and is representative of five experiments, ns = not significant, $^{**}p < .01$, Dunnett's multiple comparison test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)