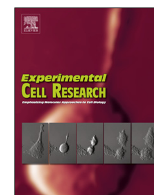
Experimental Cell Research 347 (2016) 1–13

Contents lists available at ScienceDirect

Experimental Cell Research

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



Research Article

Differential gene expression in human, murine, and cell line-derived macrophages upon polarization

Kara L. Spiller [a,1], Emily A. Wrona [b,1], Saly Romero-Torres [c], Isabella Pallotta [b], Pamela L. Graney [a], Claire E. Witherel [a], Leelamma M. Panicker [d], Ricardo A. Feldman [d], Aleksandra M. Urbanska [e], Laura Santambrogio [f], Gordana Vunjak-Novakovic [e], Donald O. Freytes [b,1,*]

[a] Drexel University, School of Biomedical Engineering, Science, and Health Systems, Philadelphia, PA, United States
[b] The New York Stem Cell Foundation Research Institute, New York, NY, United States
[c] Bio-Hyperplane LLC, Berkeley Heights, NJ, United States
[d] University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD, United States
[e] Columbia University, Departments of Biomedical Engineering and Medicine, New York, NY, United States
[f] Albert Einstein College of Medicine, Department of Pathology, Microbiology, and Immunology, Bronx, NY, United States

ARTICLE INFO

Article history:
Received 18 July 2015
Received in revised form
11 October 2015
Accepted 16 October 2015
Available online 21 October 2015

Keywords:
Classically activated
Alternatively activated macrophages
Wound healing
In vitro characterization
Angiogenesis
Tissue engineering
Induced pluripotent stem cells
Biomaterials

ABSTRACT

The mechanisms by which macrophages control the inflammatory response, wound healing, biomaterial-interactions, and tissue regeneration appear to be related to their activation/differentiation states. Studies of macrophage behavior *in vitro* can be useful for elucidating their mechanisms of action, but it is not clear to what extent the source of macrophages affects their apparent behavior, potentially affecting interpretation of results. Although comparative studies of macrophage behavior with respect to cell source have been conducted, there has been no direct comparison of the three most commonly used cell sources: murine bone marrow, human monocytes from peripheral blood (PB), and the human leukemic monocytic cell line THP-1, across multiple macrophage phenotypes. In this study, we used multivariate discriminant analysis to compare the *in vitro* expression of genes commonly chosen to assess macrophage phenotype across all three sources of macrophages, as well as those derived from induced pluripotent stem cells (iPSCs), that were polarized towards four distinct phenotypes using the same differentiation protocols: M(LPS,IFN) (aka M1), M(IL4,IL13) (aka M2a), M(IL10) (aka M2c), and M(-) (aka M0) used as control. Several differences in gene expression trends were found among the sources of macrophages, especially between murine bone marrow-derived and human blood-derived M(LPS,IFN) and M(IL4,IL13) macrophages with respect to commonly used phenotype markers like CCR7 and genes associated with angiogenesis and tissue regeneration like FGF2 and MMP9. We found that the genes with the most similar patterns of expression among all sources were CXCL-10 and CXCL-11 for M(LPS,IFN) and CCL17 and CCL22 for M(IL4,IL13). Human PB-derived macrophages and human iPSC-derived macrophages showed similar gene expression patterns among the groups and genes studied here, suggesting that iPSC-derived monocytes have the potential to be used as a reliable cell source of human macrophages for *in vitro* studies. These findings could help select appropriate markers when testing macrophage behavior *in vitro* and highlight those markers that may confuse interpretation of results from experiments employing macrophages from different sources.

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Abbreviations: APC, allophycocyanin; bFGF, basic fibroblast growth factor; β-ME, beta-mercaptoethanol; BM, Bone Marrow; CCL, Chemokine (C-C) ligand 17; CCR7, Chemokine (C-C) receptor 7; CD, Cluster of differentiation; CO2, carbon dioxide; CXCL, C-X-C motif chemokine; DEPC, Diethylpyrocarbonate; DMEM-F12, Dulbecco's modified eagle medium/nutrient mixture F12; EDTA, ethylenediaminetetraacetic acid; FBS, Fetal Bovine Serum; FGF2, Fibroblast growth factor 2; hrs, hours; IFN, Interferon; IgG2 a, immunoglobulin G 2a; IL, Interleukin; iPSC, Induced Pluripotent Stem Cells; KLF4, Kruppel-like factor 4; LPS, Lipopolysaccharide; M-CSF, Monocyte colony stimulating factor; MDM, monocyte differentiation medium; MEF, Mouse embryonic fibroblasts; MMP9, Matrix metallopeptidase 9; MRC, Mannose receptor, C; NEAA, non essential amino acids; NOS2, Nitric oxide synthase 2; OCT-4, Octamer-binding transcription factor 4; PB, Peripheral Blood; PBMC, Peripheral blood mononuclear cell; PBS, Phosphate-buffered saline; PDGF, Platelet-derived growth factor; PLS-DA, Partial Least Square Discriminant Analysis; PMA, Phorbol-12-myristate 13-acetate; RNA, ribonucleic acid; RPMI, Roswell Park Memorial Institute; TGF-β, Transforming growth factor beta; TIMP, Tissue inhibitor of metalloproteinase; TNF, Tumor necrosis factors; VEGF, Vascular endothelial growth factor; VIP, Variables important for projection

* Corresponding author. Fax: +1 212 851 5423.
E-mail address: dfreytes@nyscf.org (D.O. Freytes).

[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.yexcr.2015.10.017
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1. Introduction

Macrophages, the primary cells of the innate immune system, are major regulators of tissue development, homeostasis, repair, and disease [1,2]. The diverse functions of macrophages in tissue homeostasis appear to be related to their activation state or phenotype, which can shift rapidly in response to local environmental stimuli [3–5]. For example, macrophages found in different organs throughout the body exhibit specific transcriptional profiles, and transplantation of peritoneal macrophages to the lungs in mice caused their upregulation of lung macrophage-specific genes [5]. Moreover, removal of polarizing signals does not necessarily return macrophages to a resting state, but rather enhances their response to a second exposure to the same stimulus [6,7].

Rapid and dramatic changes in macrophage phenotype in response to injury have been well-described. At early stages of injury, macrophages first exhibit a predominantly pro-inflammatory phenotype, also referred to as “classically activated” or M1 [8–11]. At later stages of injury and healing, the predominant phenotype of the macrophage population switches to an “alternatively activated” (M2) phenotype. Importantly, it is understood that macrophages exist on a spectrum of phenotypes, including hybrid phenotypes with characteristics of M1 and M2 macrophages as well as those activated by signals that are not easily defined, such as micro-environmental cues that affect the behavior of tissue-specific macrophages [5,12,13]. Related to their role in wound healing and angiogenesis, macrophages are an important player in the host tissue response to biomaterials and tissue engineering constructs [14–16]. Although the sequential M1-to-M2 transition is conserved in wound healing of mice and humans [10,11,17,18], conflicting reports have surfaced surrounding the relative contributions of M1 and M2 macrophages to the healing process. For example, several studies have shown that M2 macrophages are the dominant phenotype promoting angiogenesis [19,20], while other studies have shown that M1 macrophages are more angiogenic [16,21]. In addition, two different subtypes of M2 macrophages, those polarized using IL4 with or without IL13 (often called M2a) and those polarized with IL10 (often called M2c), have been shown to have very different roles in angiogenesis and tissue regeneration [16,22], but few studies have directly compared them. Notably, studies are typically conducted with macrophages derived from multiple sources, with human peripheral blood, murine bone marrow, and the leukemic cell line THP-1 being the most common sources. Although there is a great deal of information to be learned from *in vitro* studies of macrophage behavior, such as the response of macrophages to biomaterials (Table 1) or the interactions between macrophages and other cells such as endothelial cells [16], fibroblasts [23], neurons [24], mesenchymal stem cells [25,26] and cardiomyocytes [27], it is not clear to what extent, if any, the cell source of macrophages has on data interpretation. Due to the lack of standardization while characterizing macrophages *in vitro*, it has been difficult to compare results from previous studies (Table 1) and to develop bench top assays of macrophage-biomaterials interactions with predictive power.

Previous studies have reported differences in gene expression between mouse and human macrophages cultured *in vitro*, including thorough microarray or deep sequencing studies that compared unactivated macrophages with those polarized using lipopolysaccharide (LPS) or IL4 [28,29]. However, the mouse-human comparisons in these studies were made only with unactivated controls, obscuring patterns of gene expressions upon polarization to more than one phenotype. In addition, gene expression across multiple studies cannot be compared because of different experimental conditions including cell culture/sampling conditions, probe set design, and donor-to-donor variability. Finally, differences in gene expression of macrophages have also been reported between human PB- and THP-1-derived macrophages [30], but not after polarization to different phenotypes [31,32]. Thus, there is a need to compare gene expression changes of macrophages polarized to multiple phenotypes cultured and analyzed under the same conditions.

Recently, macrophages differentiated from monocytes derived from induced pluripotent stem cells (iPSCs) were shown to exhibit similar patterns of gene expression to PB monocyte-derived

Table 1
Example of biomaterials tested using different macrophage sources and the markers used to differentiate between macrophage states.

Cell source	Biomaterial	Markers			Ref.
		Gene expression	Cytokine secretion	Staining	
Peripheral blood (human)	Polylactic acid	–	TNFα, CCL18	CD206, stabilin-1	[51]
	Poly-ε-caprolactone bisurea strips	MCP1, IL6, TNF, IL10, CXCL12	–	CD68, CCR7, CD206, CD163	[52]
	Silk	IL1β, IL6	IL1β, IL6	–	[53]
	Collagen I and sulfated hyaluronan	–	IL6, IL8, IL10, MCP1, RANTES, TNFα, IL12(p40)	CD14, CD16, CD71, HLA-DR, CD163	[54]
	Hydrogel-coated PLGA nanofibres	–	IL8, CCL4, IL1β, TNFα, IL6,CCL2, MPO, sCD163	27E10, CD163	[44]
THP-1	Multidomain peptide hydrogel	IL1, CD36	–	CCR7, CD163	[55]
	2-hydroxyethyl methacrylate and water-soluble oligomers	–	–	CD54, CD86	[56]
	Poly(glycerol-sebacate) tubular scaffolds	–	ILβ, TNFα	–	[57]
	Metal mesh/tissue hybrid heart valve leaflets	–	TNFα	–	[58]
	Dextran/silver nanoparticles hybrid film coatings	–	ILβ, TNFα, PGE_2	–	[59]
Bone marrow-derived (mouse)	Poly(ethylene glycol)-based hydrogel	IL1β, TNFα, iNOS, IL12β, Arg1, VEGFA, IL10	–	–	[60]
	Electrospun polydioxanone scaffolds	iNOS, Arg1	TNFα, IL6, VEGF, TGF	–	[61]
	LPS-coated polystyrene microparticles	–	TNFα, IL6	CD11b, F4/80	[62]
	Poly-lactic co-glycolic acid-based hydrogel particles	–	IL1β, TNFα, IL6	–	[63]
	Mammalian extracellular matrix	–	–	F4/80, iNOS, Fizz1	[64]

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