Phenotypic Comparison between Adherence and Magnetic Beads Isolation of Monocyte Derived Dendritic Cells

Thesis

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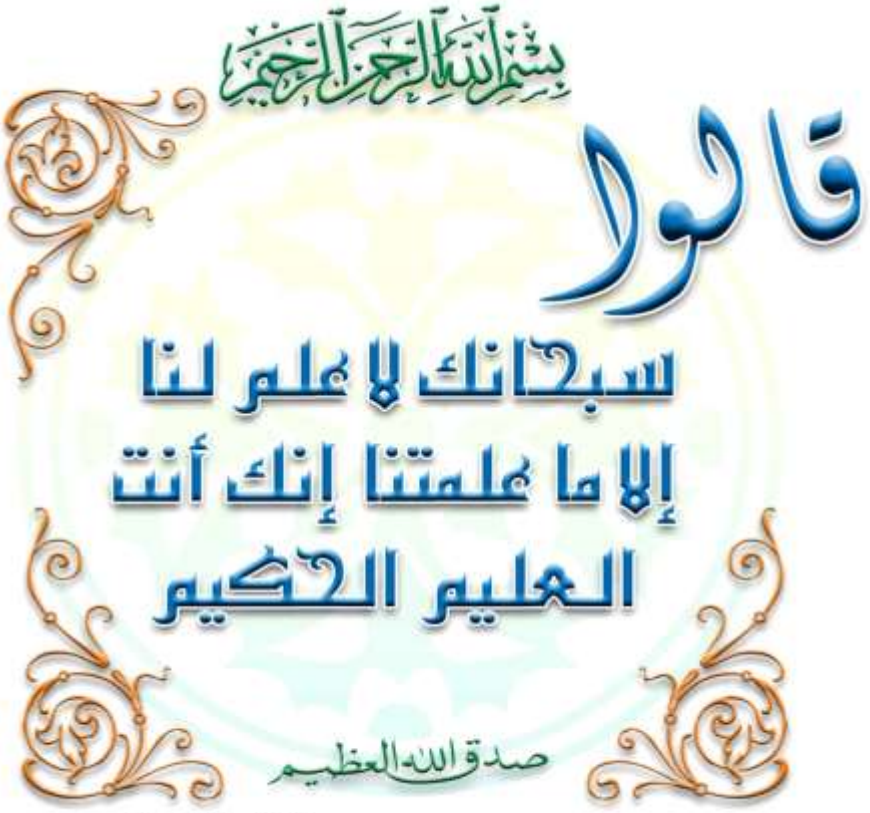

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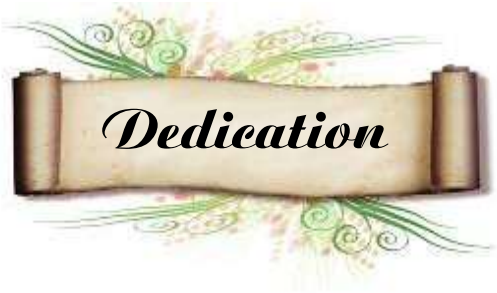

This thesis is dedicated to:

The soul of my dad and my great mom. . your kindness and extensive support, unconditional love have been ever present in my life, for which I am eternally grateful.

My beloved brothers (Ahmed and Hiatham)

All the people in my life who touch my heart,
I dedicate this research.

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List of Abbreviations

ATRA	All-trans retinoic acid
α-GalCer	Alpha galactosylceramide
Ab	Antibody
Ag	Antigen
APCs	Antigen presenting cells
AD	Autosomal dominant
AR	Autosomal recessive
BATF3	Basic leucine zipper transcriptional factor ATF-like 3
Bcl-6	B-cell lymphoma 6 protein
BDCA	Blood dendritic cell antigen
BBB	Blood-brain barrier
BM	Bone marrow
BrdU	Bromodeoxyuridine
CDH1	Cadherin-1
CMF-PBS	Calcium- and Magnesium-Free Phosphate-Buffered Saline
CCR	C-C chemokine receptor
CNS	Central nervous system
CCL	C-C chemokine ligand
CQ	Chloroquine
CD	Cluster of differentiation
CSF	Colony stimulating factor
CSF-1R	Colony stimulating factor-1 receptor
cMoP	Common monocyte progenitor
cDC-Ps	Conventional DC precursors
cDCs	Conventional or classical DCs
CD	Crohn's disease
CTLA-4	Ctotoxic T-lymphocyte-associated protein 4
CLEC	C-type lectin domain
CLA	Cutaneous leucocyte-associated antigen
COX-2	Cyclooxygenase-2
CTL	Cytotoxic T cell
DAMPs	Damage-associated molecular patterns
DCG	DC generation medium

DEC205	Dendritic and epithelial cells 205 or dendritic cell receptor for endocytosis 205
DCs	Dendritic cells
DCML	Dendritic cell, monocyte, lymphoid
E2-2	E protein
ELISA	Enzyme-linked immunosorbent assay
E-cadherin	Epithelial cadherin
EpCAM	Epithelial cell adhesion molecule
EAE	Experimental autoimmune encephalomyelitis
EAM	Experimental autoimmune myocarditis
FXIIIa	Factor XIIIa
FcεR1	Fc epsilon receptor I
FCS	Fetal calf serum
FRCs	Fibroblastic reticular cells
FACS	Fluorescent activated cell sorting
FITC	Fluorescin isothiocyanate
FLT3	FMS-like tyrosine kinase 3
FLT3L	FMS-related tyrosine kinase 3 ligand
Foxp3	Forkhead box P3
FSC	Forward scatter
GGT5	Gamma-glutamyl transferase 5
GM-CSF	Granulocyte monocyte-colony stimulating factor
GATA2	Guanine-adenine-thymine-adenine 2
HSC	Haematopoietic stem cell
HPC	Hematopoietic progenitor cells
HEV	High endothelial venules
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papilloma virus
IPCs	IFN-producing cells
iDC	Immature DC
ILT	Immunoglobulin-like transcript
IDO	Indole amine 2,3-dioxygenase
iNOS	Inducible nitric oxide synthase
ICOS	Inducible T-cell costimulator
IBDs	Inflammatory bowel diseases
IDECs	Inflammatory dendritic epidermal cells
ICAM	Intercellular adhesion molecule
IFN	Interferon

IRF	Interferon-regulatory factor
IL	Interleukin
ID/SC	Intradermal\subcutaneous
IEL	Intraepithelial lymphocyte
IN	Intranodal
iNKT	Invariant natural killer T
JAK/STAT	Janus kinase/Signal Transducer and Activator of Transcription
LCs	Langerhans cells
LPS	Lipopolysaccrides
LNs	Lymph nodes
LY	Lymphocyte antigen
LFA	Lymphocyte function-associated antigen
LTβR	Lymphotoxin β receptor
LTβ	Lymphotoxin β
LAMP	Lysosomal-associated membrane protein
MDP	Macrophage and DC precursor
MACS	Magnetic activated cell sorting
MHC	Major histocompatibility complex
MICA	MHC-class-I-polypeptide-related sequence A
MICB	MHC-class-I-polypeptide-related sequence B
MAPK	Mitogen-activated protein kinases
MLR	Mixed lymphocyte reaction
mAb	Monoclonal antibody
M-CSF	Monocyte- colony stimulating factor
MCM	Monocyte-conditioned medium
MC	Monocyte-derived cells
MoDC	Monocyte-derived DC
MonoMAC	Monocytopenia with Mycobacterium avium complex
MPS	Mononuclear phagocyte system
MLPs	Multi-lymphoid progenitors
MS	Multiple sclerosis
MOG	Myelin oligodendrocyte glycoprotein peptide
mDCs	Myeloid DCs
MDSCs	Myeloid derived suppressor cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
NKT	Natural killer T

NCAM	Neural cell adhesion molecule
NBS	Newborn Bovine calf serum
NKG2D	NK group 2, member D
NKp30	NK-cell protein 30
NLRs	NOD-like receptors
NLT	Non-lymphoid tissue
PLNs	Pancreatic lymph nodes
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
PBMCs	Peripheral blood mononuclear cells
PI3K	Phosphatidylinositide 3-kinases
PE	Phycoerythrin
PC5	Phycoerythrin-Cyanin 5
pDCs	Plasmacytoid DCs
PD-L	Programmed cell death ligand
PD-1	Programmed death protien1
PGE2	Prostaglandin E2
RELMα	Resistin-like molecule-α
RALDH	Retinaldehyde dehydrogenase
RA	Retinoic acid
RA	Rheumatoid arthritis
RNA–Seq	RNA sequencing
SP	Single-positive
SCF	Stem cell factor
SLAN	6-Sulpho LacNAc
SOCS1	Suppressor of cytokine signaling-1
SF	Synovial fluid
SLE	Systemic lupus erythematosus
SSC	Side scatter
TCM	Central memory T cells
TCR	T-cell receptor
Th	T helper
Tfh	Follicular T helper
TPO	Thrombopoietin
Tr1	Regulatory type 1 T cells
Tregs	Regulatory T cells
TSLP-R	Thymic stromal lymphopoietin receptor
TiP-DCs	TNF and inducible nitric oxide synthase-producing DCs

TRAIL	TNF-related apoptosis-inducing ligand
tDCs	Tolerogenic dendritic cells
TLR	Toll-like receptor
TGF-β1	Transforming growth factor β1
TNF	Tumor necrosis factor
TAAs	Tumor-associated antigens
TACSTD1	Tumor-associated calcium signal transducer 1
T1D	Type 1 diabetes mellitus
TKIs	Tyrosine kinase inhibitor
UC	Ulcerative colitis
Wnt	Wingless-Type MMTV Integration Site Family gene
XCR1	X-C Motif Chemokine Receptor 1
1MT	1-methyl-tryptophan
4-1BB	Type 2 transmembrane glycoprotein belonging to the TNF superfamily

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Abstract

Background: Dendritic cells are professional APCs able to modulate adaptive responses through recognition of pathogens, directly or indirectly, by sensing perturbations in the microenvironment such as infectious agents, cell damage, or inflammation. **Aim of the work:** to investigate which method of monocyte isolation, adherence method or magnetic activated cell sorting is the best as regards generation of DCs. **Subjects and Methods:** Samples in the present study were collected from healthy blood donors from the central blood bank at Ain Shams University Hospitals. All the donors aged between twenty and forty years, of both sexes (18 males and 2 females). They were found healthy in an orienting physical examination. All blood products were negative for common blood-borne pathogens (HBsAg, HIV Ab, HCV Ab), as detected by standard assays. Work area was at Clinical Pathology Department, Ain Shams University Hospital. **Results:** The present study revealed a statistically significant difference between Method 1 and Method 2 as regards the rate of decrease in $CD14^+$ cell population % from preculture to day 2, being higher in method 1. There was also a statistically significant difference between Method 1 and Method 2 as regards the rate of increase in $CD14^-$ cell population %, from preculture to day 2 with method 1 having higher rate of increase, whereas method 2 has shown a significantly higher rate of increase from day 2 to day 4 of culture. **Conclusion:** This study has shown that adherence method gave higher results of cell count and viability along the whole culture duration in comparison to MACS method. Despite of that, MACS method demonstrated higher results than plastic adherence in generating monocyte derived DCs, as it has shown significantly higher % of $CD14^-$ $CD11c^+$ $HLA\text{-}DR^+$ DCs population inspite of having a relatively lower mean % of $CD14^-$ population. A finding which could suggest that cells separated by MACS were much more capable of being readily transformed into DC than those separated by adherence method. **Recommendations:** Experiments on induction of further maturation of DCs generated from peripheral blood monocytes using TNFα, LPS, or CD40L are warranted for further use of mature DCs in preparation of DC vaccines for cancer therapy and for treatment of some infectious diseases.

Key words: dendritic cells, APCs, CD14, MACS, plastic adherence, HBsAg