بسم الله الرحمن الرحيم

« وعلمك ما لم تكن تعلم

وكان فضل الله عليك عظيما »

صدق الله العظيم

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EVALUATION OF DIFFERENT METHODS OF T- AND B-LYMPHOCYTE SEPARATION

THESIS

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Presented By

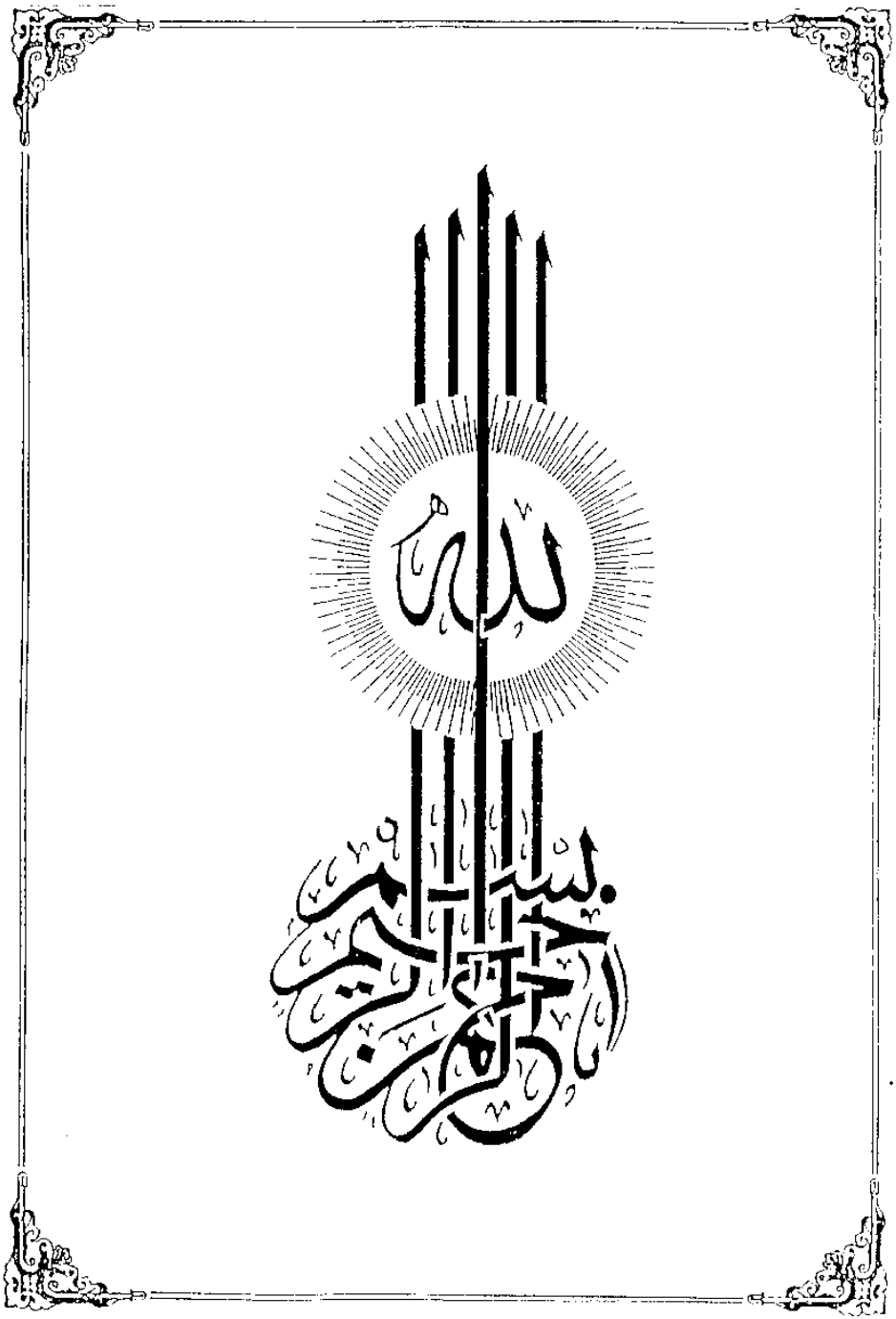
Azza Abd El Fattah Hegazi

Supervised By

Dr. O.S. Rasslan
Assistant Professor of Microbiology and Immunology
Faculty of Medicine
Ain Shams University

Dr. A.M. Zaki
Lecturer of Microbiology and Immunology
Faculty of Medicine
Ain Shams University

Faculty of Medicine
Ain Shams University
1992



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Dedication ...

To all members of my family for their great help and support.

CONTENTS

ABBREVIATIONS

SRBCs	Sheep red blood cells
PBMCs	Peripheral blood mononuclear cells
FH	Ficoll-hypaque
NK cells	Natural killer cells
CMI	Cell-mediated immunity
MHC	Major histocompatibility complex
TCR	T-cell receptor
Ig	Immunoglobulin
sIg	Surface immunoglobulin
CD	Cluster determinant
PMN cells	Polymorphonuclear cells
GVHR	Graft versus host reactivity
MCAs	Monoclonal antibodies
IL	Interleukin
AIDS	Acquired immune deficiency syndrome
HIV	Human immunodeficiency virus
CMV	Cytomegalovirus
T_H cells	T-helper cells
T_S cells	T-suppressor cells
HLA	Human leukocytic antigen
LPS	Lipopolysaccharide
APCs	Antigen presenting cells
C	Complement
iC	Inactive complement
LFA	Lymphocyte function antigen
ICAM	Intercellular adhesion molecule
PVP	Polyvinyl-pyrrolidone
EBV	Epstein Barr virus
PHA	Phytohemagglutinin
PWM	Pokeweed mitogen
HIFCS	Heat-inactivated fetal-calf serum

FCS	Fetal-calf serum
MIF	Migration inhibition factor
nSRBCs	Neuraminidase-treated SRBCs
AET-SRBCs	2-S aminoethylisothiouronium-treated SRBCs
pSRBCs	Papain-treated SRBCs
EDTA	Ethylene-diamino-tetra-acetic acid
HBSS	Hanks` balanced salt solution
BSA	Bovine-serum albumin
CLL	Chronic lymphocytic leukemia
EAC	Sheep erythrocyte coated with antibody and complement
MRBC	Mouse red blood cells
t-test	Hypothesis test
SD	Standard deviation
rpm	Rotations per minute
g	A unit of a rotation force
ed.	Editor

Introduction and Aim of the Work

INTRODUCTION AND AIM OF WORK

Introduction

The capacity to respond to immunologic stimuli rests principally in cells of the lymphoid system which include T-lymphocyte population that is human thymus-derived and responsible for cell-mediated immunity, and B-lymphocyte population that is bone marrow-derived and concerned in the synthesis of circulating antibodies (*Wigzell et al.*, 1972).

Analysis of immune responses involving distinctive cell types has been greatly facilitated by development of technology for separating and purifying different lymphocyte populations (*Schlossman and Hudson*, 1973).

Lymphocyte separation is useful in the analysis of an increased number, or of morphologically abnormal, peripheral blood lymphocytes with the objective of determining whether a malignant proliferation is present. Also, it helps in the analysis of bone marrow aspirates or lymphocytes obtained from lymph node biopsies to supplement the classic histopathological methods. A third function of lymphocyte separation includes evidence of some alteration in immune

system in different acute and chronic diseases. In some diseases such as infectious mononucleosis, the objective of the study is to characterize the cellular response to a known organism. In other infections such as leprosy, the objective is to characterize the elements of an ineffective immune response. In diseases of unknown etiology such as rheumatoid arthritis, systemic lupus erythematosus, and sarcoidosis, the studies are primarily made with a view toward uncovering features of the abnormal immune response that might lead to a better understanding of the nature of these diseases (*Ross and Winchester*, 1980).

Human peripheral blood T-cells can be identified by their formation of rosettes with sheep red blood cells (SRBCs) (E-rosettes: a phenomenon mediated by receptors on the T-cells specific for the SRBCs). This property, under normal conditions, is not shared by monocytes, granulocytes or B-lymphocytes, hence this method is a basis for separating T-cells from other peripheral blood mononuclear cells (PBMCs) (*Froland*, 1972; *Jondal et al.*, 1972). Since this method allows for the recovery of both the E-rosetted T-cells and the non-E-rosetted population, therefore it can be used for obtaining purified B-cells, provided that the monocyte cellular component of the peripheral blood mononuclear cell fraction obtained following Ficoll-hypaque density

gradient centrifugation, is eliminated or reduced prior to E-rosetting procedure (*Jondal et al.*, 1972).

The observation has been made that dextran, at optimal concentrations, added to lymphocyte-SRBC mixture can serve to enhance rosette formation. This may be in part due to a reduction in the electric charges at the cell surface which in turn allows a better cell to cell contact (*Brown et al.*, 1975).

The nylon wool separation of B-lymphocytes is based on the empirical observation that B-lymphocytes adhere preferentially to nylon wool from which they can be eluted, whereas T-lymphocytes do not adhere to the wool (*Eisen et al.*, 1972).

Aim of Work

This work is intended to evaluate three different, commonly used, methods for separation of T- and B-lymphocytes. These methods are E-rosetting using Ficoll-hypaque, rosetting with dextran and rosetting using nylon wool. The work aims at finding out the simplest, most rapid, most reliable and cheapest technique for lymphocyte separation.

Review of Literature