Viability of Interferon-β Supplemented Human Dendritic Cells after Infection with Mycobacterium Tuberculosis and Bovis

Thesis

Submitted for Partial Fulfillment of Master Degree in Clinical Pathology

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2016

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, I feel always indebted to ***Allah,*** *the Most Beneficent and Merciful.*

My most sincere gratitude is also extended to **Prof. Dr. Ghada Abdel Wahed Ismail,** *Professor of Clinical Pathology, Faculty of Medicine - Ain Shams University, for her enthusiastic help, continuous supervision, guidance and support throughout this work. I really have the honor to complete this work under her supervision.*

Words fail to express my appreciation to **Dr. Sally Mohamed Saber,** *Assistant Professor of Clinical Pathology, Faculty of Medicine - Ain Shams University, for her great help, valuable suggestions and directions throughout the whole work.*

Last but not least, I can't forget to thank all members of my ***Family****, for pushing me forward in every step in my life.*

Nada Ahmed Abo EL-fotouh

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List of Abbreviations

Abbr.	Full-term
ADA	: Adenosine deaminase
AFB	: Acid/Alcohol fast bacilli
ANOVA	: Analysis of variance
AO	: Auramine-O
APCs	: Antigen presenting cells
ASU	: Ain Shams University
BCG	: Bacillus Calmette-Guérin
BSC	: Biological safety cabinet
BSL-2	: Biosafety level 2
CDC	: Centers for disease control and prevention
CFU	: Colony forming unit
CIS	: Carcinoma in situ
CTL	: Cytotoxic T- lymphocytes
DAMPs	: Damage - associated molecular pattern
DCs	: Dendritic cells
DOT	: Directly observed therapy
EMB	: Ethambutol
FCS	: Fetal calf serum
FDA	: Food and drug administration
GI	: Growth index
GM-CSF	: Granulocyte macrophage- colony stimulating factor
IFN-β	: Interferon Beta
IFN-γ	: Interferon Gamma
IGRAs	: Interferon-gamma release assays
IL	: Interleukin

INH : Isoniazid
LAM : lipo-arabinomannan
LFA-1 : Lymphocyte function – associated antigen 1
LPS : Lipopolysaccarides
LTBI : latent tuberculosis infection
M T.B : Mycobacterium tuberculosis
M.Bovis : Mycobacterium bovis
MAC : Mycobacterium avium complex
MDR : Multidrug-resistant
MOHP : Ministry of Health and Population
MOI : Multiplicity of infection
MRC : Medical research counsil
MTBC : Mycobacterium tuberculosis complex
MVA85A : Modified Vaccina Ankara 85 A
NAAT : Nucleic acid amplification techniques
NALC : N-acetyl-L-cysteine
NHS : National health organization
NTM : Non-tuberculous mycobacteria
PAMPs : Pathogen - associated molecular pattern
PCR : Polymerase chain reaction
PPE : Personal protective equipment
PRRs : Pattern- recognition receptors
PZA : Pyrazinamide
QFT-G : QuantiFERON-TB Gold
QFT-GIT : QuantiFERON-TB Gold in–tube test.
RIF : Rifampin
RPT : Rifapentine
SCID : Severe combined immunodeficiency
SD : Standard deviation

SPSS : Statistical program for social science
T.B : Tuberculosis
Th : T- helper
TLR : Toll like receptors
TNF : Tumor necrosis factor
TST : Tuberculin skin test
TUR : Transurethral resection
UV : Ultra violet
VLA-4 : Very late antigen-4
WHO : World Health Organization.
XDR : Extensively drug-resistant
ZN : Ziehl- Neelsen

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Introduction

The World Health Organization (WHO) considered Egypt one of the high burden countries for tuberculosis (T.B) infection in Eastern Mediterranean region. The National Tuberculosis Control Program of the Ministry of Health and population (MOHP), registered that 12,000 new T.B patients were diagnosed each year and more than 50% of them were found to be sputum smear positive pulmonary T.B. Additionally, it is estimated that about 8000 people receive a diagnosis of T.B at facilities other than those of MOHP ***(Helal et al., 2009).***

The number of new T.B cases continues to increase, despite intensive global efforts ***(Lee et al., 2010).*** One-third of the world's population is thought to have been infected with Mycobacterium tuberculosis (M T.B), and new infections occur in about 1% of the population each year. In 2007, an estimated 13.7 million chronic cases were active globally, while in 2013, an estimated 9 million new cases occurred, most of which occurred in developing countries, the number reduced due to availability of T.B vaccines ***(WHO, 2014a).***

The only available vaccine against T.B is Bacillus Calmette-Guérin (BCG). The estimated efficacy of BCG vaccination was found to be up to 80%. In children it decreases the risk of getting the infection by 20% and the risk

of reactivation by nearly 60%. It is the most widely used vaccine worldwide, with more than 90% of all children being vaccinated. The immunity it induces decreases after about ten years ***(Roy et al., 2014).***

Given the variable protective efficacy provided by Mycobacterium bovis (M.bovis) BCG there is an urgent need to develop new vaccines against T.B ***(Giacomini et al., 2009).***

The beneficial effects of BCG vaccine could be the result of either strengthening of pro-inflammatory mechanisms, helping neonates to fight infections, or the induction of an immune- regulatory network restricting overt inflammation and intense pathology ***(Madura et al., 2007).***

Protective immunity against M T.B is associated with antigen presentation by the antigen presenting cells (APCs) to CD_4 and CD_8 T cells which in turn initiate a specific cellular immunity against the intracellular pathogens. Dendritic cells (DC) are the most efficient APC, which are highly represented on the sites of M T.B infection at the onset of the inflammatory response. DC are a central component of the immune system for their extraordinary capacity to initiate and modulate the immune responses elicited upon recognition of infectious agents ***(Pereira and Paiva, 2011).***

The development of interferon- γ (IFN- γ) secreting CD4 T cells is dependent on the secretion of IL-12 by infected DC, and this can be markedly enhanced by the stimulation of CD40 on infected- DC which occurs early after mycobacterial infection of DC ***(Britton and Palendira, 2003).***

IFN- γ is the most important cytokine for inducing the macrophage killing activation mechanism ***(Moura et al., 2004).*** The study conducted in 2012, confirmed this fact, where children with active T.B showed significant increase in mean IFN- γ levels using Quantiferon 2 tubes test ***(Abdel Rahman et al., 2013).***

Taking into consideration these facts, DC had generated from peripheral blood, as an initial step for comparing the effect induced by BCG and M T.B infection on the DC immunophenotype indicated that BCG is less efficient in inducing DC maturation than M T.B. In addition, BCG-infected DC poorly expressed interferon-β (IFN-β) and displayed a reduced production of IL-12 as compared with M T.B-stimulated cells. The impaired expression of IL-12p35 and IFN-β is likely a result of the inability of BCG to induce the activation of the IFN regulatory factor-3. Taking into account these data, investigation of the exogenous addition of IFN-β, a cytokine that exerts important effects on the immune system, could enhance the Th1-polarizing capacity of BCG-infected DC. Interestingly, when DC infected by

BCG were pretreated in vitro with IFN-β, they displayed a fully mature phenotype and released a significant amount of bioactive IL-12p70, which resulted in an enhanced Th1 response. This demonstrates that IFN-β potentiates DC immunological functions following BCG infection, thus suggesting INF-β as a possible candidate as vaccine adjuvant ***(Giacomini et al., 2009).***

Aim of the Work

This work aims to assess the viability of human mononuclear DC after infection with M T.B and M. bovis with and without interferon-β supplementation as a preliminary step for evaluation of cytokines production.