Effect of Tuberculin inhalation on the lung of Guinea pigs after experimental induction of allergic asthma

Thesis
Submitted for partial fulfillment of medical doctor degree in
Internal Medicine

By
Sylvia Talaat Kamal Abd Elsayed
MB. B. Ch. M. Sc.

Under supervision of

Prof. Dr. / Maged Mohamed Refaat
Professor of internal medicine, allergy and clinical immunology.
Faculty of Medicine-Ain Shams University.

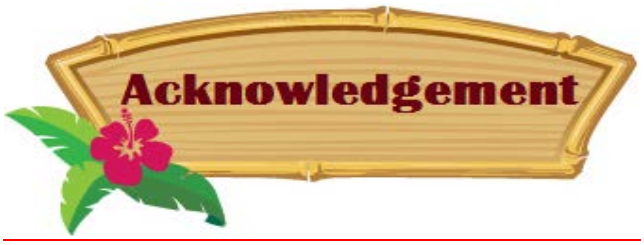
Professor Dr/Amel Ali Soliman Abo-Amer
Professor of Histology
Faculty of Medicine-Ain Shams University.

Ass. Professor Dr/Hala Salah Abd El Kawi
Assistant Professor of Pharmacology
Faculty of Medicine-Ain shams university.

Dr/ Nermine Abd Elnour Melek
Assistant Professor of internal medicine, allergy and clinical immunology.
Faculty of medicine-Ain Shams University.

Dr/Eman Elsayed Ahmed
Lecturer of internal medicine, allergy and clinical immunology.
Faculty of Medicine-Ain Shams University.

Faculty of Medicine
Ain Shams University
2014



*I humbly submit my sincere gratitude to **GOD** our creator who has guided me to every success I had in my life.*

*Special gratitude is offered to **Prof.– Dr.– / Maged Mohamed Refaat**,- Professor of Internal Medicine,- Allergy and Clinical Immunology,— Faculty of Medicine,— Ain Shams University,– for his constructive criticism,– generous help and encouragement.*

*Sincere appreciation is extended to **Prof.- Dr.- / Amel Ali Soliman**,– Professor of Histology,– Faculty of Medicine,– Ain Shams University.*

*Special recognition and sincere thanks to **Dr.- / Hala Salah abd elkawy**,– Assistant Professor of pharmacology,– Faculty of Medicine,- Ain Shams University,- for giving personal assistance, encouragement and valuable advice.*

*Special gratitude is offered to **Dr.- Nermine Abd El Nour Melek**,– Assistant Professor of Internal Medicine,– Allergy and Clinical Immunology,— Faculty of Medicine,— Ain Shams University for being extremely helpful to make this work succeed.*

*Special thanks are offered to **Dr.- Eman El Sayed Ahmed**, lecturer of Internal Medicine,- Allergy and Clinical Immunology, Faculty of Medicine,- Ain Shams University.*

Finally special gratitude to my family especially my mother & my husband who were very supportive to me.

Sylvia Talaat Kamal Abd Elsayed

I humbly submit my sincere gratitude to **GOD** our creator who has guided me to every success I had in my life.

Special gratitude is offered to **Prof. Dr. / Maged Mohamed Refaat**, Professor of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University, for his constructive criticism, generous help and encouragement.

Sincere appreciation is extended to **Prof. Dr. / Amel Ali Soliman,** Professor of Histology, Faculty of Medicine, Ain Shams University.

Special recognition and sincere thanks to Dr. / Hala Salah abd elkawy, Assistant Professor of pharmacology, Faculty of Medicine, Ain Shams University, for giving personal assistance, encouragement and valuable advice.

Special gratitude is offered to **Dr. Nermine Abd El Nour Melek,** Assistant Professor of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain

Shams University for being extremely helpful to make this work succeed.

Special thanks are offered to **Dr. Eman El Sayed Ahmed**, lecturer of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University.

Finally special gratitude to my family especially my mother & my husband who were very supportive to me.

Sylvia Talaat Kamal Abd Elsayed

List of Contents

List of Abbreviations

ACQ : Asthma control questionnaire

AHR : Airway resistance

AMC : Acid mammalian chitinase

APCs : Antigen presenting cells

ASM : Airway smooth muscle

BAL : Broncho-alveolar lavage

BALF : Broncho-alveolar lavage fluid

BCG : Bacillus Calmette–Guérin

CCL : Chemokine (C -C motif) ligand

CCR3 : C-C chemokine receptor type 3

Cdyn : Dynamic compliance

CI 95% : 95% confidence interval

CRTH : Chemo-attractant homologous receptor

CXCL : Chemokine (C-X-C motif) ligand

cys-LTs : Cysteinyl leukotrienes

DCs : Dendritic cells

EAR : Early allergic response

EDN : Human eosinophil-derived neurotoxin

FcεRI : Fc receptor for IgE

List of Abbreviations (Cont.)

FEV1 : Forced expiratory volume in the first second

FOXP3 : Forkhead box P3

FVC : Forced vital capacity

GATA3 : GATA-binding protein 3

GM-CSF : Granulocyte-macrophage colony-stimulating factor

~~List of Abbreviations~~ (Cont..))

H&E : Haematoxylin and eosin

HR : Histamine receptors

i.p. : Intraperitoneal

I.U. : International unit

i.v. : Intravenous

ICS : Inhaled corticosteroid

IFN-γ : Interferon-gamma

IgE : Immunoglobulin E

IL : Interleukin

IL-4Rα : IL-4 alpha chain receptor

L.M. : Light microscopy

LABAs : Long acting beta 2 agonists

LAR : Late allergic response

LTC4 : Leukotriene C4

M. vaccae : Mycobacteria vaccae

MAPK : Mitogen-Activated Protein Kinases

MCs : Mast cells

List of Abbreviations (Cont.)

MHC : Major histocompatibility complex

NFAT : Nuclear factor of activated T cells

NKT cells : Natural killer T cells

OVA : Ovalbumin

PAMPs : Pathogen-associated molecular patterns

PBS : Phosphate buffered saline

PEF : Peak expiratory flow

PG : Prostaglandin

PPD : Purified protein derivative

~~List of Abbreviations~~ (Cont..))

RL : Pulmonary resistance

RORγt : Retinoic acid orphan receptor-γt

RT-PCR : Real- Time PCR

SCIT : Subcutaneous immunotherapy

SD : Standard deviation

SEM : Standard error of the mean

SEM : Scanning electron microscopy

SIT : Specific immunotherapy

SLIT : Sublingual immunotherapy

sRaw : Specific airway resistance

STAT6 : Signal Transducer And Activator Of Transcription 6

TCR : T-cell receptor

List of Abbreviations (Cont.)

TGF-β **:** Transforming growth factor beta

Th cell **:** T-helper cell

TLR **:** Toll-like receptor

TNF : Tumor necrosis factor

Tregs : T regulatory cells

TSLP **:** Thymic stromal lymphopoietin

V_T : ——————Tidal volume

WT animal: ——————Wild type animal

List of Tables

List of Figures

List of Figures (Cont.)

List of Figures (Cont.)

Fig.	Subject	Page
23	A scanning electron micrograph of an asthmatic guinea pig	

List of Figures (Cont.)

Introduction

Asthma is a heterogeneous inflammatory disorder of the airways characterized by chronic airway inflammation, airway hyper-reactivity (AHR) and by symptoms of recurrent wheezing, coughing and shortness of breath. Asthma is a major public health problem, affecting 300 million people worldwide, and has increased considerably in prevalence over the past three decades (**Wilson et al., 2006**).

Clinically, although several distinct forms of asthma are recognized, the major focus of treatment and research over the past ~~25~~ few years has been on allergic asthma, the most common form of asthma. Allergen-specific T helper type 2 (Th2) cells are thought to be present in the lungs of almost all patients with asthma, particularly patients with allergic asthma **(Robinson et al., 1992)**.

~~Th2 cells produce cytokines that regulate the allergen-specific synthesis of immunoglobulin E (IgE), the recruitment of eosinophils, the recruitment and growth of mast cells, and AHR, a cardinal feature of asthma **(Holgate and Polosa, 2008).**~~

Also, it has been assumed that Treg cells play an important role in controlling ~~such~~ T~~-helper type~~h 2~~-~~biased

responses both in animal models and studies in humans **(Strickland et al., 2006; Gogishvili et al., 2007).**

At the present, the most effective standard anti-asthmatic drugs available include inhaled β2-agonists and gluco-corticoids, these control asthma in about 90-95% of patients, **(Caramori et al., 2008).** The remaining 5% to 10% of asthma patients have severe disease that is difficult to control despite optimum management of co-morbid conditions and environmental triggers, optimum therapy, and good adherence to conventional treatment regimens **(Stewart et al., 2010).**

Hence, there is a great need for new therapies of allergic asthma. In the last few years many studies were concerned by the role of BCG in asthma management, it showed promising results in animal models. However, ~~m~~ its mode of action is still controversial. **Zuany-Amorim et al. (2002),** stated that BCG regulates the allergic host immune responses through the induction of Treg cells, rather than affecting the Th1/Th2 balance.

Based on the good results of BCG in animal models of asthma, the present thesis worked upon inhaled tuberculin as a new suggested therapy to asthma. Asthma animal model is