Assessment of IL17 Producing neutrophiles in Bronchial Asthma Patients with Fungal Allergy

Thesis
Submitted for partial Fulfillment of Master Degree in
Internal Medicine

By
Hazem Ezzat Abd Elbadie
M.B.BCh
Faculty of Medicine
Ain Shams University

Supervised by

Prof. Dr. Mohmmed Kamel Sabry

Professor of Allergy and clinical Immunology,
Faculty of Medicine - Ain Shams University.

Dr. Nermine Abd Elnour Melek

Assisstant Professor of Allergy and clinical Immunology
Faculty of Medicine - Ain Shams University.

Dr. Eman El Sayed Ahmed

Assisstant Professor of Allergy and clinical Immunology
Faculty of Medicine - Ain Shams University.

Faculty of Medicine
Ain Shams University
2016

Acknowledgement

*First of all, all gratitude is due to **Allah** almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.*

*Really I can hardly find the words to express my gratitude to **Prof. Dr. Mohmmed Kamel Sabry**, Professor of Internal Medicine and immunology, Faculty of Medicine. Ain Shams University. for his supervision, continuous help, encouragement throughout this work and tremendous effort he has done in the meticulous revision of the whole work. It is a great honor to work under his guidance and supervision.*

*I would like also to express my sincere appreciation and gratitude to **Dr. Nermine Abd Elnour Melek**, Assisstant Professor of Internal Medicine and immunology, Faculty of Medicine. Ain Shams University, for her continuous directions and support throughout the whole work.*

*I cannot forget the great help of **Dr. Eman El Sayed Ahmed**, Lecturer of Internal Medicine and immunology, Faculty of Medicine. Ain Shams University. for her invaluable efforts, tireless guidance and for his patience and support to get this work into light.*

*Words fail to express my love, respect and appreciation to **my wife** for her unlimited help and support.*

Last but not least, I dedicate this work to my family, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.

Hazem Ezzat Abd Elbadie

List of Contents

Page

Acknowledgment ..... --

List of Abbreviations i

List of Figures iii

List of Tables v

Introduction 1

Aim of The Work 3

Review of Literature 4

Chapter 1: Fungal allergic asthma 4

Chapter 2: Interleukin 17 24

Chapter 3: Interleukin 17 in allergic asthma 28

Chapter 4: Neutrophil in allergic asthma 32

Subjects and Methods 42

Results 53

Discussion 69

Summary 76

Conclusion 78

Recommendations 79

References 80

Arabic Summary ..... --

List of Abbreviations

AA	:	Arachidonic acid
ABPA	:	Allergic bronchopulmonary aspergillosis
ABPM	:	Allergic bronchopulmonary mycosis
APCs	:	Antigen presemting cells
BEC	:	Bronchial epithelial cells
CF	:	Cystic fibrosis
COPD	:	Chronic obstructive airways disease
CTLA	:	Cytotoxic T-Lymphocyte Antigen
CXCL1	:	Chemokine (C-X-C motif) ligand 1
CXCR4	:	Chemokine receptor type 4
DCs	:	Dendritic cells
EAACI	:	The European Academy of Allergy and Clinical Immunology
ECP	:	Eosinophil cationic protein
EPO	:	Eosinophil peroxidase
G-CSF	:	Granulocyte colony stimulating factor
GM-CSF	:	Granulocyte Macrophage Colony- Stimulating Factor
GPI	:	Glycosyl-phosphatidylinositol
ICS	:	Inhaled corticosteroids
IFN	:	Interferon
Ig	:	Immunoglobulin
IgE	:	Immunoglobulin E
IL	:	Interleukin
ILCs	:	Innate lymphoid cells
iNKT	:	invariant natural killer T
LPS	:	Lipopolysaccharide
LT	:	Leukotriene
LTi	:	Lymphoid tissue inducer
MAPKs	:	Mitogen activated protein kinase
MDC	:	Macrophage-Derived Chemokines
MHC	:	Major histocompatibility complex
MIP	:	Macrophage inflammatory protein

List of Abbreviations (Cont.)

MMP	:	Matrix metalloproteinase
NE	:	Neutrophil elastase
NF-kB	:	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	:	Natural killer
NO	:	Nitric Oxide
OA	:	Occupational asthma
PAF	:	Platelet Activating Factor
PAMPs	:	Pathogen associated molecular patterns
PCR	:	Polymerase chain reaction
PG	:	Prostaglandin
PRRs	:	Pattern recognition receptors
SAFS	:	Severe asthma with fungal sensitization
SDF-1	:	Stromal cell-derived factor 1
SPT	:	Skin-prick test
TARC	:	Thymus and Activation-Regulated Chemokines
TCR	:	T cell receptor
Th	:	T helper
TH2	:	T helper 2
TNF	:	Tumour necrosis factor
VLA-4	:	Very late antigen-4

List of Figures

Fig.	Title	Page
1	Patterns of fungal interactions with humans, illustrating different host pathogen interactions, based on the host damage response framework.	7
2	Two contrasting disease models (A and B), with common elements of risk and exposure, but different outcomes.	9
3	Sputum samples of asthma phenotypes.	16
4	Factors that affect the size of the marginated and circulating granulocyte pools.	36
5	Effects of local and systemic inflammation on neutrophil kinetics.	39
6	Skin prick test.	46
7	CD177 by flowcytometry.	50
8	Sex among Bronchial asthma patients.	54
9	Description of laboratory data among bronchial asthma patients.	55
10	Sex in control group.	56
11	Description of laboratory data in control group.	57
12	Comparison between cases and controls as regard fungal allergy.	58
13	Comparison between cases and controls as regard CD-177 %.	60
14	Comparison between cases and controls as regard IgE.	60
15	Comparison between positive and negative fungal asthmatic cases as regard FEV1 %.	61
16	Comparison between positive and negative fungal asthmatic cases as regard laboratory data.	62

List of Figures (Cont.)

Fig.	Title	Page
17	Comparison between mild, moderate and severe asthmatic cases as regard CD-177 %.	64
18	Correlation between IgE and CD-177 among both study groups.	66
19	Correlation between FEV1 % and CD-177 among both study groups.	66
20	Correlations between IgE and CD-177 among case groups	68

List of Tables

Table	Title	Page
1	Description of personal and medical data among patients (bronchial asthma).	53
2	Description of laboratory data among bronchial asthma patients.	55
3	Description of personal and medical data in control group.	56
4	Description of laboratory data in control group.	57
5	Comparison between cases and controls as regard personal and medical.	58
6	Comparison between cases and controls as regard laboratory data.	59
7	Comparison between positive and negative fungal asthmatic cases as regard demographic, pulmonary function results and severity grading.	61
8	Comparison between positive and negative fungal asthmatic cases as regard laboratory data.	62
9	Comparison between mild, moderate and severe asthmatic cases as regard age.	63
10	Comparison between mild, moderate and severe asthmatic cases as regard sex and fungal allergy.	63
11	Comparison between mild, moderate and severe asthmatic cases as regard laboratory data.	64
12	Correlations between different variables and CD-177 among both study groups.	65

List of Tables (Cont.)

Table	Title	Page
13	Correlations between different variables and CD-177 among bronchial asthma patients	67
14	Correlations between different variables and CD-177 among control group	68

Assessment of IL17 Producing Neutrophiles in Bronchial Asthma Patients with Fungal Allergy

ABSTRACT

Prof. Dr. Mohmmed Kamel Sabry; Dr. Nermine Abd Elnour Melek; Dr. Eman El Sayed Ahmed; Hazem Ezzat Abd Elbadie
Faculty of Medicine – Ain Shams University

Introduction: Asthma is a heterogeneous chronic inflammatory respiratory disease characterized by overproduction of mucus and airway-wall remodeling that leads to bronchial hyperactivity and airway obstruction. Allergens and some pathogens have been implicated in the worsening of asthma, For many years, allergic asthma has been considered a T helper 2 (TH2)-biased disease, characterized by eosinophil infiltration and the production of the cytokines interleukin (IL)-4, IL-5, and IL-13. **Subjects and Methods.** This study was conducted on 40 asthmatic patients and 20 healthy control subjects. Patients were selected from the allergy and immunology outpatient clinic at Armed Forces Hospital, Alexandria during the period from November 20l4 to December 20l5, **Results**
The present study comprised three groups, group l included l8 asthmatics with positive skin prick test to fungi, group 2 included 22 asthmatics with positive skin test to other allergens and the control group included 20 healthy volunteers. All groups were matched in age and sex. **Conclusion**.We identified a subpopulation of CD177% neutrophils in peripheral blood of allergic asthmatic patients and healthy controls with statistically significant difference between both groups being higher in asthmatics (especially those with mild and moderate asthma). Therefore, we can conclude that this cell population might be contributing during the initial phase asthmatic disease and/or during disease progression but its role has not yet been established. Also it is not possible to define any relation between this cell subpopulation and fungal allergy or severity of asthma.

Key words: AA: Arachidonic acid; ABPA: Allergic bronchopulmonary aspergillosis; ABPM: Allergic bronchopulmonary mycosis.

Introduction

Asthma is a heterogeneous chronic inflammatory respiratory disease characterized by overproduction of mucus and airway-wall remodeling that leads to bronchial hyperactivity and airway obstruction. Allergens and some pathogens have been implicated in the worsening of asthma **(Murray, 2006).**

For many years, allergic asthma has been considered a T helper 2 (TH2)-biased disease, characterized by eosinophil infiltration and the production of the cytokines interleukin (IL)-4, IL-5, and IL-13 **(Woodruff et al., 2009)**. A TH17-biased response has also been observed in patients that exhibit chronic inflammation **(Pène et al., 2008)** and particularly in those with severe asthma who respond poorly to steroids, where inflammatory cellular infiltration in the airway is primarily due to CD4+ TH17 cells and neutrophils **(Al-Ramli et al., 2009; Green et al., 2002)**.

It is known that IL-17 is increased in BAL fluid, sputum and blood from patients with asthma **(Wong et al., 2001).** The role of IL-17 in asthma is an area of intense current investigation. As the role of IL-17 in neutrophil recruitment to the airways is well known **(Fei et al., 2011),** in the last years several studies found that the numbers of neutrophils in the sputum **(Green et al., 2002)**, bronchoalveolar lavage **(Lommatzsch et al., 2006),** bronchial biopsies **(Qiu et al., 2007)** and also in peripheral blood **(Asman et al., 1997)** of allergic asthmatic patients have been shown to increase concomitantly with IL-17 levels **(Zhao et al., 2010).** Multiple lines of evidence show a link between an increase in neutrophil numbers and the exacerbation, progression, severity, and difficulties in the control of asthmatic disease **(Green et al., 2002).**

IL-17 is mainly produced by TH17 cells, but recent studies have begun to uncover non-CD4 T cells as important sources of IL-17A (the most common form of IL-17), such as CD8+ T cells, γδ T cells, natural killer cells, and granulocytes **(Korn et al., 2009).** In addition, it has been shown that murine neutrophils release IL-17 **(Ferretti et al., 2003).**

It has been reported that neutrophils regulate the infiltration of CD8+ T cells to the inflammation site in an animal model for fungal airway allergy **(Park et al., 2006)**. Neutrophils could also act as antigen-presenting cells to promote IL-17 production by CD4 and CD8+ T cells **(Abi et al., 2011)** but no further studies have investigated the expression and release of IL-17A from human peripheral blood neutrophils in fungal airway allergy.

Aim of the Work

In this study, we aim to analyze CD-177% in the peripheral blood of atopic asthmatic patients those with fungal allergy and those without, compared to healthy individuals, and if this neutrophil subpopulation could be related to any disease variable.

Fungal allergic asthma

Fungal exposure is a daily fact of human existence, which infrequently results in disease. Yet fungal allergy drives asthma severity in very large numbers of people affected by severe asthma. Available statements from different medical associations are unequivocal in declaring that fungi are sensitizers and exacerbate allergic asthma (American College of Occupational and Environmental Medicine, Institute of Medicine, American Academy of Allergy, Asthma and Clinical Immunology and American College of Medical Toxicology). Increasing rates of fungi-associated occupational asthma are also of concern **(Bush et al., 2006)**.

While fungal exposure is universal, sensitisation and disease are not. Very early life airborne contact with fungi is well demonstrated by studies with Pneumocystis serology and pneumonia in healthy children and those with cancer **(Pifer et al., 1978)**.

Broadly speaking, fungi can cause problems to the lung in two ways; either by acting as aeroallergens or as a pathogen causing infection. Some fungi can do both, often simultaneously. To cause infection in the lung the fungus has to be able to grow at body temperature and this property is restricted to a relatively narrow range of fungi, particularly yeasts and members of the Aspergillus and Penicillium genera. The commonest fungus causing lung infections is Aspergillus fumigatus, although other Aspergillus spp. are also implicated. Fungal allergens, which can cause rhinitis and asthma, but rarely cause infection, include spores from the plant pathogens Cladosporium and Alternaria spp. A third potential cause of ill-health from fungi are volatile organic compounds and mycotoxins released by moulds such as Stachybotrys spp,

which remains controversial and will not be discussed here in depth **(Hedayati et al., 2007)**.

Sensitization and allergy:

Allergy is an inflammatory response caused by an environmentally delivered and often non-pathogenic agent and is caused by an exaggerated immune response rather than the pathogenic, pharmacological or toxic properties of the primary agent. As fungi are complex eukaryotes, all forms of allergic immune response should be considered as potentially leading to fungal allergy, although the most well-recognised clinical responses, such as asthma and rhinitis caused by Alternaria alternata, are mediated in a straightforward immunoglobulin (Ig) E/TH2 manner. The stipulation on including evidence of an inflammatory response in the definition of allergic disease is to distinguish allergy from sensitisation. Many people with elevated specific serum (s) IgE (or for that matter other intermediates of immune response) against a certain agent (sensitisation) do not develop symptoms when exposed to that agent. However, this is not a fixed difference as sensitisation can evelove into allergy depending on the level of exposure, co-factors present at the time of exposure and the age of person, with periods in their life when they develop symptoms and periods when they have sub-clinical disease or are in complete remission induced by immune tolerance **(Johansson et al., 2001)**.

Infection:

Viable microorganisms including fungi can have a range of interactions with their human host. Infection refers to the presence of a microorganism, which leads directly to ill-health as a result of its pathogenic properties. Colonisation refers to any situation where a microorganism becomes established in a new environment and doesn't